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Benzothiadiazole (BTH)-induced resistance against *Botrytis cinerea* is inversely correlated with vegetative and generative growth in bean and cucumber, but not in tomato

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Abstract Greenhouse-cultivated tomato, bean and cucumber suffer great economic losses due to grey mould, caused by *Botrytis cinerea.* Benzothiadiazole (BTH) is a chemical analogue of salicylic acid that induces resistance in a variety of plants by activating the systemic acquired resistance (SAR) pathway. Here, we investigated the effects of foliar applications of different concentrations of BTH on resistance to *B. cinerea* in these plants and on plant vegetative and generative growth. Leaf treatments with 1 up to 50 mg/l BTH resulted in increased protection of tomato against *B. cinerea.* However, on bean and cucumber, only concentrations of 250 mg/l and higher, strongly reduced susceptibility against *B. cinerea*. Moreover, BTH concentrations above 100 mg/l had a significant negative effect on plant height, flower and fruit numbers in bean and cucumber plants under pathogen-free conditions, whereas in tomato only the highest BTH dose (1000 mg/l) resulted in a significant negative effect on vegetative and generative growth. We hypothesize that the protective effects observed on bean and cucumber plants treated with higher levels of BTH are due to a general stress response, which is distinctly different from the BTH-induced resistance observed in tomato at lower concentrations of the compound without negative effects on plant growth.

Keywords Acibenzolar-S-methyl · Actigard · Bion · Grey mould · Phytotoxicity · Induced resistance · SAR · *Solanum lycopersicum* · *Phaseolus vulgaris* · *Cucumis sativus*

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The ascomycete *Botrytis cinerea* has been recently nominated as the most scientifically/economically important necrotrophic fungal plant pathogen (Dean et al. 2012). The pathogen causes grey mould on flowers, leaves, fruits and stems of a wide range of important crops and vegetables. Tomato, bean and cucumber are amongst economically important vegetables that are highly susceptible to the pathogen.

The plant activator benzo(1,2,3)-thiadiazole- 7-carbothioic acid S-methyl ester (BTH, also called acibenzolar-S-methyl) is a functional analogue of salicylic acid that induces resistance against a broad spectrum of phytopathogens by activating the systemic acquired resistance (SAR) pathway (Tally et al. 1999; Vallad and Goodman 2004). Although resistance to necrotrophic pathogens is usually controlled by the plant hormones jasmonic acid and ethylene (Thaler et al., 2012), BTH has repeatedly been shown to be effective against *B. cinerea* in various plants including tomato, Arabidopsis, grape and grapevine (Achuo et al. 2004; Zimmerli et al. 2001; Iriti et al., 2004, 2005). Interestingly, the effect of BTH treatment on plant defense activation strongly depends on the concentration used. At low concentrations, BTH does not induce defense responses directly, but rather sensitizes plants for a faster and/or stronger response to successive pathogen invasion, a phenomenon commonly referred to as priming (Conrath, 2011). High concentrations of BTH on the other hand, often result in direct activation of defenses and sometimes adverse effects on plant growth. Such growth defects triggered by high BTH doses are partly explained by the energy cost associated with inducing plant defense responses (Heil et al. 2000; Walters and Heil, 2007), but also appear to depend on host genetics, since some plants are much more sensitive to BTH-induced toxicity than others (Louws et al. 2001; Walters and Bingham, 2007). In addition, abiotic conditions have an influence on both disease resistance and adverse growth effects triggered by BTH (Dietrich et al. 2005).

