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Effect of conditions during storage of infested soil on infection of bait plants by Polymyxa betae and beet necrotic yellow vein virus

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Abstract

Infectivity of resting spores of *Polymyxa betae* in soil stored air-dry or moist was determined by assessing infection of bait plants that were exposed to the soil. Storage of soil under air-dry conditions at room temperature resulted in a delayed onset of germination of resting spores compared to germination in soil stored under moist and cool conditions, as inferred from the infection of the bait plants. Bait plants had to be exposed for more than 12 h to flooded infested soil before germination and infection had occurred. However, when soil was prewetted for 24 h before exposing bait plants, germination, infection and transmission of beet necrotic yellow vein virus (BNYVV) were accomplished within 12 h, but only with the moistly stored soil. When resting spores isolated from roots were stored for 4 and 8 weeks under dry conditions at 22 °C, germination of viruliferous spores, as measured by detection of BNYVV in bait plants exposed for 48 h to the spores, was less than that of spores stored in moist soil at 22 °C. Approximately 100% of bait plants were infected after exposure to resting spores that were frozen in demineralized water or stored cool (5 °C) in water or moist soil for 42 weeks. Air-dry cool storage for 42 weeks resulted in a low percentage of infection. Storage conditions of soil influence the results of bioassays for detection of rhizomania when short baiting periods are applied, whereas differences in infectivity were not detected using a bioassay with long duration.

Additional keywords: BNYVV, sugar beet, bioassay, germination, dormancy, resting spores.

Introduction

Polymyxa betae Keskin (Plasmodiophoromycetes) is the soil-borne fungal vector of beet necrotic yellow vein virus (BNYVV), the causal agent of rhizomania disease of sugar beet. Resting spores of this fungus can survive in moist or dry soil for at least 15 years and still transmit the virus (Abe and Tamada, 1986). Resting spores germinate in the presence of host roots, releasing primary zoospores that infect epidermal root cells (Keskin, 1964). Storage conditions of soil can affect germination of fungal resting spores (Sussman, 1976). At two institutes participating in a co-operative trial (Tuitert and Hofmeester, 1992), soil samples were stored under different conditions, air-dry at 20 °C and moist at 5 °C, prior to assessment of BNYVV and *P. betae* by bioassay. Consequently it was considered important to determine what effect these storage conditions had on the resting spores of *P. betae*. The effect on quantitative assessment of *P. betae* and BNYVV using a 6-week bioassay was reported previously (Tuitert, 1990). In this paper, effects of different storage conditions of soil or of isolated resting spores on the onset and rate of germination of the resting spores of *P. betae*, as inferred from infection of bait plants, were investigated.

Materials and methods

Effect of storage conditions of naturally infested soil on infectivity of resting spores of P. betae

BNYVV-infested soil (sandy clay, pH-KCl 7.5, organic matter 5%) was collected in spring from a field in Tholen, the Netherlands, where sugar beet had been grown in the three preceding years. The moist soil was sieved (5 mm), mixed and stored under different conditions:

MC = moist (pF 2.2–2.5, gravimetric water content c. 16%) and cool (5 °C);

DW = air-dry at room temperature (c. 20 °C).

