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# A greenhouse test for screening sugar beet (*Beta vulgaris*) for resistance to *Rhizoctonia solani*

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**Abstract**

*Rhizoctonia solani* Kühn is a serious plant pathogenic fungus, causing various types of damage to sugar beet (*Beta vulgaris* L.). In Europe, the disease is spreading and becoming a threat for the growing of this crop. Plant resistance seems to be the most practical and economical way to control the disease. Experiments were carried out to optimise a greenhouse procedure to screen plants of sugar beet for resistance to *R. solani*. In the first experiment, two susceptible accessions were evaluated for root and leaf symptoms, after being grown in seven different soil mixtures and inoculated with *R. solani*. The fungus infected all plants. It was concluded that leaf symptoms were not reliable for the rating of disease severity. Statistically significant differences between the soil mixtures were observed, and there were no significant differences between the two accessions. The two soil mixtures, showing the most severe disease symptoms, were selected for a second experiment, including both resistant and susceptible accessions. As in the first experiment, root symptoms were recorded using a 1–7 scale, and a significant expression of resistance was observed. The average severity of the disease in the greenhouse experiment generally was comparable with the infection in field experiments, and the ranking of the accessions was the same in the two types of experiments. It was concluded that evaluation procedures in the greenhouse could be used as a rapid assay to screen sugar beet plants for resistance to *R. solani*.

## Introduction

*Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is a serious plant pathogenic fungus, which can cause severe and economical damage to many crop plants. In sugar beet (*Beta vulgaris* L.), the yield reduction caused by this fungus varies greatly (up to 50%) from field to field (Herr, 1996). The disease has been reported from the USA for many years and, currently, there is a growing concern in Europe regarding the spread of the disease and the related increase of the damage to the crop (Westerdijk et al., 1998).

In sugar beet, *R. solani* can cause several types of damage, including seedling damping-off, crown and root rot, as well as dry rot canker in older plants. Crown and root rot are the most devastating forms of the disease (Herr, 1996). Crown rot begins with invasion of the fungus in older petioles, which are in contact with the soil, followed by the development of black lesions on the petioles. Rotting proceeds towards the crown and roots of the plant, and is accompanied by wilting and yellowing of the leaves. Eventually, the disease may lead to plant death. Dry rot cankers develop on the surface of the beet root and consist of numerous, defined, alternating dark and light coloured concentric rings. Beneath the lesions are deep cankers, filled with mycelium and dry remains of host tissue. The cankers are clearly separated from the adjacent healthy tissue.

*Rhizoctonia* spp. have been classified by means of hyphal anastomosis reactions between isolates. Thus, more homogeneous groups were defined, the so-called

anastomosis groups (AGs). These AGs are subdivided based on different reactions regarding host range, morphology of colonies, thiamine requirement, and biochemical and molecular characteristics (Ogoshi, 1987; Sneh et al., 1991). In sugar beet, AG2-2 is the major AG world-wide, causing crown and root rot (Herr, 1996), but other AGs have been isolated from this crop species (Naito et al., 1976; Herr and Roberts, 1980; Windels and Nabben, 1989; Rush et al., 1994).

The chemical control of soil-borne fungi like *Rhizoctonia* is difficult. Therefore, plant resistance, in combination with crop rotation, offers the most practical and economical way to control the disease. Breeding sugar beet for resistance to *Rhizoctonia* root rot started in the late 1950s in the USA. The first result was the release of two resistant cultivars (Gaskill, 1968), followed by a series of releases of improved germplasm (see Panella and Ruppel, 1996; Campbell and Bugbee, 1993).

Sugar beet is an outcrossing crop species. Resistance to *R. solani* was found to be polygenic, conditioned by at least two major genes and possibly modifying genes (Hecker and Ruppel, 1975). For breeding, mass or recurrent selection was used, as well as visual evaluation in the field, after a uniform and heavy disease pressure had been build up through an artificially induced epiphytotic (Ruppel et al., 1979; Schneider et al., 1982). However, field tests have the disadvantages that only one generation per year can be evaluated, and that the environmental variation cannot be controlled. This may result in a considerable variation in results between years, and thus many replicates must be included in the experiments.

