European Journal of Plant Pathology **110:** 53–62, 2004.

Characterisation of *Rhizoctonia solani* anastomosis groups causing bottom rot in field-grown lettuce in Germany

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Accepted 19 August 2003

Key words: anastomosis group, AG 1-IB, *Lactuca sativa*, pathogenic potential

Abstract

Bottom rot caused by *Rhizoctonia solani* is an increasing problem in field-grown lettuce in Germany. During the growing seasons of 1999 and 2000, 95 isolates of *R. solani* from lettuce plants with bottom rot symptoms were collected from eight locations. The isolates were characterised using hyphal anastomosis, pectic zymograms and morphological characteristics. Ninety-three isolates were identified as anastomosis group (AG) 1-IB, one as AG 1-IC and one as AG 2-1. Optimum hyphal growth was measured over a temperature range of 20–30 °C with an optimum at 25 °C. Aggressiveness of the AG 1-IB isolates varied from weak to strong when tested on detached lettuce leaves. The pathogenic potential of six AG 1-IB isolates was determined on 14 plant species in comparison with lettuce under conditions favourable for the fungus. Radish, broccoli, kohlrabi, spinach and millet seedlings were as severely infected as lettuce seedlings. The same isolates caused little symptoms on maize, tomato and onion. Knowledge about the host range of AGs of *R. solani* are important for planning an effective crop rotation as part of a control management system.

Introduction

Rhizoctonia solani Kühn the anamorph of *Thanatephorus cucumeris*, causes bottom rot disease of lettuce (*Lactuca sativa*). Typical symptoms appear as small rust-coloured necrotic spots on leaf midribs and leaf parts in contact with soil. The lesions expand into a rot that rapidly spreads to the adjacent leaves. The mycelium grows from the lower infected leaves mainly over the leaf surfaces to the inner leaves and finally, under favourable conditions, the head becomes a decayed mass. The disease has been known since 1900 when Stone and Smith (1900) described *Rhizoctonia* as a disease of greenhouse grown lettuce in Massachusetts. Since then bottom rot disease has been observed in all lettuce-producing areas (Davis et al., 1997).

In Germany, *R. solani* in lettuce is an increasing problem. Within a period of 4 years, 19 major lettuce-producing areas became severely infected (Kofoet et al., 2001). The use of fungicides was the main control strategy of bottom rot in lettuce in the past. At present, no fungicides are registered with an indication against *R. solani* on lettuce and alternative control methods do not exist.

Rhizoctonia solani is a ubiquitous soil-borne fungus comprising plant parasites and saprophytes. The species *R. solani* affects many agricultural and horticultural crops (Ogoshi, 1987) and is composed of genetically isolated groups (Adams, 1988). The identification and classification of these groups is primarily based on anastomosis behaviour (Anderson, 1982; Ogoshi, 1972). To date, 12 anastomosis groups (AGs), AG 1 to AG 12 are recognised (Carling et al., 1999, 2002). Isolates of *R. solani* from different AG generally do not anastomose with each other (Carling et al., 1987; Carling, 1996). Many of these AGs have been subdivided on the basis of host range, cultural

morphology, biochemical or molecular characteristics (Ogoshi, 1987). A certain degree of host specificity may occur amongst AGs.

Bottom rot in field-grown lettuce and the AGs causing the disease are not well documented. In previous studies, AG 1, AG 2 and AG 4 were shown to cause bottom rot disease in greenhouse-grown lettuce in the Netherlands (Kooistra, 1983) and in the UK (Wareing et al., 1986), whereas the same AGs plus AG 5 were detected in field-grown lettuce in the US (Herr, 1992). The occurrence of AGs and subgroups causing bottom rot in field-grown lettuce in Germany has not been documented. The objective of this study was to characterise isolates of *R. solani* causing bottom rot in field-grown lettuce in Germany. Isolates were studied for anastomosis behaviour, cultural appearance, nuclear conditions, hyphal growth rate at different temperatures, pectic zymograms and potential pathogenicity on different plant species.

Materials and methods

Pathogen isolation

During the 1999 and 2000 growing seasons (June to August), lettuce plants with symptoms typical of bottom rot were collected from eight commercial fields in south, central, northern and eastern Germany. Soil types found in the fields included sandy loam, loam, loamy sand, loamy silt and loamy clay (Table 1). Diseased lettuce plants were selected randomly at several sites in each field.

