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Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar

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Many studies have examined effects of nutrient availability on constitutive herbivore resistance of plants, but few have addressed effects on expression of rapid induced resistance (RIR). We quantified effects of two levels of nutrient availability on growth, biomass allocation, photosynthesis, and constitutive secondary metabolism of black poplar (*Populus nigra*). We also examined effects of nutrient availability on expression of constitutive resistance of poplar to gypsy moth (*Lymantria dispar*) and whitemarked tussock moth (*Orgyia leucostigma*), as well as RIR to both folivores in response to localized herbivory by gypsy moth.

The high nutrient treatment had no effect on photosynthetic rate of poplar, but dramatically increased relative growth rate, total biomass, and total leaf area, while foliar phenolic concentrations and root:shoot ratio decreased. Plant growth was negatively correlated with foliar phenolic concentrations, which is consistent with predictions of the Growth/Differentiation Balance Hypothesis when increased nutrient availability increases growth without affecting photosynthesis. These responses of root:shoot ratio and constitutive secondary metabolism to nutrient availability are consistent with those proposed by models of adaptive phenotypic plasticity in resource allocation patterns.

Nutrient availability affected constitutive resistance of poplar to first and fifth instar gypsy moth larvae, which grew much faster on high fertility plants. However, nutrient availability had no effect on constitutive resistance to whitemarked tussock moth. Localized herbivory elicited systemic RIR in poplar within 72 hours. However, the magnitude of RIR was dependent on nutrient availability, with differing effects on the two insect species. Expression of RIR to gypsy moth was most dramatic in the high fertility treatment. In contrast, RIR to whitemarked tussock moth was expressed only in the low fertility treatment. The idiosyncratic nature of effects of nutrient availability on constitutive and induced resistance challenges the value of using insect bioassays as surrogate measures of secondary metabolism for testing allocation models of plant defense, as well as the value of generalized plant defense models for predicting effects of environmental variation on resistance to specific herbivores. These results also suggest that the effects of nutrient availability on the expression of RIR may represent a largely over-looked source of variation in plant/herbivore interactions.

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Responses of woody plants to defoliation have been shown to decrease host quality for herbivores in a variety of systems (Schultz 1988, Karban and Myers 1989, Haukioja 1990, Karban and Baldwin 1997). These herbivore-induced responses have been classified as rapid or delayed (Haukioja 1980, 1990), with rapid induced resistance (RIR) occurring within hours or days of damage, and delayed induced resistance (DIR) expressed in the year(s) following defoliation.

Differential effects of resource availability on the expression of constitutive and induced resistance are a potential source of variation in herbivore response to environmentally induced changes in host quality (Lombardero et al. 2000). However, only a few studies have addressed environmental effects on the expression of herbivore-induced resistance of woody plants to folivores. Effects of nutrient availability on the expression of DIR of trees have received most attention (Tuomi et al. 1984, Bryant et al. 1993, Ruohomäki et al. 1996), while only a few studies have addressed effects of nutrient availability on expression of RIR, with divergent results. Hunter and Schultz (1995) found that fertilization may suppress expression of RIR to sap-sucking insects in red and chestnut oak (*Quercus rubra* and *Q. prinus*, respectively), and to gall-forming insects on red oak. On the other hand, Mutikainen et al. (2000) found that RIR of silver birch (*Betula pendula*) to *Epirrita autumnata* was expressed only in fertilized trees.

Many more studies have addressed effects of nutrient availability on expression of constitutive resistance of woody plants to herbivores (Kytö et al. 1996). In their review, Kytö et al. (1996) found that fertilization of woody plants increased host quality in almost every case by increasing the nutritional quality of plants and/or decreasing secondary metabolite production. The Growth/Differentiation Balance Hypothesis (GDBH) (Loomis 1932, Lorio 1986, Herms and Mattson 1992) attributes effects of nutrient availability on constitutive secondary metabolism to a resource-based, physiological trade-off between primary and secondary metabolic pathways, because both are resource-demanding processes which compete for limited resources within the plant. Recent studies have increased evidence for the existence of a trade-off between growth and secondary metabolism (Han and Lincoln 1994, Hwang and Lindroth 1997, Zangerl et al. 1997, Baldwin 1998). The GDBH, as extended by Herms and Mattson (1992), predicts a non-linear, parabolic response of constitutive secondary metabolism to variation in nutrient availability. Hence, GDBH predicts that increased nutrient availability can either increase or decrease secondary metabolite concentration, depending on the initial nutrient status of the plant (Herms and Mattson 1992, Herms 1999). Both responses have been observed (Kytö et al. 1996, Koricheva et al. 1998).

The parabolic response predicted by GDBH emerges from integration of the physiological trade-off between growth and secondary metabolism with documented effects of nutrient availability on source/sink interactions (Herms and Mattson 1992). Woody plants have been observed to increase their growth rates in response to increased nutrient availability by increasing total leaf area, with much less effect on the photosynthetic rate of individual leaves (Luxmoore 1991, Ericsson 1995, Samuelson 1998). The growth of immature leaves, which are strong photosynthetic sinks (Patrick 1988, Marcelis 1996), is supported by carbohydrates exported from neighboring, mature leaves (Harper 1989, Dickson 1991, Kozlowski 1992). If increased nutrient availability has little effect on the photosynthetic rate of existing leaves, it will not increase their carbon budget. Therefore, production of new leaves can be supported only if existing leaves export a greater proportion of photosynthate to growing meristems, retaining less carbon to support other processes, including production of secondary metabolites. Thus, rapidly growing plants in fertile soils are predicted to have lower concentrations of constitutive secondary metabolites (Herms and Mattson 1992, Jones and Hartley 1999).

