Effect of the Plant Growth Retardant Pydanon on Verticillium Wilt of Cotton and Tomato

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ABSTRACT

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Root drench treatment of cotton and tomato plants with Pydanon (200 mg/plant) before inoculation with *Verticillium dahliae* (microsclerotial strain) reduced the severity of foliar and vascular discoloration symptoms of Verticillium wilt. The protective effect of Pydanon was more marked than its curative effect. Foliar treatments with Pydanon (2,000 and 4,000 μg/ml) were less effective than soil treatments against Verticillium wilt. Pydanon was not toxic to growth of *V. dahliae* on potato-dextrose agar or to the formation of spores in a shaken synthetic nutrient solution. No ethanol-extractable antifungal compounds could be detected in xylem tissue of treated cotton plants. Pydanon interfered with gibberellic acid induction of α-amylase activity in barley endosperm.

Our interest in the possible role of plant growth regulant chemicals for the control of vascular diseases was initiated by the report by Sinha and Wood (18) that chlorocholinechloride (CCC) reduced the severity of Verticillium wilt on tomato. Subsequent studies by Buchenauer also indicated that preinfection treatment of tomato plants reduced the severity of both Verticillium and Fusarium wilt diseases of tomato (5). More recently, Buchenauer and Erwin (6, 7) reported that the growth retardants *N,N*-dimethylpyrrolidinium iodide (DPYI) and *N,N*-dimethylpiperidinium iodide (DPII) reduced the severity of Verticillium wilt in greenhouse experiments. Correlated with reduction in severity of disease, there was also a reduction in the number of propagules of *V. dahliae* in plant tissue. In recent field studies, the growth regulant tributyl [(5-chloro-2-thienyl)methyl] phosphonium chloride, when applied by foliar spray to cotton in June, reduced the numbers of Verticillium propagules in plant tissue and increased the yield (12).

The altered resistance to insect pests of plants treated with growth retardants also has been reported; CCC reduced the incidence of the gall mite *Cecidophyopsis ribis* on black currant (19) and Van Emden (11) reported that the rate of increase of the aphid *Brevicoryne brassicae* on cabbage was decreased when plants were treated with CCC. Investigations of Karas et al. (14) showed that applications of the growth retardant, *N*-dimethylamino-succinamic acid (Alar), reduced the number of tobacco ringspot virus lesions on tobacco. Crossan and Fieldhouse (8) reported that Alar reduced the incidence of bacterial spot caused by *Xanthomonas vesicatoria* on peppers. Amo-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate) inhibited growth of *Bacillus subtilis* in culture (10). When wheat plants were treated with CCC (20), they became more resistant to *Cercosporella herpotrichoides* (4, 9) but more susceptible to *Septoria, Fusarium, Alternaria,* and *Cladosporium* (20). Chlorocholinechloride and Alar increased the resistance of cucumber to *Cladosporium cucumerinum* (1, 2).

In this investigation, the effect of the growth retardant Pydanon (4-hydroxy-3,6-dioxo-hexa-hydropyridazinyl-4-acetic acid) (16) on Verticillium wilt will be reported.

MATERIALS AND METHODS

Cotton plants cultivar SJ-1 and tomato plants cultivar Bonny Best were grown in 10-cm (4-inch) plastic pots containing either sterilized sand or steamed soil (50% peat moss and 50% fine sandy loam). Cotton plants about 4-5 weeks old were inoculated with a microsclerotial, severely defoliating isolate of *Verticillium dahliae* Kleb. (V3H) by either stem puncture at the first node or by drenching roots on the exterior of the root ball with 100 ml of a spore suspension (about 10^6 spores/ml). Tomato plants were inoculated by root drench with isolate V47 since V3H induced a mild reaction on tomato.

Pydanon (H1244, 20% active ingredient from Spiess and Sohn, Kleinkerlbach, W. Germany) was applied either as a root drench or a foliage spray. Contamination of the soil with the chemical during foliar spraying was prevented by placing paper towels on the top of each pot before spraying. Plants were sprayed in the evening to delay drying as long as possible. Dosages were calculated as active ingredient.

The foliar symptom index (FSI) was based on the degree of severity rated on a 0-4 scale and the vascular browning index (VBI) was based on the degree of discoloration of a cross-section of xylem tissue at each internode rated as zero (white) to 3 (completely brown) (7, 12).