Infected leaves were washed for 2 min with tap water, surface-sterilised in sodium hypochlorite (1%) for 1 min and rinsed in sterile physiological sodium chloride solution (0.3%). Small pieces of leaves were cut from the margin of infected tissue. Four pieces from each plant sample were placed on water agar (WA, 1.2%) amended with 50 μg ml^{-1} streptomycin and 50 μg ml^{-1} penicillin. The agar plates were incubated in the dark at 25 °C. After 1–3 days, hyphal tips from colonies possessing characteristics of *Rhizoctonia* were isolated under a light microscope (100×) and transferred to potato-dextrose agar (PDA, Merck 1.10130). Cultures were stored at 10 °C until further use. Each pure culture was maintained on cereal grains at −20 °C (Sneh et al., 1986). From each lettuce plant one isolate was kept in pure culture.

Anastomosis group typing

Field isolates of *R. solani* (Table 1) were assigned to AG according to hyphal anastomosis with tester isolates from AG 1 through AG 5 (Table 2). Each isolate was paired with two tester isolates of each AG on 2% WA-coated slides with two replications (Tu et al., 1969). Mycelial disks (5 mm diameter) of field isolates and tester isolates growing on PDA were spaced 2–3 cm apart. The slides were placed on moist filter paper in Petri dishes (20 cm diameter) and incubated at 25 °C in the dark until the advancing hyphae from opposite disks overlapped slightly (2–3 days). The area of overlap was stained with lactophenol blue solution (Merck, 1.13741.0100) and examined microscopically at 100× for hyphal anastomosis. The reactions were grouped into categories in which category C0 is no reaction and C2 is fusion of hyphal cells that usually results in plasmolysis. Anastomosis grouping

Table 1. Characteristics of *R. solani* AG 1-IB isolates from diseased lettuce plants analysed in this study

Year of isolation	Location	Soil type	Number of isolates	Mean microsclerotia size [μm] ± SD
1999	Großbeeren	Sandy loam	9	363 ± 130
			1 (AG 1-IC)	
1999	Straubing	Loamy sand	4	372 ± 156
1999	Stockach	Loessal loam	9	363 ± 133
1999	Maikammer	Loamy sand	2	395 ± 82
1999	Ruchheim	Loamy sand	12	443 ± 117
			1 (AG 2-1)	
1999	Hannover	Loamy silt	18	365 ± 170
2000	Großbeeren	Sandy loam	8	407 ± 188
2000 (summer)	Golzow	Loamy clay	23	376 ± 125
2000 (autumn)	Golzow	Loamy clay	6	378 ± 129
2000	Wien	—	2	375 ± 85

Table 2. Rhizoctonia solani tester isolates used

AG	AG subgroup	Isolate code	Host	Source
1	IC	BV – 7	Sugar beet	Ogoshi
1	IC	F – 1	Sugar beet	Ogoshi
1	IA	C – 325	Rice	Ogoshi
1	IA	PRG 97 – 1	Rice	Hyakumachi
1	IB	TR – 22	Bent grass	Hyakumachi
1	IB	CS74 – 22	Sugar beet	Ogoshi
1	IB	B – 19	Sugar beet	Ogoshi
2	2–1	PS – 4	Pea	Ogoshi
2	2–2IV	K 1	Sugar beet	Sneh
2	2–2IV	Pf – 28	Sugar beet	Hyakumachi
3		B 15	—	Sneh
3		ST 11 – 6	Potato	Ogoshi
4		H1 521 – 21	Soil	Hyakumachi
4	4 HG-II	RH – 165	Sugar beet	Ogoshi
5		GM – 10	Soybean	Ogoshi
5		SH – 4	—	Ogoshi

— Indicates that all isolates were isolated in Japan.

was assessed positively (confirmed at 400×) when five or more anastomosis reactions were observed (Carling, 1996).

Morphological characteristics

Isolates of *R. solani* AG 1 were further subdivided into subgroups using Sherwood's (1969) morphological description of AG 1, the sclerotial type on WA according to Yang et al. (1989) and pectic zymograms (Schneider et al., 1997). Actively growing young cultures on PDA were placed in the centre of 9 cm Petri dishes containing 1.2% WA. Cultures (three dishes per isolate) were maintained at room temperature. After 3 weeks, the microsclerotia (size $<700\,\mu m$) were measured microscopically at 100× magnification. The size of 6–12 sclerotia were measured per isolate. Only isolates belonging to AG 1-IB produced microsclerotia after 3 weeks on WA.

The number of nuclei of 16 isolates was counted after staining with one drop of alkaline safranin solution and one drop of 3% KOH (Bandoni, 1979). Nuclei were counted within 20 hyphal cells per isolate of actively growing cultures.

