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Identification of sugar beet germplasm EL51 as a source of resistance to post-emergence Rhizoctonia damping-off

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Abstract Resistance of sugar beet seedlings to Rhizoctonia damping-off caused by *Rhizoctonia solani* has not been described. A series of preliminary characterisations using a single susceptible host and four different *R. solani* isolates suggested the disease progression pattern was predictable. Two AG-4 isolates and a less virulent AG-2-2 isolate (W22) showed a comparable pattern of disease progression in the growth chamber where disease index values increased for the first 5–6 days, were relatively constant for the next 7–8 days, and declined thereafter. Seedlings inoculated with a highly virulent AG-2-2 isolate (R-1) under the same conditions showed similar patterns for the first 4 days post-inoculation; however disease index values continued to increase until seedling death at 13–14 days. Similar results were observed in the greenhouse, and a small expanded set of other germplasm lines were screened. One tested germplasm accession, EL51, survived seedling inoculation with *R. solani* AG-2-2 R-1, and its disease progress pattern was characterised. In a field seedling disease nursery artificially inoculated with *R. solani* AG-2-2 R-1, seedling persistence was high with EL51, but not with a susceptible hybrid. Identification of EL51 as a source of resistance to Rhizoctonia damping-off may allow investigations into the *Beta vulgaris–Rhizoctonia solani* pathosystem and add value in sugar beet breeding.

Keywords Disease screening · Disease progress · Germplasm screening · Virulence

Abbreviations

AG	Anastomosis group
CRR	Crown and root rot
DI	Disease Index
DPI	Days post-inoculation

Introduction

Beet (*Beta vulgaris*) is a globally important food and fodder crop; 25% of the world's sucrose is supplied by sugar beet (Draycott 2006). A recurring theme in many parts of the world is low emergence and poor stand establishment, which ultimately reduce sucrose yield due to the loss of beets. This problem is particularly acute in Michigan where only ca. 60% of planted seed ultimately develops into beets for sucrose production (Anonymous 2007). In contrast to poor emergence, which in Michigan is a more serious concern and largely results from various abiotic stresses (De los

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Reyes and McGrath 2003; McGrath et al. 2000), stand loss in fields with otherwise good emergence appears largely due to biotic stresses that almost certainly include interactions of beets with species of *Aphanomyces*, *Pythium*, *Fusarium*, nematodes, and *Rhizoctonia*. Our observations over the past 10 years suggest that early-season crop failures result in large part from Rhizoctonia damping-off. Rhizoctonia diseases of sugar beet are increasingly important in the Great Lakes growing region, and elsewhere (Panella 2005).

Rhizoctonia solani causes foliar, crown and root rot, and seedling diseases. It is a biologically complex species with many anastomosis groups (AGs) subdivided into subgroups (Carling 1996; Sneh et al. 1991), and its genetics are complicated and poorly understood (Adams 1996; Anderson 1982; Cubeta and Vilgalys 1997). Whereas *R. solani* AG-2-2 (specifically subgroups IIIB and IV) is the most serious pathogen subgroup contributing to crown and root rot of mature sugar beet, subgroup AG-4 has been implicated as a serious pathogen only on seedlings (Herr 1996; Leach 1991; O'Sullivan and Kavanagh 1991; Rush et al. 1994; Windels and Nabben 1989). Pre-emergence damping-off does not seem to be as important as post-emergence stand reductions, mainly because growers plant into cooler soils (8–12°C) where both AG4 and AG2-2 activity is largely reduced (Engelkes and Windels 1996). Typical post-emergence Rhizoctonia damping-off symptoms begin with slight browning of the stem at or just above the soil surface. As deterioration progresses, the seedling tissue appears water-soaked, collapses, and the seedlings shrivel and die.

Genetic resistance is available for the chronic phase of the disease (e.g., crown and root rot), and methods are widely applied for screening germplasm and breeding lines (Buttner et al. 2004; Ruppel et al. 1979; Scholten et al 2001). A number of crown and root rot (CRR) *Rhizoctonia*-tolerant sugar beet germplasm lines have been released over the past 30 years, although additional sources of resistance are needed (Luterbacher et al. 2005), and current resistance can trace ancestry to a narrow germplasm base (Panella 2005). To date, no host × isolate interactions in the resistance or susceptibility to different *R. solani* AG2-2 or AG4 isolates have been reported, although different degrees of virulence have been noted (Engelkes and Windels 1994, 1996; Ruppel 1972).