When moderate nutrient deficiency decreases growth without affecting photosynthesis, carbohydrates that would otherwise be exported to growing meristems accumulate in source leaves (Körner 1991, Luxmoore 1991, Geiger et al. 1996), and the pool of carbohydrates available for partitioning to secondary metabolism is predicted to increase (Waterman and Mole 1989, Herms and Mattson 1992, Jones and Hartley 1999). If nutrient deficiency is substantial enough to limit photosynthesis, then both growth and secondary metabolism may be carbon limited. In this case, increased nutrient availability is predicted to increase both growth and secondary metabolism. Herms and Mattson (1992), p. 296) concluded that this pattern of phenotypic plasticity predicted by GDBH "results from highly regulated changes in biosynthetic pathways in response to environmental cues rather than incidental responses to environmental variation."

The lack of empirical studies has stymied the emergence of models of how resource availability may impact expression of RIR. However, it has been suggested that RIR may be strongest in fast growing plants because strong photosynthetic sinks have the metabolic machinery and draw the resources needed to drive rapid de novo biosynthesis of proteins and secondary metabolites (Schultz 1988, Herms and Mattson 1992, Karban and Baldwin 1997, Arnold and Schultz 2002). Hence, our a priori prediction is that that the expression of RIR will be strongest in plants growing in high fertility environments.

It should be emphasized that the GDBH is a phytocentric model, advanced to predict patterns of constitutive secondary metabolism in response to

environmental effects on source-sink interactions. Extending the model to effects on herbivore resistance requires system-specific knowledge of effects of changes in secondary metabolism on herbivore behavior and physiology (Larsson 1989), as well as complex responses of herbivores to often co-varying changes in plant nutrient content (Simpson and Raubenheimer 2001).

The objective of this study was to quantify effects of nutrient availability on growth, biomass allocation (above and below ground), nitrogen content, photosynthesis, and secondary metabolism of *Populus nigra* L. (black poplar). By quantifying larval growth in bioassays, we also examined effects of nutrient availability on expression of constitutive resistance of poplar to gypsy moth, *Lymantria dispar* (L.), and whitemarked tussock moth, *Orgyia leucostigma* (J.E. Smith), as well as RIR to both generalist folivores in response to localized gypsy moth herbivory.

Materials and methods

Experimental plants

Inherently fast-growing plants are predicted to exhibit greater phenotypic plasticity in response to resource availability than slow-growing plants (Herms and Mattson 1992), and thus are most appropriate for testing those predictions of GDBH that pertain to effects of resource availability on phenotypic variation in growth and secondary metabolism. Therefore, we selected the hybrid poplar clone NC5271 (*P. nigra* 'Charkowiensis' × *P. nigra* 'Caudina') for testing effects of nutrient availability on expression of constitutive and induced resistance. In comparisons with other hybrid clones, NC5271 was found to be among the fastest growing (Robison and Raffa 1994), as well as the most inducible in response to leaf feeding by gypsy moth (Havill and Raffa 1999).

On 29 May 1998, 400 dormant hardwood cuttings (15 cm in length) were obtained from stock plants maintained at the Univ. of Wisconsin. Cuttings were rooted by making an incision at the base the stem, dipping the stem in 1.0% indole-3-butyric acid and 0.5% 1-naphthaleneacetic acid (Dip'N Grow Root Inducing Concentrate®) that had been diluted with 20 parts distilled water, and then planting them in Pro-Mix potting medium. Cuttings were maintained in a growth chamber at 20°C (12L:12D) for two weeks, then transferred to mist beds where they were watered daily. On 23 June, 100 cuttings (selected for uniform root density) were planted in plastic pots (24 cm diam by 22 cm deep) in a commercial nursery medium (3:3:1:1 pine bark, hardwood bark, sand, composted sewage sludge), and transferred to an outdoor nursery under 25% shade cloth. Plants were watered daily and fertilized weekly with N,P,K (3:1:2) at a low rate (50 ppm N).

On 15 July, the 80 most uniform plants from this population were selected for inclusion in the study, and were further divided into four blocks of 20 based on uniformity in size and vigor. Of the 20 plants in each block, 16 were selected randomly for continued use in the experiment, and the other four were harvested immediately to estimate initial size for use in relative growth rate calculations.

The 64 remaining plants were transferred to the Nursery Crop Research Laboratory (Ohio Agricultural Research and Development Center, Wooster, OH). Pots were placed on a gravel bed exposed to ambient weather conditions, and were arranged in four blocks of 16, with each block consisting of 4 rows and 4 columns, with 0.25 m between pots. Blocks were separated by 0.75 m.

Experimental design

The experiment was designed as a randomized complete block with two levels of fertilization (high and low) applied in a factorial combination with two levels of herbivory (localized gypsy moth defoliation and no herbivory) as main effect factors. Eight of the pots in each of the four blocks were randomly assigned to the high fertility treatment, with the remaining eight pots receiving the low fertility treatment. Defoliation treatments were also assigned randomly, with half of the plants in each nutrient level exposed to herbivory, and the other half left untreated. Thus, each of the four treatment combinations was replicated four times in each of four blocks, for a total of 64 plants (the experimental unit).

Fertility treatment

From 15 July until mid-September, two fertility levels were maintained via fertigation: 50 and 200 ppm N, with N, P, and K supplied in a ratio of 3:1:2. Sources of nutrients were calcium nitrate, potassium nitrate, and mono-potassium phosphate. Other essential nutrients were supplied in non-limiting quantities by mineralization of composted sewage sludge incorporated in the container medium. Plants exhibited no visible symptoms of nutrient deficiency.

Nutrients were applied automatically with each irrigation event by means of a computer-controlled fertigation (fertilization and irrigation) system. The fertigation schedule was designed to maintain optimal water levels (i.e. between 75–100% container capacity, White and Mastalerz 1966). Fertigation events were automatically triggered when container moisture levels reached 75% of capacity as estimated by a model of evapotranspiration calculated from weather station data collected on site and logged by a Q-COM GEM3 software system

(Lee et al. 2000). To confirm that targeted water levels were maintained through the experiment, a computer-monitored tensiometer was inserted to a depth of eight cm in one pot per each treatment combination in each of the four blocks. The nutrient treatments were dispensed from tank solutions via two output lines connected to the fertigator. One emitter positioned in each pot delivered water and the appropriate nutrient treatment. Soluble salt concentrations in container leachate were monitored throughout the study, and did not reach excessive levels.