To overcome these problems, screening young plants of sugar beet for resistance to *R. solani* in the greenhouse is desirable. A number of greenhouse tests have been proposed, e.g., a toothpick method for inoculating partly or fully developed sugar beets (Schuster et al., 1958). Campbell and Altman (1976) assumed that the percentage of young seedlings showing damping-off, after being grown in *R. solani*-contaminated soil, could be a preliminary indication of the susceptibility to root rot. However, in more recent studies, Campbell and Bugbee (1993) stated that the screening of young seedlings for damping-off in the greenhouse could not be considered a reliable substitute for field-testing.

At the USDA Crop Research Laboratory, Fort Collins, Colorado, USA, a greenhouse test was developed and is being used by one of the authors of the present study (LWP) to evaluate the aggressiveness of fungal isolates. In this test, 8–12 week old plants are inoculated and grown for four weeks at about 25 °C, followed by visual evaluation of the disease symptoms on the roots. When using a 1–7 scale for the assessment of the severity of the disease, often most of the plants showed either low or high values. This appeared good enough as an indication of the aggressiveness of the pathogen. However, if breeding material is studied, the observed under representation of the intermediate classes probably leads to an over estimation of the percent susceptibility of the material. Because of this, the test seems less suitable for quantifying the level of resistance. Therefore, it was decided to further develop this test, and to try to improve it for use in breeding and (molecular) genetic studies.

## Materials and methods

Plant material consisted of three releases from the breeding programme of the USDA Agricultural Research Service (ARS): FC703 (Hecker and Ruppel, 1977), FC709-2 (Hecker and Ruppel, 1988) and FC718 (Panella et al., 1995). These accessions have different levels of resistance to *R. solani*. In addition, two susceptible accessions were used: cultivar 'Univers' and the F6-selection CPRO-9701 (from the CPRO sugar beet research programme on breeding for round shape and smooth skin). Seeds were sown in trays and seedlings were transplanted into 10 × 10 cm pots. Plants were grown in a greenhouse at 22 °C at day (10 h) and 17 °C at night (14 h), using extra artificial light if the natural light intensity reached a level less than 10 $Wm^{-2}$. Differences in photoperiod, caused by differences in natural light intensity during the growing season, could not be prevented.

Experiments were carried out using one isolate of *R. solani*, named 'Breda', which was collected from a field near Breda, The Netherlands. The 'Breda' isolate belongs to AG2-2 and is very aggressive on sugar beet in the field. The fungus was grown on PDA (Potato Dextrose Agar, Oxoid) in Petri dishes. For small-scale multiplication, the cultures were grown in a growth cabinet at 20 °C, with no light. For greenhouse experiments, large-scale multiplication of the fungus was done using dehulled seed of Pearl millet (*Panicum* spp.), as recommended by J.H.M. Schneider (IRS). The Pearl millet seed was soaked overnight in tap water, using 2 l preserving jars (Weck flasks). The surplus of water was removed and the wet millet was autoclaved three times at 120 °C for 20 min. To inoculate the millet, pieces of agar with actively growing hyphae of *R. solani*

were placed 2–3 cm below the surface of the millet. The flasks were shaken every 2–3 days, to avoid the formation of large, sticky clumps of millet and fungus. After two weeks of incubation at 20 °C, the millet was completely colonised with the fungus and used as inoculum. The sugar beet plants were inoculated by replacing about 0.6 g of soil around the plants with the same amount of inoculum, and cover it with soil.

In the first experiment, eight plants of each of the two susceptible accessions were transplanted in each of the seven different soil mixtures (Table 1). Plants were inoculated nine weeks after sowing. Leaf symptoms were recorded at two, five, and eight weeks after inoculation. The general criterion at two weeks after inoculation was leaf senescence, and plants were assessed according to a 1–5 scale: 0 = plant healthy, 1–4 = 1–4 senescent leaves, respectively, and 5 = whole plant senescence. At five and eight weeks after inoculation only three classes were applied: 1 = plant nearly healthy, 2 = plant diseased, showing both normal and wilting, yellowing, and dying leaves, and 3 = plant (nearly) dead. Root symptoms were recorded at five and eight weeks after inoculation, by estimating the proportion of the root surface infected by the fungus, using a 1–7 scale (Figure 1). Since none of the plants proved to be undamaged, the class 0 = 'healthy plants' could not be used. For assessing the root symptoms, plants were dug up and cleaned. Therefore, these symptoms were recorded five weeks after inoculation on four plants and eight weeks after inoculation on the other four plants.

