Relationship of Plant Age, Cultivar, and Isolate of *Rhizoctonia solani* AG-2-2 to Sugar Beet Root and Crown Rot

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ABSTRACT

Engelkes, C. A., and Windels, C. E. 1994. Relationship of plant age, cultivar, and isolate of *Rhizoctonia solani* AG-2-2 to sugar beet root and crown rot. Plant Dis. 78:685-689.

Roots of cultivars Maribo Ultramono (MU, susceptible) and ACH 184 (moderately resistant) of sugar beet (*Beta vulgaris*) were inoculated with two AG-2-2 isolates of *Rhizoctonia solani* (from sugar beet and pinto bean [*Phaseolus vulgaris*]) at 6, 8, 10, and 12 wk after planting. Two procedures were followed to inoculate roots at four ages. Seeds were planted on the same day and roots were inoculated at 2-wk intervals (consecutive inoculations); and seeds were planted at 2-wk intervals and roots were inoculated on the same day (simultaneous inoculations) in 1990–1991 field trials. From 2 to 8 wk after inoculation, roots were rated for disease (0–7 scale) at 2-wk intervals. Root rot severity was about two disease ratings higher at each evaluation in 1991 than in 1990. In three of four trials, disease decreased as root age at time of inoculation increased. In four trials, MU had a higher root rot rating (4.5) than ACH 184 (3.6); and in three trials, the *R. solani* isolate from pinto bean gave a higher root rot rating (4.9) than the isolate from sugar beet (3.8). In culture, the former isolate grew faster by 0.01–8.0 mm/24 hr at 25–35 C than the latter isolate. Average weekly air temperatures were between 25 and 35 C for at least 4 of 8 wk following inoculations in the field. Overall, the least amount of Rhizoctonia root and crown rot occurred on the moderately resistant sugar beet cultivar as plant age increased.

Rhizoctonia solani Kühn AG-2-2 causes damping-off of seedlings and root and crown rot of sugar beet (*Beta vulgaris* L.) in Minnesota and North Dakota (19). Aboveground symptoms of root and crown rot include yellowing, sudden wilting of leaves, and blackening of petioles (18). Below ground, a dark brown-gray rot starts near the crown and spreads over the root surface. Disease symptoms first appear in fields in mid- to late-June, about 6–8 wk after planting, and continue to develop throughout the season. *R. solani* grows from 12 to 35 C, with AG-2-2 having an optimum of 25–28 C (8). The severity of Rhizoctonia diseases on various crops has been positively correlated with temperatures favorable for growth of the pathogen (1,7,11,17).

Rhizoctonia root and crown rot was more severe in field plots at the Northwest Experiment Station in 1989 when sugar beet roots were inoculated 8 wk after planting than in 1988 when the roots were inoculated 12 wk after planting (2). Some researchers have observed an increase in resistance to Rhizoctonia diseases on sugar beet (3,12), corn (11), bean (11,17), oilseed rape (20), and canola (20) with increasing plant age at time of inoculation. Others have observed that sugar beet (4) and carrot (7) remained susceptible throughout the season.

The objectives of this study were to determine 1) the effects of sugar beet age at time of inoculation, sugar beet cultivar, and isolate of *R. solani* AG-2-2 on severity of root and crown rot in the field and 2) the temperature range most favorable for growth of the AG-2-2 isolates tested in the field, and to relate these growth rates to air temperatures during the field trials.

MATERIALS AND METHODS

Isolates. The two AG-2-2 cultures of *R. solani* originally were isolated from sugar beet and pinto bean (*Phaseolus vulgaris* L.) in 1987. The isolates were stored on autoclaved barley grain at 5 ± 0.5 C (13) in tubes sealed with cigarette paper (16) and stainless steel caps, and transferred annually.