In this study, we investigated whether foliar applications of different concentrations of BTH can induce resistance to *B. cinerea* in tomato (cv. Moneymaker), bean (cv. Prelude) and cucumber (cv. Jessica); while simultaneously monitoring any potential negative effect of BTH on vegetative and generative growth of these vegetables. Plants were sprayed until run-off with an aqueous solution containing different concentrations of BTH (from 10 up to 1000 mg active ingredient/l in bean and cucumber and from 1 up to 1000 mg/active ingredient in tomato), 4 days before inoculation. Infection trials were carried out with 6-week-old tomato, 2-week-old bean and 4-week-old cucumber plants grown in potting soil under greenhouse conditions (22±2 °C with 16/8 h light/dark photoperiod and 70 % humidity). Tertiary leaves of tomato, primary leaves of bean and secondary leaves of cucumber plants were used for inoculations with *B. cinerea* isolate *R16*. Inoculation suspensions were prepared according to Asselbergh et al. (2007) and the conidial concentration was adjusted to 10^5 spores ml^{-1} in 0.01 M glucose and 6.7 mM KH_2PO_4 for bean and cucumber inoculations, and 0.02 M glucose and 13.4 mM KH_2PO_4 for tomato. Following Audenaert et al. (2002), all plants were inoculated by carefully applying several 10 µL droplets of conidial suspension on the adaxial surface of the detached leaves. Disease development was evaluated 3 days post inoculation (dpi) in tomato and 4 dpi in bean and cucumber by scoring the lesions beneath the droplets in four categories (0, non-spreading lesion; 1, slightly spreading lesion; 2, moderately spreading lesion; and 3, severely spreading lesion) and calculating a disease index as described previously (Curvers et al., 2010).

In tomato, all lower levels of BTH (below 100 mg/l) were found to be effective in inducing statistically significant levels of resistance (from 13 % for 1 mg/l up to 29 % for 50 mg/l) against the pathogen (Fig. 1 a). This result is also consistent with the finding that low levels of BTH induced resistance against *B. cinerea* in tomato by means of foliar spray (Achuo et al., 2004, Malolepsza 2006) and soil treatment (Audenaert et al. 2002).

In bean and cucumber, dose-dependent disease suppression was only observed at BTH concentrations above 250 mg/l, with 90 % and 43 % protection at 1000 mg/l, for bean and cucumber respectively. Lower concentrations (10 and 100 mg/l) were not able to significantly protect bean and cucumber plants against grey mould (Fig. 1b, c; Supplemental Fig. 1B, C). However, previous work of Bigirimana and Höfte (2002) showed already increased resistance in bean against *Colletotrichum lindemuthianum* by very low concentrations (0.1–100 µM or 0.021–21 mg/l) of BTH. Likewise, Iriti and Faoro (2003) demonstrated that a single 300 µM BTH spray 7 days before inoculation was sufficient to fully control bean rust disease caused by the fungus

