Seed Treatment with L-Sorbose to Control Damping-Off of Cotton Seedlings by Rhizoctonia solani

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ABSTRACT

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The keto sugar, L-sorbose, inhibits hyphal extension and promotes profuse branching in *Rhizoctonia solani*. When applied to cotton seed planted in soil infested with the pathogen, L-sorbose protected the seed against damping-off. It is fungistatic and not fungicidal. Because L-sorbose is metabolized by soil microorganisms, it does not persist or accumulate in the soil. When applied to seed in crystalline form with sticker, L-sorbose is highly water soluble and may be leached from the spermosphere. Use of a less soluble formulation consisting of L-sorbose mixed with cellulose acetate in acetone alleviated this problem.

Additional key words: *Gossypium hirsutum*, emergence, survival.

The keto sugar, L-sorbose, first was reported to be a fungus growth inhibitor by Barnett and Lilly in 1951 (1). Subsequently, L-sorbose has been used by a number of investigators to retard hyphal extension in fungi (2, 5, 7). The mechanism of L-sorbose inhibition appears to be interference with oligosaccharide formation (8) and thus disruption of cell wall synthesis in sensitive fungi (3). *Rhizoctonia solani* Kühn, an important pathogen of cotton seedlings that can grow through the soil to reach its host, is sensitive to L-sorbose (7).

The purpose of this study was to determine the efficacy of L-sorbose as a seed treatment to inhibit the growth of *R. solani* in the soil near developing cotton seedlings.

MATERIALS AND METHODS

Inoculum.—Sand cornmeal cultures of *R. solani* prepared according to Papavizas and Ayers (6) were incubated at room temperature for 3 wk and then incorporated into nonsterile soil taken from a cotton field at a rate of 100 g per 12 liters of soil. The inoculum level of *R. solani* in the soil after incorporation was determined according to the method of Weinhold (10).

Seed treatment.—Cottonseed (*Gossypium hirsutum* L. 'Deltapine-16'), were coated with methyl cellulose sticker in water and 0, 5, 10, or 20 mg of crystalline L-sorbose per seed. For each treatment 30 seeds were planted individually in 18 × 150-mm capped culture tubes containing 10 g of moist soil infested with *R. solani*. The soil tubes were incubated in the dark at 22 C for 10 days, then harvested and the number of emerged seedlings was recorded.

Seeds treated with L-sorbose at 10 mg/seed or with methyl cellulose sticker alone were planted in flats of *R. solani* infested or noninfested soil and placed in a growth chamber set for 14-hr days and 10-hr nights at 22 C. Each treatment consisted of 30 seeds. Flats were watered daily with 800 ml distilled water per flat and incubated for 14 days. Counts were made of pre- and postemergence damped-off seedlings and of healthy survivors.

Seeds also were coated with a mixture of 10% L-sorbose and 5% cellulose acetate in acetone (0.1 ml/seed) and air dried. Seeds treated with L-sorbose and cellulose acetate or cellulose acetate alone were planted in flats of soil infested with *R. solani* or noninfested soil and compared with seed coated with sorbose by the methyl cellulose method. They were treated the same as in the first growth chamber test, except that the flats were watered with 1,200 ml per flat. Infected and healthy seeds and seedlings were recovered from infested soil and surface sterilized by washing them for 30-sec intervals in 1% sodium hypochlorite, 70% ethanol, and sterile water. Sections were plated on 2% water agar and examined after 24 and 48 hr for evidence of mycelial growth.

Inhibitory effect and persistence of L-sorbose in soil.—The L-sorbose was mixed into moist, nonsterile soil at a concentration of 1% (w/w), and 100-g samples were packed into 9-cm-diameter glass petri dishes. Controls consisted of moist, nonsterile soil without L-sorbose added. Five seeds were planted in each plate at points equidistant from each other. The soil plates were incubated at 22 C for 8 days. At the start of the experiment, and at 2-day intervals thereafter, 0.2-cc soil plugs were removed from around the seeds. One set of plugs was placed in metal cylinders on the surface of soil extract agar (the filtrate of autoclaved soil and an equal volume of distilled water with 2% agar added). Sterile water (0.05 ml) was added to each cylinder and the agar surface between the cylinders was inoculated with PDA plugs of *R. solani*. The plates were incubated at 24 C and

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TABLE 1. Emergence and survival of cotton seedlings in *Rhizoctonia solani*-infested soil flats after treatment with L-sorbose applied with methyl cellulose sticker or cellulose acetate

Treatment	Emergence[a] (%)	Survival (%)
Lightly watered		
Methyl cellulose		
Nontreated seed in noninfested soil	93[b]	93
L-sorbose-treated seed in noninfested soil	97	97
Nontreated seed in infested soil	20	20
L-sorbose-treated seed in infested soil	80	73
Heavily watered		
Methyl cellulose		
Nontreated seed in noninfested soil	90	90
L-sorbose-treated seed in noninfested soil	87	87
Nontreated seed in infested soil	17	7
L-sorbose-treated seed in infested soil	67	43
Cellulose acetate		
Nontreated seed in noninfested soil	90	90
L-sorbose-treated seed in noninfested soil	93	93
Nontreated seed in infested soil	37	23
L-sorbose-treated seed in infested soil	83	73

[a]Values are expressed as a percentage of the total number of seeds planted.
[b]Values are averages of three replications of 30 seeds each.

