

**RECOVERY OF MICROSKLEROTIA OF *VERTICILLIUM DAHLIAE* FROM SOIL AS SUBJECTED TO VARIOUS TREATMENTS**

**PEMULIHAN MIKROSKLEROTIUM *VERTICILLIUM DAHLIAE* DARI TANAH SEBAGAI AKIBAT BERAGAM PERLAKUAN**

**Loekas Soesanto<sup>\*)</sup>**

*Department of Plant Pests and Diseases, Faculty of Agriculture  
University of Jendral Soedirman, Purwokerto*

**A.J. Termorshuizen**

*Department of Biological Farming System, University of Wageningen  
Wageningen, the Netherlands*

<sup>\*)</sup> *Corresponding author, E-mail: lukas\_262@yahoo.com*

**INTISARI**

Pengaruh beragam kondisi terhadap daya tahan *Mikrosklerotium Verticillium dahliae* Kleb. dikaji dengan menggunakan tiga sumber batang kentang dari lapangan yang tertutup dengan *Mikrosklerotium* yang kompak. *Mikrosklerotium* bertahan hidup lebih dari 2 tahun pada batang kentang yang tidak dipendam di dalam tanah. Pengaruh suhu, pF, termasuk keragaman suhu dan atau pF setiap minggu, dan beragam cara pemendaman jaringan batang kentang terhadap daya tahan *Mikrosklerotium* lebih dari 1 tahun di tanah berpasir yang tidak disterilkan, juga dikaji untuk *Mikrosklerotium* dari sumber yang berbeda. Sumber inokulum berpengaruh tidak nyata. Secara luar biasa, beberapa *Mikrosklerotium* terpulihkan sehari setelah dimulainya penelitian yang beragam antara 5,5 dan 31%. Pemulihan ada pada tingkat ini atau bahkan menurun untuk bulan lainnya, untuk beberapa perlakuan, juga setelah 3 dan 6 bulan. Hanya setelah 3 – 12 bulan, pemulihan meningkat mencapai 5 kali lebih tinggi daripada di awal penelitian, tetapi pemulihan tidak dapat melebihi jumlah *mikrosklerotium* awal yang dipendam di dalam tanah. Perubahan dalam pemulihan diduga karena keragaman tingkat mikostasis tanah yang dipengaruhi oleh laju eksudasi nutrisi dari *Mikrosklerotium*.

*Kata kunci: daya tahan, Mikrosklerotium, Verticillium dahliae*

**ABSTRACT**

The influence of various conditions on the survival of microsclerotia of *Verticillium dahliae* Kleb. was studied using three sources of field-collected potato stems densely covered with naturally-formed microsclerotia. Microsclerotia were found to survive for up to two years in potato stems not incorporated in soil. The effects of temperature, pF, including weekly variations in temperature and or pF, and various modes of incorporating potato stem tissue on the survival of microsclerotia for up to 1 year in a sandy unsterilised soil was also studied for different microsclerotia sources. Inoculum source had no significant effect. Remarkably few microsclerotia were recovered one day after the start of experiments varying between 5.5 and 31%. Recovery remained at this level or even decreased for another month and for several treatments, also after 3 and 6 months. Only after 3 to 12 months, recovery increased to values up to 5 times higher than that of one day after the start of the experiment, but recoveries did not exceed the number of microsclerotia initially incorporated into the soil. Changes in recovery may be due to variation in the level of soil mycostasis which is affected by the rate of nutrient exudation from microsclerotia.

*Key words: survival, microsclerotia, Verticillium dahliae*



## INTRODUCTION

*Verticillium dahliae* Kleb. is the causal agent of wilt disease in many crops, including potato, cotton, olive, and strawberry (Schnathorst, 1981). In the absence of host plants the fungus can survive in soil for many years as microsclerotia (Wilhelm, 1955), which are formed predominantly in the senescent host tissue. Crop rotation using non-hosts is an important management strategy to reduce yields losses (Bollen *et al.*, 1989).