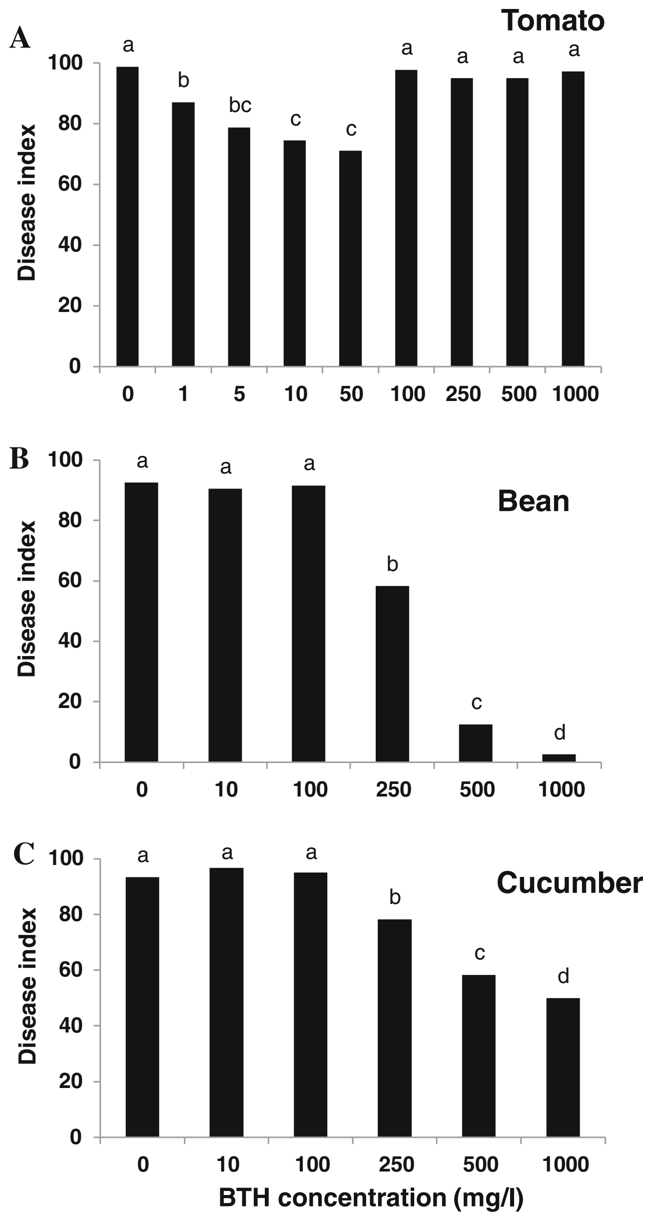

Fig. 1 Effect of foliar application of BTH at several concentrations to control *B. cinerea* in tomato (**a**) bean (**b**) and cucumber (**c**) leaves. Twelve leaves from 12 different plants were used per treatment. The experiments were repeated at least twice. Disease index was calculated based on four scoring categories (*0* non-spreading lesion; *1* slightly spreading lesion; *2* moderately spreading lesion; and *3* severely spreading lesion) and data were analyzed using non-parametric statistics (Kruskal-Wallis test). Differences between treatments were detected based on Mann–Whitney comparisons. *Bars* with a common letter do not differ significantly at $P=0.05$

Uromyces appendiculatus. Concentrations in the same range (1–300 μM) induced defense genes and disease resistance in other plants such as rice, tobacco, wheat and Arabidopsis (Bovie et al., 2004; De Vleesschauwer et al. 2008; Friedrich et al. 1996; Gorlach et al. 1996; Lawton et al. 1996).

Several concentrations of BTH (0, 10, 100, 250, 500 and 1000 mg/l) were sprayed on tomato, bean and cucumber plants under pathogen-free conditions to evaluate possible phytotoxic effects. In tomato no evidence of phytotoxic symptoms was observed until BTH doses exceeded 500 mg/l, which is in agreement with previous reports (Tally et al. 1999; Louws et al. 2001; Abo-Elyousr and El-Hendawy 2008). A low level of leaf stunting and a slight leaf scorching could be observed in tomato plants treated with higher concentrations (500 and 1000 mg/l) (Supplemental Fig. 1a). In bean and cucumber, however, significant differences in plant height were detected at concentrations of 100 mg/l BTH. Moreover, at 250 mg/l or above severe phytotoxic damage was observed in both plants (Fig. 2b, c). Most notably, high concentrations of BTH caused disruption of plant growth, leaf stunting, deformation and blockage of apical bud formation and symptoms appeared within days following treatment application (Supplemental Fig. 1B, C). We also assessed possible negative effects of BTH application on generative growth of tomato, bean and cucumber by counting the accumulative number of flowers and fruits in a time-course experiment. In case of tomato, only 1000 mg/l BTH significantly reduced flower and fruit numbers (Fig. 3a). In bean and cucumber, though, significant negative impacts on plant reproduction were observed at BTH concentrations of 100 mg/l and higher, with reductions in flower and fruit numbers and serious fruit deformations (Fig. 3b, c). These findings confirm previous