Pectic zymograms

Analysis of pectic zymograms was used as a routine method for rapid assignment of *R. solani* isolates to AG and AG subgroups (Cruickshank, 1990; MacNish et al., 1993; Schneider et al., 1997). Pure cultures were grown on pectin medium with citrus pectin (Sigma P9135) as a carbon source. Twenty-seven arbitrarily chosen field isolates from lettuce were analysed with pectic zymograms. Isolates were incubated for 10 days at 23 °C in the dark. Ten microlitre of the culture filtrate was then loaded on a native polyacrylamide gel, with 1.5 M Tris/HCl, pH 8.8, in the separating gel and 0.5 M Tris/HCl, pH 6.8, in the stacking gel. These native gels were run for 70 min in running buffer and were subsequently incubated in 0.1 M DL-malic acid for 1 h followed by staining with 0.02% ruthemium red for 2 h. The AG 1-IB anastomosis tester isolate was loaded on each gel as a reference isolate for comparison. The gels were fixed in 3 mM Na_2CO_3, scanned for permanent storage and sealed in cellophane (Schneider et al., 1997).

Rate of hyphal growth

The hyphal radial growth of 49 arbitrarily selected isolates was measured at 10, 15, 20, 25, 30 and 35 °C on PDA and compared with the radial growth of AG 1-IA, 1-IB and 1-IC tester isolates. A mycelial disk from the margin of a 7-day-old culture was placed in the centre of a Petri dish (9 cm diameter) containing PDA. The average colony diameter of the two replications of each isolate was assessed at 24-h intervals until the colony had reached the edge of the Petri dish. The average hyphal growth rate per isolate was calculated per temperature. Data from 0 to 24 h after inoculation were excluded.

Aggressiveness

The aggressiveness of *R. solani* isolates was tested according to the method of Thornton et al. (1999) two times. Detached leaves of 7-week-old lettuce plants of cv. Daguan were placed in plastic containers lined with moistened tissue paper. Mycelial disks of a 14-day-old PDA culture of *Rhizoctonia* isolates were placed on the leaf surface. Each leaf sample had three inoculation sites. Three inoculated leaves (three replicates) were tested for each isolate. A control set of lettuce leaves was inoculated with a sterile disk of PDA. After an incubation of 7 days at 20 °C lesion size was assessed with software 'KS 400 Imaging System' from Carl Zeiss Vision GmbH (1997).

Pathogenic potential

The pathogenic potential of three arbitrarily chosen isolates (7/3/14, H20, O4/10) high in aggressiveness to lettuce (lesion size $>30\,cm^2$) and three isolates (O8/14, H26, H10) low in aggressiveness (lesion size $<10\,cm^2$) was determined on different plant species using the method described by Carling et al. (1999). The following crops were included in the test: radish (*Raphanus sativus* var. *sativus*) cv. Sirri, broccoli (*Brassica oleracea* convar. *botrytis* var. *italica*) cv. Marathon, kohlrabi (*B. oleracea* convar. *acephala* var. *gongylodes*) cv. Eder, spinach (*Spinacia oleracea*) cv. Ballett, millet (*Panicum miliaceum*), bean (*Phaseolus vulgaris*) cv. Paulista, carrot (*Daucus carota*) cv. Napoli F1, pea (*Pisum sativum*) cv. Evita, Sudan grass (*Sorghum sudanense*) cv. Bicolor, wheat (*Triticum aestivum*) cv. Theresea, tomato (*Lycopersicon esculentum*) cv. Counter, maize (*Zea mays conv. Saccharata*) cv. Indira, onion (*Allium cepa* var. *cepa*) cv. Ishikura LiWhite and lettuce (*L. sativa*) cv. Nadine. Plates with 2% WA were inoculated with a PDA disk (5 mm diameter) of the fungal isolate. The control treatment was seeded with a sterile PDA disk. One day after transfer, six surface-sterilised (1% NaOCl for 1 min) seeds were placed on the agar surface in a circle. The agar plates were incubated in the dark at 21 °C for 8 days. The pathogenic potential of tester isolates belonging to AG 1-IA (isolates C-325 and PRG 97-1), 1-IB (TR 22, CS74-22) and 1-IC (F-1, BV-7) was tested against lettuce cv. Nadine using the same methodology.

For each isolate–host combination, three replicates were tested. The experiment was replicated two times. Disease severity (DS) was assessed 3 and 8 days after placement of the seeds on agar according to the following scale: 1 = healthy, no lesions on hypocotyl; 2 = minor discolouration of hypocotyl; 3 = discolouration plus small (<1 mm diameter) necrotic lesions on stem, hypocotyl or root; 4 = discolouration plus large (>1 mm diameter) necrotic lesions on stem, hypocotyl or root; and 5 = seedling dead.

