Efficacy of Sulfur for Controlling Rhizoctonia Root Rot in Sugar Beet

E. G. RUPPEL, Plant Pathologist, and R. J. HECKER, Research Geneticist, Agricultural Research Service, U.S. Department of Agriculture, Crops Research Laboratory, Colorado State University, Fort Collins 80523

ABSTRACT

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Soil application of wettable sulfur and two flowable sulfurs was tested in the greenhouse for the control of seedling damping-off of sugar beet caused by *Rhizoctonia solani*. Wettable sulfur did not control the disease. The flowables increased seedling survival over that of the control, but only in unautoclaved soil, which indicated that the effect of sulfur may have been on some other biotic system with indirect effects on the pathogen. Another biotic system was not identified; however, a biocontrol mechanism involving antagonism by *Trichoderma* spp. seemed unlikely. In a 1980 field test of the two flowables, preplant broadcast incorporated applications of both significantly reduced root rot intensity over untreated controls. In 1981, no control was obtained with one flowable at three rates and two methods of application. If the action of sulfur is on some other biotic system as greenhouse tests indicated, conditions were not conducive for such a system to be operative in the 1981 field test.

Additional key words: *Beta vulgaris*

No economical chemical is currently available to control root rot in sugar beet (*Beta vulgaris* L.) caused by *Rhizoctonia solani* Kühn, and disease incidence and severity has increased annually for the last decade (9). Potter and Schneider (15) and Schneider et al (19) reported significant reduction of rot in beet plots treated with chlorothalonil and triphenyltin hydroxide; however, as many as six weekly applications were made. In addition, their disease evaluations were based entirely on aboveground symptoms, which we have found to underestimate root rot incidence (*unpublished*), and chlorothalonil is not registered for use on sugar beet.

In 1978, sugar beet growers in western Nebraska and eastern Colorado observed an apparent decrease in the incidence of Rhizoctonia root rot wherever a flowable sulfur was used as a preplant soil-acidifying amendment (*personal communications*). Studies were therefore initiated to determine the efficacy of sulfur as a practical and relatively inexpensive control of root rot in sugar beet.

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MATERIALS AND METHODS

Greenhouse experiments. Two preliminary experiments were conducted to determine whether sulfur could control damping-off of sugar beet seedlings by *R. solani* (root rot isolate R-9; AG 2). All tests were conducted in a greenhouse maintained at 26–28 C with supplemental fluorescent light at night. The percentage of surviving seedlings was recorded 14 days after planting.

In the first experiment, field soil (pH 7.4) was mixed with peat and sand (3:1:1, v/v/v) and was used to fill 15-cm-diameter clay pots to within 5 cm of the rim; half of the pots were autoclaved for 2 hr at 1.1 kg/cm^2. After the autoclaved soil had cooled, the autoclaved and unautoclaved soils were bulked separately and ground barley-grain inoculum of *R. solani* (17) was added to both soils at a rate of 1 g/kg soil (about 82 pathogen propagules per gram of inoculum). The soil was repotted and 60 seeds of sugar beet cultivar Mono-Hy A1 were distributed over the soil surface of each pot. Additional autoclaved or unautoclaved soil infested with *R. solani* and amended with sulfur (92% wettable powder) at either 11, 22, or 44 kg of product per hectare was used to cover the seeds to a depth of 1.5 cm. The pots were irrigated immediately, and thereafter as needed. A randomized complete block design was used with four replicates; the experiment was repeated once.

In experiment 2, all procedures were similar to experiment 1, except inoculum was added at 2 g/kg soil, sugar beet hybrid (642027sl CMS × 662119s1) × FC 703 (MM) with intermediate resistance to *R. solani* was used, and two flowable sulfurs were tested. Flowable sulfur A contained 52% sulfur, whereas B contained 50% sulfur and 4.4% copper. Both flowables were mixed at a rate of 36 ml in 946 ml of water; the suspensions were sprayed over the soil surface to provide a uniform and visible coverage of sulfur. Sixty untreated sugar beet seeds were distributed over the soil surface of each pot; additional autoclaved or unautoclaved soil was used to cover the seed to a depth of 1.5 cm. The pots were irrigated immediately and thereafter as needed. A randomized complete block design was used with three replicates.

