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Effects of different temperature treatments on dormancy of sclerotia of ten isolates of Sclerotium cepivorum

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Abstract

The variability of dormancy of sclerotia of ten isolates of *Sclerotium cepivorum* was investigated. Of all isolates tested, the freshly harvested sclerotia were dormant. After drying for 48 hours the sclerotia of six isolates were able to germinate, two isolates stayed dormant and two isolates were infested by hyperparasitic fungi. After storage in soil at 5 °C or 20 °C, the sclerotia of the different isolates exhibited considerable differences in respect to germination capability, but all isolates showed highest germination after a treatment of 8 weeks at 20 °C followed by 8 weeks at 5 °C. The sclerotia of all isolates showed an increased capacity to germinate without *Allium* extracts at 10 °C after pretreatment at 30 °C for 28 days.

Additional keywords: white rot, onion, *Allium*.

Introduction

White rot of onion caused by *Sclerotium cepivorum* is maintained in soil by sclerotia of the fungus. One factor in the incidence of white rot is the size of the initial population of sclerotia in the field. There are, however, large differences in the effect of different sizes of sclerotial populations on white rot incidence (Entwistle, 1987). Another important factor is the composition of the population regarding germination ability of the sclerotia. Differences in germination ability of the sclerotia depend on the particular isolate of the fungus and on the life history of the sclerotia. Newly formed sclerotia have a period of constitutive dormancy lasting several weeks or months (Coley-Smith, 1960; Coley-Smith et al., 1987), but some isolates are able to germinate without conditioning (Brix, 1990).

Sclerotia of *S. cepivorum* may survive in soil for over 20 years, but some batches show poor survival after 1 year (Leggett et al., 1983; Coley-Smith et al., 1990). Both the rate and final degree of germination of sclerotia produced on an artificial medium (Coley-Smith and Holt, 1966) or on onion bulbs (Brix, 1990; Brix and Zinkernagel, 1992) appeared to be different for different isolates. Eruptive germination of the sclerotia is stimulated by root exudates that are specific of *Allium* spp. (Coley-Smith and Holt, 1966; Coley-Smith and King, 1969). Germination without *Allium* extracts or diallyl disulphide was reported to occur at c. 20% (Crowe and Hall, 1980; Merriman et al., 1981); conditioning sclerotia by a low temperature treatment at 5 °C favoured germination without *Allium* to 95% (Gerbrandy, 1989).

In a previous paper (Gerbrandy, 1989) the effect of different temperatures during

storage of sclerotia of one isolate on subsequent germination was described. The present paper describes the effects of different storage conditions on germination ability of sclerotia of different isolates.

Material and methods

Isolates. The isolates 2, 3, 4, 5, and 6 were obtained from Dr Q. van der Meer (IVT Wageningen). Isolate 5 originates from Egypt, the isolates 2, 3, 4 and 6 are of Dutch origin, the isolates G, J412 and A were obtained from Dr T. H. Abt-El-Moity (Giza, Egypt), Prof. Dr J. R. Coley-Smith (Hull, U.K.) and Dr I. Porter (State of Victoria, Australia) respectively. The isolates Z and F were recovered from the Dutch provinces of Zeeland and Friesland respectively.

Production of sclerotia. Sclerotia were produced by infection of onion bulbs with mycelium of *S. cepivorum*, grown on PDA. The bulbs were incubated in moist sand at 15 °C. The newly formed sclerotia were harvested after about 5 weeks, mixed with silver sand and stored in nylon pouches in soil (Gerbrandy, 1989) or dried in an exsiccator with silicagel for 48 hours.

Temperature treatments of the isolates. In order to keep the sclerotia of the ten different isolates under the same experimental conditions, all experiments concerning differences between isolates were performed on the same day. Freshly harvested sclerotia of each isolate were mixed with sand, divided into four portions and put into nylon pouches. Two pouches of each isolate were stored just beneath the soil surface in a container (45 × 30 × 8 cm) and kept at 5 °C, one for 16 weeks and one for 8 weeks, followed by 8 weeks at 20 °C; the other two pouches of each isolate were stored in the same way at 20 °C (16 weeks) or 8 weeks at 20 °C followed by 8 weeks at 5 °C. Treatment at 30 °C was performed by placing Petri dishes with moist sand and sclerotia on top of it in an incubator at 30 °C. The Petri dishes were incubated in a closed container for wet treatment. Dry treated sclerotia were incubated in an open container, which caused drying within 2 days.

