Danish Research Service for Plant and Soil Science
Research Centre for Plant Protection
Department of Botany
DK-2800 Lyngby

Report no. 2017

A method for the detection of Rizomania in soil

En metode til påvisning af Rizomania i jord

LILIAN KLOSTER, JENS BEGTRUP and BENT ENGSBRO

Summary

The purpose of this work was to obtain a sensitive method for routine testing of soil samples in the Danish survey of Rizomania, a disease caused by beet necrotic yellow vein virus (BNYVV).

A method testing soil samples for the presence of BNYVV and the vector *Polymyxa betae* where sugar beet are used as bait plants has been developed by *Beemster* and *de Heij* in 1987.

With few minor modifications the method has been carried out on soil from West Germany infested with Rizomania. Bait plants showed high infection with BNYVV. Therefore, the method will be used in the future work.

Key words: Rizomania, soil test, BNYVV, ISEM, *Polymyxa betae,* sugar beet.

Resumé

Som et led i den danske overvågning for bederoesygdommen Rizomania undersøges jordprøver for viruset (beet necrotic yellow vein virus), der forårsager sygdommen og for dets vektor *Polymyxa betae.* Hidtil er jordprøver blevet undersøgt ved hjælp af fangplanter dyrket direkte i jordprøven.

Beemster og *de Heij* har i 1987 beskrevet en metode, ved hvilken jordprøver er undersøgt for forekomst af viruset og vektoren ved hjælp af fangplanter, der blot i 4 dage bringes i kontakt med jordprøven. Herefter er planterne videredyrket i sterilt sand under optimale betingelser for opformering af viruset og vektoren.

I dette arbejde er metoden med få mindre ændringer blevet afprøvet med en vesttysk jord inficeret med Rizomania, med godt resultat. Metoden vil fremover blive anvendt ved rutineundersøgelse af jordprøver for forekomst af Rizomania.

Nøgleord: Rizomania, jordprøveundersøgelse, BNYVV, ISEM, *Polymyxa betae,* sukkerroer.

Introduction

Rizomania in sugar beet is caused by beet necrotic yellow vein virus (BNYVV) and transmitted to the host by the soil-borne fungus *Polymyxa betae* Keskin. The virus has not yet been detected in Denmark but is widely spread in the south and middle of Europe. In 1987 Rizomania was also found in England (4).

The disease can rise problems for the cultivation of sugar beet depending on a number of factors such as climate, level of infestation and cultivars. In order to spread and to survive the virus is dependent of the fungal vector *P. betae,* which has proven to be very common in Danish beet grown areas (5).

A current disease survey in beet grown regions is necessary to avoid an undetected spread. For a reliable survey of the disease it is not adequate only to inspect visually the sugar beet fields in the growing season and testing suspicious looking plants for BNYVV. The Danish spring and summer climate normally does not reach the optimal conditions for visible attacks of Rizomania (i.e. 20–25°C together with high water content in the soil). With this in mind the survey should be accompagnied by soil sampling and testing using bait plants grown in greenhouse under optimal conditions for possible infection.

In the preliminary work, soil samples were tested using sugar beet seedlings, which were grown directly in soil samples in pots for up to 4 months (5). Subsequently the roots were rinsed under tap water and the presence of the virus and the vector was determined. The method was practicable but required a rather long growth period to ensure satisfactory infection of the roots, as the concentration of the resting spores of *P. betae* was low in the soil samples.

A quick and reliable method for testing soil samples using sugar beet bait plants has been developed by *Beemster* and *de Heij* (1). The method has the advantage that the period for infection is during the seedling stage, known to be the most susceptible one. The total growth period can be considerably shortened, achieving faster results.

The Dutch method was used with small modifications on a Rizomania infested soil from West Germany. The purpose of this experiment was to obtain a more sensitive method for testing soil samples in the Danish Rizomania survey.

Materials and methods

The method described by *Beemster* and *de Heij* (1) is used with few modifications, such as use of younger seedlings, time for heating of soil samples in relation to water supply and a shorter period of infection (baiting period). The aim of the modifications was primary to simplify the working process.

The method will be described below.

The soil used in the experiment was taken from a Rizomania infested field in Wölfersheim, and was kindly provided by Professor Dr. *E. Schlösser,* Giessen, West Germany.

1. Bait plants: Dressed seeds of the sugar beet variety »Magnamono«, susceptible to Rizomania were used. The seeds were germinated at 20°C under humid conditions in germinating boxes using pleated gray stripes no. 3236, 110/50/20 mm and covering stripes no. 0858, 110 × 580 mm, (Allesen Holm's Eftf. I/S). Seven days old seedlings were gently rinsed in destilled water and used as bait plants.