The effect of these storage conditions on germination of resting spores in the soil was determined indirectly by assessing infection of bait plants exposed to the soil. The transmission of BNYVV by zoospores released from the resting spores was determined by ELISA of the exposed bait plants. The detection procedure was adapted from bioassay methods described by Westerlund et al. (1978) for *Olpidium brassicae* and Beemster and De Heij (1987) for *P. betae*. Samples of 5 g of dry soil or an amount of moist soil corresponding to 5 g of dry soil were placed in Petri dishes (diameter 14.5 cm), mixed with 45 g of sterilized coarse sand and wetted by adding 60 ml of demineralized water. Sixteen 2–3 week-old sugar beet seedlings cv. Regina, were used as bait plants in the Petri dishes with their roots spread out over the soil surface. The dishes were partly covered with an opaque lid to reduce evaporation and to keep the soil slurry in darkness. Water was supplied when necessary to compensate for evaporation. The dishes were placed in a growth chamber at a constant temperature of 22 °C with 16 h light. Bait plants were removed from the soil after 12, 24, 48 or 72 h of exposure. Their roots were carefully washed in running tap water and 12 of the 16 bait plants were planted separately into individual pots containing 200 ml of sterile coarse sand. The plants were placed, randomized, in the greenhouse with day (16 h) and night temperatures of c. 23 and 15 °C, respectively. After 6 weeks, the plants were checked for the presence of *P. betae* by light microscopy and of BNYVV by ELISA (Tuitert, 1990). Controls consisted of Petri dishes containing 50 g of sterilized coarse sand, on which bait plants were exposed for periods identical to the infested series. Experiments 1 to 4 were performed consecutively after storage periods of the infested soil of 12, 24, 30 and 40 months, respectively. Modifications to this general procedure for each Experiment (1–4) are given separately.

Expt 1. A comparison of two inoculum densities. In a preliminary experiment two amounts of infested soil were tested, either 5 g or 0.5 g, both made up to 50 g with sterile sand. The exposure period of bait plants in the Petri dishes was 48 h, with alternating temperatures of 22 and 15 °C (16/8 h, respectively). Treatments *MC*, *DW* and one extra treatment (*MCa*), where moist soil was air-dried for 1 day prior to baiting, were tested. One Petri dish per treatment was used for exposing bait plants to the soil-borne inoculum.

Expt 2. A comparison of exposure periods and the effect of two drying treatments. Three different periods of exposure of bait plants to infested soil (5 g) were compared. Bait plants were incubated in the Petri dishes for 24, 48 or 72 h. Beside the treatments *MC* and *DW*, two extra treatments, *MCa* and *MCb*, were included. For treatments *MCa* and *MCb*, moist soil was air-dried for 1 and 7 days, respectively, prior to baiting. One Petri dish per treatment was used for exposing bait plants to the soil-borne inoculum.

Expt 3. A comparison of three exposure periods. Exposure periods of 12, 24 and 48 h were compared for treatments *MC* and *DW*. All treatments were performed in duplicate:

two Petri dishes per treatment were used for exposure of bait plants.

Expt 4. A comparison of four exposure periods and the effect of prewetting. Expt 3 was repeated with the addition of a 72-hour exposure period. Also, a prewetting treatment was included where 60 ml water was added to the soil 24 h before bait plants were applied (*MC'* and *DW'*). The latter treatment was only investigated with a 12-hour exposure period of bait plants to the soil. Every treatment was performed in duplicate, as described for Expt 3.

Effect of storage conditions on infectivity of resting spores isolated from roots
A suspension of resting spore clusters was prepared from lateral roots of 5-month-old sugar beet plants, cv. Regina, which had been grown in a mixture of 15% (v/v) BNYVV-infested soil with coarse sand. The roots were cut up, macerated for 2 min in a Waring blender and ground with a pestle and mortar. After addition of water, the suspension was sieved through Monodur gauze (50 μm) by vacuum filtration to remove large root debris. This root debris was once more ground in the mortar, resuspended and filtered. In order to increase the density of resting spores, the filtrate was centrifuged (at approximately 2000 *g* for 3 min) and the pellet was resuspended in demineralized water. Five ml of the suspension, containing approximately 10^6 resting spore clusters, was pipetted gradually onto cellulose-nitrate microfilters (Sartorius, pore size 3 μm and 5 cm filter diameter), while the water was removed by vacuum filtration. Zoosporangia were not observed in the filtrate. Filters with spores were left to air-dry. A thin layer of Glisseal laboratory grease was applied along the margins of the filters and a spore-free filter was stuck on top, to create resting spore 'sandwiches'. For the non-infested controls, water was applied to the filters in stead of the suspension. The 'sandwiches' were stored under the following conditions:

1. in a mixture (50/50) of non-infested moist field soil and coarse sterile sand (pH-KCl 7.7, gravimetric water content 13%);
2. in a similar mixture, but with autoclaved field soil;
3. in demineralized water;
4. dry in the air.

