Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation costs?

MARTIN HEIL, ANDREA HILPERT, WERNER KAISER* and K. EDUARD LINSENMAIR

*Zoologie III, Theodor Boveri Institut, Biozentrum, Am Hubland, D-97074 Würzburg, Germany; and *Botanik I, Julius von Sachs Institut, Julius von Sachs-Platz 2, D-97082 Würzburg, Germany*

Summary

1 Although most theories on plant defence assume that costs will result from the production and maintenance of defensive traits, studies on the costs of induced defence against pathogens are comparatively rare.

2 We focus on fitness costs resulting from the chemical induction of systemic acquired resistance (SAR), a rather unspecific form of defence, which can be induced by and is effective against a broad spectrum of bacteria, fungi and viruses.

3 We used a model system in which we treated wheat plants that were protected against fungi by 'traditional' fungicides with BION® (a benzothiadiazole which induces pathogen resistance). Treated plants were therefore compelled to invest in defence without gaining any profit from the induction.

4 Treated plants achieved lower biomass than untreated controls, and developed fewer shoots and ears and therefore produced fewer seeds. The effects were most pronounced in plants that suffered from a shortage of nitrogen, and were observed only when pathogen resistance was induced during lateral shoot production. Later treatment revealed no significant effects.

5 We discuss whether the differences between treated and control plants can be interpreted as a consequence of allocation costs. Such costs could result from metabolic competition between processes involved in plant growth and the synthesis of defence-related compounds.

Key-words: benzothiadiazole, BION®, fitness costs, plant–pathogen interaction, *Triticum aestivum*

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Introduction

Numerous studies have demonstrated that a plant, once challenged by one single pathogen at one single spot, can develop systemically a resistance against further infections by a broad spectrum of different pathogens, including viruses, bacteria and fungi (for overviews, see Hunt *et al.* 1996; Ryals *et al.* 1996; Schneider *et al.* 1996; van Loon 1997). Though a few studies have demonstrated that such systemic acquired resistance (SAR) can increase fitness of induced plants (Caruso & Kuc 1977; Kuc 1982; Dann *et al.* 1998), and thus represents indeed an induced defence (Karban & Baldwin 1997), most studies have focused on underlying signalling pathways and on possible influences on various plant–pathogen interactions. Few studies have been conducted under field conditions (but see those cited by Hatcher & Paul 2000), and none has, to our knowledge, been designed specifically to estimate the fitness costs of SAR (Agrawal 1999; Heil 1999).

Consideration of the low specificity and the high efficiency of acquired resistance led us to ask why this resistance has to be induced by an initial (probably severe) 'challenging infection' rather than being a constitutive trait. A simple ecological explanation may be that the fitness costs of SAR may be too great (i.e., in terms of reproductive curtailment; Bazzaz *et al.* 1987; Simms & Rauscher 1987; Marquis 1991) for it to be established continuously. Fitness costs are often hard to quantify directly (Chapin

Correspondence: M. Heil (e-mail: heil@biozentrum.uni-wuerzburg.de).

1989; Gershenzon 1994), but several studies have demonstrated that they may be estimated indirectly by measuring plant growth rates (Coley 1986; Skogsmyr & Fagerström 1992; Sagers & Coley 1995; Steinberg 1995). Such costs mostly result from allocation costs, i.e. from a metabolic competition for limited resources (Herms & Mattson 1992). Several studies have compared growth rates or other fitness-related parameters of cultivars or races differing in their intrinsic pathogen resistance to evaluate the costs of constitutive defences (see, for example, Simms & Rausher 1987; Simms & Vision 1995; Hoffland et al. 1996). However, although circumstantial evidence exists that induction of pathogen resistance may reduce plant fitness in the absence of pathogens (reviewed by Agrawal 1999), there are no equivalent studies published for SAR.

