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Possible mechanisms influencing the dynamics of rhizoctonia disease of tulips

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Abstract

In two observation fields, where six sites were artificially infested with *Rhizoctonia solani* AG 2-t, bare patches developed. These patches did not re-occur at the site of infestation in three successive years. In fields with and without artificial infestation, natural infection of tulip bulbs by *Rhizoctonia* spp. occurred. The spatial distribution of infected tulip bulbs was visualised in maps after kriging. The influence of sampling intensity was evaluated by stepwise reduction obtained in the observed data set of the first year. Omnidirectional semivariogram characteristics did not change when sampling intensity was reduced down to 10%. The average maximum prediction error was minimised at sampling intensities varying from 7% to 25%. Naturally occurring bare patches slowly vanished during successive cropping of flower bulbs and did not re-appear in the fourth growing season. A high frequency of isolation of *R. solani* AG 2-t in one field (Lisse-2) in the fourth consecutive crop did not result in bare patches in that year. It is hypothesised that a reduction in aggressiveness may account for this observation. In contrast, bulb rot due to *Rhizoctonia* spp. increased during the observation period. *R. solani* AG 5 isolates were seldom isolated before the bulbs flowered, but were the dominant isolate from bulbs at harvest. In a growth chamber experiment, it was demonstrated that AG 5 did not account for replacement of AG 2-t. However, it was demonstrated that competition may partially explain replacement of AG 2-t isolates during the growing season. At 18 °C, but not at 9 °C, an AG 4 isolate prevented AG 2-t colonising and infecting iris bulbs when both isolates were introduced together to soil. *Rhizoctonia* populations develop in relation to soil temperature and plant development. It is hypothesised that a 'temporal niche differentiation' may be one of the mechanisms affecting the dynamics of rhizoctonia bare patch of tulips.

Introduction

The soil-borne fungus *Rhizoctonia solani* (Kühn) (teleomorph: *Thanathephorus cucumeris* Frank (Donk)) seriously threatens flower bulb production in the Netherlands. *R. solani* is a heterogeneous species composed of genetically isolated groups (Anderson, 1982) designated as anastomosis groups (AG). Isolates of *R. solani* are assigned to these AG according to hyphal fusion with AG tester isolates (Sneh et al., 1991; Carling, 1996). Rhizoctonia disease of field grown tulips in the Netherlands may comprise a complex of AG (Dijst and Schneider, 1996; Schneider et al., 1997b; Schneider, 1998). Tulip bulbs are planted from early October till early December at a depth of 8 cm. In autumn, roots develop rapidly and the shoot grows slowly. In spring, all mother bulb organs senescence and daughter bulbs develop rapidly after flowering. *R. solani* AG 2-t infects the shoots growing through low temperature soil (Schneider et al., 1997a,b). Symptoms include retarded shoot emergence, leaf blight and lesions on the stem. With severe infection, shoots often fail to emerge, causing typical rhizotonia bare patch. Severe infection of the shoot hampers the development of daughter bulbs and causes bulb rot. In glasshouse grown iris, rhizoctonia lesions and rot develop on the

neck of the bulb (Sonderman and MacLean, 1949; Doornik, 1981; Schneider et al., 1997b). In addition to AG 2-t, isolates belonging to AG 2-2, AG 4 and AG 5 caused bulb rot in tulip and iris in greenhouse (Doornik, 1981; Schneider et al., 1997b) and in open-air experiments (Schneider et al., 1999). The impact of AG 2-2, AG 4 and AG 5 isolates causing bulb rot in commercial flower bulb production fields, however, is unknown.

Rhizoctonia disease in flower bulbs is mainly controlled by full field application of fungicides or by soil disinfestation. For environmental reasons, the input of fungicides has to be reduced. In order to develop effective management practices for rhizoctonia disease in flower bulbs, insight into the mechanisms causing disease dynamics is essential. The patchy nature of black scurf in potato (Jager and Velvis, 1995), root and crown rot of sugar beet (Hyakumachi et al., 1990) and rhizoctonia bare patch of cereals (MacNish and Neate, 1996) in the field is well documented. The spatial analysis of disease data, however, has received little attention (Neate and Schneider, 1996). Displaying disease distribution on maps is relatively inexpensive, allows rapid visualisation of spatial patterns (Campbell and Madden, 1990) and provides a quick insight in to disease dynamics over time. Lannou and Savary (1991) applied geostatistics for mapping disease incidence of rhizoctonia blight in groundnut plots. Geostatistics is based on the theory of regionalised variables (Matheron, 1963), meaning that the value of the variable under study depends only on its spatial position. Geostatistics uses an optimal interpolation method, kriging, with a minimised and known variance and is thus highly valuable for mapping.

The dynamics of rhizoctonia bare patch in flower bulbs has not been documented. According to farmers, patches may vary in size and number within one field between successive years. The objective of our study was (1) to visualise the spatial distribution of rhizoctonia disease in maps with reduced sampling effort in four consecutive years, (2) to unravel the complex of *R. solani* anastomosis groups causing disease in experimental fields and (3) to propose hypotheses to explain the mechanisms behind the changing disease patterns.

Materials and methods

Observation fields

To study disease dynamics, observation fields were established in 1991 at the Bulb Research Centre (LBO), Lisse, and at the experimental station 'Proeftuin Zwaagdijk', Zwaagdijk, The Netherlands. Flower bulbs had been grown before at the two locations, but the history of *R. solani* in previous (bulb) crops was not documented. At each location the two fields consisted of seven beds of 10×1.5 m each. In the autumn of 1991, bulbs were planted four rows per bed in Lisse and in five rows per bed in Zwaagdijk. In 1991, within-row distances were 22.5 cm in Lisse and 20 cm in Zwaagdijk. In 1992, 1993 and 1994 all fields were planted with four rows of bulbs, with an intra-row distance of 22.5 cm. Planting density was approximately 100 bulbs per m^2 for all fields in the four years. The soil is a sandy soil with a clay content (fraction $<2\mu$m) of 1.5% at Lisse and a loamy soil with a clay content of 15.4% at Zwaagdijk. Crop husbandry, fertilisation, application of fungicides to control fire blight of tulips caused by *Botrytis tulipae* Lind., and weed control were performed according to farmers' practices. For accurate mapping, and to avoid disturbance and spread of inoculum by machinery, all bulbs were harvested by hand.

Soil infestation

One field per location was used to allow natural development of rhizoctonia bare patch and rhizoctonia bulb rot. These fields are referred to as Lisse-1 (L-1) and Zwaagdijk-1 (Z-1). To encourage the development of patches in at least one field at each location, six areas were artificially infested in the second field at each location in 1991. These fields are referred to as Lisse-2 (L-2) and Zwaagdijk-2 (Z-2). The even beds were infested at 2.5 m and at 7.5 m in the middle of each bed (Figures 1 and 2). Inoculum was prepared by growing *R. solani* AG 2-t isolate 2tR002 (Schneider et al., 1997b) for 2–3 weeks on autoclaved oat kernels at 20 °C in the dark. Between the inner two rows, soil was removed over an area of 20×20 cm^2 until the neck of the bulbs became visible. The soil was infested by evenly spreading 10 g inoculum, over this area, followed by replacing the soil.

