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Competition mediates costs of jasmonate-induced defences, nitrogen acquisition and transgenerational plasticity in *Nicotiana attenuata*

N. M. VAN DAM* and I. T. BALDWIN†

Max Planck Institute for Chemical Ecology, Carl-Zeiss-Promenade 10, 07745 Jena, Germany

Summary

1. Plants adjust their phenotype in response to environmental signals, but little is known about the interaction of plastic responses to simultaneously occurring environmental stresses.

2. To examine the costs of induced resistance on reproductive performance in plants subjected to other important environmental variables, resistance was elicited with a jasmonate treatment (MeJA) to one, both or neither of two *Nicotiana attenuata* plants growing competitively in either high- or low-N soils. Half the plants were subjected to leaf removal (LR). $K^{15}NO_3$ was used to quantify differences in N acquisition and allocation. Transgenerational effects were measured with seed germination and seedling performance tests.

3. An induced plant competing with an uninduced plant produced significantly fewer seeds, acquired less ^{15}N and allocated less ^{15}N to seed production. Uninduced plants competing with induced plants realized a comparable fitness benefit.

4. The costs of induction were greater under high N. Plants grown under low N minimized costs by allocating significantly more N to seeds. LR decreased seed production independently of any other effect. Low N and LR both reduced germination rates.

5. The effects of MeJA on seed germination depended on competition and N supply. The differences in germination rates resulted in dramatic fitness differences among offspring.

6. *N. attenuata* plants appear to use N availability and their induced status to alter their current phenotype and their offspring's phenotype to adjust to environmental changes that occur predictably over time in their natural environment.

Key-words: Costs of induction, intraspecific competition, MeJA-induced responses, nitrogen allocation, offspring fitness

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Introduction

Phenotypic plasticity allows plants and other sedentary organisms to maximize their potential fitness in variable environments (Fenner 1986; Sultan 2000; Via *et al.* 1995). Plants can reduce – or even negate – the negative fitness effects of nutrient stress (Sultan 1996; Sultan 2000) and competition (Nicotra & Rodenhouse 1995) or leaf loss (de Visser *et al.* 1997) by changing internal resource allocation patterns. Similarly, many plant species increase defence traits after an initial herbivore attack (Karban & Baldwin 1997). These changes may provide a selective advantage if the fitness of the plastic genotypes, on average, exceeds that of fixed genotypes under a range of different environmental conditions (Padilla & Adolph 1996; Via *et al.* 1995).

Whether or not phenotypic responses may increase a plant's fitness depends largely on how reliably environmental cues predict environmental change, and on the phenotypic and genetic costs associated with the responses (DeWitt *et al.* 1998; Karban *et al.* 1999; Scheiner 1993). Predictability of environmental change and reliability of cues are particularly germane for phenotypic responses that are transferred to the next generation (Donohue & Schmitt 1998; Hill 1965). Transgenerational phenotypic responses involve the transmission of information to the next generation, initiated by an environmental stimulus in the parental generation, which is not exclusively attributable to parental genotypes (Lacey 1998). Transgenerational phenotypic plasticity

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†Author to whom correspondence should be addressed. E-mail: baldwin@ice.mpg.de

*Present address: Netherlands Institute of Ecology, Centre for Terrestrial Ecology, Multitrophic Interactions Department, PO Box 40, 6666 ZG Heteren, The Netherlands.

in response to nutrient deficiency, competition or herbivore pressure may affect seed dormancy, seedling size and growth rates, and herbivore resistance in the next generation (Agrawal *et al.* 1999a; Fenner 1991; Hill 1965; Sultan 2000; Thomas & Raper 1975). Optimality models predict that environments that are highly variable, but temporally predictable, and provide reliable cues predicting the conditions in the following year, enhance selection for phenotypic plasticity in both parental and offspring generations (Donohue & Schmitt 1998; Scheiner 1993).

Various costs may constrain the adaptive value of within- and transgenerational phenotypic responses. First, the expression of phenotypic responses may divert resources from processes directly involved in reproduction (DeWitt *et al.* 1998; Tollrian & Harvell 1999). Second, genes necessary for phenotypic plasticity may have negative pleiotropic effects, or can be linked to genes that decrease fitness (DeWitt *et al.* 1998; Scheiner 1993). Finally, a phenotypic response to one environmental stress may limit a plant's ability to respond optimally to others. These costs are most likely to be seen when plants deploy several phenotypic responses simultaneously, so that internal resource trade-offs between competing functional demands may limit fitness optimization (DeWitt *et al.* 1998). We propose that such trade-offs among the competing responses may shape phenotypic responses, because simultaneously occurring stresses are the rule rather than the exception in nature.

Here we examine the effect of a suite of important environmental variables (induced resistance, leaf removal, soil nitrogen supply and intraspecific competition) on the reproductive performance of *Nicotiana attenuata*. This is an annual plant of the Great Basin desert of the USA, which mass-germinates from long-lived seed banks after fire destroys the dominant vegetation (Preston & Baldwin 1999). The combustion of cellulose produces cues (Baldwin *et al.* 1994) which the seeds use to synchronize their germination with the N-rich soils that ephemerally follow fires (Lynds & Baldwin 1998).

Grazing, particularly from browsing herbivores, tends to increase during this 3-year window (Baldwin 1998). Attack from generalist and specialist herbivores induces a response that dramatically increases (i) resistance to further attack (induced resistance *sensu* Karban & Baldwin 1997); (ii) the plant's endogenous cascade in the jasmonate wound signal; and (iii) a suite of chemical defences (reviewed by Baldwin 1999). All three components can be elicited by exogenous jasmonate treatment, which mimics the endogenous changes caused by herbivore attack.

