Inhibition of Soybean Hypocotyl Elongation by *Rhizoctonia solani*

C. R. Grau and C. A. Martinson

Former research assistant and associate professor, Department of Botany and Plant Pathology, Iowa State University, Ames, 50011.
Present address of senior author: Department of Plant Pathology, University of Wisconsin, Madison 53706.
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ABSTRACT

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Rhizoctonia solani reduced rates of hypocotyl elongation in some soybean cultivars and consequently delayed seedling emergence in artificially-infested soil:sand:vermiculite and vermiculite growth media. Inhibition of hypocotyl elongation differed with soybean cultivars, isolates of *R. solani*, and temperature. Maximum reduction occurred at 25 C, but none at 30 C. Short, swollen hypocotyls with a brownish discoloration were the symptoms associated with the condition; however, lesion formation on the lower region of the hypocotyl was not associated closely with it. Reduced hypocotyl elongation and increased time for seedling emergence was not related to the effect of temperature on the initial rate of germination. It was theorized that seed coat invasion by *R. solani* near the meristematic region in the hypocotyl hook of the seedling influenced hypocotyl elongation. Removal of the seed coat before planting increased the rate of hypocotyl elongation compared with that of seedlings with seed coats present. Water extracts from *R. solani*-infected seed coat tissue but not from healthy tissue reduced hypocotyl elongation.

Additional key words: Glycine max, Thanatephorus cucumeris, seedling diseases.

Rhizoctonia solani Kuehn (*Thanatephorus cucumeris* [Frank] Donk) causes seed decay, pre-emergence and postemergence damping-off, root rot, basal stem rot, and foliage blight of soybean (*Glycine max* [L.] Merr. (4). Seedling stand reduction in soybean due to *R. solani* has averaged 10%, and occasionally has reached 50%, in Iowa (16).

During studies of soybean damping-off caused by *R. solani*, we observed that apparently noninfected seedlings emerged more slowly in soil lightly infested with *R. solani* than in noninfested soil. This occurred even with low inoculum densities and few, if any, sunken, water-soaked lesions were evident on the hypocotyls. Klein (10) observed delayed emergence of soybean seedlings in *R. solani*-infested soil but did not study cause-and-effect relationships. Delayed seedling emergence without lesions is not part of the syndrome reported for diseases caused by *R. solani*. Strissel and Dunleavy (15) observed stunting of soybean cultivars by *Pythium debaryanum*. Both Klein (10) and Strissel and Dunleavy (15) reported that epicotyls of seedlings were completely destroyed. Reduction of rates of hypocotyl elongation increases the time of exposure of seedlings to soil-borne plant pathogens and increases the probability of damping-off and seedling root rots. Our purposes were to test the hypothesis that *R. solani* can delay seedling emergence in the absence of significant symptoms and to ascertain factors that contribute to this delay.

MATERIALS AND METHODS

Anastomosing group (AG) determinations of *Rhizoctonia solani* isolates were made by N. A. Anderson, University of Minnesota. The *Rhizoctonia solani* isolates used in these experiments were: AG-4a (from *Capsicum frutescens* L.), AG-4b (host unknown), and AG-1 (host unknown) from Lois H. Tiffany; isolate AG-4c (from *Phaseolus vulgaris* L.) from Krishna Prasad; and AG-4d (from *G. max*) from J. C. Horton. All isolates were capable of causing sunken necrotic lesions on soybean hypocotyls. The criteria established by Parmeter and Whitney (12) were used to confirm the identity of isolates.

Rhizoctonia solani was grown on potato extract-sucrose broth (PSB). Erlenmeyer flasks (250 ml) containing 50 ml of PSB, were seeded with a 7-mm diameter inoculum disk taken from the margin of a *R. solani* colony growing on Difco potato-dextrose agar (PDA).

Two-year-old Hawkeye and Ford soybean seed was used in the experiments. Seedling vigor tests indicated that the 2-yr-old seed were comparable to new seed.

The procedure used for the infestation of the growing medium with *R. solani* was as follows: Seven-day-old mycelial mats of *R. solani*, grown on PSB, were washed three times (~30 sec each time) under running tap water, blotted uniformly between paper towels, and weighed. Weighed portions of the mycelium (~4.0 g fr wt) were fragmented 30 sec at high speed in 800 ml of distilled-deionized water in a Waring Blendor. The hyphal suspension was mixed thoroughly in 3,200 cm^3 of commercial dry vermiculite to give a uniformly infested planting medium. Controls were prepared by adding 800 ml of distilled-deionized water only to vermiculite.