Induction treatment

Localized herbivory by gypsy moth larvae was used in an attempt to elicit systemic, rapid induced resistance (RIR) in plants assigned to the defoliation treatment. On 28 August, half the plants from each fertility treatment (8 plants per block) were randomly selected for inclusion in the induction treatment. Two fifth-instars (reared from egg hatch in the laboratory on river birch, *Betula nigra*, foliage) were confined in sleeve cages (light-weight polyester chiffon) on each of the plants designated to receive the herbivory treatment. The bags enclosed leaves 7–10 on the terminal shoot, with the youngest apical leaf at least 2 cm long designated as leaf one. To control for potential effects of bagging on induced responses, the corresponding four leaves on the other half of the plants were also bagged (with no larvae) as described above. Larvae were allowed to feed for 72 hours, which for all plants resulted in at least 75% defoliation of leaf area enclosed within the sleeves.

Plant measurements

Initial plant biomass estimates

On 20 July, four plants were selected at random from the 20 in each block, and harvested to estimate plant biomass at the time the fertility treatment was initiated. After carefully rinsing roots to remove container medium, plants were dried at 60°C to constant weight and their total dry mass determined. In subsequent calculations of plant relative growth rate, the mean biomass of the four plants initially harvested from each block was used as the value for initial mass of all plants in that block.

Growth and dry mass allocation

Only the 32 plants not subjected to the defoliation treatment were harvested to determine effects of nutrient availability on plant growth and carbon allocation. For each of these plants, we quantified total leaf area and average area of individual leaves, as well as total leaf, stem, root, and whole-plant biomass. On 9 September, the area (cm^2) of every other leaf on each plant was measured in situ using a portable CID leaf area meter (CI-203, CID, Inc., Vancouver, WA). These leaves were then immediately harvested for phytochemical analysis (see below). They were subsequently freeze-dried and weighed, and their area and mass included in the total for each plant.

Whole plants were then harvested by block beginning 11 September, with an entire block harvested on a single day. Roots were submerged in water and container medium gently removed, after which individual plants were divided into root, stem, and leaf fractions. Leaf area was quantified for each plant using digital image analysis (CI-400 Computer Image Analysis System and software, CID, Inc., Vancouver, WA) and was added to the area of leaves previously harvested for phytochemical analysis to calculate total leaf area for each plant. Leaf, stem, and root fractions were then dried at 60°C for 48 hrs and weighed. The mass of oven-dried and freeze-dried leaves were summed for each plant to calculate total leaf mass. Total plant mass was calculated as the sum of leaf, stem, and root mass. Root:shoot ratio was calculated as [root mass/(stem mass + leaf mass)]. Relative growth rate (mg mg^{-1} d^{-1}) was calculated as [ln(final dry mass) − ln(initial dry mass)/time)], with initial mass of each plant estimated as described above.

Photosynthesis

Light-saturated photosynthesis was measured on 20 August using a portable photosynthesis meter (LI-6200, LI-COR, Inc., Lincoln, NE). For all 64 plants, measurements were made on the youngest mature leaf (fully expanded, dark green) on a shoot on the south side of each tree. To ensure that any differences in photosynthetic rate reflected effects of the nutrient treatment on physiological potential of the plants, rather than stomatal limitations imposed by other environmental factors, measurements were made on a cloud-free morning (0830-1030 EST) of moderate temperature (23°C) and humidity (41%), with moisture levels near container capacity. Data are reported on a leaf area basis (μmoles CO_2 m^{-2} s^{-1}). Since measurements were made prior to implementation of the herbivory treatment, only the nutrient treatment was included in the statistical model for analysis.

Phytochemistry

On 9 September, every other leaf on the 32 plants subsequently harvested for biomass measurements (non-defoliated plants) were sampled to determine effects of nutrient availability on canopy-wide concentrations of foliar nitrogen and total phenolic compounds. Immediately upon removal from the plant, leaves were flash-frozen in liquid nitrogen, placed in a cooler under dry ice, and transported to

the laboratory where they were stored at −80°C until late October, when they were lyophilized while being held at −4°C, weighed (which was included in total leaf weight for each plant). After they were dried, samples were milled to pass a 40-mesh screen, thus creating a homogenized sample representative of the entire canopy. These samples were stored frozen until February 1999, when they were analyzed to determine foliar total phenolic and nitrogen concentrations.

Following extraction of a 70 mg aliquot in 50% methanol for 1.5 hr, total foliar phenolic concentrations were quantified using the Folin-Denis assay modified for use with a continuous flow analyzer as documented in detail by Nitao et al. (2001). Purified tannic acid was used as a standard, with concentrations reported as tannic acid equivalents (TAE) on a percent dry weight basis.

Foliar nitrogen concentration was determined for each plant by analyzing duplicate aliquots (10 mg) of the foliage samples described above with a Carbo Erba CNH analyzer, model NA 1500 (Daun and DeClerq 1994). Foliar nitrogen concentration was expressed on both a dry mass (mg/g) and leaf area (mg/cm^2) basis.

Insect bioassays

To determine the effect of nutrient availability on the growth of the two fifth instar gypsy moths used to implement the herbivory treatment on each plant, we weighed them as a pair immediately before and after confining them on the plant in bags with intact foliage. Since larvae were placed only on the plants receiving the herbivory treatment, only those plants were used in the analysis of fertility effects on growth of fifth instars.

