Effects of Benzothiadiazole on Induction of Resistance in Cucumber to Infection by *Cladosporium cucumerinum*

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Abstract: Tests of induction of resistance by benzothiadiazole (BTH) against scab disease, caused by *Cladosporium cucumerinum*, were conducted on etiolated cucumber (*Cucumis sativus* L.) seedlings. The results showed that 0.05-0.7mmol/L BTH could induce resistance of seedlings to the disease, with the concentration of 0.5mmol/L BTH being the best. The disease index decreased from 90.58 (Control) to 28.43, while the disease incidence decreased from 100% (Control) to 58.82%. However, BTH has no direct inhibition effect on the spore germination and mycelial growth at concentrations from 0.05 to 0.7mmol/L.

Introduction: Cucumber scab, caused by *Cladosporium cucumerinum*, is a worldwide disease on cucumber (*Cucumis sativus* L.), especially in greenhouse cucumber plants. Currently, the disease is controlled mainly by fungicide applications. As the problems of residues and pollution have been becoming increasingly serious, alternative protection methods are essential.

Systemic acquired resistance （SAR） can be induced in plants by abiotic or biotic elicitor(s) (4,6-7,9-10). Among the abiotic compounds, salicylic acid (SA) was found to induce systemic resistance to fungal, bacterial, and viral pathogens. Benzothiadiazole (BTH), a mimic of SA, is capable of inducing SAR. In 1996, BTH was introduced in Germany and is now available as a commercial product BION®. Resistance inducing effects of this product have been demonstrated in plants against different crop diseases (1-3), but for the cucumber plants against *C. cucumerinum*, the reports are rare.

Methods: The pathogen, *C. cucumerinum*, was obtained from our Plant Pathology Laboratory. The cultures were maintained on potato-dextrose agar (PDA) medium at 4°C; and fresh cultures were grown for 10 days on PDA plates at 22°C before experimentation.

The cucumber (*Cucumis sativus* L. cv. Jingyan 4) seeds examined in this study were purchased from a local seed company. The culturing method of etiolated cucumber seedlings followed Li (5). The benzothiadiazole (BTH, BION®) was obtained from Novartis Agro-Chemistry Co., Ltd.

The concentrations of BTH used in the experiment were 0.05， 0.1， 0.3， 0.5 and 0.7 mmol/L diluted in water. Etiolated cucumber seedlings of five days were sprayed with different concentrations of BTH. Three days later, the etiolated cucumber seedlings were inoculated with the pathogen by spraying with conidial suspensions of *C. cucumerinum*. Spore suspensions were prepared from ten-day-old PDA cultures by dislodging spores from the surface of the cultures with a sterile bacteriological loop in sterile distilled water. The concentration was adjusted to 2×10^5 spores mL^{-1}. Control plants were treated by spraying tap water. There were at least 30 etiolated seedling in each treatment, and three replicates

per treatment. The plants were maintained in a dark growth chamber at 22°C and disease incidence and index were recorded after 4 days.

The effects of BTH on spore germination of the pathogen were assessed on concave slides. The spore suspension of $1x10^6$ spores ml^{-1} was kept at 22°C for 24h with BTH concentrations of 0.05, 0.1, 0.3, 0.5, 0.7 mmol/L. Five fields of vision were observed microscopically to record germination rate.

Four-mm-diameter callus from 10-day-old cultures of *C. cucumerinum* on PDA plates were taken and placed on PDA plates, which contained different concentrations of BTH. The mycelial diameters were measured every other day. This experiment was repeated three times. Disease assessment followed Li (5).

Results: Etiolated seedlings were sprayed with different concentrations of BTH (0.05, 0.1, 0.3, 0.5, and 0.7mmol/L) 3 days before inoculation with *C. cucumerinum*. Four days after inoculation, black brown lesion appeared both on the cotyledons and the hypocotyls. Some etiolated seedlings shrank and perished. The disease developed very quickly; only one to two days were needed from lesion appearance to death for the seedlings.

Table 1 shows that BTH with concentrations varying from 0.05 to 0.7 mmol/L had different effects, with 0.3-0.7 mmol/L better than others. The incidence and index of disease on the etiolated seedlings treated with 0.5 mmol/L BTH decreased dramatically, from 100% and 90.58 (control) to 58.82% and 28.43. The effects treated with 0.05 and 0.1 mmol/L BTH were not as good as with 0.3-0.7 mmol/L BTH, but also showed significant difference compared with control. Table 2 shows that no inhibitory activity was observed on conidial germination of *C. cucumerinum* at different concentrations of BTH. Fig. 1 shows that BTH has no direct inhibition to the mycelial growth of the pathogen at the concentrations from 0.05 to 0.7 mmol/L.