2. Soil samples: The soil samples was mixed by hand and subsamples of 50 g were placed in Petri dishes (diameter = 9 cm). The soil samples were heated for 30 minutes at 40°C in an oven and 25–35 ml of distilled water was then added to each Petri dish. The soil/water mixture should reach a consistency like a thick soup. The consistency is of greater importance than identical water supply to all samples.

3. Procedure: Thirty seedlings were placed round the rim of the Petri dish containing the soil/water suspension and with the roots immersed in the suspension. To reduce the evaporation a piece of tin foil with a diameter of 8 cm was cautiously placed over the suspension, Fig. 1. For further protection of the delicate seedlings, the whole Petri dish was covered with tin foil.

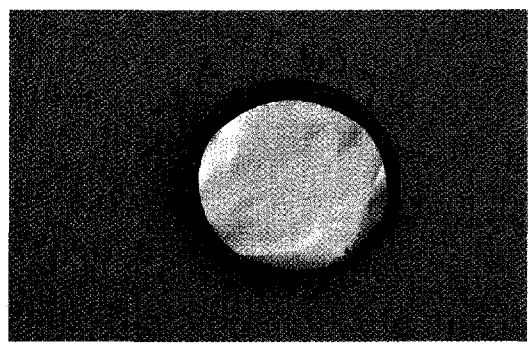

Fig. 1. One week old seedlings of sugar beet baiting in the soil/water suspension for infection.
En uge gamle kimplanter af sukkerroe lagt i jordopslemning for infektion.

Seedlings were baited in the suspension for a period of 3 days and distilled water supply was given if necessary. After this the seedlings were planted in plastic pots (7 × 7 cm) with Vermiculite: a mixture of grade 1 and grade 4 in the ratio of 1:2 (Skamol A/S), 5–6 seedlings in each pot and with 5 replications for each soil sample of 50 g. The soil/water suspension was added to one of the 5 pots, Fig. 2. This procedure was done to ensure the detection of differences in infection between seedlings in contact with the soil for 3 days only and plants in contact with the soil in the whole period of growth. Each pot was placed in a plastic tray.

Water with a compound fertilizer was added to the plastic tray when required. The seedlings were grown for a period of 6–18 weeks.

The baiting and subsequent cultivation were carried out in an unheated greenhouse in the summer 1987. The temperatures were fluctuating between 10 and 25°C the first two months and around 10°C the next two months.

Results

Sugar beets cultivated in pots representing 5 subsamples of the same soil were tested for the presence of BNYVV. Plants from each subsample, but grown in different pots, were tested after a short growth period of 6–9 weeks and after a longer period of 18 weeks. Root sap from plants with or without leaf symptoms grown in the same pot were tested separately. Sap from leaves with possible symptoms of Rizomania was tested for the presence of BNYVV.

The presence of beet necrotic yellow vein virus was detected by immunosorbent electron microscopy, ISEM, on sap from roots mainly and from leaves using BNYVV-antiserum. The antiserum