Here we present data from a model system on the costs of induced defence against pathogens in order to provide a basis for further studies. We wished to include fitness parameters in this initial, explorative study, and therefore chose a fast growing annual, wheat (*Triticum aestivum*, cv. 'Hanno'), for which we could measure biomass increase and seed set. The plant activator BION® (Novartis, Basel, Switzerland) was originally designed to treat wheat and has also been shown to induce SAR in several other plant species (see, for example, Friedrich et al. 1996; Görlach et al. 1996; Lawton et al. 1996; Molina et al. 1998; and Discussion section, below). Comparison of BION® treated plants and untreated controls may therefore be of more general ecological relevance. Detailed information on the biochemistry of chemically induced defence is available for this species (Görlach et al. 1996). Both because costs of a defensive trait become more obvious in plants that grow under resource-limited conditions (Karban & Baldwin 1997; van Dam & Baldwin 1998) and because plants growing under natural conditions are often in competition with neighbouring plants, the growing conditions were designed to impinge upon plant growth by limiting nitrogen, light and soil available to the plants to some extent. We discuss whether BION®-induced reductions in growth, morphological changes and reduced seed set could be indicative of fitness costs of induced pathogen resistance.

Materials and methods

Spring wheat (*Triticum aestivum*, cv. 'Hanno') was obtained from K.-L. Nau, Novartis Germany, Frankfurt. Where appropriate, BION® (active component benzo(1,2,3) thiadiazole-7-carbothioic acid-S-methyl ester: CGA-245 704, also known as BTH, see Schurter et al. 1987) was applied as a solution of 300 mg L^{-1} de-ionized water (equivalent to recommended concentration for field use). BTH has been shown to induce resistance in wheat against several pathogens, and to induce a set of so-called 'wheat chemically induced' (WCI) genes (Görlach et al. 1996). Plants were grown both in hydroponic chambers and in the field and under a range of nutrient conditions.

EXPERIMENT 1: PLANTS IN HYDROCULTURE

Plants were cultivated in two nutrient solutions that differed in nitrate content (solution 1a and 1b in Table 1). Hydroponic pots (4 L in volume) were kept under constant conditions in a growth chamber (10 h light per day, PFD about 250μE, air temperature 15 °C at night and 25 °C during day) and solutions, which were continuously mixed by circulating external air, were replaced weekly. Each pot (four for 50 μM N and two for 1 mM N treatment; Table 1) contained about 20 plants. Four weeks after sowing, when most of the seedlings had reached the two-node stage (developmental stage EC22, see Ciba-

Table 1 Nutrient solutions; composition (in mmol L^{-1}) of nutrient solutions used for hydroponic culture (Experiment 1) or for fertilization (Experiment 2 and 3)

	Solution 1a 50 μM N Experiment 1	Solution 1b 1 mM N Experiment 1/2	Solution 2a 0 N Experiment 3	Solution 2b 5 N Experiment 3	Solution 2c 10 N Experiment 3
KNO$_3$	–	0.4	–	2.0	4.0
Ca(NO$_3$)$_2$	*	0.3	–	1.5	3.0
NaH$_2$PO$_4$	0.5	0.5	0.5	0.5	0.5
MgSO$_4$	1.5	1.5	1.5	1.5	1.5
KCl	2.0	1.6	2.0	–	–
CaCl$_2$	1.5	1.2	1.5	–	–
H$_3$BO$_4$	0.05	0.05	0.05	0.05	0.05
FeEDTA	0.2	0.2	0.2	0.2	0.2
Micronutrients	After Johnson et al. (1957)				

*2 mL of a 0.1-M Ca(NO$_3$)$_2$ solution were added daily to each pot with solution 1a to maintain about 50 μM N.

Geigy 1996), two pots with 50 μM N and one with 1 mM N were chosen randomly and sprayed with 100 mL BION® solution per pot, while the others were sprayed only with water. Direct comparisons were made between paired pots with each pair consisting of one treated and one untreated pot containing the same nutrient solution.

At this time, and weekly thereafter, the two largest and the two smallest plants from each pot were harvested. Fresh and dry weight of shoots and roots were measured separately for each plant and means were calculated for each value for the pair of similar-sized plants that had been harvested from the same pot at the same time. There were two replicates of the entire experiment.

EXPERIMENT 2: FIELD EXPERIMENT 1997

On 21 May 1997, wheat seeds were sown into 108 plastic pots (14 cm diameter) containing homogenized field soil, which were then immediately returned to field conditions in three separate, but adjacent areas. Three weeks after germination (11 June), plants were thinned to four per pot, and from 18 June were treated twice per week with 200 mL, 50 mL or 0 mL of solution 1b (1 mM N, see Table 1) per pot to obtain high nutrient, medium nutrient and no nutrient treatments. Plants were watered daily with about 200 mL water per pot and were protected against fungal infections by application of Gladio® (a combined fungicide consisting of Propiconazole, Tebuconazole and Fenpropidine, provided by K.-L. Nau, Novartis Germany, Frankfurt).

