Oecologia (1998) 115:541 – 552

Ian T. Baldwin · Dawn Gorham · Eric A. Schmelz
Carrie A. Lewandowski · Gladys Y. Lynds

Allocation of nitrogen to an inducible defense and seed production in *Nicotiana attenuata*

Received: 5 June 1997 / Accepted: 2 March 1998

Abstract Resource-based tradeoffs in the allocation of a limiting resource are commonly invoked to explain negative correlations between growth and defense in plants, but critical examinations of these tradeoffs are lacking. To rigorously quantify tradeoffs in a common currency, we grew *Nicotiana attenuata* plants in individual hydroponic chambers, induced nicotine production by treating roots with methyl jasmonate (MJ) and standardized leaf puncturing, and used ^{15}N to determine whether nitrogen-based tradeoffs among nicotine production, growth, and seed production could be detected. Plants were treated with a range of MJ quantities (5, 45 or 250 μg plant^{-1}) to effect a physiologically realistic range of changes in endogenous jasmonic acid levels and increases in nicotine production and accumulation; MJ treatments were applied to the roots to target JA-induced nicotine production, since nicotine biosynthesis is restricted to the roots. Leaf puncturing and 5 μg MJ treatments increased de novo nicotine synthesis and whole-plant (WP) nicotine pools by 93 and 66%, while 250 μg MJ treatments increased these values 3.1 and 2.5-fold. At these high rates of nicotine production, plants incorporated 5.7% of current nitrogen uptake and 6.0% of their WP nitrogen pools into nicotine. The ^{15}N-labeled nicotine pools were stable or increased for the duration of vegetative growth, indicating that the N-nicotine was not metabolized and re-used for growth. Plants with elevated nicotine production grew more slowly and the differences in plant biomass gain between MJ-treated plants and controls were linearly related to the differences in nicotine accumulation. Despite the reductions in rosette-stage growth associated with nicotine production, estimates of lifetime fitness (cumulative lifetime seed production, mass/seed, seed viability) were not affected by any treatment. Only two treatments (leaf puncturing and 250 μg MJ) increased the allocations of ^{15}N acquired at the time of induction to seed production. On average, plants used only 14.9% of their WP nitrogen pool for seed production, indicating that either the nitrogen requirements for seed production or the reproductive effort of these hydroponically-grown plants are low. To determine if seed production is strongly influenced by the amount of vegetative biomass attained before reproduction, the experiment was repeated with plants that had 44% of their leaf area (or 29% of their WP biomass) removed before MJ treatments with a removal technique that minimized the nicotine response. MJ treatments of these plants dramatically increased nicotine production and accumulation, but these plants also suffered no measurable fitness consequences from either the leaf removal or MJ treatments. We conclude that when *N. attenuata* plants are grown in these individual hydroponic chambers, their allocation to reproduction is sufficiently buffered to obscure the large increases in nitrogen allocations to an inducible defense. To determine whether soil-grown plants are similarly buffered, we grew two genotypes of plants in the high-nutrient soil from a 1-year-old burn in a piñyon-juniper forest (the plants' natural habitat) and in low-nutrient soil from an adjacent unburned area, and induced nicotine production in half of the plants with a 500 μg root MJ treatment. Plants grown in burned soils had an estimated lifetime fitness that was on average 2.8-fold greater than that of plants grown in unburned soils. MJ treatment reduced fitness estimates by 43% and 71% in the burned and unburned soils, respectively. We

I.T. Baldwin(✉)[1] · C.A. Lewandowski · G.Y. Lynds
Department of Biological Sciences, SUNY,
University at Buffalo, Buffalo, NY 14260-1300, USA

D. Gorham
Department Comparative Biosciences,
University of Wisconsin, Madison, WI 53706, USA

E.A. Schmelz
Department of Entomology, University of Arizona,
Tucson, AZ 85711, USA

Present address:
[1]Max-Planck-Institute für Chemische Ökologie,
D-07745 Jena, Germany Tel: 011-49-3641-643660;
e-mail: Baldwin@ChemOek-Jena.mpg.de

conclude that while hydroponic culture allows one to rigorously quantitate nitrogen allocation to growth, reproduction and defense, the allocation patterns of plants grown in hydroponic culture differ from those of plants grown in soil. Under hydroponic conditions, plants have low reproductive allocations and reproductive-defense tradeoffs are not detected. Reproductive-defense tradeoffs are readily discernible in soil-grown plants, but under these growing conditions, the nitrogen-basis for the tradeoff is difficult to quantify.

Key words Allocation · Nitrogen · Nicotine · Seed · *Nicotiana attenuata*

Introduction

Explanations of tradeoffs between growth, defense, and reproduction as based on resources play a central role in most plant defense theories (McKey 1974, 1979; Rhoades 1979, 1983; Feeny 1976; Rhoades and Cates 1976; Coley et al. 1985; Herms and Mattson 1992), which view the construction of chemicals used by plants for defense as limiting the availability of resources (e.g. carbon, nitrogen) that otherwise could be used for growth and reproduction. However, as Mole (1994) pointed out in his insightful review of allocation theory, the requirements for establishing a resource-based tradeoff are absent from all studies that have tried to examine this topic. While many studies have documented negative relationships between plant growth and defense (reviewed in Herms and Mattson 1992), none have demonstrated that a resource-based tradeoff between the different plant functions is responsible for the negative growth-defense relationship.

Mole (1994) outlines the difficulties of demonstrating a resource-based tradeoff. First, allocation to the different plant functions (growth, reproduction, and defense) must be quantified in the currency of a fitness-limiting resource. Researchers have attempted to solve this difficulty by introducing the concept of exchange ratios for the conversion of one currency into another (Bloom et al. 1985; Reekie and Bazzaz 1987); however, this approach has not been tested for the allocation to defensive metabolites. A related problem is the question of whether an allocation "decision" can be revoked; can, for example, a plant metabolize its defense metabolite pool and re-utilize the limiting resource invested in this pool for another plant function – such as growth or reproduction?

A second major difficulty lies in defining the total budget of the limiting resource, the budget within which the tradeoffs are supposed to occur. The problem is conceptually simple, for the allocations must be quantified at the level of the whole organism (the unit at which the putative tradeoff occurs), however empirically difficult, particularly for plants which have so many different routes for the entry and egress of fitness-limiting resources (Mole 1994). Hence, for a formal or uncircumventable tradeoff to exist, the total quantity of the fitness-limiting resource in a plant must be determined and found to equal the sum of the amounts allocated to the different functions. The allocations must be quantified with an accuracy that would allow one to detect the consequences of small changes in allocations among functions. For many plant secondary metabolites, which may comprise less than 1% of a plant's total biomass, the amount of resource allocated to other plant functions may be so large as to easily obscure even a many-fold increase in the allocation to defense. Moreover, even large tradeoffs may be obscured by compensatory intake of the limiting resource; hence resource intake must be carefully monitored, if not controlled, if the total resource budget is to be accurately determined. For most studies of allocational tradeoffs, large unquantified allocations to other function(s) within the organism or to other undefined components of the total resource budget are not determined. Mole (1994) calls these unquantified allocations "third party tradeoffs" and it is these tradeoffs which have undermined previous attempts to establish the existence of allocational tradeoffs (Mole 1994).

