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Growth responses and fitness costs after induction of pathogen resistance depend on environmental conditions

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ABSTRACT

Fitness costs of resistance are among the most widely discussed explanations for the evolution of induced resistance, but studies on induced resistance to pathogens are scarce and contradictory. In the present study the influence of nitrogen supply, length of the growing period and competition on the seed production of *Arabidopsis* in response to treatment with the chemical resistance elicitor BION® was investigated. BION® treatment elicited resistance to the bacterial pathogen *Pseudomonas syringae*, and biochemical changes after BION® treatment were similar to those observed after bacterial infection. Induced plants grew more slowly during the first week after resistance induction, for which they then compensated by exhibiting faster growth than controls. Whether or not induced plants produced less seeds than controls depended on growing conditions. Costs, no costs and even higher seed production by induced plants were observed in experiments differently combining abiotic conditions. A higher seed production by induced plants arose particularly when the vegetation period was short, most probably a consequence of senescence-related processes that had been activated by resistance elicitation. Induced plants, however, produced less seeds when competing with controls and experiencing a full growing period. Studies controlling only some of the critical environmental factors can easily lead to apparently contradictory results, which in fact represent different outcomes of a complex interplay of factors.

Key-words: compensation; induced systemic resistance; overcompensation; phenotypic plasticity; plant–pathogen interaction; salicylic acid; SAR (systemic acquired resistance); senescence.

INTRODUCTION

Induced resistance is characterized by a time lag between the inducing attack and the phenotypic expression of resistance traits, a constraint that has to be counterbalanced by positive effects in order to make induced resistance an evolutionarily stable alternative to constitutive mechanisms. Fitness costs, that is, the negative effects on a plant's genetic contribution to the next generation, can result from resistance expression under conditions not actually requiring an active resistance. They are among the most widely discussed explanations for the evolution of induced resistances (Heil & Baldwin 2002), although alternatives that focus on the co-evolution among plants and their enemies (Rausher 2001) must also be considered.

While there is increasing evidence that induced resistance to herbivores is costly (Baldwin 1998; Agrawal 2000; Purrington 2000; Cipollini 2002; Zavala *et al*. 2004), the question whether or not induced resistance to pathogens causes relevant costs is still matter of discussion. Reduced seed production due to expression of a specific, R gene-mediated resistance to pathogens in *Arabidopsis* has been reported (Tian *et al*. 2003), while several other studies did not detect costs of pathogen resistance (reviewed by Bergelson & Purrington 1996). Physiological costs can result from the allocation of limited resources to defence, which then cannot be used for other fitness-relevant processes (Herms & Mattson 1992). Whether such allocation phenomena lead to net costs might strongly depend on the actual supply of the resources in question – varying growing conditions thus are likely to affect the existence and magnitude of detectable costs. Based on this assumption, Bergelson & Purrington (1996) predicted that the probability of measuring fitness costs becomes greater with increasing limitation (limited resources – higher fitness costs hypothesis).

Interactions among abiotic stresses and resistance are just now starting to be investigated (Cipollini 2004; Mopper *et al*. 2004; Thaler & Bostock 2004). No framework currently exists to confidently predict the conditions under which fitness costs are most likely to occur, and studies on the relevance of such effects for induced pathogen resistance are generally scarce and contradictory. Costs of chemically induced resistance of wheat to pathogens indeed were strongest when plants suffered from a shortage of nitrogen (Heil *et al*. 2000); a result most probably explained by the need to allocate N to the *de-novo* synthesis of pathogenesis-related (PR) proteins. In contrast, Cipollini (2002) reported no detectable effects of competition on the occurrence of fitness costs of resistance elicitation, although such interactions have been reported for jasmonate-mediated induced resistance to herbivores in tobacco (van Dam & Baldwin 1998, 2001). Siemens *et al*. (2002) detected costs

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for resistance of *Brassica rapa* to herbivores only in a non-competitive but not in a competitive environment.

We used a chemical resistance elicitor, BION® (Syngenta, Basle, Switzerland), to investigate whether or not a dosage-controlled resistance induction leads to different effects on subsequent plant growth and seed production when conducted under different, yet pathogen-free growing conditions. The active component of BION® (benzo (1,2,3) thiadiazole-carbothioic acid *S*-methylester, BTH: CGA 254–704) acts in the disease-related signal pathway at or downstream of salicylic acid (SA), the plant hormone usually involved in the signalling cascade that leads to induced systemic resistance (ISR) to pathogens (Ryals *et al*. 1996). We chose BION® as a chemical elicitor because it (1) can be applied externally to the plants (Oostendorp *et al*. 2001); (2) allows induction of resistance-related enzymes in an exactly dosage-controlled manner (Dietrich, unpublished); and (3) allows to induce resistance without any effects of infecting pathogens. As discussions continue concerning whether or not BION® treatment leads to a biologically relevant resistance induction, additional experiments were conducted to (a) test whether BION® successfully induces resistance to bacteria in our particular plant–pathogen system and (b) compare BION®-induced changes in resistance-relevant enzymes to the responses after biological infection.

Fitness hardly can be quantified directly. However, *Arabidopsis thaliana* is a highly selfing species. Hoffmann *et al*. (2003) found a rate of outcrossing of less than 1% under natural conditions, even when counting all flower visitations by insects as successful outcrosses. Lifetime seed production thus appears a suitable estimate of fitness of *Arabidopsis* plants. Parameters that were varied in different experiments are nitrogen supply, competition, resistance induction, and length of the growing period.

MATERIALS AND METHODS

Cultivation of plants and resistance induction

Seeds of *Arabidopsis thaliana* (L.) Heynh. accession Col-0 (H. Zimmer, Botanical Institute, University of Cologne, Germany) were germinated on moistened filter paper for 5 d and then transferred to 0.4 L pots (two to four seeds per pot) containing commercially available soil without nitrogen (80% 'Nullerde' Archut Erzeugnisse GmbH, Vechta, Germany; 10% sand; 10% vermiculite 1–2 mm, Isola Mineralwollewerke, Sprockhövel, Germany). Conditions in a growth chamber (York Industriekälte GmbH & CoKG, Mannheim, Germany) were 10 h photoperiod, 50% relative humidity and 21 °C. The plants were watered three times a week. Following earlier results (Heil *et al*. 2000) we regarded nitrogen supply as a critical parameter and varied it in all experiments. The levels of nitrogen received by plants in low (N1), medium (N3), high (N10) and highest (N30) nitrogen treatment groups were manipulated by fertilizing plants with mineral fertilizer containing different concentrations of N (10 mL every second day of an aqueous 1, 3, 10 and 30 g L^{-1} solution of ammonium nitrate). All other mineral nutrients were provided *ad libitum*. There was a significant correlation of plant growth with N supply, indicating that N was the mineral limiting plant growth under the chosen cultivation conditions (unpublished data). After 10 d plants were thinned to one or two plants per pot and assigned to pairs according to rosette diameter (two plants forming a pair were grown in individual pots in most experiments, yet in the same pot when 'competitive' conditions had to be applied). After 25 d the plants were subjected to induction treatment.

