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Benzoic acid induces tolerance to biotic stress caused by *Phytophthora cinnamomi* in *Banksia attenuata*

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Abstract

Banksia attenuata plants were treated with soil drenches or foliar sprays of benzoic acid (BZA) to determine induced resistance to *Phytophthora cinnamomi*. Stems of *B. attenuata* were inoculated with the pathogen 1 week after treatment with BZA. Resistance was estimated by measuring *P. cinnamomi* lesions on stems. Treatment with 0.10 mM, 0.25 mM or 0.50 mM BZA caused a reduction in lesion size with 0.50 mM BZA applied as a soil drench being the most effective treatment at suppressing the development of lesions. This is the first report of BZA induced host resistance in any plant species to any pathogen.

Abbreviations: BZA – benzoic acid; SA – salicylic acid

Introduction

Phytophthora cinnamomi occurs worldwide and has a host range in excess of 1000 plant species (Zentmeyer 1980). The soil-borne plant pathogen causes severe losses in species richness and foliage cover in susceptible forest, heathland and coastal plant communities of Western Australia (Weste and Marks 1987; Wills and Keighery 1994). The use of induced host resistance by certain natural and synthetic chemical compounds holds great potential for the control of *P. cinnamomi*. Certain salicylates including salicylic acid (SA) and its derivative acetyl salicylic acid (aspirin) have been shown to stimulate natural defence responses in host plants including the induction of resistance against a variety of plant pathogens (Kessmann et al. 1994; Durner et al. 1997). BZA, known to provide abiotic stress tolerance similar to that reported for SA (Senaratna et al. 2003), was investigated as an inducer of resistance in *Banksia attenuata* to *P. cinnamomi*, as a foliar spray or soil drench.

Material and methods

A randomised complete block design was employed with three replications. Plants were blocked according to size.

Six- to ten-month-old *B. attenuata* plants were treated with BZA (Sigma®) at concentrations of 0.00 mM (control), 0.10 mM, 0.25 mM or 0.50 mM. Foliar sprays of BZA were applied to plants using a hand-held sprayer. For spray treatment, each plant received enough BZA (~3 mL) to ensure total leaf surface coverage. Soil was drenched with ~75 mL of BZA per 375 mL soil volume (Senaratna et al. 2000).

One week after treatment with BZA, a small incision was made midway up the stem of each plant. One square (~2 mm^2) of Miracloth® (Calbiochem®), a thin gauze-like material which had been colonised with *P. cinnamomi*, was inserted into the incision. As a control, Miracloth® without any pathogen was inserted into plants that did not receive treatment with BZA. Lesions were observed and measured every 2–3 days for 45 days. Disease progression curves were constructed as lesions developed. Lesion lengths recorded on stems 1, 15, 30 and 45 days after symptoms were first observed were transformed ($\log_{10}$), prior to statistical analysis by ANOVA.

Results and discussion

Negative control plants (inoculated with Miracloth® without *P. cinnamomi*, 0.00 mM BZA) did not develop lesions on their stems. The majority of susceptible plants inoculated with *P. cinnamomi* developed characteristic lesions within 5 days. All positive control plants (inoculated with Miracloth® with *P. cinnamomi*, 0.00 mM BZA) developed severe lesions. At the conclusion of the experiment, several BZA treated plants did not develop lesions on their stems.

Lesions (Figure 1) generally varied in colour from pink to brown. Tangential spread of lesions (all plants) ranged from 90° to 360°. As lesions developed, they girdled stems or were confined tangentially with varying levels of extension above and below the stem wound.

Construction of disease progression curves revealed a large amount of variability in the rate and severity of lesion development between individual plants of a treatment. Dixon et al. (1984) also reported a wide range in lesion sizes observed on stems of 10 different species of field grown *Banksias* inoculated with *P. cinnamomi*. Overall, stem lesions were smaller: (i) on plants subjected to soil drenching; and (ii) at concentrations of 0.50 mM. Generally, all BZA foliar spray and soil drench pre-treatments caused a reduction in the size of lesions compared to control plants.

Lesions were largest on stems of plants not treated with BZA (mean 9.33 cm at day 45). A significant ($p<0.05$) concentration effect of the compound on lesion size was observed on day 45. In soil drench

Figure 1. Typical stem lesion caused by *Phytophthora cinnamomi* on *Banksia attenuata*, 8 weeks after inoculation. Arrow indicates point of inoculation.

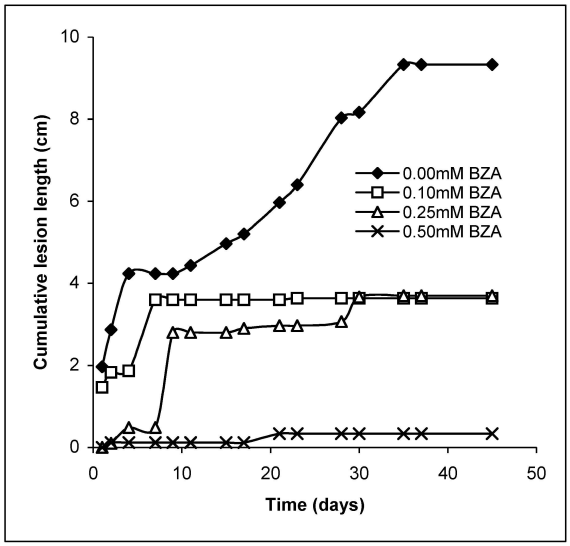

Figure 2. Disease progression curves: Lesion development on stems of *Banksia attenuata* following soil drench treatment with benzoic acid (BZA) and stem inoculation with *Phytophthora cinnamomi*. Lesions were measured for 45 days from when symptoms first became visible (day 0).

treatments, lesions became progressively smaller as the concentration of BZA applied increased. Lesions were smallest on stems of plants pre-treated with 0.50 mM BZA as a soil drench with an average lesion length of 0.33 cm at day 45 (Figure 2).

For plants pre-treated with foliar sprays of BZA, 0.50 mM was not as effective as concentrations of 0.10 mM or 0.25 mM BZA (disease progression curves not shown). This phenomenon is difficult to explain at this time. Recently, Senaratna et al. (2003) demonstrated that higher dosage of BZA (0.50 mM) was less effective than 0.10 mM and 0.25 mM in inducing tolerance to biotic stresses such as heat, chilling and drought. Rao et al. (1997) reported that high dosage of SA is detrimental to plant tissues and that overproduction of hydrogen peroxide (H_2O_2) is the major cause for the injury. A discrepancy in absorption and translocation of BZA between the two application methods may have caused the differences in effective dosage observed in the present study. Further investigation is warranted to ascertain the exact reason for these observations.

Since BZA is the common group of several SA derivatives associated with systemic acquired resistance, it may in fact be the active molecule that triggers defence mechanisms in the host and subsequently confers resistance. BZA may induce SAR to plant pathogens other than *P. cinnamomi*.

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