Experiment 2 consisted of the three resistant and two susceptible accessions, as well as the soil mixtures 5 and 6. For each accession, 24 plants were transplanted into the two soil mixtures, and inoculated eight weeks after sowing. Root symptoms were recorded at three and five weeks after inoculation (12 plants per accession per soil mixture per date).

*Table 1.* Description of the seven soil mixtures used in the experiments to improve a greenhouse test for screening sugar beet for resistance to *Rhizoctonia solani*

1. 80% river sand + 20% clay (as powder) + 2 g Osmocote[1] per pot
2. 85% river sand + 15% potting soil + 2 g Osmocote per pot
3. 80% river sand + 15% vermiculite + 5% dried cow-manure + 2 g Osmocote per pot
4. 80% river sand + 10% clay + 10% potting earth + 2 g Osmocote per pot
5. 80% quartz (silver) sand + 10% clay + 10% potting earth + 2 g Osmocote per pot
6. 100% potting earth type Lent 4 (somewhat richer than type Klasman)
7. 100% potting earth type Klasman

[1]Slow release artificial fertiliser.

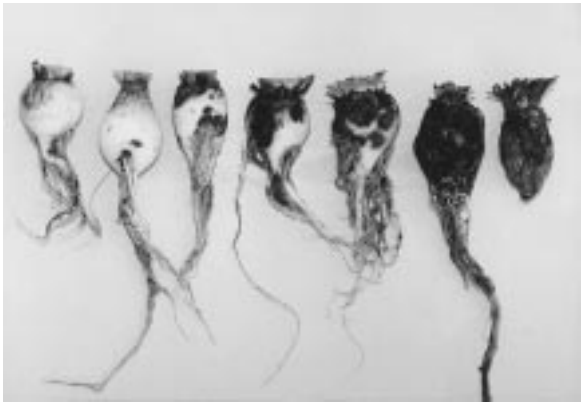

*Figure 1.* The seven disease classes used for assessing the root symptoms in experiments to improve a greenhouse test for screening plants of sugar beet for resistance to *R. solani*. 1 = only superficial damage of the skin, 2–6 = up to 5, 25, 50, 75, 100% rot of the skin, respectively, and 7 = 100% rot of skin and root.

## Results and discussion

### *Experiment 1*

The scoring of leaf symptoms (wilting, yellowing and dying of the leaves) was compared with the rating based on root symptoms of individual plants. Leaf symptoms often were more erratic than root symptoms, and were also not correlated with root symptoms. It was concluded that leaf symptoms should not be used for disease assessments.

Root symptoms consisted of the formation of lesions, followed by rotting of the root tissue. Results of the classification of root symptoms are summarised in Table 2. The fungus clearly infected all plants in the experiment. The variation between the four plants per soil mixture per screening date appeared to be large. An analysis of variance showed statistically significant differences at the main effects, i.e., 'soil mixture' and 'date', (LSD-values: 0.92 and 0.47, respectively; $P = 0.05$). The third main effect, 'plant material', was not significant. Also the interactions between the main effects were not statistically significant. This was perhaps due to the observed level of variation between the plants per treatment combination.

The average values for the two susceptible accessions were very similar, indicating that 'Univers' and

*Table 2*. Experiment 1. Average levels of infection by *R. solani*, in two accessions of sugar beet ('Univers' and CPRO-9701), tested in seven different soil mixtures under greenhouse conditions. Root symptoms were assessed at five and eight weeks after inoculation (four plants per accession per soil mixture per date), using a scale of 1–7[1]

| Soil mixture[2] | 'Univers' | | CPRO-9701 | | Mean over accessions | | Total mean[3] |
|---|---|---|---|---|---|---|---|
| | Week 5 | Week 8 | Week 5 | Week 8 | Week 5 | Week 8 | |
| 1 | 4.00 | 4.87 | 2.75 | 4.50 | 3.37 | 4.69 | 4.03 a |
| 2 | 2.62 | 2.25 | 3.75 | 4.25 | 3.19 | 3.25 | 3.22 a |
| 3 | 3.00 | 2.87 | 2.25 | 4.75 | 2.62 | 3.81 | 3.22 a |
| 4 | 4.75 | 6.87 | 5.37 | 5.12 | 5.06 | 6.00 | 5.53 b |
| 5 | 4.62 | 6.87 | 5.12 | 6.12 | 4.87 | 6.50 | 5.69 b |
| 6 | 5.83 | 6.00 | 6.00 | 6.87 | 5.91 | 6.44 | 6.18 c |
| 7 | 5.25 | 4.75 | 4.75 | 5.50 | 5.00 | 5.12 | 5.06 b |
| Total mean[3] | 4.30 | 4.93 | 4.29 | 5.30 | 4.29 x | 5.12 y | 4.70 |

[1]See Figure 1 for description of classes.
[2]Soil mixtures are described in Table 1.
[3]Numbers followed by different letters are significantly different, $P = 0.05$. LSD-values: 0.92 for soil mixture and 0.47 for date.