Data on the long-term persistence of microsclerotia of *V. dahliae* in field soils are scarce. Wilhelm (1955) reported that *V. dahliae* may survive in field soils for at least 14 years. Long-term observations on the persistence of *V. dahliae* in the field are, however, confounded by production of new microsclerotia on non-hosts (Malik and Milton, 1980). Probably only a minor fraction of microsclerotia remains viable during several years since yield losses in susceptible potato cultivars are nullified in a crop rotation with 3 years of non-hosts (Bollen *et al.*, 1989). Slattery (1981) studying the survival of microsclerotia of *V. dahliae* in potato stems buried in field soil during winter for 7 months found survival rates as low as 23%. In other studies on the survival of microsclerotia, total mortality was found after 5 years (Green, 1980). Green (1980) concluded that temperature and soil moisture had less influence than soil type on survival of microsclerotia. Hawke and Lazarovits (1994) found that survival of *in vitro* produced microsclerotia less than 53 – 75  $\mu\text{m}$  diam. stored without soil declined quickly after 16 weeks incubation, while those measuring 75 – 106  $\mu\text{m}$  diam. survived fully for 36 weeks. Mortality of these larger microsclerotia buried in soil depended on soil type and varied between 0 and 24% after 4 weeks of exposure.

In this paper we report on the recovery of microsclerotia of *V. dahliae* as a

function of temperature, moisture, and the incorporation of organic debris. Our hypothesis was that fluctuating temperature and/or pF would cause an initially rapid decrease in survival.

## MATERIALS AND METHODS

*Incubation of microsclerotia without soil: effect of temperature and relative humidity (Experiment 1).* Inoculum of *V. dahliae* consisted of naturally formed microsclerotia on dead potato stems collected from three potato fields in the Province of Drenthe, The Netherlands, in October 1996. The three sources of inocula were designated as V1, V3 and V4. After collection, the stems were cut into 10-cm-long pieces, allowed to air-dry for about 10 days under ambient conditions at room temperature, and subsequently stored in plastic bags until further use. For each inoculum source, 30 pieces of dried 10-cm-long potato stems covered with microsclerotia were placed in a plastic box (l/w/h, 25/10/9 cm) on a plastic screen table (height 6 cm) and loosely covered with a lid. Stem pieces were incubated at relative air humidity of 100 or 70%. To establish a relative air humidity of 100%, a 3-cm-layer of water was added into the plastic box. A saturated NaCl solution was added to maintain a relative air humidity of 70% (Winston and Bates, 1960). Boxes were incubated in a climate chamber at 5 or 20 °C for 0, 3, 6, 12, or 24 months. Each temperature  $\times$  relative humidity combination was replicated three times.

At each sampling occasion, three potato stems were taken randomly from each box, ground using a mortar and pestle, and sieved using meshes of 106 and 20  $\mu\text{m}$ . Material remaining on the 20  $\mu\text{m}$  sieve was placed on filter paper under a dissecting microscope and 75 microsclerotia were picked and plated onto Modified Soil Extract Agar (MSEA, Harris *et al.*, 1993)



amended with 50 ppm oxytetracyclin using an insect pin 000 (Emil Arlt, Australia). Each plate contained 25 microsclerotia. Plates were placed upside down and incubated in the dark at 23 °C for three weeks. The numbers of colonies of *V. dahliae* were subsequently counted. The germination percentages of the three inoculum sources plated three weeks prior to the start of the experiment were 67, 67, and 89% for V1, V3, and V4, respectively.

**Incubation of microsclerotia in soil (Experiment 2 – 4).** Inoculum of *V. dahliae* in dead potato stems collected from three different potato fields in the Province of Drenthe, The Netherlands, in October 1997 and were designated K1, K2, and K3. After collection, the stems were cut into 10-cm-long pieces, allowed to air-dry for about 10 days at room temperature, and subsequently stored in plastic bags until further use. Germinability determined 3 – 5 weeks prior to the start of the experiment was 94 – 95%.

A sandy agricultural soil from Meterik (Province of Limburg, The Netherlands) was checked for presence of microsclerotia of *V. dahliae* largely following the method described by Harris *et al.* (1993). Twelve and a half grams of soil was air-dried for 14 days, and sieved over 106 and 20- $\mu$ m screens. The material remaining on the 20- $\mu$ m sieve was suspended in 50mL 0.08% water agar and 10 0.8 mL-subsamples from the soil suspension were plated onto 10 Petri dishes containing MSEA. Plates were incubated in the dark at 23 °C for three weeks and subsequently the number of colonies of *V. dahliae* was counted.