Statistical analysis

For statistical analysis we used the STATISTICA program (StatSoft Inc., Tulsa, Oklahoma, USA). The statistical analysis of the aggressiveness of the tested isolates on detached leaves measured as differences in lesion size of isolates were compared with the control after ANOVA using the Tukey's procedure (HSD) with $P = 0.05$. Differences in hyphal growth rate were analysed using the least-significant-difference test (LSD) with $P = 0.05$. The pathogenic potential of lettuce isolates on different plant species was tested after non-parametric analysis, using the Kruskal–Wallis test with $P < 0.05$. The correlation was calculated for hyphal growth rate and lesion size on detached leaf, and between hyphal growth rate and DS on lettuce seedlings.

Results

Anastomosis group typing and colony morphology

Ninety-four *Rhizoctonia* isolates from diseased lettuce plants anastomosed with the tester isolates belonging to *R. solani* AGs 1-IA, 1-IB and 1-IC (Table 1). Pairing of isolates with each tester isolate of AG 1 gave C2 reactions. One isolate (2/37) showed C2 anastomosis reaction with AG 2 tester isolates. Pairing of lettuce isolates with other tester isolates gave C0 anastomosis reactions.

The 94 isolates of AG 1 were further divided into subgroups based on microsclerotial production on WA. The tester isolates of AG 1-IA and AG 1-IB were included in the investigation for comparison. Ninety-three lettuce isolates of AG 1 formed microsclerotia and no or few sasakii-type sclerotia on WA, whereas isolates of AG 1-IA produced only sasakii-type sclerotia. The microsclerotia of the 93 lettuce isolates of AG 1-IB had a lateral growth type of development, which was different from the loose growth of sasakii-type sclerotia of IA subgroup isolates. These observations are in accordance with those of Yang et al. (1989). Microsclerotia appeared as tiny white spots and later were dark brown to nearly black spots on WA. The size of mature microsclerotia of AG 1-IB isolates on WA varied from 241 to 650 µm. On PDA all field isolates and tester isolates (AG 1-IA and 1-IB) also produced brown to dark-brown sclerotia, which were variable in size and often aggregated into compound sclerotia. The occurrence of microsclerotia was also observed microscopically on diseased lettuce leaves, especially individually in lesions on midribs at harvest time, whereas the production of macrosclerotia was mainly observed on the surface of the dead lower leaves in the field. The diameter of 20 mature microsclerotia selected from leaf midribs was measured at an

average of 50.7 μm, whereas sclerotia produced on WA measured 383 μm on average.

Only one isolate from the location Großbeeren was identified as AG 1-IC (Table 1), which can readily be distinguished from AG 1-IA and AG 1-IB according to colony morphology. AG 1-IC isolates developed on PDA the 'salt and pepper' colony type, conforming to Sherwood's (1969) type 3 with sclerotia a uniform size of 0.5–1.6 mm. The hyphal cells were multinucleate and contained 3–21 nuclei with an average of six per cell.

Rate of optimal hyphal growth

The optimal hyphal growth rate of 49 *R. solani* lettuce isolates was measured over a temperature range of 20–30 °C (Figure 1). The hyphal growth rate of single isolates varied from 13 to 39 mm day^{-1}. The optimum temperature for hyphal growth rate of tester isolates ranged also from 20 to 30 °C with an optimum at 30 °C for AG 1-IA and AG 1-IB or at 25 °C for AG 1-IC. The growth rates of lettuce isolates with a high standard deviation at each temperature were greater at 10–30 °C in comparison with the tester isolates, but corresponded more to those of tester isolate AG 1-IC. Statistically significant differences in growth rate were found between lettuce isolates and AG 1-IA tester isolates at 10 °C and between lettuce isolates and AG 1-IB tester isolates at 15–25 °C. No growth was observed at 5 °C. All isolates tested, produced sclerotia on PDA at 10, 15, 20 and 25 °C, but not at 30 or 35 °C.

Pectic zymograms

Twenty-six lettuce field isolates were assigned to AG 1-IB, according to their similarity with the AG 1-IB tester isolate. Little variation in pectic enzyme patterns was observed among these 26 isolates (Figure 2). No relationship between aggressiveness and pectic enzyme pattern was found. Isolate 2/37 was identified as AG 2-1 when compared with the anastomosis tester isolate.

Aggressiveness

Seventeen isolates of *R. solani* AG 1-IB from different locations were tested for aggressiveness on detached leaves (Table 3). Irregular circular lesions developed initially at the inoculation site. Later, lesions enlarged and produced tan to dark brown discoloured areas on the leaf.

