

Effect of Soluble Aluminum on Growth and Pathogenicity of *Verticillium albo-atrum* and *Whetzelinia sclerotiorum* from Sunflower

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ABSTRACT

Verticillium albo-atrum from sunflower was nearly suppressed by 8 $\mu\text{g/g}$ Al^{+3} in vitro and was characterized by hyaline, apparently unpigmented mycelia and few, if any, microsclerotia. This Al-sensitivity was related to the toxicity of soluble Al in the culture substrate at pH 4.7 or below. *Whetzelinia sclerotiorum* was tolerant to Al because it grew with as much as 32 $\mu\text{g/g}$ Al^{+3} even though the acidity of the substrate increased to pH 3.7. Sclerotial development was, however, inhibited by Al above 16 $\mu\text{g/g}$. The Al-sensitive *V.*

albo-atrum was more harmful to sunflower plants grown in acid, Al-toxic Bladen soil amended with 3,000 $\mu\text{g/g}$ CaCO_3 (pH 5.4) than with 750 $\mu\text{g/g}$ (pH 4.4). The Al-tolerant *W. sclerotiorum* was extremely harmful to sunflower plants grown in acid, Al-toxic Tatum soil amended with 750 $\mu\text{g/g}$ CaCO_3 (pH 4.4). These host-pathogen interactions might have been influenced by the extent of detoxification of soil Al by CaCO_3 .

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Toxic levels of exchangeable aluminum (Al^{+3}) in acid soils critically retard plant growth and limit crop production (3, 4, 5, 6). Differential Al tolerance of sunflower genotypes has been shown in Al-toxic Bladen clay loam in the greenhouse and in nutrient solution (7). Aluminum toxicity can be corrected by liming, which reduces acidity and Al solubility in the soil (4, 5).

Zwarun et al. (22) and Zwarun and Thomas (23) showed that Al reduces exponentially the survival of cells of *Pseudomonas stutzeri* (Lehmann & Neumann) Kluver in vitro. Similarly, Ko and Hora (12) found that less than 1.0 $\mu\text{g/g}$ Al inhibited germination of ascospores of *Neurospora tetrasperma* Shear & Dodge. Johnson (10) showed that Al inhibited growth of *Verticillium albo-atrum* Reinke & Berthold in nutrient culture, Lewis (14) reported that Al salts reduced the growth of *Aphanomyces euteiches* Drechsler on peas under greenhouse conditions, and we have made a preliminary report of Al tolerance of certain sunflower-pathogenic fungi (17). We are unaware of other studies on Al fungitoxicity.

This paper presents results of our studies of the effect of soluble Al on growth in vitro and on the pathogenicity of *V. albo-atrum* and *Whetzelinia sclerotiorum* (Lib.) Korf & Dumond (13) (= *Sclerotinia sclerotiorum* (Lib.) De Bary) on sunflowers grown in acid Al-toxic soil, amended with 750 or 3,000 $\mu\text{g/g}$ CaCO_3 .

MATERIALS AND METHODS.—*Sunflower pathogens.*—Isolate V-39, a microsclerotial form of *V. albo-atrum* (*Verticillium* wilt) from Rosemount, Minnesota (16), and isolate S-EP of *W. sclerotiorum* (stalk and head rot) from El Paso, Texas, were obtained from field-infected sunflowers. According to the species concept of Isaac (9) and Schnathorst (20) and usage of Tolmsoff (21), the microsclerotial form of *V. albo-atrum* is synonymous with *V. dahliae* Klebahn. The

pathogenicity of these isolates was verified in controlled inoculations of susceptible sunflowers by the method described under "Pathogenicity tests."

