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Inactivation of sclerotia of Rhizoctonia solani on potato tubers by Verticillium biguttatum, a soil-borne mycoparasite

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Abstract

Experiments in the laboratory and on farms with potato tubers in storage are described in which sclerotia of *Rhizoctonia solani* were inactivated after inoculation of infected tubers with a suspension of conidia and hyphal fragments of *Verticillium biguttatum*.

Sclerotia on freshly harvested tubers can be killed in a period of six to eight weeks, provided that (1) a direct contact between sclerotia and conidia of *V. biguttatum* is obtained, (2) the temperature during the storage period is at least 15 °C, but preferably closer to 20 °C during the first weeks, and (3) the relative humidity of the air between the tubers is at least 99%.

Seed tubers are only certified as export quality if the infection with *R. solani*, visible as sclerotia on the tubers, is assessed as below a specified incidence. To restore the economical value of tubers with many sclerotia, living sclerotia can be inactivated by *V. biguttatum*. However, also dead sclerotia have to be removed, as dead and living sclerotia cannot be distinguished visually by inspectors. A satisfactory way to remove dead sclerotia from tubers has not yet been found.

Additional keywords: biological control, black scurf, certification of seed tubers.

Introduction

Sclerotia of *Rhizoctonia solani* (Kühn) grown in soils with a pH lower than about 6.5 are frequently colonized by *Verticillium biguttatum* (W. Gams). Jager and Velvis (1983) found that the mycoparasite was present on 30-96% of the sclerotia on tubers grown in the soils of more than sixty plots in the northern part of the Netherlands. Among five mycoparasites tested for their effectiveness in destroying sclerotia when incubated at 20 °C for 7 weeks, *V. biguttatum* was the only one that killed almost all sclerotia (Velvis and Jager, 1983). The effectiveness was independent of the presence of other soil microorganisms. *V. biguttatum* was effective also at 15 °C, but not at 10 °C. Therefore, besides its use for biological control during crop growth (Jager and Velvis, 1985, 1986), *V. biguttatum* looks promising for restoration of the value of infected seed potatoes during storage.

Aluko (1968) found that the parasitism of *Gliocladium virens* on sclerotia of *R. solani* was strongly influenced by the relative humidity of the air. To determine whether the same might be true for *V. biguttatum*, experiments were conducted in which sclerotia on tubers were inoculated with the parasite and kept at different temperatures and relative humidities in the laboratory and under actual storage conditions on farms.

Materials and methods

V. biguttatum inoculum was produced as described earlier (Jager and Velvis, 1986); isolate M73 was used. The relation between the relative humidity (r.h.) and the rate of killing of sclerotia was studied using the agar disk isopiestic equilibration technique of Harris et al. (1970). Sclerotia (2-6 mm in diam.) from tubers were dipped into a spore suspension of *V. biguttatum* (about 10^7 spores per ml), and dried on filter paper in an air current for two hours. The sclerotia were placed on the bottom of a Petri dish, and an agar disk with the appropriate NaCl concentration to create a given r.h. was attached to the inside of the lid. At each r.h. level 50 sclerotia were used. Petri dishes having the same r.h. levels were put in a sealed plastic bag. After 32 days at 20 °C the experiment was terminated. The various fungi present on the sclerotia were recorded. The viability of sclerotia was assessed by placing them on water agar for at least 48 hours and counting the number of emerging hyphae of *R. solani*. As a measure of the viability of the sclerotia the index given below was used. Depending on the number of emerging hyphae the sclerotia were divided into five classes numbered 0-4, viz., without emerging hyphae (0), with 1-5 emerging hyphae (1), with 6-10 emerging hyphae (2), with 11-25 emerging hyphae (3) and with more than 25 emerging hyphae (4).

The sclerotium viability (v.i.) index was calculated as follows:

$$\text{v.i.} = \frac{\Sigma\,(\text{number of sclerotia in one class} \times \text{class number}) \times 100}{4 \times \text{total number of sclerotia involved}}$$

The effectiveness of *V. biguttatum* in killing sclerotia on tubers was studied in two experiments in the laboratory. The tubers were washed before the experiments were started.