Field plot conditions. Experimental plots were at the Northwest Experiment Station, University of Minnesota, Crookston, in a Bearden silty clay loam soil in 1990 and in a Glyndon very fine sandy loam soil in 1991. Seeds (provided by American Crystal Sugar Co., Moorhead, MN) of cultivars Maribo Ultramono (susceptible to *R. solani*) and ACH 184 (moderately resistant) were treated

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Contribution No. 19,687 of the Minnesota Agricultural Experiment Station based on research supported by the Station and by a grant from the Sugarbeet Research and Education Board of Minnesota and North Dakota, Fargo 58105.

Accepted for publication 11 April 1994.

with metalaxyl (Apron, 325 mg a.i./kg). Three hundred seeds of each cultivar were planted per 9-m row in four-row plots (56 cm apart) with a cone planter. Plots were fertilized according to soil-test recommendations for a yield goal of 44.8 t/ha (10). To control the sugar beet root maggot, chlorpyrifos (Lorsban 15G, 2.24 kg a.i./ha) was applied in-furrow at planting. Plants were thinned to 20 cm at 4 wk after seeding.

Inoculation. Inoculum was grown on kernels of dent corn that were soaked in distilled water for 12 hr, drained, and autoclaved at 121 C for 60 min on two consecutive days (7,15). Two 1.5-cm-diameter disks from the margin of a 5-day-old colony of *R. solani* on acidified potato-dextrose agar (APDA) were placed in each jar (473 ml) containing about 200 kernels. Two disks of APDA were placed in jars containing kernels for the control. Inoculum was incubated at 25 ± 5 C for 10–21 days and shaken every 2 days.

Two procedures were followed in 1990 and 1991 to inoculate sugar beet roots at four ages. In one procedure, seeds were planted on 14 May 1990 and 9 May 1991, and roots were inoculated at 6, 8, 10, and 12 wk after planting (consecutive inoculation). In the other procedure, seeds were planted at 2-wk intervals, and roots were inoculated on 30 July 1990 and 1 August 1991 at 6, 8, 10, and 12 wk after planting (simultaneous inoculation). A multiple split-plot design with three replicates was used. Ten plants were inoculated per treatment by placing two *Rhizoctonia*-infested corn kernels adjacent to the root, 3 cm below the soil surface. Two sterile corn kernels were placed adjacent to roots of control plants in 1990 but not in 1991, because no disease developed on controls in 1988–1989 field trials (2). Soil was hilled around crowns of sugar beet to favor disease development (14). The entire plot was irrigated with 2.5 cm of water each week by overhead sprinkler in 1990 and by irrigation tape in 1991. Daily minimum and maximum air temperatures were recorded by a weather station at the Northwest Experiment Station.

Evaluation. The roots were hand-harvested and evaluated at approximately 2, 4, 6, and 8 wk after inoculation. Soil was removed and percent root surface area rotted was rated on a 0–7 scale, where 0 = no visible lesions; 1 = superficial, arrested lesions at point of inoculation; 2 = shallow, dry rot canker, <5%; 3 = deep, dry rot canker, 5–24%; 4 = extensive rot, 25–49%; 5 = rot extending well into root interior, 50–74%; 6 = most foliage dead, 75 to <100%; and 7 = plant dead, 100% (E. G. Ruppel, USDA-ARS Crops Research Laboratory, Fort Collins, CO, *personal communication*).

Root rot occurred on a few control plants in 1990. Four 1.5-cm-diameter cores were removed from each of these roots, surface-treated in 0.5% NaOCl for 30 sec, rinsed twice in sterile distilled water, and placed on APDA. *R. solani* isolates then were hyphal-tipped and morphologically identified to AG by colony appearance on APDA. For confirmation, 10% of the reisolated cultures (randomly selected) were paired with AG test cultures (9,19) provided by N. A. Anderson (University of Minnesota, St. Paul) and A. Ogoshi (Hokkaido University, Sapporo, Japan). At least two slides were examined to verify each identification.