More than two-thirds of the plants had reached at least the two-node stage by 25 June, and the 36 pots grown under the same nutrient conditions (i.e. within the same area) were arranged according to visually judged above-ground biomass. Pots were divided into 18 pairs with similar biomass, and one, chosen randomly, was treated with BION®, while the other was sprayed with water (control). To exclude any small-scale site effects confounding those resulting from BION®-treatment, the two pots forming one pair were arranged side by side in the field for further cultivation.

One pair was harvested from each nutrient treatment at the time of treatment with BION® and every week for seven weeks thereafter, and plant fresh and dry weight was measured separately for shoots and roots. Since plants that were cultivated in the same pot could not be regarded as independent, only total biomass per pot was recorded. At the end of the experiment, the remaining 10 pairs per nutrient level were harvested and total numbers of ears and of earlets per pot, as well as the number of earlets per ear, were counted. The Mann–Whitney (U-) test was applied separately for each

nutrient condition to compare treated to control pots.

EXPERIMENT 3: FIELD EXPERIMENT 1998

This experiment was conducted at a site with better (less sandy) soil than Experiment 2. Seeds were sown on 18 March 1998 into 150 earthen pots (20 cm diameter), and wheat was sown directly into the soil between the pots to simulate typical field conditions. Different nitrogen conditions (0 N, 5 N and 10 N) were created in three areas by applying 1.5 L m^{-2} of one of solutions 2a–c (Table 1). Fertilization corresponded to about 100 mL per pot and was carried out twice per week. Six weeks after germination, the number of plants was reduced to seven per pot, and 2 weeks later, plants were sprayed with the same fungicide as in Experiment 2. More than 75% of the plants had reached the two-node stage by 30 April and pots were then sorted as in Experiment 2 and allocated to groups of four pots each. Within each group, pots were assigned randomly to four treatments: immediate treatment with BION® (A), treatment 3 weeks later on 27 May (B), treatment after the end of lateral shoot production on 26 June (C), and no treatment (control) (D). To exclude any small-scale site effects, the four pots forming a group were kept together.

Five pot groups per nutrient condition were selected randomly and were harvested at the beginning of August (60 pots overall); above-ground biomass was weighed, the total number of shoots, ears and earlets per pot was counted, and the mean number of earlets per ear was calculated. The number of ears and earlets were counted in three additional, randomly selected pot groups per N-treatment (36 pots overall) to increase the number of samples for these variables. The remaining 54 pots were used for other studies and are not considered further in this context.

STATISTICAL EVALUATION

Statistical evaluation was based on nonparametric tests and followed the experimental design as closely as possible. Wilcoxon tests for matched pairs were used to evaluate data in the first two experiments, which had been set up in a pairwise design, while Kruskal–Wallis (H-) tests (followed by *post hoc* pairwise Mann–Whitney tests, *P*-values corrected according to the Bonferroni method for multiple comparisons; see, for example, Sachs 1992) were conducted to compare the four different treatments in Experiment 3. In all experiments, independent sets of plants were cultivated under different nutrient conditions and statistical comparisons are, therefore, conducted within, but not between, single nutrient conditions. All statistical evaluations were

conducted with SPSS for Windows 8.0 (SPSS Inc., Chicago, IL).

Results

EXPERIMENT 1: PLANTS IN
HYDROCULTURE

Untreated controls grew faster and achieved higher biomass than treated plants (Fig. 1). In both replicates, the effect on total plant biomass became visible 3–4 weeks after treatment (Fig. 1a, b for both nutrient levels combined). In the first replicate, BION® treatment had a significant effect on root dry weight and total plant dry weight under 50 μM N conditions ($P < 0.05$, Wilcoxon pair test), while only root dry weight showed a significant effect under 1 mM N conditions. The differences between treatment and control plants were slightly higher in the second replicate (Fig. 1b), where dry weight of shoots as well as roots and total plants showed significant effects under 50 μM N conditions. In both replicates, the absolute and relative differences

between treated and control plants were higher under 50 μM N than under 1 mM N conditions.

