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Temperature dependence of the pathogenicity of several isolates of Rhizoctonia solani in some bulb crops as an intrinsic property of the isolate

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Abstract

Several isolates of *Rhizoctonia solani* obtained from tulip, iris or lettuce, infected various bulb crops either only at soil temperatures of 13 °C or higher ('warmth preferring isolates'), or mainly at soil temperatures below 13 °C ('cold preferring isolates'). Infection of the host by cold preferring isolates at temperatures above 13 °C was possible when the inoculum was in close contact with the host. Pathogenic activity under unfavourable temperature conditions was relatively brief but could recur if the temperature became favourable. Differences in the rate of sprout growth due to preplanting temperature treatment of bulbs influenced infection, but the main characteristic of the isolates with respect to the temperature-pathogenicity relationship prevailed.

Additional keywords: Tulipa, Hyacinthus, Lilium, Anemone, Iris.

Introduction

In 1928, Van Poeteren reported that a disease in tulips originating from California was caused by *Rhizoctonia solani* Kühn. Sonderman and MacLean (1949) described the infection of irises by *R. solani* in the USA. The first observation of the disease in Dutch-grown tulips was reported by Jaarsveld (1952). Muller reported the presence of the disease in the Netherlands in *Anemone coronaria* (1968), *Iris* (1969a), *Hyacinthus* (1970), and *Lilium* (1973). He also demonstrated (Muller, 1969b) that infection of tulips originates either from diseased bulbs or from contaminated soil; although cross-infection with isolates from different bulb crops was possible, he found considerable differences in the pathogenicity between isolates.

During the testing of several isolates for pathogenicity in tulips and irises at the start of the present investigations, some of them were found to be highly pathogenic in the glasshouse, but non-pathogenic at relatively low temperatures in the field, and others caused hardly any infection in the glasshouse whereas in the field almost all plants became heavily infected. On the other hand, it was known from field observations that new infections in tulips could be found from early March, when the soil temperature fluctuates around 5 °C, until harvesting in July, when the soil temperatures are around 17 °C. These findings made it important to investigate the relationship between soil temperature and pathogenicity to bulb crops.

In the literature three views predominate as to the influence of soil temperature on host infection by some soil-borne pathogens. According to Leach (1947), the degree of pre-emergence damping-off caused by *Pythium ultimum* and *R. solani* is correlated with the ratio between host and pathogen growth rates at different temperatures. Griffin (1958) argued that the vigour of the host, expressed in its growth rate during the susceptible period, is more determinative for infection by *P. ultimum* than is the growth of the pathogen or the ratio between host and pathogen growth rates. In Richards' (1923) opinion the optimum temperature for pathogenicity of *R. solani* is determined by an intrinsic physiological characteristic of the pathogen. Each of these views is supported by several reports. This paper presents some evidence that Richards' view is applicable to the host-parasite relationship between *R. solani* and certain bulb crops.

Materials and methods

Isolates of *R. solani* originating from various crops were maintained in sand culture (Schneider, 1958) at 10 °C. In the experiments presented here, mainly isolates from tulip, iris and lettuce (indicated as T, I, and S, respectively) were used. Three weeks before the start of an experiment, the isolate was transferred first to potato-dextrose agar (PDA) and then to a medium containing 100 ml of oat grains and 65 ml water which had been sterilized for one h on two successive days. A 2-week-old oat culture grown at 20 °C was used as inoculum. For all experiments a dune sand soil (organic matter 2,4%, pH-KCl 6.7, $CaCO_3$ 0.8%, mineral constituents $<16\,\mu m$ about 3.5%) was used. Unless otherwise mentioned, soil was contaminated with 20 g inoculum per m^2 at bulb level at the time of planting. Bulbs and inoculum were covered with approximately 3 cm of soil. Trials were carried out in three replicates of 15 bulbs each. Usually, infection was evaluated six weeks after planting. Disease percentages were determined on the basis of macroscopical symptoms.

Iris and tulip bulbs to be planted in the field in the autumn at soil temperatures varying between 5 °C and 10 °C, were stored at 30 °C and 20 °C, respectively. If bulbs were to be planted under controlled temperature conditions, iris bulbs were pre-treated at 17 °C for six weeks and tulip bulbs at 5 °C for 13 weeks. Roots and sprouts started to grow out immediately after planting. Bulbs taken from storage either before planting in the field or before the cooling treatment were disinfected in 4% (v/v) formalin for 15 minutes to eliminate external contamination. Disinfection after cooling would have been preferable, but might have damaged the root primordia.

Mycelium growth in moist unsterilized soil was observed in perspex containers measuring 4 × 10 × 60 cm. One colonized oat grain was inserted about 5 cm deep at one of the short ends of each container and mycelium growth was followed microscopically on both of the long sides.