Intraspecific competition and soil N supply are likely to influence the expression of jasmonate-induced responses. Such responses incur large fitness costs (20% reductions in lifetime seed production) when plants are grown with conspecific competitors (van Dam & Baldwin 1998), and in natural populations when plants are protected from herbivores (Baldwin 1998). Moreover, they utilize substantial quantities of N; a jasmonate-induced *N. attenuata* plant will incorporate 6% or more of its whole-plant N in nicotine alone (Baldwin *et al.* 1998), an investment which cannot be recouped by metabolism (Baldwin & Ohnmeiss 1994; Baldwin *et al.* 1998).

Here the costs of jasmonate-induced responses in *N. attenuata* plants subjected simultaneously to different levels of N, leaf removal and intraspecific competition were assessed on two different levels. First, we measured the effects of jasmonate induction on plant fitness, i.e. seed production and seedling performance. Differences in seedling performance may eventually be a more important determinant for final reproductive success than the absolute amount of seed production, because the competition between conspecifics is often asymmetrical (Schwinning & Weiner 1998). Second, by adding $K^{15}NO_3$ to soil, the outcome of competition for N between plants and patterns of N allocation to seed production was measured directly. Because N is a limiting nutrient for these plants, we expect that the physiological patterns reflected the ecological consequences of jasmonate-induced responses in competing plants.

Materials and methods

EXPERIMENTAL PROCEDURES

Nicotiana attenuata Torr. ex. Wats (Solanaceae) seeds, a bulk collection from 10 plants in a natural population in Utah, were germinated and grown for 2 weeks in peat soil soaked in 50× diluted liquid smoke (House of Herbs, Passaic, NY, USA) to synchronize germination (Baldwin *et al.* 1994). Seedlings were transferred to communal hydroponic boxes filled with Peters Hydrosol nutrient solution (Baldwin *et al.* 1998; Ohnmeiss & Baldwin 1994) to synchronize growth before being transferred 7 days later to 2 l pots containing a 1 : 1 peat–Perlite mixture (van Dam & Baldwin 1998) in which the plants grew until senescence. Each pot received 1·19 g N, 1·22 g P, 1·25 g K in the high-N treatment, and 0·21 g N, 0·80 g P, 1·32 K in the low-N treatment. High-N pots contained 1·5 times more P than low-N pots, but this relatively small difference was overshadowed by the effects of the sixfold increase in N (van Dam & Baldwin 1998). The quantities of N were designed to provide growth under high and low N-supply rates that mimic NO_3^- supply rates typically found in 1- and 3-year-old burns (Lynds & Baldwin 1998). Two seedlings of similar size and appearance were planted 7 cm apart in each pot. The pots were placed on individual saucers in a growth room with a 16 : 8 h day : night photoperiod at 32/27 °C, 65% RH, and 1000–1500 $\mu mol\ m^{-2}\ s^{-1}$ PAR at plant height. Growth chambers were kept free of insects, and flowers were not disturbed during seed set so that all seeds were produced by self-pollination.

In the first 2 weeks after transfer, each pot received 200 ml demineralized water every other day. From the third week onwards, the pots were supplied with

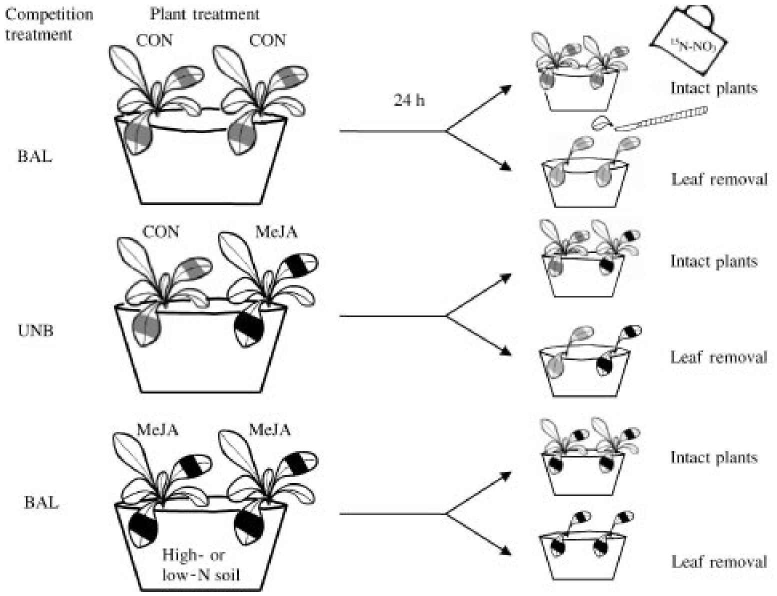

Fig. 1. Overview of treatments and schedule of MeJA and ^{15}N–NO$_3$ applications and leaf removal. BAL, balanced competition (both plants received the same treatment); UNB, unbalanced competition; CON, control plants treated with 20 µl lanolin; MeJA, plants treated with 250 µg MeJA in 20 µl lanolin on two fully expanded leaves. Leaf removal (on average 58·5% of all leaves) took place immediately before the addition of 10·2 mg ^{15}N as K^{15}NO$_3$ per pot.

200 ml water each day for two consecutive days, while on the third day they were not watered. This schedule provided plants with sufficient water to grow, but not so much that pots would lose nutrients. Six weeks after transfer, watering was stopped to mimic the drought that typically terminates growth in the plant's natural environment (I.T.B., personal observation).

Eight days after transfer, 35 high-N and 33 low-N pots with two plants of equal rosette size and appearance were selected and randomly assigned to one of the treatments (Fig. 1): two N levels (high-N and low-N), each with three jasmonate treatment–competition combination groups, which in turn were subdivided over two levels of leaf removal. Methyljasmonate (MeJA) (250 µg in 20 µl lanolin) was applied to two fully expanded leaves. This quantity of MeJA does not influence lifetime seed production in hydroponically grown plants, yet it induces a full nicotine response after 5 days (Baldwin *et al.* 1998). MeJA is de-esterified to JA by plant esterases after application, and is a convenient way introduce JA into plants. MeJA (Sigma, St Louis, IL, USA) was close to its thermodynamic equilibrium (90·1% 1R, 2R MeJA and 8·3% 1R, 2S MeJA) for the two naturally occurring epimers. On control plants (CON), two fully expanded leaves were treated with 20 µl pure lanolin. Pots in which both plants underwent the same treatment (either CON or MeJA) were designated BAL (for balanced competition), while pots with one MeJA plant and one CON plant were designated as UNB (unbalanced competition).