For experiments 2 and 3, microsclerotial suspensions were prepared by grinding dried inoculum in water using mortar and pestle, sieving over 106 and 20- $\mu$ m screens, and suspending the material remaining on the lower screen in a small, measured amount of water. The numbers of microsclerotia  $\text{ml}^{-1}$  suspension were

directly counted in small subsamples under the dissecting microscope. The suspension was diluted to achieve numbers of microsclerotia in an amount of water that would lead, after thoroughly hand-mixing with the soil, to 100 microsclerotia  $\text{g}^{-1}$  air-dry soil and a pF of 2.0 or 2.8. Small plastic pots (h/diam., 5/3 cm) were filled with 75 g of the infested soil, and incubated under different conditions as described below. The pots had lids with a small opening to allow for oxygen exchange. The pots were kept at constant weight by hand watering. Samples were taken after 0, 1, 3, 6, or 12 months. The first sample ( $t = 0$ ) was taken 1 day after incorporation of the microsclerotia into the soil. At each sampling occasion, the whole 75 g of soil was used, so for every sample different pots were used. The density of microsclerotia in soil was determined as described above (Harris *et al.*, 1993). All experiments were carried out in a completely randomized block design with 3 blocks.

**Effect of temperature (Experiment 2).** Small plastic pots were filled with soil containing 100 microsclerotia  $\text{g}^{-1}$  air-dry soil of inoculum sources K1, K2, or K3 at a pF of 2.0. Treatments included constant temperature (-28, 5, or 25 °C), or weekly varying temperature (-28/+5 °C; or -28/+25 °C).

**Effect of temperature and moisture content (Experiment 3).** Small plastic pots were filled with soil containing 100 microsclerotia  $\text{g}^{-1}$  air-dry soil of inoculum K3 at pF of 2.8. Additional water was added to part of the pots to reach a pF of 2.0. Treatments included temperature (constant 5, 15, or 25 °C, or weekly alternating 5/25 °C) and soil moisture (constant pF 2.0 or 2.8, or monthly alternating pF 2.0/2.8) regimes in all possible combinations, giving a total of 12 different treatments. The pots treated with



alternating 5/25 °C and/or pF 2.0/2.8 started at 5 °C and pF of 2.8.

**Effects of adding plant tissue (Experiment 4).** The three *V. dahliae* inocula K1, K2, and K3 were incorporated in four ways: (1) 3 g of 1-cm-long pieces of potato stems containing microsclerotia were buried in the soil, (2) the material as in (1) was ground using mortar and pestle, (3) as (2) but the ground material was sieved over 106 and 20- $\mu$ m screens, and the material remaining on the upper sieve was autoclaved (121 °C for 20 min) before incorporation in the soil, and (4) as (2) but without incorporation of the potato tissue remaining on the 106  $\mu$ m sieve in soil. Stem pieces in treatment (1) were incorporated after soil had been moistened to pF 2.0 by careful hand-mixing. Ground materials (treatments 2 – 4) were incorporated into soil by suspending in an amount of water that after thorough hand-mixing resulted in a pF of 2.0. The density of microsclerotia in soil was determined as described above (Harris *et al.*, 1993). The remaining stem segments were ground in a mortar, sieved on meshes of 106 and 20  $\mu$ m, suspended in 50 mL 0.08% water agar and 10 0.8 mL-subsamples from the suspension were plated onto 10 Petri dishes containing MSEA. Plates were incubated in the dark at 23 °C for three weeks and subsequently the number of colonies of *V. dahliae* was counted and merged with those from soil in the same treatment.