All samples were stored in the dark, with alternating temperatures of 22 and 15 °C (16/8 h, respectively), except for two sandwiches which were tested immediately after preparation.

After 4 and 8 weeks storage, two sandwiches per treatment were taken for assessment of germination of viruliferous resting spores by indirect bioassay. The sticky margin of the sandwiches was cut off and both filters of each sandwich were placed on the bottom of a Petri dish (diameter 8.5 cm) with 25 ml of diluted Steiner nutrient solution (10%). Twenty 2–3 week-old sugar beet seedlings cv. Regina were placed in each dish, with their roots covering the filters. The dishes were partly covered and placed for 48 h in a growth chamber with alternating temperatures of 22 and 15 °C (16/8 h, respectively). Plants were removed from the dishes, their roots were washed with running tap water and they were individually planted in pots containing 200 ml of sterile sand. These pots were placed under greenhouse conditions as described before. After 6 weeks, roots of the bait plants were tested for the presence of BNYVV. BNYVV was the indicator of infection by viruliferous *P. betae*.

To investigate the effects of a long storage period, two filters per treatment were assayed after storage at 5 °C for 42 weeks. In addition, two filters from treatment 3 were kept frozen at –18 °C for 42 weeks.

Statistical analyses

The frequency tables of infected plants from the different treatments and exposure periods were analyzed by generalized linear modelling with a logistic link function and a binomial error distribution, using GENSTAT 5 (Payne et al., 1988). The deviances of factors were compared with χ^2-values to determine their significances. In Expt 1, the effect of increasing the period of drying the soil before the assay on the number of plants infected by *P. betae* or BNYVV was tested for each of the two amounts of soil separately. In Experiments 3 and 4, the main effects analyzed were storage conditions (two) and exposure period (three and four, for Expt 3 and 4, respectively). For the 12-hour exposure period in Expt 4, the effect of prewetting the soil on numbers of infected plants was tested separately.

Results

Experiments with naturally infested soil. Expt 1 showed that 5 g was an adequate amount of soil to use in this type of experiment (Table 1). Exposure to dry soil, air-dried for 1 day (*MCa*) or stored dry for 12 months (*DW*), yielded lower numbers of infected plants (*P. betae* and BNYVV) than exposure to the moist (*MC*) soil. In Expt 2, with an additional air-drying treatment, a decreasing number of infected plants with increasing time of drying of soil prior to exposure was found, but only for the 24-hour exposure period (Table 2). At longer periods of exposure, the number of bait plants was not adequate to detect differences in infection between treatments.

In Experiments 3 and 4, significantly higher numbers of bait plants were infected when exposed to *MC* than to air-dried (*DW*) soil. The numbers of *P. betae*- and BNYVV-infected plants increased significantly with increasing exposure period (Tables 3 and 4). Exposure of roots for 12 h to either *MC* or *DW* soil was too short to detect any infection of bait plants.

Not only was the time of onset of germination of spores delayed when infested soil had been stored dry and warm, also the rate of increase of germination (inferred from the rate of progress of infection) appeared to be slowed down. For *MC* soil, a 79% increase of

Table 1. Effect of storage conditions of infested soil on germination of resting spores of *Polymyxa betae* and transmission of BNYVV, as measured by the infection of bait plants exposed for 48 h to two quantities of infested soil (Expt 1).

Storage conditions [a]		Numbers of infected bait plants ($N = 12$)			
		P. betae		BNYVV	
		5 g soil	0.5 g soil	5 g soil	0.5 g soil
MC	Moist, 5 °C	12	6	6	2
MCa	Moist, 5 °C air-dried 1 day	6	4	1	0
DW	Air-dry, 20 °C	0	1	0	0
Significance [b]		$P < 0.001$	$P < 0.10$	$P < 0.001$	$P < 0.10$

[a] Duration of storage of the soil was 12 months.