One day after the application of the lanolin, five or six pots in each treatment group were assigned to a leaf-removal treatment (LR). All but the two leaves with lanolin were removed from both plants in the pots. The leaves were removed by cutting the petiole with a sharp scalpel, a technique that elicits only a minimal nicotine response (Baldwin *et al.* 1998), and thereby allowed us to partially separate the effects of leaf removal from the effects of induced resistance. Only leaves that had a clear petiole were removed to avoid damage to the apical meristem. On average, we removed 58·5% of the number of visible leaves from the both high- and low-N rosette plants assigned to the LR treatment. Immediately after LR, each pot was supplied with 10·2 mg ^{15}N in 200 ml of a 0·347 g l^{-1} K^{15}NO$_3$ solution (99·6–99·9 at. %; Isotec Inc., Miamisburg, OH, USA). The plants in the other pots were left undamaged and received the same amount of ^{15}N per pot. In summary, the individual plants were assigned to 16 different environments (five or six replicates per environment), characterized by two N levels (high-N or low-N), two MeJA treatment levels (MeJA or CON), two competition levels (BAL or UNB), and two LR treatments (LR or intact).

Stalk lengths were measured 7 days after LR, and the first day of flowering was recorded to estimate the progression from vegetative to reproductive growth on all plants. To measure lifetime reproductive output, all mature seeds were collected separately for each plant and weighed. The last seeds ripened and were collected 4 weeks after watering had ceased. The dry above-ground parts (leaves, stems and empty capsules) were combined, air-dried and weighed. Although leaf removal and induction of defences may also affect male fitness components (Agrawal *et al.* 1999b), we used seed production as our main fitness estimate. *Nicotiana attenuata* is a self-compatible species with a high selfing rate, and the pollen produced by a single anther (of five per flower) is sufficient to fertilize all the ovules in one ovary (Baldwin *et al.* 1997). In our experiment all the seeds resulted from self-pollination, thus only an 80% reduction in the number of pollen grains per flower would have reduced seed production. Given that Agrawal *et al.* (1999b) reported that 50% LR and induction by herbivores reduced pollen production in wild radish by only 12%, such a large reduction seems unlikely.

^{15}N ANALYSIS

Three pots from each treatment were selected for total N and ^{15}N analysis by Dumas combustion–mass spectrometry at the Research Institute for Agrobiology and Soil Fertility (AB-DLO, Wageningen, The Netherlands). We selected plants from those pots whose lifetime seed masses were closest to their group mean. For the BAL pots we randomly selected the seeds of one plant per pot, as both plants in these pots were treated the same. Seeds from both plants in UNB pots were used. The above-ground plant parts of these pots were ground in a coffee mill, and an aliquot was passed through a 60-mesh (0·25 mm) sieve and collected in a 5 ml glass vial. Similarly, an aliquot of the total seed mass of each plant was ground in a mortar and passed through a 60-mesh sieve. Total N and total ^{15}N in seeds and above-ground parts were calculated as (% N/100) × total mass and (% ^{15}N enrichment/100) × mg N, respectively (Baldwin *et al.* 1998). To determine the mass per seed, 20 seeds per plant were weighed to the nearest µg. The amount of N per seed was calculated as (% N/100) × mass per seed.

SEED MASS AND GERMINATION RATES

Seeds of those plants selected for ^{15}N analysis were used to determine mass per seed and germination rates, which were used as proxies of offspring fitness. Seed germination rates were determined in trials in seed cups, under conditions that maximize germination rates for this species (Baldwin *et al.* 1994). Each cup contained 20 seeds, and was placed in a growth cabinet (Snijders, Tilburg, The Netherlands) at 32 °C/16 h, 28 °C/8 h. Seeds were examined for germination under a 10× binocular microscope, starting on the third day after hydration and ending on day 7 when more than 95% of the seeds had germinated.

SEEDLING COMPETITION

To determine if the transgenerational effects, seen as delays in seed germination, translated into effects on seedling growth and reproductive performance under competition in soil, we conducted the following experiment. We selected seed from four different plants: two that had been in one pot from high-N, UNB, no LR treatment; and two from the low-N, UNB, no LR treatment. As the number of replicates required to examine the seed from all plants could not fit into a single growth chamber, we selected the seed from plants whose germination was closest to the average of their treatment group. Hence the experiment was intended only to determine if the delays in germination observed in the seed cups translated into fitness effects for the seedlings. We placed two seeds from two different parents 2·5–3 cm apart on the soil surface in 200 ml pots. Four combinations were created (with 20 replicate pots per combination): (i) one seed of a CON plant versus one seed from its MeJA-treated neighbour from a high-N, UNB treatment; (ii) same as in (i), but seeds came from a low-N, UNB treatment; (iii) competition between CON plants from different N supply rates, namely one seed from a high-N UNB plant versus one seed of a low-N UNB plant; (iv) competition between MeJA-treated plants from different N supply rates. After placing seeds on the soil, the soil was saturated with dilute liquid smoke before each pot was covered with clear plastic and randomly placed on communal plastic trays for bottom watering. The trays were placed in a climate room with a 16 : 8 h day : night photoperiod at 32/27 °C, 65% RH and 375–475 µmol m^{-2} s^{-1} PAR. After 4 days, when the first seeds started to germinate, the plastic covering was removed from all pots. Two weeks after the start of the experiment the trays were transferred to a high-light growth room (same photoperiod and temperature regime, but 1000–1500 µmol m^{-2} s^{-1} PAR). Germinating seeds with visible cotyledons were counted and watered daily. When one of the plants in a pot had one filled seed capsule, the amount of water given was gradually reduced over a 5 day period. Two weeks later, the number of filled seed capsules was counted and the above-ground parts weighed.