To determine effects of fertility on the expression of constitutive and rapid induced resistance, we used laboratory bioassays with neonate gypsy moth and whitemarked tussock moth larvae (eggs of which were obtained from the Canadian Forest Service, Insect Production Laboratory, Sault St. Marie, Ontario). Seventy-two hours after the fifth instar gypsy moths and mesh bags were removed from plants, the two leaves immediately distal to (younger than) the four leaves that had been bagged were detached and directly used in the bioassays. One leaf from each plant was offered to either first instar gypsy moth or whitemarked tussock moth, which were then allowed to feed for 48 h. Because these leaves did not receive any feeding damage themselves, any reduction in growth of larvae feeding on a leaf sampled from defoliated relative to control plants would indicate elicitation of a systemic induced response to herbivory.

Four neonate whitemarked tussock moth were weighed collectively and placed in a polystyrene petri dish (15 cm diam × 2.5 cm deep, containing a base of plaster mixed with activated charcoal) with a leaf that had just been harvested from one of the 64 experimental plants. Four neonate gypsy moth larvae were confined in another dish with the other leaf from the same plant. To control for ontogenetic variation in leaf quality, half the replicates of each insect species received the oldest leaf of the pair (closest to the bagged leaves), and the other half received the youngest. The plaster base of each dish was saturated with distilled water to maintain turgor of detached leaves over the course of the bioassay. Petri dishes were then placed in a growth chamber (25°C, 16L:8D) for the duration of the 48 h bioassay. Larval growth was calculated as (final mass − initial mass), with data expressed as the mean for each individual larva in the replicate group.

Statistical analyses

Effects of nutrient availability and herbivory on plant and insect response variables were assessed by analysis of variance (PROC GLM, Type III sums of squares, SAS Institute, Inc. 1998), with data reported as least square means ± one standard error. No data transformations were required, as all data met assumptions of normality of residuals and homogeneity of variance. Only the fertility treatment was included in the model for testing treatment effects on photosynthesis, plant variables, and growth of fifth instar gypsy moth. Defoliated plants were not included in analysis of plant variables because the herbivory treatment was not imposed until the very end of the experiment. Hence, there would have been insufficient time for the herbivory treatment to impact plant allocation patterns, beyond direct effects on leaf area due to defoliation. Herbivory was not included in the model for testing treatment effects on photosynthesis (because the treatment had yet to be imposed when plants were measured), or growth of fifth instar gypsy moth (because larvae were only exposed to the 32 plants receiving the herbivory treatment).

Both nutrient availability and herbivory (and their interaction) were included in the model for testing treatment effects on first instar gypsy moth and whitemarked tussock moth growth. The PDIFF option ($\alpha = 0.05$) following the LSMEANS statement (PROC GLM, SAS Institute, 1998) was used to make pre-planned, pairwise comparisons of means for defoliation effects within each fertilization treatment. Pearson's correlation coefficients were used to quantify the relationship between dependent variables. When inspection of bi-variate plots revealed non-linear relationships, correlation coefficients were calculated using non-linear correlation analysis (PROC NLIN, SAS Institute, Inc. 1998).

Table 1. Summary of ANOVA and treatment means (±SE) of effects of nutrient availability on growth, photosynthesis, and biomass allocation of *Populus nigra*. N,P, and K were applied in a ratio of 3:1:2 in both treatments.

Plant response variable	ANOVA statistics		Treatment means	
	F	*P*	50 ppm N	200 ppm N
Relative growth rate (g g $^{-1}$ d $^{-1}$)	54.1	0.0001	0.034 ± 0.001	0.046 ± 0.001
Total plant mass (g)	82.4	0.0001	16.80 ± 1.21	32.07 ± 1.17
Total leaf mass (g)	185.2	0.0001	4.23 ± 0.33	10.56 ± 0.32
Total stem mass (g)	61.5	0.0001	6.78 ± 0.49	12.07 ± 0.47
Total root mass (g)	30.3	0.0001	5.72 ± 0.48	9.43 ± 0.47
Root:shoot ratio	24.7	0.0001	0.51 ± 0.01	0.41 ± 0.01
Total leaf area (cm^2)	157.6	0.0001	490.2 ± 36.2	1134.3 ± 36.2
Individual leaf area (cm^2)	37.7	0.0001	16.36 ± 0.760	22.83 ± 0.73
Foliar N (mg g^{-1})	311.2	0.0001	21.4 ± 0.4	31.1 ± 0.4
Foliar N (mg cm^{-2})	191.5	0.0001	0.187 ± 0.005	0.287 ± 0.005
Foliar phenolics (% TAE)	36.8	0.0001	5.12 ± 0.06	4.61 ± 0.06
Photosynthesis (μmol m^{-2} s^{-1})	3.1	0.0858	13.9 ± 0.5	15.1 ± 0.5

Results

Plant growth and biomass allocation

Nutrient availability had dramatic effects on plant growth and biomass allocation. Relative growth rate of poplar was 35% greater in the high than in the low fertility treatment (Table 1). Over the course of the study this increased the mass of all plant fractions, including leaf, stem, and root, which resulted in plants with 91% more total biomass (Table 1). While total root mass was greatest in high fertility plants, root:shoot ratio was greatest in the low fertility treatment, indicating that nutrient availability altered patterns of above and below ground biomass allocation. On average, low fertility plants allocated 24% more of their total biomass to root growth than did high fertility plants (Table 1).

Nutrient availability substantially impacted total leaf area, which was much higher (131%) in the high fertility than in the low fertility treatment. There were strong, nearly identical, non-linear relationships between total leaf area and both relative growth rate ($r = 0.94$, $P < 0.0001$, n = 32), and total plant biomass (Fig. 1). Nutrient availability also affected the area of individual leaves, which was 40% greater in the high fertility treatment (Table 1). However, nutrient availability had no effect on specific leaf mass (Table 1).