Between the two experiments no significant differences were identified. The average size of healthy lettuce leaves was 90.2 cm^2. All tested isolates were able to infect the lettuce leaves within 7 days of inoculation, but the results showed considerable variation

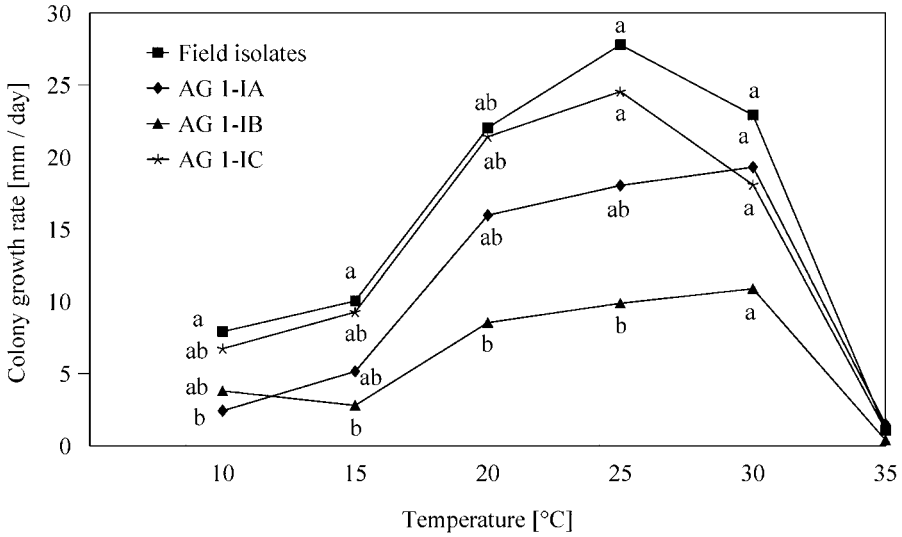

Figure 1. Average hyphal growth rate of *R. solani* isolates from diseased lettuce plants belonging to AG 1-IB on PDA at six temperatures in comparison to tester isolates belonging to AG 1-IA, AG 1-IB and AG 1-IC. Averages per temperature followed by the same letter are not significantly different according to the LSD test ($P = 0.05$).

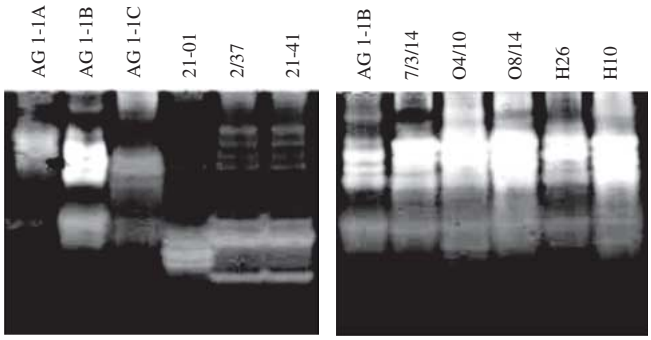

Figure 2. Pectic zymograms of *R. solani* anastomosis tester isolates AG 1-IA, AG 1-IB, AG 1-IC, AG 2-1 (21-01, 2/37, 21-41) and isolates from lettuce (7/3/14, O4/10, O8/14, H26, H10) obtained with a commercially available vertical gel-electrophoresis system.

Table 3. Lesion size (cm^2) caused by *R. solani* AG 1-IB isolates on 7-week-old detached leaves of lettuce cv. Daguan, inoculated with PDA disks overgrown with the fungus

Isolate code	Lesion size [cm^2]	Standard deviation
Control	0.06 a	0.05
1/3/22	0.96 a	1.08
2/3/31	1.42 b	1.07
3/3/13	4.38 a	6.43
6/3/21	81.12 c	29.58
7/3/14	82.23 c	32.91
H5	3.61 b	3.72
H10	2.81 a	3.98
H17	0.97 b	0.78
H20	87.40 c	26.40
H26	4.86 a	5.34
O1/1	1.68 b	0.94
O2/17	0.68 b	0.46
O4/10	42.88 d	38.01
O6/6	0.48 b	0.28
O8/14	2.77 b	2.15
PF2/4	2.83 a	6.01
PF2/14	0.62 b	0.55

*Average lesion sizes followed by the same letter are not significantly different according to Tukey's test ($P = 0.05$).

in colonisation of leaf tissue between isolates. Isolates O4/10, 6/3/21, 7/3/14 and H_20 were the most aggressive and caused severe infection with a lesion size of 42.9–87.4 cm^2. Aggressiveness of these isolates was significantly different from that of all other isolates tested. Lesion size caused by isolates 1/3/22, 3/3/13, H10, H26 and PF2/4 was not significantly different from the control. In these treatments lesion sizes were highly variable with standard deviations varying from 1.08 to 6.43. Most isolates tested were weak to moderately aggressive, with a lesion size of 0.62–4.8 cm^2 on average, within 7 days. No correlation ($r = 0.049$) was found between lesion size and hyphal growth rate.

