Neth. J. Pl. Path. 95 (1989) 319-326

The effects of various temperatures during storage in soil on subsequent germination of sclerotia of Sclerotium cepivorum

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Accepted: 20 May 1989

Abstract

The effects of various storage temperatures on germination of sclerotia of *Sclerotium cepivorum* Berk. were investigated. Sclerotia buried in soil for 10 weeks at temperatures of 5 and 10 °C were conditioned to a fast germination. When germination was performed at 15 °C and induced by *Allium* extracts, 50% of these sclerotia germinated within 10 days and the total of germination was over 90%. Sclerotia buried at temperatures of 15, 20 and 25 °C were conditioned to a slow germination. About 50% of these sclerotia could be induced to germinate at 15 °C by *Allium* extracts. The conditioning by high or low temperatures proved to be reversible.

The optimum temperature for germination of the cold (5 °C) conditioned sclerotia was 10-20 °C. The optimum temperature for germination of sclerotia conditioned at 20 °C was about 10 °C. Without *Allium* extracts 90%, 80%, 50% and 40% of the sclerotia stored at 5 °C, germinated at temperatures of 10, 5, 15 and 20 °C respectively. Sclerotia stored at 20 °C did not germinate without *Allium* extracts at any temperature.

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

Introduction

White rot disease of onions, caused by *Sclerotium cepivorum* Berk., is an important problem in the Netherlands, particularly in the south western part of the country (Tichelaar, 1965; Blikman, 1986). The pathogen has a narrow host range, suitable hosts being restricted to the genus *Allium* (Coley-Smith, 1959). During disease development numerous small (0.2-0.5 mm diameter) sclerotia are produced which function as the survival structures of this pathogen and are a severe threat to ensuing crops. Newly formed sclerotia undergo a period of constitutive dormancy, which breaks down after a period in soil from one to three months (Coley-Smith, 1960; Coley-Smith et al., 1987). In the absence of suitable host plants, these sclerotia may survive for many years (Coley-Smith and Sansford, 1986). Although germination of sclerotia has been reported to occur at a low level in the absence of host plants (Merriman at al., 1981; Crowe and Hall, 1980), it is generally a very specific response to compounds released by *Allium* species (Coley-Smith and Holt, 1966; Coly-Smith and King, 1969). This germination response can be induced by treatment of sclerotia with *Allium* extracts (Crowe and Hall, 1980), artificial onion oil (Merriman et al., 1981) or diallyl disulphide (DADS) (Coley-Smith and Parfitt, 1986). The germination of sclerotia induced by DADS showed a seasonal response: summer treatments with DADS during June, July and August gave

a poor germination, but a high level of germination was recorded for treatments in the other months of the year. This seasonal response was interpreted as a result of the rapid disappearance of DADS from soil at higher temperatures (Coley-Smith and Parfitt, 1986). However, the germination of sclerotia may be influenced by the temperature during the period preceding germination. Sclerotia of *Claviceps purpurea* e.g. require a chilling period of several weeks at 0-10 °C to activate germination (Mitchell and Cooke, 1968).

The effects of various temperature treatments on germination of sclerotia of *Sclerotium cepivorum* were investigated.

Materials and methods

Production of sclerotia. The method of producing sclerotia of *Sclerotium cepivorum* was similar to that described by Coley-Smith (1985). *S. cepivorum* was isolated from infected onion bulbs at Berlikum in the province of Friesland, the Netherlands. Onion bulbs were inoculated with four 5 mm plugs of mycelium from the edge of *S. cepivorum* colonies grown on PDA by placing the plugs on top of the bulbs. The bulbs were incubated in moist sand at 13-15 °C. After eight weeks the newly formed sclerotia were mixed with sand and stored in nylon pouches just beneath the surface of a sandy garden soil in a greenhouse with a minimum temperature of 15 °C.

Temperature conditioning. Sclerotia buried for four weeks were thoroughly mixed, divided into five portions and, with the adhering sand, put into nylon pouches. The pouches were buried just beneath the surface in a moist sandy garden soil in pots of 15 cm in diameter. The pots were stored in the dark at temperatures of 5, 10, 15, 20 and 25 °C respectively.