[b] Significance of the decrease in numbers of infected plants from treatment *MC* to *MCa* to *DW*, per quantity of soil.

Table 2. Effect of storage conditions and air-drying treatments of infested soil on germination of resting spores of *Polymyxa betae* and transmission of BNYVV, as measured by the infection of bait plants exposed for 24, 48 or 72 h to the wetted infested soil (Expt 2).

Storage conditions [a]		Numbers of infected bait plants ($N = 12$)					
		P. betae			BNYVV		
		24 h	48 h	72 h	24 h	48 h	72 h
MC	Moist, 5 °C	12	12	12	6	12	11
MCa	Moist, 5 °C air-dried 1 day	5	11	12	4	11	11
MCb	Moist, 5 °C air-dried 7 days	2	12	12	1	12	12
DW	Air-dry, 20 °C	0	12	12	0	9	12
Significance [b]		$P < 0.001$	n.s.	n.s.	$P < 0.01$	n.s.	n.s.

[a] Duration of storage of the soil was 24 months.
[b] Significance of the decrease in numbers of infected bait plants with increasing time of drying of soil prior to baiting (from *MC* to *DW*).
n.s. = not significant.

Table 3. Effect of moist/cool and dry/warm storage of infested soil on germination of resting spores of *Polymyxa betae* and transmission of BNYVV, as measured by the infection of bait plants exposed for 12, 24 or 48 h to the wetted infested soil (Expt 3).

Storage conditions[a]		Mean numbers of infected plants ($N = 2 \times 12$) [b]					
		P. betae			BNYVV		
		12 h	24 h	48 h	12 h	24 h	48 h
MC	Moist, 5 oC	0	11	12	0	8.5	10.5
DW	Air-dry, 20 oC	0	0	8.5	0	0	3

[a] Duration of storage of the soil was 30 months.
[b] Each treatment consisted of an assessment of two lots of 12 plants. The main effects, storage condition and exposure period, were analyzed separately for *P. betae* and BNYVV. The two storage conditions resulted in different numbers of infected plants, $P < 0.001$. Numbers of infected plants increased with increasing period of exposure ($P < 0.01$), for both *P. betae* and BNYVV. Interaction of the two treatments was not significant.

infection by *P. betae* (from 0 to 9.5 out of 12 test plants) required less than 12 h, for *DW* soil a 38% increase (from 4.5 to 9 out of 12 test plants) occurred in 24 h (Table 4). This apparent effect on the rate of increase was not detected as a statistically significant interaction between storage treatment and exposure period.

Pre-wetting of infested soil for 24 h before exposure of bait plants caused an increase in infection with *MC* soil, but not with *DW* soil at the 12-hour exposure period (Table 4).

After air-drying the baited soil samples of Expt 4, to kill zoospores released by the first baiting, all were baited a second time, with a 72-hour exposure period. On every soil sample, infection of bait plants occurred during the second baiting.

Table 4. Effect of moist/cool and dry/warm storage of infested soil on germination of resting spores of *Polymyxa betae* and transmission of BNYVV, as measured by the infection of bait plants exposed for 12, 24, 48 or 72 h to the wetted infested soil. The effect of prewetting of soil for 24 h before baiting was tested with a 12-hour exposure period (Expt 4).

Storage conditions[a]	Mean numbers of infected bait plants ($N = 2 \times 12$)[b]							
	P. betae				BNYVV			
	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h
MC Moist, 5 °C	0	9.5	11.5	11.5	0	7	9.5	11
DW Air-dry, 20°C	0	0.5	4.5	9	0	0	1	2
MC' Moist + prewetted	8.5				7			
DW' Dry + prewetted	0				0			

[a] Duration of storage of the soil was 40 months.

[b] Each treatment consisted of an assessment of two lots of 12 plants. Effects of storage condition and exposure period were analyzed for *P. betae* and BNYVV separately. The difference in numbers of infected plants between the two storage conditions of soil was significant at $P < 0.001$. Numbers of infected plants increased with increasing exposure period ($P < 0.001$). Interaction of the two treatments was not significant. For the 12-hour exposure period, the effect of prewetting was analyzed separately: prewetting of moist soil resulted in an increase in the numbers of infected plants ($P < 0.001$) compared to moist soil that was not prewetted.