EXPERIMENT 2: FIELD EXPERIMENT 1997

Control plants grew faster and achieved higher above-ground as well as root biomass than plants treated with BION®. These differences became visible about 5–6 weeks after treatment (Fig. 2). Because treated plants and untreated controls differed in their growth rate, the difference between them increased during the experiment (Fig. 2). Wilcoxon pair tests demonstrated that the detrimental effect of the treatment on all biomass parameters was significant under high and medium nutrients ($P < 0.01$, $P < 0.05$, respectively). However, only root biomass differed significantly ($P < 0.05$) under no nutrient conditions (see Table 2).

BION® treatment also caused significant differences in fitness parameters for fertilized plants (Table 3). Under medium and high nutrients, treated plants produced significantly less ears per pot than corresponding controls (Table 3). Under high nutrients the numbers of ears and of earlets per pot was nearly twice as high in the controls. However, mean numbers of earlets per ear did not differ significantly between BION® treated and untreated plants in any of the nutrient conditions (Table 3).

EXPERIMENT 3: FIELD EXPERIMENT 1998

The developmental stage at the time of BION® treatment markedly influenced the effect on seed set (Fig. 3), as well as on other growth and fitness parameters (Table 4). Effects of treatment time were significant for all parameters measured under 0 N conditions, while only numbers of ears and of earlets per pot were influenced significantly at 5 N, and

(a)

(b)
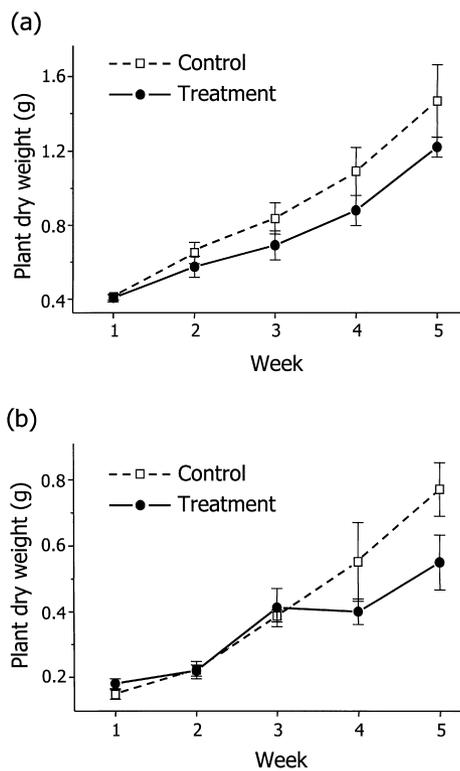
Fig. 1 Growth of BION®-treated and of untreated control plants in experiment 1 (hydroculture). Means ($\pm$ SE) of total plant dry weight (g) are calculated for five harvests, starting at the date of BION® treatment. Data from both nitrogen treatments are combined and each point represents 12 individual plants. The panels (a) and (b) show data from two replicate experiments.
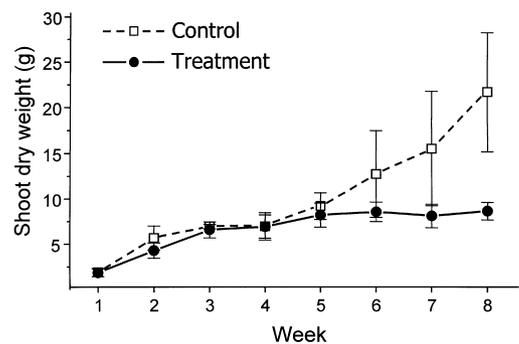
Fig. 2 Above-ground growth of treated and untreated plants in experiment 2 (first field experiment). Mean ($\pm$ SE) shoot dry weights (g) are shown for all nutrient conditions combined ($n = 12$ pots for each data point with each pot containing four plants).

Table 2 Results of Wilcoxon pair tests on biomass parameters in Experiment 2. Pairs consisting of one BION®-treated pot and one control pot harvested at the same time were used as matched pairs in these tests, sample size is therefore eight pot pairs (each pot containing four plants) for each test. dw, dry weight; ** $P < 0.01$, * $P < 0.05$, NS = not significant

	No nutrient treatment	Medium nutrient treatment	High nutrient treatment
Shoot biomass [g dw]	NS	*	**
Root biomass [g dw]	*	*	**
Whole plant biomass [g dw]	NS	*	**

Table 3 Medians and results of Mann–Whitney tests on fitness parameters in Experiment 2. Data analysis was conducted separately within each nutrient condition; $n = 10$ pots (each containing four plants) per treatment and nutrient treatment. ** $P < 0.01$, NS = not significant