Severe infection symptoms were distinguished from symptoms in general, when at least half of the iris bulb and/or the basal part of all three sheat leaves were affected. Infection in tulips was recorded as severe when many or large lesions were found on the lowest leaf (forming the outside of the outgrowing sprout) and/or basal part of the stem.

Results

Influence of temperature on infection. The isolates T4 and I2 were used for experiments done with irises at controlled temperatures ranging between 2 °C and 20 °C. The standard amount of inoculum was placed at bulb level in the centre of a container, i.e., at a distance of about 7 cm from the surrounding circle of planted bulbs. The experiment was repeated six times at different dates in one year. Although the infection percentages differed per experiment, all of them showed the same tendency. In one of the experiments for example the T4 isolate infected irises for 33, 80, 98, 32, 2 and 2% at 2, 5, 9, 13, 17 and 20 °C respectively. The isolate I2 caused 7, 90 and 100% infection at 13, 17 and 20 °C respectively, while no infection occurred at temperatures below 13 °C. In another experiment a possible effect of distance between host and inoculum was eliminated by pressing a single oat grain overgrown by mycelium against iris bulbs and a second one against their outgrowing sprouts. Each of the 30 bulbs per treatment was planted in a separate pot. Three isolates caused infections in iris bulbs and/or sprouts at all temperatures, but severe symptoms were found mainly at temperatures below 17 °C. Three other isolates did not cause infections at temperatures below 13 °C, and heavy infections on bulbs and/or sprouts were found mainly at 17 °C and 20 °C (Table 1).

Table 1. Relationship between temperature and iris infection after exposure to inoculum pressed against the bulb and outgrowing sprout.

Temperature (°C)		Cold preferring isolates			Warmth preferring isolates		
		T4	T7	T8	I2	S1	S2
2	A[1]	100	76	76	0	0	0
	B[1]	17	0	0	0	0	0
5	A	93	98	100	0	0	0
	B	20	40	53	0	0	0
9	A	97	100	100	0	0	0
	B	17	61	75	0	0	0
13	A	93	91	89	79	52	73
	B	17	0	14	21	5	9
17	A	67	77	82	100	100	93
	B	3	2	2	45	51	53
20	A	0	44	67	87	100	100
	B	0	0	0	63	100	100

[1] A: Plants with infections on bulbs and/or sprouts (%); B: plants showing severe infection symptoms (%).

Tabel 1. Relatie tussen temperatuur en infectie van irissen wanneer het inoculum tegen de bol en spruit is gedrukt.

The isolates that infected irises mainly at low temperatures were indicated as 'cold preferring isolates' (coded cp), those that infected only at temperatures above 13 °C were indicated as 'warmth preferring isolates' (coded wp).

Similar experiments were done with other bulb crops at 9 °C (representing low temperatures) and at 17 °C (representing high temperatures). Wp isolates were usually most pathogenic at 20 °C, but occasionally an infection caused by *Fusarium* sp. or secondary bacteria obscured the *Rhizoctonia* symptoms.

Because bulb infections in tulip, hyacinth, lily and anemone may be absent or difficult to recognize, the infection percentages shown in Table 2 represent only sprout symptoms. The cp isolates infected these crops at 9 °C and 17 °C, the severest symptoms occurring at 9 °C. The infection by wp isolates at 9 °C was almost negligible compared with infection at 17 °C, especially when it is taken into account that the inoculum was pressed against the outgrowing sprouts. Similar results were obtained for other isolates belonging to both categories.

Table 2. Relation between temperature and sprout infection in various bulb crops after exposure to inoculum pressed against the outgrowing sprouts.

Temperature		Hosts							
		tulip	hya-cinth	lily	ane-mone	tulip	hya-cinth	lily	ane-mone
Cold pref. isolates:			T4				T8		
9 °C	A[1]	100	47	50	27	100	64	73	—
	B[1]	31	0	0	0	51	0	0	—
17 °C	A	100	20	3	4	100	82	30	—
	B	0	0	0	0	20	0	0	—
Warmth pref. isolates:			I2				S1		
9 °C	A	0	4	0	1	0	11	0	0
	B	0	0	0	0	0	0	0	0
17 °C	A	100	100	97	82	100	100	73	71
	B	16	36	0	0	44	16	0	0

[1] A: Diseased sprouts (%); B: sprouts with severe symptoms (%).

Tabel 2. Relatie tussen temperatuur en infectie van verschillende bolgewassen wanneer het inoculum tegen de spruit is gedrukt.

Influence of temperature on mycelial growth. A number of cold or warmth preferring isolates were plated on PDA and stored at 5, 9, 17 and 25 °C. Optimal growth was observed at 25 °C for all isolates. The cp isolates grew out on PDA at all temperatures tested, growth of the wp isolates did not occur below 9 °C. At 9 °C, growth of the cp isolates was distinctly better than that of the wp isolates.