Hyphal growth of *R. solani* AG-2-2. A 0.9-cm-diameter disk from the margin of a 5-day-old colony of *R. solani* (same AG-2-2 isolates tested in the field) on APDA was transferred to the edge of a 100 × 15 mm petri dish containing 20 ml of PDA, placed in plastic boxes (40 × 27 × 17 cm), and incubated at 15, 20, 25, 30, and 35 C. After 24 hr (to allow the agar and fungus to equilibrate to incubator temperatures), a baseline was drawn on the bottom of a petri dish at the colony margin. Hyphal growth then was measured from the baseline to the colony margin after 24, 48, and 72 hr. Cultures at 15 and 35 C (incubated for 72 hr after establishing the baseline) were transferred to 25 C for 24 hr to establish a new baseline. Hyphal growth was measured from the baseline to the margin of the colony 24 hr later. Each of the two incubator-growth trials was a split-plot design with four replicates.

Data analyses. For analyses of root rot ratings (6), plant age at inoculation was the main plot variable; sugar beet cultivar was the subplot variable; AG-2-2 isolate was the sub-subplot variable; and time of disease evaluation was the sub-sub-subplot variable. Analyses of variance were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, NC) general linear model (GLM) procedure for each trial. Data for control plants were near zero and therefore excluded (6). The sum of squares for each of the four plot variables

Table 1. Analyses of variance for effects of plant age, sugar beet cultivar, *Rhizoctonia solani* AG-2-2 isolate, and time of disease evaluation on root rot severity of plants inoculated consecutively and simultaneously in 1990 and 1991 field trials

		Consecutive inoculation				Simultaneous inoculation			
		1990		1991		1990		1991	
Source of variation	df	SS[a]	TSS(%)[b]	SS[a]	TSS(%)[b]	SS[a]	TSS(%)[b]	SS[a]	TSS(%)[b]
Total	1,919	14,389.70		8,354.27		7,854.67		6,943.20	
Replicate	2	2.23		25.37		121.49		17.05	
Plant age (A)	3	9,460.44**	87.5	2,226.00**	39.7	479.11*	17.3	1,408.59**	31.0
Error a	6	37.65		53.63		188.48		25.67	
Cultivar (C)	1	153.00**	1.4	300.51**	5.4	626.78**	22.6	482.00**	10.6
A × C	3	108.27*	1.0	81.69**	1.5	98.62	3.6	81.34**	1.8
Error b	8	41.72		10.14		354.73		22.23	
AG-2-2 isolate (I)	1	31.78	0.3	947.20**	16.9	422.81**	15.3	480.00**	10.6
A × I	3	189.29*	1.8	431.39**	7.7	88.00*	3.2	77.68	1.7
C × I	1	14.88	0.1	35.10**	0.6	18.41	0.7	58.80*	1.3
A × C × I	3	26.84	0.2	15.86*	0.3	27.40	1.0	0.16	0
Error c	16	273.34		17.02		116.93		144.13	
Evaluation time (E)	3	327.67**	3.0	1,229.98**	21.9	573.06**	20.7	1,601.19**	35.3
A × E	9	117.61*	1.1	71.92**	1.3	54.98	2.0	113.24**	2.5
C × E	3	57.08*	0.5	15.43	0.3	159.76**	5.8	46.28*	1.0
I × E	3	47.25	0.4	48.18**	0.9	39.30	1.4	19.02	0.4
A × C × E	9	48.79	0.5	59.70**	1.1	30.18	1.1	96.14**	2.1
A × I × E	9	126.62*	1.2	93.17**	1.7	96.47	3.5	19.75	0.4
C × I × E	3	13.62	0.1	16.00	0.3	24.93	0.9	29.58	0.7
A × C × I × E	9	86.55	0.8	40.15*	0.7	27.54	1.0	25.11	0.6
Error d	96	573.63		214.74		528.74		373.65	
Sampling error	1,728	2,651.45		2,421.24		3,776.95		1,821.60	

[a] Sum of squares, significant at ** = $P \leq 0.01$ and * = $P \leq 0.05$.