The fungitoxic effect of Pydanon against radial growth of *Verticillium dahliae* was tested in 90-mm diameter petri dishes containing 15 ml of potato-dextrose agar (PDA) amended with 0, 10, 50, 100, and 500 μg/ml of Pydanon. The effect of Pydanon (0, 10, 50, 100, and 500 μg/ml) on the formation of conidia was tested in 50-ml flasks containing 10 ml of a synthetic nutrient medium (17). The flasks were inoculated with 0.2 ml of a spore suspension (2×10^7 spores/ml) and incubated in a shaker incubator for 6 days at 25 C. Numbers of conidia were determined by turbidity with a Spectronic 20 colorimeter.

To determine whether Pydanon induced antifungal compounds in the xylem tissue of cotton plants, the method of Zaki et al. (21, 22) was employed. Plants were treated with 300 mg of Pydanon by root drench and harvested 7 days later. The xylem tissue from treated and from nontreated plants was blended with ethanol (10 ml/g xylem tissue) in a Sorvall OmniMixer for 3 minutes and filtered. The ethanol extract was dried under vacuum. The residue was redissolved in ethyl acetate (0.1 ml/g xylem tissue). Samples of 20 and 40 μliters were spotted on a thin-layer plate and the chromatogram was run using the solvents chloroform:acetone:formic acid: and concentrated HCl (95:5:2:0.3, v/v). After drying, the plate was sprayed with a spore suspension of *Cladosporium cucumerinum* in potato-dextrose agar at about 50 C. After incubation in a humid chamber for 3 days at 25 C (21, 22), zones with no growth indicated fungitoxic compounds.

To test the effect of Pydanon on the gibberellic acid (GA) induction of α-amylase in barley endosperm the method of Jones and Varner (13) was used (7).

All experiments were repeated two or three times.

RESULTS

Growth of cotton plants following root drench treatment with Pydanon (100, 200, and 300 mg/plant) was retarded about 20% when measured 16 days after treatment. Roots often become brown. Dosages of 200-300 mg/plant caused physiological wilting, after which the plants recovered after 2-3 days.

Root treatments of cotton plants with 100 mg of Pydanon 2 and 4 days before inoculation by stem puncture delayed the expression of foliar symptoms of Verticillium wilt on the leaves for 11 days after inoculation. Five days later the foliar symptom index of the treated plants was only 10 compared to 74 for the nontreated control plants. The vascular browning index also was reduced markedly in the treated plants (Table 1). In another experiment in which Pydanon (100 mg/plant) was applied 2 days before and 2 days after inoculation by stem puncture, the foliar symptom index was reduced from 67 in the control to 6 in the treated plants and the vascular browning index was reduced from 70 to 10 (Fig. 1).

When Pydanon (100 mg/plant) was applied by root drench 2 and 3 days after inoculation by stem puncture the expression of foliar symptoms and vascular discoloration was reduced; but the curative effect of Pydanon was less marked than its protective influence.

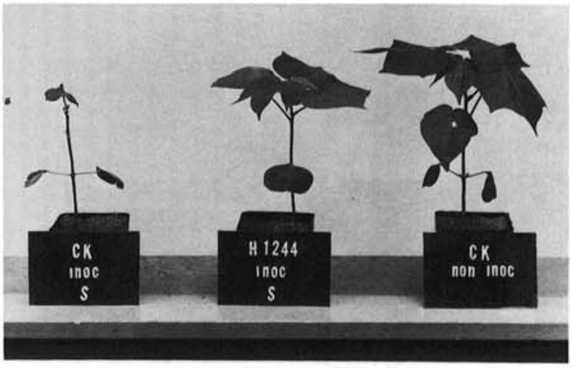

Fig. 1. Effect of root treatments with Pydanon 2 days before and 3 days after inoculation by stem puncture (5×10^{-4} spores/ml) on Verticillium wilt of cotton. Left to right: control, inoculated; treated with Pydanon (H 1244) inoculated; and control noninoculated.

TABLE 1. Effect of two root drenches (each 100 mg/pot) with the growth retardant Pydanon applied 4 and 2 days before inoculation on Verticillium wilt of cotton following inoculation by stem puncture (5×10^4 spores/ml)

	Disease assessment by days after inoculation			
	Foliar index[a]		Vascular index[b]	Height (cm)
Treatment	11	16	16	16
Control, noninoculated	0	0	0	49
Control, inoculated	36	74	68	44
Pydanon 200 mg/pot, inoculated	0	10	11	39

[a]Foliar symptom index = $\frac{\text{sum of leaf symptom values (0-4)}}{\text{number of leaves examined} \times 4} \times 100$:

in which leaf symptom values are estimated 0 = normal; 1 = epinasty; 2 = slight chlorosis; 3 = extensive chlorosis; and 4 = defoliation.