Experiment 1. About 40 kg of tubers with sclerotia was divided into two equal portions, each of them having the same number of tubers that were heavily, moderately and lightly infested with sclerotia. One portion was sprayed with a suspension of conidia and hyphal fragments of *V. biguttatum*, the other portion with water. Each portion was put in a plastic bag and kept at 15-16 °C for six weeks. The r.h. in the closed bags was 100% or very nearly so. After this period 100 sclerotia were randomly taken from tubers of each sample and tested for their viability.

Experiment 2. A large sample of tubers with sclerotia was chosen from the harvest of an experimental field on marine sandy loam. It was split into 24 subsamples, each containing about 60 tubers, which were put into plastic bags. Twelve bags contained tubers inoculated with a suspension of conidia and hyphal fragments of *V. biguttatum* and another twelve with tubers that received the fluid only. One third of the number of bags of each treatment was incubated at 14, 17 and 20 °C, respectively. After 17, 31 and 45 days of incubation at each temperature, the viability of the sclerotia was investigated. Three bags with inoculated and three with non-inoculated tubers were available to determine the effect of inoculation on the sclerotium index (s.i.), a measure of the amount of sclerotia on tubers (Jager and Velvis, 1985). We tried to remove dead sclerotia from tubers with a brush.

In experiments on farms, tubers were sprayed with a suspension of conidia and hyphal

fragments of *V. biguttatum* at the end of the conveyor belt to the store. For 1000 kg of tubers 1.5-2 litres of suspension was used, which barely wetted the tubers. The r.h. in potato heaps usually is sufficiently high to permit growth of *V. biguttatum*. The upper layer of about 50 cm thickness, however, may become too dry. Therefore, the heaps were covered with a layer of gunny sacks in later experiments.

Results

The relation between the r.h. of the air and the activity of *V. biguttatum* towards sclerotia is given in Table 1. At r.h. values below 96.7% no *V. biguttatum* was visibly present. The sclerotia were densely overgrown by *Penicillium* species, which probably had a negative effect on their viability. Isolates of *Penicillium* species growing on sclerotia were tested in previous experiments and proved to be only slow killers of sclerotia. A very marked effect became apparent at a r.h. of 98.4% and higher, coinciding with growth and sporulation of *V. biguttatum*. Together with *V. biguttatum* incidentally other mycoparasites such as *Gliocladium roseum* and *G. nigrovirens* were observed at this and higher r.h. values. *Penicillium* spp. were still present, but at a much lower level than under drier conditions. The percentage of sclerotia with visible growth (sporulation) of *V. biguttatum* increased from 12% at 98.4% r.h. to 60% at 100% r.h. At a r.h. of 99.7% and higher all sclerotia were dead after one month, i.e. no hyphae of *R. solani* emerged from sclerotia during a one-week incubation on water agar at 20 or 23 °C. The failure of germination was probably attributable principally to the activity of *V. biguttatum*. At a r.h. level of 98.4% the effect of *V. biguttatum* was too small. For successful commercial application 99% should be considered as the minimum value.

The results of the first experiment with stored tubers are presented in Table 2. The effect of inoculation of infected tubers with *V. biguttatum* was very pronounced: 92% of the sclerotia was dead and 4% nearly so. Natural infection with *V. biguttatum* was apparent in the non-inoculated sclerotia: 39% was dead.

The results of the second experiment with stored tubers are given in Table 3. At 14 °C the process of killing proceeded slowly. At 17 and 20 °C the killing rate was satisfactory. After 45 days 95 and 89% of the sclerotia did not show any germination and were

Table 1. Viability of sclerotia of *R. solani*, inoculated with *V. biguttatum* after incubation for 32 days at 20 °C and various relative humidities.