Induced defense responses are ideal systems with which to measure these putative tradeoffs because by inducing the defenses, one can alter the allocation to defense within the same genetic background of plant. Moreover, damage-inducible defense metabolite production is thought to have evolved as a cost-saving measure to reduce the "expense" of metabolite production by enabling plants to time metabolite production so it coincides with the need for defense (Haukioja and Hakala 1976; Givnish 1986; Harvell 1986, but see Karban and Baldwin 1997 for alternative explanations). We have studied an induced nitrogen-intensive defense (nicotine) in two native tobacco species (*Nicotiana sylvestris* and *N. attenuata*) and developed techniques for the (1) quantification of whole-plant (WP) allocations of a limiting resource (nitrogen) to growth and defense (Ohnmeiss and Baldwin 1994), and (2) the induction of nicotine production without wounding (which would complicate the determination of allocation patterns) by exogenously applying the plant's endogenous wound signal (jasmonic acid – Baldwin et al. 1994c, 1996, 1997a; Baldwin 1996) and (3) studied the plant's ability to metabolize nicotine and reuse the nitrogen from the alkaloid pool for growth (Baldwin et al. 1994a; Baldwin and Ohnmeiss 1994). We have developed an individual-plant hydroponic culturing technique which allows us to accurately measure WP growth, regulate nitrogen acquisition and determine WP nitrogen pools, and, using ^{15}N pulse-chase techniques, measure the allocation of nitrogen to nicotine biosynthesis, growth and seed production (Baldwin et al. 1994a). Here we use these techniques to examine putative tradeoffs among growth, defense, and reproduction in a nitrogen currency.

The examination of these tradeoffs in a nitrogen currency is particularly germane for our study organism,

N. attenuata. This herbaceous annual is native to North America and is an important but ephemeral component (usually for only three growing seasons), of the post-fire annual community in burned sagebrush, blackbrush, and piñyon-juniper forests of the Great Basin desert (Wells 1959; references in Baldwin and Morse 1994). It produces self-compatible perfect flowers, which mature into capsules, each of which dehisces 10–300 small (100–160 μg) seeds approximately 16 days after pollination. Seed production (e.g., seed number capsule^{-1} or seed mass capsule^{-1}) in self-pollinating, glasshouse grown plants is not increased by increasing pollen loads with hand-pollination, which suggests that seed production in the glasshouse is more resource- than pollen-limited (Baldwin et al. 1997b). The seeds are largely dormant until exposed to aqueous extracts of wood smoke; by synchronizing its germination with the post-fire environment, this species takes advantage of the high-nitrogen soils that characterize such environments (Baldwin et al. 1994b; Baldwin and Morse 1994). In both field and glasshouse (I.T. Baldwin, N. Diab and G. Lynds, unpublished work) experiments, nitrogen-supplementation was found to increase plant growth and seed production; hence *N. attenuata* has life history traits which allow it to germinate in high nitrogen soils and the availability of soil nitrogen represents a limiting resource for this plant.

We induced nicotine production by treating roots with three different quantities of jasmonate to induce three different levels of nicotine production which, in turn, span the 4-fold increases in nicotine concentrations observed after leaf damage in plants growing in their native habitat (Baldwin and Ohnmeiss 1993). Roots were treated with jasmonate because nicotine synthesis occurs only in the roots and after leaf wounding, root jasmonate levels are transiently elevated, due to the transport of jasmonate synthesized at the wound sites in the leaves (Baldwin et al. 1997a; Zhang and Baldwin 1997). Leaf wounding increases endogenous jasmonic acid levels 5–500 ng plant^{-1} in proportion to the amount of wounding (Baldwin et al. 1997a; Ohnmeiss et al. 1997). In addition, with the use of ^{14}C-labeled jasmonate, we have determined that the exogenous application of jasmonate in microgram quantities per plant are required to effect changes in endogenous jasmonate pools in ng quantities plant^{-1} (Baldwin et al. 1994c; Zhang and Baldwin 1997). We also induced plants with a standardized leaf-puncturing protocol which does not remove leaf tissue (Baldwin and Schmelz 1994). While the relationships among mechanical wounding, jasmonate and nicotine responses are linear and predictable (Baldwin et al 1997a; Ohnmeiss et al. 1997), they are more complicated when a specialist herbivore is responsible for the wounding. For example, herbivory by *Manduca sexta* larvae, a specialist herbivore relatively tolerant of dietary nicotine, results in a dramatic amplification of wound-induced jasmonate levels and a suppression of the wound-induced nicotine response. Both the jasmonate amplification and the nicotine suppression effects are due to salivary factors from the herbivore (McCloud and Baldwin 1997) and we are currently investigating these factors.

When plants are grown in hydroponic culture, where rigorous quantification of nitrogen allocation to growth, reproduction and defense is possible, the plants have low reproductive efforts and allocate only a small proportion of their total acquired nitrogen to seed production and do not exhibit any tradeoffs between defense and reproduction. In order to determine whether seed production is a function of vegetative biomass in these hydroponically grown plants (and hence a function of the resources acquired during vegetative growth), we repeated the experiment with plants which had half of their canopy (or 29% of their WP biomass) removed at the time of induction with a technique which minimized the nicotine response to wounding (Baldwin and Schmelz 1994). We found that neither leaf removal nor the large increases in induced nicotine production had any affect on the amount (or viability) of seed produced. Under these hydroponic conditions, seed production appears to be limited by the number of reproductive meristems activated rather than the resources acquired during vegetative growth. To determine whether the seed production is similarly buffered in soil-grown plants, we grew in the glasshouse two genotypes of plants in pots containing soil collected both from a 1-year-old burn and from an adjacent unburned area, and induced these plants with a single root methyl jasmonate (MJ) treatment. In these plants, we observed large decreases in seed production in induced plants. However, since we have yet to develop the techniques to rigorously quantify nitrogen budgets and address the problems of third-party tradeoffs in soil-grown plants, we are unable to determine whether the reductions in seed production in soil-grown induced plants are the result of allocational tradeoffs between defense and reproduction.

Materials and methods

Hydroponic experiments 1 and 2

Seeds were from the first generation of glasshouse-grown self-pollinated plants which originated from a single-plant collection made in 1993 on the DI Ranch in southwestern Utah (section T40S R19W). Seeds were soaked in an aqueous extract of wood smoke to stimulate germination (Baldwin et al. 1994b), germinated in flats of Cornell mix A potting soil, and transplanted after 12–14 days into 40-l hydroponic boxes. The hydroponic box contained a complete nutrient solution [0.292 g Peter's Hydro-sol l^{-1} (W.R. Grace, Inc., Fogelsville, Pa., USA), 0.193 g $Ca(NO_3)_2$ l^{-1}, and 70 ml of a no-nitrogen nutrient solution l^{-1} (described in Ohnmeiss and Baldwin 1994)]. The plants grew in this solution for 4–6 days, after which 400 plants for each of the two hydroponic experiments were transferred to individual opaque 1-l hydroponic containers containing the no-nitrogen nutrient solution supplemented with KNO_3 to produce a 2.0 mml^{-1} solution. Throughout both hydroponic experiments, the solutions in the 1-l containers were maintained at 900–1000 ml with additions of the no-nitrogen nutrient solution. All plants were grown in a glasshouse with supplemental lighting from 400 W sodium vapor lamps for 16 h/day^{-1}, which provided a minimum photosynthetically active radiation (PAR) of 220 μmol

m^{-2} s^{-1}. After 10–12 days of growth in the individual containers, all 400 plants in each experiment were weighed, and the 235 (experiment 1) and 195 (experiment 2) plants with the most similar masses and morphologies were assigned to treatment groups by sorting by wet mass (as described in Ohnmeiss and Baldwin 1994). This consecutive random allocation of plants produced treatment groups with plant wet mass means that were not significantly different on day zero (grand means experiment 1 = 4.03 ± 0.05; experiment 2 = 4.99 ± 0.03 g wet mass; one-way ANOVAs, Fs <= 0.002, Ps = 1.000). This high level of uniformity, especially across the harvest days, is required to accurately estimate induced changes in WP nicotine pools and in plant growth. Plants were randomly assigned to positions on the glasshouse bench independently of their treatment, and all plants were in the rosette-stage of growth at the start of the experiments.