## RESULTS AND DISCUSSION

The effects of temperature and relative humidity on the germination percentage of microsclerotia from potato stems placed on a plastic screen (experiment 1) did not differ significantly for the three inoculum sources. Therefore, the data for the three sources were merged. The germination percentage of microsclerotia gradually

decreased for all treatments with exception of incubation at 5 °C and 70% relative humidity (Figure 1), up to 6 months incubation time; then it increased ending at levels equal to those at the onset of the experiment. No microsclerotia were observed only for the 25 °C, 70% relative humidity treatment. The variation in recovery corresponds with results obtained by Wheeler and Rowe (1995), who reported strongly varying recoveries, mostly between 0 and 100%, depending on soil type and drying time of the soil samples, but the authors could not discover a certain pattern in the effect of drying on recovery. The variation in densities of microsclerotia found here may be due to real fluctuations in density by death or other events affecting the germination of microsclerotia on the agar medium. Germination and the subsequent formation of new microsclerotia are prerequisites for the recovery of a microsclerotium with the method used.

The decrease in recovery of microsclerotia incubated in the absence of soil (experiment 1) may be related to the same phenomenon of mycostasis. The prevailing conditions of high humidity may have favored the growth of microorganisms resident in the potato stems. If the mycostasis hypothesis is correct, low recoveries would correspond to high numbers of micro-organisms on the surface of microsclerotia, caused by a relatively high leakage of nutrients by the microsclerotia. This would agree with the observation that exudates from sclerotia of *Sclerotium rolfsii* decrease exponentially during 26 days in the soil (Hyakumachi and Lockwood, 1989). At sampling occasions later than 3 – 6 months, leakage is likely declined to low levels, and populations of micro-organisms on the surface of microsclerotia may decrease. In natural soils, microsclerotia germinate only, or mainly, near roots (Ben-Yephet and Pinkas, 1977; Huisman and Gerik, 1989), where



carbon supply is not limiting (Lynch and Whipps, 1990). The proportion of germinating microsclerotia in the rhizosphere may then depend on the amount of root exudation and competition with the rhizosphere microflora, including the mycostasis-inducing micro-organisms resident on the surface of microsclerotia.

The three inoculum sources also gave similar results in response to incubation in soil at varying temperatures (experiment 2), and these data were combined. To illustrate this, at the first sampling occasion one day after start of the experiment, density of microsclerotia recovered from the soil was 14, 14, and 12% relative to the 100 germinable microsclerotia added to the soil for inocula K1, K2, and K3, respectively.

After one month, densities recovered were 7.3 – 12% (Figure 2). However, later harvests consistently yielded higher recoveries, varying between 1.2 and 5.9 times more than the level observed at the start of the experiment. After 12 months of incubation, no significant differences were found between treatments. The maximum recovery of microsclerotia found was  $81 \pm 14\%$  of the added microsclerotia for inoculum K1 after 6 months of incubation at 25 °C. *V. dahliae* possesses no or only very limited competitive saprotrophic ability (Wilhelm, 1951), making the formation of new microsclerotia in unsterile soil in the absence of plant roots highly unlikely.