One plant per pair was randomly chosen as a control (sprayed only with water), the other one as induced (sprayed with an aqueous 300 mg L^{-1} BION® solution). Both plants were sprayed twice until runoff with an interval of approximately 120 min between.

Experiments 1 and 2: growth response to BION® treatment

Fourteen plants were cultivated under each N regime and treated as described above. At the time of BION® treatment and 1, 2 and 3 weeks later, rosette diameters were measured as an average of three different diameter vectors. Plants then were allowed to finish their growth period (i.e. they were watered until fruit set started, approximately 6–7 weeks after induction) and seeds were collected in 'Arasystems' plastic tubes (Beta Tech, Gent, Belgium). Similar conditions were applied during an independent experiment, yet only plant growth was registered during the first 4 weeks after BION® application.

Experiment 3: effects of competition

Plants were cultivated under competitive conditions; that is, the two plants forming a pair were cultivated in the same pot. Other cultivation conditions (different N regimes, length of the growing period and BION® treatment) were as described above. Control plants were covered to avoid unintended induction. Although some uptake of BION® by the controls via the soil could not be completely excluded, enzyme activities of chitinase and peroxidase (data not shown) and morphological data (see Fig. 5) indicate a clear difference in resistance expression between induced and control plants cultivated in the same pot. Plant growth was measured during the first 4 weeks after BION® treatment.

Experiments 4 and 5: length of growing period

Preliminary observations indicated that plants being induced and suffering from N limitation began earlier with shoot elongation and thus flowering and seed set. Shortened growing periods could therefore affect growing and seed quantity. To check for interactions among N supply, resistance induction and length of the growing period, plants cultivated under the same N conditions and treated

with BION® as described above were subjected to different levels of water stress by ceasing water supply 1, 2, 3 and 4.5 weeks after resistance induction. All plants were then cultivated until they finished fruit production and died.

In a second experiment, plants again were cultivated under competitive conditions; namely the two plants forming a pair were cultivated in the same pot. Other cultivation conditions (different N regimes and BION® treatment) were as described above, and plants were watered for 1, 2, 3, 4, 5, 6, 7 and 8 weeks after BION® application.

Cultivation of bacteria

Bacteria were used to (a) infect plants in order to compare biochemical responses to BION® treatment to those after biological infection and (b) to check whether BION® treatment leads to significant resistance to pathogens. *Pseudomonas syringae* is a natural pathogen of *Arabidopsis* (Jakob *et al*. 2002). *Pseudomonas syringae pv. tomato* (*Pst*) strain DC 3000 was obtained from Justin Lee (Institute for Plant Biochemistry, Halle, Germany) and the isogenic strain carrying the avirulence gene *avrRpt2* was obtained from Brigitte Mauch-Mani (University of Neuchatel, Switzerland). Bacteria were cultivated in LB medium (containing 50 mg L^{-1} rifampicin and for *Pst* DC 3000 *avrRpt2* additionally 25 mg L^{-1} kanamycin) at 28 °C on a shaker (shaking frequency 175 r.p.m). For bacterial inoculation, cells were grown up to a defined optical density (A_{600} = 0.4–0.8), collected by centrifugation, washed once with 10 mM $MgCl_2$ and then re-suspended in 10 mM $MgCl_2$. Cells were diluted to 10^7 colony-forming units (cfu)/cm^3 (a concentration of A_{600} = 0.2 corresponds to 10^8 cfu mL^{-1}) and one leaf per plant was infiltrated with approximately 50 μL bacterial suspension using a 1-mL syringe.

Biological tests of resistance and quantification of resistance markers

To determine the degree of bacterial infection, two leaf discs per leaf (1 cm diameter) were taken from control and induced leaves from seven independent plants. Samples were harvested from leaves 0, 1, 2 and 3 d after inoculation. Leaf discs were washed and homogenized in 500 μL 10 mM $MgCl_2$. Quantification was done by plating different dilutions on LB agar (containing 50 mg L^{-1} rifampicin).

For the quantification of resistance markers 100 mg of infected and uninfected leaf material was collected 4 d after induction from six independent plants and stored at –20 °C until enzyme analysis. The leaf material was ground using a chilled mortar and a pestle in liquid N_2 and sea sand. The suspension was collected in 1.5 mL of 50 mM Na-acetate buffer pH 5.0. After a 5-min centrifugation (Eppendorf, Hamburg, Germany) at 16 000 *g* the supernatants were desalted by gel filtration on NAP 10 columns (Amersham Biosciences, Piscataway, NJ, USA) equilibrated with 50 mM Na-acetate buffer at pH 5.0. These supernatants were used for quantification of enzyme activities.

Chitinase assay

Assays based on a method of Wirth & Wolf (1990) were conducted in 96-well microplates. A total volume of 100 μL reaction preparation contained 15 μL plant extract, 50 μL RBV-chitin (Loewe, München, Germany) and 35 μL 50 mM Na-acetate buffer at a pH of 5.0. Each reaction was replicated four times, incubated 2.5 h at 37 °C and stopped with 55 μL 0.05 M HCl. After 5 min incubation at –20 °C the plate was centrifuged at 2100 *g* at 4 °C. One hundred microlitres of the supernatant were transferred to a new microplate and measured at 550 nm in a 'Spectra Max 250' plate reader (MD, Ismaning, Germany). Previous investigations with a chitinase from *Streptomyces griseus* (Sigma, Taufkirchen, Germany) confirmed a linear time response between absorptions (A_{550nm}) of 0 and 0.4.

Glucanase assay

Glucanase assays based on a method of Wirth & Wolf (1990) were conducted in 96-well microplates. A total volume of 100 μL reaction preparation contained 30 μL plant extract, 50 μL RBB-curdlan (Loewe) and 20 μL 50 mM Na-acetate buffer at a pH of 5.0. Each reaction was replicated four times, incubated 3 h at 37 °C and stopped with 50 μL 2 M HCl. After 5 min incubation at –20 °C the plate was centrifuged at 2100 *g* at 4 °C. One hundred microlitres of the supernatant were transferred to a new microplate and measured at 600 nm in a 'Spectra Max 250' plate reader (MD). Previous investigations with laminarinase from *Penicillum* species (Sigma) confirmed a linear time response between absorptions (A_{600nm}) of 0 and 0.3.