Crop rotation during four successive years

A rotation of tulip – iris – tulip – tulip was applied to favour rhizoctonia disease development. In the week of October 27, 1991, all experimental fields were planted with *T. greigii* cultivar (cv.) 'Red Riding Hood'. These were harvested around June 20, 1992. In the

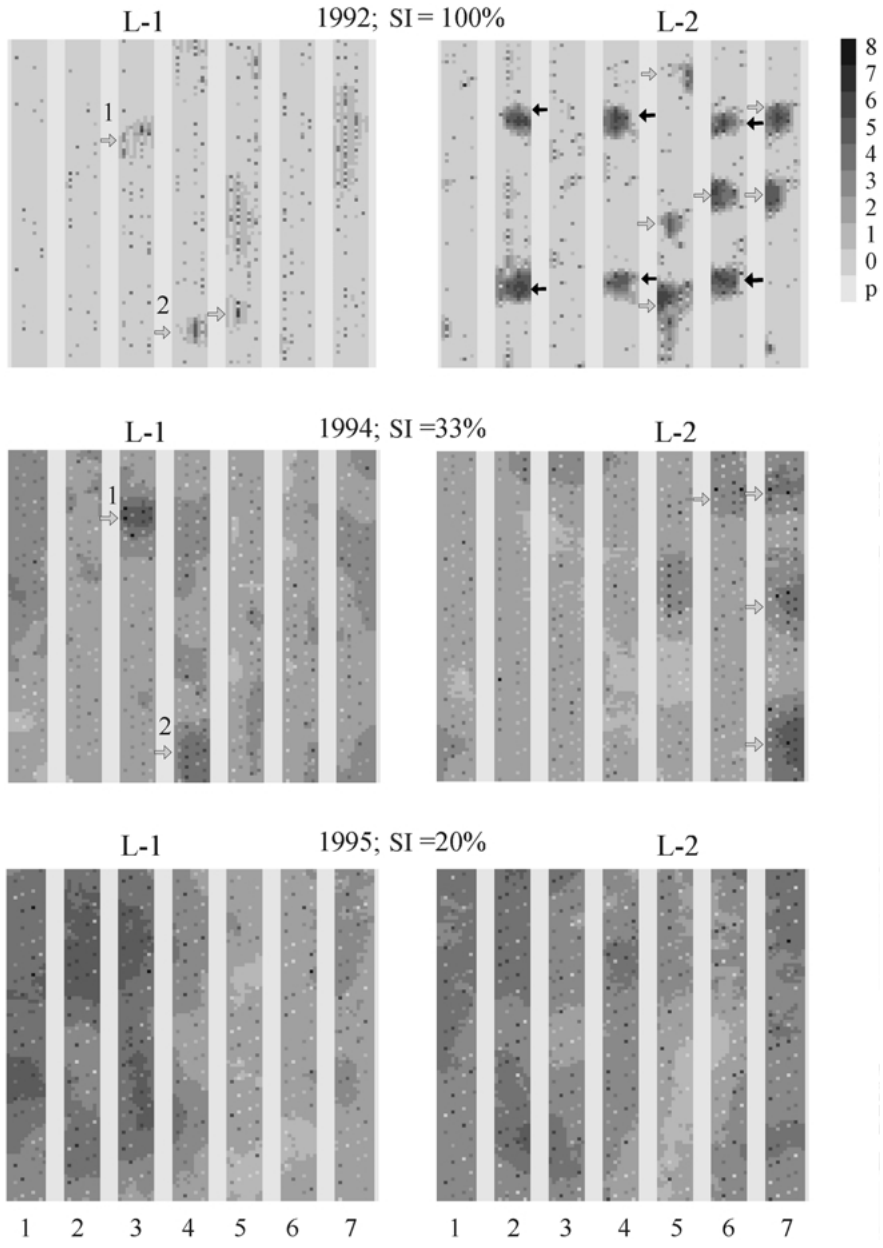

Figure 1. Spatial distribution of rhizoctonia disease at Lisse after kriging using the omnidirectional semivariogram parameters of Table 1 and a sampling intensity (SI) of 100%, 33% or 20%. Lisse-1 was used to allow natural development of the disease, whereas Lisse-2 field was artificially infested at six sites, indicated by black arrows. Legend: p = path between beds, 0 = no infected bulbs to 8 = bulbs completely decayed. The grey, right oriented arrows indicate natural infested patches.

week of November 3, 1992, all experimental fields were planted with Iris cv. 'Blue Magic'. These were harvested around August 17, 1993. In the week of November 4, 1993, all fields were planted again with *T. greigii* cv. 'Red Riding Hood'. These were harvested around June 17, 1994. In the week of November 4, 1994, all fields were planted with *T. kaufmanniana* cv. 'Giuseppe Verdi'. These were harvested around June 8, 1995. Between harvest and subsequent planting no crops were grown. Planting material was obtained

