Study on the herbicidal activity of vulculic acid from *Nimbya alternantherae*

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Summary

The detached leaves of 28 species of plants and the plants of alligator weed were tested for toxic activity of vulculic acid isolated from *Nimbya alternantherae*. Results indicated that the toxin was non-host-specific and could cause leaf blight and withering of alligator weed. The effect of the toxin on the ultrastructure of leaf tissue of alligator weed was studied by treating the mature leaves with the toxin. It was shown that the damage on leaf tissue included plasmolysis and vacuolation in cells, lamellae disorder and vacuolation in chloroplasts as well as disappearance of ridges and vacuolation in mitochondria after treatment with vulculic acid at a concentration of 50 μg/ml for 12 h. These results suggest that the target sites for vulculic acid action may be the plasma membranes, the lamellae of chloroplast and the ridges of mitochondria of the alligator weed leaf.

Keywords: mycotoxin, alligator weed, ultrastructure, pathogenic mechanism.

Introduction

Alligator weed, *Alternanthera philoxeroides* (Martius) Grisebach, is widely known as a serious exotic weed. Some progress has been achieved in the biological control of this weed with plant pathogenic fungi and their metabolites. Pathogenic fungi reported on alligator weed include *Rhizoctonia solani* Kühn (Singh and Devi, 1991), *Colletotrichum* sp. (Tan and Gu, 1992), *Cercospora alternantherae* Ellis & Langlois (Barreto and Torres, 1999) and *Nimbya alternantherae* (Holcomb & Antonopoulus) Simmons & Alcom (Xiang *et al.*, 1998; Barreto and Torres, 1999). The latter two species are considered to have a potential as biological control agents for alligator weed (Barreto and Torres, 1999; Barreto *et al.*, 2000; Xiang *et al.*, 2002a). Moreover, Wan *et al.* (2001) found that the crude preparation of the toxin from *Alternaria alternata* (Fr.) Keissler was strongly toxic to the leaves of the alligator weed. The metabolic product of *Fusarium* sp. could also induce leaf lesions and withering of the weed (Tan *et al.*, 2002).

N. alternantherae can cause purplish leaf spots and defoliation (Barreto and Torres, 1999; Xiang *et al.*, 2002a) and is a highly host-specific pathogenic fungus (Xiang *et al.*, 2002a). Xiang *et al.* (2002b) found that the filtrate of this fungus had herbicidal activity and could inhibit radicle growth in sorghum, and Zhou *et al.* (2006) isolated vulculic acid from the filtrate. To provide a foundation for developing a biochemical herbicide, the herbicidal activity of this toxin was studied and is reported in this paper.

Materials and methods

Materials

Toxin material: Vulculic acid was isolated from the filtrate of *N. alternantherae* using the method of Zhou *et al.* (2006), which was slightly modified. *N. alternantherae* was cultured in modified Fries (Xiang, 2005) at 28°C, 200 rpm for 7 days, and the culture liquid was filtered. Methanol was added into the filtrate in the volume ratio of 1:3, stirred, then filtered and evaporated at 60°C to get a mush. The crude product was extracted with ethyl acetate from the mush, and dissolved with benzene and acetone (in volume ratio of 1:1) at 60°C and filtered. After crystallizing and re-crystallizing in an ice-bath two to three times with benzene and acetone in 1:1 ratio, crystals with a few impurities were mixed with silica gel in a mass ratio of 1:1 and put into a column of 300 × 15mm, eluting with benzene and acetone (1:1). The eluting liquid was evaporated at 60°C until 30 to 40 ml remained and then re-crystallized in an ice-bath. The toxin obtained was dried in a vacuum desiccator, and its purification was evaluated by high-performance liquid chromatography and infrared spectra (IR).

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Plant material: Alligator weed was grown in nutritional liquid that is commonly used for leafy vegetable production. The other 27 species of plants tested for toxic activity were all seedlings grown in a greenhouse or taken from the field (see Table 1).