Germination of sclerotia. The course of germination was followed after placing forty sclerotia on Petri dishes filled with washed silver sand as described previously (Gerbrandy, 1989). Four Petri dishes were used in each experiment. The sand was wetted with 0.1 M phosphate buffer of pH 5.2. The effect of *Allium* on germination was studied by placing two Petri dishes with about 40 g of chopped onions and two squeezed garlic cloves in the incubation boxes (50 × 30 × 3 cm). The onions and garlic were replaced at weekly intervals. Boxes containing *Allium* were stored in incubators in a room separate from the room containing incubators with boxes without *Allium*.

Results

Dormancy of fresh sclerotia. Freshly harvested sclerotia of *S. cepivorum* produced on onion and prevented from drying were almost completely dormant during 30 days of incubation at 15 °C, in the presence of *Allium* (Table 1). After 80 days, only isolate 5 showed a substantial germination (24%). Drying of the freshly harvested sclerotia

Table 1. Germination of freshly harvested sclerotia of ten different isolates of *S. cepivorum*, either fresh or dried for 48 hours at room temperature, after 30 and 80 days at 15 °C in the presence of *Allium*; and decay of dried sclerotia of isolates with poor germination.

Isolate	% Germination of fresh sclerotia		% Germination of dried sclerotia		% Decayed after 120 days
	30 days	80 dayss	30 days	80 days	
2	0	0	0	0	72
3	4	5	34*	65*	
4	0	0	0	8	2
5	1	24	26*	28*	–
6	0	1	73	82	–
Giza	6	9	70	83	–
F	0	0	72	97	–
Z	0	1	0	1	63
J412	0	3	29	58	–
A	0	0	1	4	0

* Some germinated without plug.

for 48 hours caused a release from dormancy of isolate 6, Giza, F and J412, whereas the sclerotia of the isolates 4 and A stayed dormant. After drying, some of the sclerotia of the isolates 3 and 5 germinated by radial outgrowth of mycelium without plug formation. The sclerotia of the isolates 2 and Z were infected by hyperparasitic fungi within 14 days, and during the experiment most of them decayed without germination.

Effect of burial of the sclerotia at different temperatures. As is shown in Fig. 1, dormancy of the sclerotia of the various isolates differed considerably after temperature treatments at 20 °C and 5 °C. Of the sclerotia of the isolates F, Z and J412, 40–80% germinated after 16 weeks of burial at any temperature, whereas the sclerotia of the isolates 2, 5 and Giza stayed dormant. After 16 weeks of burial in soil the sclerotia of all isolates that underwent a treatment of 8 weeks at 20 °C followed by 8 weeks at 5 °C showed the highest germination, whereas a temperature treatment of 16 weeks at 5 °C caused similar germination as 16 weeks at 20 °C. Burial for one year at 5 °C released dormancy of the isolates 2, 3, 6, F, Z and J412, but the sclerotia of the isolates 4, 5 and Giza stayed dormant even after a year. Isolate 4 is particular in that only the treatment of 8 weeks at 20 °C followed by 8 weeks at 5 °C released dormancy.

Germination of sclerotia after pretreatment at 30 °C. The effect of high temperature treatment was further studied using sclerotia stored at 5 °C for about one year. They were incubated at 30 °C followed by an incubation at 10 °C. Incubation at 30 °C for different periods did not much affect the subsequent germination of sclerotia of isolate F at 10 °C, in the presence of *Allium* (Fig. 2A), but germination without *Allium* was enhanced, particularly after 28 and 42 days at 30 °C (Fig. 2B). Incubation at 30 °C under drying conditions had a less stimulating effect on germination without