STATISTICAL ANALYSIS

The data were analysed using STATVIEW (SAS Institute Inc., Gary, NC, USA) and SYSTAT 7·0 (SPSS Inc., Chicago, IL, USA) statistical software packages. Test values were calculated using Type III SS. The percentages of ^{15}N allocated to seed were arcsine square-root transformed, and stalk lengths were log-transformed to meet the requirements of normal distribution for parametric analysis. Data were analysed by ANOVA as in van Dam & Baldwin (1998). Data obtained from plants in one pot are technically not independent; due to the costs involved in ^{15}N addition and analysis we did not have a large enough data set to select one plant from each pot. Using separate paired tests for comparisons between plants within one pot and unpaired tests between plants from different pots would result in a patchwork of different analyses and require complicated corrections for multiple comparisons. In our opinion, using ANOVA to analyse this data set is the most parsimonious solution that does justice to the data. Stalk length, first flower day and total seed mass were analysed individually by ANOVA with N level, competition, leaf removal and MeJA treatment as main effects. Because these data were obtained from the same plants, we used a conservative Bonferroni correction to correct α for multiple comparisons ($\alpha/3 = 0{\cdot}0167$; Rice 1989). A similar procedure was applied to the analysis of ^{15}N acquisition and allocation data (mg ^{15}N per plant, % ^{15}N allocation to seeds and seed mass), which were obtained on a subset of the plants. Germination data were analysed separately with repeated-measures ANOVA. Differences in germination and seed mass produced in the seedling competition experiment were analysed by the Mann–Whitney *U*-test followed by a sequential Bonferroni correction for multiple comparisons (Holm 1979).

Results

We treat all developmental and fitness data together, but for simplicity present only data for total seed production, because the trends in the stalk lengths and first flowering day data were very similar to those in the total seed data. Any deviations from the general patterns are discussed.

Both N supply and MeJA treatment significantly delayed development and reduced seed output (Table 1). LR significantly decreased all developmental parameters,

Table 1. *F*-values of univariate ANOVAs performed on developmental parameters and total reproductive output (stalk length, days to first flower, and lifetime mg seed per plant) or N-allocation parameters (total mg ^{15}N per plant, percentage ^{15}N to seed, µg N per seed)

Effect $F_{\text{d.f.}}$	Stalk length $F_{1,69}$	First flower $F_{1,69}$	Seed per plant (mg) $F_{1,69}$	Total ^{15}N (mg) $F_{1,32}$	% ^{15}N to seed $F_{1,32}$	N per seed (µg) $F_{1,32}$	Germination $F_{1,128}$
Nitrogen (N)	**13·33****	**8·13***	**48·78*****	**42·85*****	**228·80*****	**8·94***	**21·33*****
Leaf removal (LR)	**27·53*****	**58·12*****	5·82$^{P=0·0185}$	3·09	0·10	0·63	**20·89*****
Competition (COM)	1·14	3·084	0·232	1·23	0·65	0·23	3·75$^{P=0·055}$
MeJA	**36·83*****	**88·88*****	**50·06*****	2·70	**14·39****	0·00	1·65
N × LR	2·82	3·56	0·02	0·29	0·01	0·688	**8·83****
N × COM	3·90	4·24	**7·62***	0·001	0·40	4·28	0·37
N × MeJA	2·10	2·25	**6·73***	0·06	0·00	0·01	2·89
LR × COM	**8·58***	5·92$^{P=0·0176}$	5·45	0·57	2·5	0·97	**4·49***
LR × MeJA	**8·43***	**18·85*****	0·08	0·22	**7·07***	0·14	2·71
COM × MeJA	0·64	0·14	**19·83****	**25·41*****	0·40	0·01	0·03
N × LR × COM	1·07	0·22	0·10	3·67	1·08	0·27	0·003
N × LR × MeJA	0·07	0·26	0·00	0·15	3·77	0·22	2·47
N × COM × MeJA	1·04	1·79	0·97	**7·86***	1·58	0·11	**5·83***
LR × COM × MeJA	4·23	**6·50***	0·22	0·24	0·004	0·20	0·01
N × LR × COM × MeJA	0·20	0·20	3·54	0·27	0·004	1·76	0·26

A Bonferroni correction (Rice 1989) was applied to correct for multiple comparisons in both sets of three variables ($\alpha/3 = 0·0167$, $*P < 0·0167$, $**P < 0·003$, $***P < 0·0003$). Seed germination rates were analysed separately by repeated-measures ANOVA ($*P < 0·05$, $**P < 0·01$, $***P < 0·001$). *F*-values with a *P*-value <α are in bold. MeJA = methyljasmonate treatment. Significant effects are shown in bold.