Peroxidase assay

A total volume of 200 μL reaction solution contained 10 μL plant extract, 36.6 mM H_2O_2, 40.25 mM guaiacol and 50 mM Na-phosphate buffer at pH 6.0. The oxidation of the substrate was measured spectrophotometrically at 470 nm as described previously (Hammerschmidt, Nuckles & Kuc 1982).

Statistical analysis

Statistical analyses followed the experimental setup as close as possible. *T*-tests were applied whenever effects of resistance induction were to be tested within the paired experimental design, and ANOVA was conducted to compare more than two treatments. Prior to all statistical analyses, data were tested concerning the relevant assumptions and were transformed whenever required.

RESULTS

Biological tests of resistance

Exogenous application of BION® strongly affected the development of subsequent bacterial infection. Infiltrated *Pseudomonas syringae* grew much faster in controls than in

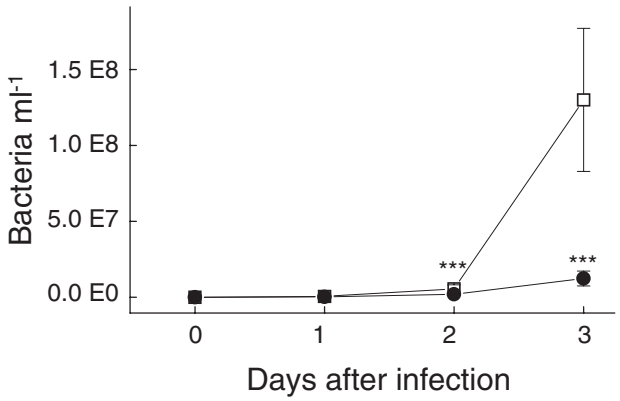

Figure 1. Bacterial growth in response to BION® treatment. Plants were infiltrated with bacterial suspension (approximately 10^4 bacteria per cm^3) and leaves were collected at day of infection (0) and after 1, 2 and 3 d. Amounts of bacteria are given per cm^3 separately for control (□) and treated (●) plants. *** Highly significant differences between induced and control plants ($P < 0.001$ according to *t*-test; $n = 7$ plants per day × treatment combination).

treated plants. One day after infection, treated plants contained 2.1×10^5 mL^{-1} bacteria, whereas untreated controls contained on average 5.5×10^5 mL^{-1}. Although this difference was not significant ($P = 0.07$ according to *t*-test comparing each seven treated and untreated plants), the effect became stronger during further bacterial development. After 2 d, treated plants contained highly significantly less bacteria than untreated controls (1.9×10^6 mL^{-1} versus 5.5×10^6 mL^{-1}), and after 3 d control plants contained almost 1000 times more bacteria than plants treated with BION® (Fig. 1).

Infection with *P. syringae* led to local and systemic increases in activities of most of the marker enzymes that characterize BION® treated plants (Dietrich, Ploss & Heil 2004). Glucanase activity was significantly increased by bacterial infection, and a significant localization–treatment interaction indicated that this response differed between infected and uninfected ('systemic') leaves of the same plant (Table 1). Direct comparisons of treated and control plants revealed significant differences only in infected, yet not in uninfected leaves (Fig. 2). Similarly, peroxidase activities were significantly induced only in the infected leaves. Chitinase activities increased both locally and systemically (Fig. 2), although the magnitude of induction differed among infected and uninfected leaves of the same plant (significant localization–treatment interaction, see Table 1).

Table 1. Results of univariate ANOVA on effects of infection with bacteria on activities of resistance markers

Enzyme	Effect	*F*	*P*
Glucanase	localization	2.729	n.s.
	induction	16.095	0.001
	loc × ind	5.294	0.032
Peroxidase	localization	9.361	0.006
	induction	0.682	0.042
	loc × ind	1.719	n.s.
Chitinase	localization	29.955	<0.001
	induction	47.485	<0.001
	loc × ind	10.440	0.004

One leaf per plant was infected or mock-inoculated ('infection' effect) and activities were quantified both in the infiltrated leaf (local induction) and in untreated leaves ('systemic' induction) of the same plant ('localization' effect). Degrees of freedom (d.f) are 1 in all cases and are thus not listed.

Effects of BION® on short-term growth and seed production

Plants cultivated under four nitrogen regimes were either sprayed with BION® or with water only as a control. Rosette diameters of induced and control plants differed in response to this treatment, yet in different ways under dif-

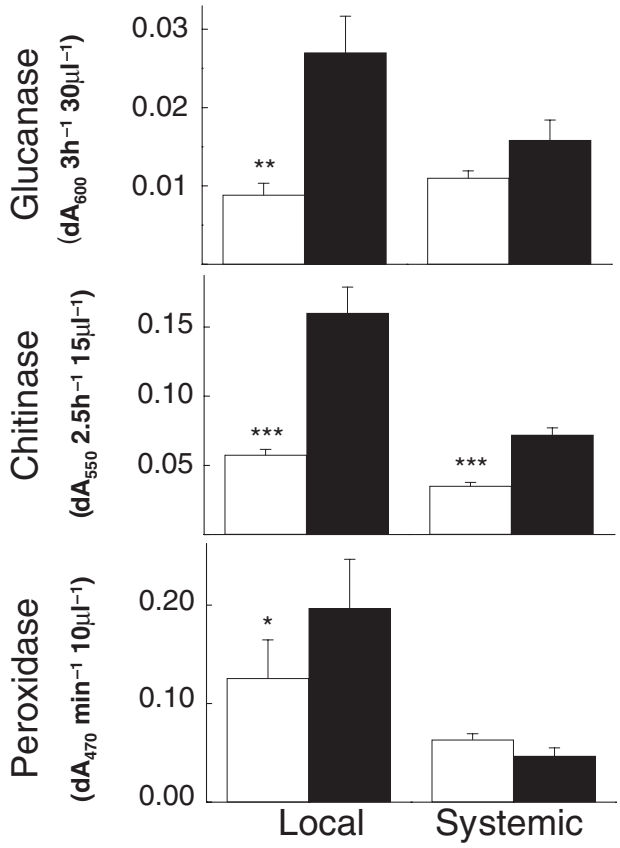

Figure 2. Response of resistance markers to bacterial infection. Activities of three resistance-related classes of enzymes were quantified in infected (black bars, ■) and in mock-inoculated (white bars, □) plants both in the treated leaves (local) and in non-treated leaves of the same plants (systemic). Activities of enzymes are given as changes in specific absorptions (dA_{550} 2.5 h^{-1} 15 μL^{-1} plant extract for chitinase, dA_{600} 3 h^{-1} 30 μL^{-1} plant extract for glucanase and dA_{470} min^{-1} 10 μL^{-1} plant extract for peroxidase). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ according to *t*-tests comparing marker activities in the same plant parts of induced and mock-inoculated plants.

ferent N regimens: One week after induction, treated plants were smaller than controls under all four nitrogen regimes. These differences were significant under three of four N conditions (*t*-tests, see Fig. 3a). Under strongly limiting 'N1' conditions this difference became smaller during further plant growth, but induced plants continuously remained smaller than controls. Plants cultivated with higher N supply showed a different pattern: Induced plants overcompensated and became larger than control plants 2 and 3 weeks after induction. While only tendencies could be detected under 'N3' and 'N10' conditions, this difference was significant (*t*-test, see Fig. 3a) when plants received very high amounts of nitrogen (N30). In the same experiment, induced plants produced less seeds than controls under all N conditions, but this difference was significant only under medium N supply (N3 and N10, see Fig. 3b).