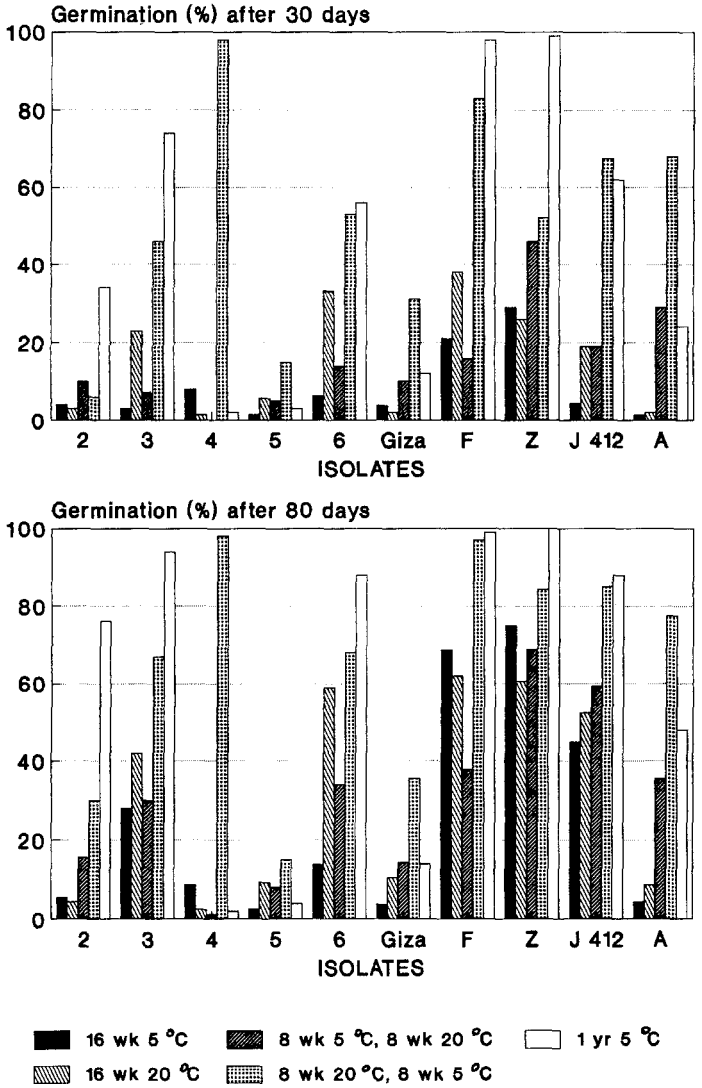

Fig. 1. Germination of sclerotia of ten isolates of *S. cepivorum* preincubated in soil, at 5 and 20 °C for 8 weeks (8 wk), 16 weeks (16 wk) and 1 year (1 yr), after 30 days and 80 days at 15 °C in the presence of *Allium*.

Allium (Fig. 2C). Also all other isolates tested exhibited a stimulation of the germination at 10 °C without *Allium* after prior incubation for 28 days at 30 °C, either wet or dry. Isolate 2 showed higher germination after a dry treatment, isolates 5 and A after a wet treatment at 30 °C (Fig. 3). The wet sclerotia of isolate 4 started with germination after about 100 days of incubation at 10 °C, therefore the percentage of germination after 140 days is also given (Fig. 3).