Concentration NaCl solution (mol/l)	Relative humidity at 20 °C (%)	Viability index of sclerotia[1]
3.0	89.3	53
2.0	93.2	53
1.5	95.0	57
1.0	96.7	88
0.5	98.4	36
0.1	99.7	0
0.05	99.8	0.5
0	100	0

[1] The initial viability index was 81.

Table 2. Effect of inoculation of sclerotia of *R. solani* on potato tubers with *Verticillium biguttatum*, after incubation at 15-16 °C for six weeks.

Treatment	Percentage of sclerotia according to the number of emerging hyphae					Viability index
	0	1-5	6-10	11-25	>25	
Inoculated	92	4	0	3	0	4
Non-inoculated	39	11	2	6	42	66

Table 3. Percentage of dead sclerotia of *R. solani* on potato tubers with and without inoculation with *V. biguttatum*. Incubation at 14, 17 and 20 °C for 17, 31 and 45 days.

Incubation temperature (°C)	Inoculation	Percentage dead sclerotia after various periods of incubation (days)			Sclerotium index after 47 days
		17	31	45	
14	–	5	8	10	61
	+	5	20	22	56
17	–	22	20	27	59
	+	79	86	95	58
20	–	12	20	38	56
	+	67	85	89	59

considered to be dead. Part of the sclerotia from non-inoculated tubers apparently had already been infected in the field, and about one third of them proved to be dead after a 45-day incubation period at 17-20 °C. Due to inoculation an additional 60% was killed. After 47 days of incubation the amount of sclerotia on the tubers had not changed, because living and dead sclerotia cannot be distinguished visually. Brushing the tubers to remove the dead ones, in such a way that the peel was not damaged, was not successful.

The first experiment on a farm was carried out in 1983 with tubers of the smooth-peeled cultivar Spartaan, grown in a sandy loam soil. They were harvested under dry weather conditions and the tubers were free of adhering soil. The average sclerotium index was such that the lot had to be rejected as certified seed potatoes. The complete lot was inoculated and stored in the beginning of August. During August and September the temperature was sufficiently high for growth of *V. biguttatum* and consequently for killing sclerotia. Sorting of the tubers started in the first half of December. During transport from the heap to the sorting table the tubers were forced to roll towards each other as much as possible in order to rub off the sclerotia. A sorting machine with fine screens was very successful in removing sclerotia. The movement of the screens forces the tubers forward in small jumps. The majority of the sclerotia adhering to the tubers was rubbed off in this process. The sclerotium index of the tubers treated in this way was 12, which is sufficient to meet the export quality requirements of the

N.A.K. (General Netherlands Inspection Service for Agricultural Seeds and Seed Potatoes) for seed tubers (class A). Sixty percent of the sclerotia still present on the tubers was dead, 25% nearly dead and only 15% was in good condition. The amount of rejected tubers was negligible.

Weather conditions are not always like those in the experiment just described. A high incidence of sclerotia on the tubers is often due to rather long periods of wet weather. Then, after haulm destruction, harvest can be seriously delayed, and the harvested tubers often carry far more sclerotia than when they are harvested without delay. Besides, under wet conditions soil adheres to the tubers covering the sclerotia and protecting them against parasitism by *V. biguttatum* sprayed on the soil. For successful parasitism contact between *V. biguttatum* and a sclerotium or a hypha of *R. solani* is a prerequisite.

An experiment under wet conditions, but with fairly clean tubers (free from soil), was started at the end of September 1984. The average initial sclerotium index was very high, viz. 63. Mid-December the sclerotium index had decreased to 42, which was an insufficient reduction. The maximum sclerotium index allowed for certified seed tubers (class A) is 12.5, provided the tubers are only lightly speckled with sclerotia. Tubers moderately and heavily speckled must be discarded. All other treatments on farms which had started late in 1984 were unsuccessful in killing sclerotia because the temperature was too low and too much soil adhered to the tubers, especially to the sclerotia.