Germination of sclerotia. Forty sclerotia were randomly picked out of the sand and placed in Petri disches (4 cm diameter) filled with washed silver sand. The sand was wetted with 0.1 M phosphate buffer, pH 5.1. The Petri dishes were incubated in plastic boxes (50×30×3 cm) with moist filter paper on the bottom in order to maintain a high relative humidity. Sclerotia were considered to be germinated if a hyphal plug with mycelium appeared. The germinated sclerotia were counted weekly or, in cases of a high germination rate twice a week, and removed.

The effect of onion and garlic on germination was studied by placing two Petri dishes with c. 40 g of chopped onions, and two squeezed garlic cloves in the box. Onion and garlic were replaced at weekly intervals. For every treatment four Petri dishes were used.

Viability of sclerotia. Viability was determined according to Crowe and Hall (1980). Surface-sterilized sclerotia were cracked with forceps and placed on water agar. The sclerotia were incubated at 15 °C for 14 days and examined twice for development of mycelial growth.

Results

Temperature conditioning. Sclerotia buried at temperatures of 5, 10, 15, 20 and 25 °C respectively for 14 days showed no differences in subsequent germination at 15 °C.

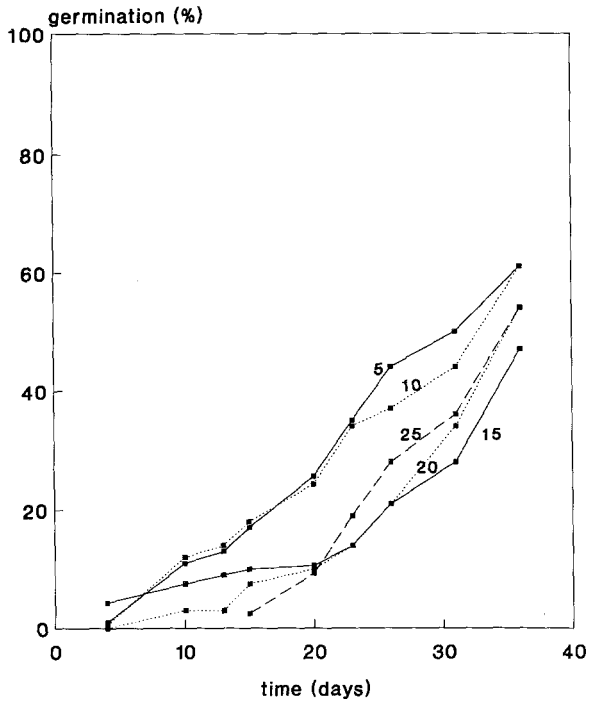

Fig. 1. Germination of sclerotia after 30 days storage at temperatures of 5, 10, 15, 20 and 25 °C. Germination was performed at 15 °C in the presence of onion and garlic.

After a burial period of 30 days, the sclerotia kept at 5 and 10 °C exhibited a faster germination rate, in the presence of onion and garlic, than the sclerotia kept at the higher temperatures (Fig. 1). After 70 days of conditioning, full germination (95%) of the sclerotia kept at 5 and 10 °C was obtained after 30 days, whereas the sclerotia kept at 15, 20 and 25 °C had germinated for about 55% after 60 days (Fig. 2). Viability of the sclerotia was not influenced by the different storage conditions because on water agar the sclerotia conditioned for 70 days at the various temperatures all germinated to an extent of 90-100%.

Germination without onion and garlic. Without temperature conditioning the sclerotia hardly germinated (less than 5%) at 15 °C. After 70 days at 5 and 10 °C, 35-40% of the sclerotia germinated at 15 °C in the absence of onion and garlic (Fig. 2). The sclerotia kept at 15, 20 and 25 °C did not germinate (less than 5%) at 15 °C in the absence of onion and garlic.