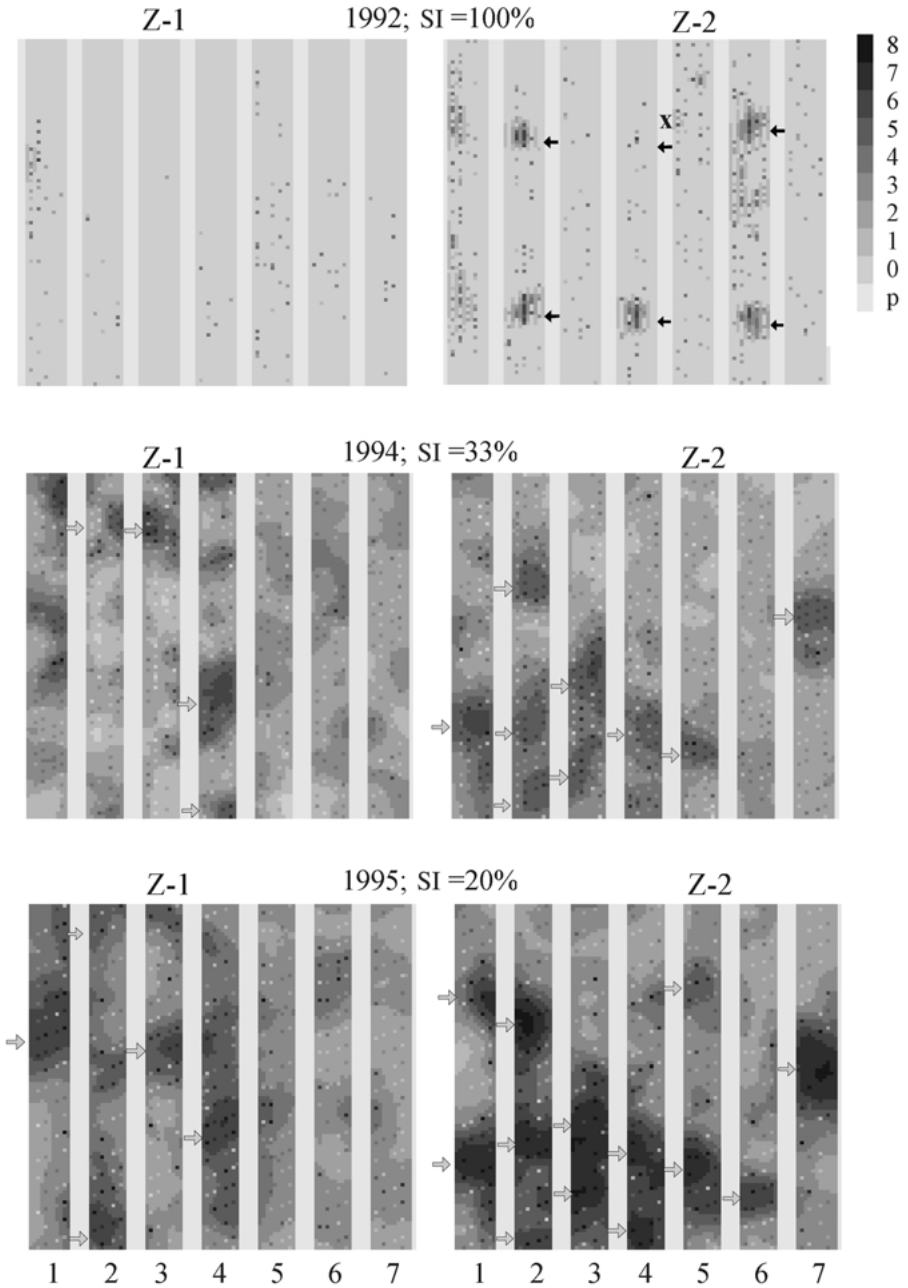

Figure 2. Spatial distribution of rhizoctonia disease at Zwaagdijk after kriging using the omnidirectional semivariogram parameters of Table 1 and a sampling intensity (SI) of 100%, 33% or 20%. Zwaagdijk-1 was used to allow natural development of the disease, whereas Zwaagdijk-2 was artificially infested at six sites, indicated by black arrows. Legend: p = path between beds, 0 = no infected bulbs to 8 = bulbs completely decayed. The grey, right oriented arrows indicate natural infested patches.

from breeders' plots where there was no rhizoctonia bare patch. The bulbs were visually inspected for disease symptoms by A. Doornik of the Bulb Research Station at Lisse. Bulbs without symptoms were used to plant experimental fields. To check for bulb-borne inoculum, some randomly selected bulbs were planted in a standardised soil mixture (Schneider et al., 1997b) and incubated for six weeks at 9 °C in the glasshouse.

Disease assessment

After harvest, bulbs were stored at 4 °C until disease assessment. Disease severity per bulb was rated on a scale from 0 to 8 (Schneider, 1998) with 0 : 0% of the bulb surface area infected, no distinct *R. solani* symptoms visible; 1: up to 5% of the bulb surface covered with small *R. solani* lesions; 2: up to 12.5% of the bulb surface covered with lesions; 3: up to 25% of the bulb surface covered with lesions; 4: up to 50% of the bulb surface covered with lesions, and/or one bulb scale disrupted; 5: >50% of the bulb surface infected and/or at least two bulb scales disrupted; 6: no daughter bulbs developed, mother bulb apparently healthy; 7: bulb almost completely decayed; 8: bulb completely decayed, plant dead. Bulbs in disease classes 0–5 had developed a cluster of daughter bulbs, whereas those in disease classes 6–8 apparently developed only one bulb. Mean disease severity (DS) was calculated per 0.1 m row length.

Disease mapping

Mapping was done using geostatistics (Matheron, 1963; Journel and Huijbregts, 1978). Experimental *semivariograms* were fitted to linear, exponential and spherical models using the FVARIOGRAM and MVARIOGRAM directives of Genstat 5, Release 3.2 (Genstat 5, 1995). The semivariance (γ) is a function of the lag, the distance between samples (h), as follows: $\gamma(h) = \frac{1}{2}E\{[Z(x+h) - Z(x)]^2\}$, with $Z(x)$ a set of regionalised variables (DS) and E the expectation of the random variable. The highest percentage variance accounted for by the model and the lowest nugget variance were both used to choose the omnidirectional semivariogram model for spatial interpolation or *kriging*. Kriging provides an interpolated value $Z^* = \sum_{i=1}^{N} \lambda_i Z_i$ where Z_i is the parameter (DS) at sample site i, N is the number of neighbouring sample sites and λ_i are the weights applied to each Z_i, so that Z^* is unbiased and with minimum variance. The KRIGE directive of GENSTAT produces DS matrices of kriging interpolators and the accompanying estimation errors. Maps (Figures 1 and 2) were produced using the sample co-ordinates for interpolated DS matrices in Corel Presentations 6.0 (CorelDRAW 6.0, 1995). The shading with various intensities of grey, within and between plant rows (four per bed), is due to kriging. In other words, kriging provides a map as if every 0.1 m of the field had been planted.

Spatial variation

Ideally, the semivariogram passes through the origin, where the distance between sample units is zero. The nugget (C_0) represents the unexplained or random semivariance caused by measurement errors or microvariability. The range is the distance between sample units (h) at which the semivariance reaches a more or less constant value, called the sill. A pure nugget effect occurs when the semivariance appears solely as a discontinuity at the origin with $\gamma_{(0)} = 0$ or $\gamma_{(h)} = C_0$ (Journel and Huijbregts, 1978) and is interpreted as no spatial dependency. Variation in the spatial distribution of DS was studied by means of omnidirectional semivariograms and directional semivariograms, along rows (90°) and across rows (180°) with a tolerance of 45°. If the semivariograms of the two perpendicular directions have approximately the same parameters, the spatial variation is considered isotropic. If the semivariograms along rows and across rows display different ranges, anisotropy prevails (Journel and Huijbregts, 1978; Burrough, 1987).

Sampling

Sampling was done in units of 0.1 m row. The positions of the sample units and the numbers of diseased and healthy bulbs in each sample unit were recorded. To gain an insight into the spatial distribution of a certain property, sampling should be done as densely as possible on a regular grid (Webster and Burgess, 1984). Therefore, at harvest in 1992 we sampled all bulbs in all four infested fields. For estimation of the spatial semivariogram, sampling on a regular triangular grid (Burgess et al., 1981) or systematic sampling (square grid) provides best semivariogram parameters (Burgess et al., 1981; Corsten and Stein, 1994).

The effect of sampling intensity on the accuracy of kriged maps was evaluated stepwise considering every 2nd, 3rd, 4th, 5th, 10th, 15th and 20th 0.1 m row length as a sample unit of the 1992 data set. The sampling intensity was thus reduced to 50, 33, 25, 20, 10, 7 and 5% of the original data. One criterion for optimal sampling is that the maximum prediction error is minimised (Webster, 1985). The most uncertain locations with the highest prediction error are the co-ordinates midway between neighbouring observations (Stein et al., 1994). The mean maximum prediction error, σ^2_{mm}, was assessed by excluding the kriging variance accompanying the observed values (value 0) from the prediction

error matrix obtained by kriging per field and per sampling distance. The quality of the kriging result was assessed by (1) graphing σ^2_{mm} to the sampling distance, and (2) comparison of omnidirectional semivariogram characteristics.

The effect of sampling distance on the accuracy of the kriged maps was not determined in 1993 and 1994. Preliminary analysis in 1992 indicated that a sampling intensity of 33–20% gave representative maps for our fields. In 1993 and 1994 therefore, every third 0.1 m of all rows was used as a sample unit, the maximum amount that could be handled to assess DS and to isolate and identify isolates causing bare patch and bulb rot. For 1995, a sampling intensity of 20%, one in five 0.1 m row length, was used. In all three years regular sampling (1 : 3 or 1 : 5) was applied, with the first sample unit varying regularly with the row to be sampled.