Direct sowing of seed into artificially pre-inoculated field nurseries with AG2-2 was determined to be ineffective for selection of resistance to CRR, with little or no survival of seedling-inoculated plants at harvest noted (Gaskill 1968). In growth chambers, and only at 26°C, the percentage of seedlings surviving 21 days post-germination in AG2-2 pre-inoculated soil was correlated with CRR resistance (Campbell and Altman 1976); however the proportion of survivors was low. In other tests, post-emergence damping-off with either AG2-2 or AG4 averaged >85% using a range of germplasm and isolates (Windels and Nabben 1989), although Ruppel (1972) showed that AG2-2 caused 70% damping-off in the CRR resistant germplasm FC701/2. Presumably based on these results, to date, a broad survey of sugar beet germplasm for Rhizoctonia damping-off resistance has not been reported.

Assuming little or no seedling resistance was present in sugar beet or related germplasm, our objective initially was to characterise the host–pathogen interaction of beet seedlings and *R. solani* by examining phenotypic responses of a (susceptible) hybrid with various *R. solani* isolates that varied in their ability to cause seedling disease, reasoning that gross differences observed between disease and non-disease outcomes might be exploited for finer discrimination, for instance at the cytological or molecular levels, and provide clues as to the nature of resistance and for disease management. However, this line of inquiry was adjusted after initial tests revealed potential seedling resistance, and the objective was changed to confirm and extend this finding. Here we describe results leading to our conclusion that sugar beet germplasm EL51 harbours resistance to post-emergence Rhizoctonia damping-off, and show that strong field resistance to Rhizoctonia damping-off exists in current sugar beet germplasm.

Materials and methods

Plant materials

Sugar beets USH20 (PI 631354) and EL51 (PI 598074) were used for most experiments (Coe and Hogaboam 1971; Halloin et al. 2000). Additional germplasm screened was obtained from the USDA-ARS breeding programme at East Lansing, MI USA [i.e., SR96=PI

628272 (McGrath 2003), Y03-384-18, Y03-384-60, Y03-384-99, Y03-384-70, and 92RM3 mm] or the U.S. National Plant Germplasm System [i.e., PI 285590, PI 285592, PI 285593, PI 285594, PI 285595, PI 546539, PI 552532, PI 558505, PI 558513=FC401 (Hecker and Lasa 1992), PI 558515, PI 546537, PI 546538, PI 546533, PI 552532, PI 546510, and PI 535826, each reported with resistance to Rhizoctonia CRR; see http://www.ars-grin.gov/cgi-bin/npgs/html/desc_form.pl?49; using Descriptor: Rhizoctonia <4].

For growth chamber and greenhouse experiments, seeds were soaked in 0.3% hydrogen peroxide for 24 h, allowed to germinate on water-soaked Whatman filter paper for 48 h prior to transplanting to BACCTO High Porosity Professional Planting Mix (Michigan Peat Company, Houston, TX, Code Number 3592). Seedlings were inoculated at 2 weeks post-germination. For field experiments, raw seed without added fungicides or chemicals was direct-seeded.

Fungal inocula

Rhizoctonia solani AG-2-2 isolates R-1 (subgroup IIIB, highly virulent) and W22 (low virulence, subgroup unidentified, ATCC # 18619) and AG-4 isolates R6 (lower virulence) and R7 (higher virulence) were used (kindly provided by Drs. Lee Panella and Linda Hanson, Ft. Collins, CO). Fungal isolates were grown on corn meal agar (CMA) in Petri dishes at room temperature. De-hulled millet seeds, sterilised at 120°C for 20 min and cooled to room temperature (repeated 3X), were placed as a single layer on 3 day-old CMA fungal cultures and incubated at room temperature for an additional 4 days. The infested millet seeds were scraped from the CMA plates, air dried in a bio-safety hood, and used directly as inoculum.

Disease screening protocols

Growth chamber To ascertain the disease progression of *R. solani* AG-2-2 and AG-4 isolates on a susceptible beet, the four fungal isolates were tested against the legacy hybrid USH20. USH20 seedlings were grown in pots (9 cm diam by 8 cm deep) in the growth chamber (20°C, 20 h light, 4 h dark), watered daily, and thinned to three plants per pot. Five pots (arranged in a complete randomised block, 15 plants total) of four- to six-leaf stage seedlings were inoculated with one of the four *R. solani* isolates by distributing ten fungus-infested millet seeds on the soil surface around the plant at a distance of 2 cm from each seedling. Control plants were mock-inoculated with sterilised millet seeds in the same manner. Symptoms on each seedling were recorded at 1-day intervals (days post-inoculation, DPI) according to a rating system based on the observed disease progression (Table 1). The mean seedling disease score at each DPI was reported as the Disease Index (DI).