	No nutrient treatment			Medium nutrient treatment			High nutrient treatment		
	BION®	Control	*P*	BION®	Control	*P*	BION®	Control	*P*
Ears per pot	4.5	5.0	NS	3.0	6.0	**	5.5	9.5	**
Earlets per pot	59.5	61.0	NS	60.1	82.0	**	83.0	140.5	**
Earlets per ear	13.5	13.0	NS	13.4	14.3	NS	13.7	15.1	NS

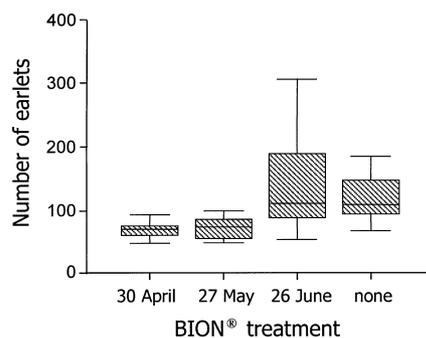no significant differences could be detected at 10 N (Table 4).

The effects observed under 0 N conditions were, however, mainly due to treatments A and B showing different values to those in C and D. For example, total shoot biomass per pot in treatments C and D (medians of 5 pots each: 42.8 and 53.1 g dry weight, respectively) was about three times as high as in treatments A and B (12.9 and 14.8 g dry weight per pot, see Table 4), while the number of earlets per pot in treatments C and D (both medians higher than 120) was more than twice as high as in treatments A and B (both medians lower than 60). The much smaller differences seen at 10 N, although not significant, still showed a tendency towards increasing values with later BION® treatment for both

Fig. 3 Influence of timing of BION® treatment on seed set (second field experiment). Total numbers of earlets produced by the seven plants of a single pot are presented and data from all nitrogen conditions are combined ($n = 24$ pots for each treatment). Boxes indicate the first and third quartile, whiskers represent the 5% and 95% percentile, and medians are indicated by lines within boxes.

shoot biomass and the number of earlets. Apart from data for earlets under 10 N (where values for treatment B were exceptionally high), treatments A and B always gave lower values than treatments C and D (Table 4).

Following the Kruskal–Wallis tests, pairwise comparisons of single treatments were conducted within single nitrogen conditions to localize the sources of significant treatment effects (Table 5). This confirmed that significant effects at 0 N conditions (Table 4) resulted mainly from significant differences between BION®-treatments A and B on the one hand and treatment C and D on the other (Table 5). For example, for the fitness parameters (last three columns in Table 5) only differences between A and B and between C and D were not significant. A similar pattern could be detected at 5 N, but only one fitness comparison proved to be significant at 10 N. For the vegetative parameters, significant differences were generally only observed between treatments A and D, although treatments B and D also showed some differences at 0 N. Treatments A and B never differed significantly, nor did treatments C and D.

Discussion

In all three experiments, chemical induction of pathogen resistance under pathogen free conditions had negative effects on plant growth and/or on seed production (see Figs 1–4), despite the differences in growing conditions and fertilizer treatments. Induction of systemic resistance by benzothiadiazoles has previously been described for several plant species, in most cases without phenotypic alterations (e.g. see Lawton *et al.* 1996; Molina *et al.* 1998 for

Table 4 Medians and treatment effects in Experiment 3. Sample sizes for biomass parameters are five pots and those for fitness parameters are eight pots (containing seven plants each) per median. Kruskal–Wallis tests on treatment effects were conducted for all variables and separately for each N treatment. See Materials and methods for the timing of BION® application (A–C; D = untreated control). dw, dry weight; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS = not significant

BION®-treatment:	0 N treatment					5 N treatment					10 N treatment				
	A	B	C	D	P	A	B	C	D	P	A	B	C	D	P
Shoot number	7.5	9.0	14.0	13.0	*	7.0	9.0	9.0	8.0	NS	7.0	7.0	7.0	11.0	NS
Shoot biomass (g dw)	12.9	14.8	42.8	53.1	*	18.9	18.0	40.9	24.3	NS	21.8	29.9	32.2	33.9	NS
Mean dw per shoot (g)	1.8	2.1	6.1	7.6	*	2.7	2.6	5.9	3.5	NS	3.1	4.3	4.6	4.8	NS
Ears per pot	7.0	7.0	10.5	11.5	***	7.0	7.0	11.0	9.5	**	7.0	7.0	8.0	8.0	NS
Earlets per pot	59.5	54.0	124.5	148.0	***	71.5	74.5	147.5	111.5	*	77.0	95.0	94.0	96.0	NS
Earlets per ear	8.5	7.7	11.9	12.7	**	10.2	10.6	14.2	12.4	NS	11.0	13.6	13.4	12.2	NS