Disease development within one growing season

In 1995, each bed in L-2 was subdivided in quadrats of 1 m^2, thus resulting in 10 quadrats per bed or 70 per experimental field. Each month, from February to May, four tulip plants, one per row, from each quadrat were randomly sampled. Disease incidence (DI) per field was assessed as the mean percentage of rhizoctonia infected tulip bulbs per quadrat. Disease prevalence (DP) gives information on the disease distribution over the field and was assessed as the mean percentage of quadrats per field with at least one rhizoctonia infected tulip. From all sampled bulbs, four per quadrat, *Rhizoctonia* spp. were isolated and identified as described below. The disease parameters were analysed by ANOVA after an arcsine transformation.

Isolation and identification of Rhizoctonia *spp.*

R. solani-like isolates were obtained by plating out, on water-agar amended with 250 ppm chloramphenicol and 250 ppm metalaxyl (WACM) (Schneider et al., 1997b), bulb bottoms from all sampled bulbs. Agar plugs taken from the edge of *R. solani*-like colonies were transferred again to WACM and stored on PDA at 4 °C until they could be identified. Isolates were grouped according to colony morphology. From each group, representative isolates (25–50%) were assigned to AG according to anastomosis with tester strains of AG 1 to AG 5 (Schneider et al., 1997b) and pectic zymography (Schneider et al., 1997a). The number of nuclei of the isolates that did not anastomise with AG 1 to AG 5 was counted after staining with 4′, 6′-diamidino-2-phenylindole (DAPI) (Martin, 1987).

Competition between R. solani *AG 2-t and AG 4 or AG 5*

During the research period we observed that AG 2-t isolates were abundant on infected tulip shoots in early spring when soil temperatures were low, but rarely isolated at harvest. In contrast, AG 5 was found at low incidence in early spring and at high incidence at harvest. The hypothesis that a high incidence of AG 2-t in early spring and a low incidence at harvest could be explained by competition between AG 2-t and AG 5 or other soil microorganisms was tested using *R. solani* AG 5, AG 4 and iris as a model. Iris bulbs can be used in potting experiments year-round, but not tulips. Moreover, glasshouse grown tulip bulbs and field grown tulip bulbs are physiologically different (Le Nard and De Hertogh, 1993). Iris bulbs were planted in pots and inoculated with either AG 2-t, AG 4 or AG 5 or dually with AG 2-t + AG 4 or AG 2-t + AG 5. Two colonised oat kernels of each isolate were placed in the corner of each pot. Inoculum was prepared by growing isolates 2tR002, 4R22 (Schneider et al., 1997a,b) and 5R40, obtained from iris in L-2 in 1993, on autoclaved oat kernels (Doornik, 1981; Schneider et al., 1997b) for 2–3 weeks at 20 °C in the dark. One iris bulb cv. 'Blue Magic' was planted per 10 × 10 × 9 cm pot filled with a standard soil mixture (Schneider et al., 1997b). Six pots (six replications) with one bulb were used per treatment. The experiment was carried out in two climate chambers, one at constant 9 °C and the other at constant 18 °C, favourable to AG 2-t and AG 4 or AG 5, respectively (Doornik, 1981; Schneider et al., 1997b). After 6 weeks of incubation, DS of iris bulbs was assessed on a scale from 0: 0% of the bulb area infected, no symptoms; 1: 5% of the bulb area infected; 2: 20% of the bulb area infected; 3: 50% of the bulb area infected; 4: 75% of the bulb area infected, and 5: 100% rotted or plant dead. For each plant, two pieces of bulb bottom, bulb and stem (irrespective of the presence of lesions) were plated out to re-isolate *R. solani.* Isolation and identification was done as described above. The experimental design was a complete randomised block. Statistical analysis was done by ANOVA in GENSTAT.

Results

Disease maps

The spatial distribution of infected tulip bulbs in our observation fields is visualised in kriged maps (Figures 1 and 2). Sample units can be seen as regularly appearing pixels along the rows. Each pixel represents 0.1 m. The darker the pixels, the more severe the infection of the bulbs at harvest. Bulbs with a DS of 6, 7 or 8 (dark grey to black pixels) were mostly found in bare patches, but also occurred at random in the field. A DS of 0, light grey pixels, indicates that no symptoms due to *R. solani* were found on the bulbs. DS maps for 1994 and 1995 (Figures 1 and 2) show areas varying in intensity of grey as a result of interpolation between white pixels (healthy sample units) and black pixels (DS = 8; plant severely affected or dead).

Development of bare patch, 1992

Upon emergence of the tulip shoots in the first week of March 1992 most shoots had been severely infected by *R. solani* in all six artificially infested patches in L-2 and in five out of six artificially infested patches in Z-2 (Figures 1 and 2). Infection was light in the sixth patch of Z-2 (indicated with **x** in bed 4). At harvest all 12 artificially infested patches were visible as distinct bare patches. In addition, a few small (2–3 dm^2) bare patches (indicated by short sequences of black pixels in Figure 1), probably due to natural soil infestation, had developed in L-1 and L-2. The infested area of artificially and naturally developing patches was approximately 1 m^2, which was in accordance with patch sizes frequently observed in farmers' fields (Schneider, 1998). No natural bare patch occurred in Z-1. Upon harvest in mid-June, tulip bulbs with *R. solani* symptoms were found outside bare patches in all four experimental fields. On average, DS ($\pm$S.D.) was 0.35 ± 1.0, 0.69 ± 1.52, 0.07 ± 0.45, and 0.25 ± 0.89 for L-1, L-2, Z-1 and Z-2, respectively (Table 1).

Occurrence of bare patches and bulb rot, 1993–1995

In 1993 (iris), 1994 (tulip) and 1995 (tulip) bare patches did not re-occur at the areas which were artificially infested in 1992. However, bare patches and bulb rot did occur outside artificially infested sites in 1992 (Figures 1 and 2). At harvest in August 1993, iris bulbs did not show any disease symptoms and therefore no disease maps are shown for 1993.

Lisse-1. In 1994, the size of two bare patches, coded 1 and 2 (Figure 1), had increased compared to 1992. In addition, a few scattered individual severely infected tulip plants were found (indicated by a single black pixel). In June 1995, no distinct bare patch occurred and only a few severely infected plants (with DS = 6, 7 or 8) were randomly distributed in the field (indicated by individual dark pixels). On average, DS had increased from 0.35 in 1992 to 3.0 in 1995 (Table 1).

Lisse-2. In June 1994, small bare patches (indicated by grey arrows in Figure 1) had developed in beds 5, 6 and 7. In June, 1995, no bare patches occurred, but there were some severely infected bulbs, apparently distributed at random (scattered dark pixels in Figure 1). Since a mean DS per sample unit (0.1 m) is displayed, individual, severely infected bulbs are not clearly visible. On average DS had increased from 0.69 in 1992 to 3.1 in 1995 (Table 1).