*Table 3*. Experiment 2. Resistance to *R. solani* in five accessions of sugar beet tested in two different soil mixtures under greenhouse conditions. Root symptoms were assessed at three and five weeks after inoculation (12 plants per accession per soil mixture per date), using a scale of 1–7[1]

| Plant material | Soil mixture 5[2] | | | Soil mixture 6[2] | | | Mean | | Total mean[3] |
|---|---|---|---|---|---|---|---|---|---|
| | Week 3 | Week 5 | Mean | Week 3 | Week 5 | Mean | Week 3 | Week 5 | |
| FC703 | 4.17 | 4.58 | 4.38 | 3.08 | 3.08 | 3.08 | 3.63 | 3.83 | 3.73 b |
| FC709-2 | 1.92 | 2.25 | 2.08 | 1.00 | 1.25 | 1.13 | 1.46 | 1.75 | 1.60 a |
| FC718 | 2.42 | 3.33 | 2.88 | 1.67 | 1.92 | 1.79 | 2.04 | 2.63 | 2.33 a |
| 'Univers' | 5.58 | 6.67 | 6.13 | 5.33 | 6.08 | 5.71 | 5.46 | 6.38 | 5.92 c |
| CPRO-9701 | 6.42 | 7.00 | 6.71 | 6.25 | 5.50 | 5.88 | 6.33 | 6.25 | 6.29 c |
| Total mean[3] | 4.10 | 4.77 | 4.43 p | 3.47 | 3.57 | 3.52 q | 3.78 x | 4.17 y | 3.98 |

[1]See Figure 1 for description of classes.
[2]Soil mixtures are described in Table 1.
[3]Numbers followed by different letters are significantly different, $P = 0.05$. LSD-values: 0.75 for plant material, 0.48 for soil mixture, and 0.34 for date.

CPRO-9701 have the same level of susceptibility. The general observation, that the value recorded after eight weeks was larger than that recorded after five weeks, was expected, and is a reflection of the time period between the assessments. Although the ranking of the soil mixtures across the two screening dates was not fully consistent, the soil mixtures 1, 2, and 3 always had the lowest level of infection, whereas the average infection in soil mixture 6 was the most severe. Soil mixtures 1, 2, and 3 also showed the poorest plant development, as recorded just before inoculation (data not shown). This is an indication that the test should be carried out in richer soils. Also, the comparison between the two mixtures of potting soil (soil mixtures 6 and 7) showed a significantly higher level of infection in the richer soil mixture 6, than in soil mixture 7. From the mean values at the two screening dates, it was not possible to conclude which one of the dates was the best for the assessment. However, the higher level of disease after five weeks resulted in a slightly decreased variation between plants within the treatment-combinations.

*Experiment 2*

Disease ratings of root symptoms are summarised in Table 3. Again, the fungus infected all plants of the experiment. Analysis of variance showed statistically significant differences at the main effects, i.e., 'plant material', 'soil mixture', and 'date' (LSD-values: 0.75, 0.48, and 0.34, respectively; $P = 0.05$), whereas the interactions between the main effects were not significant. The ranking of the three resistant accessions across the soil mixtures and screening dates was

always the same. In experiments 1 and 2, the level of infection on the two susceptible accessions across soil mixtures and screening dates, was of the same order of magnitude, with total means of 5.94 and 6.11, respectively, indicating that the two experiments are comparable.

In nearly all cases, the level of infection after five weeks was slightly higher than after three weeks, and more consistent than in experiment 1. As in experiment 1, a significant difference was observed between the two soil mixtures, but the ranking was opposite in the two experiments. This cannot be explained. The differences between the two susceptible accessions on the one side and the resistant accessions on the other were highly significant, indicating that the experimental procedure reveals the resistance of plant accessions. Among the resistant accessions, FC703 showed a significantly lower level of resistance than FC709-2 and FC718. The difference between the mean values of the latter accessions was considerable (0.73), and close to the LSD-value of 0.75 ($P = 0.05$). The ranking of the three resistant accessions agreed well with the previously obtained ranking in field experiments (Table 4).