In the same year, however, we conducted an experiment on a farm with tubers grown on light, loamy sand. Tubers were inoculated at the end of August, except a small lot which was stored in bags amidst the treated lot. At the end of November the sclerotium index of the untreated lot was still very high, viz., about 66, in spite of brushing. Brushing to remove dead sclerotia of the treated lot led to an index of 25. After the tubers had passed through the sorting machine (mainly for removal of oversized tubers) the sclerotium index of the remaining lot was 12, sufficiently low to meet the quality demands for seed potatoes class A.

Discussion

Killing of sclerotia of *R. solani* was studied by Aluko (1968), who used *Gliocladium virens* as a biological control agent. During a six-week incubation period 85-94% of the sclerotia was killed at 15 °C. The inactivation was attributed to antibiotics, viz., gliotoxin and viridin, produced by *G. virens* (Aluko and Hering, 1970). The mechanism of killing of sclerotia of *R. solani* by *V. biguttatum* is still obscure. We often observed signs of parasitism as described by Barnett (1964), and Barnett and Binder (1973) on or in hyphae (Jager et al., 1979), but we seldom observed parasitism of monilioid cells of sclerotia, possibly because this phenomenon is difficult to observe, the cells being dark and thickwalled. When a sclerotium that fails to germinate due to the activity of *V. biguttatum* is squashed, intercellular hyphae of *V. biguttatum* are found, but no hyphae that are attached to, or protruding from, monilioid cells. It would appear that the destruction of sclerotia of *R. solani* is achieved through the production of toxic substances (Jager et al., 1979) rather than through parasitism by *V. biguttatum*. The abundant sporulation of *V. biguttatum* growing on sclerotia points to a good supply of nutrients. However, the competitive saprophytic ability of *V. biguttatum* is very weak. Thus, the withdrawal of nutrients by *V. biguttatum* from a sclerotium seems to take place without any serious competition from other microbial species. This situation can

be achieved by poisoning sclerotial cells from the interior of other cells. *V. biguttatum* can invade the interior of sclerotial cells via parasitized hyphae leading to or emerging from sclerotia.

Monilioid cells are present in chains. The protoplasm in these cells is connected via septal pores between the monilioid cells (Butler and Bracker, 1970). Chains of monilioid cells at the base in contact with a parasitized hypha could be poisoned from here. The solubilized contents of killed monilioid cells might be absorbed by the parasite without leaving any trace of parasitic action in the killed cells. Not all monilioid cells of a parasitized sclerotium are necessarily killed (Saat, pers. comm.). We observed that when non-germinating sclerotia were suspended in water and fragmented, part of the fragments germinated when spread on water agar. All monilioid cells in a non-germinating sclerotium were not necessarily dead. Germination may be inhibited, probably by one or more water-soluble substances. The phenomena observed are not in disagreement with the hypothesis that sclerotia of *R. solani* are killed by *V. biguttatum* via parasitized hyphae from the interior of sclerotial cells. The mechanism of destruction is under investigation by P.H.J.F. van den Boogert et al.

Certified seed tubers are allowed to contain a specified maximum amount of tubers lightly speckled with sclerotia, the amount depending on the quality class of the seed tubers. The amount of living sclerotia can be reduced by inoculation of infected tubers with *V. biguttatum*. However, dead and living sclerotia cannot be distinguished visually by the inspectors, so dead sclerotia have to be removed from the tubers to restore their economical value as seeds. Brushing to remove dead sclerotia met with varying success, depending on the way the sclerotia were attached to the peel. Removal of dead sclerotia from smooth and firm peels is probably easy, but detachment will be difficult or impossible from rough peels with small cracks to which sclerotia are attached. Chand and Logan (1984) demonstrated that depth of penetration of sclerotia into the periderm depended on the length of the period during which tubers are left in the soil after haulm destruction, on the length of the storage period and on growth into scab lesions and lenticells.

The removal of dead sclerotia from tubers needs further study.