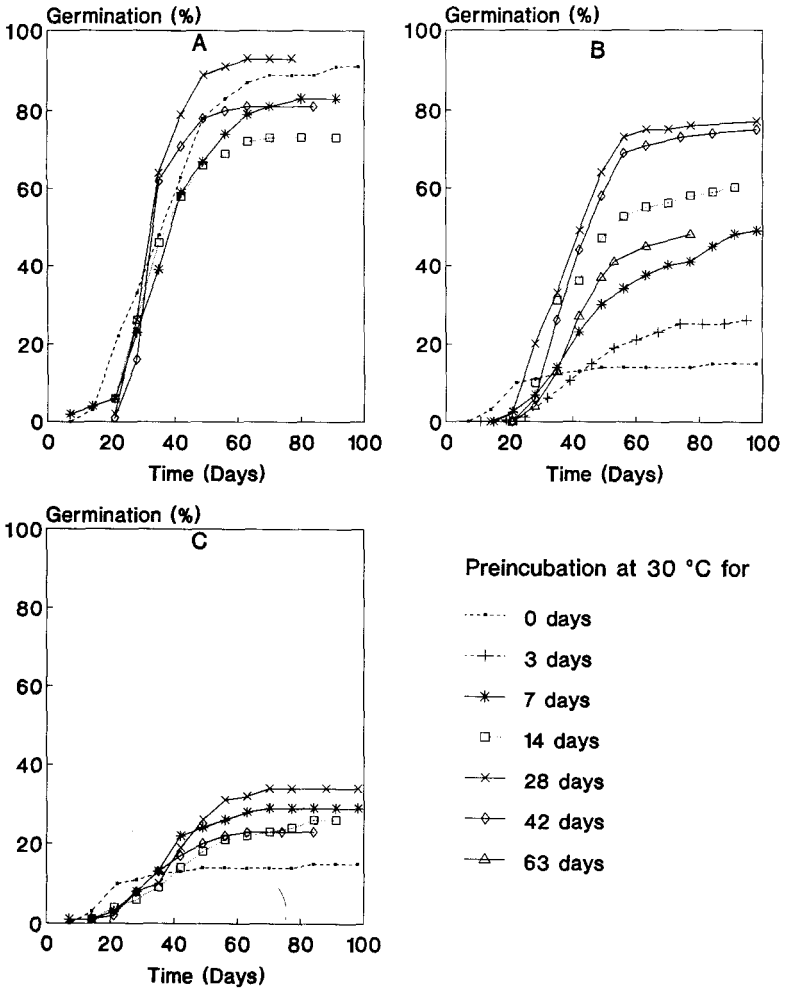

Fig. 2. Germination of sclerotia of isolate F at 10 °C in the presence (A) or absence (B,C) of *Allium*. The wet (A, B) or dry (C) sclerotia were preincubated for 0, 3, 7, 14, 28, 42 or 63 days at 30 °C.

Discussion

The collection of isolates of this study showed considerable differences in dormancy of freshly harvested and dried sclerotia and in the release from dormancy of sclerotia buried in soil due to different temperature conditions.

Newly formed sclerotia of some isolates (6, Giza, F and J412) lost their constitutive dormancy after drying, whereas the sclerotia of the isolates 4 and A stayed dormant (Table 1). The sclerotia of the isolates 4, 5 and Giza stayed dormant after one year in soil at 5 °C, but over 75% of the sclerotia of the isolates 2, 3, F, Z and J412 germinated (Fig. 1).

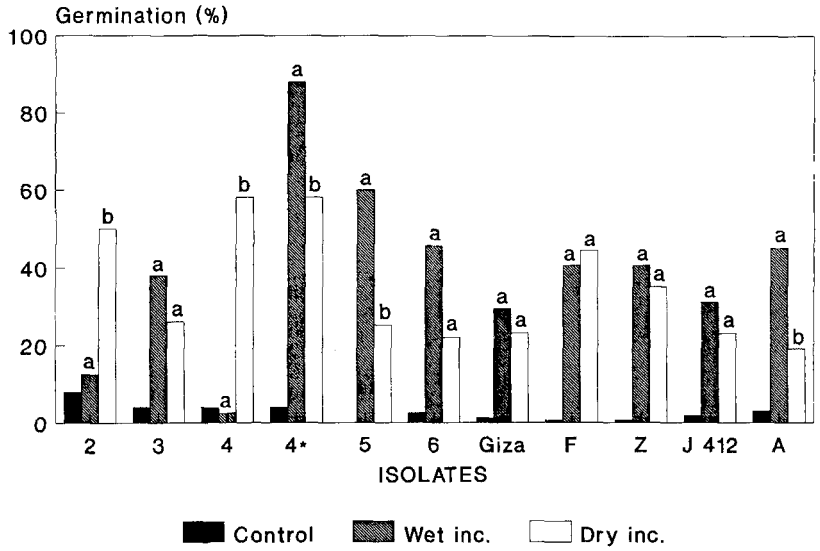

Fig. 3. Germination of sclerotia of ten isolates of *S. cepivorum*, after 80 days at 10 °C without *Allium*. The sclerotia were preincubated at 30 °C for 28 days under wet (wet inc.) or dry (dry inc.) conditions. Different letters indicate significant differences. For isolate 4 percentage of germination is also given after 140 days (4*).

The sclerotia of the different isolates also show similarities in their dormancy and germination. The sclerotia of all isolates were dormant just after harvest, if prevented from drying. Germination of sclerotia of all isolates was stimulated after a period of high temperature followed by a period of low temperature; in the present case, 8 weeks at 20 °C followed by 8 weeks at 5 °C (Fig. 1), or 28 days at 30 °C followed by an incubation at 10 °C (Figs 2, 3). Isolate 4 seemed to be exceptional because 97% of the sclerotia germinated after a treatment of 8 weeks at 20 °C followed by 8 weeks at 5 °C and all other treatments resulted in less than 10% germination (Fig. 1). After wet treatment at 30 °C isolate 4 needed an incubation time of more than 100 days before starting with germination (Fig. 3).

The large differences in the effect of different sizes of sclerotial population on white rot incidence were supposed to be due to unfavourable conditions for sclerotial germination, either because of high temperatures or because germination stimulants were not produced or were retained in soil (Entwistle, 1990). However, in comparing white rot incidence in different countries, it should be taken into account that climatic differences, especially temperature and drying, cause differences in germination ability. In addition, the populations might differ markedly from each other concerning dormancy of the sclerotia.