Soybean seeds were surface-disinfested in 0.5% NaOCl for 15 min and then rinsed under running distilled water for 2 min. Soybean seeds with cracked seed coats and those that had imbibed excessive amounts of water during surface disinfestation were discarded. About 800 cm^3 of infested planting medium was placed in polystyrene boxes (18 × 26 × 10 cm deep) and leveled to form a seedbed. Seeds were planted in five rows per box and 10 seeds per row. A uniform planting depth of 5 cm was obtained by covering seeds with 2,400 cm^3 of the planting medium. The boxes were covered with polystyrene lids, which were not air tight. Germination was allowed to proceed during incubation in growth chambers at 25 C and continuous darkness for 120 hr. The seedlings were removed from the planting medium, and hypocotyl lengths (from the cotyledonary node to the first lateral root) were measured. The point of the first lateral root coincided with the upper border of the region where 0.1% trypan blue in 10% acetic acid ceased to stain the root tissues.

The experimental data were analyzed by the standard error of the mean and the "D" test (14) at $P = 0.05$.

RESULTS

Soybean hypocotyl symptoms associated with decreased rates of seedling emergence. Hypocotyl symptoms associated with the slower rate of soybean seedling emergence in the presence of

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Rhizoctonia solani (AG-4d) were reduced length, increased thickness, superficial brown discoloration, and occasional twisting (Fig. 1). Formation of necrotic lesions on the hypocotyl ranged from extensive to none; symptoms associated with slower emergence developed independently of the typical sunken hypocotyl lesions normally produced by *R. solani*. Brownish surface lesions that involved only epidermal and outer cortical cells occasionally developed on the underside of the hypocotyl hook and extended basipetally, which suggested a prolonged association of the pathogen with that tissue. Seed coat tissue invaded by *R. solani* had a brownish discoloration and was macerated. Infected seed coats remained attached to cotyledons after emergence.

Factors associated with reduced hypocotyl elongation of soybean seedlings grown in the presence of Rhizoctonia solani. *Fungal isolates.* Hawkeye and Ford soybean cultivars were grown at 25 C for 120 hr in continuous darkness and in the presence or absence of five isolates of *R. solani*. Hypocotyls of Hawkeye soybean seedlings exposed to *R. solani* isolates AG-4a, AG-4b, AG-1, AG-4c, and AG-4d elongated 10, 29, 41, 42, and 47% less, respectively, than the uninoculated controls (Fig. 2). For Ford soybean, the mean reduction in hypocotyl lengths (compared with uninoculated controls) caused by the five isolates ranged from 32 to 53%.

Soybean cultivars. Boosalis (2) reported that soybean cultivars do not differ in resistance to hypocotyl infection by specific isolates of *R. solani*. The cultivars Hawkeye, Hark, Harosoy, and Corsoy and a black seed-coat mutant of the cultivar Clark were evaluated for resistance to the hypocotyl effect caused by *R. solani*. Seeds from each cultivar were planted in vermiculite in the presence or absence of *R. solani* (AG-4d) and grown at 25 C for 120 hr in continuous darkness. *Rhizoctonia solani* caused a 26 and 34% reduction in hypocotyl lenth of the cultivars Hawkeye and Hark, respectively; average measurements for Clark (black and seed coat mutant), Harosoy, and Corsoy were not statistically different from those of the control seedlings (Table 1). Seed coats of all cultivars were colonized by *R. solani*.

Field soil in the growing medium. Isolates AG-4a and AG-4d of *R. solani* were compared in a growing medium containing sand:field loam soil:vermiculite (1:2:1, v/v). Vermiculite was infested with 4 g fr wt of fragmented mycelium suspended in 200 ml of distilled deionized water. The length of hypocotyls of Hawkeye seedlings grown in infested and noninfested (control) growing medium for 120 hr at 25 C and continuous darkness was compared. No damping-off occurred in the noninfested controls. The length of hypocotyls for seedlings grown in the presence of *R. solani* isolate AG-4d were reduced in hypocotyl length by 17% as compared with uninoculated controls, whereas hypocotyls of seedlings grown in the presence of isolate AG-4a were not reduced in growth.