Experiments 2, 3 and 4 were performed at the same temperature (22 °C) during exposure of the bait plants. These experiments gave corresponding numbers of infected plants for similar treatments and exposure times, indicating that the duration of storage of soil had no effect on the behaviour of the resting spores and/or the released primary zoospores, at least between 24 and 40 months of storage.

Table 5. Effect of various storage conditions for periods of 4, 8 or 42 weeks on germination of viruliferous resting spores of *Polymyxa betae* isolated from roots, as measured by BNYVV infection of bait plants. Bait plants were exposed to wetted resting spores on microfilters for 48 h.

Storage conditions[a]	Mean numbers of BNYVV-infected bait plants ($N = 2 \times 20$)[b]			
	4 weeks, 22 °C	8 weeks, 22 °C	42 weeks, 5 °C	42 weeks, –18 °C
Moist soil	15	19.5	19	n.t.
Moist soil, autoclaved	17.5	20	20	n.t
Water	3	10.5	20	18.5
Air (dry)	0	9	3	n.t.

[a] Temperatures during storage for 4 and 8 weeks were 22/15 °C (16/8 h) and during 42 weeks constant 5 °C or –18 °C.

[b] Each treatment consisted of an assessment of two lots of 20 plants. Analysis of the data for storage periods of 4 and 8 weeks showed that the number of infected plants was significantly affected by storage conditions ($P < 0.001$), period of storage ($P < 0.001$) and the interaction of these factors ($P < 0.05$).

n.t. = not tested.

Experiment with resting spores isolated from roots. The fresh resting spores on filters were able to cause infection; an average number of 15.5 out of 20 bait plants became infected when spore sandwiches were baited directly after preparation. Storage of resting spores either in water or air-dry, resulted in less infection of bait plants than with storage in soil, either non-sterile or autoclaved (Table 5). With an increase in storage time from 4 to 8 weeks, infection of bait plants increased, but this increase was dependent upon the storage conditions (interaction between storage treatment and period).

When the spore 'sandwiches' were stored cool for 42 weeks, infection percentages were approximately 100% for all moist treatments, either soil or water. The spores that were stored under dry conditions gave rise to low percentage infection. The level of infection resulting from frozen spores was the same as that from spores stored in water at 5 °C.

Discussion

Onset and rate of germination of resting spores in naturally infested soil. Irrespective of the preceding storage conditions of soil, bait plants had to be exposed for more than 12 h to flooded naturally infested soil in order to become infected, when no specific pre-treatments were applied. When bait plants were exposed to infested soil for 24 or 48 h, higher numbers of *P. betae*- and BNYVV-infected plants were detected for soil stored moist and cool than for soil stored air-dry at room temperature. With *DW* soil, the onset of germination was delayed; infected plants were rarely detected after 24 h exposure. Prewetting the moistly stored soil showed that germination of and infection by *P. betae*, with transmission of BNYVV, can take place within 12 h.

Effects of different treatments on germination of resting spores in soil is difficult to study on the germination process itself. The effects of storage conditions of soil on the incidence of infected bait plants was probably mainly due to an effect on the resting spores, rather than on the zoospores after they are released from the resting spores. Consequently, treatment effects on germination of resting spores are inferred from the indirect assessment, i.e. bait plant infection. Dry or moist storage conditions were at different storage temperatures. However, in the experiment with isolated resting spores, four storage treatments were compared at the same temperature. As under these conditions a dry treatment resulted in a lower infectivity than moist soil treatments (Table 5), moisture during storage is assumed to be more important than temperature in determining infectivity or rate of germination of resting spores by bioassay, in Experiments 1–4.

The numbers of bait plants and exposure periods tested were adequate to detect differences in the onset of germination of resting spores between different treatments. However, differences could only be detected with short periods of exposure. A smaller amount of infested soil, a higher number of bait plants and a number of intermediate exposure times, in various combinations or individually, would have been necessary to allow an accurate determination of response curves and infection rates.