We harvested plants at three intervals to quantify growth and WP nicotine responses; an additional set of treatments were grown to maturity to permit us to quantify seed production. At the time of puncturing and MJ treatments, plants were given $^{15}NO_3$ to measure the amount of nicotine produced from nitrogen that was acquired, reduced, and assimilated in response to induction, and to determine how the allocation of nitrogen to seed production is influenced by MJ induction and puncturing. For simplicity, we refer to the ^{15}N-nicotine values as *de novo* nicotine production.

Experiments 1 and 2 both consisted of five treatment groups with four harvests for each treatment: three destructive harvests on days 7, 9, 13 (experiment 1) or on days 7, 11, 15 (experiment 2) each with 10 (experiment 1) or 8–9 (experiment 2) replicate plants in each harvest and a set of treatments–with 15 (experiment 1) or 10 (experiment 2) replicate plants in each of the five treatments – which were allowed to grow to flowering and complete seed set, and from which all seeds produced were collected, weighed and tested for viability. In addition, each experiment had a group of 10 replicate plants which were harvested at time 0 immediately before the treatments were started. Experiment 1's five treatment groups consisted of: (1) an unwounded control group; (2) a damaged plant group where six fully-expanded leaves were damaged with four rolls from a fabric pattern wheel (as described in Baldwin and Schmelz 1994); and three MJ groups which received (3) 5, (4) 45, or (5) 250 μg of MJ to the hydroponic solution of the individual growth chambers. experiment 2's five treatment groups consisted of: (1) an unwounded control group; and four damaged plant groups which had every other leaf removed by pinching the leaves from the stem at the base of the petiole – a leaf removal technique which results in only a minimal nicotine response in *N. sylvestris* (Baldwin 1989; Baldwin and Schmelz 1994) – and received (2) 0, (3) 5, (4) 45, or (5) 250 μg of MJ to the hydroponic solution of the plants' individual growth chambers. The removed leaves were traced for area determination and freeze-dried for biomass and nicotine quantification. The start of the experiment was designated as day 0, when all plants received either 7.0 mg of $[^{14}N]KNO_3$ or 7.5 mg $[^{15}N]KNO_3$ and were given their MJ and/or damage treatments. All plants in experiment 1 received $[^{15}N]KNO_3$, but to save costs in experiment 2, only the plants slated for seed production and harvests on day 15 were given $[^{15}N]KNO_3$; all others were given $[^{14}N]KNO_3$. In experiment 2, leaves were removed from plants in treatments 2–5 just before the addition of the $[^{15}N]KNO_3$ and the MJ treatments so as not to lose any ^{15}N from the plants in the removed leaves. Hence, all plants in both experiments received the same molar amount of nitrogen (e.g., 28 mg ^{14}N before day 0 and 7 mg of ^{14}N or 7.5 mg of ^{15}N after day 0 for a total of 2.5 mml^{-1} for each plant).

At the time of harvest, plants in both hydroponic experiments were separated into roots and shoots, frozen in liquid nitrogen, lyophilized, weighed (to 0.1 mg), and ground to a fine powder (850 μm mesh) in a Wiley mill (Thomas Scientific, Swedesboro, N.J., USA). In experiment 2, the leaf area was measured on the plants harvested on day 0 and the height of the flowering stalk was measured on all plants on day 25. In both experiments, plants were examined daily for mature seeds once the capsules started maturing (after day 30). Seeds were air dried at 27–29°C and were pooled for each plant, and the pooled seed mass was weighed every 3–5 days until the plants had completely senesced.

Soil experiment

Seeds were from the first generation of glasshouse-grown self-pollinated plants which originated from two single-plant collections made in 1995 from Motoqua Mountain, 12 miles east of St. George, Utah, and from the Kearsley Ranch, Arizona, 3.2 km north of Flagstaff, Arizonia. Seeds were soaked in an aqueous extract of wood smoke to stimulate germination (Baldwin et al. 1994b), germinated in flats of Cornell mix A potting soil, and transplanted after 18 days into 1-l pots each containing 1 kg of either burned soil collected from a 1-year-old burn at Brine Pond, Utah, (BLM fire R201, T20S R19W section 30), or unburned soil collected from the intact piñyon-juniper habitat immediately adjacent to the burned area. Since the burned and unburned soil collections consisted primarily of the A_0 and A_1 horizons, each soil type was mixed 2:1 (v:v) with sand to provide a mineral component to the soil and better simulate the composition of the native soils.

Fifteen days after transplanting, ten plants of each genotype growing in each soil type were randomly assigned to either control or MJ treatments. Plants in the MJ treatment received 500 μg of MJ dispersed by sonication in 10 ml of water and applied to the soil at the base of the plant, while the plants of the control treatment received only water (day 0). On day 5, one leaf occupying position 8 was harvested for nicotine analysis from each plant growing in burned soil. Plants were examined daily for mature seeds once the capsules started maturing. Genotype 1 began to set seed on day 39, while genotype 2 began to set seed on day 29. Seed was collected every 3 days till day 67 after MJ treatment when plants in the unburned soil had senesced. Seeds were air-dried at 27–29°C and were pooled for each plant, and the pooled seed mass was weighed every 3–5 days.

Nutrient contents of the soils were analyzed at the Cornell Nutrient Analysis Laboratory (Cornell University, Ithaca, N.Y., USA) both before and after plant growth. Before plant growth, the burned soil contained 130 mg of extractable N kg^{-1}(as either ammonia or nitrate), 495 mg available K kg^{-1}, 583 mg Mg kg^{-1}, 8340 mg Ca kg^{-1}, 3.5 mg Fe kg^{-1}, 19 mg Al kg^{-1}, 43 mg Mn kg^{-1}, 0.62 mg Zn kg^{-1}, 8.8 mg Cu kg^{-1}, and 7.24% organic matter. After plant growth, the extractable nitrogen in burned soil had decreased by 70 mg kg^{-1}, and levels of available K, Mg, Ca, Fe, Al, Mn, Zn and Cu had decreased to 219, 151, 5481, 1.9, 11, 25, 0.33, and 4.7 mg kg^{-1}, respectively. Before plant growth, the unburned soil contained no extractable ammonia or nitrate N, 275 mg available K kg^{-1}, 238 mg Mg kg^{-1}, 4441 mg Ca kg^{-1}, 4 mg Fe kg^{-1}, 8.95 mg Al kg^{-1}, 21 mg Mn kg^{-1}, 0.43 mg Zn kg^{-1}, 4.3 mg Cu kg^{-1} and 9.23% organic matter. Since all the pots were top-watered and had individual bottom-trays to recycle leached nutrients back into the pots, we infer that the difference between plant growth values before and after reflect plant acquisition.

Quantification of chemical and fitness parameters

Nicotine analysis was modified from Baldwin (1988) to obtain WP nicotine measures (hydroponic experiments) or leaf nicotine concentration (soil experiments). A weighed portion (approximately 50 mg) of lyophilized and homogenized whole plant or leaf was extracted for 3 days in 10.0 ml of aqueous alkaloid extraction solution (40% methanol 0.1% HCl). Nicotine contents of these extracts were determined by HPLC with nicotine standards analyzed every 30 samples. WP nicotine pools and nicotine concentrations were calculated from biomass values, and nicotine was expressed as micrograms of N in nicotine to facilitate comparison with the measures of *de novo* nicotine production (^{15}N in nicotine). Since the WP nicotine concentration data for the two hydroponic experiments can be simply calculated by converting the WP N-nicotine data (Figs. 1C and 4C) into WP nicotine and dividing it by the WP biomass data (Figs. 1A and 4A), these data are not presented.