Effect of conditioning upon germination at different temperatures. Sclerotia stored at 5 °C for 58 days exhibited a fast germination at 15 °C and 20 °C in the presence of chopped onion and garlic. At 10 °C the germination started later. At 5 °C the germination was much slower. The differences in the total amount of germinating sclerotia were not statistically significant and varied between 82 and 95% (Fig. 3), and no germination was observed at 25 °C. Without onion and garlic germination also occurred, but the rate was slower (Fig. 4). At 20 °C and 15 °C the sclerotia did not germinate over a level of 40% and 52% respectively. Although the germination at 10 °C and particularly at 5 °C was slower than at 20 °C and 15 °C, more than 80% of these sclerotia

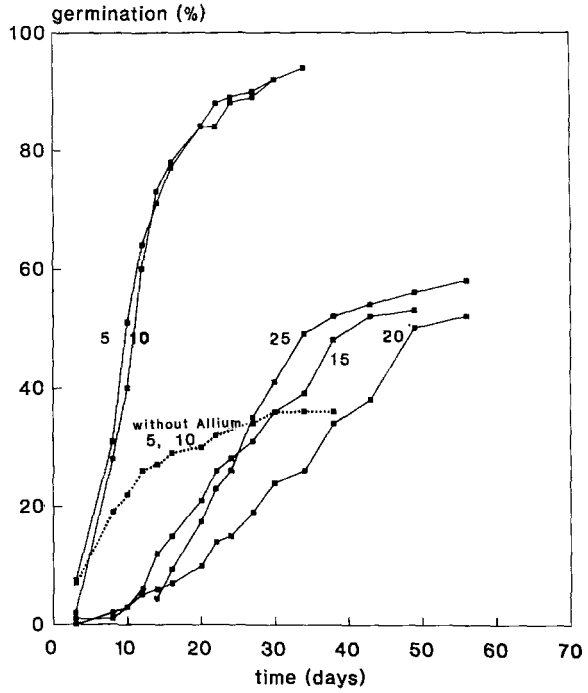

Fig. 2. Germination of sclerotia after 70 days storage at temperatures of 5, 10, 15, 20 and 25 °C. Germination was performed at 15 °C, in the presence of onion and garlic. The dotted line represents germination of sclerotia stored at 5 and 10 °C in the absence of onion and garlic.

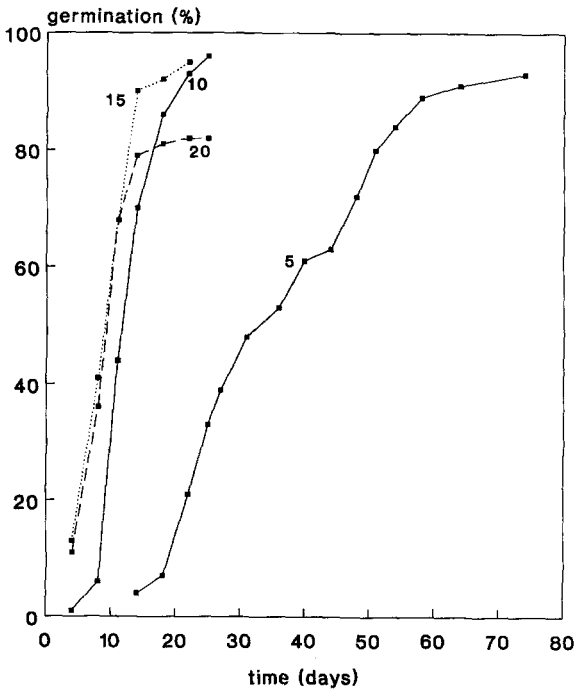

Fig. 3. Germination of sclerotia at temperatures of 5, 10, 15 and 20 °C in the presence of onion and garlic. Sclerotia were stored at 5 °C for 58 days prior to germination.