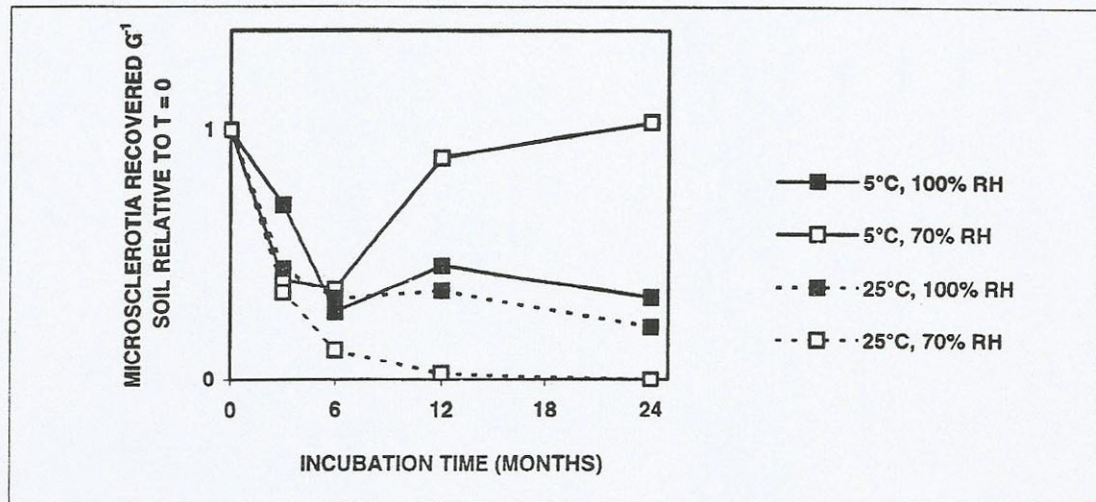


Figure 1. Effect of temperature, relative humidity, and time on the recovery of microsclerotia of *V. dahliae* in field-collected 10-cm-long, dead potato stems incubated on plastic screens. The recovery is expressed as the proportion of germinating microsclerotia of *V. dahliae* relative to the germination at  $t = 0$ . Germination percentage was determined by plating 75 single microsclerotia to Modified Soil Extract Agar plates. The experiment included three different sources of inoculum. Since no differences were observed among inocula, data were merged. Error bars indicate standard deviations of the means for the three inocula. At  $t = 0$ , average germination percentage was 74%.



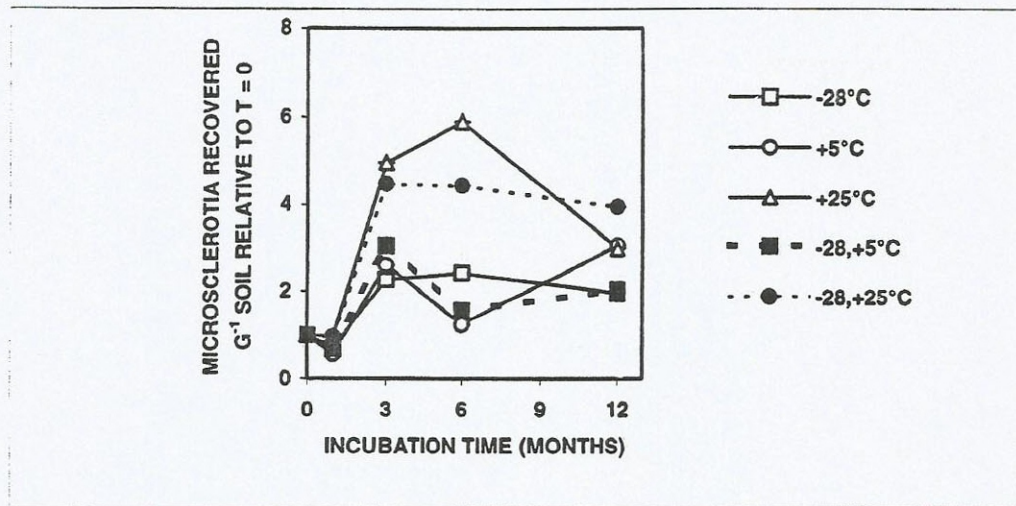


Figure 2. Effect of constant temperatures (-28, +5, or +25 °C) or weekly changing microclimates (-28/+5 °C or -28/+25 °C) on the recovery of microsclerotia of *V. dahliae* in an unsterilized sandy soil. The number of dry soil relative to the number at  $t = 0$  is shown. At  $t = 0$ , 100 germinable microsclerotia  $g^{-1}$  air-dry soil were incorporated. Prior to incubation, germination percentage as determined by plating single microsclerotia was 94 – 95%. The experiment included three different inoculum sources. Since no differences were observed among inocula, data were merged. Average recovery of microsclerotia one day after incorporation was 13% (see text). Error bars indicate standard deviations of the means of the three inocula.

Formation of new microsclerotia in soil in the absence of roots has been reported only after amendment of glucose to the soil (Menzies and Griebel, 1967; Emmatty and Green, 1969; Green and Papavizas, 1968). In our experiments no nutrients were added nor was the soil sterilized. In addition, formation of new microsclerotia in soils exposed continuously to -28 °C (experiment 2) cannot have occurred and therefore cannot explain the apparent two-fold increase of microsclerotia after 3, 6, and 12 months. If the measurements of densities of microsclerotia in soil by Wheeler and Rowe (1995) reflected the amount of viable microsclerotia, it would have been impossible to measure 0% microsclerotia recovered at one sampling occasion and 100% at a subsequent date. Thus, we

conclude it was unlikely that formation of new microsclerotia played a major role.