Table 1. Sensitivity of the plants tested to vulculic acid.

Plant species	Concentration of the toxin (μg/ml)				
	50	30	10	5	CK
Red pepper (*Capsicum annuum* L.)	+	–	–	–	–
Tomato (*Lycopersicon esculentum* Mill)	+	+	+	–	–
Eggplant (*Solanum melongena* L.)	–	–	–	–	–
Spinach (*Spinacia oleracea* L.)	+	–	–	–	–
Water cress (*Nasturtium officinale* R.Br.)	–	–	–	–	–
Radish (*Raphanus sativus* L.)	–	–	–	–	–
Gynura (*Gynura bicolor* L.)	+	+	+	–	–
Yerbadetajo (*Eclipta prostrate* L.)	+	+	+	+	–
Red tasselfpower [*Emilia sonchifolia* (L.) DC.]	–	–	–	–	–
Crowndaisy oxeyedaisy (*Chrysanthemum coronarium* L.)	–	–	–	–	–
Lettuce (*Lactuca sativa* var. *crispa* L.)	–	–	–	–	–
China crabdaisy [*Wedelia trilobata* (Osb.) Merr.]	+	+	–	–	–
Japanese youngia [*Youngia japonica* (L.) DC.]	–	–	–	–	–
Celery (*Apium graveolens* L.)	+	+	+	–	–
Coriander (*Coriandrum sativum* L.)	+	+	–	–	–
Carrot (*Daucus carota* L. var. *sativus* DC.)	+	–	–	–	–
Pea (*Pisum sativum* L.)	+	–	–	–	–
Sour dallisgrass (*Paspalum conjugatum* Berg.)	+	–	–	–	–
Corn (Zea mays L.)	–	–	–	–	–
Sweet potato (*Ipomoea batatas* Lam.)	+	+	+	–	–
Dashen [*Colocasia esculenta* (L.) Schott]	+	+	–	–	–
Cucumber (*Cucumis sativus* L.)	+	+	–	–	–
Nutgrass cypressgrass (*Cyperus rutundus* L.)	+	+	+	–	–
Red woodsorrel (*Oxalis corymbosa* DC.)	–	–	–	–	–
Asia plantain (*Plantago asiatica* L.)	+	+	–	–	–
Alligator weed [*A. philoxeroides* (Martius) Grisebach]	+	+	+	+	–
Thorny amaranth (*Amaranthus spinosus* L.)	–	–	–	–	–

Note: "+" means sensitive, "-" means insensitive

Methods

Effective range of the toxin: The purified toxin was diluted to give concentrations of 50, 30, 10 and 5 μg/ml with double-distilled water. The healthy leaves of the plants were washed with tap water and then three times with double-distilled water, dried on sterile filter paper, cut into 0.5 ×0.5 cm pieces and then were placed into test tubes with 2 ml of the toxin solution. Ten plant pieces were added to each tube, and then was a duplicate for each concentration. The tubes with solution and pieces were put into an illuminated incubator at 25°C for 24 h, and pathological changes were recorded.

Effect of the toxin on alligator weed plant: The purified toxin was diluted to concentrations of 300, 200, 100 and 50 μg/ml with distilled water. Alligator weed stems that had just begun to develop roots were dug up in the field, washed in tap water and grown in nutrient solution for 3 to 4 days. Then, the plants were washed three times with distilled water, dried on sterile filter paper and transplanted into tubes containing 10 ml of the different toxin dilutions. Three plants were included in each tube, and tubes were duplicated for each concentration. Distilled water was used in control tubes. The treated plants were grown at room temperature for 24 h, and then pathological changes were recorded.