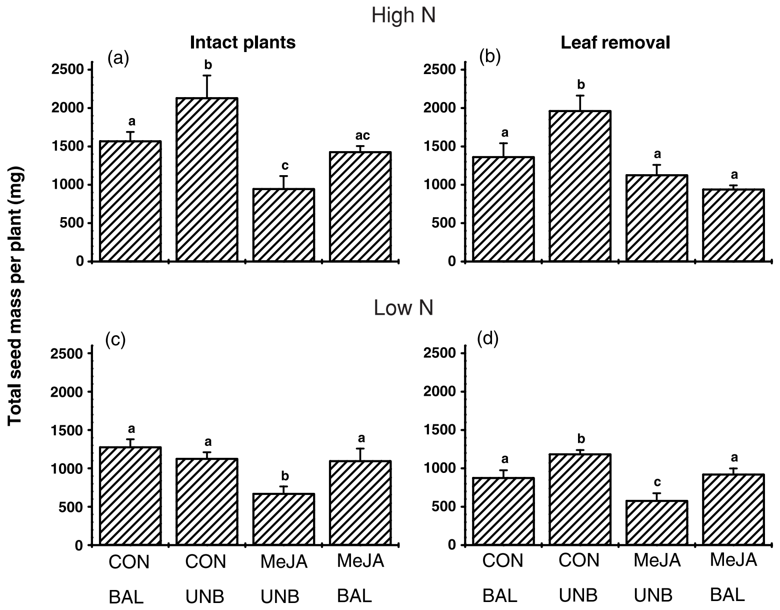

Fig. 2. Total seed mass produced by plants in the different treatment combinations (Fig. 1) grown under high- and low-N supply. Letters indicate significant differences between treatments within N and leaf removal level (Fisher's LSD, $P < 0·05$).

but eventually had only a marginally significant effect on total seed mass per plant. Type of competition alone had no significant effect on any of the plant parameters, which indicates that, on average, development and seed output per pot were similar for all balanced and unbalanced pots in our experiment (Table 1).

In all N and LR treatment combinations, MeJA-treated plants in unbalanced competition pots produced significantly less seed mass than their untreated neighbour (Fig. 2). This effect, however, is not a direct effect of MeJA application, because in none of the balanced competition treatments did MeJA-treated plant pairs produce significantly less seed than was produced by pairs of control plants (Fig. 2). The strongly significant MeJA–competition interaction term (Table 1) supports the conclusion that the costs of MeJA induction are opportunity costs that are realized only when a plant competes with an uninduced neighbour.

The uninduced neighbour, in turn, benefited from competing with an induced plant. Control plants in competition with MeJA-treated plants under high N supply rates produced significantly more seeds than control plants with control neighbours, and thus obtained an opportunity benefit of a 35% (intact plants) and 43% (LR) increase in seed production (Fig. 2a,b). The opportunity benefit was smaller when plants were grown under low N supply. Intact control plants in balanced and unbalanced designs produced similar seed masses, while control plants in unbalanced designs that were clipped produced 35% more seed mass than control plants in balanced designs (Fig. 2c,d). This N-dependent decrease in productivity of control plants was probably the reason that total productivity of plants in unbalanced pots was, overall, affected more by smaller N availability (42% decrease in total seed production per pot) than that of plants in balanced designs (a 22% decrease in seed production). The effect of N supply on average plant fitness partly depended on whether the competition for resources was balanced or unbalanced (N–COM interaction, Table 1).

Seed output of MeJA-treated plants was buffered slightly better against low N supply than that of control plants (Table 1, N × MeJA). Low-N control plants produced 35% less seed mass than under high N, while MeJA-treated plants produced only 27% less seed mass per plant.

Although LR reduced seed output, the effect was independent of any other treatment effect (no significant

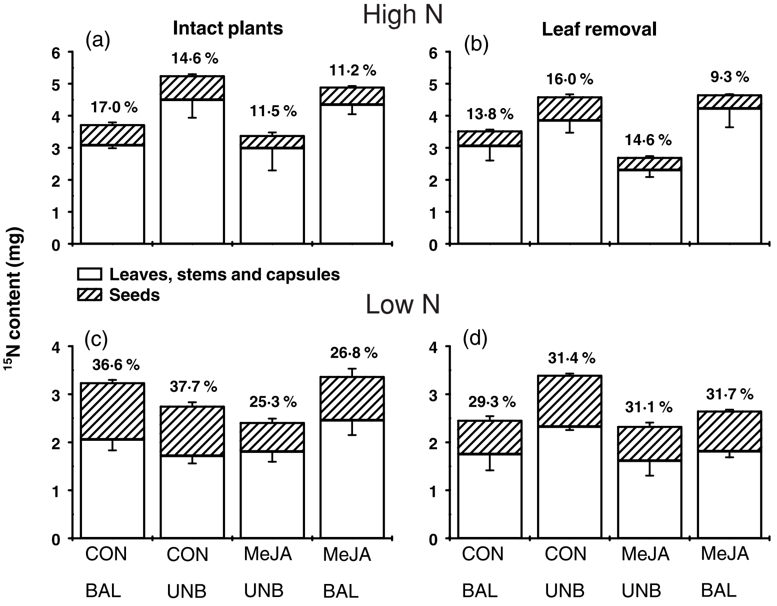

Fig. 3. Mean (± SE) ^{15}N content of leaves/stems and seeds of plants grown in different treatment combinations under high and low N supply. Values over bars indicate the percentage of total ^{15}N in the above-ground parts that was allocated to seeds. Each pot contained two plants and was supplied with 10·2 mg $K^{15}NO_3$. See Fig. 1 caption for treatment abbreviations.

interactions with LR; Table 1). Interactions between LR and MeJA or competition, however, affected the transition from vegetative to reproductive growth (Table 1). LR on MeJA-treated plants reduced stalk length to 22% of unclipped controls and delayed the onset of flowering by 4·3 days. Control plants subjected to LR had flower stalks that were 57% the length of unclipped controls and flowered only 1·2 days later. Delay of development due to LR was stronger in balanced than in unbalanced pots, hence the LR–COM interaction. These effects, however, did not translate into significant changes in total seed output (Table 1).

^{15}N ALLOCATION PATTERNS

The allocation of ^{15}N added to the plants at time of LR was a good predictor of final N allocation within the plant under both N-supply regimes. Total amounts of N and ^{15}N in the same organ were strongly correlated (regression analysis, high-N: $R^2 = 0·91$, $F_{1,22} = 214·23$, $P < 0·001$; low-N: $R^2 = 0·81$, $F_{1,22} = 96·03$, $P < 0·001$; seeds, high-N: $R^2 = 0·92$, $F_{1,22} = 251·54$, $P < 0·001$; low-N: $R^2 = 0·87$, $F_{1,22} = 147·17$, $P < 0·001$).