Relation of seedling emergence to rate of seed germination. The effect of *R. solani* on soybean seed germination was investigated to determine whether reduced hypocotyl length could be attributed to a delay in seed germination rather than to a reduction in hypocotyl elongation. Hawkeye soybean seeds were planted in vermiculite infested with *R. solani* (AG-4d) and noninfested vermiculite in 720-ml plastic cups (20 seeds per cup). The seeds were incubated at 25 C in constant darkness, and the percent germination in five-cup samples was determined periodically. A seed was considered germinated when the radicle had emerged through the seed coat. The percentages of germination 24, 30, 36, and 48 hr after planting were 7, 31, 66, and 95, respectively, for controls and 5, 37, 69, and 95, respectively, in the presence of *R. solani* (AG-4d). Thus, the delay in emergence associated with *R. solani* was not attributed to a slower rate of seed germination.

Temperature. Initial studies indicated there was no inhibition of soybean hypocotyl elongation by *R. solani* at 30 C. Hawkeye soybean seedlings were grown in infested and noninfested vermiculite for 120 hr at 23, 25, 27, 30 C and alternating temperatures of 12 hr at 25 C and 12 hr at 30 C. At 20 C the growth period was 192 hr. Maximum inhibition of hypocotyl elongation caused by *R. solani* was at 25 C and none occurred at 30 C (Fig. 3); statistically significant inhibition of hypocotyl elongation by *R.*

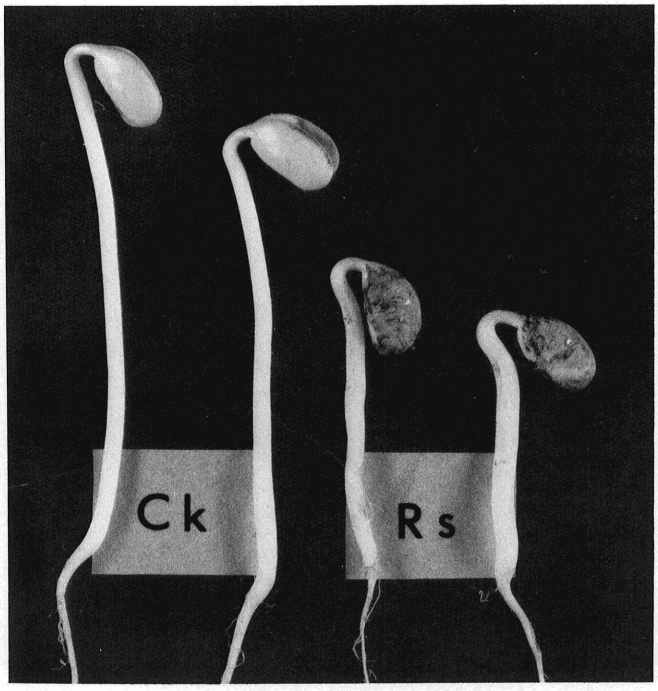

Fig. 1. Hypocotyl symptoms on Hawkeye soybean seedlings grown in the presence (Rs) and absence (Ck) of *Rhizoctonia solani* (AG-4d) at 25 C.