[b] Percent variation in root rot severity accounted for by partitioning total treatment sum of squares (TSS) into main plot and interaction treatment variables (5).

and interactions was divided by the total treatment sum of squares in each of the four trials to determine the percent variation in root rot severity that was accounted for by each variable (5). For the plant-age and evaluation-time variables, the sum of squares of each was partitioned into linear, quadratic, and residual trend components. For the trend component with the highest contrast sum of squares and F value, the regression equation and coefficient of determination (r^2) were calculated based on average root rot ratings of each variable.

For analyses of hyphal growth, temperature was the main plot variable, and AG-2-2 isolate was the subplot variable. Analyses of variance were performed with the Statistical Analysis System GLM procedure. Data from the two incubator-growth trials were combined for analyses because error b variances of the trials were homogeneous (Bartlett's test criterion $F = s_1^2/s_2^2$, where s_1^2 is the larger error mean square).

RESULTS

Analyses of variance of root rot data of sugar beet cultivars of four ages inoculated consecutively or simultaneously with *R. solani* AG-2-2 isolates in 2 yr are presented in Table 1. There were statistically significant effects ($P \leq 0.05$) of plant age, cultivar, AG-2-2 isolate, disease evaluation time (plot variables), and interactions among these variables on root rot severity (marked with asterisks). When the total treatment sum of squares (% TSS) for each trial was partitioned (5), greater than 5% variation in root rot severity was accounted for by plant age in all four trials; by cultivar, AG-2-2 isolate, and evaluation time in three trials; and by an interaction between plant age and AG-2-2 isolate, and between cultivar and evaluation time in one trial.

Plant age. Plant age significantly affected root rot severity and accounted for 88 and 40% of the variation in root rot in the 1990 and 1991 consecutive inoculations, respectively, and for 17 and 31% of the variation in the 1990 and 1991 simultaneous inoculations, respectively (Table 1). Conclusive statements about the effect of plant age cannot be made in the 1991 consecutive inoculation because of the significant interaction between plant age and AG-2-2 isolate (Fig. 1A), which accounted for 8% of the variation (Table 1). However, the same linear trends occurred in the consecutive inoculations in 1990 and 1991 (Fig. 1A) and in the simultaneous inoculation in 1991 (Fig. 1B). Root rot ratings were highest for 6-wk-old plants, intermediate for 8- and 10-wk-old plants, and lowest for 12-wk-old plants. The decrease in root rot severity with increasing plant age at the time of inoculation was less when roots were inoculated with the *R. solani* AG-2-2 isolate from pinto bean than with the isolate from sugar beet in the consecutive inoculation in 1991 (Fig. 1A).

In the 1990 simultaneous inoculation, root rot ratings were low (Fig. 1B). Sugar beet plants inoculated at 6 and 12 wk after planting had similar and lower root rot ratings than those inoculated at 8 and 10 wk after planting. A quadratic regression line accounted for 95% of the variation in the effect of plant age on root rot severity in this trial.

Evaluation time. The time of disease evaluation accounted for 22% of the variation in the 1991 consecutive inoculation and for 21 and 35% of the variation in the simultaneous inoculations in 1990 and 1991, respectively (Table 1). Root rot ratings increased linearly from 2 to 8 wk after inoculation for the consecutive inoculations in both years (Fig. 2A) and for the simultaneous inoculation in 1991 (Fig. 2B). Conclusive statements about the effect of evaluation time on root rot cannot be made in the 1990 simultaneous inoculation because of a significant interaction between evaluation time and sugar beet cultivar (Fig. 2B) which accounted for 6% of the variation (Table 1). The increase in disease severity was greater for the susceptible cultivar Maribo Ultramono than for the moderately resistant cultivar ACH 184 in the simultaneous inoculation in 1990 (Fig. 2B).

AG-2-2 isolate. The two isolates of *R. solani* AG-2-2 accounted for 17% of the variation in root rot severity in the 1991 consecutive inoculation and for 15 and 11% of the variation in the 1990 and 1991 simultaneous inoculations, respectively (Table 1). There was a significant interaction between plant age and isolate in the 1991 consecutive inoculation, as previously described. The isolate from pinto bean caused more root rot than did the isolate from sugar beet in three trials (Table 2). For plants that were inoculated consecutively in 1990, the isolate of AG-2-2 from sugar beet caused a higher average root rot rating than did the isolate from pinto bean, but the difference was not significant.