Growth of both isolate types in unsterilized soil was assessed at 9 °C and 17 °C, the temperatures used in most of the trials. Development of all isolates was best at 17 °C (Table 3). Growth of the cp isolates in this soil at 9 °C was again better than that of the wp isolates.

Table 3. Mycelial growth in unsterilized sandy soil after 4 weeks at 9 °C or 9 days at 17 °C.

Isolate	Soil temperature	
	9 °C	17 °C
Cold preferring		
T1	5.3[1]	10.3[1]
T4	4.9	8.1
T8	5.3	7.0
Warmth preferring		
I2	1.3	7.8
I4	0.9	4.6
I5	0.3	3.1
I7	1.4	7.7
S1	0.6	7.9

[1] Expressed as mm per day, values are means of 3 replicates.

Tabel 3. Myceliumgroei in niet-gesteriliseerde zandgrond na 4 weken bij 9 °C of 9 dagen bij 17 °C.

Disease incidence in relation to isolate type and gradual changing soil temperatures. To follow the symptom expression due to each of the two isolate types throughout the growth season, iris bulbs were planted in early December in an unheated glasshouse in soil contaminated with cp T8 or wp S1. Soil temperatures rose from 3-7 °C in December and January to 7-15 °C in February and 15-20 °C in March and the temperature fluctuated between 15 °C and 28 °C from April till final harvest in July. Every month a sample of 50 plants was taken and examined for symptoms on shoots, planted bulbs, and later on offspring bulbs. Under the prevailing low

Table 4. Influence of changing soil temperature on symptom expression in irises.

Isolate	Mother plants[1] with symptoms on bulbs and/or sprouts (%)					Daughter bulbs[2] with symptoms (%)	
Sampling date[3]	25/1	25/2	28/3	26/4	24/5	24/5	22/6
Temperature prior to sampling:	3-7°C	7-15°C	15-20°C	15-28°C		15-28°C	
cp T8	7	100	100	100	90	0	25
wp S1	0	0	14	35	97	47	82

[1] Date of planting December 17 th.
[2] No daughter bulbs developed before early May.
[3] About 50 plants and 70-100 daughter bulbs sampled per date.

Tabel 4. Invloed van veranderende bodemtemperatuur op de symptoomexpressie in irissen.

temperatures, wp S1 did not cause disease symptoms in January or February (Table 4). Only after the temperature rose the percentage of diseased iris plants increased; the percentage of diseased offspring bulbs was high in the last sample at the end of the observations. Under the same temperature conditions, all of the plants grown in the cp T8 plot showed visible symptoms in the first samples in winter, but these symptoms were not sufficiently severe to render the plants left unable to produce offspring. The percentages offspring bulbs with disease symptoms were much lower and the symptoms were by far less severe than among the offspring of the wp S1 plot.

Influence of temperature on the persistence of parasitism. When hyacinths were grown during one of two successive 7-weeks periods in soil contaminated with cp T7 or cp T8, 73 and 82% of the plants, respectively, showed infection at 17 °C in the first period, whereas no infection was found in the second planting. At 9 °C, 71 and 64% of the plants were infected in the first period and 36 and 22% in the second, respectively. Similar results had been obtained previously with irises (Doornik, 1980).

Influence of growth rate of the host on infection. After storage with or without precooling, tulip and iris bulbs were planted at 9 °C and 17 °C. The sprout lengths associated with different storage conditions, as measured six weeks after planting, are shown in Table 5. The main characteristic of both isolate types prevailed: there was no infection at 9 °C by the warmth preferring isolates, and symptoms induced by cold preferring isolates were more frequent and in particular more severe at 9 °C than at 17 °C (Table 5). Sprout infection in iris was significantly ($p = 0,05$,

Table 5. Influence of sprout growth rate on sprout infection.

Soil temp. (°C)	Bulb treatment before planting	Sprout length after 6 w (cm)		Irises				Tulips			
				cold preferring isolates		warmth preferring isolates		cold preferring isolates		warmth preferring isolates	
				T4	T8	I2	S1	T4	T8	I2	S1
9	not cooled	3	A[1]	9	27	0	0	100	100	0	7
			B[1]	0	0	0	0	0	42	0	0
	cooled	12	A	82	82	0	2	100	100	0	0
			B	16	41	0	0	31	51	0	0
1 7	not cooled	3	A	0	30	64	29	91	98	100	100
			B	0	0	0	0	2	41	47	57
	cooled	25	A	27	40	100	95	100	100	100	100
			B	0	2	96	81	0	20	16	44

[1] A: sprouts with symptoms (%); B: sprouts with severe symptoms (%).