TABLE 2. Persistence of L-sorbose in field soil and the effect of soil leachate on *Rhizoctonia solani*

Time (days)	L-sorbose (mg/cm^3 soil)	Inhibition (*R. solani*)
0	13.1[a]	+++[b]
2	13.1	+++
4	10.4	++
6	4.1	+
8	0	0

[a]Values are the averages of three replications with the average values of the untreated controls subtracted from them.
[b]Symbols: +++ = strong inhibition; ++ = moderate inhibition; + = slight inhibition; 0 = no inhibition.

the resulting colonies of *R. solani* were observed for signs of depressed hyphal extension and profuse branching.

Another set of soil plugs removed from around the seeds was diluted with distilled water, centrifuged, and 1-ml aliquots of the supernatant solutions were assayed for reducing sugars by the method of Nelson (4) and Somogyi (9). The results were compared to a standard curve prepared from L-sorbose to determine the concentration of the sugar in the soil.

RESULTS

L-sorbose treatments of 0, 5, 10, or 20 mg resulted in 37, 57, 87, or 67% emergence, respectively, in soil infested with *R. solani*. Thus, the most effective concentration for optimum emergence was 10 mg/seed. Examination of the nongerminated seed showed that those treated with 0 and 5 mg L-sorbose/seed were in an advanced state of decay, whereas those treated with 10 or 20 mg/seed were still firm. The number of plants emerging from soil tubes infested with *R. solani* was more than doubled by L-sorbose at 10 mg/seed, but 20 mg/seed appeared to have an adverse effect on seedling emergence. Assay of the soil from flats infested with *R. solani* showed the population to be 80 propagules per 100 g of soil.

The data obtained on the emergence and survival of L-sorbose-treated cotton seedlings planted in flats of soil infested with *R. solani* (Table 1) agreed with the results of the tube test. Treatment with 10 mg L-sorbose/seed did not depress the rate or percentage of seed germination in noninfested soil, and increased approximately fourfold the number of surviving seedlings in infested soil. A subsequent experiment showed that excessive watering of the soil flats could reduce the number of treated seedlings that survived in infested soil by 30% (Table 1).

Treatment of the cottonseed with a cellulose acetate and L-sorbose slurry in acetone left cellophane envelopes around the seed with L-sorbose crystals embedded in the matrix. This treatment gave good control of damping-off by *R. solani* even with heavy watering (Table 1). The mycelium of *R. solani* grew out of virtually all infected seeds and seedlings recovered from infested soil flats and plated on water agar. *Fusarium* sp. grew only rarely from infected sections and *Pythium* sp. were not recovered at all. No mycelium was found in plates containing healthy seeds and seedlings.

The inhibitory effect of L-sorbose on *R. solani* in soil decreased with time. Initially, inhibition was strong and characterized by extreme retardation of hyphal extension and by profuse, closely spaced branching. The inhibitory effect became less and less marked during the test period (Table 2). By the 8th day, no inhibitory effects were observed. This loss of inhibitory activity coincided with a progressive decrease in the L-sorbose concentration in the soil as measured by the reducing sugar assay.

After several days' incubation on soil extract agar, all of the soil plugs containing L-sorbose had the mycelia of several *Fusarium* sp. radiating from them. Growth and

sporulation of these fungi was normal and inhibition by L-sorbose was not apparent. In fact, growth was stimulated from soil plugs with L-sorbose compared to growth from plugs without L-sorbose.

DISCUSSION

At the concentrations used in this study, L-sorbose was not lethal to *R. solani*. However, it caused a shortening of hyphal extension and profuse branching with little distance between branches. The results of this study indicate that suppression of hyphal extension is the best explanation for the protection of cotton seedlings by L-sorbose. In addition, the L-sorbose treatment would give the saprophytic *Fusarium* sp. a selective advantage that might enable them to actively compete with *R. solani* for available nutrients in the soil surrounding the seed.

The main disadvantage to the use of L-sorbose as a seed protectant is its water solubility. It is easily leached from the critical area by percolating water. This problem can be alleviated by the cellulose acetate treatment that retards the leaching process.

L-sorbose does not persist in the soil. It is metabolized by the soil microflora and disappears quickly. Because it is nontoxic to humans and has a short residual life, it does not appear to constitute a threat to the environment. Under laboratory and greenhouse conditions it effectively deters damping-off of cotton seedlings by *R. solani*. Currently its performance in the field is being evaluated.

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