In an independent replication, induced plants again were significantly smaller than controls 1 week after BION® treatment under three of for N conditions (Fig. 4a) and then compensated for this growth depression by exhibiting higher growth rates than controls during the second week (Fig. 4b). Rosette growth was strongly affected by nitrogen supply: While all control plants showed continuously decreasing relative rosette growth (grey lines in Fig. 4b), growth rates of induced plants increased from week 1 to week 2 under all nitrogen conditions except 'N1' (black lines in Fig. 4b). Relative rosette growth of induced as well as of control plants then decreased and was slightly above zero during week 3 and negative during week 4 after BION® treatment. Overcompensation (i.e. induced plants being larger than controls) was not observed during this experiment.

Under competitive conditions, rosette growth of induced

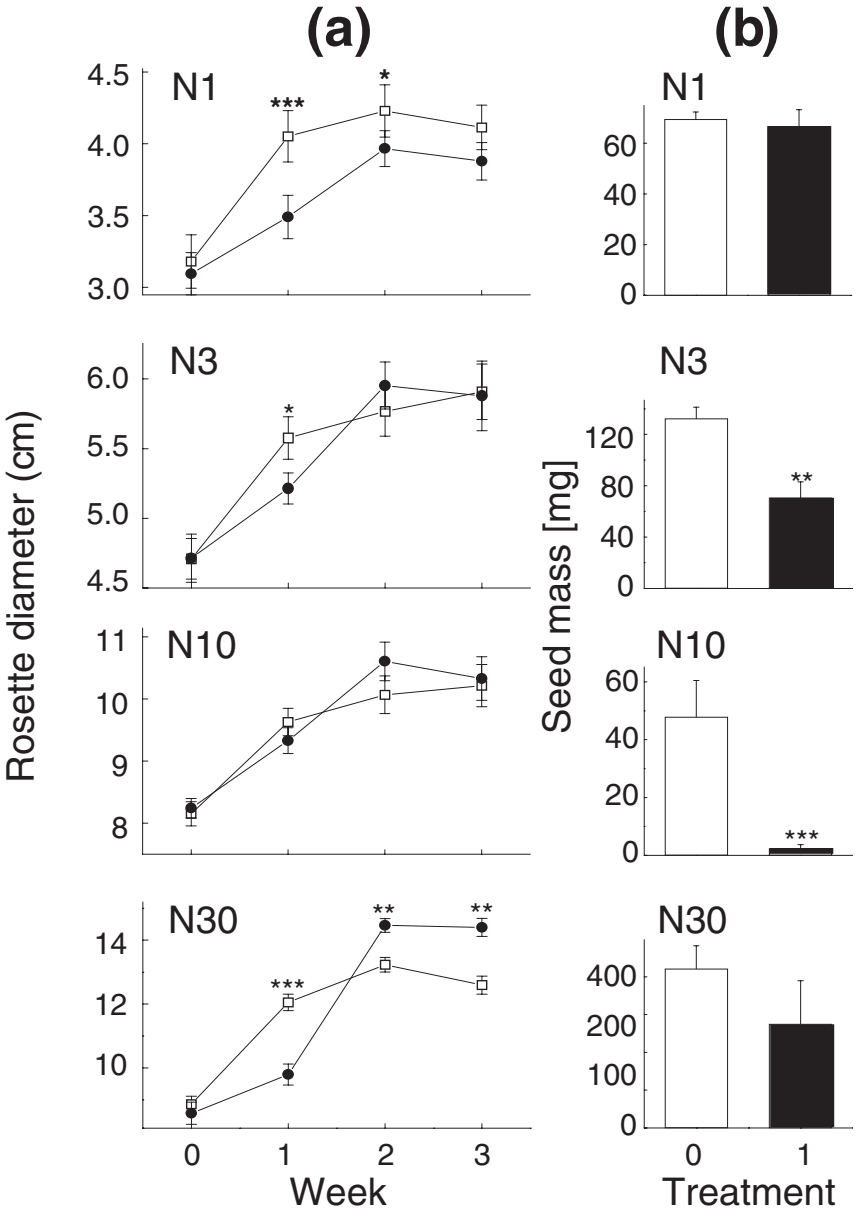

Figure 3. Responses in growth during 3 weeks after BION® treatment (a) and seed production (b) in response to BION® treatment under different nitrogen conditions. (a) Rosette diameters were measured for 14 plant pairs at the time of BION® treatment and after 1, 2 and 3 weeks. Note that *Y*-axes differ among panels. (b) Seed production was quantified for seven plants each at the end of the plants' growing period (i.e. approximately 9–10 weeks after induction) and is given as seed mass (mg per plant). Open symbols and bars (□) indicate control plants; black symbols (●) and bars treated plants. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ according to paired *t*-tests (plant pairs defined by rosette diameter before BION® treatment).