Bioassays of Al-tolerance of fungal pathogens.—The basal medium contained the following amounts per liter: $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.5 g; KCl, 0.5 g; K_2HPO_4 , 0.05 g; NaNO_3 , 3.0 g; sucrose, 30.0 g; FeSO_4 , 0.02 g; and Al-free Hoagland solution, 0.5 ml. Aluminum as $\text{Al}_2(\text{SO}_4)_3 \cdot \text{H}_2\text{O}$ was added to give concentrations of 4, 6, 8, 18, 24, 26, and 32 $\mu\text{g/g}$. Because of the intrinsic buffering activity of Al, no standard buffer was added. Before sterilization, the media were adjusted to pH 4.7 with HCl or NaOH. They were then sterilized either by autoclaving for 15 minutes at 1 atmosphere or by filtering with a Nalge millipore filter (Nalge Sybron Corp., N.Y.). All assays were run with an Al-free control medium at its original unadjusted pH of 6.3. The sterilized medium, in 250-ml Erlenmeyer flasks, with 50 ml of medium/flask, was inoculated with single 5-mm-diameter plugs cut from young Difco potato-dextrose-agar cultures of the test fungi. Plugs of *W. sclerotiorum* excluded sclerotia. The inoculated media were incubated for about 5 weeks at 26 C in stationary cultures in the dark. The fungal mass was removed by centrifugation at ca. 6,000 g for 3 minutes, and weights were determined on oven-dry basis. The pH of the culture filtrate was measured during the assays. Each experiment consisted of four to five replicates/Al-concentration and was conducted at least three times.

Soils, sunflower, and pathogenicity tests.—Acid soils used in this study were Bladen clay loam from the Tidewater region near Fleming, Georgia, and Tatum clay loam from the Piedmont region near Orange, Virginia, both having an initial pH of 4.1. Both soils have been thoroughly characterized in previous publications and shown to be reliable media in which to screen plant genotypes for Al tolerance (7, 19). A typical analysis of

TABLE 1. Effect of soluble aluminum on relative growth of *Verticillium albo-atrum* and *Whetzelinia sclerotiorum* of sunflowers (expressed as percentage of weight of fungal mass in Al-free basal control medium having unadjusted pH of 6.3), and effect of fungal growth on pH of medium

Al (μ g/g)	Original pH of medium ^a	<i>V. albo-atrum</i>		<i>W. sclerotiorum</i>	
		Relative growth (%) ^b	pH of culture filtrate	Relative growth (%) ^b	pH of culture filtrate
0	6.3	100 (0.138)	6.9	100 (0.490)	4.6
0	4.7	89 (0.123)	4.6	98 (0.480)	4.0
4	4.7	62 (0.086)	4.5	96 (0.470)	4.0
6	4.7	24 (0.033)	4.1
8	4.7	4 (0.005)	4.5	86 (0.420)	4.0
16	4.7	0 0	...	63 (0.310)	3.9
24	4.7	0 0	...	77 (0.380)	3.9
26	4.7	0 0
32	4.7	0 0	...	22 (0.110)	3.7

^aAl-free basal control medium had original unadjusted pH of 6.3. All other media had original pH adjusted to 4.7.

^bFigures in parentheses are average dry weights in grams (oven-dry-weight basis) of fungal mass in three experiments.

TABLE 2. Effect of $CaCO_3$ on soil pH, disease severity index (DSI), and growth of CM 144 and CM 162 sunflowers inoculated when 8 days old with *Verticillium albo-atrum* and grown in Bladen Al-toxic clay loam soil in the greenhouse

$CaCO_3$ (μ g/g)	Soil pH	CM 144		CM 162		
		<i>V. albo-atrum</i>	DSI ^y	Fresh weight of aerial parts ^z (g/pot)	DSI ^y	Fresh weight of aerial parts ^z (g/pot)
750	4.4	—	—	1.97 b	—	0.93 c
3,000	5.4	—	—	2.63 a	—	0.93 c
750	4.4	+	2.9	1.10 c	4.7	0.43 d
3,000	5.4	+	4.2	0.70 cd	5.0	0.41 d

^yDSI = (number of infected plants \times severity class) \div (number of inoculated plants). Severity classes range from 0 (no symptoms) to 5 (all plants killed).

^zPlants weighed 2 weeks after inoculation; any two values within a vertical column, followed by a letter in common, are not significantly different, $P = 0.05$, by Duncan's multiple range test.

Bladen soil showed a cation exchange capacity (1 N CH_3COONH_4 at pH 7.0) of 12.1 meq/100 g and 4.3 meq/100 g of KCl-extractable Al. The Tatum soil had a cation exchange capacity of 12.6 meq/100 g and 5.93 meq/100 g of KCl-extractable Al.

