Herbicidal Properties of the Thaxtomin Group of Phytotoxins

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The thaxtomins are a group of phytotoxins generated by the bacterium *Streptomyces scabies* (the main causal organism of potato common scab). Available members of the group were assessed for herbicidal activity by a variety of standard tests. Test results indicated that thaxtomin A, the predominant member, was also the most physiologically active. Injury symptoms in most instances were similar to those caused by known cellulose biosynthetic inhibitors such as dichlobenil and isoxaben. Although test results indicated that the thaxtomins had many of the biological properties desirable in a commercial herbicide, they nevertheless lacked the systemic phytotoxicity critical to deliver reliable weed control at low rates.

Keywords: *Thaxtomin A; phytotoxins; herbicides; dichlobenil; isoxaben; Streptomyces scabies*

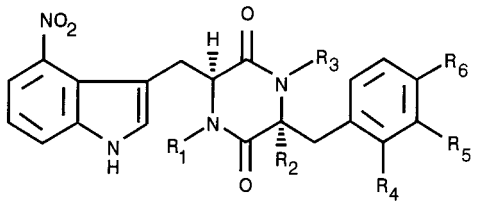
INTRODUCTION

Investigations of phytotoxins generated by the soil bacterium *Streptomyces scabies* (the main causal organism of potato common scab) (*1*) have resulted in the isolation and characterization of a series of unique 4-nitroindol-3-yl-containing 2,5-dioxopiperazines (*2*−*4*). These phytotoxins, named thaxtomins, cause plant cell necrosis at nanomolar concentrations, but their mode of action is still unknown (*5*). Thaxtomin A (**1**) is the predominant phytotoxin, but minor amounts of 10 other related compounds have also been isolated and characterized (Figure 1).To evaluate the utility of thaxtomins as leads for new commercial herbicide development, thaxtomin A and available analogues were tested for activity against a variety of indicator plant species in various assay formats. In the process it was projected that more insight into the precise mode of action of the thaxtomins might be forthcoming. Such findings could prove to be of prime importance in future endeavors at changing potato genotypes to increase their resistance to potato common scab disease.

MATERIALS AND METHODS

Chemicals. Thaxtomin A and analogues were acquired from microbial sources as described previously (*3, 4*).

***Lemna*/*Agrostis* Assays.** Approximately 25 mL of bentgrass (*Agrostis palustris*, AGRPA) seed was surface-sterilized by adding 75 mL of 50% (v/v) bleach solution and shaking for 15 min. The seeds were rinsed three times with sterilized water and added to 2 L of sterile medium [3.1 g L^{-1} Gamborg B-5 (Sigma G-5768) and 4.4 g L^{-1} Murashige and Skoog (Sigma M-8280) salts at pH 5.5]. Seeds were uniformly suspended in solution using a stir bar and magnetic stirrer, and 2 mL of the seed/medium suspension was added to each well of a 24-well tissue culture plate (i.e., four replicates) (Falcon, model 3043) using a peristalic pump. A 5000 ppm stock solution of thaxtomin A (**1**) was prepared in dimethyl sulfoxide, and subsequent solutions were prepared by making 5-fold serial dilutions of the initial 5000 ppm stock. Aliquots (10 μL) of the appropriate stock solutions were added to the 2 mL of seed/medium for a dose−response analysis (*6*). Final treatment concentrations ranged from 25 to 0.008 ppm. Untreated controls and solvent checks were also added.



Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	Me	OH	Me	H	OH	H
2	Me	OH	Me	OH	H	H
3	Me	H	H	H	H	H
4	Me	OH	Me	H	H	H
5	Me	H	Me	H	H	H
6	Me	OH	H	H	H	H
7	Me	OH	Me	H	H	OH
8	Me	OH	Me	H	OH	OH
9	Me	OH	H	H	OH	H
10	H	OH	Me	H	OH	H
11	H	H	H	H	H	H

Figure 1. Structural formulas of compounds **1**−**11**.

Lemna (*Lemna minor*, LEMMI) was cultured in 35 mL of sterile medium (as described above plus 10 g L^{-1} sucrose). One colony of *Lemna* (3−4 frond stage) was added to each well of the tissue culture plate using a sterile inoculating loop.

Plates were placed in the growth chamber (24 h photoperiod,

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Table 1. Activity of Thaxtomin A in the *Lemna/Agrostis* Assay

	visual injury (%)	
concn (ppm)	LEMMI	AGRPA
25	100	100
5.0	100	100
1.0	100	100
0.2	95	100
0.04	70	80
0.008	30	50

26 °C, average photosynthetic photon flux duration 250 μmol m^{-2} s^{-1}) and evaluated 9 days after treatment. Visual injury ratings were taken for both *Lemna* and bentgrass on a 0−100 scale, with 0 being no visual injury and 100 being complete necrosis.