De novo nicotine production was determined with a pulse-chase technique using $K^{15}NO_3$ as the sole nitrogen source and the quantification of the incorporation of ^{15}N into nicotine by mass

spectrometry (Baldwin et al. 1994a). These measures of nicotine production reflect the amount of nicotine produced from nitrogen that is acquired, reduced, and assimilated after the introduction of the $^{15}NO_3$ into the hydroponic solution (Day 0). Nicotine production was measured as WP quantities of ^{15}N in nicotine at harvests on days 7, 9, 13 (experiment 1) and on day 15 (experiment 2), which was in turn calculated from the measures of mg N in nicotine and the measures of ^{15}N incorporation into nicotine. Calculations of single and double ^{15}N incorporations into the nicotine molecule were based on the relative abundances of fragment ions 133, 134, and 135 as described in Baldwin et al. (1994a).

Analysis of %N and atom%^{15}N of the pooled seed samples was performed by Dumas Combustion-Mass Spectrometry by Metabolic Solutions Inc. (Merrimack, N.H. USA). Seed and WP total N and ^{15}N pools were calculated as (%N/100) cumulative seed mass or WP mass and (%^{15}N enrichment/100) × mg N, respectively. The percentages of total nitrogen in nicotine and seed and total ^{15}N in nicotine and seed were calculated as (mg N-nicotine or N-seed/plant)/(mg N/plant) 100% and (mg ^{15}N-nicotine or ^{15}N-seed/plant)/(mg ^{15}N /plant) 100%, respectively.

Four replicate aliquots of 100 seeds from each plant were weighed to determine the average mass seed^{-1}. The pooled seed collections from each plant were pooled by treatment, and ten replicate samples of 20 seeds from each treatment were tested in a seed germination protocol which provided the optimum conditions for germinating all viable seeds, as described in Baldwin et al. (1994b) and Baldwin and Morse (1994). Briefly, for each germination trial, 20 seeds were soaked for 1 h in a 1:300 dilution (v:v) of "liquid smoke" (House of Herbs, Inc., Passaic, N.J., USA) in 1 mml^{-1} KNO_3 and transferred to souffle cups (Solo 1 oz, P100) containing approximately 5.5 g sterile sand. The sand in the seed cups was saturated with 1 mml^{-1} KNO_3 so that seeds were just touching the water layer. Cups were sealed with transparent lids (Solo PL1 lids) and placed in a growth chamber (Percival Boone, Iowa, USA, Model E-54U) which provided 14 h day:10 h night photoperiod with 200 μM m^{-2} s^{-1} PAR and 30°C day:22°C night temperature cycle. Seeds were examined for germination for up to 20 days after the initial transfer to the cups.

Chemicals

MJ was obtained from Bedoukian Research (Danbury, CT) which we determined by GC-MS analysis to be close to the thermodynamic equilibrium for the epimers of MJ (Mueller and Brodschelm 1994): 90.1% 1R, 2R MJ and 8.3% 1R, 2S MJ. All plant nutrients were purchased from Sigma Chemical Co. (St. Louis, Mo., USA) except ^{15}N-labeled (99.9 atom %) KNO_3, which was purchased from Isotec Inc. (Miamisburg, Ohio, USA).

Statistical analysis

Percentage and proportion data were arcsin-transformed before statistical analysis. Two-way ANOVAs with treatments and harvest days as main effects were performed for all parameters measured during the three destructive harvests of each experiment, and contrasts among treatment groups were made from these ANOVAs to test the following *a priori* null hypotheses: that MJ-treated or leaf-removal (LR) treatment plants did not differ among themselves or from controls (experiments 1 and 2) and that punctured plants differed from controls (experiment 1). All other *post hoc* contrasts were performed with the Bonferroni correction of significance values for multiple comparisons. Since only the day 15 harvest of experiment 2 was given $^{15}NO_3$, the analysis of ^{15}N-nicotine for this experiment was performed with a one-way ANOVA. Seed production data from all experiments were analyzed with repeated measures one- and two-way ANOVAs. All experiments were analyzed separately because they were conducted sequentially in the glasshouse. All data analysis was performed with the SYSTAT statistical package (Evanston, Illinois, USA).

Results

Hydroponic experiment 1: (no leaf removal)

All measures of nicotine production (WP N-nicotine, ^{15}N-nicotine and % nicotine) revealed that both stan-

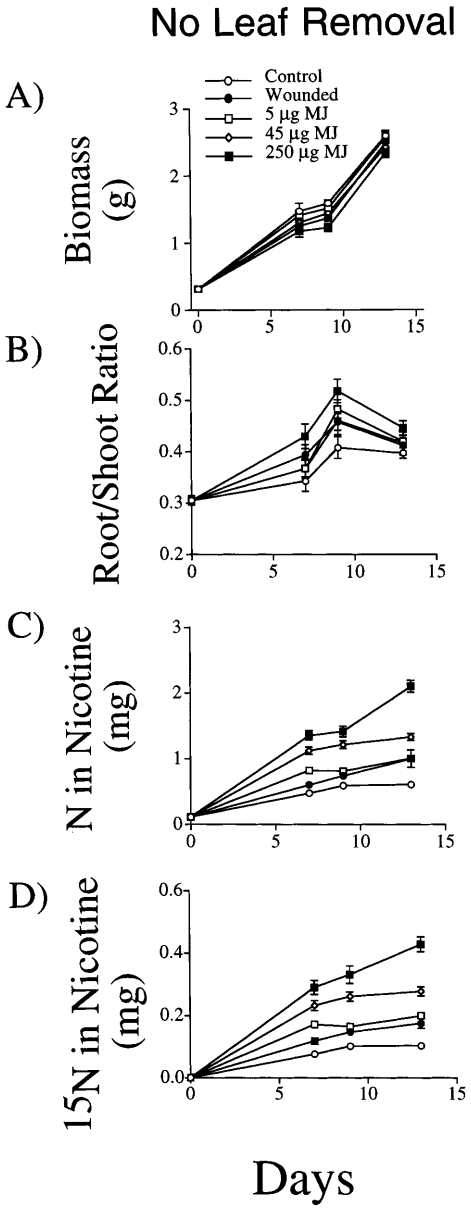

Fig. 1 Mean (±SEM) **A** whole-plant (WP) biomass (g), **B** root:shoot ratio, **C** WP N in nicotine (mg) and **D** WP ^{15}N in nicotine from plants in 4 harvests (on days 0, 7, 9, 13) from hydroponic experiment 1 (in which no leaves were removed). Plants were treated with 0 (*Controls*), 5, 45, or 250 μg of methyl jasmonate (*MJ*) applied to their roots or had leaves punctured with a standardized leaf puncturing protocol on day 0. All plants were given 7.5 mg of ^{15}N as $K^{15}NO_3$ on day 0 and the measures of ^{15}N in nicotine (**D**) quantify the nicotine synthesized *de novo* with N accumulated and assimilated since day 0 – the day of puncturing and MJ treatments. See Table 1 for statistical analysis. *Plot symbols* obcure *error bars* at some harvests

dardized leaf puncturing and root MJ treatments significantly (Table 1) increased WP nicotine pools (Fig. 1C) and *de novo* nicotine production (Fig. 1D). The response to leaf puncturing was comparable to that of the 5 μg MJ treatment which increased *de novo* nicotine production (Fig. 1D), WP nicotine pools (Fig. 1C) and percentage nicotine contents (calculated by dividing WP nicotine pools by WP biomass – Fig. 1A) by 93, 66 and 65%, respectively, above the values measured in control plants by the day 13 harvest. The nicotine response increased with increasing amounts of MJ applied to the plant's roots. Plants treated with 250 μg of MJ had values that were 3.1-fold, 2.5-fold and 2.8-fold higher than those of control plants (Fig. 1C, D) while plants treated with 45 μg of MJ synthesized and accumulated nicotine in quantities intermediate to those observed in the 5 and 250 μg MJ treatments (Fig. 1C, D).

