# Decrease In Disease Severity in Sugarbeet in an Established Rhizoctonia Crown and Root Rot Nursery†

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## ABSTRACT

**A disease nursery used for more than 20 years to evaluate resistance of sugarbeet (*Beta vulgaris*) to crown and root rot caused by *Rhizoctonia solani (AG 2-2)* has failed in recent years to produce disease suitably severe to discriminate between resistant, partially resistant, and susceptible lines. Experiments were done to determine if biological control of the pathogen was responsible for the reduced disease severity. Highly susceptible to highly resistant sugarbeet lines were planted in the established nursery and in adjacent fields with no prior history as sugarbeet root rot disease nurseries. In each of 3 years, the disease was more severe at the new sites than in the established nursery. The weight of non-inoculated roots was similar at the two sites. Microbiological assays of soil from the sites revealed no quantitative differences in populations of microorganisms antagonistic to *R. solani* that were consistent with biological control of disease. Greenhouse experiments revealed no difference in seedling growth as a result of the**

† Names are necessary to report factually on available data; however, the USDA neither guaranties nor warrants the standard of the product, and the use of the names by the USDA implies no approval of the product to the exclusion of others that may be suitable.

**site of soil origin, andautoclaving of soil did not produce the increase in disease severity that would be anticipated with biological control of the pathogen. Soil analyses of likely disease suppressing edaphic factors revealed no consistent differences between sites. Thus, artificial epiphytotics of crown and root rot caused by *R. solani* were more severe in new sites than in an established disease nursery, but the reasons for this difference remain obscure.**

**Additional Key Words:** *Beta vulgaris, Rhizoctonia solani* AG-2-2, biological control, suppressive microorganisms.

In recent years a disease nursery used for more than 20 years to evaluate resistance of sugarbeet (*Beta vulgaris* L.) to crown and root rots caused by *Rhizoctonia solani* Kühn AG-2-2 in East Lansing, MI, failed to produce disease severe enough to differentiate between resistant, partially resistant, and susceptible lines. The nursery employed a 2-year rotation between sugarbeet and alfalfa (*Medicago sativa* L.); during test years, sugarbeet plants were inoculated with the fungus. Rotation of sugarbeet with alfalfa was intended to increase *Rhizoctonia* inoculum in the soil; Schneider and Robertson (1975) observed that the incidence of crown rot was higher when sugarbeet followed alfalfa in a rotation. Similar results were reported in Texas by Rush and Winter (1990).

The decline in disease severity in the East Lansing nursery resembled the decline in severity of take-all of wheat caused by *Gaeumanomyces graminis* (Sacc.) Arx & D. Oliver, attributed to control of the pathogen by *Pseudomonas* spp. (Cook and Rovira, 1976). A similar situation was observed with Rhizoctonia crown and root rot of sugarbeet in Japan (Hyakumachi and Ui, 1982a), where severity of the disease first increased and then decreased under sugarbeet monoculture. Disease decline also was observed in experiments in which successive cycles of sugarbeet seedlings were subjected to disease pressure from *R. solani* (Hyakumachi and Ui, 1982b); a reduction in *R. solani* microsclerotia accompanied the decline in disease severity. Although the cause of the disease decline under monoculture has not been determined, possible contributing factors included the appearance of isolates of the pathogen having reduced pathogenicity (Hyakumachi and Ui, 1984), non-self-anastomosing isolates that failed to produce microsclerotia (Hyakumachi and Ui, 1987), and parasitism of microsclerotia by *Trichoderma* spp. (Hyakumachi et al., 1990). Overall populations of soil microorganisms were unrelated to disease severity (Hyakumachi and Ui, 1982a). We evaluated our East Lansing, MI plots to

determine if biological control of *R. solani* was a plausible explanation for the low disease severity.

## MATERIALS AND METHODS

### Field Experiments

Twelve sugarbeet lines ranging from highly susceptible to highly resistant to *R. solani* were planted in 1992, 1994, and 1995 in an established nursery (L1) and in nearby fields (within 200 m) with no prior histories as Rhizoctonia root rot nurseries (L2). The same 12 lines were not used in all years because of seed availability constraints. Six replicates were planted in 7.5 m plots and randomized complete blocks at each location each year. Plants at the L2 site appeared to be smaller than those at the L1 site in 1992 and 1994, thus, a split plot design, split into inoculated and non-inoculated groups, was used in 1995 to determine if there was a site-specific effect on plant growth. This required an additional six replicates of each variety in blocks that were not inoculated but were harvested at the same time as inoculated roots to determine root weight.