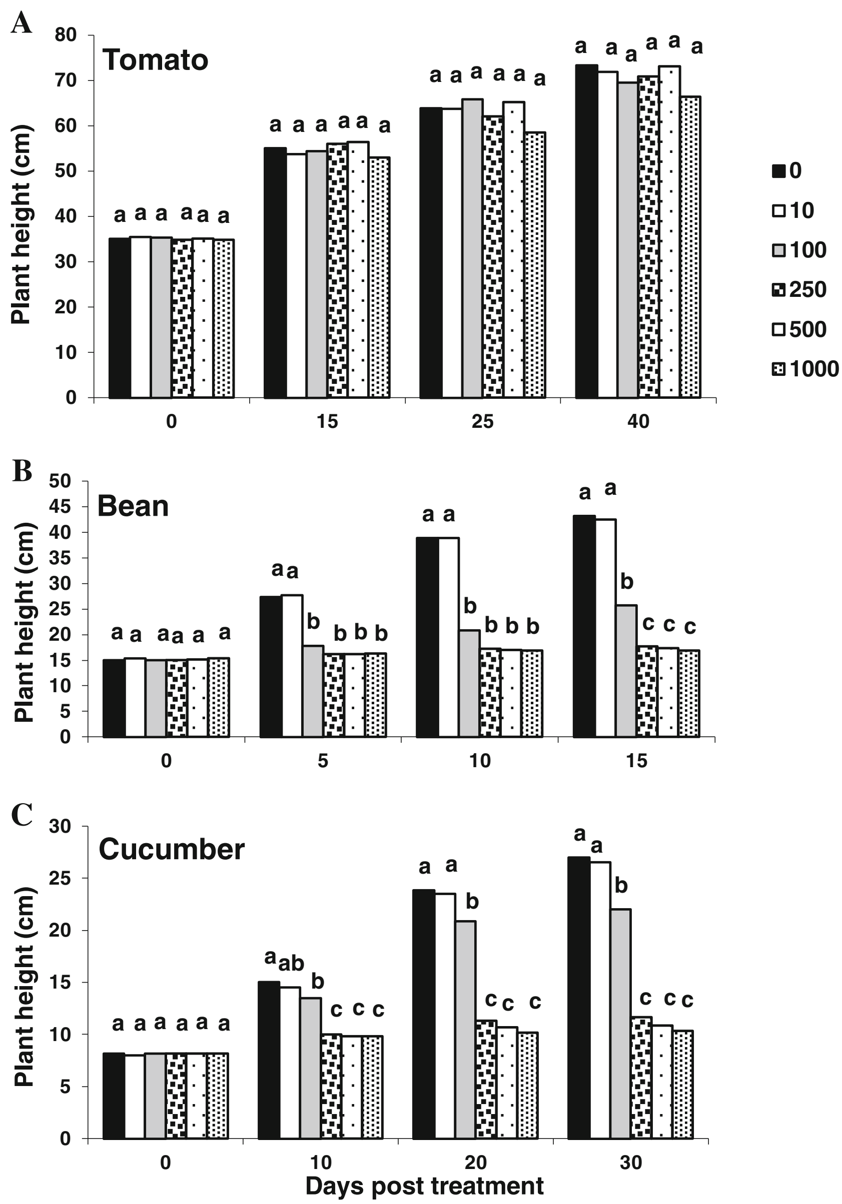

Fig. 2 Effect of different concentrations of BTH (mg/l) on plant height in tomato (**a**), bean (**b**) and cucumber (**c**). Each experiment was repeated at least twice with six plants per treatment and results of a representative experiment are shown. Data were analysed by ANOVA. Within each crop, different letters indicate significant differences among the treatments ($P<0.05$)