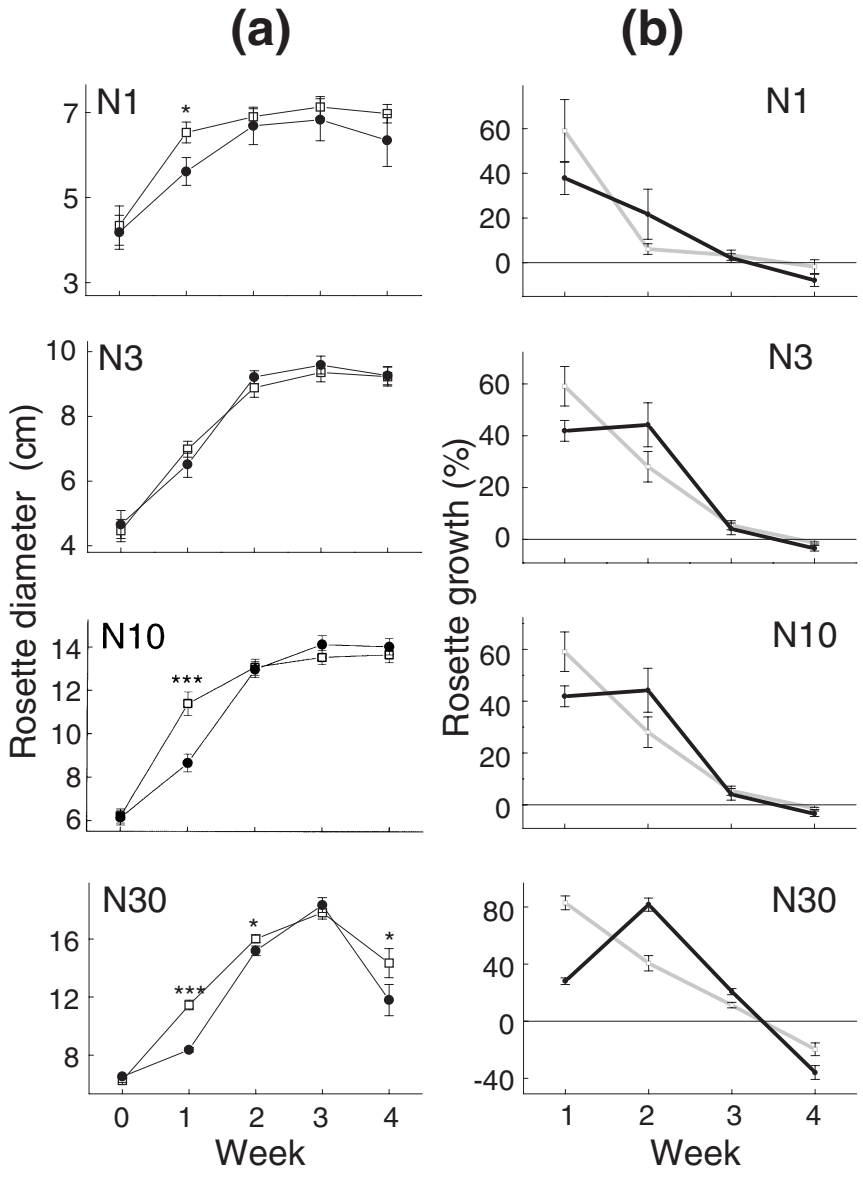

Figure 4. Compensatory growth of Arabidopsis in response to BION® under different nitrogen conditions. (a) Rosette diameters of controls (□) and treated (●) plants were quantified in (cm) at time of BION® treatment (week 0) and after 1, 2, 3 and 4 weeks. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ according to paired *t*-tests (plant pairs defined by rosette diameter before BION® treatment). Note that *Y*-axes differ among panels. (b) To illustrate rosette growth, relative increases in rosette diameter as compared with their diameter 1 week earlier were calculated for all individual plants after 1, 2, 3 and 4 weeks and are presented as (percentage) for controls (grey lines) and induced plants (black lines).

plants never exceeded that of controls (Fig. 5). During the first week, rosettes of induced plants grew significantly less than those of controls under all four nitrogen conditions. Under 'N1' conditions, rosettes of induced plants already shrunk during the second week. Similarly, rosettes of induced plants cultivated under 'N3' conditions continuously grew less (and shrunk faster) than controls. Only induced plants cultivated under high 'N10' and very high 'N30' conditions reached again similar growth rates as controls, which was the case, however, at the earliest 3 weeks under 'N30' and even 4 weeks under 'N10' conditions (Fig. 5).

Effects of shortened growing period on seed set

Two experiments investigated whether and how shorter growing periods (caused by a ceasing of water supply) affected seed production of induced and control plants. In the first experiment plants growing individually were treated with BION® and were watered for the following 1, 2, 3 and 4.5 weeks. These plants' growth responses paralleled those observed in earlier experiments: Rosette growth of plants cultivated under low N supply (N1 and N3) was lower than those of controls at all times after BION® treatment. In contrast, induced plants cultivated under high 'N10' and very high 'N30' conditions compensated and exhibited higher growth rates than controls during the second and third week after resistance elicitation (Fig. 6a).

When watering ceased already 1 week after resistance elicitation, only treated plants grown under the most severe shortage of N produced seeds at all (Fig. 6b). Plants that were watered over 2 weeks after BION® treatment produced seeds when cultivated under low 'N1' or 'N3' conditions, whereas plants cultivated under high 'N10' and 'N30'