Plants were thinned to a 20-cm spacing 6 wk after planting, counted, and inoculated with *R. solani* AG-2-2 (Ruppel et al. 1979). Ten weeks post-inoculation, disease severity on roots was evaluated on a scale of 0 (no disease) to 4 (75-100% rot); dead plants were noted and scored 4. Roots from non-inoculated plots were weighed in 1995. Results were subjected to analysis of variance (ANOVA) (Dowdy and Wearden, 1983).

### Microbiological Experiments

Soil samples were collected in 1994, at both L1 and L2, between inoculated rows and within 1 cm of both healthy and diseased roots; six subsamples of each sample type were collected. Population densities of microorganisms antagonistic to *R. solani* were evaluated in the samples by the methods of Johnson, et al. (1967). Means were evaluated by the Student-Newman-Keuls test (Dowdy and Wearden, 1983).

### Greenhouse Experiment

A greenhouse experiment was designed to identify soil factors antagonistic to growth and parasitism by *R. solani*. Soil samples were collected from between rows of the 1995 field plots using soil from the top 15 cm. Soil was air-dried and sieved through a 6 mm screen before use. The experiment included six treatments: non-treated soils of L1 and L2; a 1:1 mixture of L1 and L2 soils; and samples of L1, L2, and the 1:1 mixture that were moistened and autoclaved for 1 hr before use. Air-dried soil (2 kg per sample) was placed in 28 x 20 x 6 cm plastic trays, moistened, and allowed

to equilibrate and drain for 24 hr. Thirty seeds of the *Rhizoctonia*-susceptible hybrid American Crystal 185 were planted in two 28-cm rows spaced 10-cm apart. Two weeks after planting, seedlings were thinned to five uniformly spaced seedlings per row. One half gram of *R. solani*/millet inoculum was distributed into a 1-cm deep furrow between the rows and covered with soil. Seedlings were removed 4 wk after planting, washed, and scored for disease severity on a scale of 0 (healthy) to 4 (dead or completely girdled). Eight replicates arranged on greenhouse benches in randomized complete block designs were repeated once, and results were analyzed by ANOVA (Dowdy and Wearden, 1983).

**Soil Assays**

Soil samples were collected in the autumn of 1995 from the fields that had been in sugarbeets in 1992, 1994, and 1995. Five samples were collected from different areas of each field. Each sample was a composite of five sub-samples collected within an area 10 m in diameter. Samples were air dried and sieved through a 6 mm screen. Soil samples were assayed by the Michigan State University Soil Testing Laboratory, East Lansing, MI. Means were separated by LSD analysis (Dowdy and Wearden, 1983).

## RESULTS AND DISCUSSION

Disease was more severe in roots at the L2 sites than at the L1 site for all 3 years (Table 1). When the 12 test lines were grouped into the six most susceptible and the six most resistant lines, increased disease severity at L2 was evident in both the "susceptible" and the "resistant" lines (Table 2). Increased disease severity at L2 also was apparent from the greater number of plants that died at L2 (Table 2).

Surviving roots at the L1 site in 1992 and 1994 appeared, visually, to be slightly larger than those at the L2 sites. Engelkes and Windels (1994) demonstrated that resistance to crown and root rot increases with plant age. If plants at the L1 site were larger than those at L2 they might have been more physiologically mature, irrespective of age, and might therefore be more likely to survive. Therefore, the 1995 plantings included plots that were not inoculated, from which mature, non-infected roots were harvested and weighed. None of the 12 tested lines exhibited significant differences in root weights between the two sites. Thus, increased disease severity at L2 could not be accounted for by differences in size of the roots.

Antagonism of soil microorganisms to growing pathogen hyphae is a mechanism of biological control (Cook, 1990), that could explain field observations. However, the only significant difference observed in populations of microorganisms that were antagonistic to *R. solani* in 1994 field

**Table 1.** Rhizoctonia crown and root rot ratings of inoculated sugarbeets produced in an established disease nursery (L1) and in nearby fields (L2) not previously used as Rhizoctonia disease nurseries.