Root rot occurred on a few control

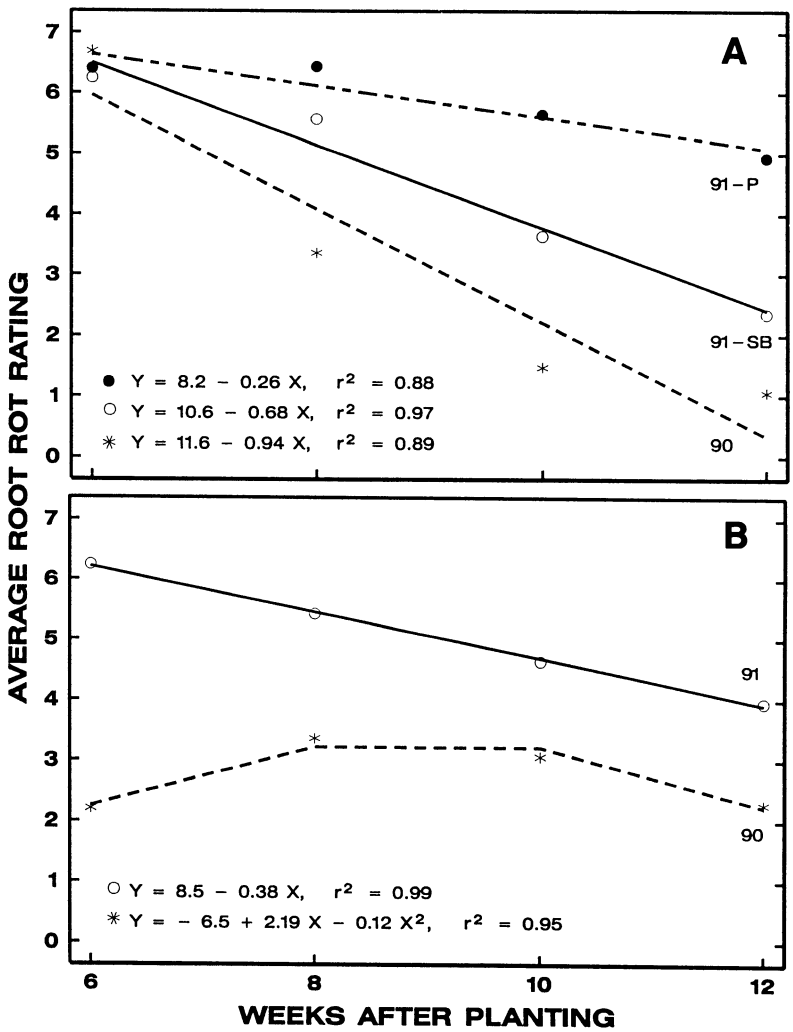

Fig. 1. Regression lines of sugar beet age at time of inoculation (6, 8, 10, and 12 wk after planting) with *Rhizoctonia solani* AG-2-2 on root rot severity in 1990 (90) and 1991 (91) field trials. **(A)** Consecutive inoculations with an interaction in 1991 between plant age and AG-2-2 cultures originally isolated from pinto bean (91-P) and sugar beet (91-SB). **(B)** Simultaneous inoculations. Root rot rating is based on a 0–7 scale (0 = healthy, 7 = plant dead). Each datum point for plant age and interaction effects is based on 480 and 240 plants, respectively.