Exposure periods longer than 72 h were avoided, because of the risk of secondary infection of plants by zoospores released from neighbouring plants. The minimum time needed for resting spores to germinate in the presence of host roots has not yet been reported. However, the results in Table 4 indicate that, with a pre-treatment of wetting the soil, spores can germinate and lead to infection within 12 h. From initial zoospore infection to release of secondary zoospores takes approximately 70 h at 22 °C (Ivanović et al., 1983). Once secondary zoospores are released, they can cause new infections within 30 min (Peters and Godfrey-Veltman, 1989).

As for the time of onset of germination; Bouhot (1988) mentioned that penetration into root hairs occurred the second day after contact between roots and *P. betae*. He did not

state whether the inoculum was infested soil or resting spores isolated from roots. Experiments carried out by Slykhuis (1974) on WSSMV transmission by *Polymyxa graminis*, showed that after exposure for 1 day to infested soil stored under dry conditions, none of the bait plants were infected, and after exposure for 2 days only 1% of bait plants were infected.

Observations made with the baiting procedure are not independent, as has been discussed earlier (Tuitert, 1990). In theory, zoospores from one resting spore cluster might be able to infect more than one bait plant. However, as the number of bait plants was not very high, resulting in a low density of roots, the chance that zoospores from one resting spore cluster infected more than one bait plant will have been small.

The results for BNYVV reflect those for *P. betae*. Transmission of BNYVV by viruliferous spores in soil can be accomplished within 12 h. There is no information on the rate of germination of viruliferous versus non-viruliferous resting spores. The present study does not allow to draw conclusions on this aspect either.

All soil samples from Expt 4 gave rise to infection when baited for a second time. From the point of view of survival strategy, it is advantageous that not all resting spores had germinated within 72 h.

Effects of drying and pre-treatment of soil on infectivity. Dry/warm storage conditions probably induced dormancy of the resting spores, whereby spores needed a longer period of hydration before they were able to germinate and also showed a greater variation in germination (resulting in a lower rate of infection) than when they were stored under moist/cool conditions. The longer the soil was dried, the more spores became dormant or the longer the period necessary for hydration; drying for only 1 or 7 days reduced the infectivity of the *MC* soil compared with direct assessment of this soil (Tables 1 and 2).

With another fungal virus vector, *Olpidium brassicae*, comparison of germination of resting spores in freshly collected and air-dried soil (Westerlund et al., 1978) yielded results similar to those for *P. betae*. With *O. brassicae*, the dormancy period after air-drying appeared to be longer. Six to eight days of exposure of bait plants to soil previously stored under dry conditions were needed for infection by *O. brassicae*, whereas exposure for 48 h to moist soil caused 100% infection. Somerville (1894) mentioned that soil infested with *Plasmodiophora brassicae* had to be kept in a moist condition because he had experienced that soil 'became useless for purposes of infection if it was allowed to become over-dry'.

Slykhuis (1975) studied the effect of drying of soil on the transmissibility of WSSMV by *P. graminis*. He observed an increase in infectivity in a 90–120 day bioassay after air-drying the soil samples for 4–5 months, as compared to the infectivity directly after collecting the soil. This increase should, however, not merely be ascribed to the drying of soil. Duration of storage might have been responsible for maturation of spores; storage under moist conditions might also have resulted in higher infection, but this was not tested.

Prewetting the *MC* soil for 24 h altered the germination of spores in such a way that a high incidence of infection by *P. betae* was observed after only 12 h exposure (Table 4). It is generally assumed that root exudates are required as a stimulus for infection (Habibi, 1969). Apparently, the continuous presence of plant roots is not required to induce a germinable state. This may be an example of Hawker and Madelin's (1976) statement that germination of spores often depends on two sequential signals, the first is a dormancy-breaking one (in this case hydration), priming the spore for receipt of the second specific signal that actually evokes germination. The results of Table 4 suggest that not only in *DW* soil, but also in the moistly stored soil (*MC*) some of the spores were dormant.