| Variety | 1992 | | 1994 | | 1995 | |
|---|---|---|---|---|---|---|
| | L1 | L2 | L1 | L2 | L1 | L2 |
| | Crown and root rot rating[a] | | | | | |
| USH23 | 2.7 | 3.1 | 3.6 | 4.0 | 3.0 * | 3.8 |
| 85320-0 | 2.2 * | 2.8 | 3.6 | 3.9 | 3.0 * | 4.0 |
| 88B12 | 2.8 * | 3.8 | 2.8 * | 3.7 | 1.8 * | 2.9 |
| 88B22-00 | 2.5 * | 3.1 | 3.8 | 3.9 | 2.7 * | 3.7 |
| FC712 | 1.7 | 1.9 | 2.2 * | 3.1 | 1.4 * | 2.2 |
| WC90318 | 2.1 * | 2.9 | 2.0 * | 3.5 | 1.6 * | 2.4 |
| 86B18-1 | 2.0 | 2.0 | - | - | - | - |
| 86B18-30 | 3.5 | 3.9 | - | - | - | - |
| 86B18-124 | 2.6 * | 3.7 | - | - | - | - |
| 87B3-33 | 2.1 * | 2.9 | - | - | - | - |
| 87B3-26 | 2.9 * | 3.6 | - | - | - | - |
| 85250-80 | 2.3 | 2.7 | - | - | - | - |
| Univers | - | - | 3.7 | 4.0 | 3.0 * | 3.9 |
| WC91270M | - | - | 3.8 | 3.8 | 2.9 * | 4.0 |
| WC89263A | - | - | 3.5 | 3.9 | 2.4 * | 3.7 |
| 88B24-02 | - | - | 3.5 | 3.9 | 2.1 * | 3.6 |
| 89B6-1 | - | - | 2.9 * | 3.7 | 2.0 * | 2.9 |
| 89B9-i4 | - | - | 2.2 * | 3.1 | 1.5 * | 2.7 |
| Means | 2.5 * | 3.1 | 3.1 * | 3.7 | 2.3 * | 3.3 |

An asterisk between two values indicates that they differ significantly at $P<0.05$.

[a]Disease ratings are on a linear scale of 0 (no rot) to 4 (75-100% of the crown and root rotted).

soils, as measured by inhibition of growth of *R. solani*, was a higher population from around healthy roots at the L2 site than at other sites (Table 3). *Streptomyces* spp. were the most prevalent antagonistic microorganisms identified.

**Table 2.** Means of Rhizoctonia crown and root rot ratings and percentages of plants killed by Rhizoctonia crown and root rot in an established disease nursery (L1) and in nearby fields (L2) not previously used as *Rhizoctonia* disease nurseries. Lines were grouped into the six most resistant and the six most susceptible to crown and root rot.

| Group | 1992 | | 1994 | | 1995 | | 3 yr. av. | |
|---|---|---|---|---|---|---|---|---|
| | L1 | L2 | L1 | L2 | L1 | L2 | L1 | L2 |
| | Crown and root rot rating[a] | | | | | | | |
| 6 "susc." lines | 2.8 * | 3.5 | 3.3 * | 3.7 | 2.8 * | 3.9 | 3.0 * | 3.7 |
| 6 "res." lines | 2.1 * | 2.6 | 3.0 * | 3.7 | 1.7 * | 3.0 | 2.3 * | 3.1 |
| Overall means | 2.5 * | 3.1 | 3.1 * | 3.7 | 2.3 * | 3.3 | 2.6 * | 3.4 |
| | Percentage of plants killed | | | | | | | |
| 6 "susc." lines | 14 * | 40 | 46 * | 77 | 12 * | 83 | 24 * | 67 |
| 6 "res." lines | 4 | 5 | 4 * | 24 | 1 * | 25 | 3 * | 18 |
| Overall means | 9 * | 23 | 25 * | 50 | 7 * | 54 | 14 * | 42 |

An asterisk between two values indicates that they differ significantly at $P<0.05$.

[a]Disease ratings are on a linear scale of 0 (no rot) to 4 (75-100% of the crown and root rotted).

**Table 3.** Numbers of microorganisms antagonistic to *Rhizoctonia solani* in soil samples from an established disease nursery (L1) and in a nearby field (L2) in 1994. The L2 field was not used as a Rhizoctonia disease nursery prior to 1994.

| Soil source | Antagonistic microorganisms, Propagules/g soil X $10^{-3}$ |
|---|---|
| L1 between rows | 279 b* |
| adjacent to rotted root | 203 b |
| adjacent to healthy root | 318 b |
| L2 between rows | 472 b |
| adjacent to rotted root | 203 b |
| adjacent to healthy root | 1230 a |

*Means followed by the same letter do not differ significantly based on the Student-Newman-Keuls test *(P=0.01)*.