Effect of the toxin on the ultrastructure of alligator weed leaf: The purified toxin was diluted to a concentration of 50 μg/ml with double-distilled water. Healthy mature leaves of alligator weed were washed with tap water and then three times with double-distilled water, dried on sterile filter paper and cut into pieces of 2 to 3 mm transversely. The pieces were placed into test tubes with 2 ml of the toxin solution and decompressed for 20 min. Then, the tubes with solution and pieces were put into an illuminated incubator at 25°C for 12 h. Double-distilled water was used in the control tube.

The samples were fixed with 2.5% glutaraldehyde, then with 1% osmic acid. After being dehydrated using a standard method, the samples were embedded in Epon 812. Microtome sections were dyed with uranium acetate, then lead citrate and observed through FEI-Tecnai 12 transmission electron microscope.

Results

Effective range of the toxin

Vulculic acid was a non-host-specific toxin. It had toxic activity towards many plants from different families or genera, including alligator weed, after treatment for 24 h under almost all of the concentrations tested (Table 1). It caused brown blight on the leaf pieces.

Effect of the toxin on alligator weed plant

The toxin inhibited root growth and induced wilt of alligator weed plants after treatment for 24 h under all of the concentrations tested (Fig. 1A, B).

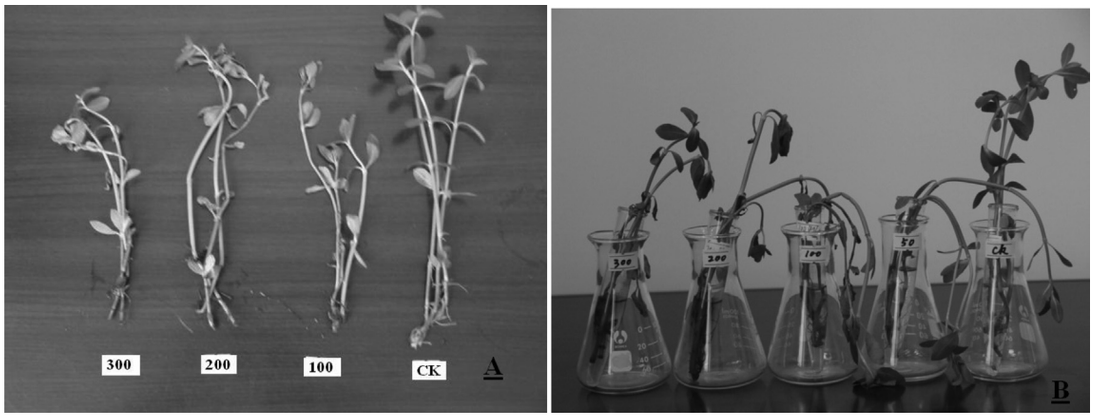

Figure 1. **A** Roots of alligator weed treated with the toxin for 24 h. **B** Plants of alligator weed treated with the toxin for 24 h.