The rate of variation of the susceptible accessions within the treatment-combinations was less in experiment 2 than in experiment 1, and again, the second recording (5 weeks after inoculation) showed a slightly lower rate of variation than at the first one (at 3 weeks; data not shown). The rate of variation within the treatment-combinations of the resistant accessions was relatively high, especially in FC703 and FC718. This is the result of the occurrence of some plants showing a disease rating as high as the plants of the susceptible accessions. Such susceptible plants in this material can be explained, since the resistant accessions used are part of a breeding programme and are still under selection. The process of genetical segregation, which was induced by the last cross between resistant and susceptible breeding material, has not yet been finalised. Therefore, these sugar beet accessions are not yet genetically homogeneous and thus segregate for resistant and susceptible individual plants. The further the selection for resistance proceeds, the more resistant individual plants per accession will be obtained. In general, the testing of 16–24 plants per accession will present a good impression of the level of resistance of the material.

In experiment 2, inoculation and screening of symptoms was carried out earlier than in experiment 1. Despite the difference in time between inoculation and disease evaluation, the levels of infection were comparable in the two experiments. Plants developed faster in experiment 2 than in experiment 1. Most likely, this was the result of the time of the year in which the experiments were carried out, and the specific weather conditions. Experiment 1 was carried out in spring under rather cloudy weather conditions, whereas experiment 2 took place in full summer, and the weather generally was clear and sunny. The greenhouse was regulated for temperature, but not for photoperiod. During cloudy days a minimum light intensity of 10 $Wm^{-2}$ was obtained, which is far below the natural light intensity that was reached during sunny days.

## Concluding remarks

Results indicate that the greenhouse test for screening individual sugar beet plants for resistance to crown and root rot caused by *R. solani* is useful. All plants showed some infection, indicating that the methods

*Table 4.* Mean disease indices of *R. solani* in sugar beet accessions, using a scale of 1–7, obtained in field experiments carried out at Fort Collins, Colorado, USA, in comparison to mean disease indices obtained in a greenhouse test (experiment 2 of the present study)

| Plant material | Results of field experiments | | | | | | | | Mean results of experiment 2 |
|---|---|---|---|---|---|---|---|---|---|
| | 1990[1] | 1993[1,2] | 1994[2] | 1995[2] | 1996[3] | 1997[2] | 1998[2] | 1999[2] | |
| FC703 | 1.3 | 1.2 | 1.8 | 1.9 | 1.4 | 3.5 | 3.2 | 3.8 | 3.73 |
| FC709-2 | 1.1 | 1.0 | 1.0 | 1.5 | 0.9 | 2.5 | 2.6 | 2.0 | 1.60 |
| FC718 | 1.5 | 1.1 | 1.4 | 1.5 | 1.3 | n.d. | n.d. | 3.8 | 2.33 |
| Susceptible check | 4.8 | 3.0 | 4.9 | 3.4 | 3.0 | 6.6 | 5.5 | 5.9 | 6.11 |
| LSD ($P = 0.05$) | 0.8 | 0.4 | 0.8 | 0.8 | 0.5 | 1.2 | 1.1 | 0.9 | 0.75 |

[1]Panella et al. (1995).
[2]Unpublished results from LWP.
[3]Panella et al. (http://www.crl.ars.usda.gov/rhzcgerm.htm).

of producing the inoculum and of inoculation were successful. In experiment 2, the level of infection in susceptible plant material was 2.4 times higher than the mean level in the three resistant accessions. The rate of variation within the treatment-combinations was influenced by the date of screening, the specific weather conditions, and, perhaps, the photoperiod. The ranking of the mean level of resistance in the greenhouse test was the same as observed in field experiments. However, the observations regarding the resistant accessions were biased by the occurrence of some susceptible plants, due to the fact that the resistant accessions were not genetically homogeneous. It was observed that the test was most successful when richer soil mixtures, in which the plants developed well were used. It is concluded that the greenhouse test can be used as a rapid assay for screening sugar beet plants for selection and research purposes.

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