An additional experiment was done to determine whether antagonism to *R. solani* by soil microorganisms could account for the greater disease severity in L2 compared to L1. Sugarbeet seedlings were grown in non-treated and autoclaved soils from the 1995 sites. *R. solani* was forced to grow through the soil to parasitize the seedlings. Autoclaving should have killed any soil microorganisms antagonistic to the pathogen. The only difference ($P = 0.05$) in pathogenesis observed between non-treated and autoclaved soils was an increase in disease in seedlings growing in autoclaved soil from L2 (Table 4). Our observations on populations of antagonistic microorganisms and severity of seedling disease are inconsistent with the hypothesis that antagonistic microorganisms are responsible for the differences in disease severity between fields.

**Table 4**. The effect of soil source and soil biota on severity of seedling rot caused by *Rhizoctonia solani*. Soils were collected from the top 15 cm, between rows of sugarbeets in an established crown and root rot disease nursery (L1) and in a new nursery site (L2) in 1995. The L2 field had not been used as a Rhizoctonia disease nursery prior to 1995.

| Soil and treatment | Experiment 1 | Experiment 2 | Mean |
|---|---|---|---|
| | | Disease rating[a] | |
| L1, non treated | 1.4 | 2.6 | 2.0 |
| L1, autoclaved | 1.4 | 1.9 | 1.6 |
| L1/L2 (1/1), non treated | 1.6 | 2.1 | 1.8 |
| L1/L2 (1/1), autoclaved | 1.9 | 2.0 | 2.0 |
| L2, non treated | 1.6 | 1.8 | 1.7 |
| L2, autoclaved | 2.4 | 2.4 | 2.4 |
| L.S.D. (0.05) | 0.54 | 0.68 | 0.48 |

[a]Seedling disease was evaluated on a scale of 0 (= healthy) to 4 (= dead).

Plant nutrition has been implicated as a factor in resistance to many diseases. Manganese availability is of special significance in this regard. Huber and Wilhelm (1988) reviewed numerous instances in which disease severity was reduced by manganese fertilization and decreased soil pH; both increase availability of Mn to plants. Additionally, control of take-all of wheat caused by *Gaeumanomyces graminis*, a disease for which biological control frequently has been reported (reviewed in Cook, 1990), consistently was improved by conditions that enhanced manganese availability (Huber and Wilhelm, 1988). In our study, soil chemical factors likely to contribute

to overall disease resistance expression were measured. Significant differences in all determinations existed between locations (Table 5); however, values for all determinations from L1 except magnesium were among values for determinations on samples from the three L2 sites. Whereas these findings do not preclude critical differences in nutrient uptake among the sites, such differences seem unlikely.

**Table 5**. Chemical analyses of soil samples from between rows of sugarbeets in fields used in evaluations of Rhizoctonia crown and root rot severity. The fields were an established crown and root rot nursery (L1) and three nearby fields (L2) not used as Rhizoctonia disease nurseries prior to 1992, 1994, and 1995.

| Characteristic | Field Sampled | | | | L.S.D. |
|---|---|---|---|---|---|
| | L1 | 92L2 | 94L2 | 95L2 | (P=0.05) |
| pH | 7.0 | 6.4 | 6.9 | 7.5 | 1.07 |
| P, mg/kg | 64 | 39 | 34 | 123 | 10.2 |
| K, mg/kg | 135 | 132 | 164 | 275 | 13.1 |
| Ca, mg/kg | 1710 | 1690 | 2510 | 2160 | 31.6 |
| Mg, mg/kg | 267 | 502 | 357 | 287 | 16.4 |
| Fe, mg/kg | 41 | 36 | 29 | 52 | 5.0 |
| Mn, mg/kg | 40 | 25 | 43 | 56 | 5.7 |

The results of field experiments on crown and root rot severity in an established disease nursery and in new nursery locations demonstrate that the disease is more severe at new sites. Plausible explanations for these observed differences between L1 and L2 sites include enhanced root growth that provides disease escape, biological control of *R. solani* AG-2-2 through microbial antagonism, and enhanced host resistance through different nutrient availability. Although none of these mechanisms were demonstrated in experiments designed to implicate them, the experiments cannot preclude them as likely causes of reduced disease severity in our crown and root rot nursery. The similarity between our findings and those in the parallel situation of crown and root rot decline in sugarbeet monoculture in Japan (Hyakumachi and Ui, 1982a) suggests that decline in severity of crown and root rot under continued disease pressure may be a generalized phenomenon. This may be related to anecdotal reports by Michigan sugarbeet growers that the occurrence of crown and root rot often is more severe in fields that

have not previously been used for sugarbeet production, or that have been out of sugarbeet production for many years, compared to fields in which sugarbeets are a regular component in the crop rotation sequence. Seemingly in contrast to the reports of Schneider and Robertson (1975) and Rush and Winter (1990) that rotation with alfalfa increased the incidence of *Rhizoctonia* crown and root rot, Pinckard, et al. (1996) reported that composted alfalfa hay nearly eliminated *Rhizoctonia* from soils, and the parasite could not be reestablished for at least 3 years. It is unknown if repeated rotation with alfalfa contributed to the disease decline we observed. We recommend that to obtain disease severity that provides useful differentiation between highly resistant, partly resistant, and susceptible germplasms, disease nurseries should include crop rotation cycles of at least 4 years, and not include alfalfa. Transfer of nurseries to new locations after several cycles of crop rotation may also prove desirable.