Foliar nitrogen

Not surprisingly, nutrient availability also affected foliar nitrogen concentration, which was substantially higher in the high fertility treatment when measured on both a leaf mass (mg/g) and leaf area (mg N/cm^2) basis (45% and 53% greater, respectively) (Table 1). Foliar nitrogen concentration was strongly correlated with poplar relative growth rate on both a dry mass ($r = 0.71$, $P < 0.0001$, n = 31) and leaf area basis ($r = 0.76$, $P < 0.0001$, n = 31). The correlation between total leaf area and foliar nitrogen concentration was even stronger, for nitrogen on both a leaf mass ($r = 0.90$, $P < 0.0001$, n = 31) and leaf area basis ($r = 0.84$, $P < 0.0001$, n = 31). Root:shoot ratio was negatively correlated with foliar nitrogen concentration on both a dry mass ($r = -0.73$, $P < 0.0001$, n = 31) and leaf area basis ($r = -0.61$, $P < 0.0001$, n = 31).

Photosynthesis

Despite the large effects on foliar nitrogen concentration, nutrient availability had no significant effect on net photosynthetic rate per unit leaf area (Table 1). Photosynthetic rate was weakly correlated with foliar nitrogen on both a leaf mass ($r = 0.38$, $P = 0.03$, n = 31) and leaf area basis ($r = 0.35$, $P = 0.06$, n = 31). However, there was no relationship between photosynthetic rate and plant relative growth rate ($r = 0.26$, $P = 0.17$, n = 32) or total plant biomass (Fig. 2).

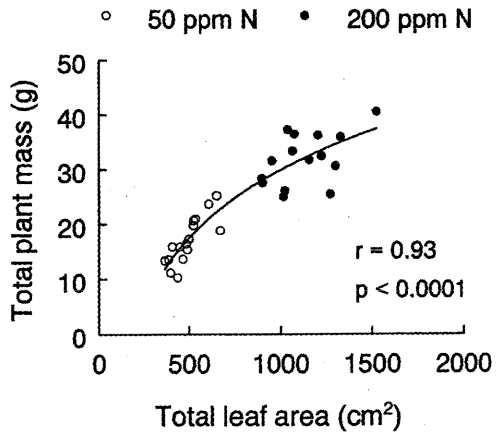

Fig. 1. Relationship between total leaf area and total dry mass of black poplar (*Populus nigra*) in response to low (50 ppm N) and high (200 ppm N) nutrient regimes, with N,P, and K applied in a ratio of 3:1:2.

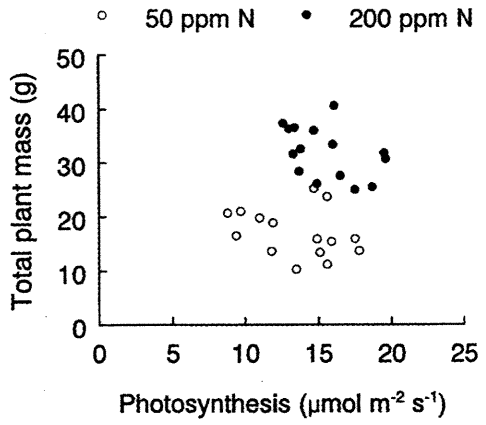

Fig. 2. Lack of relationship between net photosynthetic rate per unit leaf area and total dry mass of black poplar (*Populus nigra*) in response to low (50 ppm N) and high (200 ppm N) nutrient regimes, with N,P, and K applied in a ratio of 3:1:2.

Plant secondary chemistry

Nutrient availability also affected foliar total phenolic concentration, which was 11% greater in the low than in the high fertility plants (Table 1). Total phenolic concentration was inversely related to plant growth, as indicated by a negative correlation between total phenolic concentration and both relative growth rate of poplar ($r = -0.41$; $P = 0.02$, $n = 32$) and total plant biomass (Fig. 3).

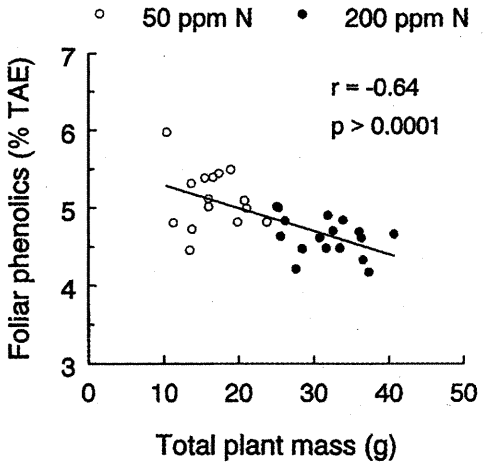

Fig. 3. Negative correlation between total plant dry mass and percent total foliar phenolic concentrations (expressed as tannic acid equivalents, TAE) in black poplar (*Populus nigra*) in response to low (50 ppm N) and high (200 ppm N) nutrient regimes, with N,P, and K applied in a ratio of 3:1:2.

Constitutive and rapid induced herbivore resistance

The growth of the fifth instar gypsy moths confined in mesh bags on the plants receiving the defoliation treatment was also dramatically affected by nutrient availability. Larval growth was 80% greater on high fertility than on low fertility plants (298.3 ± 36.1 vs 162.7 ± 39.2 mg, respectively; $F = 6.51$; df = 1,31; $P = 0.018$).

The growth of first instar gypsy moths, which were exposed to all experimental plants in laboratory bioassays, was also affected by the fertility treatment. As was the case with fifth instars feeding on the intact plant foliage, first instars feeding on detached foliage grew much faster on the high fertility than on the low fertility plants ($F = 11.9$; df = 1, 53; $P = 0.001$, Fig. 4). On plants that were not exposed to herbivory, growth of first instar gypsy moth was negatively correlated with total phenolic concentrations ($r = -0.44$, $n = 32$, $P = 0.019$) and positively correlated with foliar nitrogen concentration ($r = 0.46$, $n = 32$, $P = 0.013$).