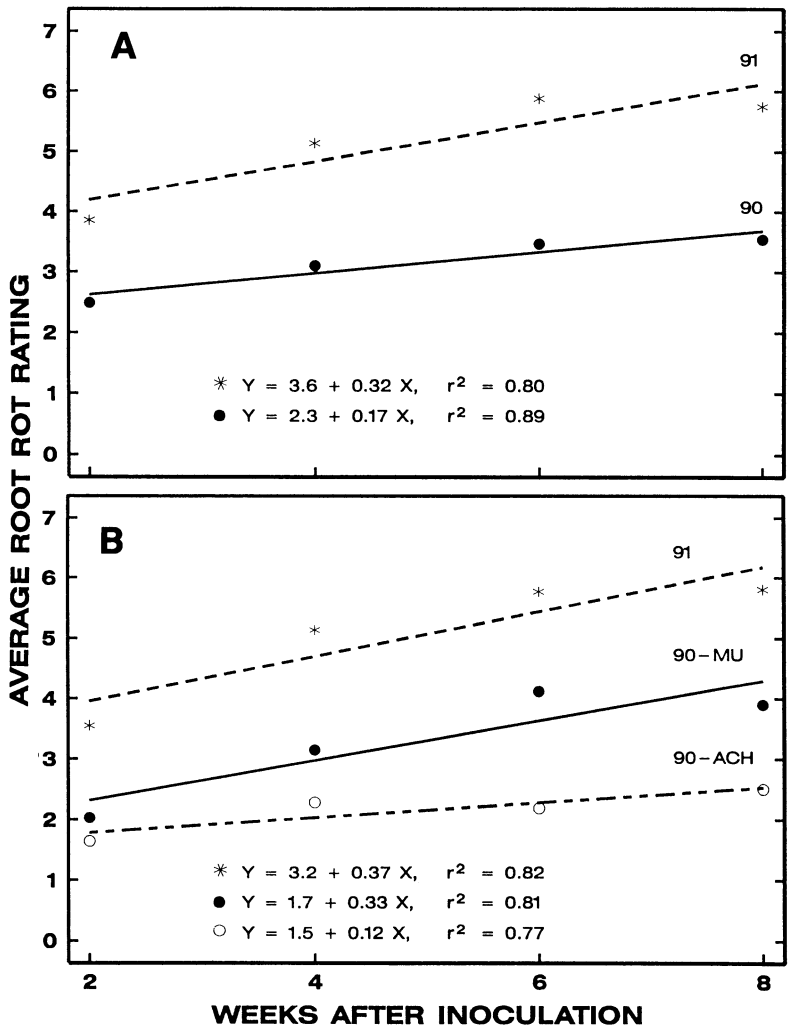

Fig. 2. Regression lines of time of disease evaluation of sugar beet roots at 2, 4, 6, and 8 wk after inoculation with *Rhizoctonia solani* AG-2-2 on root rot severity in 1990 (90) and 1991 (91) field trials. **(A)** Consecutive inoculations and **(B)** simultaneous inoculations with an interaction in 1990 between evaluation time and sugar beet cultivars Maribo Ultramono (90-MU, susceptible) and ACH 184 (90-ACH, moderately resistant). Root rot rating is based on a 0–7 scale (0 = healthy, 7 = plant dead). Each datum point for evaluation time and interaction effects is based on 480 and 240 plants, respectively.

Table 2. Average root rot ratings of two sugar beet cultivars inoculated consecutively and simultaneously in 1990–1991 field trials with two cultures of *Rhizoctonia solani* AG-2-2 originally isolated from pinto bean (P) and sugar beet (SB)

	Average root rot rating[a]			
	Consecutive inoculation		Simultaneous inoculation	
Variable	1990	1991	1990	1991
AG-2-2 isolate				
P	3.0	5.9[b]	3.2*	5.6**
SB	3.3	4.5	2.3	4.6
Cultivar				
Maribo Ultramono	3.4**	5.6**	3.3[b]	5.6**
ACH 184	2.9	4.8	2.2	4.6

[a] Root rot rating is based on a 0–7 scale (0 = healthy, 7 = plant dead). Each value based on 960 plants. Significant within trials at ** = $P \leq 0.01$ and * = $P \leq 0.05$.
[b] Significance levels cannot be presented because of statistically significant interactions (isolate × plant age, cultivar × evaluation time) which accounted for >5% variation in root rot severity.

roots in 1990, but none occurred in 1991 (data not shown). All cultures of *R. solani* isolated from infected control plants were identified as AG-2-2.