Greenhouse All accessions above were screened in the greenhouse (18–24°C, 16 h light, 8 h dark) and watered daily in the same manner as for the growth chamber. Additionally, USH20 and EL51 were compared quantitatively using the same protocol as the growth chamber; however only *R. solani* AG-2-2 isolate R-1 and mock-inoculated treatments were performed. Damping-off disease progressions were scored at 1-day intervals using the rating system in Table 1. Experiments were managed in a double-blind fashion, and were conducted in September 2003 and July 2004.

Field Trials were planted at the Michigan State University Plant Pathology Farm in East Lansing, MI USA in 2004 and 2005 in plots that have been used for assessment of Rhizoctonia crown and root rot for >25 years, in a rotation of sugar beet, corn, and soybean (Ruppel et al. 1979). A split-plot design was used and each replicate (ten replicates in 2004 and 14 reps in 2005) was thinned to ca. 30 beets per 6 m plot (75 cm row spacing) within 2 weeks after emergence. Within 1 week after thinning, seedlings were inoculated with either *R. solani* AG-2-2 R-1 or sterilised

Table 1 Key to Rhizoctonia damping-off disease scoring of sugar beet seedlings

Score	Phenotypic symptom
0	Healthy plant
1	Shallow penetration scar at soil surface, visible to naked eye
2	Deep penetration scar, wound margins brown to black
3	Petioles lacking turgor and rigidity, hypocotyls with water-soaked lesions
4	Leaf blades wilting
5	Plant dead

millet seeds by placing 0.2 g inoculum on the soil surface 2 cm away from each plant. Stand counts were taken at inoculation and at four consecutive weekly intervals. The number of diseased plants at each weekly interval relative to the total number of plants present at that interval was used to calculate the proportion of diseased seedlings.

Re-isolation of *R. solani* from diseased sugar beet seedlings and inoculated soil

Re-isolation of *R. solani* AG 2-2 isolates R-1 or W22 from USH20 and EL51 was conducted in a separate experiment to address whether USH20 and EL51 differed with respect to colonisation by the *R. solani* AG-2-2 or whether the pathogen colonised tissues of both genotypes equally. In this case, wooden boxes (40×58×18 cm) were filled to within 2 cm of the top with BACCTO planting mix. Forty 3 day-old seedlings of USH20 or EL51 were planted per wooden box (five boxes per treatment, in randomised complete blocks) and thinned to 30 well-spaced seedlings after 2 weeks. These were inoculated with *R. solani* AG-2-2 R-1 or W22 by adding 0.1 g of inoculum (ca. 20 infested millet seeds) to the soil surface on opposite sides of each plant at a distance of 2 cm from each seedling. Ten seedlings were randomly selected, two seedlings per wooden box, at each day post-inoculation for pathogen re-isolation from asymptomatic tissues adjacent to lesions. Harvested seedlings were washed in running water for 2 h, and the leaves, two thirds of the hypocotyl, and the narrow tail of the root were excised. The remaining 2 cm of hypocotyl and root tissue was washed for 10 min in sterile water (repeated 3X), blotted to remove excess water under sterile conditions, and a sterile blade was used to section the hypocotyl into 1 mm slices adjacent to the diseased tissue, carefully avoiding direct contact with rotted tissue. Explants were transferred to water agar containing 0.05% lactic acid (*V*/*V*), incubated at 28°C in the dark, and after 24–72 h incubation they were mounted on microscope slides, stained with cotton blue and viewed at ×40 magnification. *Rhizoctonia solani* was identified by its distinctive mycelial morphology (Sneh et al. 1991). Presence of *R. solani* in the soil was also followed daily using 1.0 g soil samples taken from the top 3 cm of the wooden box soil profile at the site of each harvested seedling, and plated on selective media as described (Ko and Hora 1971).

Growth of *R. solani* on different media

Growth of four *R. solani* isolates was compared on five different media; 1.7% distilled water agar, 1.7% corn meal agar (Hardy Diagnostics, Santa Maria, CA, Item #C5491), potato dextrose agar (DIFCO, Detroit, MI), 1.5% BACCTO planting mix extract agar, and 1.7% sugar beet extract agar. Planting mix extract agar was made by autoclaving 400 *g* of air-dried BACCTO planting mix in 1.0 l of tap water for 1 h at 121°C and then centrifuged at 400×*g* for 5 min. Fifteen g of agar was added to 1.0 l of the supernatant (Rajendran et al. 1991). Plant extract agar was made from 2 week-old sugar beet seedlings. Five g of whole seedlings was crushed in 20 ml sterile water, filtered through muslin cloth, and centrifuged at 400×*g* for 5 min. The supernatant was diluted to a final volume of 1.0 l with distilled water, and 17 *g* agar was added. Media were sterilised at 121°C for 20 min, then dispensed (20 ml) into 9 cm diam Petri plates. Mycelial plugs, 5 mm diam, cut from the margin of an actively growing CMA plate, were placed on the centre of the test plates, and plates were incubated at 25°C in the dark. For each media, each fungal isolate was incubated at room temperature, in triplicate, and fungal growth was measured using a caliper after 4 days. The point of maximal growth from the centre of the inoculum plug to the edge of the mycelia was recorded.