In experiment 3, the density of microsclerotia recovered directly after the start varied between 5.5 and 31%. The density of microsclerotia recovered from soil varied markedly in time and for the different treatments (Figure 3). For all treatments the density recovered decreased after 1 month. For some treatments the recovery then increased, whereas for others it further decreased. The maximum and minimum densities recorded were 44 and 1.1%, respectively. Even after initially low observed densities, considerable increases were recorded at subsequent sampling occasions. For example, percentages of microsclerotia recovered at incubation of pF 2.0 and 5 °C were found to be  $2.9 \pm 1.7\%$  after 3 months, and after 6 and 12



months, they were  $21 \pm 11$  and  $32 \pm 9.4\%$  respectively. No obvious pattern was found. Only in the drier soil (pF 2.8) densities sometimes exceeded those originally introduced. The problems with quantifying *V. dahliae* population densities in soil are well-known (Wheeler and Rowe, 1995; Termorshuizen *et al.*, 1998). Recovery is generally 30 – 40% at best, depending on soil characteristics; it may vary considerably between soil sample replicates and complete failure of an assay may even occur (Termorshuizen *et al.*, 1998). Thus, low recovery rates could have been due to limitations in the detection procedure. We therefore conclude that the consistently observed low recoveries in the first two or three sampling occasions were not affected by random 'noise' of the detection procedure.

In experiment 4, when whole stems were incubated, densities recovered after 1 day incubation were  $3.7 \times 10^3$ ,  $4.7 \times 10^3$ , and  $4.2 \times 10^3$  microsclerotia  $g^{-1}$  soil for inoculum sources K1, K2, and K3 respectively. With the other treatments some losses may have occurred during handling of the potato stems and consequently densities recovered after 1 day incubation were lower: on average  $1.5 \times 10^3$ ,  $1.2 \times 10^3$ , and  $2.7 \times 10^3$  microsclerotia  $g^{-1}$  soil for inoculum sources K1, K2, and K3 respectively. Inoculum sources K1 - K3 also responded similarly to the different treatments and the data were combined. The treatment with intact pieces of potato stems incorporated into the soil resulted in a sharp decrease in recovery after 6 months; after 12 months the fungus could not be recovered as all (Figure 4). By contrast, density of microsclerotia recovered from soils where potato stem

tissue was excluded remained high and increased to values higher than those found at the start of the experiment ( $P = 0.10$ ).

The observed variation in density of microsclerotia in time may also be caused by variation in germinability of viable microsclerotia. In other words, microsclerotia may acquire a reversible non-constitutive dormancy, which is released in time. This relates to the phenomenon of mycostasis, the inhibition of germination of fungal propagules in natural soils (Lockwood, 1977). Evidence exists that deficiency of nutrients needed for germination is a significant factor involved in this phenomenon (Lockwood, 1977). Thus, it may be postulated that microorganisms resident on the surface of spores/microsclerotia consume exudates from the propagules of which a certain threshold concentration is necessary for their germination (Hsu and Lockwood, 1973; Bristow and Lockwood, 1975). In line with this hypothesis is the observation of Toyota and Kimura (1993), who isolated bacteria from the surface of chlamydospores of *Fusarium oxysporum* f.sp. *raphani* that inhibited germination but were not antagonistic to fungal growth *in vitro*. This phenomenon may occur on agar plates as well since the MSEA-medium used to plate soil samples is semi-selective only and antagonism may hinder the germination of microsclerotia. Bacteria residents on the surface of microsclerotia do not need to be affected by the antibiotic oxytetracyclin in the agar media, because the microsclerotia are spread on, and not into the medium. Thus, as treatments and incubation time may affect the presence of the saprotrophic microorganisms on the surface of microsclerotia, the ability of microsclerotia to germinate may vary accordingly.