Discussion: In order to reduce pollution and strive for a cleaner environment, efforts are being made to develop alternatives to pesticides for the control of plant diseases. BTH has been found to be active in inducing systemic resistance against a wide range of pathogens in a diverse group of plants(8,11). This paper investigates the potential of this chemical for inducing systemic resistance in cucumber plants against *C. cucumerinum*. The results showed that 0.05-0.7 mmol/L BTH expressed effects on induced resistance, among which the induced resistance in seedlings treated with 0.3-0.7 mmol/L BTH had better effects.

According to Li(5), spray inoculation on etiolated seedlings for resistance identification is equally accurate for comparing with that on true leaf inoculation, and has advantages of time saving and symptom distinctiveness。BTH has no direct inhibition to the spore germination and mycelial growth at concentrations from 0.05 to 0.7 mmol/L, which means that the decreases of disease index and incidence are due to the resistance induction. As for the effect of BTH on the field plants, further research is needed.

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Table 1. Effects of BTH with various concentrations upon cucumber resistance induction to scab

Treatment (mmol/L)	Disease incidence (%)	Disease index	Disease severity
Control	100a	90.58a	5.43
0.05	100a	65.63bc	3.94
0.1	87.50a	46.88cd	2.81
0.3	75.00ab	32.50d	1.95
0.5	58.82b	28.43d	1.71
0.7	77.78ab	36.11d	2.17

Note: Significant difference at P=0.05.

Table 2. Effects of BTH on the spore germination of *C. cucumerinum*

Treatment (mmol/L)	Germination rate (%)
CK	76.85a
0.05	77.88a
0.1	80.35a
0.3	72.22a
0.5	72.08a
0.7	78.57a

Note: Significant difference at P=0.05.

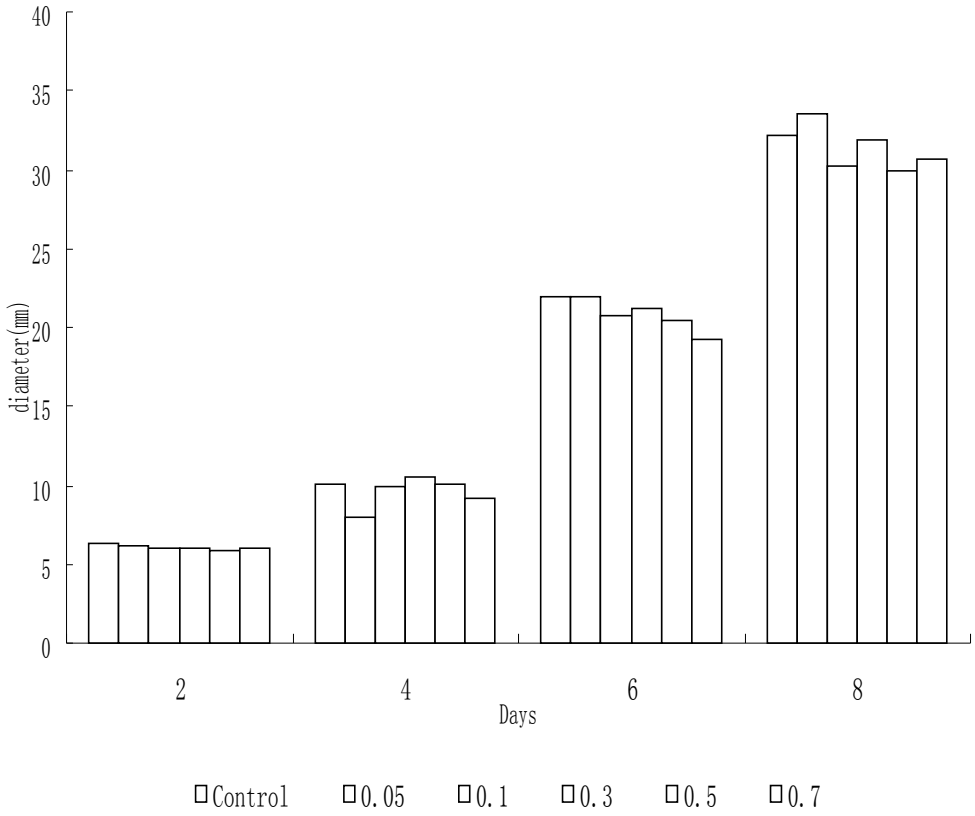

Fig.1 Effects of BTH upon the growth of *C. cucumerinum*