Before planting, the soils were autoclaved for 1 hour, allowed to stand for 2 weeks, and fertilized with 50, 55, and 69 μ g/g of, respectively, N, P, and K from NH_4NO_3 and K_2HPO_4 . The soils were treated with either 750 or 3,000 μ g/g $CaCO_3$, with resulting pH values of 4.4 and 5.4 for each soil, respectively. Nonlimed acid soils were not used because sunflowers grown in these soils were severely stunted and had root injury which killed the plants, and would have interfered with pathogenicity tests. Sunflowers tested were *Verticillium*-resistant CM 144 and *Verticillium*-susceptible CM 162 grown in the Bladen soil, and *Whetzelinia*-susceptible Romania HS 52 grown in the Tatum soil.

From surface-disinfested seed, plants were grown in metal cans (three to four plants/can) lined with waterproof polyethylene bags of 2-kg capacity without drainage (7). In some experiments, test plants were 8- to 9-day-old seedling transplants grown in a steamed sand-soil mixture. The pathogenicity of *V. albo-atrum* was determined by root-dip inoculation at transplanting (16). That of *W. sclerotiorum* was determined on maturing 4-

to 5-week-old plants when susceptibility to stalk rot is highest (18) by placing a PDA-plug of the fungus on the intact lower hypocotyl and covering it with soil. Soil experiments were conducted twice in the greenhouse, with day and night temperatures about 23 and 20 C, respectively. Only distilled water was used to irrigate the plants. Disease reaction was expressed by a disease

TABLE 3. Effect of $CaCO_3$ on soil pH, disease severity index (DSI), and growth of Romania HS 52 sunflower inoculated when 4 weeks old with *Whetzelinia sclerotiorum* and grown in Tatum Al-toxic clay loam soil in the greenhouse

$CaCO_3$ (μ g/g)	Soil pH	Romania HS 52		
		<i>W. sclerotiorum</i>	DSI ^a	Fresh weight of whole plant ^b (g/plant)
750	4.4	—	—	3.05
3,000	5.4	—	—	5.40
750	4.4	+	4.3	2.53
3,000	5.4	+	0.8	4.43

^aDSI = (number of infected plants \times severity class) \div (number of inoculated plants). Severity classes range from 0 (no symptoms) to 5 (all plants killed).

^bPlants weighed 1 week after inoculation.

severity index (DSI) calculated for each host-pathogen interaction, as described in Tables 2 and 3.

RESULTS.—Effect of Al on fungal growth.—The highest Al concentration that allowed slight although detectable growth of *V. albo-atrum*, as compared to growth in the basal Al-free medium at either pH 6.3 or 4.7, was 8 $\mu\text{g/g}$ Al. Still higher Al concentrations suppressed growth completely (Table 1). The Al-sensitivity of this pathogen was apparently related to the presence of soluble Al in the culture substrate at pH 4.7 or below. Growth of this fungus in the presence of Al was characterized by thin-walled, thread-like, hyaline, apparently unpigmented mycelium in association with strands of hyaline, torulose, thick-walled mycelium, few if any microsclerotia, absence of hyphal anastomoses, and profuse conidial development. Mycelial fragments, drawn from cultures grown with Al and transferred to PDA without Al, produced dark mycelia and microsclerotia. *W. sclerotiorum* was highly tolerant to Al in vitro (Table 1) as demonstrated by development of abundant to moderate mycelial growth with 4–32 $\mu\text{g/g}$ Al and numerous black sclerotia which ranged in size from 0.5 to 5.0 mm, in basal medium containing up to 16 $\mu\text{g/g}$ Al, even though the fungus increased the acidity of the substrate at all Al concentrations. Although the tolerance of this fungus to Al varied with individual experiments, the average relative growth (Table 1) of the fungal mass in three experiments decreased consistently with the increasing Al content of the basal medium.