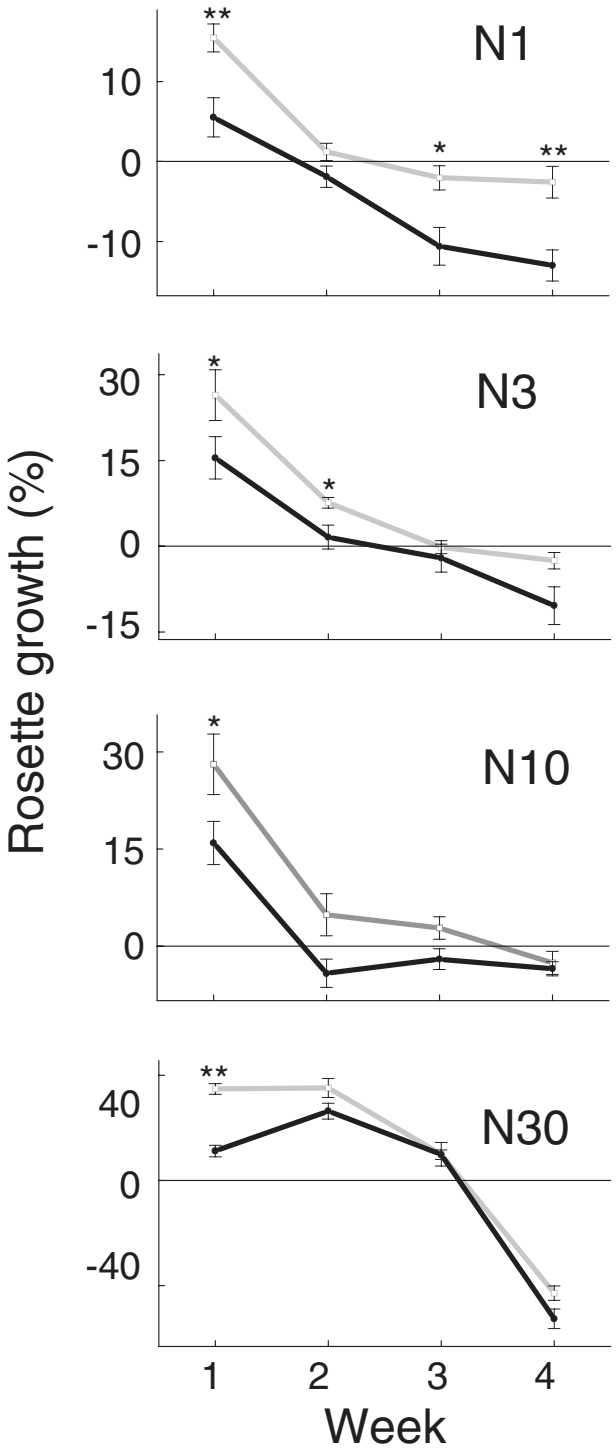

Figure 5. Compensatory growth of Arabidopsis in response to BION® under competition. Each one control and one treated plant were cultivated in the same pot. Rosette growth was calculated 1, 2, 3 and 4 weeks after treatment as relative increases in rosette diameter as compared to the same plant's diameter 1 week earlier and are presented as (percent) for controls (grey line) and induced plants (black line). Note that *Y*-axes differ among panels. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ according to paired *t*-tests using plants growing in the same pot as pairs, sample size $n = 7$ plant pairs for each N condition.

nitrogen supply required at least 3 weeks of watering after BION® application to produce seeds at all (Fig. 6b). When tested separately, seed masses of these plants were almost significantly ($P = 0.07$ according to paired *t*-test) higher for induced than for control plants. Whether or not these plants successfully produced seeds could be predicted from the plants' relative growth rates at the time of start of drought. Only those plants that on average exhibited a relative growth of <10% at this time finally had produced seeds (dashed line in Fig. 6a).

Treating plants growing in competition to untreated controls and ceasing watering at different times after BION® application in general confirmed these results (Fig. 7). The seed mass produced by such plants was significantly affected by N supply, BION® treatment and the time at which watering ceased (Table 2). Significant 'N × week' and 'BION®–week' interactions indicate that the beginning of water stress significantly affected the effects of BION® application and N supply on seed masses produced (Table 2): Plants cultivated under 'N30' conditions again produced no seeds at all when watering was stopped already 1 week after BION® treatment, and treated plants produced more seeds than controls when watering of plants cultivated under 'N1' conditions was stopped earlier than 5 weeks after resistance elicitation. Longer growing conditions (plants being watered for at least 5 weeks after BION® treatment) resulted in negative effects of resistance elicitation on seed production under all four nitrogen conditions (Fig. 7).

DISCUSSION

Although increasingly being investigated, the influence of abiotic conditions on the extent of resistance induction and its ultimate effects on plant growth and fitness is still poorly understood (Cipollini 2004; Mopper *et al.* 2004; Thaler & Bostock 2004). We induced resistance to pathogens chemically by applying BION® to *Arabidopsis* plants under different conditions to investigate whether resistance

Table 2. Results of univariate ANOVA on effects of nitrogen supply (N), resistance elicitation with BION® and time after resistance elicitation at which watering was ceased (week) on seed set of plants growing under competition (i.e. each one induced and one control plant cultivated in the same pot)

Factor	d.f.	*F*	*P*
N	3	106.4	<0.001
BION®	1	30.5	<0.001
week	7	107.6	<0.001
N × week	21	10.2	<0.001
BION® × week	7	3.2	0.003

Sample size $n = 7$ plants per N–BION®–week combination, induced and control plants were cultivated under four N regimes and watering was ceased 1, 2, 3, 4, 5, 6, 7 and 8 weeks after induction. Overall, 448 plants cultivated in 224 pots were considered. Only significant interactions are reported.

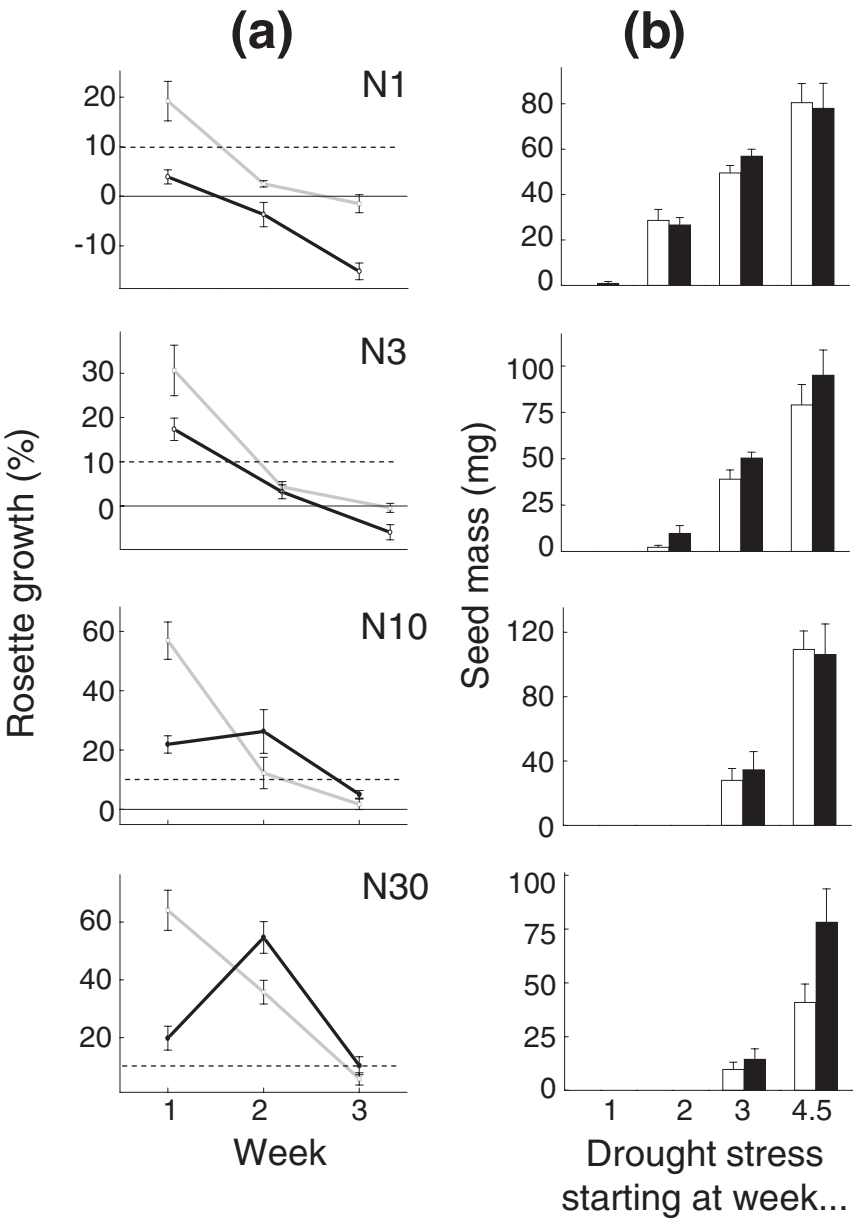

Figure 6. Plant growth (a) of Arabidosis in response to BION® under different nitrogen supply and seed production (b) in dependence of length of growing period. (a) Rosette growth of controls (□) and treated (■) plants was calculated as relative increases in rosette diameter as compared to the same plant's diameter 1 week earlier and are presented as (percentage) for controls (grey line) and induced plants (black line). Dashed lines (– –) indicate relative growth rates of 10%. (b) Seed production in (mg per plant) in response to shorter growing periods achieved by stopping watering after 1, 2, 3 and 4.5 weeks ($n = 7$ plants per treatment).*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ according to Wilcoxon pair test. Note that *Y*-axes differ among panels.