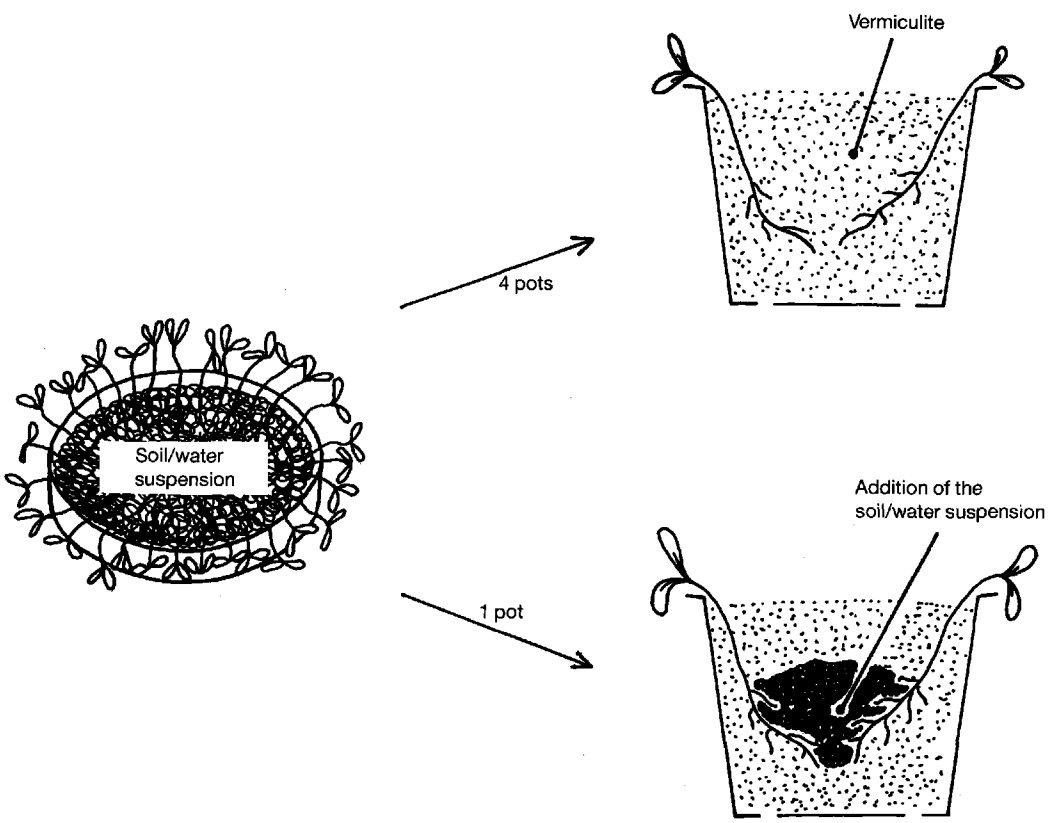

Fig. 2. Ten days old sugar beet seedlings being potted in five pots after three days in the soil/water suspension.
Ti dage gamle roekimplanter fordeles i fem potter efter tre dage i jordopslemning.

was kindly provided by Dr. *R. Köenig,* Braunschweig, West Germany.

Roots from 18 of 23 pots were tested for the presence of *Polymyxa betae.* The presence of the vector was determined by light microscopy (5).

The results are presented in Table 1 and show that BNYVV was detected in root sap of plants from all the pots except one. A growth period of 6–7 weeks was sufficient for the detection of the virus, even if the temperatures were periodically

Table 1. Infection of beet necrotic yellow vein virus (BNYVV) and the vector *Polymyxa betae* in roots of sugar beet bait plants after immersion in 5 subsamples of the same soil infested with Rizomania.
Infektion med Rizomaniavirus og vektoren Polymyxa betae *i rødder af sukkerroefangplanter bragt i kontakt med 5 delprøver af den samme Rizomaniainficerede jord.*

Sample no.	Pot no.	Tested after X weeks	Reaction achieved		Leaf symptoms
			BNYVV	*P. betae*	
	A *	6	+	–	N
	C	6	++	–	N
	B a **	7	++	+	YP
1	B b	7	++	++	N
	D	9	0	–	N
	E	18	+++	–	LT
	A	7	+	+++	N
	B a	7	++	+	YP
2	B b	7	+	++	N
	C	18	+++	0	YP
	D	18	++	+	LT
	E	18	+	+	LT
	B	9	++	–	YP
3	A	18	++	+	YP
	C	18	++	+	LT
	D	18	++	+	LT
	B	7	+	+	N
	C	9	+	–	N
4	A	18	+++	+	LT
	D	18	+	+	LT
	E	18	++	+	LT
	B	9	+	–	YP
5	A	18	++	0	YP
	C	18	+	+	LT
	D	18	+	+	LT

* The soil/water suspension is added to all pots signed A.
** Pot 1B and 2B divided in plants a) with or b) without indications of symptoms in the leaves.

Quantitative estimation of the presence of BNYVV and *P. betae:*
0 = no virus particles or resting spores of the vector observed
\+ = low concentration
++ = medium concentration
+++ = high concentration
– = not tested

Leaf symptoms:
N = none
YP = faint yellowish patches
LT = low tension light yellow-green; on the date of the test the leaves without symptoms had grown up.

under the optimal for the development of Rizomania. After 18 weeks, a greater part of the plants showed high concentration of virus in the roots. No direct correlation between the occurrence of leaf symptoms and the degree of infection with BNYVV in the roots has been revealed, (Table 1, pots no. 1B and 2B).

In the first 9 weeks of the growth period a part of the plants showed faint yellowish patches on the leaves but no distinct colouring along the vein which is characteristic for systemic infection with Rizomania. Sap from leaves with faint yellowish patches from plants in the pots no. 2C, 3A and 5A were tested for BNYVV. No virus particles were observed. At the end of a warmer period in August and September the foliage with a low tension turned yellowish/green followed by necrosis. Later in the cultivation period during October and November the temperatures were below 15°C and the growth of the foliage was normal.

The secondary roots of all plants were beard-like in appearance, very dense, with a poor development of the storage root.