Since plants were grown with a defined nitrogen supply, the proportion of the plant's total acquired nitrogen pool that was incorporated into nicotine and the proportion of the nitrogen that the plants acquired, reduced, assimilated, and incorporated into newly synthesized nicotine after the MJ treatments (as determined with the $^{15}NO_3$ pulse and designated as the proportion of "current uptake" hereafter) could be readily determined. Thirteen days after the treatments, control plants and plants treated with 5, 45, or 250 μg of MJ and subjected to leaf puncturing had incorporated an average of 1.37, 2.65, 3.68, 5.69, 2.32% of the current uptake and 1.74, 2.88, 3.80, 6.00, 2.86% of the total nitrogen into nicotine, respectively.

Plant growth was significantly influenced by the treatments (Table 1; Fig. 1A), but patterns of allocation between root and shoot, as discernible from the root:-shoot ratio (Fig. 1B), were unaffected by the treatments (Table 1). Contrasts were performed on the ANOVAs presented in Table 1 to determine which treatments affected plant growth at which harvests. Plants with leaf puncturing and plants treated with 45 and 250 μg of MJ had biomass values significantly lower than control plants at the day 9 harvest ($F_{1,45}$s > 8.29; Ps < 0.01). The decreases in plant biomass between the MJ-treated plants and the controls (biomass lost) were positively correlated with the amount of nicotine that treated plants synthesized *de novo* (^{15}N-nicotine induced) and accumulated (N-nicotine induced) above control plants (Fig. 2 for day 13 harvest). For all three harvest dates the relationship was highly linear (all r^2s > 0.968) and positive (day 7: mg ^{15}N-nicotine = 0.498 g biomass lost + 0.067 and mg N-nicotine = 2.232 g biomass lost + 0.233; day 9: mg^{15}N-nicotine = 0.523 g biomass lost + 0.048 and mg N-nicotine = 1.840 g biomass lost + 0.199; see legend of Fig. 2 for the regression coefficients for the day 13 harvest). Hence, an increase in one mg of

Table 1 Two- and one-way ANOVAs for **A** biomass, **B** root/shoot ratios, **C** N-nicotine, **D** % nicotine, and **E** ^{15}N-nicotine and **F** repeated measures one-way ANOVAs for cumulative lifetime seed production for plants harvested from hydroponic experiment 1 (no leaf removal) and experiment 2 (leaf removal). Means and SEMs are presented in Figs. 1–5

Source of variation	No leaf removal			Leaf removal		
	df	*F*	*P*	*df*	*F*	*P*
A Biomass (g):						
Treatment	4	7.31	< 0.001	4	27.92	< 0.001
Day	2	251.42	< 0.001	2	60.63	< 0.001
Day × Treatment	8	0.24	0.982	8	0.711	0.6
Error	135			120		
B Root/Shoot ratio:						
Treatment	4	1.21	0.308	4	12.63	< 0.001
Day	2	7.38	< 0.001	2	46.83	< 0.001
Day × Treatment	8	0.62	0.760	8	0.780	0.621
Error	135			120		
C N-nicotine (mg plant^{-1})						
Treatment	4	144.82	< 0.001	4	105.91	< 0.001
Day	2	42.56	< 0.001	2	4.52	0.013
Day × Treatment	8	6.19	< 0.001	8	2.54	0.014
Error	135			120		
D % Nicotine (%dry mass)						
Treatment	4	135.58	< 0.001	4	143.52	< 0.001
Day	2	33.08	< 0.001	2	13.64	< 0.001
Day × Treatment	8	1.62	0.124	8	1.30	0.250
Error	135			120		
E ^{15}N-nicotine (mg plant^{-1})						
Treatment	4	138.59	< 0.001	4	29.22	< 0.001
Day	2	20.28	< 0.001			
Day × Treatment	8	2.9	0.005			
Error	135			37		
F Cumulative seed lifetime production (mg plant^{-1})						
Treatment	4	2.31	0.066	4	0.257	0.904
Error	70			45		

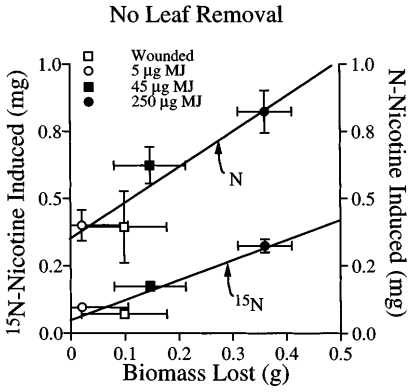

Fig. 2 Mean (±SEM) mg of ^{15}N in nicotine (left axis) and total mg N in nicotine (right axis) induced above control plants in plants treated with 5, 45, or 250 µg of methyl jasmonate (*MJ*) 13 days prior in hydroponic experiment 1 (in which no leaves were removed). Induced increases in ^{15}N-labeled and total nicotine are plotted against the differences in whole-plant biomass (g) between plants treated with MJ and untreated control plants. The regression equations for MJ treated plants are: mg^{15}N-nicotine = 0.674 g biomass lost + 0.079 and mg N-nicotine = 1.210 g biomass lost + 0.403. The values for plants whose leaves were wounded with a standardized mechanical wounding protocol are also depicted but are not included in the regression equation. Regression equations for harvests on days 7 and 9 are given in the text

total N-nicotine plant^{-1} is associated with a reduction in 2.232 grams of biomass 7 days after induction, but this association decreases to 1.210 g biomass by day 13. Over the same time interval, the synthesis of 1 mg of ^{15}N-nicotine from N that was acquired and assimilated at the time of induction is associated with smaller but increasing amounts of biomass lost, from 0.49 g on day 7 to 0.67 g on day 13. The use of previously stored reserves of N (i.e., unlabeled N) for the synthesis of nicotine may have greater negative consequences for growth than the use of nitrate-N acquired from the environment at the time of induction. The values from plants with wounded leaves were below the induced nicotine-biomass lost regression lines (Fig. 2). In other words, they showed a greater reduction in biomass per mg of nicotine accumulated and synthesized than did the MJ-treated plants, which is not surprising given that leaf puncturing induces many other responses in addition to the production of nicotine (i.e., wound repair; Baldwin 1994) which, in turn, are likely to be associated with reductions in plant growth.

Plant root:shoot ratios (Fig. 1B) increased up to the day 9 harvest as plants depleted their N reserves and allocated proportionally more biomass to root production. Plants had started to elongate their floral stalks by day 13 and by this harvest root:shoot ratios declined, corresponding to the initiation of reproductive growth.

Interestingly, despite the significant reductions in growth, cumulative seed production was not significantly affected by any of the treatments (Table 1; Fig. 3A). Plants with wounded leaves tended to produce the largest amount of seed, suggesting that puncturing had activated a compensatory response, but the increase was not significant. The viability of seeds did not differ among treatments ($F_{4,40} = 0.092$, $P = 0.7$); all were more than 90% viable. Similarly, the average mass seed^{-1} (115.3 ± 1.6 µg), and the average amount of N in seed plant^{-1} (5.313 ± 0.186 mg) was not significantly affected by the treatments ($F_{4,70}$s < 2.1; Ps > 0.09). On average, plants used 14.9% of their total acquired N pools for seed production. The amount of N acquired after MJ and puncturing treatments that was used for seed production was determined from the amount of ^{15}N in seed; treatments significantly influenced these values ($F_{4,70} = 2.50$, $P = 0.050$) . Since all plants had acquired by day 13 the entire 7.5 mg of ^{15}N in NO_3 that they were given on day 0, the ^{15}N in seed values are expressed as a percent of the total given (Fig. 3B). Contrasts from the ANOVA revealed that plants in the leaf-puncturing and 250 µg MJ treatments had significantly higher allocations of ^{15}N to seed (16.0%) than did the other treatments (13.0%; $P = 0.045$).