induction under enemy-free conditions can lead to negative effects on plant growth and, ultimately, seed production. Chemical elicitation has been used in many studies on induced resistance (Brown 1988; Baldwin 1998; Stout *et al*. 1998; Thaler 1999a, b; Heil *et al*. 2000; Cipollini & Bergelson 2001; Redman, Cipollini. & Schultz 2001; van Dam & Baldwin 2001; Cipollini 2002) since it allows induce resistance under enemy-free conditions and thus without any putative effects of infections or feeding animals.

Biological relevance of BION® treatment

How similar are resistance phenomena observed after BION® treatment to those after biological infection? BION® application elicited significant resistance to the bacterial pathogen *Pseudomonas syringae* (Fig. 1), and biochemical changes observed after bacterial infection were similar to those after BION® treatment (described in detail in Dietrich *et al*. 2004): Activities of glucanases and chitinases increased in leaves that were infected with *P. syringae* (Fig. 2: local responses), and even 'systemically', that is, in leaves that were not infected themselves. Only peroxidases did not respond systemically to bacterial infection and thus appear to represent a strictly local response. BION® treatment leads to a plant-wide elicitation of combined local and systemic responses, thus mimicking an intensive plant-wide infection. The similarities among chemical and biological resistance elicitation (Figs 1 and 2 and Dietrich *et al*. 2004) were high enough to make BION®-treated plants a suitable model to study responses to resistance elicitation.

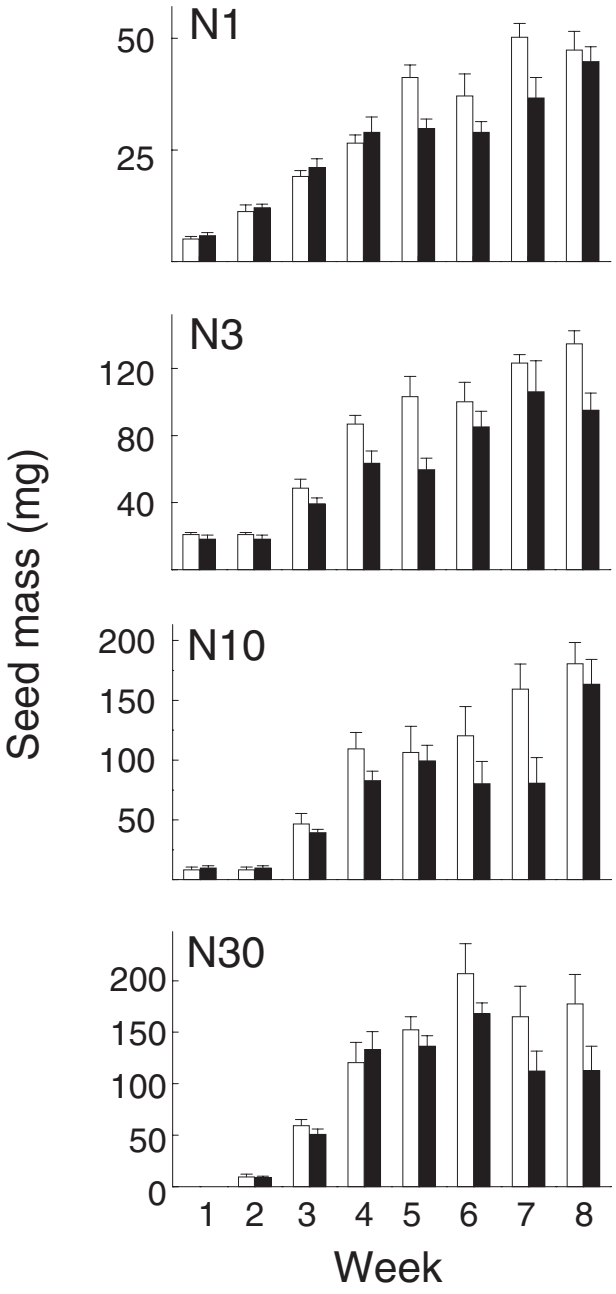

Figure 7. Combined effects of competition, N supply and length of growing period on seed set of Arabidopsis in response to BION®. Each one untreated control (□) and one treated (■) plant were cultivated in the same pot and treated with BION® at time 0. Watering was stopped 1, 2, 3, 4, 5, 6, 7 and 8 weeks after BION® application and seed set was quantified for all plants as (mg per plant). Note that *Y*-axes differ among panels.

Growth reduction after resistance elicitation

Induced plants were commonly smaller than untreated controls 1 week after BION® treatment. This effect was significant under each three of four nitrogen regimes in two independent experiments (Figs 3a & 4a). Reduced rosette growth during the first week after induction (Figs 4b, 5 & 6a) could be caused by resource allocation to the synthesis of resistance compounds. This apparently mirrors 'the dilemma of plant: to grow or to defend' (Herms & Mattson 1992). Rosette diameter is a relevant parameter for growth, since dry weight and rosette diameter of the same plants are proportionally related (unpublished data).

These phenotypic observations are in line with described physiological and genetic processes, since both primary and secondary metabolism are dramatically affected in response to natural or chemical induction: An intensive metabolic 're-programming' during resistance elicitation has been described (Somssich & Hahlbrock 1998; Hahlbrock *et al*. 2003) and includes reductions in the expression of genes related to primary metabolism (Kombrink & Hahlbrock 1990; Logemann *et al*. 1995; Somssich & Hahlbrock 1998). Salicylic acid has a central role in the elicitation of pathogen resistance and also affects nitrate reductase activity (Jain & Srivastava 1981). Such down-regulation of primary metabolism might be required to free up resources needed for *de novo* synthesis of defensive compounds (Heil 2002) and apparently has the phenotypic consequence of a growth depression, which was observed in all independent experiments conducted in the present study.