Zwaagdijk-1. In June 1994, no bare patches due to *R. solani* were found at harvest. As of February 1994, however, a severe infection due to *R. tuliparum* developed causing bare patches (shown as dark flecks in Figure 2). *R. tuliparum* forms thick black sclerotia on infected bulbs, whereas *R. solani* does not (Boerema, 1963). Both fungi are therefore readily distinguishable in the field. Bulb rot, mainly caused by *R. tuliparum*, occurred in patches, but it was also found outside bare patches (Figure 2). In 1995, no bare patch due to *R. solani* was found. By contrast, bare patches due to *R. tuliparum* had increased in abundance and severity in comparison to 1994. On average, DS increased from 0.07 in 1992 to 3.5 in 1995.

Zwaagdijk-2. As of February 1994, bare patches due to *R. solani* did not occur, but bare patches due to *R. tuliparum* were abundant as indicated by the dark shaded roundish patches. Bulb rot occurred in patches, but was also found scattered through the field (Figure 2). At harvest in June, 1995, no bare patches but some bulb rot due to *R. solani* was found. Bare patches due to *R. tuliparum*, however, had increased in abundance and severity compared to 1994, as in Z-1. Outside the bare patches bulb rot was also found (Figure 2).

Table 1. Disease severity (DS) statistics and semivariogram parameters at different sampling intensities for four experimental fields during three tulip growing seasons

	Field	SI[1]	DS[2]		Omnidirectional				Along				Across			
			Mean	SD	Model[3]	Nugget[4]	Sill[5]	Range[6]	Model[7]	Nugget	Sill	Range	Model[7]	Nugget	Sill	Range
1992	L-1	100	0.35	1.00	L	0.96	PN	—	S	0.77	0.97	8.5		0.95	PN	—
		50	0.36	1.01	L	0.98	PN	—		0.60	1.00	7.4[a]		0.93	1.06	14.0[a]
		33	0.35	1.01	L	0.98	PN	—		0.93	PN	—		0.99	PN	—
		25	0.36	1.00	L	0.93	PN	—		0.94	PN	—		0.87	PN	—
		20	0.33	0.97	L	0.82	PN	—		0.94	PN	—	S	0.59	1.02	13.8
		10	0.40	1.06	L	1.01	PN	—		0.85	PN	—		0.74	PN	—
		7	0.41	1.02	L	1.07	PN	—		1.20	PN	—		1.01	PN	—
		5	0.39	1.02	L	1.14	PN	—		0.65	PN	—		2.61	PN	—
1994	L-1	33	2.50	1.20	S	1.03	1.44	12.3	S	1.03	1.39	20.6	L^n	1.40	PN	—
1995	L-1	20	3.00	1.80	L	2.33	0.03	—	L	2.30	PN	—	L	2.40	0.02	—
1992	L-2	100	0.69	1.52	S	0.57	2.62	12.6		0.49	2.76	12.2[a]		0.36	2.45	11.8[a]
		50	0.70	1.52	S	0.65	2.59	12.5		0.37	2.71	11.8[a]		0.32	2.43	11.4[a]
		33	0.68	1.51	S	0.73	2.53	12.8		0.46	2.71	11.4[a]		0.27	2.36	11.9[a]
		25	0.67	1.49	S	0.78	2.52	13.9		0.85	2.72	15.3[a]		0.37	2.31	12.3[a]
		20	0.71	1.52	S	0.06	2.55	11.3		0.53	2.56	11.0	L^n	1.48	PN	—
		10	0.79	1.57	S	1.01	3.18	20.6	L	2.20	PN	—	L^n	0.72	PN	—
		7	0.66	1.41	S	0.01	2.17	10.7	L^n	3.53	PN	—		0.41	2.14	14.9
		5	0.62	1.45	S	1.07	2.65	23.3	L^n	1.32	PN	—	L^n	−2.09	PN	—
1994	L-2	33	2.3	1.4	S	1.79	1.84	13.9	S	1.28	1.91	13.2	L^n	1.67	PN	—
1995	L-2	20	3.1	1.9	L	3.03	0.02	—	L	2.86	0.02	—	L	2.99	0.03	—

1992	Z-1	100	0.07	0.45	L	0.18	PN	—		0.19	0.00	—		0.15	PN	—
		50	0.06	0.40	L	0.14	PN	—		0.15	PN	—		0.11	PN	—
		33	0.07	0.46	L	0.19	PN	—	S	0.17	0.23	16.6		0.16	PN	—
		25	0.06	0.39	L	0.14	PN	—	S	0.14	0.17	14.2		0.10	PN	—
		20	0.06	0.41	L	0.15	PN	—		0.17	PN	—		0.10	PN	—
		10	0.07	0.44	L	0.18	PN	—		0.19	PN	—	S	0.02	0.18	17.3
		7	0.03	0.28	L	0.07	PN	—		0.09	PN	—		0.08	PN	—
		5	0.09	0.53	L	0.21	PN	—		0.23	PN	—		0.03	PN	—
1994	Z-1	33	2.7	1.70	S	1.38	2.78	14.0	S	0.98	2.59	11.8[a]	S	1.17	2.81	10.5[a]
1995	Z-1	20	3.5	2.20	S	3.33	4.97	23.2	S	3.59	4.44	21.4[a]	S	3.15	5.12	21.3[a]
1992	Z-2	100	0.25	0.28	L	0.66	0.01	—	S	0.44	0.86	10.8[a]	S	0.51	0.90	19.7[b]
		50	0.25	0.91	L^n	0.68	0.01	—	S	0.44	0.89	10.4[a]	S	0.40	0.94	17.3[b]
		33	0.22	0.84	L^n	0.56	0.01	—	S	0.27	0.76	8.0	L	0.48	PN	—
		25	0.27	0.93	L	0.68	0.02	—	S	0.02	0.89	7.7	L^n	0.70	PN	—
		20	0.25	0.88	L	0.66	PN	—	L^n	0.70	PN	—	S	0.21	0.87	15.0
		10	0.27	0.90	S	0.68	0.89	19.6	L^n	1.01	PN	—	L^n	0.67	PN	—
		7	0.17	0.69	L	0.38	PN	—	L^n	0.67	PN	—		0.16	PN	—
		5	0.23	0.84	L	0.54	PN	—	L^n	1.09	PN	—	S	0.19	0.74	20.5
1994	Z-2	33	2.9	1.80	S	1.91	2.95	19.9	S	1.69	3.16	20.6[a]	S	1.44	2.82	14.2[b]
1995	Z-2	20	4.0	2.50	S	3.15	5.79	22.5	S	2.76	6.20	23.3[a]	S	2.90	5.30	16.1[b]

[1]Sampling intensity in % of the observed data set.
[2]DS: disease severity. Mean and standard deviation (SD) per field.
[3]Best fitting model for the experimental semivariogram, based on the % variance accounted for by the model and minimal nugget variance. S: spherical model; L: linear model. L^n: indicates negative semivariogram parameters for non-linear models.
[4]Nugget: the variance near the origin representing microdistributional and measurement bias.
[5]Sill for a spherical and exponential model, slope for a linear model, PN for 'pure nugget effect'.
[6]Range: zone of influence for non-linear models; ranges for along and across rows followed by the same letter are not significantly different (t-test; $p \leq 0.05$).
[7]Model when different from the omnidirectional model.