Cultivar. Sugar beet cultivar accounted for 5% of the variation in root rot in the 1991 consecutive inoculation and for 23 and 11% of the variation in the 1990 and 1991 simultaneous inoculations, respectively (Table 1). Cultivar accounted for only 1% of the variation in root rot severity in the 1990 consecutive inoculation. There was a significant interaction between sugar beet cultivar and time of disease evaluation in the 1990 simultaneous inoculation, as previously described. However, the susceptible cultivar Maribo Ultramono had higher root rot ratings than ACH 184 in the four trials, particularly in 1991 (Table 2).

Hyphal growth of *R. solani* AG-2-2. Similar daily growth rates occurred from 15 to 35 C at 0–24, 24–48, and 48–72 hr; so only data for 0–24 hr are presented (Fig. 3). The isolate from sugar beet grew slightly faster than the isolate from pinto bean at 15 and 20 C, whereas the isolate from pinto bean grew faster than the isolate from sugar beet at 25, 30, and 35 C. Optimal growth of the isolate from sugar beet was at 25 C and of the isolate from pinto bean was at 30 C. When cultures at 15 and 35 C were transferred to 25 C, the isolate from pinto bean grew faster than the isolate from sugar beet. Hyphal growth was greater when cultures were transferred from 15 to 25 C than from 35 to 25 C.

Temperatures after inoculations in field. Weekly minimum and maximum air temperatures following all inoculations in 1990 and 1991 were within the favorable temperature range (25–33 C) for Rhizoctonia root and crown rot (4) for at least 4 of 8 wk following inoculations in the field (data not shown). Average maximum air temperatures during the first week after inoculations in 1990 were 2–5 C higher than in 1991.

DISCUSSION

Rhizoctonia root and crown rot of sugar beet was less severe as plant age increased at time of inoculation in three of four field trials. Although the effect of plant age (main plot variable) was measured with much less precision than the effects of cultivar, AG-2-2 isolate, and evaluation time (subplot variables) in the four multiple split-plot designs, plant age accounted for the most variation in root rot severity (17–88% of the variation in the total sum of squares). These results are consistent with previous reports where inoculation of older bean and corn plants resulted in higher survival at harvest compared to inoculation of younger plants (11). Ruppel and Hecker (12) and Ruppel et al (13) also suggested that timing of inoculation in relation to sugar beet plant age was more effective than dosage of *R. solani* inoculum for varying the intensity of root rot in field experiments.

Environmental conditions likely affected interactions between sugar beet and *R. solani* AG-2-2 and thus confounded the results of increased plant age on root rot severity. In simultaneous inoculations, seeds were planted on four dates (May through June), and plants grew under conditions atypical of commercial production in the last three plantings. In consecutive inoculations, seeds

were planted on the same day in early- to mid-May, and plants developed under more typical environmental conditions. In both inoculation procedures, however, it is uncertain how soil temperature and moisture differed with plant age, as affected by differences in the plant canopies.

Sugar beet did not show increasing tolerance to root rot with increasing plant age in the 1990 simultaneous inoculation. Instead, less root rot developed in 6- and 12-wk-old roots than in 8- and 10-wk-old roots. These results can be attributed to very dry soil conditions at the time of inoculation. The amount of irrigation water (2.5 cm) applied after inoculation likely was insufficient, and growth of inoculum was inhibited. Van Bruggen et al (17) suggested that disease incidence of *R. solani* on dry bean was dependent solely on temperature, while lesion size was determined primarily by soil moisture. Root rot ratings were low for the 1990 simultaneous inoculation and, when averaged across all evaluation times, were 2.2 for plants inoculated at 6 wk, 3.4 for 8 wk, 3.1 for 10 wk, and 3.4 for 12 wk. A rating of 2 means that <5% of the root surface was rotted, and 3 means that 5–24% of the root surface was rotted. Although the root rot ratings were statistically different, they represented low amounts of disease that may not be biologically significant.