Statistical analysis

Data from growth chamber and greenhouse experiments were analysed using mixed model ANOVA with repeated measures, results from each seedling being a discrete measure (dead seedlings recorded as value=5, Table 1). Data were analysed by SAS software (version 9.1.2; SAS Institute, Cary, NC). Tests were adjusted by the Tukey method. Adjusted *P*-values (<0.05) were considered as significant on the response variable (DI) between different treatments (i.e., USH20/R-1, USH20/W22, USH20/MOCK, EL51/R-1, EL51/W22, and EL51/MOCK). Field data compared variety (USH20 or EL51), treatment (AG-2-2 R-1 or sterilised millet seeds),

and week after inoculation (2, 3, or 4) using ANOVA (version JMP 5.1, SAS Institute, Cary, NC).

Results

We initially assumed that Rhizoctonia damping-off resistance was unavailable in beet germplasm, and reasoned that a careful comparison of the disease progression between a susceptible host and high- or low-virulence pathogen isolates would suggest opportunities for further study. Post-emergence seedling inoculation appeared to be an unexploited option whereby the damping-off disease progress could be visualised, and we initially applied this approach to a qualitative screen of 24 Plant Introductions and breeding materials in the greenhouse. Survival among these 24 germplasm lines was monitored daily for 14 days. Most plants from one germplasm release, EL51 that was previously reported with high resistance to CRR, survived infection from each fungal isolate, as did variable proportions of FC 401 (PI 558513) and an East Lansing breeding line, Y03-384-60, although these latter two were not characterised further. All other germplasm, including the legacy hybrid USH20, died under these conditions.

Growth chamber Rhizoctonia damping-off reactions

Concomitantly with the greenhouse screening of the 24 germplasm lines above, we monitored disease progress patterns in a growth chamber using the susceptible sugar beet hybrid USH20 inoculated with one of each of the four *R. solani* isolates. Disease progression of different *R. solani* isolates showed comparable features. A clear infection scar was observed at the soil surface, suggesting that all fungal isolates penetrated the host. Disease progression was similar through to the second DPI; (Fig. 1), and thereafter damping-off symptoms increased through to 5 DPI. For all but *R. solani* AG-2-2 R-1 inoculated seedlings, there was a reduction in DI at 7 DPI, followed by worsening symptoms through to 9 DPI, at which time disease progression was static through to 13 DPI. At 14 DPI, there was a marked drop in disease severity (except those infected with *R. solani* AG-2-2 R-1) and most seedlings survived. By this time, all USH20 seedlings inoculated with *R. solani* AG-2-2 R-1 had died. Slightly greater seedling

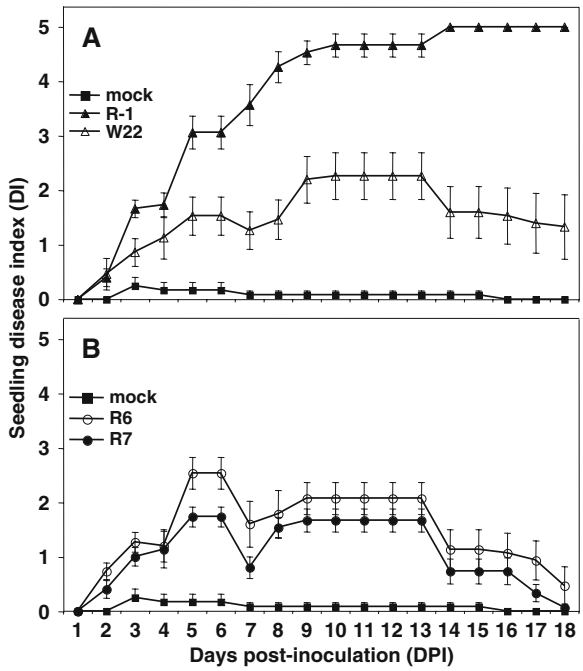

Fig. 1 Rhizoctonia damping-off disease progress curve in susceptible beet seedlings of USH20 using three plants per pot replicated five times, in the growth chamber (20°C, 20 h light). Values are the average of the 15 plants rated at each day post-inoculation (DPI). **a** Inoculation with AG-2-2 isolates R-1 (high virulence) and W22 (low virulence). **b** Inoculation with low virulence AG-4 isolates R6 and R7. *Error bars* are standard errors

disease was observed with *R. solani* AG-2-2 W22 than with either AG-4 isolates, and this difference was statistically significant (at the 5% level) only at DPI 5 and 6 (Fig. 1a, b); however these differences were not characterised further. These results established the competence of all tested *R. solani* isolates to cause symptoms on USH20. The disease reaction of EL51 was not monitored in the growth chamber.