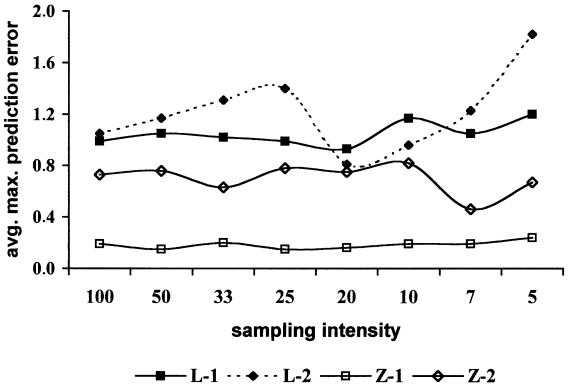

Figure 3. The average maximum prediction error (σ^2_{mm}) for DS plotted against the sampling intensity in per cent for four experimental fields.

Mean DS had increased from 0.25 in 1992 to 4.0 in 1995 (Table 1). The majority of short sequences of black pixels indicate bare patches due to *R. tuliparum* and a minority due to bulb rot due to *R. solani*.

No bare patch and bulb rot symptoms developed in planting stock grown in the greenhouse at 9 °C from bulbs randomly sampled per location for each of the growing seasons.

Sampling intensity

For the four experimental fields in 1992, a reduction of the observed data set (SI = 100%) did not influence the general omnidirectional semivariogram characteristics distinctively with decreasing sampling intensities (Table 1). The σ^2_{mm} was graphed over the sampling intensity for all fields (Figure 3). The sampling intensities with the lowest σ^2_{mm} indicate the most accurate prediction values. An optimal sampling intensity appeared to be 20% in L-1 and L-2. In Z-1 only few diseased bulbs occurred and, hence, the effect of reduced sampling was non-informative. For Z-2 a sampling intensity of 7% gave the lowest σ^2_{mm}.

Spatial variation

Lisse-1. For 1992, a linear model best fitted the omnidirectional semivariance and the semivariance across rows, whereas a spherical model best fitted the semivariance along rows (Table 1), thus suggesting anisotropy. The nugget values for omnidirectional and across-rows semivariograms were 0.96 and 0.95, whereas the sill for along row was 0.97. The nugget value for along rows was 0.77; anisotropy therefore seems to be caused by less bias in the along-row data and not reflecting spatial dependency. In June 1994, bulb rot occurred aggregated as indicated by a spherical semivariogram (Table 1) and comparison of semivariograms for along and across rows suggests no spatial variation. For 1995, a linear model best fitted the data for omnidirectional and directional semivariograms.

Lisse-2. For 1992, a strong isotropic spatial dependency was observed (Table 1). The ranges for along rows (12.2) and across rows (11.8) were not significantly different (t-test; $p \leq 0.05$). In June, 1994, bulb rot occurred in clusters as indicated by the spherical semivariogram (Table 1).

Zwaagdijk-1. For Z-1 a pure nugget effect was observed for DS for omnidirectional semivariograms (Table 1). Along rows, the slope for the linear model (0.002), though significantly different from 0 (t-test; $p \leq 0.05$), was considered too small to account for anisotropy. Bulb rot, mainly caused by *R. tuliparum*, occurred in clusters as indicated by a spherical semivariogram, but it was also found outside bare patches (Figure 1).

Zwaagdijk-2. For Z-2, a linear model best fitted the observed data over DS in all directions, whereas a spherical model better described the semivariances for DS along and across rows. The ranges along (10.8) and across rows (19.7) were significantly different (t-test; $p \leq 0.05$). Bulb rot occurred clustered (Table 1), but was also found outside bare patches (Figure 1). Spread of bulb rot was more prevalent along rows than across rows, as indicated by a significant difference (t-test; $p \leq 0.05$) between the ranges with values of 20.6 and 14.2, respectively. In 1995, bulb rot was aggregated (Table 1). Spread of bulb rot was more prevalent along than across rows, as indicated by a significant difference (t-test; $p \leq 0.05$) between the ranges, with values of 23.3 and 16.1, respectively.

Occurrence and spatial distribution of R. solani *AG 2-t isolates: 1993–1995*

In all fields AG 2-t isolates were isolated in low frequency at harvest in 1993, 1994 or 1995. The frequency of isolation varied from 0.3% in August 1993 in Lisse-1, to 17% in 1995 in Lisse-2 (Table 2). In all fields the spatial distribution of AG 2-t isolates seemed

Table 2. Mean percentage of *Rhizoctonia solani* and *R. solani*-like isolates from bulbs per sample unit per field in three consecutive years

	SI[1]	Total[2]	AG 2-t	AG 5	BNR[3]	AG 4	N.i.[4]
1993	33						
L-1		758	0.3	59	14	0	27
L-2		1373	10	64	24 (0.2)	0.3	9 (0.1)
Z-1		845	3	52 (0.5)	27	3 (0.2)	15
Z-2		700	3	45 (0.2)	28	0.3	23
1994	33						
L-1		1176	1	90 (9.4)	1	0	9
L-2		424	3	80 (11)	2	0	13
Z-1		648	0	83 (0.3)	6	0	7
Z-2		508	0	79	5	0.4	15
1995	20						
L-1		254	11	81 (1.3)	6	0	0
L-2		188	17	74 (1.8)	8	0	11
Z-1		238	0	60	21	0	20
Z-2		220	0	65	14	0.5	21

[1]SI: sampling intensity. [2]Total: total number of isolates. [3]BNR: binucleate rhizoctonia. [4]N.i.: not identified; *R. solani* (-like) isolates not identified to AG. [5](): percentage of AG 2-t isolates in dual occurrence with other isolates.

random (figures presented elsewhere (Schneider, 1998)). *R. solani* AG 2-t isolates were not isolated in 1994 and 1995 in the Zwaagdijk fields.

Spatial distribution of AG 4, AG 5 and BNR isolates: 1993–1995

Most isolates from iris and tulip bulbs in all fields and cropping seasons belonged to AG 5 (Table 2). In L-1, *R. solani* AG 5 constituted 59%, 90% and 81% of the total numbers of *Rhizoctonia* spp. isolates in 1993, 1994 and 1995, respectively. Similarly, AG 5 constituted 64%, 80% and 74% in L-2, 52%, 83% and 60% in Z-1, and 45%, 79% and 65% in Z-2, respectively. On average, relatively more AG 5 was found in Lisse than in Zwaagdijk.

Isolates of AG 4 occurred only sporadically throughout all fields (Table 2). AG 4 isolates were only found once at Lisse. In 1993, 0.3% of the total isolates in L-2 were AG 4. In Z-1, AG 4 isolates were found in 3% in 1993 and absent in 1994 and 1995, while in Z-2 AG 4 was found in 0.5% of total isolates in 1995.

Binucleate *Rhizoctonia* spp. (BNR) were isolated primarily at harvest in June 1993 (Table 2). Frequency of isolation varied from 1% in L-1 in 1994 to 28% in Z-2 in 1993. On average, BNR was isolated more frequently in Zwaagdijk than in Lisse.