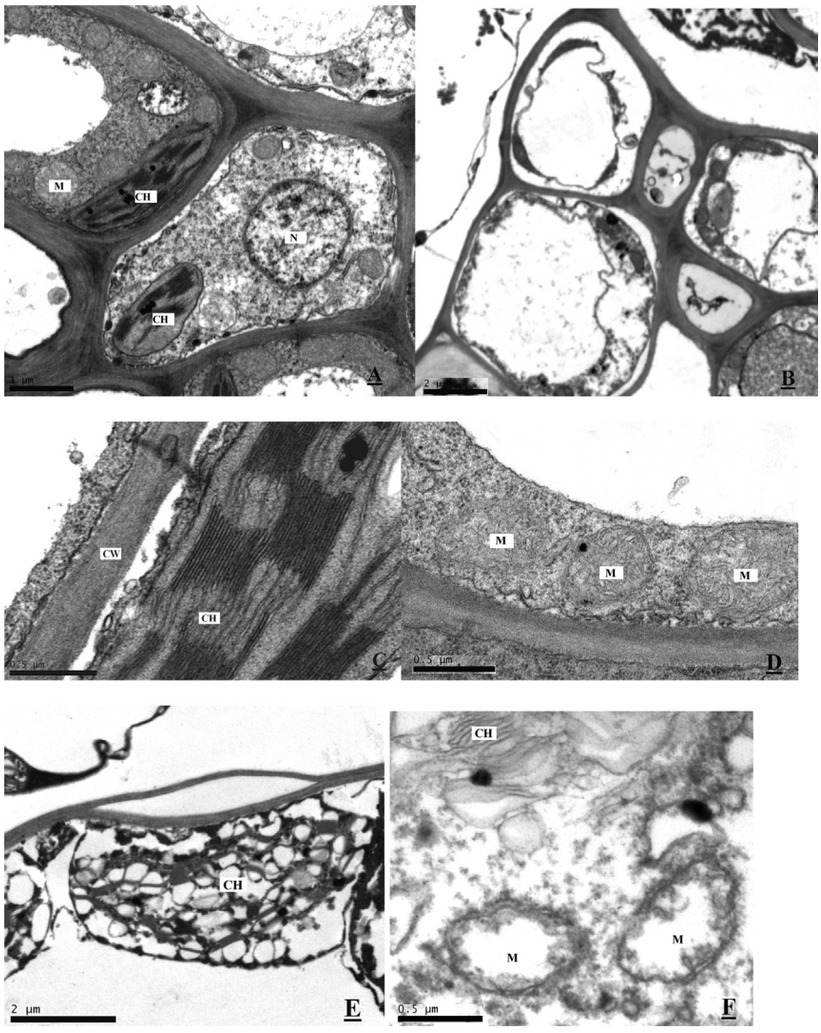

Figure 2. **A** Normal ultrastructure in the alligator weed control leaf. **B** Plasmolysis and vacuolated cells in the treated leaf with the toxin. **C** Normal lamellae of chloroplast in the control leaf. **D** Normal structures of mitochondria in the control leaf. **E** Disordered lamellae and vacuolated chloroplasts in the treated leaf with the toxin. **F** Vacuolated mitochondria and disordered lamellae of chloroplast in the treated leaf with the toxin. *CH* Chloroplast, *M* mitochondria, *N* nuclei, *CW* cell wall.

Effect of the toxin on ultrastructure of alligator weed leaf

In tissue samples after treatment with vulculic acid, at a concentration of 50μg/ml for 12h, the plasmalemma of leaf cells was invaginated and detached from the cell wall in almost all places, and the cells became vacuolated. However, the plasmalemma did not appear ruptured (see Fig. 2B). Chloroplasts were severely damaged with disorder lamellae and a lot of vacuoles in the treated tissue with the toxin (Fig. 2E). Mitochondria from leaf cells treated with the toxin showed significant disorganization, such as the decrease of ridges and the vacuolation of mitochondria (Fig. 2F).

Discussion

The toxin, vulculic acid, isolated from *N. alternantherae*, was reported to inhibit the pollen germination of black pine, *Pinus thunbergii* Parl., by up to 85.3% at a concentration of 10 mg/l (Kimura *et al.*, 1991). Before our study, its toxicity to other plants had not been reported. The preliminary screening results showed that vulculic acid is a non-host-specific toxin and could inhibit root growth and induce wilt of alligator weed. Thus, the advantage of vulculic acid as an herbicide compared to *N. alternantherae* lies in its wider host range and better prospect for product development.

In this study, vulculic acid was toxic to the plasmalemma, the lamellae of chloroplast and the ridges of mitochondria of alligator weed leaf cells after treatment at a concentration of 50 μg/ml for 12 h. These results suggest that the target sites for the toxin action may be on the plasma membranes, the lamellae of chloroplast and the ridges of mitochondria of alligator weed leaf. However, this is the first and preliminary study on the ultrastructural effect of vulculic acid on plant tissues. Further studies are required to determine which one of the three target sites is damaged first and the minimum concentration of toxin needed to cause the damage.

Acknowledgements

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