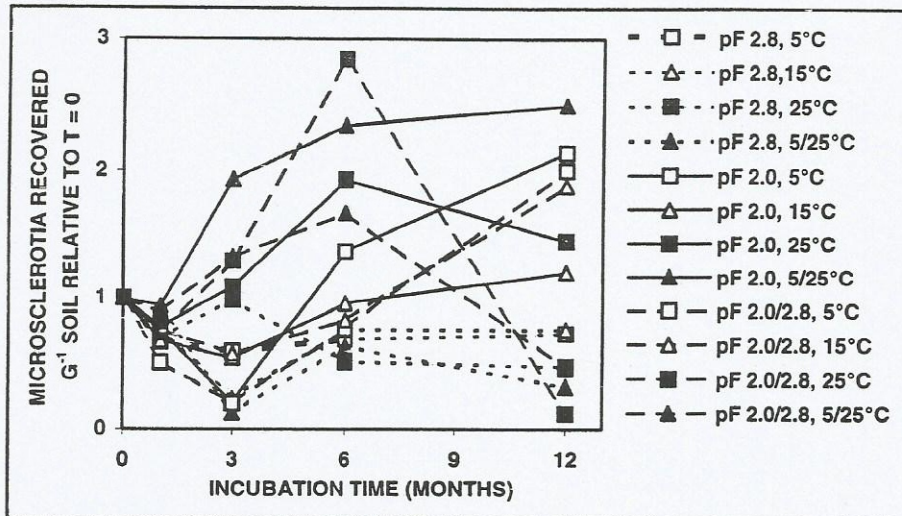


Figure 3. Effect of constant or weekly changing temperature (5, 15, 25, or 5/25 °C) and pH (2.0, 2.8, or 2.0/2.8) on the recovery of microsclerotia of *V. dahliae* in an unsterilised sandy soil. The number of microsclerotia  $g^{-1}$  air-dry soil relative to the number at  $t=0$  is shown. At  $t=0$ , 100 microsclerotia  $g^{-1}$  air-dry soil were incorporated. Prior to incubation, germination percentage as determined by plating single microsclerotia was 94 – 95%. The experiment included only one source of inoculum. Average recovery of microsclerotia one day after incorporation in soil was 7.7 – 22% (see text).

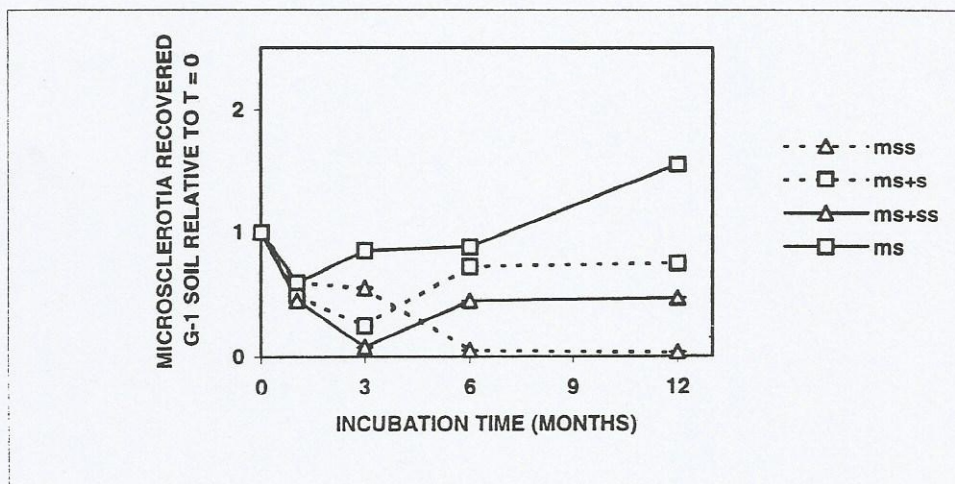


Figure 4. Effect of incorporation of plant material on the survival of microsclerotia of *V. dahliae* in an unsterilised sandy field soil. The number of microsclerotia recovered  $g^{-1}$  air-dry soil relative to the number at  $t=0$  is shown. As inoculum, field-collected potato stems (FPS) containing microsclerotia was used. Treatments: mss = incorporation of 3 g of 1-cm-long FPS containing microsclerotia; ms+s = separation of microsclerotia from 3 g of FPS by grinding and sieving, followed by incorporation of both microsclerotia and FPS into the soil; ms+ss = separation of microsclerotia of 3 g of FPS by grinding and sieving, sterilisation of the ground FPS, followed by incorporation of both microsclerotia and the sterile FPS; ms = separation of microsclerotia of 3 g of FPS by grinding and sieving, followed by incorporation of the microsclerotia alone. Data for three different sources of inoculum were merged. Error bars indicate standard deviations of the means of the three inocula.