Pathogenic potential

No significant differences in DS were apparent between the two experiments carried out with 14 plant species 3 and 8 days after seed had been transferred to the plate. All seedlings without fungal inoculation remained symptomless. Lettuce seedlings were more severely damaged by *R. solani* than the other plant species within 3 days (Table 4). Radish, broccoli and kohlrabi were infected by all isolates, but more severely by isolate 7/3/14, whereas other isolates caused low to moderate DS on these plant species. Few or no symptoms were observed on carrot, millet, pea, Sudan grass, wheat, tomato, maize and onion. All lettuce, radish, broccoli, kohlrabi, spinach and millet seedlings were severely infected (DS > 3.5) by all isolates within 8 days. The DS of all other plant species were significantly lower (DS < 3.0). Bean, carrot, pea, Sudan grass and wheat were moderately to severely infected and tomato, maize and onion seedlings were less damaged (DS < 2).

Few symptoms developed on lettuce when confronted with isolates O8/14 and H10 within 3 days. The DS on lettuce incited by isolates 7/3/14, O4/10 and H10 were significantly higher compared with the DS caused by isolates H20, H26 and O8/14 within 8 days. The mean DS including all crops caused by isolate 7/3/14 was significantly higher compared with the mean DS caused by the other isolates (Figure 3).

Isolates TR 22 and CS74-22, belonging to subgroup AG 1-IB, caused more severe damage (DS $= 4.5$) on lettuce seedlings than isolates C-325 and PRG-97-1 of

Table 4. DS of 14 crop seedlings 3 and 8 days after inoculation with *R. solani* AG 1-IB isolates (7/3/14, H20, O4/10, O8/14, H26, H10) and mean DS of all isolates per crop in an average of two experiments

Plant species	3 days after inoculation[a]						8 days after inoculation						Mean DS	
	7/3/14	H20	O4/10	O8/14	H26	H10	7/3/14	H20	O4/10	O8/14	H26	H10	DS-3	DS-8
Lettuce	3.1	2.3	2.7	1.1	2.0	1.1	4.7	3.2	4.7	2.7	3.8	4.4	2.1	3.9
Radish	3.0	1.6*	2.1*	1.7*	1.2*	1.7*	4.6	4.2*	4.2*	4.0*	4.2*	4.4	1.9	4.3
Broccoli	3.1	2.3	1.4*	1.1	1.2*	1.2	3.9*	4.1*	4.1*	4.2*	4.3*	4.1	1.7	4.1
Kohlrabi	3.9*	1.6*	1.6*	1.1	1.5	1.3	4.6	3.5	3.9*	3.3*	4.4*	3.7	1.8	3.9
Spinach	2.0*	1.0*	1.5*	1.1	1.0*	1.0	4.2*	3.4	3.0*	3.2	3.8	3.9	1.3*	3.6
Millet	1.2*	1.0*	1.0*	1.0	1.0*	1.0	4.0*	3.4	3.7*	3.5	3.9	3.3*	1.0*	3.6
Bean	1.8*	1.5*	1.1*	1.1	1.0*	1.0	3.3*	3.3	2.8*	2.8	2.9*	3.0*	1.2*	3.0*
Carrot	1.3*	1.0*	1.0*	1.0	1.0*	1.0	3.9*	2.6*	2.6*	2.8	3.1*	1.8*	1.1*	2.8*
Pea	1.3*	1.0*	1.1*	1.0	1.0*	1.0	2.8*	2.4*	2.0*	2.0*	1.9*	2.4*	1.1*	2.3*
Sudan grass	1.0*	1.0*	1.0*	1.0	1.0*	1.0	3.3*	2.9*	2.8*	2.4	2.5*	2.5*	1.0*	2.7*
Wheat	1.0*	1.0*	1.0*	1.0	1.0*	1.0	3.5*	3.3	3.5*	2.8	3.5	3.1*	1.0*	3.3*
Tomato	1.1*	1.0*	1.0*	1.0	1.0*	1.0	2.9*	1.6*	1.6*	1.6*	2.1*	1.6*	1.0*	1.9*
Maize	1.1*	1.0*	1.0*	1.0	1.0*	1.0	2.5*	1.2*	1.2*	1.4*	1.7*	1.6*	1.0*	1.6*
Onion	1.0*	1.0*	1.0*	1.0	1.0*	1.0	1.4*	1.4*	1.4*	1.4*	1.5*	1.5*	1.0*	1.4*
Mean value	1.9	1.3	1.3	1.1	1.1	1.1	3.5	2.9	3.0	2.7	3.1	2.9	—	—

[a]Mean value of DS of each isolate assessed on a scale ranging from 1 to 5, in which 1 = plant healthy and 5 = plant dead.
*Significantly different from lettuce according to the Kruskal–Wallis test ($P < 0.05$).