Brix (1990) concluded that dormancy of sclerotia of *S. cepivorum* is not a general characteristic as postulated by Coley-Smith (1960) but a characteristic of a particular isolate. In his experiments the sclerotia were routinely dried for 24 hours before germination and, as is shown in Table 1, the sclerotia of some isolates are able to germinate after drying. None of the isolates produced sclerotia without constitutive dormancy,

which confirms the conclusion of Coley-Smith (1960) that dormancy is a general characteristic of sclerotia of *S. cepivorum*.

Sclerotia of all isolates were able to germinate without *Allium* after incubation at 30 °C (Fig. 3). It was stated in a previous paper (Gerbrandy, 1989) that this kind of germination was caused by a low temperature treatment (5 °C) of the sclerotia. It was then obviously overlooked that the sclerotia were stored in a non-conditioned greenhouse with a minimum temperature of 15 °C before storage at 5 °C. The data presented here indicate that a shift from high temperature to low temperature, from 30 to 10 °C (Figs 2 and 3) conditioned sclerotia to germinate without *Allium*.

It may be concluded that the dormancy pattern differs for sclerotia of different isolates of *S.cepivorum*, that previous temperature is an important factor for subsequent germination, and that the sclerotia are able to germinate without *Allium*. These observations might be important for the interpretation of field observations concerning resistance of *Allium* spp. to white rot, biological control of white rot and longevity of sclerotia.

Acknowledgement

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References

Brix, H.D., 1990. Untersuchungen zur Biologie von *Sclerotium cepivorum* Berk. und zur Resistenz von Alliumarten gegen die Mehlkrankheit. Thesis, Technische Universität München.

Brix, H.D. & Zinkernagel, V., 1992. Effects of cultivation, conditioning and isolate on sclerotium germination in *Sclerotium cepivorum*. Plant Pathology 41: 13–19.

Coley-Smith, J.R., 1960. Studies of the biology of *Sclerotium cepivorum* Berk. IV. Germination of sclerotia. Annals of Applied Biology 48: 8–18.

Coley-Smith, J.R. & Holt, R.W., 1966. The effect of species of *Allium* on germination in soil of sclerotia of *Sclerotium cepivorum* Berk. Annals of Applied Biology 58: 273–278.

Coley-Smith, J.R. & King, J.E., 1969. The production by species of *Allium* of allyl sulphides and their effect on germination of sclerotia of *Sclerotium cepivorum* Berk. Annals of Applied Biology 64: 289–301.

Coley-Smith, J.R., Parfitt, D., Taylor, I.M. & Reese, R.A., 1987. Studies of dormancy in sclerotia of *Sclerotium cepivorum*. Plant Pathology 36: 594–599.

Coley-Smith, J.R., Mitchell, C.M. & Sansford, C.E., 1990. Long-term survival of sclerotia of *Sclerotium cepivorum* and *Stromatinia gladioli*. Plant Pathology 39: 58–69.

Crowe, F.J. & Hall, D.H., 1980. Soil temperature and moisture effects on sclerotium germination and infection of onion seedlings by *Sclerotium cepivorum*.
Phytopathology 70: 74–78.

Entwistle, A.R., 1987. Relationships between soil sclerotial populations of *Sclerotium cepivorum* and the incidence of Allium white rot. In: A.R. Entwistle (Ed.), Proceedings of the Third International Workshop on Allium White Rot, 17-19 September 1986, Wellesbourne UK, Section 6, pp. 21–29.

Entwistle, A.R., 1990. Measuring net changes in populations of *Sclerotium cepivorum* sclerotia to evaluate the long-term potential of control measures. In: A.R. Entwistle & P. Mattusch (Eds), Proceedings of the Fourth International Workshop on Allium White Rot, 5-7 June 1990, Neustadt/Weinstrasse, Germany, pp. 59–68.

Gerbrandy, Sj.J., 1989. The effects of various temperatures during storage in soil on subsequent germination of sclerotia of *Sclerotium cepivorum*. Netherlands Journal of Plant Pathology 95: 319–326.

Leggett, M.E., Rahe, J.E. & Utkhede, R.S., 1983. Survival of sclerotia of *Sclerotium cepivorum* Berk. in muck soil as influenced by drying and the location of sclerotia in soil. Soil Biology & Biochemistry 15: 325–327.

Merriman, P.R., Samson, I.M. & Schippers, B., 1981. Stimulation of germination of sclerotia of *Sclerotium cepivorum* at different depths in soil by artificial onion oil. Netherlands Journal of Plant Pathology 87: 45–53.