Accounts from sugarbeet growers commonly report unusually severe occurrences of crown and root rot in plantings on land that has been out of sugarbeet production for at least 10 years. Suppression of crown and root rot may be a normal aspect of the 3 to 4 year rotations used in Michigan, and this suppressiveness may be lost during long intervals without sugarbeet production. The 1997 crown and root rot nursery at East Lansing, MI, was planted in a portion of the established nursery land that was kept fallow for three years following use for the 1993 nursery. High intensity of disease occurred on susceptible materials, and useful differentiation between resistant and susceptible materials occurred throughout the nursery, indicating diminishment of factors suppressive of disease.

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## LITERATURE CITED

Cook, R. J. 1990. Twenty-five years of progress toward biological control. Pages 1-14. *In*: D. Hornby and R. J. Cook (eds.). Biological Control of Soil-Borne Plant Pathogens. C.A.B. Int., Wallingford, England. 479 pp.

Cook, R. J., and A. D. Rovira. 1976. The role of bacteria in the biological control of *Gaeumanomyces graminis* by suppressive soils. Soil Biol. Biochem. 8:267-273.

Dowdy, S., and S. Wearden. 1983. Statistics for Research. John Wiley & Sons, New York. 537 pp.

Engelkes, C. A., and C. E. Windels. 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.

Huber, D. M., and N. S. Wilhelm. 1988. The role of manganese in resistance to plant diseases. Pages 155-173. *In*: R. D. Graham, R. J. Hannam, and N. C. Uren (eds.). Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht. 344 pp.

Hyakumachi, M., K. Kanzawa, and T. Ui. 1990. *Rhizoctonia* root rot decline in sugarbeet monoculture. Pages 227-247. *In*: D. Hornby and R. J. Cook, eds. Biological Control of Soil-Borne Plant Pathogens. C.A.B. Int., Wallingford, England. 479 pp.

Hyakumachi, M., and T. Ui. 1982a. Decline of *Rhizoctonia* root rot during sugarbeet monoculture. Mem. Fac. Agric., Hokkaido Univ., Sapporo 13:445-454.

Hyakumachi, M., and T. Ui. 1982b. Decline of damping-off of sugarbeet seedlings caused by *Rhizoctonia solani* AG 2-2. Ann. Phytopath. Soc. Japan 48:600-606.

Hyakumachi, M., and T. Ui. 1984. Properties of diseased isolates of *Rhizoctonia solani* isolated from soil. Ann. Phytopath. Soc. Japan 50:255-262.

Hyakumachi, M., and T. Ui. 1987. Non-self-anastomosing isolates of *Rhizoctonia solani* obtained from fields of sugarbeet monoculture. Trans. Br. Mycol. Soc. 89:155-159

Johnson, L. F., G. C. Papavizas, C. B. Davey, T. E. Freeman, L. J. Herr, C. Leben, and P. E. Tsao. 1967. Naturally occurring soil actinomycetes antagonistic to plant pathogenic fungi. Pages 285-287. *In*: A. Kelman (ed.). Sourcebook of Laboratory Exercises in Plant Pathology, W. H. Freeman and Co., San Francisco. 387 pp.

Pinckard, J. A., R. G. Davis, and P. B. Richey. 1996. The natural process for cleansing soils of unwanted entities. Plant Dis. 80:963.

Ruppel, E. G., C. L. Schneider, R. J. Hecker, and G. J. Hogaboam. 1979. Creating epiphytotics of Rhizoctonia root rot and evaluating for resistance to *Rhizoctonia solani* in sugarbeet field plots. Plant Dis. Rep. 63:518-522.

Rush, C. M., and S. R. Winter. 1990. Influence of previous crops on Rhizoctonia crown and root rot of sugar beet. Plant Dis. 74:421-425.

Schneider, C. L., and L. S. Robertson. 1975. Occurrence of diseases on sugarbeet in a crop rotation experiment in Saginaw county, Michigan in 1969-1970-1971. Plant Dis. Rep. 59:194-197.