Field experiments. Irrigated field experiments were conducted in 1980 and 1981 in areas heavily infested with *R. solani* (AG 2) and, to ensure uniformity, an additional preplant broadcast application of ground barley-grain inoculum at 56 kg/ha was incorporated 10 cm deep into the experimental sites. The soil pH was 7.4 in the 1980 site and 7.8 in the 1981 site.

Randomized complete block designs with three replicates were used. Cultural procedures, plot size, and disease index (DI) calculations were as described previously (18). Briefly, a DI of 0–7 was used with 0 = no rot and 7 = plant dead. Additionally, roots in disease classes 0–3 were combined to calculate percent harvestable roots. Such roots would be recovered in the harvest.

In 1980, flowable sulfurs A and B described under greenhouse experiment 2 were preplant broadcast and incorporated at a rate of 8 L of product per hectare (5 gal/acre; 33 and 34 kg/ha, respectively). Test sugar beet cultivars included *Rhizoctonia*-resistant FC 703 and intermediately resistant commercial hybrid HH32. Untreated plots served as controls.

In 1981, three rates of flowable sulfur A were applied either broadcast or on a 10-cm band over the row 1 wk before planting; both treatments were incorporated 10 cm deep. Rates of the flowable were 2, 3, and 8 L of product per hectare (7, 13, and 34 kg/ha), respectively. Test cultivars were susceptible commercial hybrid Mono-Hy D2 and HH32. Plots not treated with sulfur served as controls.

Population density assays. Soil application of sulfur has been shown to stimulate *Trichoderma* spp. (14), some of which are reported to be biocontrol agents of *R. solani* (2,5,7). We determined the population density of *Trichoderma* spp. in the unautoclaved soil-peat-sand mix by soil dilution assay on a selective medium (6) before planting and in

sulfur-treated and untreated soil at the end of experiment 2. The population density of *R. solani* in previously autoclaved and unautoclaved sulfur-treated and untreated soil was assayed with a soil-pelleting device (10) on Ko and Hora's (11) selective medium at the end of experiment 2. Assays for both fungi also were performed preplant and postharvest on soil samples from sulfur-treated and untreated field plots in 1980 and 1981.

The arc sine transformation was used for statistical analyses of all percent data, but actual percentages are presented in the tables.

RESULTS

Greenhouse experiments. Few seedlings survived in either trial of experiment 1, regardless of sulfur concentration (Table 1). The data were quite variable, and frequent zero values of emergence precluded meaningful statistical analyses. There was a trend toward slightly greater survival in unautoclaved soil compared with that in autoclaved soil, but no rate effect was evident.

In the second experiment, a highly significant difference in seedling survival was obtained across sulfur treatments between autoclaved and unautoclaved soil, with a mean of 21% more survival in the unautoclaved soil (Table 2). Separate analyses of variance also showed that differences among treatments in autoclaved soil were not significant, whereas they were significant in unautoclaved soil. Significantly greater survival occurred where the S-Cu flowable was used compared with the untreated control, but there was no significant difference in seedling survival between the fungicides. Survival with application of flowable sulfur without copper (A) was not significantly better than the control, although 46% more seedlings survived in the treated soil than in the control. Application of sulfur at the rates used did not affect soil pH in either experiment.

Field experiments. In 1980, a significant increase in percent harvestable roots was obtained in cultivar HH32 treated with either flowable sulfur over the untreated control (Table 3). A similar but not significant trend was also observed in cultivar FC 703. The DI was reduced significantly only in HH32 by the S-Cu flowable; however, both flowables tended to lower the DI in the test cultivars compared with the appropriate controls. Across cultivars, there was no significant difference between fungicides, as measured by DI and percent harvestable roots, but both significantly reduced disease severity over the untreated controls.