P. betae was detected in roots of 16 out of 18 investigated samples. Higher concentrations of the vector were detected after 6 to 7 weeks compared with 18 weeks of cultivation.

No differences in the degree of infection of the virus and the vector were demonstrated between sugar beets in pots with or without addition of the soil/water suspension.

Discussion and conclusion

The described method, slightly modified after *Beemster* and *de Heij* (1), was found to be very efficient for the detection of Rizomania in soil samples where sugar beet were used as bait plants. Beet necrotic yellow vein virus, BNYVV, was detected in roots of the sugar beets baited in all 5 subsamples of a soil from Germany infested with Rizomania. In only one pot no virus could be detected in the root sap (Table 1, pot no. 1D) but plants from the four other replications showed good infection. The results show that a number of 30 seedlings used as bait plants for each 50 g soil sample were sufficient to detect the virus.

All the beets in the experiment showed an abnormal and dense growth of the secondary roots and poorly developed storage roots as characteristic for an attack of BNYVV. The observed leaf symptoms, which were weak yellowish irregular patches, were apparently caused by the partial systemic root infection of BNYVV as no virus particles could be detected in the leaf sap. Virus moves only slowly from infected roots to the top of the plants (4–10 weeks after infection) while the virus easily moves from the top to the roots (6). It was not possible to reveal any differences in the concentration of the virus in root sap from plants with or without leaf symptoms.

The vector *Polymyxa betae* was detected in roots from 16 out of 18 pots. Nevertheless, the roots from two of the pots (Table 1, pots no. 2C and 5A) in which *P. betae* was not observed, showed high degree of infection with the virus. The fact that a fungal attack is required for virus infection suggests that the fungal vector must have been overlooked in the dense root mass. Where the cultivation period extended 18 weeks the conditions were obviously not optimal for the development of the fungus. Whether this is due to low temperatures in the last part of the period, unfavourable changes in the roots as a result of the attack of BNYVV or changes in the microflora in the growing media with time is not jet provable.

The used method increases obviously the development of the fungal resting spores into zoospores, which subsequently can infect the very susceptible beet seedlings in the soil/water suspension. A baiting period of 3 days was sufficient for an infection. The succeeding growth of the seedlings was accomplished at conditions only periodically reaching optimum for the development of BNYVV and the virus was detectable after 6 weeks. A reduction of the growth period to less than 6 weeks should be possible using temperatures constantly near optimum.

The degree of infection was obviously not higher in plants grown in pots supplied with the soil/water suspension.

Other methods for testing soil samples using bait plants are known (2, 3) but the method described by *Beemster* and *de Heij* seemed to be reliable and suitable for a Danish survey of Rizomania.

In this work, a quantitative estimation of the fungal vector or the virus has been made visually on the root mass and in the root sap respectively. If a more standardized method for a quantitative estimation of the level of infestation in soil is wished, *Beemster* and *de Heij* proposed to use a higher number of bait plants, potting them separately and basing the estimation on the number of

infected plants proportional to the total number of plants (1).

Because the method described in this paper was found to be very effective for the establishment of beet necrotic yellow vein virus in sugar beet bait plants, it will be used in future work for the survey of Rizomania in Denmark.

References

1. *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. Neth. J. Pl. Path. 93, 91-93.
2. *Bouhot, D.* 1985. Distribution en France du *Polymyxa betae* Keskin, vecteur de la rhizomanie de la betterave sucrière. International Institute for Sugar Beet Research, IIRB. Proceedings of the 48th winter congres. Brussels. 13-14 Feb. 399-404.
3. *Goffart, J. P., van Bol, V. & Maraite, H.* 1987. Quantification du potentiel d'inoculum de *Polymyxa betae* Keskin dans les sols. International Institute for Sugar Beet Research, IIRB. Proceedings of the 50th winter congres: II B.N.Y.V.V. Brussels. 11-12 Feb. 295-306.
4. *Hill, S. A.* 1988. The outbreak of rhizomania in England in 1987. Programme, abstracts and participants. European and Mediterranean Plant Protection Organization. Conference on soil-borne viruses and their vectors. OEPP/EPPO. Malmö, 27-29 Oct. 1988, 20.
5. *Kloster, L., Begtrup, J. & Engsbro, B.* 1989. Rizomania i sukkerroer. Undersøgelser over mulig forekomst af viruset og dets vektor *Polymyxa betae* i Danmark 1985-1986. Tidsskr. Planteavl 93, 73-81.
6. *Paludan, N.* 1985. Spread of viruses by recirculated nutrient solutions in soilless cultures. Tidsskr. Planteavl. 89, 467-474.

Manuscript received 1 May, 1989.