To ascertain if the aggressiveness of *R. solani* AG-2-2 R-1 on USH20 could be explained in part by a faster growth rate of this isolate relative to the others, each isolate was plated on five different media. Significant differences in growth rate were apparent (Fig. 2), but none suggested that the aggressiveness of *R. solani* AG-2-2 R-1 was due to increased saprophytic growth. Indeed, by day 4, *R. solani* isolate AG-4 R6 mycelia had grown significantly larger than AG-2-2 R-1 on each medium except water agar. All tested organic media supported sclerotial formation.

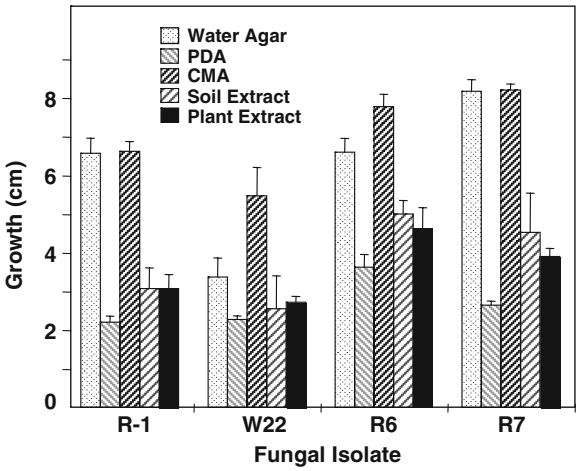

Fig. 2 Saprophytic growth of *R. solani* on different media. Agar plugs of inoculum were plated on each medium and the point of maximum growth was measured after 4 days of culture in the dark at 25°C. *PDA* = Potato dextrose agar, *CMA* = Corn meal agar. *Error bars* are standard deviations

Thus, saprophytic growth rates were not predictive of the disease outcome.

Disease progress patterns in the greenhouse and reaction of EL51

Since *R. solani* AG-2-2 R-1 was the only isolate to cause mortality in the susceptible host USH20, subsequent experiments focused on the interactions between this *R. solani* isolate and EL51, as a germplasm candidate for Rhizoctonia damping-off resistance, and the susceptible USH20 hosts. Damping-off disease progress patterns in the greenhouse were similar to those in the growth chamber for USH20 (Fig. 3), and the disease progress of EL51 mirrored that of USH20 through to 12 DPI (Fig. 3a) or 13 DPI (Fig. 3b), at which time EL51's DI decreased significantly. Most EL51 seedlings survived at the end of the experiment, and most USH20 seedlings had died. It should be noted that the greenhouse disease progression was delayed relative to the growth chamber and the aggressiveness of *R. solani* AG-2-2 R-1 appeared reduced. However, the striking difference in survival between USH20 and EL51 was repeatable in the greenhouse, and it was desirable to examine the extent of tissue ramification by the fungus in both hosts, as well as the applicability of these results to *bona fide* field conditions.

