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Flooding causes loss in viability and pathogenicity of sclerotia of Rhizoctonia tuliparum

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Rhizoctonia tuliparum Whetzel & Arthur causes grey bulb rot, a destructive disease of tulip and bulbous iris. The pathogen can attack a wide range of other monocotyledonous plants, but it has never proved serious on any of these (Moore, 1979). Grey bulb rot was first described by Wakker in 1884 and has been known in the Netherlands for many years as 'kwadegrond'. Usually in the soil, but also on affected bulbs or sprouts, sclerotia are formed which vary in form and size (1-10 mm). Sclerotia are white when young but later become brown to almost black (Bergman et al., 1983). A long-term experiment on the survival of *R. tuliparum* showed that about 10 per cent of sclerotia can remain viable in the field for at least 10 years (Coley-Smith et al., 1979).

Sclerotia of some fungi can be killed by immersion in water for a period of several weeks. Brooks (1942) described flooding experiments on control of celery pink rot caused by *Sclerotinia sclerotiorum* in Florida. Moore (1949) demonstrated complete decay of sclerotia of *S. sclerotiorum* in soil after flooding for 23-45 days. A single rotation with paddy rice controlled verticillium wilt of cotton for 2-3 years in experiments by Pullman and De Vay (1981).

From 1982 on, flooding for 6 weeks in summer is a common practice at several Dutch flower bulb farms to control diseases and weeds. Preliminary experiments in practice on the effect of flooding on sclerotia of *R. tuliparum* and some other bulb pathogens had yielded promising results. We decided to study such effects in detail and to start with *R. tuliparum*.

Sclerotia of *R. tuliparum*, freshly collected from diseased tulips, were put in nylon pouches (60 per pouch) and buried in soil from bulb fields in plastic containers of 10 l. The soil in the containers was flooded about 2 cm above soil level and the containers were kept at 17 °C. Control soils were not flooded. The trial was in triplicate.

After 2, 4 and 6 weeks of flooding pouches with sclerotia were taken out of the soil and rinsed with tap water. Sclerotia were disinfected in 2.5% formaldehyde for 30 min, thoroughly rinsed with sterile water and dried on filter paper. They were cut in halves and from 56 sclerotia the halves were placed on 2% malt-extract agar (Oxoid CM59) with 10 mg oxytetracycline l^{-1} in Petri dishes. After two weeks of incubation at 24 °C germination of sclerotia, which had been buried in unflooded soil for 2-6 weeks, varied between 86 and 100%. Two weeks of flooding resulted in percentages of germination of 16, 27 and 29 in the three replicates, respectively. Sclerotia from soil flooded for 4 or 6 weeks did not germinate.

Table 1. Effect of flooding for 1 to 4 weeks at 17 °C on viability and pathogenicity of sclerotia of *Rhizoctonia tuliparum*.

Flooding time (weeks)	Germinated sclerotia (%)[1]		Infected tulips (%)[2]	
	control	flooded	control	flooded
1	98	68	n.t.[3]	96
2	91	0	96	52
3	93	0	96	23
4	89	0	96	0

[1] Per treatment, 56 half sclerotia were transferred to malt agar.
[2] Per treatment, 48 tulip bulbs were inoculated with one sclerotium each.
[3] n.t.: not tested.

In a second experiment sclerotia in soil were flooded as described above, but for 1, 2, 3 and 4 weeks; viability was tested on malt agar (only one pouch), and pathogenicity by inoculation of tulip bulbs (cv. Apeldoorn) in small pots. Per treatment, 48 bulbs were planted separately and inoculated between the brown skin and the white scale with one sclerotium per bulb. Potting soil was added and the pots were incubated at 9 °C and over 95% r.h. Control bulbs were inoculated with unflooded sclerotia. Plants were examined for symptoms of grey bulb rot two months after inoculation.

Sclerotia that had been flooded for two or three weeks and then transferred to agar did not germinate (Table 1), but if inoculated onto tulip bulbs such sclerotia could establish infection. The discrepancy may have been caused by the disinfection in formaldehyde before transfer to agar, although sclerotia from unflooded soil that were disinfected in the same way germinated for over 90% (Table 1). A similar phenomenon was reported by Gladders and Coley-Smith (1980), who studied the behaviour of sclerotia of *R. tuliparum* after invasion by a number of soil fungi. Invasion was associated with an apparent rapid loss in viability when measured by germination on agar, but sclerotia were able to infect tulip bulbs.

After flooding for 3 weeks, most sclerotia had lost their pathogenicity. Four weeks were required to kill them (Table 1).

According to Moore (1949), flooding experiments with sclerotia in the field yielded similar results as trials under laboratory conditions. Flooding experiments with grey bulb rot in the field are in progress.

Samenvatting

Sclerotiën van Rhizoctonia tuliparum verliezen hun kiemkracht en pathogeniteit door inundatie

Rhizoctonia tuliparum veroorzaakt kwadegrond in tulp en iris. Sclerotiën van deze schimmel kunnen in grond zeer lang levensvatbaar en pathogeen blijven. Van sclerotiën van enkele schimmels is bekend, dat ze gevoelig zijn voor inundatie die een aantal weken duurt. Inundatie wordt in Nederland vanaf 1982 door verscheidene bollentelers

's zomers toegepast ter bestrijding van ziekten en onkruid.

Sclerotiën van *R. tuliparum* werden verpakt in nylon zakjes, ingegraven in emmers met grond en 1-6 weken geïnundeerd bij 17 °C. Op verschillende tijdstippen werden geïnundeerde en niet-geïnundeerde sclerotiën hetzij ontsmet in 2,5% formaldehyde en vervolgens uitgeplaat op moutagar met oxytetracycline, hetzij aangebracht tussen de bruine huid en de buitenste bolrok van in kleine potjes geplante tulpebollen (cv. Apeldoorn); iedere bol werd geïnoculeerd met een sclerotium. Na twee maanden werden de planten beoordeeld op symptomen van kwadegrond.

In de eerste proef, waarbij sclerotiën werden uitgeplaat op agar, bleek er uit sommige sclerotiën die 2 weken waren geïnundeerd nog mycelium te groeien, terwijl dit na 4 weken inundatie niet meer plaatsvond. In de tweede proef kiemden de sclerotiën op agar niet meer na 2 weken inundatie, maar meer dan de helft van de geïnoculeerde tulpebollen vertoonde wel symptomen. Pas als de sclerotiën 4 weken geïnundeerd waren bleken ze zodanig geïnactiveerd te zijn, dat er geen symptomen van kwadegrond werden waargenomen. Resultaten van veldproeven moeten nog worden afgewacht.

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