In Vitro Symptomology. Detailed symptomological observations were made on seeds from *Arabidopsis thaliana* (var. Columbia, ARATH) and bentgrass (*Ag. palustris*), cultured as described previously (*7*). Symptom development was observed through a 9 day period over a wide dose range for each test compound.

Postemergence and Preemergence Tests. Whole plant activity was assessed on sunflower (*Helianthus annuus*, HELAN), morningglory (*Ipomoea hederacea*, IPOHE), barnyardgrass (*Echinochloa crusgalli*, ECHCG), and wild oats (*Avena fatua*, AVEFA) using foliar-applied (postemergence) and soil-applied (preemergence) treatments exactly as described previously (*7*).

Visual injury ratings were taken 9 and 16 days after treatment for postemergence and preemergence applications, respectively, on a 0−100 scale, with 0 being no injury and 100 being complete necrosis.

Systemic Phytotoxicity Assays. Compounds were prepared as 5000 ppm stocks in methanol, which were subsequently diluted in methanol to form 500 and 50 ppm solutions. A 20 μL aliquot of the appropriate stock solution was removed, added to 20 μL of 0.2% (v/v) Triton X-77 in water, and applied to the bioassay. Test compounds were applied to either the apical meristem or cotyledons of 4-day-old sunflower and morningglory or the apical meristem or first true leaf of 5-day-old velvetleaf at rates of 100, 10, and 1 μg per seedling. Plants were returned to the greenhouse after treatment (conditions described above), and visual injury ratings were taken 1 week after treatment on a 0−100 scale, with 0 being no visual injury and 100 being complete necrosis.

RESULTS AND DISCUSSION

***Lemna/Agrostis* Assay.** Thaxtomin A (**1**) was highly active in the initial phytotoxicity evaluations (Table 1). Using regression analysis of log-transformed dose response data, GR_{50} values on *Lemna* and *Agrostis* were estimated to be 12 and 5 ppb, respectively. This level of activity is greater than that of many commercial herbicides, including many inhibitors of acetolactate synthase (ALS) (*8*).

In Vitro Symptomology. The high level of phytotoxicity exhibited by thaxtomin A in the *Lemna*/*Agrostis* assay was also observed in agarose-based tests of arabidopsis and bentgrass (Table 2). Thaxtomin A had plate test I_{50} values on *Arabidopsis* and *Agrostis* of approximately 10 and 25 ppb, respectively. Again, this level of activity is relatively high compared to that observed with most commercial herbicides. Levels of biological activity differed among the thaxtomin A analogues tested, and no analogue had greater activity than thaxtomin A. Thaxtomin B (**4**), thaxtomin C (**3**), hydroxythaxtomin C (**6**), and hydroxy thaxtomin A (**8**) were relatively active in the assay with I_{50} values of ≤50 ppb. In contrast, thaxtomin A *p*-isomer (**7**), thaxtomin A *o*-isomer (**2**), and des-*N*-methylthaxtomin C (**11**) were relatively inactive in the assay, with I_{50} values of ≥100 ppb.

Table 2. Agarose-Based Test Results

compound	plate test I_{50} (ppb)
thaxtomin A (**1**)	ARATH: 10 AGRPA: 25
thaxtomin B (**4**)	ARATH: 28 AGRPA: N/A
thaxtomin C (**3**)	ARATH: 50 AGRPA: 40
thaxtomin A *p*-isomer (**7**)	ARATH: 190 AGRPA: 100
thaxtomin A *o*-isomer (**2**)	ARATH: 100 AGRPA: 90
hydroxythaxtomin A (**8**)	ARATH: 10 AGRPA: 40
hydroxythaxtomin C (**6**)	ARATH: <40 AGRPA: <40
des-*N*-methylthaxtomin C (**11**)	ARATH: 400 AGRPA: 600

Table 3. Postemergence Activity of Thaxtomin A and Selected Analogues

		visual injury (%)			
compound	rate (kg ha^{-1})	HELAN	IPOHE	ECHCG	AVEFA
thaxtomin A (**1**)	4	85	85	85	80
	2	85	80	40	20
	1	85	80	20	30
	0.5	75	70	10	0
thaxtomin C (**3**)	1	70	80	40	0
	0.5	30	80	20	0
	0.25	30	80	0	0
	0.125	30	70	0	0
thaxtomin A (**2**) *o*-isomer	2	75	40	20	0
	1	75	40	0	0
	0.5	10	10	0	0
	0.25	0	0	0	0

Table 4. Preemergence Activity of Thaxtomin A

		visual injury (%)			
compound	rate (kg ha^{-1})	HELAN	IPOHE	ECHCG	AVEFA
thaxtomin A (**1**)	4	100	100	100	100
	2	100	100	100	100
	1	100	100	98	100
	0.5	70	100	98	100

Thaxtomin A and all analogues tested caused similar symptoms. These included stunting and root tip puffing. These symptoms were remarkably similar to those caused by known cellulose biosynthesis inhibitors (CBIs) such as dichlobenil and isoxaben (*9*, *10*). On the basis of this information, it was hypothesized that the phytotoxicity caused by thaxtomin A and its analogues is due to inhibition of cellulose biosynthesis. The *Streptomyces* species generated phthoxazolins (*11*) and epopromycins (*12*) have also been reported to inhibit cellulose biosynthesis in plants.