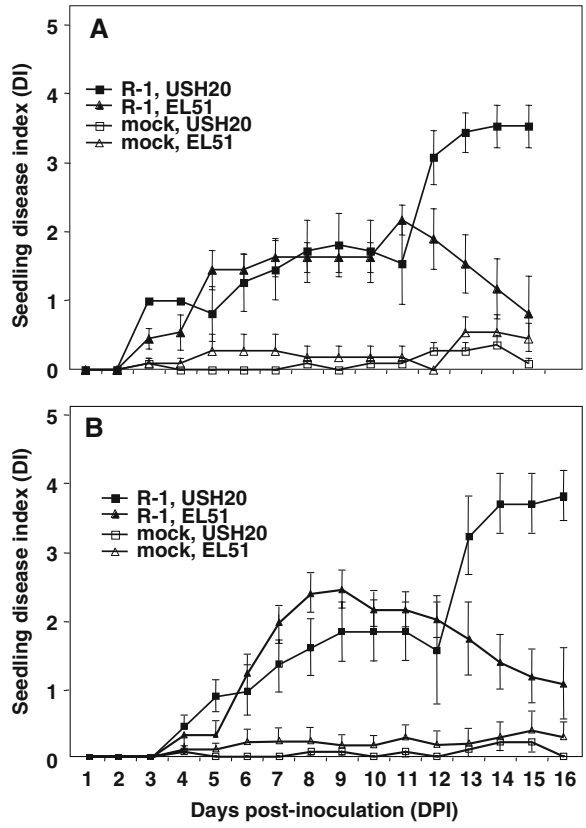

Fig. 3 Disease reaction of USH20 and EL51 sugarbeets inoculated with *R. solani* AG-2-2 R-1 in the greenhouse (18–24°C, 16 h light). The mean Disease Index (DI) was calculated from disease ratings of 15 individual seedlings (five pots each with three seedlings) at each day post-inoculation (DPI). **a** Experiment of September 2003. **b** Experiment of July 2004. *Error bars* are standard errors

Recovering *R. solani* from diseased seedlings and soil

We wished to know whether *R. solani* was present and active in all seedlings at all time points, or whether the unaffected seedlings were, in effect, cured of the fungus. In a separate greenhouse experiment, USH20 and EL51 were inoculated with either *R. solani* AG2-2 R-1 or W22. Non-symptomatic tissues were excised from roots and lower hypocotyls adjacent to diseased tissues, and were plated on selective media. Using AG-2-2 R-1, *R. solani* was isolated from all USH20 (susceptible) seedlings at each DPI (Table 2). In contrast, *R. solani* was only consistently re-isolated at 2 and 3 DPI in the putatively resistant EL51, after which only some of the tested samples harboured culturable fungus through to 9 DPI, and no *R. solani* was recovered

Table 2 Re-isolation of *R. solani* AG-2-2 from diseased seedlings

Sugar beet	*R. solani*	Days post-inoculation (DPI)											
		1	2	3	4	5	6	7	8	9	10	11	12
USH20	R-1	0	90	100	100	100	100	90	100	100	100	100	100
EL51	R-1	0	100	100	50	40	60	40	50	40	0	0	0
USH20	W22	0	100	100	100	100	100	100	40	60	60	0	0
EL51	W22	0	100	100	100	100	0	100	0	60	60	10	0

Seedlings of USH20 and EL51 were inoculated with *R. solani* isolates AG-2-2 R-1 (high virulence) or W22 (low virulence), and 10 seedlings were randomly selected at each day post-inoculation for pathogen re-isolation on water agar plates containing 0.05% lactic acid from asymptomatic tissues adjacent to lesions to determine if *Rhizoctonia* was ramifying the beet hypocotyl. Values are the % of sections with *R. solani* growing from the excised tissue

from 10 to 12 DPI. Using *R. solani* AG-2-2 W22, both USH20 and EL51 were similar in their ability to yield *R. solani*; however results were equivocal after day 5 with EL51 since between 0% and 100% of explants yielded culturable *R. solani* with no apparent pattern over time. *Rhizoctonia solani* was re-isolated from the soil in all instances.

Field validation

Field tests were performed in 2004 and 2005 to examine the reactions of USH20 and EL51 to seedling inoculation with *R. solani* AG-2-2 R-1. Stand counts were taken prior to inoculation, after thinning stands to no more than 30 plants per plot. Seedlings were inoculated at the two- to four-leaf stage of plant growth in 2005, but weather prevented inoculation until the four- to six-leaf stage in 2004. The number of wilted and dead plants were scored at weekly intervals, after a preliminary trial in 2003 demonstrated the timing of field disease progression.

The proportion of afflicted vs. total plants per plot was determined at each time interval (graphically represented in Fig. 4). In mock inoculations, no significant overall difference (i.e., summed across weekly scoring intervals) in the proportion of diseased plants was observed in 2004, but there was in 2005, and data were not combined. In 2004, but not 2005 or 2003 (data not shown), mock-inoculated USH20 showed extremely high variability in damping-off symptoms, especially pronounced at the last weekly interval (Fig. 4), suggesting other problems were also present in this nursery (although not specifically characterised, emergence and stand establishment in this field nursery has been problematic historically and *Aphanomyces* is known to be present. Both USH20 and EL51 have resistance to Aphanomyces seedling disease, and the wet conditions in 2004 would have exacerbated an *Aphanomyces* problem. However, Rhizoctonia damping-off in EL51 was not significantly affected by such confounding influences).