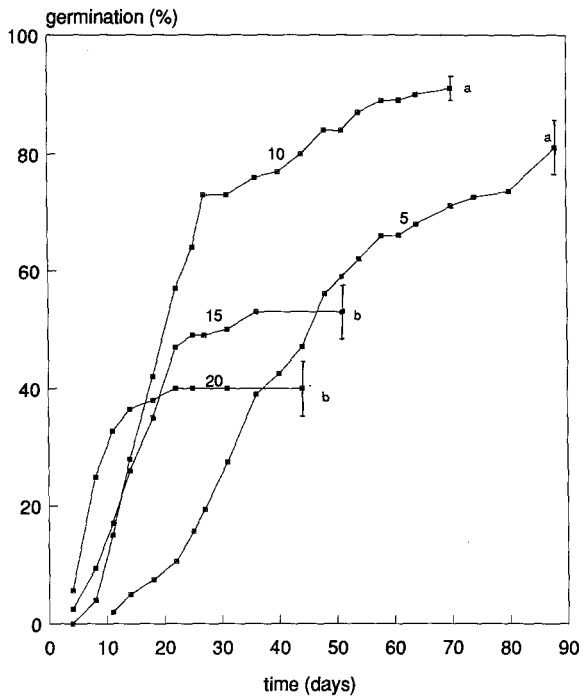

Fig. 4. Germination of sclerotia at 5, 10, 15 and 20 °C in the absence of onion and garlic. Sclerotia were stored at 5 °C for 58 days prior to germination. Vertical bars represent standard errors. Different letters indicate significant differences (P = 0.05).

eventually germinated (Fig. 4). Sclerotia kept at 20 °C for 74 days exhibited a lower germination as compared with those stored at 5 °C (Fig. 5). The total amount of sclerotia that could be induced to germinate was reduced by conditioning at 20 °C. At 10 °C, 83% of the sclerotia germinated, at 5 °C, 80%, at 15 °C, 50% and at 20 °C only 16% (Fig. 5). Sclerotia stored at 20 °C showed hardly any germination in the absence of chopped onion and garlic (less than 2%).

Reversibility of the temperature conditioning. Part of the sclerotia kept at 5 °C for 70 days were kept additionally at 20 °C for 72 days and sclerotia stored at 20 °C for 70 days were kept at 5 °C for 72 days. In both cases the effect of the final temperature treatment was dominant over the effect of the previous conditioning (Fig. 6).

Discussion

The data presented in this paper indicate that temperature conditions in soil are of major importance for germination behaviour of sclerotia of *S. cepivorum*. This pertains to the rate of germination, to the ultimate level of germination as well as to the susceptibility of sclerotia to induction of germination by *Allium* spp. Low temperature conditions (5 and 10 °C) appeared to be stimulatory for the subsequent germination process. This effect can be observed to a slight degree after a period of 30 days (Fig. 1) and is far more pronounced after 70 days (Fig. 2).

The lag before *Allium*-induced germination was c. 10 days shorter in low-temperature-conditioned sclerotia than in high-temperature-conditioned sclerotia (Fig. 2). The level of germination in high-temperature-conditioned sclerotia was reduced to c. 50% at 15

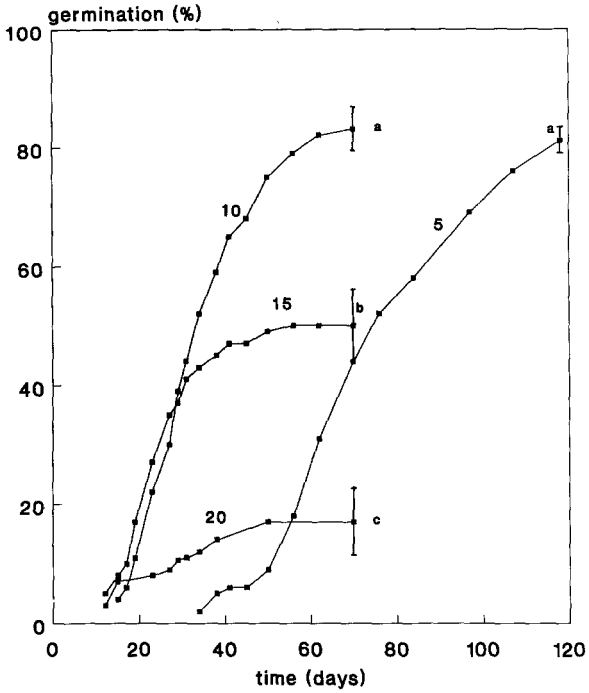

Fig. 5. Germination of sclerotia at 5, 10, 15 and 20 °C in the presence of onion and garlic. The sclerotia were kept at 20 °C for 74 days prior to germination. Vertical bars represent standard errors. Different letters indicate significant differences ($P = 0.05$).