Postemergence and Preemergence Testing. Thaxtomin A had moderate to good postemergence activity on dicotyledon species, with GR_{50} values on sunflower and morningglory of <500 g ha^{-1} (Table 3). Thaxtomin A was less active on the monocotyledon species, with GR_{50} values on barnyardgrass and wild oats between 2 and 4 kg ha^{-1}. Postemergence symptoms included

Table 5. Activity of Thaxtomin A and Selected Analogues Applied to the Apical Meristem or Leaf/Cotyledon of Three Bioassay Species

		visual injury (%)					
		ABUTH		IPOHE		HELAN	
	rate (μg)	meristem	leaf	meristem	cotyledon	meristem	cotyledon
thaxtomin A (**1**)	100	80	0	80	0	90	0
	10	75	0	80	0	90	0
	1	20	0	75	0	50	0
thaxtomin C (**3**)	100	50	0	nd[a]	nd	75	0
	10	0	0	nd	nd	30	0
	1	0	0	nd	nd	0	0
14-hydroxythaxtomin C (**6**)	100	70	0	nd	nd	100	20
	10	20	0	nd	nd	95	0
	1	0	0	nd	nd	80	0
N-desmethylthaxtomin C (**11**)	100	10	0	nd	nd	50	0
	10	0	0	nd	nd	0	0
	1	0	0	nd	nd	0	0

[a] nd, not determined.

stunting, meristematic effects, and additional symptoms consistent with a CBI mode of action. However, thaxtomin A also caused substantial wilting on all species following postemergence applications, a symptom dissimilar to that caused by known CBIs. This may indicate an additional herbicidal mode of action or a perturbation of cellulose biosynthesis that is different from that caused by known CBIs that manifests itself with an additional set of symptoms.

Thaxtomin C (**3**) and thaxtomin A *o*-isomer (**2**) were also more active on dicotyledonous than on monocotyledonous species postemergence. Of the analogues tested, thaxtomin C (**3**) was the most active, with a postemergence GR_{50} value on morningglory of <125 g ha^{-1}.

Thaxtomin A was more active when applied preemergently (Table 4) than postemergently, with GR_{50} values on all bioassay species of <500 g ha^{-1}. Preemergence symptoms included a lack of emergence at high rates, whereas symptoms at lower rates included stunting and meristematic effects. Symptoms in the preemergence assay were also consistent with a CBI mode of action.

Systemic Phytotoxicity Assays. Phloem mobility and systemic activity are desirable properties for a herbicide because they can impart suppression of regrowth and more reliable weed control. The majority of known CBIs have extremely limited phloem mobility. To determine whether thaxtomin A and its analogues also have limited systemic phytotoxicity, the activity of selected compounds was assessed following applications to either the apical meristem or cotyledon/leaf of three bioassay species. Although thaxtomin A caused substantial injury to velvetleaf, morningglory, and sunflower when applied to the apical meristem region, it caused no detectable injury when applied to either the cotyledons or first true leaf (Table 5). Because no injury was detected in the meristem from the cotyledon/leaf treatment at 100 mg plant^{-1}, compared to the direct meristem application at 1 mg plant^{-1}, this suggests that $<1\%$ of thaxtomin A applied to the cotyledons or first true leaf reached the apical meristem and indicates that thaxtomin A has extremely low levels of systemic phytotoxicity. The systemic phytotoxicity of thaxtomin A analogues evaluated in the systemic phytotoxicity assay was also limited. All analogues caused substantially greater injury when applied to the meristems than when applied to the cotyledons or first true leaf of the bioassay species.

In summation, results of the testing protocols indicated that the thaxtomins are potent phytotoxins that induce symptomology consistent with inhibition of cellulose biosynthesis in plants. Although thaxtomin A and analogues demonstrated many of the biological properties desirable in potential herbicides, they lacked the systemic phytotoxicity critical to deliver reliable weed control in the field at low herbicide rates. However, the findings that the phytotoxicity caused by thaxtomin A and its analogues appeared to be due to the inhibition of cellulose biosynthesis in plant cells may prove to be a major discovery for disease researchers and could inevitably help yield strategies for engineering scab resistance in potatoes and other affected crops.

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