Hydroponic experiment 2: (leaf removal).

The LR treatment on day 0 removed an average of 0.127 ± 0. 004 (n = 109) g of leaf mass from each plant, which represented 28.9% of the average WP mass

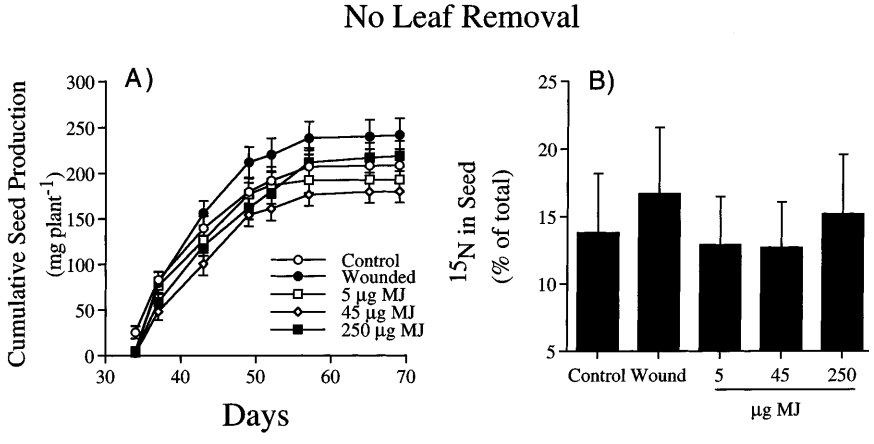

Fig. 3 Mean (±SEM) **A** cumulative life-time seed production and **B** percentage of ^{15}N given on day 0 (7.5 mg of ^{15}N as KNO_3) which was used in seed production of plants from the 5 treatments of hydroponic experiment 1 (in which no leaves were removed). Plants were treated with 0 (Controls), 5, 45, or 250 µg of methyl jasmonate (*MJ*) applied to their roots or had leaves punctured with a standardized leaf puncturing protocol on day 0

or 44% of the shoot area of the plants on day 0. This leaf removal significantly reduced the WP masses of all LR treatments to values 30–41% of unwounded controls for all harvests through day 15 (Fig. 4A; Table 1). The growth effects of MJ treatments on LR plants could be seen only in the first harvest (day 5), when all MJ-treated LR plants had WP masses approximately 14% lower than untreated LR plants ($F_{1,44} = 5.781$; $P = 0.02$). At this harvest MJ-treated plants did not differ among each other ($F_{1,37} = 0.477$; $P = 0.477$). Leaf removal alone modestly increased nicotine production (Fig. 4D), but did not increase WP nicotine pools (Fig. 4C) or concentrations ($F_{1,37}$s < 0.4). MJ treatments, however, significantly increased all measures of nicotine production and accumulation. WP nicotine pools, percentage nicotine contents, and *de novo* nicotine synthesis were 25, 25, and 48% higher in LR plants treated with 5 μg of MJ than in LR-untreated plants. Again, the nicotine response increased with increasing amounts of MJ. LR plants treated with 250 μg of MJ had values that were 3.1-fold, 3.3-fold, and 4.2-fold higher than those of LR untreated plants (Fig. 4C, D), while plants treated with 45 μg of MJ synthesized and accumulated nicotine in quantities intermediate to those observed in the 5 and 250 μg MJ treatments (Fig. 4C, D). Fifteen days after the treatments, plants had incorporated an average of 1.02, 1.39, 2.05, 3.92, 7.24% of their current (^{15}N-labeled) N uptake and 0.88, 0.87, 1.13, 1.95, 3.54% of the total acquired N into nicotine in unwounded control plants and in LR plants treated with 0, 5, 45 or 250 μg of MJ, respectively.

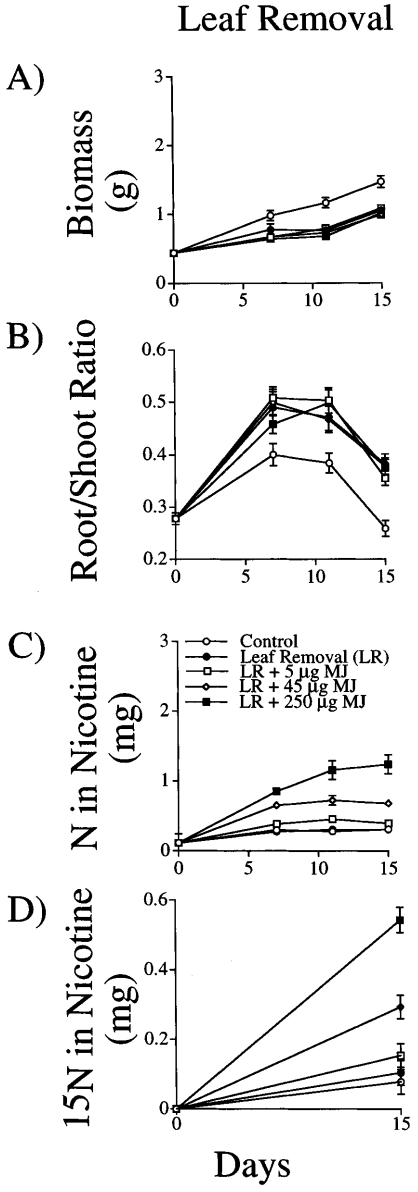

Fig. 4 Mean (±SEM) **A** whole-plant (WP) biomass (g), **B** root:-shoot ratio, **C** WP N in nicotine (mg) and **D** WP ^{15}N in nicotine from plants in 4 harvests (on days 0, 7, 11, 15: **A–C**) or 2 harvests (on days 0 and 15: **D**) from hydroponic experiment 2 (in which 44% of the leaf area was removed on day 0 from plants in *LR* treatments). LR plants were treated with 0, 5, 45, or 250 μg of methyl jasmonate (*MJ*) applied to their roots on day 0. Plants in the control treatment were neither wounded nor treated with MJ. All plants were given 7.5 mg of ^{15}N as $K^{15}NO_3$ on day 0 and the measures of ^{15}N in nicotine (**D**) quantifies the nicotine synthesized *de novo* with N accumulated and assimilated since day 0 – the day of leaf removal and MJ treatments. See Table 1 for statistical analysis. *Plot symbols* obsure *error bars* at some harvests. The values from the LR treatment plants are obscured by the values from the control treatment in **C**, demonstrating that the leaf removal technique did not increase WP nicotine pools

Plants had started to elongate their floral stalks by day 15 with the corresponding decrease in root:shoot ratios (Fig. 4B). The height of the floral stalk was measured on day 25 when all plants had produced at least one mature flower. The floral stalk of undamaged control plants (46.7 ± 1.7 cm) was significantly ($F_{4,37} = 7.79$; $P = 0.0001$) taller than all of the LR treatments (33.3 ± 2.0 cm), which did not differ among the MJ-treatments ($F_{3,37} = 0.09$; $P = 0.76$). Interestingly, despite reductions in growth resulting from leaf removal, cumulative seed production did not differ significantly among any of the treatments (Table 1; Fig. 5A). As was observed in experiment 1, plants with leaf puncturing tended to produce more seed than those not wounded, but the difference was again not significant. The viability of seeds did not differ among treatments ($F_{4,40} = 0.23$, $P = 0.5$); all were more than 93% viable. Similarly, the average mass seed^{-1} (119.6 ± 0.8 μg) and the average amount of N in seed plant^{-1} (5.003 ± 0.160 mg) were not significantly affected by the treatments ($F_{4,45}$s < 0.38; Ps > 0.82). On average, plants used 14.1% of their total nitrogen pools for seed production. Contrasts from the ANOVA analyzing the amount of ^{15}N in seed and the percentage of total ^{15}N in seed revealed that unwounded control plants allocated less ^{15}N to seed production (17.1% of current uptake) than did the LR treatments (21.7% of current uptake; $F_{1,45} = 3.50$, $P = 0.001$), which, in turn, did not differ among themselves in the amount allocated ($F_{3,45} = 0.16$, $P = 0.6$; Fig. 5B).