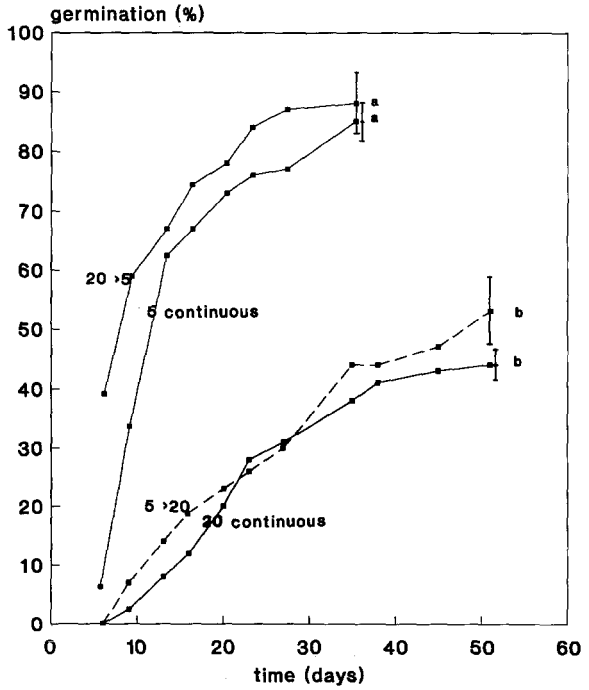

Fig. 6. Germination at 15 °C, in the presence of onion and garlic, of sclerotia stored at alternating temperatures: 20 > 5, sclerotia stored at 20 °C for 70 days and then at 5 °C for 72 days; 5 continuous, sclerotia stored at 5 °C for 142 days; 5 > 20, sclerotia stored at 5 °C for 70 days and then at 20 °C for 72 days; 20 continuous, sclerotia stored at 20 °C for 142 days. Vertical bars represent standard errors. Different letters indicate significant differences ($P = 0.05$).

°C and to c. 20% at 20 °C (Fig. 2 and Fig. 5), whereas the low-temperature-conditioned sclerotia germinated to a level of above 90%. Low temperature conditioning enables the sclerotia to germinate without *Allium* extracts. The germination without *Allium* extracts at 10 °C and 5 °C was of the same level as the induced germination, although it occurred at a slower rate (Fig. 4). Germination of sclerotia of *S. cepivorum* without *Allium* spp. was observed previously (Crowe and Hall, 1980; Merriman et al., 1981; Coley-Smith and Parfitt, 1986). In all these studies germination did not exceed the level of 20%. This is probably due to the fact that a high germination level, c. 90%, occurs only at a low germination temperature when sclerotia are conditioned at low temperature (Fig. 4). It is obvious that low temperature conditioning is not the only prerequisite for germination without *Allium* extracts, for it is well documented that sclerotia persist in soil for many years in temperate climates with extended periods of soil temperatures below 15 °C.

Optimum temperature for *Allium*-induced germination appeared to depend on pre-germination temperature. After storage at 5 °C the fastest rate of germination occurred at 15 and 20 °C (Fig. 3) whereas with sclerotia conditioned at 20 °C the highest level was obtained at 10 and 5 °C, higher germination temperatures giving progressivily less germination.

The data presented in this paper suggest that two mechanisms are involved in germination of sclerotia devoid of constitutive dormancy: first a low temperature effect that brings the sclerotia into a condition of easy germination, and an additional effect of *Allium* extracts by which germination is induced (Coley-Smith, 1960) Low and high temperature conditioning was shown to exhibit a reversible effect (Fig. 6). This phenomenon might also occur in field conditions in which Coley-Smith and Parfitt (1986) observed a seasonal response of germination of sclerotia treated by diallyl disulphide (DADS). Our data suggest that part of this seasonal effect might be caused by high and low temperature conditioning of the sclerotia in soil.