Compensation of growth depressions

In most cases the induced plants later compensated, at least in part, for the initial growth depression. Resources obviously were shifted again to growth. However, plants growing under limiting 'N1' conditions only partly compensated and continuously remained smaller than untreated controls, whereas those receiving high 'N30' nitrogen supply overcompensated and were significantly larger than controls 2 and 3 weeks after resistance elicitation (Figs 3a & 4a). The capacity to compensate depended on growing conditions: Although Cipollini (2004) has discussed that and why positive linkages among plant responses to competitors and to herbivores or pathogens might occur, induced plants in our experiments never achieved higher rosette growth rates than controls when they directly competed with untreated plants (Fig. 5). Similarly, induced plants cultivated without competition in another independent experiment grew faster than controls only when receiving medium (N10) or high (N30) nitrogen supply, yet not under limiting 'N1' and 'N3' conditions (Fig. 6a). The capacity to (over)compensate for negative effects of resistance induction on plant growth thus was clearly constrained by the amount of N available to the induced plant.

Life history, growing period and (over)compensation of seed mass

Arabidopsis shifts resources from its rosette leaves to the production of a main, flower-bearing shoot – and thus ultimately reproduction – at a given time of its development. 'Negative growth' (i.e. a reduction in average rosette diameters) was observed in all experiments of the present study and resulted from dying of leaves during the re-allocation

of resources to the flower-bearing shoot. This is a typical trait of 'monocarpic' plants, which are characterized by a coupling of whole-plant senescence to reproduction (Hildebrand 1881; Hensel *et al*. 1993). Whether a plant can successfully produce seeds is thus dependent on the time at which this shifting starts, on biotic and abiotic factors affecting this time, on the plant's physiological state at this given time, and on factors that influence the plant during the remaining growing period.

The length of the growing period was varied in two experiments by ceasing watering at different times after resistance elicitation. N conditions again determined the plants' capacity to exhibit (over)compensatory growth rates (Fig. 6a). However, high growth rates ultimately could have negative rather than positive effects on seed set when the growing period was limited. In contrast to predictions of the 'limited resources – higher fitness costs' hypothesis, only induced plants growing under most strongly limiting N conditions produced seeds at all when watered just for 1 week after resistance elicitation. Plants watered over 2 weeks after BION® treatment produced seeds under 'N1' or 'N3' conditions, while plants receiving 'N10' or 'N30' nitrogen supply required watering at least for a third week to successfully produce seeds. Plants cultivated under five among 16 combinations of water stress and nitrogen supply did not produce seeds at all, and in eight of the remaining groups induced plants produced more seeds than controls (Fig. 6b). Although the induction effect was only marginally significant ($P = 0.07$ for plants watered over 3 weeks, univariate ANOVA were conducted on effects of N supply and induction separately for each water stress condition) it indicates a fitness advantage of resistance induction when the vegetation period is short. Such overcompensatory effects have rarely been described for herbivore-induced plants (Lennartson, Tuomi & Nilsson 1987; Paige & Whitham 1987), and the present study appears to be the first observation of beneficial effects of induction of resistance to pathogens under pathogen-free conditions.

Senescence, resistance and reproduction

Seed production was predicted by rosette growth at the time at which watering ceased: Only plants that at this particular time had started to shift from rosette growth to shoot production (indicated by a relative rosette growth of <10%, see dashed lines in Fig. 6a) finally produced seeds (Fig. 6b). Induced plants obviously started earlier to shift resources from vegetative to reproductive growth (see Fig. 6b). Senescence-related genes are induced by water stress (Becker & Apel 1993), heat stress (John *et al*. 1997), or light limitation (Oh *et al*. 1996). Different environmental stresses thus may activate senescence processes (Nam 1997; Weaver *et al*. 1998) and thereby act as signals to the plant to invest earlier in reproduction. Similar responses are elicited by resistance-related plant hormones such as ethylene, JA or SA (Morris *et al*. 2000). Therefore, Obregon *et al*. (2001) discussed whether defence induction and senescence are inter-related.

Our study provides further evidence that resistance induction might induce senescence as well and may increase rather than decrease a plant's seed production under limited growing periods: BION® treatment elicited an earlier shift from rosette growth to shoot production particularly in plants growing under limiting N supply (Fig. 6a). *Arabidopsis* is typically growing on sandy soils and in highly ephemeric environments (Hensel *et al*. 1993), and it is characterized by comparably low indicator values for both soil nitrogen content and water supply (Ellenberg *et al*. 2001). Its responses under N limitation and water stress thus might evolutionarily be as (or even more) relevant than those observed under optimal conditions.

Methodical implications

In several cases higher growth rates observed at a given time after resistance induction ultimately resulted in smaller seed masses (Figs 3 & 6). Growth rates or dry masses appear valuable phenotypic parameters to observe and quantify proximate responses to an induction, yet in several cases failed to be valid predictors of effects on lifetime seed production. Studies quantifying only plant growth (Siemens *et al*. 2002) might fail to detect costs due to these phenomena rather than an absence of costs of the factor in question. Fitness costs thus should be quantified as directly as possible, for example by quantifying seed capsules (van Dam & Baldwin 1998), seed number (Agrawal 2000), seed mass (Cipollini 2002), germination success (Redman *et al*. 2001), the performance of seedlings (van Dam & Baldwin 2001), or ultimately the genetic contribution to the next generation. In the present study, no effects of resistance induction on germination rates could be detected (unpublished data), and lifetime seed production thus appears a suitable estimate of plant fitness.

CONCLUSIONS

Reduced lifetime seed production due to resistance induction was detected under certain conditions. Under all conditions tested, induced plants experienced a growth depression during the first week after resistance induction. Most probably, this phenomenon was caused both by the allocation processes required for the synthesis of resistance-related compounds and by an earlier allocation to reproduction. However, the effect of resistance induction on seed production was determined by environmental conditions such as nitrogen supply, water stress, and whether or not the induced plants competed with non-induced neighbours: Costs, no costs and even higher seed production by induced as compared with control plants were observed under different combinations of these factors. Studies controlling – or varying – only some of these factors thus can easily lead to apparently contradictory results, which in fact represent different outcomes of a complex interplay of factors. Many more growing conditions than usual have to be tested to investigate whether resistance

induction can lead to significant effects on growth and fitness of a particular plant species.

In summary, competition increased the probability of detecting fitness costs in particular when the growing periods were longer and when the plants experienced a weakly limiting N supply. The limited resources – higher fitness costs hypothesis thus was supported only for plants growing in a competitive environment, since limited supply of nitrogen and water alone did not increase the chance to detect fitness costs of induced plants.

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