[b]Vascular browning index = $\frac{\text{sum of vascular browning values (0-3)}}{\text{number of nodes examined}} \times 100$:

in which leaf symptom values are estimated 0 = no discoloration of a cross section of xylem tissue; 1 = 1-33% of xylem discolored; 2 = 34-67% discolored; and 3 = 68-100% discolored.

The effect of foliage treatments was investigated. Pydanon (2,000-4,000 μg/ml) was sprayed on foliage 2 and 4 days before inoculation by both stem puncture and root drench. Pydanon reduced the foliar symptom and vascular browning indices markedly, but not so great as when used as a soil treatment (Table 2).

Root application with 100 mg Pydanon per plant on tomato plants 2 and 4 days following inoculation by root drench reduced the foliage symptom index from 48 to 7 and the vascular discoloration index from 87 to 13.

In vitro tests showed that Pydanon at concentrations up to 500 μg/ml was neither toxic to the radial growth of *V. dahliae* on PDA nor to the formation of spores in the synthetic nutrient solution.

When ethanol extracts of xylem tissue from Pydanon-treated cotton plants were bioassayed on thin-layer plates, no antifungal compounds were detected.

Pydanon (at concentrations of 100 and 1,000 μg/ml) intefered with the GA-activated induction of α-amylase activity in barley endosperm (Table 3).

TABLE 3. Effect of Pydanon on the induction of α-amylase by gibberellic acid (GA) in the barley endosperm test

Treatment[a]	Pydanon concn. (μg/ml)	α-amylase activity (% of control)
GA (2 μg/ml)	0	100
Control, nontreated	0	8
GA + Pydanon	100	74
GA + Pydanon	1,000	54

[a]GA was used at the rate of 2 μg/ml.

DISCUSSION

The results indicated that a soil drench treatment with the growth retardant, Pydanon, reduced the severity of foliar symptoms and vascular browning caused by *V. dahliae* in cotton and tomato plants. Foliar spray treatments also were effective, but less so than root drench. The wilt-reducing effect of Pydanon decreased with increasing the period of time following inoculation (Table 1).

Since Pydanon was not fungitoxic to *Verticillium*, its action appeared to be due to an interaction with the plant. Since the bioautography method did not indicate that Pydanon induced an ethanol-extractable fungitoxic compound in the xylem tissue, possibly Pydanon alters other metabolic pathways of the host plant which then become unfavorable for propagation of the pathogen in the xylem or induces internal morphological changes which inhibit invasion and/or movement of the pathogen in the plant. Keyworth and Dimond (15) and Biehn and Dimond (3) demonstrated that chemically induced root injury of tomato plants decreased the severity of Fusarium wilt. Since root treatments with high concentrations of Pydanon (200-300 mg/plant) caused a reduction of root size and a browning of the cortex, the wilt-reducing factor may be associated with the roots. However, in our tests, since some plants were inoculated by root drench and others inoculated by stem puncture, it did not appear that the wilt-reducing effect of Pydanon was solely caused by injury per se of the root system.

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TABLE 2. Effect of foliage treatments with Pydanon 2 and 4 days before inoculation by stem puncture (5×10^4 spores/ml) and by root drench (10^6 spores/ml) on Verticillium wilt of cotton

Inoculum placement and treatment	Disease assessment by days after inoculation: Foliar index[a] 19	Vascular index[b] 19
Stem inoculation:		
Control, noninoculated	0	0
Control, inoculated	98	64
Pydanon 2,000 μg/ml inoculated	50	30
Pydanon 4,000 μg/ml inoculated	75	42
Root inoculation		
Control, noninoculated	0	0
Control, inoculated	92	67
Pydanon 2,000 μg/ml inoculated	50	30
Pydanon 4,000 μg/ml inoculated	61	36

$$^{a}\text{Foliar symptom index} = \frac{\text{sum of leaf symptom values (0-4)}}{\text{number of leaves examined} \times 4} \times 100$$

in which leaf symptom values are estimated 0 = normal; 1 = epinasty; 2 = slight chlorosis; 3 = extensive chlorosis; and 4 = defoliation.

$$^{b}\text{Vascular browning index} = \frac{\text{sum of vascular browning values (0-3)}}{\text{number of nodes examined}} \times 100$$

in which leaf symptom values are estimated 0 = no discoloration of a cross section of xylem tissue; 1 = 1-33% of xylem discolored; 2 = 34-67% discolored; and 3 = 68-100% discolored.

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