Within 72 hours, feeding by fifth instar gypsy moth had systemically induced resistance in younger leaves to first instar gypsy moth, as evidenced by decreased larval growth on plants exposed to herbivory ($F = 3.82$; df = 1,53; $P = 0.056$). Averaged across the two fertility treatments, larval growth was 38% lower on plants receiving herbivory than on the nondefoliated plants (0.46 ± 0.09 vs 0.72 ± 0.09 mg, respectively).

Although the interaction between the fertility and defoliation treatments was not statistically significant ($F = 2.02$; df = 1,53; $P = 0.16$), it is apparent from Fig. 4 that the magnitude of rapid induced resistance was much greater in the high fertility treatment. In preplanned, pairwise statistical comparisons, the effect of herbivory was significant in the high fertility treatment ($P = 0.014$), where larval growth was 56% lower on the defoliated than on the non-defoliated plants (Fig. 4). However, herbivory clearly had no effect on larval growth in the low fertility treatment ($P = 0.71$, Fig. 4).

Nutrient availability and defoliation interacted to affect the growth of first instar whitemarked tussock moth ($F = 5.1$; df = 1, 57; $P = 0.028$). In contrast to gypsy moth, rapid induced resistance to whitemarked tussock moth was only expressed in the low fertility plants (Fig. 4). Herbivory had no effect on the growth of larvae feeding on high fertility plants ($P = 0.18$). However, in the low fertility treatment, herbivory decreased the growth of larvae by 30% ($P < 0.0001$, Fig. 4).

The significant interaction depicted in Fig. 4 also indicates that nutrient availability had no effect on constitutive resistance of poplar to whitemarked tussock moth. There was no difference in larval growth between the high and low nutrient plants that were not exposed to herbivory ($P = 0.86$). Furthermore, on con-

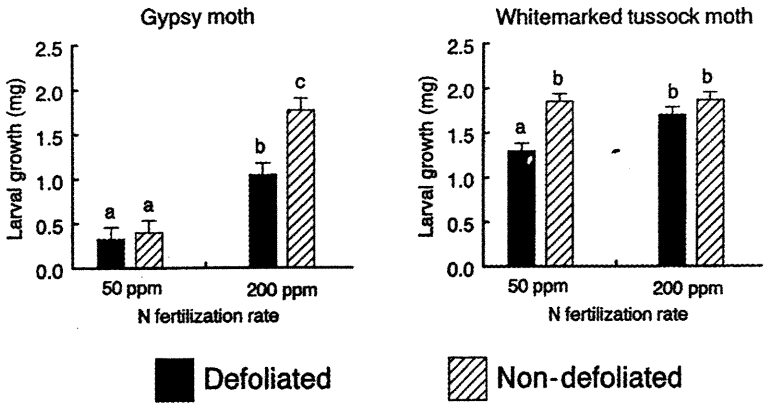

Fig. 4. Effects of nutrient availability and localized defoliation by fifth instar gypsy moth (*Lymantria dispar*) on growth of first instar gypsy moth and whitemarked tussock moth (*Orgyia leucostigma*) on black poplar (*Populus nigra*). Plants were grown under low (50 ppm N) and high (200 ppm N) nutrient regimes, with N,P, and K applied in a ratio of 3:1:2.

stitutive plants there was also no correlation between larval growth and foliar concentrations of nitrogen ($P = 0.94$) or phenolics ($P = 0.39$). However, nutrient availability did impact growth of larvae on plants that were exposed to herbivory, which was 31% greater on high fertility than on low fertility plants ($P = 0.002$, Fig. 4).

Discussion

The GDBH as extended by Herms and Mattson (1992) predicts that if increased nutrient availability results in an increased rate of photosynthesis, then growth and secondary metabolite concentrations will both increase, resulting in a positive correlation between them. However, if increased nutrient availability increases growth without impacting photosynthesis rate, then secondary metabolite concentrations are predicted to decline, resulting in a negative correlation between growth rate and secondary metabolism. The results of this study are consistent with the latter prediction. Increased nutrient availability dramatically increased the growth of poplar, but had no effect on photosynthesis. As predicted, increased nutrient availability resulted in lower concentrations of total foliar phenolic concentrations, resulting in a negative correlation between growth rate and secondary metabolism.

The 35% greater relative growth rate of plants in the high fertility treatment resulted in plants with almost twice the total biomass. However, photosynthetic rate per unit area was not affected by nutrient availability, nor was there any relationship between photosynthetic rate and relative growth rate or total plant biomass. Rather, plants responded to increased nutrient availability with a dramatic increase in total leaf area, which was highly correlated with previous observations that phenotypic differences in growth rates are due more to differential investment of photoassimilates into new leaf area than variation in photosynthetic rate (Potter and Jones 1977, Körner 1991, Lambers and Poorter 1992). Furthermore, these results are consistent with previous observations that growth rate of plants is much more sensitive to nutrient availability than is photosynthesis (Chapin 1980, Luxmoore 1991, Samuelson 1998), which is a cornerstone premise of GDBH (Herms and Mattson 1992).

The lower foliar phenolic concentrations and root:shoot ratios we observed in high fertility plants are also consistent with another cornerstone premise of GDBH: increased growth receives allocation priority for plant resources in high resource environments (Bazzaz et al. 1987, Herms and Mattson 1992). At the beginning of the experiment there were no differences in the average size of plants in the high and low nutrient treatments. Furthermore, nutrient availability had no effect on the photosynthetic rate of existing leaves. Therefore, the increased growth of plants in the high fertility treatment could have resulted only from increased allocation of current photosynthate to support growing meristems, which as net importers of carbon (Dickson 1991) are strong photosynthetic sinks (Marcelis 1996). Any current photosynthate exported from source leaves to support production of new leaf area is by necessity not available to support secondary metabolism in the source leaves, or to support root growth. The strong positive correlation observed between total leaf area and plant growth rate, as well as the negative correlations between total leaf area and both secondary metabolism and root:shoot ratio, are consistent with these allocation trade-offs.