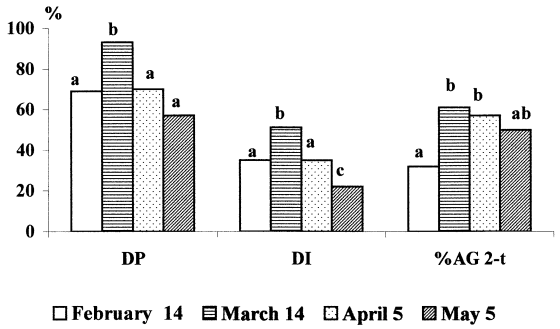

Figure 4. Within-season development of rhizoctonia disease in L-2 at four sample dates in 1995. Mean percentage of quadrats per field with rhizoctonia disease (DP), mean percentage of infected plants per quadrat (DI) and mean percentage of *R. solani* AG 2-t (%AG 2-t) isolated from the sampled (healthy and diseased) plants. For each parameter bars marked by the same letter are not significantly different (LSD $\leq$ 0.05) after an arcsine transformation.

Spatial distribution of R. solani *AG 2-t in one field*

On February 14, 1995, tulips in L-2 showed symptoms due to *R. solani* with a disease prevalence (DP) of 69% (Figure 4). A significant ($p \leq 0.05$) increase of DP to 93% was found on March 14, but later DP decreased significantly to 57% on May 3, 1995. Disease incidence (DI) showed the same tendency. In February DI was 35% and significantly ($p \leq 0.05$) increased to 50% on March 14, whereafter DI significantly ($p \leq 0.05$) decreased to 22% on May 3. The percentage of plants from which AG 2-t was isolated was low (32%) in February, increased significantly ($p \leq 0.05$) to 61% in March and decreased somewhat to 50% in May.

Surprisingly, the relatively high incidence of tulips infected by AG 2-t did not result in bare patches in 1995. The remaining isolates were not identified to AG.

Competition between R. solani *AG 2-t and AG 4 or AG 5*

At 9 °C, AG 2-t alone caused severe infection (DS = 4.2) of iris bulbs (Table 3). In dual inoculation of iris bulbs with AG 2-t + AG 4 or AG 2-t + AG 5 disease severity was 3.2 and 4.8 respectively. With single inoculation of AG 4 and of AG 5, no infection occurred (DS = 0). From iris bulbs grown at 9 °C, after single or dual inoculation, only AG 2-t was re-isolated.

At 18 °C, iris bulbs were severely infected by single inoculations of AG 2-t or AG 4 and by dual inoculations

Table 3. Mean disease severity (DS) and mean percentage of re-isolation of *R. solani* AG 2-t, AG 4 and AG 5 from iris bulbs after 6 weeks of incubation at 9 and 18 °C. Soil was infested with AG 2-t, AG 4 or AG 5 separately and with AG 2-t + AG 4 or AG 2-t + AG 5 combined. DS on a scale from 0 (healthy) to 5 (plant dead)

AG	DS (9 °C)	% Isolation (9 °C)			DS (18 °C)	% Isolation (18 °C)		
		2-t	4	5		2-t	4	5
2-t	4.2	100			4.1	100		
4	0.0		0		4.5		100	
5	0.0			0	0.5			100
2-t + 4	3.2	100	0		4.5	0	100	
2-t + 5	4.8	100		0	4.0	100		0
LSD (0.05)	0.7				0.7			

of AG 2-t+AG 4 or AG 2-t+AG 5 with a DS of 4.1, 4.5, 4.5 and 4.0 respectively (Table 3). Single inoculation with AG 5, in contrast, did not cause symptoms on iris bulbs (DS was 0.5). In dual inoculation of AG 2-t+AG 4, only AG 4 isolates were re-isolated from the iris bulb, while in dual inoculation of AG 2-t + AG 5, only AG 2-t could be re-isolated (Table 3).

Isolates of AG 2-t, AG 4 and AG 5 could readily be re-isolated from the bulbs after single inoculation and thus all three isolates could grow actively through the soil and colonise the bulb when the soil temperature was 18 °C.

Discussion

Occurrence and decline of bare patches

Our study shows that rhizoctonia bare patch in tulip can be induced by artificial soil infestation using an isolate of *R. solani* AG 2-t. Patches ranging from 0.2 m^2 to 1–2 m^2 occur in commercial fields (Schneider, 1998). The resulting bare patches were more pronounced on the sandy soil in L-2 than on the loamy soil in Z-2. In both fields inoculum from the same batch was used. Abiotic and biotic differences may account for less pronounced patches in Z-2. Decline of various rhizoctonia diseases is well documented (Flentje and Saksena, 1957; MacNish, 1985, 1988, 1996; Lucas et al., 1993; Hyakumachi, 1983; Jager and Velvis, 1995), but poorly understood. Various mechanisms have been suggested: antagonistic microorganisms and a rapid decrease of the number of viable sclerotia (Hyakumachi et al., 1990), the combination of both a susceptible host and a virulent isolate of AG 8 (Lucas et al., 1993), a general microbial antagonism (Wiseman et al., 1996) and differences in seasonal suppressiveness (MacNish, 1996). For AG 3 in potato the mechanism for disease decline remains to be solved. Neither *Verticillium biguttatum*, an effective mycoparasite (Van den Boogert et al., 1990), nor replacement of AG 3 by AG 5 were responsible for disease decline in field-grown potato. Disease decline may be irrespective of the amount of initial inoculum (Hyakumachi, 1983). In contrast, MacNish (1996) found that bare patches became evident in the second and third year after infestation and he suggested a 'lag phase' in patch development. It is unknown whether the introduced inoculum in our infested fields failed to survive after the first year, persisted in soil without causing disease, or grew saprophytically through soil, thus causing diseased bulbs scattered through the field in succeeding years.

Mapping with reduced sampling intensity

Our data show that reducing sampling intensity down to 20% did not change the general characteristics (spherical or linear) of the omnidirectional semivariograms when compared with all samples in our observation fields. The average maximum prediction error was minimised at a sampling intensity of 20% for L-1 and L-2. A sampling intensity of 7% seemed optimal for Z-2 whereas for Z-1 no optimal sampling grid could be determined due to absence of disease patches. However, sampling intensities less than 10% altered the general characteristics of the semivariogram, disease severity on average and provided worthless maps (Schneider, 1998) in general and for Z-2 in particular. We showed that for mapping rhizoctonia disease in our observation fields, a sampling intensity of 20% can be as informative as sampling 100% of the field.

When a disease has a patchy character, a pronounced spatial distribution is expected. Indeed for L-2 and Z-2, with artificial and naturally occurring patches, a pronounced spatial dependency was found. It is anticipated that the shape of the omnidirectional semivariogram in L-2 and Z-2 is largely determined by the focal character of the artificially created patches. This focal character may account for small nugget values and a spherical semivariogram. By contrast, a pure nugget effect or a slight spatial dependency of naturally occurring patches would indicate a patch with multiple infection sites within the patch and little fungal spread between

plants or little disease. Analysis and comparison of artificial and natural patches warrants further study, but is beyond the scope of this paper.

Comparison of directional semivariograms along and across rows revealed no significant anisotropy in L-1 and L-2. Isotropy indicated that the spread of disease was the same along rows, with bulbs side by side, and across rows, with the bulbs separated by approximately 23 cm. From this it can be concluded that growth of *Rhizoctonia* was not hampered by biotic and/or abiotic soil factors between rows, or that the fungus needs a nutritional source approximately every 23 cm within the row. Anisotropy was observed only in Z-2. The semivariogram for across rows for Z-2 shows considerable variation in the data. This variation may indicate spatial dependency over different ranges for across rows, meaning that elongated patches with different width across and approximately the same length along rows occur in the field.