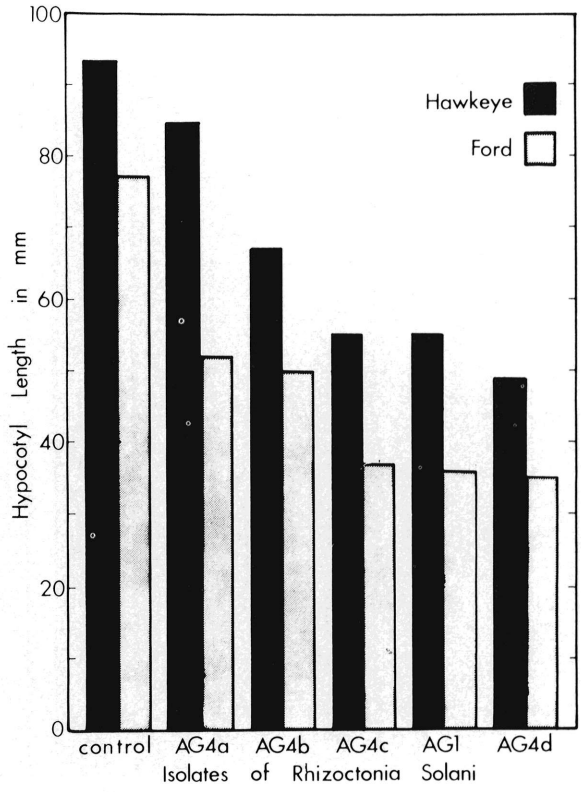

Fig. 2. Mean hypocotyl length (mm) of seedlings of soybean cultivars Hawkeye and Ford grown at 25 C for 120 hr in the presence of five isolates of *Rhizoctonia solani*. Mean hypocotyl lengths of seedlings grown in the presence of five isolates of *R. solani* were statistically different (P=0.05) from the means of the control except for isolate AG-4a with cultivar Hawkeye.

solani occurred at all temperatures below 30 C, including the regime in which the temperature alternated between 25 and 30 C.

In a separate experiment, hypocotyl lengths were measured 48, 72, and 120 hr after planting soybeans in *R. solani* (AG-4d)-infested and noninfested vermiculite which was incubated at 25 and 30 C. Soybean hypocotyl growth in the infested medium at 25 C and 48, 72, and 120 hr after planting was 38, 41, and 44%, respectively, less than the growth of the noninfested controls (Fig. 4). Reduction in hypocotyl length at 30 C was 3% at 48 and 72 hr and ~0% after 120 hr. The length of control seedling hypocotyls at 25 and 30 C was nearly identical up to 72 hr; after 120 hr, however, the growth was greater at 30 C than at 25 C.

Soybean seed coat. Seed coat tissue invaded by *R. solani* at 25 and 30 C was brown and macerated (Fig. 1); however, infected seed coats remained attached to cotyledons during seedling emergence at 25 C, but not at 30 C. Seed coats invaded by *R. solani* were stained with 0.1% trypan blue (in 10% acetic acid) to facilitate microscopic observation of hyphae. No difference in the extent of hyphal colonization of the seed coat by *R. solani* was observed at 25 and 30 C.

The effect of seed coat infection on reduction of hypocotyl length was studied by planting soybean seed with and without seed coats in infested (*R. solani* AG-4d) and noninfested vermiculite. A batch of Hawkeye soybean seeds were allowed to imbibe distilled deionized water for 30 min to facilitate removal of seed coats, then divided into two groups. Seed coats of one group of the seeds were removed without damage to the radicle, those of the other group (controls) were left with the seed coats intact. The seedlings were grown in noninfested and *R. solani* (AG-4d)-infested vermiculite at 25 C for 96 hr in darkness. In noninfested vermiculite, the rates of hypocotyl elongation were identical for seedlings grown from intact seed and those from seed with seed coats removed. However, in the presence of *R. solani*, the mean hypocotyl length for seedlings from intact seed was reduced 44% (compared to noninfested controls), whereas there was a reduction of only 13% in seedlings grown from coatless seed (Table 2). Few hyphae and lesions caused by *R. solani* were observed on the cotyledons of seedlings grown from coatless seed.

Toxins produced in soybean seed coat tissue invaded by Rhizoctonia solani. Because *R. solani* produces toxins and growth regulators (1,3,17,18), distilled water extracts were made from soybean seed coat tissues infected by *R. solani* to assay for water-soluble substances that might reduce hypocotyl elongation. Five-day-old healthy and *R. solani* (AG-4d)-invaded seed coats from Hawkeye soybean seedlings grown at 25 C were fragmented (10 g of seed coats in 25 ml of distilled deionized water) in a Waring Blendor for 1 min. The water extract was filtered through four layers of cheesecloth, through Whatman No. 2 filter paper in a Büchner funnel and then through a Seitz filter. The extracts (both pH 6.7) were stored at 4 C for 2 days.

The extracts from seed coat tissue invaded by *R. solani* were compared with deionized water and extracts from healthy seed coat

TABLE 1. Mean hypocotyl length of five soybean cultivars grown at 25 C for 120 hr in the presence or absence of *Rhizoctonia solani* isolate AG-4d in continuous darkness

	Hypocotyl length (mm)		
Cultivar	Control	*R. solani*	Studentized[b] range D =
Hawkeye	84	62	6
Hark	87	58	8
Harosoy	82	86	9
Corsoy	108	107	10
Clark	66	56	12

[a] Mean of two replicates of 83–98 seedlings per replicate.
[b] Studentized range test (14) was used to compare treatment means at $P = 0.05$.