Combined over all intervals, the proportion of diseased seedlings in plots inoculated with *R. solani* AG-2-2 R-1 was significantly different between varieties in both years (2004: mean EL51=0.093, mean USH20=0.804, $F_{0.05}$=213.3, P<0.0001; 2005: mean EL51=0.040, mean USH20=0.459; R^2=0.706,

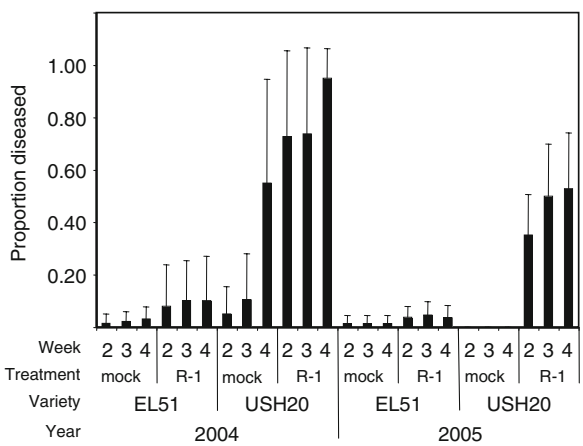

Fig. 4 Artificially-inoculated Rhizoctonia damping-off field nursery disease reactions between susceptible USH20 and resistant EL51 inoculated with virulent *R. solani* isolate AG-2-2 R-1 over 2 years. Ten replications were done in 2004, and 14 in 2005. The mean proportion of diseased plants relative to the number of healthy plants per plot is plotted. *Error bars* are standard deviations

$F_{0.05}$=359.0, P<0.0001). For EL51, most diseased plants were observed at the first time point, and little disease progression was observed subsequently. For USH20, symptoms progressed throughout the counting period, and beyond. USH20 also showed an increase in stand loss compared to EL51 in the mock-inoculated treatments. Final stand counts were not collected in 2005, but in 2004, EL51 retained virtually all plants at the end of season (20 weeks post-inoculation) despite showing mild damping-off symptoms early, while USH20 lost 39% (mock-inoculated) and 94% (*R. solani* AG-2-2 R-1-inoculated) plants at this same harvest time point.

Discussion

A Rhizoctonia damping-off screening protocol was developed for growth chamber and greenhouse conditions. The assay is novel in that a single fungal isolate is mechanically placed on the soil surface adjacent to post-emergent 2 week-old seedlings, and reproducible in displaying stages of infection that characterise the Rhizoctonia damping-off disease progression. Specific timing of disease progression events was slower in the greenhouse compared with the growth chamber, suggesting the environment plays a role in the host–pathogen interaction. Similarly, the timing of disease progression under field conditions was slower than that observed in the greenhouse. In all environments, clear discrimination between resistant and susceptible interactions was obtained.

All *R. solani* isolates successfully penetrated the hypocotyls as evidenced by an infection scar at the soil surface, and all caused at least some visible symptoms early in the infection process. With increasing time, there appeared to be two additional phases of the host–pathogen interaction. The second phase appeared to commence with a brief recovery period, suggesting a host response against the fungus or an unknown change in fungal metabolism, and was followed by a relatively static interaction. Ultimately, the third phase resolved the interaction to one where the plant either died or survived. These phases were clearest in the growth chamber, but also evident in the greenhouse. Whether the progression observed here represents the extremes of reaction for *R. solani* on sugar beet seedlings, or whether there are intermediate reactions is not known. Significant differences between non-lethal *R. solani* AG-2-2 W22 and AG4 isolates were noted, but not characterised in depth, suggesting that a range of reactions might be the norm.

The pattern of tissue ramification by the fungus was examined at a gross level of plating visibly healthy hypocotyl sections excised from areas adjacent to diseased tissues. In the case of the susceptible host USH20 infected with the virulent *R. solani* AG2-2 R1, *R. solani* was able to be cultured through to 12 DPI except the first day. In all other instances (e.g., USH20 and EL51 with low virulence isolates and EL51 with *R. solani* AG2-2 R1), the ability to culture *R. solani* from these explants was lost over time. Since, by necessity, these experiments were destructive, one conclusion is that this loss of culturability is not a uniform process in these plant populations, and, in some instances, the success or failure to recover *R. solani* may have been due to excising too close or too distant from the active lesion. However, on balance, it appears that the host is able to restrict the growth of the fungus *in planta*, and it is this ability of the host that leads to a favourable survival outcome for the plant. It is interesting to ask whether such a restriction in fungal growth is similar between a susceptible host and a low virulence pathogen *versus* the putative resistance of EL51 towards the virulent *R. solani* AG2-2 R1. Our unpublished evidence (Nagendran 2006; and in preparation) suggests that these two non-disease producing interactions occur via different as yet unknown mechanisms.