Twenty-four hour of prewetting was too short to detect any effect on the spores in the dry soil with a 12-hour exposure period. After a 4-day exposure of bait plants to soil infested with *O. brassicae* and stored under air-dry conditions, no plants became infected when prewetting of soil lasted 1 day, but all plants were infected when it lasted 6 days (Westerlund et al., 1978). Pre-treatments other than wetting were not tested, but heat treatment apparently also stimulates germination of *P. betae* (Beemster and De Heij, 1987). Various treatments can break constitutive or exogenous dormancy of fungal spores, e.g. flooding, alternate wetting and drying, prolonged drying, freezing and heat treatment (Sussman and Halvorson, 1966).

Infectivity of viruliferous resting spores isolated from beet roots. Exposure of bait plants for 48 h to microfilters covered with viruliferous resting spores of *P. betae* isolated from roots resulted in a high percentage infection with BNYVV. The amount of resting spores applied was high, to compensate for the finding that only a fraction of fresh clusters germinate (Tuitert et al., submitted), and that not all the infective clusters will be carrying BNYVV (Fujisawa and Sugimoto, 1977; Tuitert et al., submitted). The filters were dried before sealing them into a sandwich, this would have killed any zoospores that were present (Campbell and Lin, 1976). When dry conditions were prolonged for 4 weeks at 22/15 °C or spores were kept in demineralized water, the germination of viruliferous spores was reduced compared to germination of those that had been stored in moist soil (inferred from the infection of the bait plants, Table 5). The effect of dry conditions, both at 22/15 °C and at 5 °C, corresponded with the results of the experiments with naturally infested soil; apparently the hydration of dry spores requires time.

For BaYMV, transmitted by *P. graminis*, Usugi (1988) reported that the potency of dried roots or roots stored in flooded soil at 4 °C as a source of inoculum increased after they had been buried in moist soil at 23 °C for over 14 days. This finding is in line with the reduced infection from *P. betae* spores stored dry or in water for 4 weeks (Table 5).

It is known that soil extracts often stimulate fungus spores (Sussman and Halvorson, 1966). Nevertheless, it is difficult to speculate on the causes for the lower infection levels after storage of spores in water for 4 and 8 weeks than after storage in moist soil (pH, ion concentration, oxygen), because after 42 weeks storage differences between water and soil storage were not detected. Repetition of this experiment and further investigations into the germination process would be required to explain these findings.

Implications of the effects of storage conditions for detection procedures. When short baiting periods are used in methods for detection of BNYVV and *P. betae* in soil, storage conditions influence the results of assessments. This risk might be diminished when a pre-treatment of soil is applied (Beemster and De Heij, 1987). The effects of storage conditions on germination of *P. betae* and transmission of BNYVV should also be considered when infested soil is used as source of inoculum in screening for resistance.

Differences in infectivity between spores stored under different conditions were not detected when plants were exposed to the soil for long periods of time: with a 6-week bioassay on serial dilutions of *MC* and *DW* soil, estimates of the inoculum level in the soil were similar, for both *P. betae* and BNYVV (Tuitert, 1990). These equal estimates also demonstrated that in the baiting experiments a difference in decline of inoculum could be excluded as the cause of the difference observed in infectivity of the inoculum in the *MC* and *DW* soils during the 12–40 months storage period. The duration of storage of soil neither influenced the infectivity of the soil in short-lasting baiting experiments: Experiments 2, 3 and 4 showed corresponding numbers of infected plants for similar treatments and exposure times.

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References

Abe, H. & Tamada, T., 1986. Association of beet necrotic yellow vein virus with isolates of *Polymyxa betae* Keskin. Annals of the Phytopathological Society of Japan 52: 235–247.

Beemster, A.B.R. & De Heij, A., 1987. A method for detecting *Polymyxa betae* and beet necrotic yellow vein virus in soil using sugar-beet as a bait plant. Netherlands Journal of Plant Pathology 93: 91–93.