Nitrogen availability in the soil in which the plants were grown significantly determined how much ^{15}N was recovered from the above-ground parts of the plants, as well as the proportion of N allocated to the seeds (Table 1, Fig. 3). On average, we recovered 80·5% of the supplied ^{15}N from plants grown under high N supply, and only 58·1% from the low-N plants. Low-N plants allocated significantly more of their N in the above-ground parts to seeds (Table 1; Fig. 3). Greater allocation of N to seeds could not fully compensate for the fivefold smaller N supply in these pots. Low-N plants produced seeds that contained 8·3% less N per seed than those of high-N plants (Table 1; high-N: 2·80 ± 0·22 μg N per seed, low-N: 2·57 ± 0·29 μg N per seed).

MeJA treatment did not influence the amount of N in shoots, but influenced the allocation pattern within the shoots of intact plants (Fig. 3a,c): under high N supply; control plants on average allocated 15·8% of shoot ^{15}N to seeds, while 11·4% was used for seed production in MeJA-treated plants. For plants grown under low N, these figures were 37·2% (control) and 26·1% (MeJA), respectively. These differences in allocation patterns were significantly smaller in plants that were subjected to LR (Fig. 3b,d): High-N plants that were clipped allocated 14·9% (control) and 12% (MeJA) to seed production, and clipped low-N plants 30·4% (control) and 31·4% (MeJA), respectively.

The costs and benefits of growing in unbalanced competition were reflected in the N-acquisition data. In unbalanced pots, we recovered less ^{15}N from the shoots and seeds from the MeJA-treated plant than from the untreated control plant in the same pot (Fig. 3; Fisher LSD comparisons CON–BAL versus CON–MeJA all $P < 0·03$, except Fig. 3c, $P > 0·1$). MeJA treatment affected the relative differences in ^{15}N acquisition between competition treatments (Table 1; COM–MeJA interaction). We recovered more ^{15}N per plant (mean ± SEM) from control plants in unbalanced competition (3·99 ± 0·33 mg) than from controls in balanced pots (3·24 ± 0·20 mg), while the opposite was true for MeJA-treated plants (UNB–MeJA, 2·70 ± 0·23 mg versus BAL–MeJA, 3·89 ± 0·33 mg). These differences were most pronounced under high N supply rates (Table 1, N × COM–MeJA interaction). These treatment-dependent differences between balanced and unbalanced pots support our previous finding that MeJA-treated plants in unbalanced competition accrue opportunity costs, while the control plants in the same pot have an opportunity benefit compared to equally treated plants in balanced competition. Neither differences in N acquisition nor in N-allocation patterns, however, resulted in a smaller mass per seed (data not shown; ANOVA as in Table 1, no significant effects). The average mass per seed over all treatment groups was 111 μg (±1 μg SEM), similar to values found for field-grown plants (Baldwin 1998). While the amount of N per plant was positively correlated with total seed production per plant (Kendall rank correlation, $T = 0·587$, $P < 0·001$), the percentage of N allocated to seeds was not ($T = 0·168$, $P = 0·09$).

SEED GERMINATION

Seeds from plants grown under high N supply germinated significantly faster than seeds from low-N plants (Table 1; Fig. 4). LR had a much stronger effect on germination of seeds from low-N plants than on seeds from high-N plants (significant N–LR interaction in Table 1). MeJA application itself had no significant effect on seed germination, but the significant MeJA × N–COM interaction indicated that MeJA application

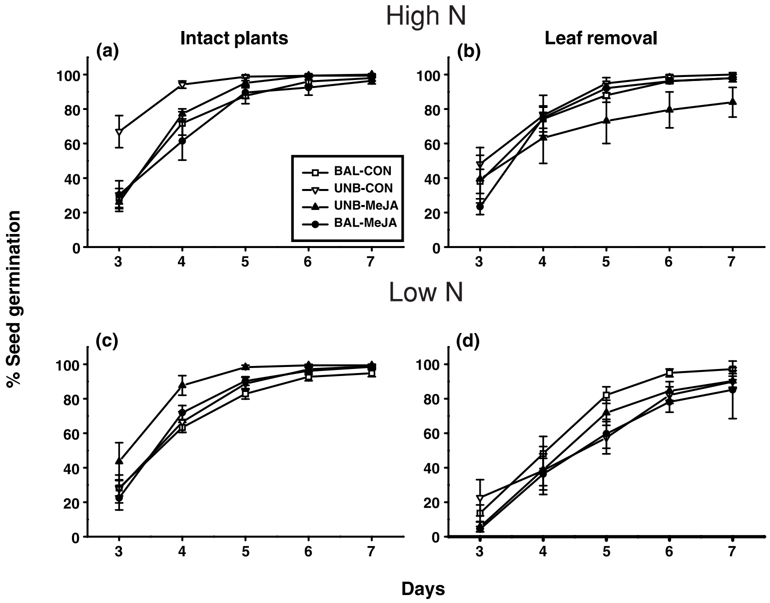

Fig. 4. Mean (± SE) percentage germination of seeds (20 per cup, three replicates per seed batch) from plants in the different treatment combinations. See Fig. 1 caption for treatment abbreviations.

influenced germination of seeds depending on competition and N level (Table 1). Individual *post hoc* analysis of the MeJA effect within N and competition level revealed that this effect was caused by a switch in the rank order of germination speed between seeds of high- and low-N plants in unbalanced competition. This is most clearly seen in seeds of undamaged plants (Fig. 4a,c): control plants in high-N–UNB pots produced seeds that germinate faster than their MeJA-treated neighbours (Fig. 4a, $F_{1,16}$ = 13·34, P = 0·002), while under low N the opposite was true (Fig. 4c, $F_{1,16}$ = 6·940, P = 0·018). This switch in germination rates between high- and low-N plants also occurred in LR plants, but because of the smaller differences in germination rates between the treatments, the effect was not statistically significant. In contrast, comparing seed germination rates of seeds produced by plants in balanced competition did not reveal any significant differences caused by MeJA treatment.