Arabidopsis, Friedrich *et al.* 1996 for tobacco, Siegrist *et al.* 1997 for *Phaseolus vulgaris*, Dann *et al.* 1998 for soybean, Jensen *et al.* 1998 for *Brassica* and Smith-Becker *et al.* 1998 for melons). Only a recent report by Godard *et al.* (1999) has described a dosage-dependent growth reduction in benzothiadiazole-induced cauliflower. However, none of these studies was designed to detect costs of pathogen resistance. In our experiments, wheat plants were protected 'passively' against fungal infections by 'traditional' fungicides, and the costs of induction of pathogen defence were therefore incurred without conferring any benefit to the plants in the form of protection against infection. The differences between treated and control plants in our experiments may therefore reflect allocation costs resulting from the induction of SAR.

Several studies hinted at the costs of pathogen resistance, as it had been already proposed by Smedegaard-Petersen & Tolstrup (1985). *Arabidopsis* plants that are transformed to express SAR constitutively have been reported to be characterized by a 'stunted phenotype', suggesting a reason why SAR is not normally constitutive (Bowling *et al.* 1994). Hoffland *et al.* (1996) compared 15 radish cultivars differing in their resistance to fungal infections and reported a negative correlation between growth rate and resistance, both of which could be explained to a high degree by particular changes in chemical composition. Stadnik & Buchenauer (1999) reported that protecting wheat by traditional fungicides results in higher yield than when using BION®, and interpreted this as a hint that "BTH might consume energy limiting plant growth and grain yield". Treating *Vicia faba* var. 'hang down' with BION® also tended to inhibit plant growth (C. Kolb and E. Pfündel, personal communication). Moreover, trade-offs in chemically induced defence against herbivores and pathogens have been reported recently (Felton *et al.* 1999; Thaler 1999; Thaler *et al.* 1999; see reviews by Bostock 1999; Maleck & Dietrich 1999; Pieterse & van Loon 1999). Although further efforts are required to understand the molecular mechanisms and ecological reasons for the 'crosstalk' between these different forms of induced resistance (Hatcher & Paul 2000), the described trade-offs might be simply a consequence of resource limitations preventing both signalling pathways from reaching maximum levels of induction when initiated simultaneously.

Absolute growth rates differed between the replicates of Experiment 1 (Fig. 1), most probably due to differing temperatures of the external air that was blown through the nutrient solution. The negative effect of BION® on growth was however clearly seen on both occasions. In all experiments, the negative effect of BION® on biomass and on fitness parameters could be mainly explained by a reduced number of shoots per plant (as in the example

Table 5 Results of pairwise comparisons between single-application BION® treatments on different dates. Tests were based on Mann–Whitney tests, with *P*-values being corrected according to the Bonferroni method because six comparisons had to be conducted for four data sets within each N treatment. Dates of BION® treatment were 30 April (treatment A), 27 May (treatment B), and 26 June (treatment C); treatment D represent untreated controls. See Table 4 for sample numbers (*n*). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS = not significant

Treatment pair	No. of shoots	Total weight	Weight per shoot	No. of ears	No. of earlets	No. earlets per ear
0 N treatment						
A↔B	NS	NS	NS	NS	NS	NS
A↔C	NS	NS	NS	**	*	**
A↔D	*	*	*	***	***	***
B↔C	NS	NS	NS	**	**	*
B↔D	NS	*	*	***	***	**
C↔D	NS	NS	NS	NS	NS	NS
5 N treatment						
A↔B	NS	NS	NS	NS	NS	NS
A↔C	NS	NS	NS	*	*	*
A↔D	NS	*	*	**	***	NS
B↔C	NS	NS	NS	*	*	NS
B↔D	NS	NS	NS	**	**	NS
C↔D	NS	NS	NS	NS	NS	NS
10 N treatment						
A↔B	NS	NS	NS	NS	NS	NS
A↔C	NS	NS	NS	NS	NS	NS
A↔D	*	*	*	NS	NS	NS
B↔C	NS	NS	NS	NS	NS	NS
B↔D	NS	NS	NS	*	NS	NS
C↔D	NS	NS	NS	NS	NS	NS

shown in Fig. 4). The number of ears per plant was affected much more than the number of earlets per