Rhizoctonia populations

The higher DS for L-1 and L-2 indicates that there was a higher natural indigenous *Rhizoctonia* population in Lisse than in Zwaagdijk. Soil samples taken from the two locations did not differ significantly in soil receptivity when tested in a bio-assay (Dijst, unpublished results). In contrast to bare patches, DS of bulbs increased during successive years of bulbs. AG 2-t isolates were found in frequencies too low to solely account for all bulb rot. AG 5 was the predominant AG found at harvest and the percentage of isolation increased in three successive growing seasons. AG 5 isolates are pathogenic to tulip in glasshouse experiments at 18 °C, but not at 9 °C (Doornik, 1981; Schneider et al., 1997b). Some AG 5 isolates, obtained from iris in 1993, caused weak symptoms on tulip shoots in glasshouse tests (Schneider, unpublished). AG 5 is generally considered a weak pathogen (Sneh et al., 1991) and it was less aggressive than AG 2-t in glasshouse experiments at 18 °C (Schneider et al., 1997b). Between flowering and harvest the daughter bulbs are formed. AG 5 may account for the majority of bulb rot symptoms on the newly formed bulbs.

Binucleate rhizoctonia (BNR) were isolated in a higher frequency from iris bulbs in 1993 than from tulip in 1994 and 1995. Different hosts may select different microorganisms around the bulb. Climatic conditions may also play a role, since iris was harvested in August 1993, while tulips were lifted in June 1994 and 1995. BNR comprise 21 AG with teleomorphs in *Ceratobasidium* and 2 AG in *Tulasnella* (Sneh et al., 1991) including pathogens and antagonists (Sneh et al., 1986; Cardoso and Echandi, 1987). The ecological niche of BNR in our observation fields is unknown and requires further study.

Grey bulb disease due to *R. tuliparum* did not occur on the sandy soil in Lisse but was more devastating than rhizoctonia disease in our fields at Zwaagdijk. The disease was known to occur in that area and was probably induced by our short rotation of bulbs. Z-2 was visited during the same sample periods as for L-2 in 1995 to assess within season development of rhizoctonia and grey bulb disease. Analogous to AG 2-t in L-2, *R. tuliparum* was found at high incidence in early spring shortly after emergence of the sprouts but was not isolated at harvest (Schneider, unpublished).

Temporal niche differentiation of AG 2-t

Our results show that *R. solani* AG 5 is a weak competitor of AG 2-t at 9 and 18 °C. It is not likely that an increase in AG 5 in soil induces a decrease of bare patch isolates. Our observations are in line with Jager and Velvis (1995). They showed that AG 5 did not replace AG 3 in potted potato experiments. Competition by microorganisms, however, perhaps induces replacement of AG 2-t isolates. In the presence of our AG 4 isolate, AG 2-t was unable to infect iris bulbs and cause disease at 18 °C in pot experiments. Neither was AG 2-t re-isolated from diseased iris bulbs after dual inoculation of AG 2-t + AG 4. As inoculum of both isolates was placed in one corner of the pot, the results suggest that AG 4 grew faster through soil than AG 2-t and colonised the iris bulb earlier, thus preventing AG 2-t from colonising the bulb and causing disease. *R. solani* AG 4 was found in too low a frequency in our fields to replace AG 2-t. Under the climatic conditions prevailing in the Netherlands AG 4 is not expected at high frequency and is unlikely to cause severe disease outbreaks in flower-bulb fields. Microorganisms other than *Rhizoctonia* spp., which are active at higher temperatures but not at lower temperatures, may cause replacement of AG 2-t. Harris and Moen (1985) noticed replacement of AG 8 on wheat seedlings by other root-rot and minor pathogens. In addition to AG 5 and other *R. solani*-like fungi, *Fusarium* spp., *Trichoderma* spp. and *Penicillium* spp. grew from healthy and diseased bulbs (Schneider, unpublished). Their role in the bulb rot complex requires further study.

The physiological condition of the tulip plant may also be of importance. Tulip shoots grow through the soil when the temperatures are low. Roots and some bulb tissues senesce between flowering in April/May and harvest in June/July and at the same period new daughter bulbs are formed (De Hertogh et al., 1983). This process, probably inducing activity of different microorganisms at different temperatures, might initiate replacement of AG 2-t between flowering and harvest. *R. solani* AG 2-t is capable of infecting tulip shoots at 18 °C and has an optimum growth rate between 20 and 25 °C (Schneider et al., 1997b). In the field, the bulbs and their immediate environment change continuously in physical, chemical and biological aspects, because seasons change and the development of the tulip is a process of growth, senescence and growth (Le Nard and De Hertogh, 1993). We hypothesise that microorganisms, locally active, change continuously in a regular succession and thus the niches available to pathogens vary in a regular pattern. We postulate a 'temporal niche differentiation' in contrast to a spatial differentiation, one niche besides the other. We propose that there are a series of niches, one after the other at the same position, depending on soil temperature and the development of the plant. Temporal niche differentiation of AG 2-t isolates in relation to bulb development needs further research, which may yield interesting microorganisms for reducing bare patch disease.

Decrease in aggressiveness

A high incidence of infected and symptom bearing tulips and a high isolation frequency of AG 2-t isolates early in February and March 1995, did not result in any bare patch at L-2. In 1995, *T. kaufmanniana* cv. 'Giuseppe Verdi' was grown, whereas in the previous three years *T. greigii* cv. 'Red Riding Hood', iris cv. 'Blue Magic' and *T. greigii* cv. 'Red Riding Hood' were grown, respectively. Iris did not show any symptoms at harvest, although it was found that iris can show severe symptoms in glasshouse experiments when inoculated with AG 2-t isolates and grown at 9 or 18 °C (Doornik, 1981; Schneider et al., 1997b). *T. kaufmanniana* cv. 'Giuseppe Verdi' is susceptible to AG 2-t (Koster and Van de Meer, 1990). From February to March 1995, the number of quadrats containing at least one infected tulip showing symptoms (DP) increased significantly (Figure 4), but no bare patch developed. AG 2-t isolates were readily isolated from tulips early in the season. Schneider et al. (1999) demonstrated that differential interaction between tulip cultivars and AG 2-t isolates can be influenced by environmental conditions. Another possible explanation for the observed phenomenon may be that aggressiveness (Bos and Parlevliet, 1995) of AG 2-t isolates had reduced. Daniels (1963) suggested that patches develop where there is a localised increase in aggressiveness. This hypothesis requires further research.

In summary it may be concluded that rhizoctonia bare patches could be induced by artificial infestation of the field with an AG 2-t isolate. Bare patches build up, re-appear and vanish. *R. solani* AG 5 was found at high incidence at harvest and may account for the majority of bulbs with light to mild (DS 2–5) rot symptoms in the field. In contrast, AG 4 seemed only of minor importance. Two mechanisms are suggested to be involved in decline of bare patch, a reduction of aggressiveness of AG 2-t isolates and temporal niche differentiation. Both hypotheses require further study.

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