SEEDLING COMPETITION

As expected, both germination percentages and speed of germination in soil were less than in the seed cups, in which over 95% of the seeds had germinated within 7 days (Fig. 4; Table 2). However, patterns of germination behaviour from the seed cup bioassay were confirmed in the seedling competition experiment. Control plants grown in unbalanced competition and high N supply produced faster germinating seeds than their MeJA-treated competitors, while MeJA-treated plants grown under low-N conditions produced faster germinating seeds than their control neighbours (Table 2). These differences in germination speed resulted in dramatic fitness differences. Seeds that germinated first invariably produced plants with significantly more biomass and seed capsules (Table 2). The competitive interaction between the two plants was clearly asymmetrical, that is, the fastest germinating seeds produced plants that pre-empted the resources by overshadowing and suppressing the slower germinating competitor. In most cases the slower germinating seeds produced plants that remained small and did not produce any seed capsules (Table 2). The competitive ability of seeds of MeJA-treated plants in unbalanced competition was not significantly affected by the soil N level in which plants were grown (Table 2).

Discussion

Although activation of the jasmonate cascade can directly influence growth and seed production (Creelman & Mullet 1997), jasmonate application itself did not significantly reduce seed production. The competitively mediated cost of jasmonate induction in unbalanced pots resulted in an opportunity benefit for uninduced plants growing adjacent to a jasmonate-induced neighbour, especially under high N supply, confirming earlier

Table 2. Second-generation fitness consequences of N supply, competition and MeJA treatment

Competitive treatment	Common parental treatment	Differential parental treatment	Germination percentage	Days to germination	Above-ground mass (g)	Number of seed capsules
1	High N	Control	80	**8·5 (0·9)****	**2·05 (0·34)****	**8·1 (1·5)****
	High N	MeJA	80	18·4 (2·5)	0·48 (0·17)	1·4 (0·7)
2	Low N	Control	40	30·8 (4·5)	0·21 (0·21)	0 (0)
	Low N	MeJA	60	**16·7 (2·8)***	**1·69 (0·23)****	**5·1 (1·3)****
3	Control	High N	85	**7·2 (0·2)*****	**2·34 (0·31)*****	**6·7 (0·9)*****
	Control	Low N	45	26·6 (2·2)	0·13 (0·09)	0·3 (0·2)
4	MeJA	High N	35	18·8 (6·0)$^{\text{ns}}$	0·69 (0·24)	3·6 (1·2)$^{\text{ns}}$
	MeJA	Low N	55	21·0 (3·3)	0·92 (0·48)$^{\text{ns}}$	2·1 (0·9)

Mean (+ SE) germination percentage, biomass and reproductive output of plants grown in four different competitive combinations. Seeds came from selfed plants that were grown in unbalanced competition under high or low N levels. Asterisks indicate P-values of Mann–Whitney U-test comparisons between offspring of plants growing in one pot (within competitive treatment). *P < 0·05, **P < 0·01, ***P < 0·001, after Bonferroni correction for correlated fitness measures (Holm 1979). ns, not significant. The indicators of statistical significance identify the treatment that germinated faster, produced larger plants, or produced more seed capsules. Significant effects are shown in bold.

work (van Dam & Baldwin 1998). These results demonstrate that intraspecific competition is greater and more asymmetrical when plants grow in highly productive environments, a result consistent with reports from other disturbance-adapted species (Casper & Jackson 1997; Nicotra & Rodenhouse 1995; Schwinning & Weiner 1998).

^{15}N allocation patterns in the above-ground plant parts are a reliable predictor for overall N allocation patterns. On average, ^{15}N recovery was high, which was surprising given the possibility of rapid immobilization of NO_3^- by soil micro-organisms present in the peat–soil mixture we used (Nadelhoffer & Fry 1994). In plants grown under low N we recovered less ^{15}N than from plants grown under high N supply. This difference in recovery could either be a result of slower uptake by these slower-growing plants, or may reflect a greater resource allocation to roots, a response commonly found in response to low N supply (Casper & Jackson 1997). As we did not harvest root biomass, we could not determine which was the case in our experiment.

The correlation between ^{15}N acquisition and seed production strongly supports the hypothesis that MeJA treatment temporarily decreases ability to compete for N and other nutrients in unbalanced pots (Fig. 3). Similarly to seed production, the reduction in ^{15}N acquisition was not a direct effect of MeJA application, because MeJA-treated plants in balanced pots had as much, or more, ^{15}N in their above-ground parts. We conclude that the primary fitness costs of MeJA-induced responses depend on the relative competitive strength of induced *N. attenuata* plants growing next to uninduced plants.

Jasmonate-induced plants allocated less N to seed than control plants, which may reflect a trade-off with N-based defensive responses triggered by MeJA application. Similarly, jasmonate induction decreased plant fitness in proportion to the amount of N used in nicotine production when induced *Nicotiana sylvestris* plants competed with uninduced plants (Baldwin & Hamilton 2000). In *N. attenuata*, jasmonate treatment increases the production of a suite of N-intensive metabolites that include nicotine, its biosynthetic enzymes, protease inhibitors and many other compounds (Baldwin 1999; Van Dam *et al.* 2001). The percentage of N allocated to defence rather than seeds could exceed the 6% that is used in induced nicotine synthesis (Baldwin *et al.* 1998), and thus may constitute a significant trade-off with N allocation to seed production.