Bouhot, D., 1988. Early stage detection of *Polymyxa betae* on sugar beet roots as a method for soil diagnosis pesticides studies and screening of new genotypes. In: Abstracts of Papers, Fifth International Congress of Plant Pathology. Kyoto, Japan, August 1988. p. 453.

Campbell, R.N. & Lin, M.T., 1976. Morphology and thermal death point of *Olpidium brassicae*. American Journal of Botany 63: 826–832.

Fujisawa, I. & Sugimoto, T., 1977. Transmission of beet necrotic yellow vein virus by *Polymyxa betae*. Annals of the Phytopathological Society of Japan 43: 583–586.

Habibi, B., 1969. Beiträge zur Biologie von *Polymyxa betae*. Dissertation, Göttingen. 83 pp.

Hawker, L.E. & Madelin, M.F., 1976. The dormant spore. In: Weber, D.J. & Hess, W.M. (Eds), The fungal spore: form and function. Wiley, New York. p. 1–70 .

Ivanović, M., MacFarlane, I. & Woods, R.D., 1983. Viruses of sugar beet associated with *Polymyxa betae*. In: Report of the Rothamsted Experimental Station, 1982. p. 189–190.

Keskin, B., 1964. *Polymyxa betae* n.sp., ein Parasit in den Wurzeln von *Beta vulgaris* Tournefort, besonders während der Jugendentwicklung der Zuckerrübe. Archiv für Mikrobiologie 49: 348–374.

Payne, R.W., Lane, P.W., Ainsey, A.E., Bicknell, K.E., Digby, P.G.N., Harding, S.A., Leech, P.K., Simpson, H.R., Todd, A.D., Verrier, P.J., White, R.P., Gower, J.C., Tunnicliffe Wilson, G. & Paterson, L.J., 1988. Genstat 5 Reference Manual. Clarendon Press, Oxford. 749 pp.

Peters, D. & Godfrey-Veltman, A., 1989. *Polymyxa betae* zoospores as vectors of beet necrotic yellow vein furovirus. EPPO Bulletin 19: 509–515.

Slykhuis, J.T., 1974. Differentiation of transmission and incubation temperatures for wheat spindle streak mosaic virus. Phytopathology 64: 554–557.

Slykhuis, J.T., 1975. Effects of drying on the transmissibility of wheat spindle streak mosaic virus in soils from wheat fields in Ontario. Canadian Plant Disease Survey 55: 106–108.

Somerville, W., 1894. An infection experiment with finger and toe. Journal of the Royal Agricultural Society of England 5: 808–811.

Sussman, A.S., 1976. Activators of fungal spore germination. In: Weber, D.J. & Hess, W.M. (Eds), The fungal spore: form and function. Wiley, New York. p. 101–137.

Sussman, A.S. & Halvorson, H.O., 1966. Spores: their dormancy and germination. Harper & Row, New York, London. 354 pp.

Tuitert, G., 1990. Assessment of the inoculum potential of *Polymyxa betae* and beet necrotic yellow vein virus (BNYVV) in soil using the most probable number method. Netherlands Journal of Plant Pathology 96: 331–341.

Tuitert, G. & Hofmeester, Y., 1992. Epidemiology of beet necrotic yellow vein virus in sugar beet at different initial inoculum levels in the presence or absence of irrigation: Dynamics of inoculum. Netherlands Journal of Plant Pathology 98: 343–360.

Usugi, T., 1988. Epidemiology and management in Japan of soil-borne cereal mosaic viruses with filamentous particles. In: Cooper, J.I. & Asher, M.J.C. (Eds), Developments in applied biology II.

Viruses with fungal vectors. Association of Applied Biologists, Wellesbourne, UK. p. 213–225.
Westerlund, F.V., Campbell, R.N., Grogan, R.G. & Duniway, J.M., 1978. Soil factors affecting the reproduction and survival of *Olpidium brassicae* and its transmission of big vein agent to lettuce. Phytopathology 68: 927–935.