Fig. 4 Reduction in biomass and lateral shoot production after treatment with BION® in experiment 3. The four pots shown here were grown in the field under the 0 N treatment. A and B treatments were applied during the period of lateral shoot production; treatment C was applied after lateral shoot production was complete; treatment D is the untreated control. See Materials and methods for further details.

ear (Tables 3 & 4). Consequently, there was less variation in the total numbers of earlets in those treatments that resulted in a severe suppression of lateral shoot production (Fig. 3, compare treatments A and B to C and D) because most of these plants had developed no lateral shoots at all and thus possessed only a single ear.

The morphogenetic effect on lateral shoot production could have been caused directly by BION® rather than by the induction of SAR. However, any such effects would probably occur regardless of nutrient treatment, and although we did not test directly for the effects of nutrients on the detrimental influence of BION®, Experiments 1 and 3 indicated that growth reduction was more severe when plants suffered from a shortage of nitrogen (Tables 4 & 5). Wheat plants only produce lateral shoots during a short period, and all BION® treatments except C in Experiment 3 were applied during this period. While treatments A and B differed significantly from untreated controls (D), no difference could be detected between treatments C and D (see Table 5 and Figs 3 & 4). This dependence both on the plants' developmental stage and on nutrient conditions provides further support for the interpretation that allocation costs are associated with induced pathogen resistance.

In most species, induction causes pathogenesis-related (PR) proteins to be produced in much less than 1 week (Ryals *et al.* 1994; Schneider & Ullrich 1994; Lawton *et al.* 1996; Schneider *et al.* 1996). The content of β-1,3-glucanase and chitinase in TMV-

infected tobacco increased within three days to about 3% of the soluble protein fraction (Vögeli-Lange *et al.* 1988), while up to 10% of the soluble protein fraction in cultured tobacco leaf tissue was found to consist of a single PR-protein (Felix & Meins 1985; see van Loon 1997 for the function of PR-proteins in SAR). The induction of BION®-induced pathogen defence in wheat is characterized by a rapid induction of at least five different proteins (Görlach *et al.* 1996).

During this initial period, the production of SAR-related proteins may compete with that of proteins which are needed for growth. The 'growth-differentiation balance' hypothesis (Herms & Mattson 1992; see also Bazzaz *et al.* 1987) assumes a metabolic competition between processes involved in plant growth and processes necessary for aspects of differentiation, such as the synthesis of defence chemicals. To date, only a single study has reported that PR-protein induction depends on leaf developmental stage (Herbers *et al.* 1996), and these data clearly fit the predictions of this hypothesis (see discussion in Heil 1999), as do those of our study. The data of Godard *et al.* (1999) also can be interpreted in the context of competition between growth and induction of resistance. Defence by means of production of specific proteins may be cheap compared to mechanisms such as increased amounts of cell wall material (Niemann *et al.* 1992; Hoffland *et al.* 1996), but its costs can increase dramatically when plant growth is limited by nitrogen supply.

Fitness costs are difficult to quantify directly (Chapin 1989), and few of those for defensive traits have been quantified (but see Gershenzon 1994; Sagers & Coley 1995; Heil *et al.* 1997; Baldwin 1998; Moore 1998; Agrawal *et al.* 1999). Most of those relating to pathogen resistance have focused on constitutive defence (Burdon & Müller 1987; Simms & Triplett 1994; Simms & Vision 1995; Hoffland *et al.* 1996; Gianoli & Niemeyer 1997), and many failed to demonstrate relevant fitness costs (but see Hoffland *et al.* 1996).

Conclusion

It has yet to be confirmed that the reduction in growth and seed set after chemical induction of pathogen defence occur as a consequence of the associated allocation costs. The biochemical mechanisms that lead to such effects also remain unclear. However, our data suggest that high allocation costs do result – at least over a short time span – from the induction of pathogen defence. These costs are most probably caused by a competition between *de novo* production of compounds leading to pathogen resistance and those proteins that are involved in growth related processes. The influence of these allocation costs on plant fitness seemed to depend strongly on nitrogen availability and on the time of induction of defence proteins. Further studies are needed to define the limited set of conditions under which allocation costs that result from the production of defence-related substances lead to reduced seed set and thus to fitness costs.

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