Nitrogen allocation patterns, however, could not account for all the observed fitness differences in reproductive output, and the plants demonstrated remarkable homeostasis in seed production and performance. For example, smaller ^{15}N allocation to seeds in jasmonate-induced plants did not result in less seed production or lower seed quality. Most remarkably, plants grown under six times lower N levels produced seeds with a similar masses and only 8% less N than high-N plants, by allocating a higher proportion of their shoot N to seed production. These changes in internal allocation patterns indicate that *N. attenuata* plants employ phenotypic plasticity to maintain seed quality in terms of percentage N when growing in the range of N conditions they normally experience (Lynds & Baldwin 1998).

Leaf removal reduced seed production in high- and low-N plants, but not as strongly as low N levels or MeJA application in unbalanced competition. In addition to inducing defences, MeJA application enhances the production of vegetative storage proteins, which may make plants more tolerant to leaf removal (Creelman & Mullet 1997). In our experiments, however, we found no evidence for MeJA-induced tolerance in LR plants, because MeJA-treated plants in balanced competition eventually produced similar amounts of seed to control plants.

Another effect of jasmonate-induced defences is that they reduce the amount of leaf loss by herbivores (Baldwin 1998; van Dam *et al.* 2000). In our experiment, induced and uninduced plants suffered equal amounts or leaf loss, and the competitive balance between them remained the same. However, selective herbivory may change the competitive balance between plants, and hence their resource acquisition (Baldwin 1998). The opportunity costs for producing induced defences may be compensated by the reduction in leaf removal, but it is not clear at which difference in damage between induced and uninduced plants the benefits will exceed the costs.

Competition, leaf removal and N supply also affected the offspring of plants. The most striking transgenerational effect was the N-dependent switch in seed germination rates of seeds from jasmonate-induced and control plants produced under unbalanced competition. Seeds from control plants grown under high-N conditions germinated significantly more quickly than those of their jasmonate-induced competitors, but when grown under low N the opposite result was found, independent of leaf removal. While these differences in germination behaviour appear to be only a matter of days in the seed cup bioassay, they consistently affected the competitive ability and fitness of the offspring. Moreover, seeds produced by plants grown on high N germinated more quickly (Table 2; Fig. 3), a phenomenon that has also been observed in *N. tabacum* (Hill 1965; Thomas & Raper 1975) and other plant species (Fenner 1991). Leaf removal decreased seed germination rates of all plants, but the effects were much stronger in low-N than in high-N plants. This indicates that the phenotypic fitness cost of leaf loss may extend at least to the next generation.

Recently, Agrawal and co-workers showed that through maternal effects (induced) resistance traits could be transferred to the next generation when *Brassica napus* plants or *Daphnia cuculata* mothers were exposed to predators. The cues experienced by the mothers resulted in offspring better prepared for the same environment

Table 3. Predictable changes in the natural environment of *N. attenuata* and cues that predict the environment for next year

	Year			
Environmental character	1	2	3	Cues
Nitrogen levels	High	Medium	Low	Nitrogen availability in soil Smoke germination cue (partly)
Herbivory	Low	Medium	High	Induced defences or leaf loss by herbivores
Competition (conspecifics)	High	High	Decreasing?	Presence of neighbouring plants
Competition (other species)	Low	Medium	High	Suppression of germination by cues in litter Presence of neighbouring plants?

(Agrawal *et al.* 1999a). Our results suggest that MeJA-induced *N. attenuata* parents growing under low-N conditions prepare their offspring for an environment they have not experienced themselves, but which they can predict from cues in their present environment. To our knowledge, this is the first example of transgenerational phenotypic plasticity that prepares offspring for an environment that differs from that of their parents.

We propose that the environmentally induced differences in germination rates are yet another example of how *N. attenuata* employs (transgenerational) phenotypic responses to adapt to its highly variable, but very predictable, habitat (Table 3). *Nicotiana attenuata* plants are typically found for only three growing seasons after a fire, although the smoke cue triggering germination remains in the soil for more than 7 years after a fire (Preston & Baldwin 1999). High N availability, and the absence of herbivore attack and its associated induced responses, are typical cues indicating the start of this 3-year window in which *N. attenuata* individuals can maximize their reproductive output (Lynds & Baldwin 1998; Preston & Baldwin 1999; Table 3). Low N conditions and the high frequency of herbivore attack, which is indicated physiologically by a highly induced status, signal the end of this window. Plants that perceive these cues (high N, no induction or low N, induction) can increase their fitness and that of their offspring by producing fast-germinating seeds. Under such conditions it has been postulated that transgenerational phenotypic plasticity is a better adaptive strategy than bet-hedging (Tollrian & Harvell 1999).

The failure of *N. attenuata* to produce fast-germinating seeds under other than the above conditions may constitute a phenotypic cost of (transgenerational) phenotypic plasticity. Plants that encounter circumstances uncommon to their natural environment, that is, high N and an induced status, or low N and no induction, produce maladapted phenotypes (DeWitt *et al.* 1998). The existence of these maladaptive phenotypes does not necessarily affect selection for phenotypic plasticity if, overall, the ability to be plastic provides a fitness advantage (DeWitt *et al.* 1998).

Such adaptive scenarios clearly need to be tested rigorously, with the appropriate control of genetic background and comparisons between plastic and non-plastic genotypes in different environments (Scheiner 1993). However, these tests are not trivial exercises: little is known about real seed-bank behaviour, and attempts to study this in the laboratory are fraught with difficulties (Cabin *et al.* 1998; Clauss & Venebale 2000; Philippi 1993).

Nicotiana attenuata is a species with a remarkable amount of phenotypic plasticity throughout its life history (Baldwin 1999). To date, most of the research has focused on the conditional nature of defence in this species, and the interaction of the defensive phenotype with the fitness costs and benefits of defence (Baldwin 1998; Baldwin *et al.* 1998; van Dam & Baldwin 1998). This study underscores how profoundly plastic responses to other environmental factors mediate not only the costs of induced resistance in the parents, but also the germination behaviour of the next generation.

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