Root rot ratings tended to be lower in 1990 than in 1991. Since air temperatures were within the range (25–28 C) reported as optimal for growth of *R. solani* (8) for 4 of 8 wk after inoculations in both years, these results may have occurred because of different irrigation methods (options differed because of plot location). The irrigation tape used in 1991 may have allowed more water to infiltrate the soil than did the overhead sprinkler system in 1990, especially in plots of 10- and 12-wk-old sugar beet with dense leaf canopies. Thus, soil moisture conditions would be more favorable for *R. solani* in the 1991 experiments.

The moderately resistant cultivar ACH 184 slowed disease development compared to the susceptible cultivar Maribo Ultramono. ACH 184 is commercially available in Minnesota and North Dakota (R. A. Steen, American Crystal Sugar Co., Moorhead, MN, *personal communication*). Disease tolerance was more clearly expressed in the simultaneous inoculations than in the consecutive inoculations. This difference is probably because of varying growth rates of the two cultivars caused by environmental conditions after the four plantings in the simultaneous inoculations.

Cool temperatures are more favorable for initial growth of sugar beet than for *R. solani* AG-2-2. Campbell and Altman (1) reported greater survival of *Rhizoctonia*-inoculated sugar beet seedlings at 16 C than at 26 C. Early planting, particularly of a sugar beet cultivar with tolerance to *R. solani* AG-2-2, also can reduce root and crown rot severity by producing older plants that are less susceptible than later-planted beets.

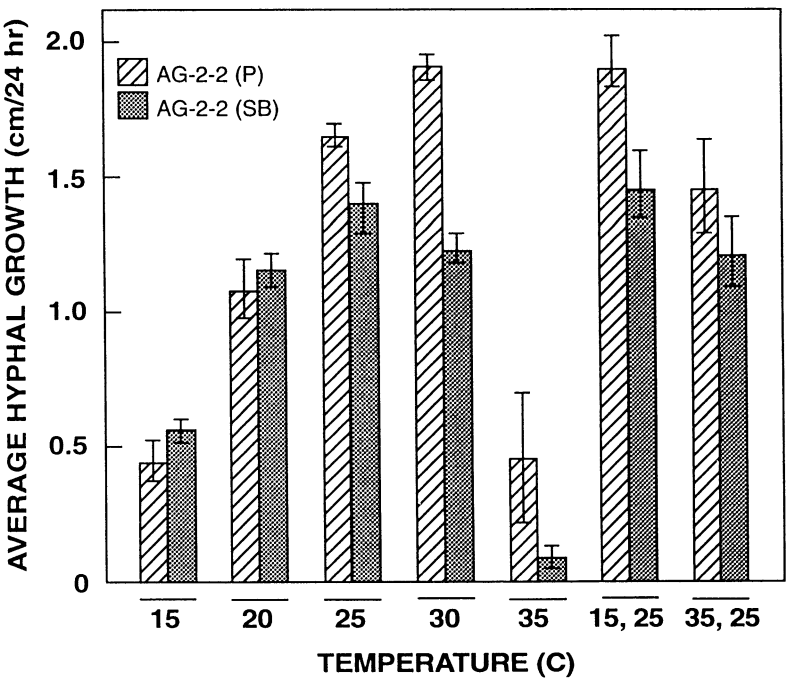

Fig. 3. Average hyphal growth of *Rhizoctonia solani* AG-2-2 cultures originally isolated from pinto bean (P) and sugar beet (SB) at various temperatures for 24 hr. Isolates at 15 and 35 C were transferred to 25 C for 24 hr (noted as 15,25 and 35,25). Each bar (with standard deviations) represents 32 petri dishes from two incubator trials.

ACKNOWLEDGMENTS

We thank F. B. Martin, University of Minnesota, and J. V. Wiersma, Northwest Experiment Station, for advice on the statistical analyses, and Rita Kuznia and Todd E. Cymbaluk, Northwest Experiment Station, for technical assistance.

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