Effect of CaCO_3 on disease reaction in Al-toxic soil.—The wilt syndrome incited by *V. albo-atrum* on plants of CM 144 and CM 162 sunflower when grown in Bladen clay-loam soil amended with CaCO_3 was not typical of the wilt syndrome that is generally observed on these genotypes when grown in neutral soils. Disease symptoms of CM 144 (epinasty, stunt, and wilt) were more severe on plants grown in soil amended with 3,000 $\mu\text{g/g}$ CaCO_3 (pH 5.4) than with 750 $\mu\text{g/g}$ CaCO_3 (pH 4.4) as indicated in Table 2 by DSI of 4.2 and 2.9, respectively. The growth response of CM 144 to CaCO_3 in this soil was apparently blocked by the disease. Weights of the aerial parts of these plants were significantly less than those of noninoculated plants. The susceptible CM 162 sunflower failed to respond to CaCO_3 with or without fungal inoculation. *V. albo-atrum* and *W. sclerotiorum* were re-isolated readily from the diseased plants.

Table 3 shows that disease reactions of Romania HS 52 in Al-toxic Tatum clay-loam soil amended with 750 $\mu\text{g/g}$ CaCO_3 and inoculated with *W. sclerotiorum* were extremely severe (DSI = 4.3); most plants were killed within 8 days after inoculation. Plants of this sunflower grown with 3,000 $\mu\text{g/g}$ CaCO_3 apparently became tolerant to the disease as shown by the low DSI (= 0.8). The growth response of Romania HS 52 to CaCO_3 was also reduced by the disease.

DISCUSSION.—Our results showed that the sunflower pathogen, *V. albo-atrum*, was much more sensitive to Al in vitro than was *W. sclerotiorum*. There was a slight tendency for growth inhibition in Al-free media of pH 4.7, compared with the original unadjusted medium of pH 6.3, particularly with *V. albo-atrum*, but this effect was not statistically significant for either pathogen. The high Al-sensitivity of *V. albo-atrum*

coincides with its inability to increase the pH of the medium that would detoxify Al by precipitating it from solution. Certain Al-sensitive varieties of wheat and barley decrease the pH of their root zones, whereas Al-tolerant varieties increase the pH (5). However, such pH/Al-solubility mechanisms cannot explain the high Al tolerance of *W. sclerotiorum*. This pathogen actually lowered the pH of the medium from 4.7 to 4.0 or below. Such a lowering would greatly increase the solubility and the expected toxicity of Al. Thus, *W. sclerotiorum* has an exceptionally high specific tolerance to the Al ion. The pH decrease produced by this organism may be related to the excretion of oxalic acid (15), other acid-forming metabolites, or both.

This investigation showed that Al interfered with pigment development, which in *V. albo-atrum* is reportedly linked to melanin synthesis (1, 2). According to Isaac (9), among other investigators, the presence of dark mycelia is the characteristic used in distinguishing this species from *V. dahliae*. Brown and Wyllie (2) suggest that hyaline mycelia of *Verticillium* spp. are nonfunctional whereas dark mycelia carry out the functions of survival.

With respect to the pathogenic behavior of these two pathogens towards sunflowers grown in acid, Al-toxic Bladen and Tatum clay-loam soils and both amended with 750 and 3,000 $\mu\text{g/g}$ CaCO_3 , our results are in agreement with the in vitro Al response of these two pathogens. Thus, the Al-sensitive *V. albo-atrum* incited a more severe disease reaction on sunflowers grown with the higher than with the lower CaCO_3 rate, indicating that this fungus was favored by the greater detoxification of the Al in the soil. The disease reactions of CM 162 were, however, not well expressed, apparently because of the extreme susceptibility of this genotype. It is possible that Al^{+3} overcame or made inoperable the resistance mechanism of CM 144 to *Verticillium* wilt. The low virulence of *W. sclerotiorum* towards Romania HS 52 when grown with 3,000 $\mu\text{g/g}$ CaCO_3 (pH 5.4) could have been affected not only by reduced Al toxicity at a low acidity level in the soil, but also by the high Al tolerance of this sunflower genotype. An increased susceptibility of tomatoes to *Verticillium* and *Fusarium* wilts in soils of low pH and low Ca have been shown by Hubbeling et al. (8) whereas Jones and Woltz (11) have shown that liming reduced *Fusarium* wilt severity in tomatoes, although additions of Zn plus Mn and Zn plus Fe increased disease incidence. The results of this investigation suggest, therefore, the need for additional work on the pathogenic activities of soil-borne fungi and other plant pathogenic microorganisms in acid soils.

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