We interpret these responses of root:shoot ratio and constitutive secondary metabolism to nutrient availability to be consistent with models of adaptive phenotypic plasticity. Theories of optimal allocation predict that plants preferentially allocate resources to processes and structures that maximize acquisition of limiting resources, including proportional increases in root growth in response to nutrient limitation (Bloom et al. 1985, Hirose 1987, Hilbert 1990, Chapin 1991, Ingestad and

Ågren 1991, Gleeson 1993), a response that has been observed in a number of studies (Ericsson 1995, Aerts and Chapin 2000).

Similarly, responses of secondary metabolism to resource availability may also represent adaptive patterns of carbon allocation and partitioning. GDBH has been characterized as a 'supply-side' substrate-driven model of incidental responses of secondary metabolism to resource availability that ignores adaptive, 'demand-side' needs for defense resulting from selection by herbivores (Lerdau et al. 1994). However, contrary to this characterization, Herms and Mattson (1992), p. 296) concluded that patterns of secondary metabolism predicted by GDBH represent highly regulated, adaptive phenotypic plasticity consistent with theories of optimal defense (Herms 1999). The regulation of metabolic pathways is under strong genetic control (Herrmann and Weaver 1999). Furthermore, there are no inherent physiological constraints on secondary metabolism in resource-rich environments as there are on growth in resource-limited environments. Rather, decreased secondary metabolism in resource-rich environments may be an inevitable consequence of increased growth that is necessary to enhance competitive ability by minimizing usurpation of resources and space by neighbors. Conversely, increased secondary metabolism in resource-limited environments may be adaptive if it increases herbivore resistance and stress tolerance in environments where growth rate is constrained (Herms and Mattson 1992, Herms 1999).

This logic is similar to that used by Coley et al. (1985) to develop their model of optimal defense as applied to interspecific patterns of growth and secondary metabolism in response to variation in resource availability. Inter- and intra-specific models of optimal defense in different environments complement optimal defense models that predict internal patterns of defense allocation based on the value of a particular tissue (McKey 1974, Rhoades 1979, Hamilton et al. 2001). Increases in both root growth (Zhang and Forde 2000) and secondary metabolism (Bongue-Bartelsman and Phillips 1995) in response to nutrient limitation have been shown to result from highly regulated gene expression, further suggesting an adaptive role for these responses.

The generality of a physiological trade-off between growth and secondary metabolism has been questioned because fertilization increased both growth and foliar terpene concentrations in some studies (Haukioja et al. 1998, Koricheva et al. 1998), and because correlations between growth and terpene levels are not always positive (Koricheva 2002). Based on the results of their meta-analyses, Haukioja et al. (1998) and Koricheva et al. (1998) concluded that phenolic compounds are generally decreased by fertilization, but that terpenes are not responsive to nutrient availability.

The Biosynthetic Pathway Hypothesis has been advanced to explain this pattern, proposing that physiological trade-offs with growth are more likely to occur for phenolic than for terpenoid biosynthesis (Muzika 1993, Haukioja et al. 1998). Their reasoning is based on the existence of direct competition between protein (necessary for growth) and phenylpropanoid biosynthesis for their common precursor phenylalanine, the supply of which is considered to be rate-limiting for both. On the other hand, the precursors of terpenoid biosynthesis are considered to be more generalized, entering many pathways. However, recent studies indicate that phenylalanine supply per se does not limit protein and phenolic biosynthesis because it is continuously regenerated from a limited nitrogen pool (Razal et al. 1996, Singh et al. 1998), and that all intermediates of the shikimate pathway serve as substrates for other metabolic pathways (Herrmann and Weaver 1999). This suggests the possibility of generalized substrate competition between growth and both terpenoid and phenolic pathways.

Furthermore, evidence suggests that terpenes in fact may be responsive to nutrient availability. Some studies have observed terpene levels to increase in response to fertilization (Björkman et al. 1991, 1998, McCullough and Kulman 1991, Lerdau et al. 1995, Honkanen et al. 1999), and others have reported decreases (Mihaliak and Lincoln 1985, Bryant et al. 1987, Johnson and Lincoln 1991, Holopainen et al. 1995, Wilkens et al. 1997, Wainhouse et al. 1998, Warren et al. 1999). A potential explanation for these divergent results is that terpenes may either increase or decrease in response to fertilization, contingent on the conditions of the study. If so, then a model such as GDBH is required that can predict either positive or negative responses of constitutive secondary metabolism to resource availability. The GDBH predicts explicitly that the correlation between growth and secondary metabolism will be positive in nutrient-limited environments but negative in nutrient-rich environments (Herms and Mattson 1992). This prediction was recently corroborated by the meta-analysis of Koricheva (2002).

Field studies in which fertilization increased growth and foliar terpene concentrations were conducted with pine trees on extremely nutrient deficient sites where tree growth was quite slow (Björkman et al. 1991, 1998, McCullough and Kulman 1991, Honkanen et al. 1999). The photosynthetic rates of nutrient-deficient conifers have been shown to increase in response to fertilization (Brix 1981, Linder and Rook 1984, Brown et al. 1996). Hence, these responses may be consistent with predictions of the GDBH. However, these studies (Björkman et al. 1991, 1998, McCullough and Kulman 1991, Honkanen et al. 1999) did not measure photosynthesis, so it is not possible to judge if the increase in terpene production they observed is truly consistent with the model. A few studies have quantified photosynthesis

and secondary metabolism. Lerdau et al. (1995) did find that when photosynthesis of Douglas-fir (*Pseudotsuga menziesii*) increased in response to increased nutrient availability, monoterpene concentrations also increased. Conversely, when nutrient availability increased growth without affecting photosynthesis of willow (*Salix aquatica*), foliar phenolic concentrations decreased (Waring et al. 1985).