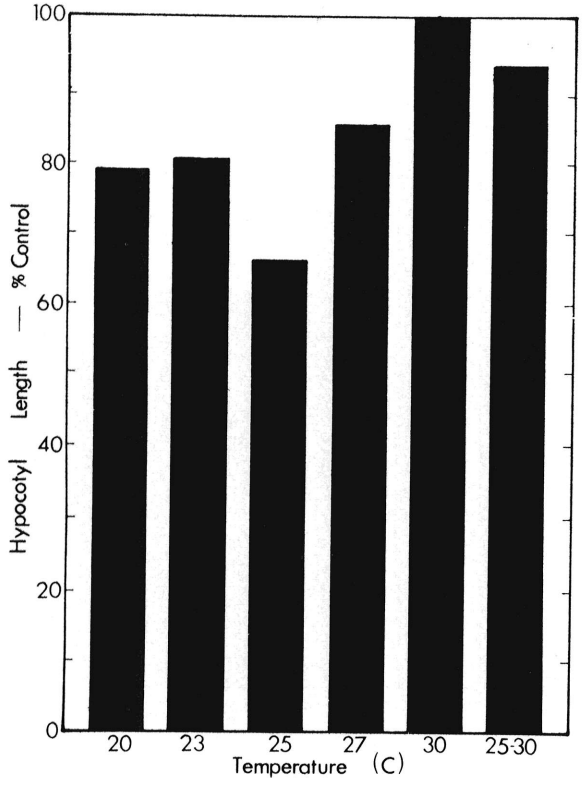

Fig. 3. Hypocotyl lengths (percentage of noninfested control) of Hawkeye soybean seedlings grown at six temperatures (C) for 120 hr in the presence of *Rhizoctonia solani* (AG-4d). Mean hypocotyl lengths of seedlings in the presence of *R. solani* were statistically different (P=0.05) from the mean of the noninfested control except for the 30 C temperature.

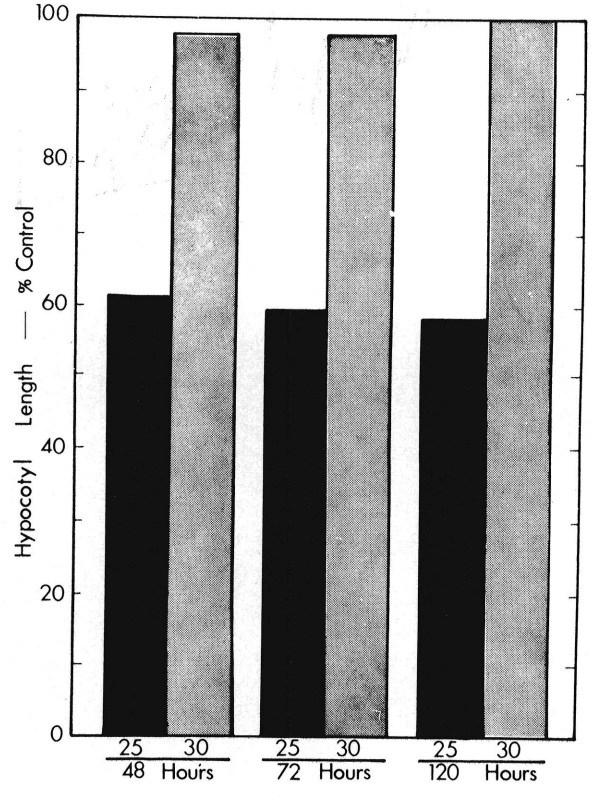

Fig. 4. Hypocotyl lengths (percentage of noninfested control) of cultivar Hawkeye soybean seedlings grown in the presence of *Rhizoctonia solani* at 25 and 30 C and measured 48, 72, and 120 hr after planting. Mean hypocotyl lengths in the presence of *R. solani* were statistically different (P=0.05) from the mean of the control at 25 C but not at 30 C at all time intervals.

controls. Soybean seeds were allowed to imbibe the test solution for 40 min at 22 C and then were planted in vermiculite and grown at 25 C for 120 hr in darkness. A control of unimbibed seed also was included.

The hypocotyl lengths of seedlings from unimbibed, water imbibed, and healthy seed coat extract imbibed seed were similar, but the lengths of hypocotyls of seedlings grown from seed that had imbibed the extract from the *R. solani*-infected seed coats were reduced 16% (Table 3).