Samenvatting

Het onschadelijk maken van sclerotia van Rhizoctonia solani op aardappelknollen door Verticillium biguttatum, een mycoparasiet uit de grond

In proeven in het laboratorium en in de praktijk werden sclerotiën van *Rhizoctonia solani* (lakschurft) gedood door met lakschurft bezette knollen te beënten met sporen van *Verticillium biguttatum*. Sclerotiën op kort tevoren gerooide knollen worden bijna volledig gedood in 6-8 weken, als aan de volgende voorwaarden wordt voldaan.
(1) Er moet een direct contact zijn tussen de sclerotiën en de sporen van *V. biguttatum*. De sclerotiën op de knollen moeten dus vrij zijn van aanhangende grond.
(2) De temperatuur moet gedurende de bewaring tenminste 15 °C zijn en gedurende de eerste weken liever 20 °C of dicht hierbij. Dit is in augustus, als de poters in de bewaarplaats worden gebracht, meestal wel het geval.
(3) De relatieve vochtigheid van de lucht tussen de knollen moet 100% zijn. Dit is meestal

het geval in een hoop pas gerooide, jonge knollen. Om de bovenste laag van 40-50 cm niet te droog te laten worden kan het oppervlak afgedekt worden met een laag grove jute zakken.

Door borstelen kunnen dode sclerotiën soms grotendeels worden verwijderd; dit is nodig voor de keuring omdat dode en levende sclerotiën op het oog niet te onderscheiden zijn. Voor de verwijdering van dode sclerotiën wordt naar een betere methode gezocht.

References

Aluko, M.O., 1968. Microbial antagonism in the control of *Rhizoctonia solani* Kühn on potatoes. Thesis University of Nottingham.

Aluko, M.O. & Hering, T.F., 1970. The mechanisms associated with the antagonistic relationship between *Corticium solani* and *Gliocladium virens*. Transactions of the British Mycological Society 55: 173-179.

Barnett, H.L., 1964. Mycoparasitism. Mycologia 56: 1-19.

Barnett, H.L. & Binder, F.L., 1973. The fungal host-parasite relationship. Annual Review of Phytopathology 11: 273-292.

Butler, E.E. & Bracker, C.E., 1970. Morphology and cytology of *Rhizoctonia solani*. In: J.R. Parmeter Jr (Ed.), *Rhizoctonia solani*, biology and pathology. University of California Press, Berkeley, Los Angeles and London. p. 32-52.

Chand, T. & Logan, C., 1984. Post-harvest development of *Rhizoctonia solani* and its penetration of potato tubers in Northern Ireland. Transactions of the British Mycological Society 82: 615-619.

Harris, R.F., Gardner, W.R., Adebayo, A.A. & Sommers, L.E., 1970. Agar disk isopiestic equilibration method for controlling the water potential of solid substrates. Applied Microbiology 19: 536-537.

Jager, G. & Velvis, H., 1983. Suppression of *Rhizoctonia solani* in potato fields. 1. Occurence. Netherlands Journal of Plant Pathology 89: 21-29.

Jager, G. & Velvis, H., 1985. Biological control of *Rhizoctonia solani* on potatoes by antagonists. 4. Inoculation of seed tubers with *Verticillium biguttatum* and other antagonists in field experiments. Netherlands Journal of Plant Pathology 91: 49-63.

Jager, G. & Velvis, H., 1986. Biological control of *Rhizoctonia solani* on potatoes by antagonists. 5. The effectiveness of three isolates of *Verticillium biguttatum* as inoculum for seed tubers and of soil treatment with a low dosage of pencycuron. Netherlands Journal of Plant Pathology 92: 231-238.

Jager, G., Hoopen, A. ten & Velvis, H., 1979. Hyperparasites of *Rhizoctonia solani* in Dutch potato fields. Netherlands Journal of Plant Pathology 85: 253-268.

Velvis, H. & Jager, G., 1983. Biological control of *Rhizoctonia solani* on potatoes by antagonists. 1. Preliminary experiments with *Verticillium biguttatum*, a sclerotium-inhabiting fungus. Netherlands Journal of Plant Pathology 89: 113-123.