The resistant sugar beet EL51, even though infected initially, restricted subsequent establishment by the virulent *R. solani* AG2-2 R-1 isolate, and recovered from the disease, reminiscent of the interaction of susceptible USH20 with non-disease-causing isolates. This was unexpected, despite EL51's history of selection under inoculated CRR pressure over at least six breeding cycles in Michigan (Halloin et al. 2000). Early work towards developing a Rhizoctonia damping-off field nursery was abandoned due to excessive seedling and plant mortality exhibited in these fields (Gaskill 1968), since there was no reason to suppose any resistance was present in cultivated germplasm. Also, resistance appears to increase with increasing age of the sugar beet plant (Engelkes and Windels 1996); thus later inoculations were preferred due to the relative ease of discriminating CRR reactions in adult plants. In at least one instance, seedling reaction in growth chambers

appeared to correlate with CRR resistance; however this was valid only at one of two temperatures tested (i.e., 26°C) and there was high (ca. 40–50%) seedling mortality observed among highly-resistant CRR germplasm (Campbell and Altman 1976).

Ruppel (1973) observed that CRR resistance of mature sugar beet roots to AG-2-2 isolates is not due to a mechanical barrier. Both resistant and susceptible sugar beet cultivars were penetrated by fungal hyphae, and the pathogen was restricted to the periderm or outer secondary cortex in resistant germplasm, whereas in susceptible roots, several vascular rings were invaded. Our evidence indicates that the *R. solani* AG-2-2 R-1 fails to colonise beyond the endodermis of EL51 seedlings (Nagendran 2006; and in preparation), consistent with experimental results reported here. Whether the seedling resistance exhibited by EL51 is qualitatively different from its resistance to CRR is uncertain. Preliminary evidence suggests EL51 is resistant to pre-emergence Rhizoctonia damping-off, at least in the greenhouse (Nagendran, unpublished data). If confirmed, seedling resistance in EL51 may be qualitatively different than other highly-resistant CRR germplasm such as FC701 and FC702 (Campbell and Altman 1976). Assuming a substantially similar mechanism of resistance in seedling and CRR etiologies, a qualitative difference could relate to the timing of expression of seedling resistance, that is, earlier in EL51 and later in other highly-resistant CRR germplasm. It is conceivable that such a trait exists in other germplasm, and that selection and breeding history has simply increased the frequency of relevant alleles in EL51. Indeed, EL51 shares parentage with FC701, and it may be expected that seedling resistance is present in FC701, albeit at a lower frequency than EL51; however this has not yet been specifically examined.

In other species, resistance to *Rhizoctonia* seedling diseases is also needed. In both soybean and canola germplasm screens (Bradley et al. 2001; Yang and Verma 1992), where resistance is unavailable commercially, all tested plants were infected; however quantitative differences in disease expression were evident, but relatively few lines showed high levels of resistance at the seedling stage. In the case of canola, at least, seedling resistance appeared to be amenable to selection (Yang and Verma 1992). Uniform susceptibility was found to seedling disease in a large germplasm screen of rice germplasm; however a small proportion of these accessions did show resistance at later stages of development (Hori et al. 1981, cited in Akino and Ogoshi 1995). Similarly, in peanut, although resistance to Rhizoctonia at the seedling stage was found, this was not correlated with resistance at later development of the stem (limb rot) (Franke et al. 1999). For sugar beet, the association between CRR and seedling resistance needs to be tested further; however evidence suggests that these should not *a priori* be considered separate responses to the pathogen. Analyses of resistance to *Rhizoctonia* seedling diseases found in bean, cotton, potato, mustard, radish and okra suggest that processes associated with maintenance of cell wall integrity, such as inhibiting *Rhizoctonia*-derived cell wall degrading enzymes, play a role in the expression of resistance (reviewed in Akino and Ogoshi 1995). In our work, microscopic examination of diseased and non-diseased transverse hypocotyl sections showed penetration of the endodermis and maceration of the stele, in particular, in susceptible plants but not in resistant plants (Nagendran 2006, and in preparation).

In summary, all tested *Rhizoctonia solani* isolates were able to infect beets, and the interaction of *Rhizoctonia* with beet followed a predictable disease progression. Establishing that resistance is available within the cultivated germplasm pool of beets is an important development. The timing of resistance expression is variable, depending on the environment; however host processes appear to limit spread of disease within the plant in the resistant reaction but not in susceptible plants. Additional insight into this pernicious host–pathogen system may now be gained by a closer examination of the morphological and molecular outcomes of seedling disease by contrasting both susceptible and resistant beets with both high- and low-virulence *Rhizoctonia* isolates in disease- and non-disease-producing *Beta vulgaris*–*Rhizoctonia solani* interactions.

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