Tabel 5. Invloed van de snelheid van spruitgroei op spruitinfectie.

regression analysis) more frequent in the fast-growing than in the slow-growing sprouts when conditions were favourable for the isolates. This was not the case in tulips. In this host symptoms of sprout infection caused by the cp isolates at 9 °C were significantly more severe in the fast growing sprouts and the wp isolates caused more severe infection at 17 ° C in slowly developing sprouts.

Discussion

It is not known whether penetration of the fungus into host tissue of ornamental bulbs is always accompanied by visible symptoms. This may mean that although infection might occur at an unfavourable temperature, symptoms can only develop if the temperature becomes favourable for the type of isolate involved (e.g., Table 4 : wp S1 in the winter and early spring). Mycelium of all isolates on PDA and in unsterilized soil grew better at 17 °C than at 9 °C, and below 9 °C no growth of the warmth preferring isolates was observed. However, the mycelial growth rate is not the only factor which influences infection. Although optimal growth of cold preferring isolates was seen at high temperatures, infection symptoms occurred most frequently at approximately 9 °C. On the other hand, warmth preferring isolates did not infect at temperatures below 13 °C, although mycelial growth was observed at 9 °C.

Blair (1943) and Henis and Ben-Yephet (1970) stated that the distance between host and inoculum influences the virulence of the mycelium and thus the degree of infection. In our experiments, the distance between inoculum of various isolates and the bulbs was same for all temperatures tested, yet the infection percentages differed.

Martinson (1963) and Benson and Baker (1974) showed that the inoculum they used lacked the potential to infect abundantly at an unfavourable temperature. When the amount of inoculum was increased, the inoculum potential ('energy of growth of a pathogen available for infection of a host at the surface of the host organ to be infected', Garrett, 1956) increased sufficiently to give 100% damping-off at an unfavourable temperature. Benson and Baker (1974) also proved that contact between mycelium of the pathogen and the host within 24 h, can result in 100% infection regardless of the soil temperature. In the experiments of Martinson (1963) and Benson and Baker (1974), the increase in the amount of inoculum in the soil may have reduced the distance between host and pathogen propagules and thus the chance of contact may have increased.

Pressing of the inoculum against the bulbs may have made conditions for infection optimal in our experiments, but nevertheless infection by the warmth preferring isolates was very rare at unfavourable temperatures, even after 10 weeks of incubation. For the cold preferring isolates the infection percentages were considerable at unfavourable temperatures, but the symptoms were usually slight. Sanford (1952) and Henis et al. (1978) showed that at a favourable temperature the pathogenic activity of *Rhizoctonia* inoculum introduced into soil decreases in successive plantings. This pattern was also seen for pathogenic activity in successive plantings of irises (Doornik, 1980) and of hyacinths at favourable temperatures. At an unfavourable temperature pathogenic activity showed a considerable stronger decrease in successive plantings.

Observations made by Leach (1947) and Griffin (1958) suggest an influence of the host growth rate on infection, either via the ratio of growth between pathogen and host or via the growth vigour of the host alone. In our experiments, however, rapidly growing sprouts did not escape infection, and less vigourous growth of the host did not result in more infection. The relationship between temperature and the pathogenicity of the various isolates prevailed over the influence of the growth rate of the host. The fact that after storage without cooling irises showed less symptoms compared with cooled bulbs producing sprouts upon planting, might be explained by the action of infection-stimulating exudates of growing plants (Hayman, 1969). The findings in tulips are difficult to explain.

The results reported in this paper support Richards' (1923) view, i.e., that the optimum temperature for pathogenicity is an intrinsic characteristic of the pathogen.

Samenvatting

De bodemtemperatuur als bepalende factor voor pathogeniteit van isolaten van Rhizoctonia solani ten opzichte van enkele bolgewassen

Enkele isolaten van *Rhizoctonia solani* verkregen uit tulpen, irissen of sla infecteerden verschillende bolgewassen óf alleen bij een bodemtemperatuur van 13 °C of hoger ('warmte-minnende isolaten') óf in hoofdzaak bij een temperatuur beneden 13 °C ('koude-minnende isolaten'). Deze koude-minnende isolaten konden gedurende een korte periode na grondbesmetting ook actief zijn bij temperaturen boven 13 °C. Daarbij was de aantasting groter, wanneer het inoculum tegen de waardplant was gedrukt. De pathogene activiteit van beide typen isolaten, die bij ongunstige temperatuur gering of geheel afwezig was kon zich herstellen wanneer de temperatuuromstandigheden gunstig werden. De aantasting van de spruiten werd beïnvloed door verschillen in snelheid van spruitgroei ten gevolge van temperatuurbehandeling van de bollen voor het planten, maar de relatie temperatuur/pathogeniteit van de isolaten had een overheersenden invloed op de mogelijkheid van aantasting.

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