All storage experiments were performed in non-sterilised soil and germination experiments were performed with non-sterilised sclerotia. Therefore it is likely that the phenomenon of temperature conditioning of sclerotia is also an important factor under field conditions.

Acknowledgements

The author wishes to thank Mr H. Fokkema for his interest and encouraging discussions, Dr E.A. Weststeyn (Phytopathological Laboratory Willie Commelin Scholten, Baarn) for valuable discussions and Prof. Dr J.R. Coley-Smith (University of Hull) for critically reading the manuscript.

Samenvatting

Het effect van verschillende temperaturen tijdens bewaring in grond op de kieming van sclerotiën van Sclerotium cepivorum

Sclerotiën van *Sclerotium cepivorum* Berk. werden onder niet steriele omstandigheden bewaard bij verschillende temperaturen. Het effect van de bewaring bij verschillende temperaturen op de kieming werd onderzocht. Het bleek dat sclerotiën, die 70 dagen bewaard werden in zakjes met zand in niet steriele grond bij een temperatuur van 5

°C of 10 °C, geconditioneerd werden tot een snelle, vrijwel volledige kieming. Onder invloed van vluchtige stoffen uit gesnipperde ui en knoflook kiemden meer dan 90% van deze sclerotiën bij een temperatuur van 15 °C. Sclerotiën die bij een hogere temperatuur bewaard werden, namelijk bij 15, 20 of 25 °C, kiemden na de bewaarperiode langzaam bij 15 °C. Ongeveer 50% van deze sclerotiën konden tot kieming gebracht worden onder invloed van ui- en knoflookextract. Sclerotiën die door een koude bewaarperiode geconditioneerd waren, namelijk bij 5 of bij 10 °C, kiemden ook zonder ui- en knoflookextract; 78% en 90% van deze sclerotiën kiemden bij temperaturen van respectievelijk 5 en 10 °C. De sclerotiën die een warme bewaarperiode ondergaan hadden, namelijk bij 15, 20 of 25 °C, kiemden niet zonder ui en knoflook. De optimum temperatuur voor de kieming was 15-20 °C voor sclerotiën die bij 5 °C bewaard waren. De optimum temperatuur voor de kieming van sclerotiën die bij 20 °C bewaard waren was lager, en wel ca. 10 °C.

De conditionering door een hoge of door een lage bewaartemperatuur bleek reversibel te zijn. Sclerotiën die eerst bij 5 °C bewaard werden en daarna bij 20 °C onderscheidden zich niet van sclerotiën die continu bij 20 °C bewaard werden. Sclerotiën die eerst bij 20 °C bewaard werden en vervolgens bij 5 °C, kiemden als sclerotiën die continu bij 5 °C bewaard waren.

References

Blikman, I., 1986. Allium white rot in Holland. Proceedings of the Third International Workshop on Allium White Rot, Wellesbourne U.K. Section I: 1-8.

Coley-Smith, J.R., 1959. Studies of the biology of *Sclerotium cepivorum* Berk. III. Host range; persistence and viability of sclerotia. Annals of Applied Biology 47: 511-518.

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., 1985. Methods for the production and use of sclerotia of *Sclerotium cepivorum* in field germination studies. Plant Pathology 34: 380-384.

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. & Kind, 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., 1986. Some effects of diallyl disulphide on sclerotia of *Sclerotium cepivorum*: Possible novel control method for white rot disease of onions. Pesticide Science 37: 587-594.

Coley-Smith, J.R. & Sansford, C.E., 1986. Survival of sclerotia of *Sclerotium cepivorum*. Proceedings of the Third International Workshop on Allium White Rot, Wellesbourne U.K. Section VI: 11-16.

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.

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.

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.

Mitchell, D.T. & Cooke, R.C., 1968. Some effects of temperature on germination and longevity of sclerotia of *Claviceps purpurea*. Transactions of the British Mycological Society 51: 721-729.

Tichelaar, G.M., 1965. Witrot in zaai-uien. Groenten en Fruit 16: 2107.