An indication that agar plating conditions are important is obtained from the observation that methods involving the plating of air-dry soil generally perform better than methods involving the plating of soil suspensions (Termorshuizen *et al.*, 1998). Under dry plating conditions the activity of microorganisms resident at the surface of microsclerotia is likely to be considerably lower than under wet conditions. The difference in germination percentage of the starting material collected in a commercial potato field (varying between 67 and 89%), and that of the material incubated for 3 and 6 months may be caused by the fact that the starting material was air-dry when the microsclerotia were plated, whereas the microsclerotia that were plated later came directly from damp potato stems.

Very few microsclerotia were recovered one day after incorporation of the material into soil, varying between 5.5 – 31% for experiments 2 and 3. After 1 – 6 months, the recovery of microsclerotia decreased even further. Decreased recovery was also observed after 3 and 6 months for experiment 1 and after 1 and 3 months for experiment 4. This decrease was followed by an increase for most treatments to levels that were always lower than the number of microsclerotia incorporated into soil. This study was designed to evaluate the effect of temperature, moisture, and organic matter on the survival curve of microsclerotia of *V. dahliae*. Since formation of new microsclerotia in unsterile soil in the absence of plants is reportedly rare, we expected to observe only decreases in densities of microsclerotia in time. However, in all experiments, the numbers of microsclerotia recovered were in many cases equal to, or considerably higher than, previous harvests. Evans *et al.* (1966) and Green (1980) observed an initial increase of inoculum density after incorporation of field-collected microsclerotia and attributed this to the dissociation of microsclerotia

that were initially clumped together in host tissue. Clumping of microsclerotia could not play a role in our experiments since in experiments 1 and 4 the material was ground before plating. In experiments 2 and 3 the inoculum consisted of material that had been ground and passed through a 106- $\mu\text{m}$  sieve. In addition, the inoculum was checked under the dissecting microscope prior to incorporation into soil for presence of clumps of microsclerotia.

Based on our results, little can be said about factors affecting the survival of microsclerotia of *V. dahliae*, since we were unable to distinguish between dead and non-germinable but viable microsclerotia. However, it is clear that nearly all treatments resulted in the survival for at least 12 or 24 months (experiment 1) of some fractions of the *V. dahliae* population. Thus we may conclude that a great portion of microsclerotia is not sensitive to temperatures between -28 and 25 °C and pF 2.0 – 2.8, and fluctuations of these values in time. These observations are largely in line with those of Green (1980), who concluded that temperature (4 and 28 °C) and soil moisture (-1/3 and -15 bars) were not the main limiting factors in survival of *V. dahliae* over two years or more. In experiment 1, decrease of recovery continued throughout the experiment for the 25 °C treatments, which may indicate a true decrease in survival. Incubation at 25 TMR 11 °C and 75% relative humidity was the only treatment that resulted in no recovery at the final sampling occasion after 24 months. This may have been due to desiccation of the microsclerotia. Pataky and Beute (1983) reported low survival rates of microsclerotia of *Cylindrocladium crotalariae*, the causal agent of black rot of peanut, when incubated on the soil surface compared to microsclerotia incorporated into the soil, and they attributed this to low soil matric potentials. More detailed studies on the recovery of microsclerotia of *V. dahliae* are necessary. Bioassays need to be



performed with a short exposure time to determine whether the germinability of microsclerotia is affected in soil. For example, the infection density of *V. dahliae* on the roots of *Arabidopsis thaliana* could be determined (Soesanto and Termorshuizen, unpublished). Also, the study of the population of microorganisms colonizing the surface of microsclerotia deserves more attention. Perhaps these organisms can be exploited to suppress the germination of microsclerotia in the rhizosphere of hosts.

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