DISCUSSION

The delayed emergence of soybean seedlings in the presence of *R. solani* originally observed by Klein (10) was caused by a reduced rate of hypocotyl elongation and not by delayed germination. *Rhizoctonia solani* caused this stunting of certain soybean cultivars primarily when it grew on the seed coat tissues of emerging seedlings. Removal of seed coats prior to planting reduced the hypocotyl-inhibiting effects of *R. solani*. The hypocotyl-stunting syndrome which was reproduced by water extracts of *R. solani*-infected seed coats suggests the possibility of phytotoxin or growth regulator activity. Some *R. solani* isolates produced auxins (3) and phenylacetic acid (PPA) (1,11), m-hydroxyphenylacetic acid (m-HPAA) (1,11), and p-hydroxyphenylacetic acid (p-HPAA) (1,11) which cause stunting in seedling bioassays for phyrorozina. Isolate AG-4d produced auxin like products and ethylene, but PPA, m-HPAA, and p-HPPA were not detected (*unpublished*). Although seed coats were colonized by *R. solani* at 25 and 30 C, seed coats did not readily separate from cotyledons during emergence at 25 C; thus, at that temperature, the apical meristem of a soybean seedling was exposed to metabolites of *R. solani* for a longer time.

Soybean cultivars differ in rates of hypocotyl elongation at 25 C (6,7,9), and cultivars have been characterized as short, intermediate, and long types with better field emergence from deep planted seeds associated with the long type (6,7,9). At 30 C, the short and intermediate types elongate at the same rate as long types (6,7,9) and all emerge well following deep-seeding. Cultivars Corsoy, Harosoy, and Hawkeye are long-hypocotyl types, but only Hawkeye was inhibited in elongation at 25 C by *R. solani*. Ford (short-hypocotyl type) and Hark (unclassified) also were inhibited in hypocotyl elongation at 25 C. The ability of *R. solani* to cause inhibition of hypocotyl elongation at 25 C but not at 30 C suggests that *R. solani* is inducing or enhancing some temperature-regulated growth phenomenon in soybean hypocotyls. Samimy and LaMotte (13) related the inhibition of soybean hypocotyl elongation at 25 C to high ethylene production by short hypocotyl types at 25 C. The symptoms induced by *R. solani* at 25 C (stunted and swollen hypocotyls) were identical to those described by Samimy and LaMotte (13).

Boosalis (2) concluded that soybean cultivars do not differ in susceptibility to a specific isolate of *R. solani* if total emergence and lesion formation on hypocotyls are the disease reactions evaluated. Because we observed soybean cultivars in which hypocotyl elongation was not inhibited by *R. solani*, selection for resistance to *R. solani* and that disease reaction may be possible. Field emergence of soybeans is a heritable trait (8). Fehr (5) also found the long, intermediate, and short hypocotyl elongation characteristics to be heritable in soybean. In our experiments, the presence of *R. solani* changed the phenotypic expression of cultivar Hawkeye but not that of cultivars Harosoy and Corsoy at 25 C. No relationship is known between hypocotyl type and resistance to hypocotyl inhibition by *R. solani*.

TABLE 2. Mean hypocotyl lengths of Hawkeye soybean seedlings from seed with seed coats present or removed and grown in the prescence or absence of *Rhizoctonia solani* (AG-4d) at 25 C for 96 hr in continuous darkness

Treatment	Hypocotyl length (mm)[a]		
	Seed coat present	Seed coat removed	Studentized[b] range D =
Control	94	93	11
R. solani	53	81	10
Studentized[b] range D =	10	11	

[a] Mean of 40–49 seedlings and repeated twice.
[b] Studentized range test (14) for the comparison of treatment means at $P = 0.05$.

TABLE 3. Mean hypocotyl length of Hawkeye soybean seedlings treated with water extracts from healthy and *Rhizoctonia solani* (AG-4d)-infected seed coat tissue and grown at 25 C for 120 hr in continuous darkness

Treatment	Hypocotyl length (mm)
No imbibition	105[a]
Water control	106[a]
Healthy seed coat extract	106[b]
R. solani infected seed coat extract	89[b]
Studentized range D = [c]	8

[a] Mean of 50 seedlings repeated once.
[b] Mean of 99 seedlings repeated once.
[c] Studentized range test (14) and for the comparison of treatment means at $P = 0.05$.

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