Our conclusions regarding the trade-off between growth and foliar phenolic levels are based on the Folin-Denis assay for total phenolics, the weaknesses of which are well recognized (Hagerman 1988, Appel et al. 2001). Specifically, it does not provide valid quantitative comparisons of samples that differ qualitatively in their phenolic profiles, such as foliage from different species. However, when comparing foliage of the same age from genetically identical plants, as in this study, the Folin-Denis assay has been considered useful for detecting relative quantitative differences among experimental treatments (Cork and Krockenberger 1991, Cipollini et al. 1993, Dudt and Shure 1994). For example, in other *Populus* species, Folin-Denis total phenolics were found to be highly correlated with quantitative measures of proanthocyanidins in *P.* × *eugeneii* ($r = 0.97$, $P < 0.0001$; Mattson, Herms, and Parry, unpubl.) and *P. tremuloides* ($r = 0.89$, $P < 0.0001$, Nitao et al. 2001), as well as phenolic glycoside (salicortin + tremulacin) concentrations in *P. tremuloides* ($r = 0.51$, $P < 0.0001$; Nitao, Mattson, and Herms, unpubl.). While this assay provides no insight into specific compounds responsible for mediating plant/herbivore interactions, it can provide a quantitative measure of biological activity by quantifying the reducing power of the compounds present (Appel et al. 2001), as phenolic oxidation is considered to be an important anti-herbivore mode of action (Appel 1993).

Effects of nutrient availability on constitutive herbivore resistance

It is important to emphasize the phytocentric nature of GDBH, which is a model of physiological responses of plants to resource availability. Due to the complex and often idiosyncratic nature of plant/herbivore interactions, measures of nutrient and secondary metabolite concentrations may not necessarily reflect herbivore preference or performance (de Jong and van der Meijden 2000), which was the case in this study. The increased growth of first and fifth instar gypsy moths on high fertility plants that were not exposed to herbivory (constitutive plants) is consistent with many studies with woody plants that show increased nutrient availability to favor insects (Kytö et al. 1996). However, whitemarked tussock moth growth on constitutive plants was not affected by nutrient availability, indicating that all herbivores do not respond in the same way to changes in host quality. Although gypsy moth growth was correlated positively with foliar nitrogen concentration and negatively with foliar phenolic concentrations, whitemarked tussock moth growth was not correlated with either measure, further emphasizing the idiosyncratic relationship between insect performance and phytochemistry.

Effects of nutrient availability on expression of rapid induced resistance

Localized gypsy moth herbivory elicited RIR in poplar within 72 hours. The effect was systemic in nature, as indicated by decreased quality of leaves that had not been damaged. However, the magnitude of RIR was dependent on nutrient availability, the effects of which varied according to the insect species tested. Expression of RIR to gypsy moth was most dramatic in the high fertility treatment. However, larval growth was quite slow in the low nutrient treatment, even in the plants that were not exposed to herbivory. Any induced responses may have little additional effect on herbivore performance in plants with high levels of background constitutive resistance (Herms and Mattson 1992). In contrast, RIR to whitemarked tussock moth was expressed only in the low fertility treatment, perhaps because nutritional benefits of nitrogen content may have offset detrimental effects of induced responses in the high fertility plants (Karban and Baldwin 1997). Considered together, the responses of gypsy moth and whitemarked tussock moth indicate that RIR occurred in both the high and low fertility plants, but that ability to detect the induced response was dependent on the herbivore species used in the bioassay. This supports the contention of Tuomi et al. (1990) that induced responses of plants are likely to have idiosyncratic effects on herbivores, and that conclusions of experimental studies of induced resistance will be dependent on the abiotic environment in which the experiment is conducted, as well as the bioassay organism used to detect the response.

No theoretical framework has emerged for predicting effects of resource availability on the expression of rapid induced resistance (RIR). However, Herms and Mattson (1992) suggested that RIR might be strongest in fastest growing plants because mechanisms of RIR are closely associated with the same high metabolic activity as plant growth processes (see also Schultz 1988, Karban and Baldwin 1997, Arnold and Schultz 2002). For example, Frischknecht et al. (1987) found that wounding did not induce alkaloid accumulation in drought stressed plants.

The occurrence of RIR in both high and low fertility plants that we observed is not consistent with this prediction. It should be noted that GDBH is a model of secondary metabolic responses, which we did not quan-

tify in response to the induction treatments. However, results of the few studies that have investigated effects of nutrient availability on induced secondary metabolism have also been inconsistent with this prediction (Ohnmeiss and Baldwin 1994, Zangerl and Berenbaum 1994/95, Darrow and Bowers 1999). The effects that we observed may have also been due to induction of defensive proteins (Hammerschmidt and Schultz 1996, Haruta et al. 2001), which are compounds not addressed by GDBH. Nutrient availability has been shown to influence the induction of defensive proteins in *Lycopersicon esculentum* (Stout et al. 1998) and *Brassica napus* (Cipollini and Bergelson 2001).

In summary, plant growth was increased in the high fertility treatment, while constitutive secondary metabolism and root:shoot ratio were decreased. Increased growth was associated with increased leaf area, but not photosynthetic rate. As predicted by GDBH when increased nutrient availability increase plant growth without affecting photosynthesis, there was a negative correlation between plant growth and secondary metabolism. These responses are consistent with those predicted by models of adaptive phenotypic plasticity in whole-plant carbon budgeting.

The idiosyncratic responses of gypsy moth and whitemarked tussock moth to the herbivory and nutrient availability treatments challenges the value of using insect bioassays as surrogate measures of secondary metabolism for testing plant resource allocation models such as GDBH. Conversely, our results hi-light the limitations of GDBH for predicting effects of resource availability on plant quality for herbivores. The results do suggest that effects of nutrient availability on the expression of rapid induced resistance represent a largely over-looked source of variation in plant/herbivore interactions.

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