In 1981, sulfur had no significant effect on root rot severity, regardless of cultivar, method of application, or dosage applied (Table 4). Treatment means across cultivars also were not significantly different.

Population density assays. The population density of *Trichoderma* spp. in the unautoclaved potting mix was <250 propagules per gram of soil before planting; after 14 days, *Trichoderma* spp. again averaged <250 propagules per gram of soil, with or without sulfur treatment. *R. solani* population densities in previously autoclaved and unautoclaved soil were about four propagules per gram of soil at the end of experiment 2, whether or not sulfur was used. Population densities of *Trichoderma* spp. and *R. solani* in the field were <500 and 0.7 propagules per gram of soil, respectively, regardless of year, sampling time, or sulfur treatment.

Table 1. Surviving Mono-Hy A1 sugar beet seedlings 14 days after planting seed in autoclaved and unautoclaved soil infested with *Rhizoctonia solani* (eight propagules per 100 g of soil) and amended with wettable sulfur

Soil treatment	92% Wettable S[a] (kg/ha)	Mean percent survival of trials 1 & 2 combined[b]
Autoclaved	0	0
	11	5.2
	22	2.1
	44	2.5
Unautoclaved	0	3.6
	11	4.8
	22	4.8
	44	3.4

[a] Soil used to cover seed was amended with wettable sulfur at rates approximating broadcast incorporated applications in the field.
[b] Four replicates per trial.

Table 2. Effect of two flowable sulfur fungicides applied as aqueous postplant sprays on survival of sugar beet seedlings 14 days after planting seed in autoclaved and unautoclaved soil infested with *Rhizoctonia solani* (eight propagules per 100 g of soil)[x]

	Percent seedling survival[z]	
Soil treatment[y]	Autoclaved	Unautoclaved
A (52% S)	2.2 a	17.7 ab
B (50% S, 4.4% Cu)	1.1 a	27.6 a
C (no fungicide)	3.2 a	8.2 b
Mean	2.2	17.8

[x] Sugar beet hybrid (642027sl CMS × 662119s1) × FC 703 (MM) having intermediate resistance to *R. solani* was used in this test.
[y] Flowable sulfurs were mixed at a rate of 36 ml in 946 ml water; the suspensions were sprayed over the soil surface to provide a uniform and visible coverage of sulfur.
[z] Survival based on percent control survival of seedlings in autoclaved or unautoclaved soil without the addition of *R. solani* inoculum. Means of three replicates; means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 3. Effect of two flowable sulfur fungicides on severity of Rhizoctonia root rot in two field-grown sugar beet cultivars (1980)[v]

	Disease index[x]			Percent harvestable roots[y]		
Soil treatment[w]	FC 703[z]	HH32[z]	$\bar{x}$	FC 703	HH32	$\bar{x}$
A (52% S)	1.6 a	2.9 ab	2.3 b	89.1 a	64.5 b	76.8 a
B (50% S, 4.4% Cu)	1.7 a	2.6 b	2.2 b	91.2 a	68.5 b	79.9 a
C (no fungicide)	2.4 a	4.0 a	3.2 a	75.2 a	41.1 a	58.2 b

[v] Values are means of three replicates. Means within columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.
[w] Fungicides were applied broadcast with incorporation at 33–34 kg/ha 1 wk before planting.
[x] Disease index on a scale of 0–7, with 0 = no rot and 7 = plant dead.
[y] Percent harvestable calculated by combining disease index classes 0–3.
[z] FC 703 = Rhizoctonia-resistant breeding line; HH32 = commercial hybrid with intermediate resistance to *Rhizoctonia*.