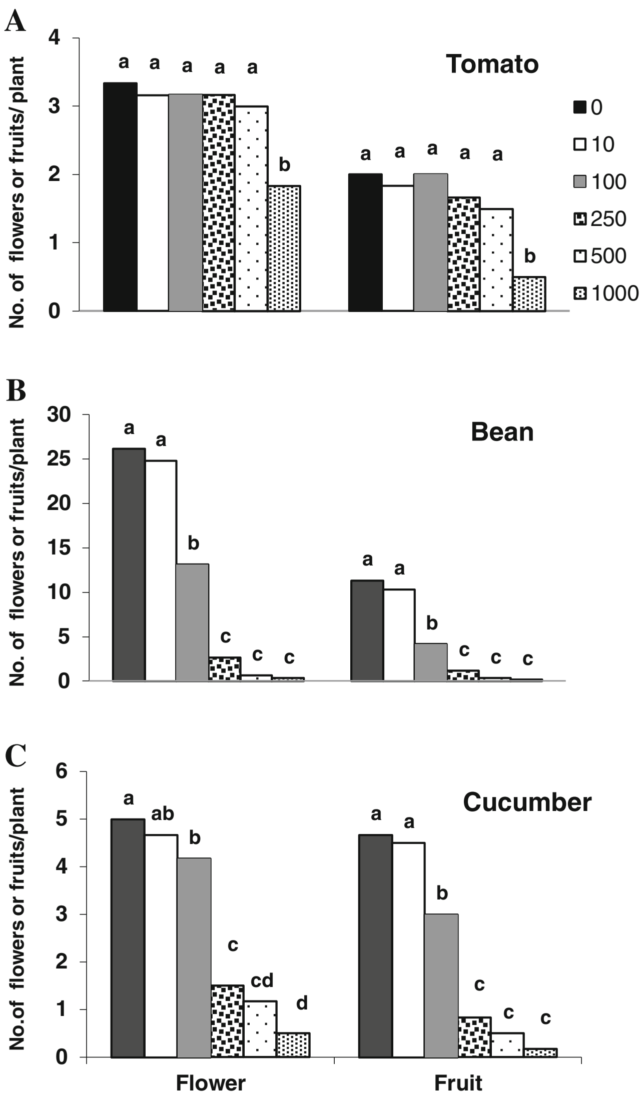

Fig. 3 Effect of different concentrations of BTH (mg/l) on plant flower and fruit numbers in tomato (**a**), bean (**b**) and cucumber (**c**). Each experiment was repeated at least twice with six plants per treatment and results of a representative experiment are shown. Data were analysed by ANOVA. Within each crop, different letters indicate significant differences among the treatments ($P<0.05$)

study on bean and cucumber in which BTH concentrations in the same range led to phytotoxicity symptoms and adverse effects on growth and yield (Iriti and Faoro, 2003; Bigirimana and Höfte 2002; Bovie et al. 2004; Bokshi et al. 2008; Wurms et al. 1999).

For both cucumber and bean, disease resistance was dose-dependent and negatively correlated with plant height and number of flowers and fruits, while there was no such correlation for tomato (Table 1). In tobacco, a positive correlation between reductions of *Tomato spotted wilt virus* by BTH and phytotoxicity has also been noted (Mandal et al. 2008).

We hypothesize that the observed disease resistance in bean and cucumber triggered by high doses of BTH is not primarily

Table 1 Pearson correlation between disease resistance and plant height, flower and fruit number in bean, cucumber and tomato. Correlations were calculated based on the data presented in Figs. 1–3.

	Disease resistance		
	Bean	Cucumber	Tomato
Plant height	−0.781	−0.890[a]	0.209
Flower number	−0.854[a]	−0.933[b]	0.189
Fruit number	−0.810	−0.888[a]	0.185

[a] Significant at $P=0.05$ level (two-tailed)

[b] Significant at $P=0.01$ level (two-tailed)

due to a bona fide SAR response, since this response is already activated at much lower concentrations (Bigirimana and Höfte 2002; Bovie et al. 2004). Rather, by directly activating defences throughout the plant and, hence, re-allocating cellular resources away from growth and development, such high BTH concentrations may trigger some sort of metabolic stress, the response to which could result in enhanced resistance to *B. cinerea*. Implicit here is the view that BTH-inducible SAR is not effective against *B. cinerea* in the bean and cucumber cultivars used in this study, as shown before for tobacco (Achuo et al. 2004). This is in clear contrast with tomato, a plant in which low doses of BTH decrease susceptibility to *B. cinerea* without adverse effects on growth and development. In keeping with our findings, there is ample evidence demonstrating the variable outcomes of BTH application on plant growth and disease resistance with studies demonstrating successful SAR induction (Bokshi et al. 2003; Cole 1999; Godard et al. 1999; Hukkanen et al. 2007), studies that found low or insignificant effects of BTH-treatment on plant resistance or yield (Louws et al. 2001; Romero et al. 2001; Stadnik and Buchenauer 1999), or reporting that effects of BTH application depend on the plant genotype used (Dann et al. 1998; Heil and Ploss 2006), on the pathogen type used (Córdova-Campos et al. 2012) and on the prior infection stage of the plant (Walters et al. 2011). It should be noted, however, that phytotoxic and SAR effects of BTH can be decoupled as Arabidopsis *nim1/npr1* mutants unable to mount SAR still showed phytotoxicity when treated with high levels of BTH (discussed in Louws et al. 2001). Further dissection of the molecular mechanisms underpinning the costs and trade-offs associated with BTH-induced plant resistance will be fundamental in harnessing the full potential of chemical plant activators for effective disease control in various agricultural contexts.

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