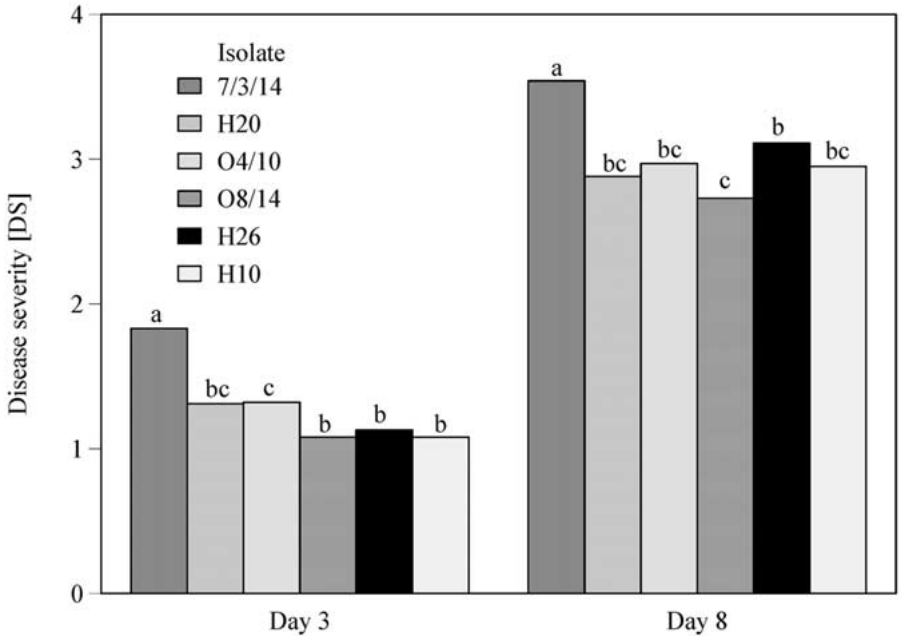

Figure 3. DS (1 = plant healthy, 5 = plant dead) caused by *R. solani* AG 1-IB isolates averaged over 14 plant species 3 and 8 days after inoculation. Means in a column followed by the same letter are not significantly different according to the Kruskal–Wallis test ($P < 0.05$).

AG 1-IA and isolates F 1 and BV 7 of AG 1-IC within 8 days (Table 5). Moderate infection (DS 2.7 and 2.1) on lettuce seedlings was observed in response to isolates of subgroups AG 1-IA and AG 1-IC.

Discussion

Identification and characterisation of *R. solani* isolates causing bottom rot in field-grown lettuce is a

Table 5. DS of seedlings of lettuce cv. Nadine 3 and 8 days after inoculation with tester isolates belonging to AG 1-IA, 1-IB and 1-IC of *R. solani*

AG subgroup	Isolate code	Day 3	Day 8
IA	C-325	1.0 a	2.2 ab
IA	PRG-97-1	1.3 bc	3.2 a
1B	TR 22	2.4 d	4.4 c
1B	CS74-22	1.2 c	4.7 d
IC	F 1	1.7 e	2.7 a
IC	BV 7	1.0 a	1.6 b

*Means in a column followed by the same letter are not significantly different according to the Kruskal–Wallis test ($P < 0.05$).

prerequisite to developing environmentally friendly and durable crop-management systems. In Germany, little information is available on which AGs of *R. solani* occur on field-grown crops in general and horticultural crops in particular, although the occurrence of AGs of *R. solani* in sugar beet (AG 2-2, AG 4) was reported (Zens and Dehne, 1997).

AG 1-IB is the predominant AG causing bottom rot of field-grown lettuce in Germany. Our results are in agreement with those of Herr (1992), who also found AG 1-IB isolates to be the major AG causing bottom rot of field-grown lettuce in Ohio. Herr also found that AG 1-IC, AG 2-1, AG 2-2 and AG 4 were involved in the lettuce bottom rot complex. Wareing et al. (1986), however, found AG 2 isolates to be the dominant AG causing lettuce bottom rot on glasshouse-grown and on some field-grown lettuce plants at two locations in the UK. In addition to AG 2 isolates, Wareing et al. (1986) found AG 1, AG 2-1 and AG 4 isolates. Kooistra (1983) predominantly isolated AG 1 isolates from diseased glasshouse-grown lettuce plants in the Netherlands. These isolates were not further divided into subgroups.