Table 4. Effect of flowable sulfur at three rates and two methods of application on severity of Rhizoctonia root rot in two field-grown sugar beet cultivars (1981)[v]

	Sulfur	Disease index[x]			Percent harvestable roots[y]		
Application[w]	(kg/ha)	D2[z]	HH32[x]	$\bar{x}$	D2	HH32	$\bar{x}$
...	0	4.6	3.7	4.2	33.4	50.5	42.0
Broadcast	7	5.6	3.4	4.5	13.0	53.1	33.1
	13	5.2	3.4	4.3	21.2	58.8	40.0
	34	5.9	2.7	4.3	13.9	69.0	41.5
Banded	7	5.0	4.3	4.7	24.6	41.6	33.1
	13	5.5	3.6	4.6	15.5	54.1	34.8
	34	4.7	3.6	4.2	30.8	56.6	43.7

[v] Means of three replicates; means within columns not significantly different from each other at $P = 0.05$ according to Duncan's multiple range test.
[w] Applications made preplant with 10-cm incorporation. For the banded application, the aqueous fungicide suspension was applied on a 10-cm band over the row.
[x] See footnotes Table 3.
[y] See footnotes Table 3.
[z] D2 = Mono-Hy D2 = commercial hybrid susceptible to *Rhizoctonia solani*; HH32 = commercial hybrid with intermediate resistance.

DISCUSSION

Wettable sulfur did not control *Rhizoctonia* seedling damping-off in the greenhouse (Table 1). Because particle size of sulfur is known to affect its toxicity (21), we tested flowable sulfurs in which particle size is less than 1 μm. We did obtain a decrease in damping-off with two flowables, but only in unautoclaved soil (Table 2); a similar effect with unautoclaved soil was observed with wettable sulfur in experiment 1. The increased efficacy of sulfur in unautoclaved versus autoclaved soil may indicate that the effect was not on *Rhizoctonia* per se, but that some other biotic system was affected, which in turn affected the pathogen. On the other hand, the inoculum potential of *R. solani* may have been much greater in unautoclaved versus autoclaved soil; however, our population density assays do not support this premise.

Partial control of sugar beet damping-off with sulfur is not easily explained. Control of Fomes root rot in *Hevea* with sulfur was thought to be caused by stimulation of antagonistic *Trichoderma* spp. by the resultant lowered soil pH (14). Several *Trichoderma* spp., known biocontrol agents of *Rhizoctonia* (1), produce antibiotics, gliotoxin and viridin, both of which are stable only in an acid medium (8). In the absence of antibiotic production, some *Trichoderma* spp. also can directly parasitize fungal pathogens (5,7), but such mechanisms seemed unlikely in our greenhouse experiments because the small additions of sulfur did not affect soil pH, and no change in *Trichoderma* populations could be detected in unautoclaved soil between the beginning and conclusion of experiment 2. Furthermore, *Trichoderma* populations were extremely low (<250 propagules per gram of soil); apparently, more than 10^5 propagules per gram are needed for effective biocontrol of *Rhizoctonia* in greenhouse tests in alkaline soil (R. Baker, *personal communication*).

The inconsistent results obtained with sulfur in the field probably are related to differences in soil environment between the 1980 and 1981 experimental sites. If the action of sulfur is on some other biotic system, as our greenhouse tests indicate, that system must be present and the environment must be suitable for its activation or proliferation. Conditions evidently were not conducive for such a system in our 1981 field test.

As in our greenhouse experiments, partial control of Rhizoctonia root rot in the field in 1980 (Table 3) probably did not involve *Trichoderma* as a biocontrol system. Soil populations of *Trichoderma* spp. were <500 propagules per gram of soil at planting and harvest. Soil reaction of pH 7.4 also was not conducive to the enhancement of *Trichoderma* as a potential biocontrol agent (12); however, other hyperparasites or antagonists of *R. solani* may be involved (2–4, 13, 16, 20). Elucidation of the biotic system that was seemingly operative in our greenhouse and 1980 field tests may provide a new approach to future control of Rhizoctonia root rot in sugar beet.

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