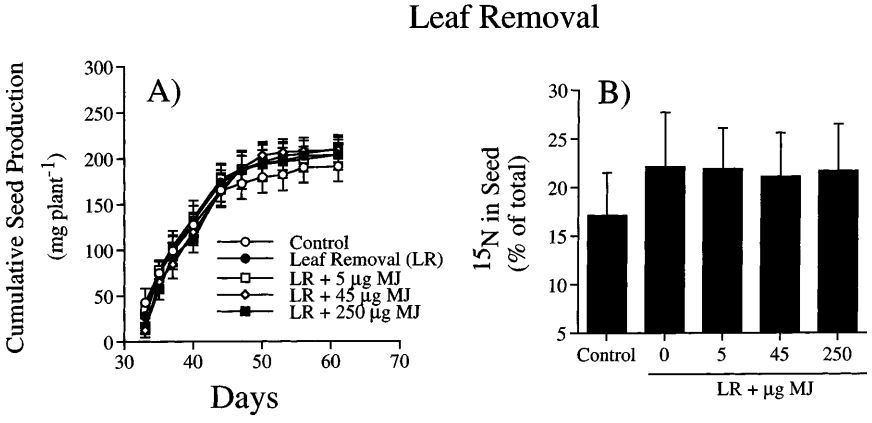

Fig. 5 Mean (±SEM) **A** cumulative life-time seed production and **B** percentage of ^{15}N given on day 0 (7.5 mg of ^{15}N as KNO_3) which was used in seed production of plants from the 5 treatments of hydroponic experiment 2 (in which 44% of the leaf area was removed on day 0 from plants in LR treatments). LR plants were treated with 0, 5, 45, or 250 µg of methyl jasmonate (MJ) applied to their roots on day 0

Soil experiment

MJ treatments of plants growing in burned soil increased the nicotine concentrations by 253% and 109% for genotypes 1 and 2, respectively (both $F_{1,34s} \leq 20.08$; $Ps > 0.001$; Fig. 6). These increases were comparable to those observed in the 250 µg MJ-treatments of the hydroponic experiments for genotype 1 and in the 45 µg MJ-treatments for genotype 2. Single-leaf nicotine concentrations are typically higher than WP nicotine concentrations because the inclusion of less defended tissues such as roots, which have concentrations 10–15% of those of shoots (Baldwin 1996) will decrease the WP values.

The rate of cumulative seed production was significantly higher in burned soil than in unburned soil for both genotype 1 (356 ± 91% higher; $F_{1,36} = 65.98$, $P < 0.0001$) and genotype 2 (213 ± 34% higher; $F_{1,35} = 111.00$, $P < 0.0001$; Fig. 6). These increases in seed production were a result of increases in capsule production per plant ($F_{1,30} = 55.98$, $P < 0.0001$ and $F_{1,35} = 88.494$, $P < 0.0001$ for genotypes 1 and 2, respectively) rather than changes in the mass of seeds produced per capsule. Soil type had no effect on the mass of seed produced per capsule ($F_{1,30} = 0.378$, $P = 0.544$ and $F_{1,35} = 3.756$, $P = 0.061$ for genotype 1 and 2, respectively). MJ-treatments significantly reduced cumulative seed production both in genotype 1 (by 48.0 ± 13.6% in burned soil and by 77.1 ± 26.5% in unburned soil; $F_{1,35} = 42.0$, $P < 0.0001$) and in genotype 2 (by 38.2 ± 11.5% in burned soil and by 65.7 ± 13.2% in unburned soil; $F_{1,36} = 29.6$, $P < 0.0001$; Fig. 6).

By analyzing soil before and after plant growth, we estimated the amount of nitrogen that the plants acquired from the soil and allocated to seed production. In burned soil, control plants of genotype 1 used 27.9 ± 2.4% of their total N for seed production and MJ-treated plants used 14.5 ± 2.7%, while control plants of genotype 2 used 60.8 ± 4.8% and MJ-treated plants used 37.6 ± 4.7%. Hence, MJ-treated plants growing in burned soil used approximately 50% less of their total acquired N for seed production as compared

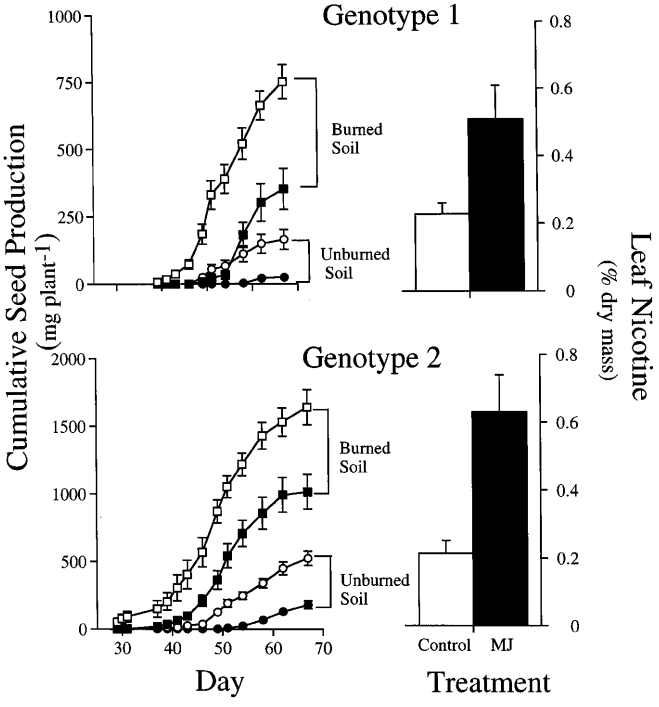

Fig. 6 Mean (±SEM) cumulative seed production of genotypes 1 and 2 growing in burned (*squares*) and unburned (*circles*) soil and treated with water (*open symbols and bars*) or one treament of 500 µg MJ delivered to the rhizosphere (*filled symbols or bars*: *left panels*), and control and MJ-induced nicotine concentrations of a leaf at node 8 for each genotype in burned soil (*right panels*) 5 days after treatment. *Error bars* are obscured by *plot symbols* in some cases

to control plants, reflecting a tradeoff between seed production and MJ-induced responses. Control plants, growing in burned soil, allocated 2–4.3 times more of their acquired N to seed production than did hydroponically-grown plants, and this greater allocation may be due to the greater numbers of floral meristems produced by soil-grown plants. Soil-grown plants tended to have a bushier growth-form than hydroponically grown plants.

Because no nitrogen could be extracted from unburned soil, the proportion of acquired N used in seed production could not be calculated. Plants in unburned

soil incorporated on average 9.41 ± 1.32 mg N into seeds in control plants and 3.33 ± 0.66 mg N into seeds in MJ-treated plants. We assume that these plants used immobilized N in the soil which was not extractable by KCl because *N. attenuata* can not fix nitrogen.