The diameter of microsclerotia of AG 1-IB on diseased soybean leaves was $240 \pm 33\,\mu m$ (Yang et al., 1989), whereas the sclerotium size of our isolates varied from 241 to 650 µm on WA and was 51 µm on lettuce leaves. The factors which influence sclerotium size *in vitro* or *in vivo* are unclear. The availability of nutrients could be a factor or perhaps the existence of physiological specialisation for soybean or lettuce. More information is needed regarding the occurrence of microsclerotia under field conditions and their importance for the survival of *R. solani*. How long microsclerotia survive in soil is unknown. Yang et al. (1988) suggested that microsclerotia may be related to the sexual stage of the pathogen and the occurrence of both microsclerotia and the teleomorph was observed frequently following extended periods of rain on soybean in Lousiana. However, we did not find the teleomorph in the field.

Our results show a high pathogenic potential of both AG 1-IB tester isolates and the field isolates from lettuce. This is in agreement with Herr (1992), who reported AG 1-IB isolates were the most virulent among those tested in a glasshouse assay. We also found AG 1-IB field isolates and tester isolates of AG 1-IB to vary in DS on lettuce seedlings. Some isolates (7/3/14, O4/10, H10) caused more severe symptoms on seedlings than other isolates (O8/14, H20). The tester strains of AG 1-IB were not isolated from lettuce, but for example from bent grass (isolate TR 22). Irrespective of variation in DS on lettuce, the tester isolates of AG 1-IB damaged seedlings more severely than the tester isolates of subgroups 1-IA and 1-IC.

The characterised isolates were sampled at random from different fields. Both highly and less aggressive isolates with a low or high hyphal growth rate occurred in a field population, and no relationship was found between aggressiveness and hyphal growth rate. Isolates 7/3/14 and 6/3/21 (highly aggressive) and isolates 1/3/22 and 2/3/31 (low in aggressiveness) from the same field population differed greatly in hyphal growth rate. The low aggressive isolates (1/3/22 and 2/3/31) had a higher hyphal growth rate than the highly aggressive isolates. In contrast, isolates H20 (highly aggressive) and H10 (low in aggressiveness) were also sampled from the same field (Hannover), but the hyphal growth rate of both was similar with no statistically significant differences. Additional research is required to show which properties are responsible for variation in aggressiveness. Plant cell wall-digesting enzymes may be related to aggressiveness of isolates. Also it is to be explored if aggressiveness on detached leaves correlates with the level of virulence.

The hyphal growth rate of AG 1-IB tester isolates was lower in comparison with the growth rate of freshly isolated lettuce isolates, but there was a range in growth rates among isolates from lettuce. Growth characteristics on PDA supported the observation that *R. solani* isolates from lettuce and tester isolates of AG 1 preferred temperature conditions between 20 and 30 °C. The optimal temperature conditions for mycelial growth were the same for all isolates. Lettuce bottom rot caused by *R. solani* is favoured by warm, wet conditions and can cause crop losses as high as 70%

(Davis et al., 1997). Problems with bottom rot exist especially in commercial fields in warmer regions such as southern Germany.

Rhizoctonia solani has a wide host range. In this study, we investigated the ability of AG 1-IB isolates originating from lettuce to infect other plant species. The experimental set up indicates that the pathogenic potential and the pathogenicity have to be verified in pot and field experiments. On average, lettuce, radish, broccoli, kohlrabi, spinach and millet were more severely infected in comparison to the other plant species. Plant species like tomato, maize and onion were only slightly susceptible to AG 1-IB isolates. All plant species of the family Brassicaceae (radish, broccoli, kohlrabi) were more severely infected, whereas the plant species of the Poaceae (maize, Sudan grass, wheat, millet) varied from slightly to high susceptibility. We hypothesise that plant species which we consider highly pathogenic in our bio-assays, may also infect by *R. solani* under field conditions. This hypothesis remains to be tested in the field. Plant species susceptible to *R. solani* AG 1-IB may maintain or increase the population level of the pathogen in the field and are disadvantageous in crop rotation. For example, a negative effect of maize in rotation with sugar beet on *Rhizoctonia* root rot in sugar beet was observed (Wolf and Verreet, 1999) and the causal agent of root rot (AG 2-2) was also isolated from wheat and barley. The pathogen caused damping-off on these crops (Scheinpflug and Heupel, 1998). In commercial lettuce production, crop rotation can be employed as part of a strategy to control *R. solani*. Therefore, knowledge about the host range of *R. solani* AG 1-IB when planning an effective crop rotation system is essential.

Acknowledgements

This work was supported financially by the Ministries of Agriculture of the Federal Republic of Germany and the States of Brandenburg and Thuringia. Our thanks to Drs. A. Ogoshi, M. Hyakumachi and B. Sneh for supplying isolates of *R. solani*. We would also like to thank Kerstin Fischer and P.M.S. Musters-van Oorschot for skilled technical assistance.

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