Discussion

The requirements for establishing a resource-based tradeoff are onerous (Mole 1994) and demand particular growth conditions to meet these requirements (see Introduction). The hydroponic culturing of individual plants makes it possible to measure the variables required to assess a resource-based tradeoff in a nitrogen currency with a non-nitrogen fixing plant species. By growing plants in individual hydroponic chambers and assigning them to treatment groups by their starting biomass, we can accurately quantify biomass changes resulting from jasmonate and wound-induced responses and thereby quantify the "third party" tradeoffs between defense and growth that might obscure tradeoffs between defense and reproduction. Not only does hydroponic culture allow for the accurate quantitation of changes in WP biomass because total root mass can be accurately measured, it minimizes microbial immobilization of N which can result in additional third party tradeoffs between the plant and the microbial community residing in the soil which, in turn, can obscure allocation patterns occurring in the plant.

In the two hydroponic experiments, we were able to increase the allocation of N to a nicotine-based defense approximately 3-fold, until plants had incorporated 6% of their total acquired N into their nicotine pool. As in our work on the sibling species, *N. sylvestris* (Baldwin et al. 1994a), we found no evidence that *N. attenuata* metabolizes the N in the nicotine it has synthesized and re-utilizes this N for growth. The ^{15}N-nicotine pools increased or remained stable for the duration of vegetative growth, a time when the plants received no additional N inputs and were under N-limited growth. Such a condition should select for N reutilization. The large increases in MJ-induced nicotine production and accumulation were linearly correlated with decreases in plant biomass (Fig. 2), demonstrating that MJ-induced responses are clearly costly to plant growth.

However, these MJ-induced reductions in vegetative growth did not affect any of the estimates of plant fitness for undamaged hydroponically-grown plants (Fig. 3A). Moreover, cumulative lifetime seed production was not reduced even when plants lost 44% of their leaf area at the time of MJ-treatments (Fig. 5A), a removal which resulted in dramatic reductions in vegetative growth (Fig. 4A) and stalk elongation. An examination of the WP patterns of N allocations revealed that the potential third party tradeoffs were large and could easily buffer any potential N-based tradeoffs between seed and nicotine productions. Plants in both hydroponic experiments utilized only 14% of their total acquired N for seed production and 6% for nicotine biosynthesis, which leaves 80% of the plant's acquired N to buffer N-based tradeoffs with seed production. The use of current N uptake (^{15}N-labeled nitrogen) for seed production (Figs. 3A and 5 A) was a more sensitive indicator of the N demands of responses induced by MJ, wounding, and leaf removal treatments. We conclude that seed production of plants grown under these hydroponic conditions is highly buffered from the nitrogen demands of wound- and jasmonate induced responses.

Given that *N. attenuata* is an annual plant that survives to the next growing season only by producing seed, it is surprising that, at the time of total senescence, these hydroponically grown plants had allocated only 14% of their total acquired N to seed production. While the estimates of N allocation to seed production in other desert annual species are two to three times higher than the estimates reported here (Williams and Bell 1981), methodological considerations make comparisons difficult. Most measures of the proportion of N used for seed production express the amount of N in the seed as a proportion of the amount of N in the residual vegetative parts of the plant at the time of sencesence. Plants inevitably lose N to the environment during senescence, and these values likely over-estimate the allocation of total acquired N used for seed production.

To determine whether seed production of soil-grown plants was similarly buffered from the responses induced by MJ, we grew 2 genotypes of plants in the high- and low-nutrient soils that characterize burned and unburned environments from the plant's natural habitat: the piñyon-juniper forests of the southwestern United States. For these soil-grown plants, MJ induction resulted in significant reductions in seed production (Fig. 6). Treating roots with 500 μg of MJ increased nicotine concentrations to levels comparable to the 45 μg and 250 μg treatments of the hydroponic experiments (depending on the genotype of plant). MJ treatments resulted in large reductions in absolute seed production in both genotypes of plant grown in both soil types (a reduction of 399 ± 99.96 and 139.4 ± 38 mg of seed per plant in genotype 1 plants grown in burned and unburned soils, and a reduction of 256 ± 181.9 mg and 343.5 ± 60.04 mg for genotype 2 plants grown in burned and unburned soils, respectively; Fig. 6). Because plants grown in unburned soils had a lower absolute seed production than the plants grown in burned soil, the proportional fitness reductions resulting from MJ treatments were higher for these smaller plants.

Our estimates of the proportional N allocation to seed production for soil-grown plants are substantially less accurate than the estimates for hydroponically-grown plants. In the hydroponic experiments, we were able to directly measure WP nitrogen budgets and accurately determine the proportion of the budget allocated to nicotine in response to the treatments. We have yet to develop the techniques to do this with sufficient accuracy for soil-grown plants. Not only is it difficult to quantify WP biomass accurately at the time of harvest,

due to difficulties in recovering the entire root system, it is not possible to assign plants to treatment groups by biomass in order to produce groups of plants with same mean biomass before the start of the experiment – a requirement for quantifying the absolute increase in allocation to a function (defense, growth or reproduction) in response to a particular treatment. Hence, for the soil-grown plants we estimated the WP N budget by the differences in extractable soil N before and after plant growth. However, the immobilization of nitrogen by the soil microbial community seriously compromised the accuracy of this approach by producing a large, unquantified, third party tradeoff. For example, cumulative seed production of plants grown in unburned soils were comparable to those of the plants grown under the hydroponic conditions. However, no detectable amounts of extractable ammonia or nitrate could be measured in these unburned soils. Since these plants are not nitrogen-fixing we must assume that they used immobilized N sources for growth.

Plants grown in the burned soils were larger and produced 3.5-2.1 fold more seed than the same genotypes grown in unburned soil. Based on the measures of extractable soil N contents before and after plant growth, we estimate that these plants allocated 30–61% (depending on plant genotype) of their total acquired N to seed production, or approximately 2–4.3 times the amount observed in the hydroponically-grown plants. MJ induction reduced these estimates of N allocations to seed production to 51–61% of levels found in control plants. We conclude that while hydroponic culture allows for a rigorous quantitation of N allocation to different plant functions, the allocation patterns of this species when grown in hydroponic culture differ from those of plants grown in their native soils. Hydroponically grown *N. attenuata* plants appear to initiate fewer reproductive meristems than soil-grown plants and consequently have lower reproductive allocations. Experiments with hydroponically-grown members of the sibling species, *N. sylvestris*, detected significant resource-based tradeoffs when plants were grown under intraspecific competition (I.T. Baldwin and W.D. Hamilton, unpublished work), so the difficulty with hydroponic culture may be species-specific.

We conclude that MJ induced responses result in significant fitness reductions when plants are grown in soil, but are unable to determine whether the reductions are a result of an allocational tradeoff. The fitness reductions measured here (38–48%) are similar to those observed in a field experiment with the sibling species, *N. sylvestris* (Baldwin et al. 1990), which used a different approach to estimating the fitness consequences of wound-induced nicotine production. In this experiment, plants were wounded with a standardized mechanical damage technique and had their wound-induced nicotine response suppressed with auxin applications to the wound site. The lifetime seed production of wounded plants which exhibited the normal wound-induced nicotine response was 32% less than that of similarly wounded plants which had their wound-induced nicotine suppressed with auxin. There is something strongly associated with wound- and jasmonate induced nicotine production in *Nicotiana* which is clearly costly, however, whether it is the nitrogen costs of nicotine production that are responsible for these costs is not clear.

Acknowledgements This research was supported by grants from the National Science (DEB-9505950) and A.W. Mellon Foundation and the Max-Planck-Gesellschaft. We thank E. Wheeler for editorial assistance, B. McNulty for assistance in preparing the figures, and Dr. D. Bowers and an anonymous reviewer for insightful comments on an earlier draft of this manuscript.

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