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Dissemination of rhizomania by soil, beet seeds and stable manure

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Abstract

Polymyxa betae, the vector of beet necrotic yellow vein virus (BNYVV), (the causal agent of rhizomania of sugar-beet), forms cystosores which are very persistent and might be dispersed by soil, beet seeds, plant material and stable manure. Research has been carried out into the risk of dissemination; relative importance was not determined. Inoculation with diseased soil in a field caused rhizomania in sugar-beets within one year. This implies that even small amounts of soil adhering to plant roots constitute a potential danger. Direct transmission of BNYVV by sugar-beet seeds could not be demonstrated, but, after processing and cleaning seed lots originating from infested fields, the seed waste proved to be contaminated. Cystosores of *P. betae* and, to a lesser extent, BNYVV could pass through the intestine of sheep in fodder experiments carried out with heavily infested sugar-beet tails.

Additional keywords: beet necrotic yellow vein virus (BNYVV), *Polymyxa betae*, sugar-beet.

Introduction

Some areas in the Netherlands have been infested with rhizomania for a long time. Until 1983 the epicentres were not recognized and from there the disease could have been disseminated. This is demonstrated by a number of fields surrounding the epicentres showing small patches of infested plants.

Since cystosores of *Polymyxa betae*, the vector of beet necrotic yellow vein virus (BNYVV), can survive a long time in the soil (Asher and Blunt, 1987), it is very likely that infections will be transferred by soil adhering to machinery and plant material. In some cases infections could be traced back to dumping of tare soil from harvesting machinery or settling ponds. If small patches are infested, rhizomania can be spread within one field very quickly (Schäufele et al., 1985), most probably by machinery for soil tillage and by spraying equipment.

Hess et al. (1984) did not detect any transmission of BNYVV by seeds from sugar-beet plants originating from infested fields, if they were processed properly. However, this research was carried out on seeds from plants which were not infested systemically by BNYVV and processing waste was not investigated.

Because of their persistent nature, cystosores of *P. betae* might possibly pass through the intestine of cattle without losing their infectivity. Hillman (1984) analysed manure samples from sheep fed with diseased sugar-beets. Cystosores of *P. betae* were detected in a small part and BNYVV in only a few of the samples. Since little was known of the dissemination of rhizomania by soil, beet seeds and stable manure, trials have been set up to estimate and, if possible, quantify the risks.

Materials and methods

Samples of soil were analysed by bio-assay. Cylinders filled with 900 ml of soil, and covered at one end with 0.5-mm gauze, were placed in containers filled with baked clay granules to provide a regular moisture supply. The soils under investigation were dried and mixed with 50% (v/v) of these granules. In this way additional moisture for movement of zoospores was combined with sufficient oxygen supply. In each cylinder one sugar-beet seedling ('Monohil') was planted and after six or more weeks (depending on the level of detection needed) at 25 °C in a climate cabinet, the root systems were collected. Part of these were homogenized in a Waring Blendor and the number of cystosores was counted in the suspension under the microscope. From the remaining roots, sap was collected with a hand press. Larger quantities were collected by means of a Pollähne press.

ELISA was applied according to the method described by Walter et al. (1979), using BNYVV antiserum obtained from the Institut National de la Recherche Agronomique (INRA) at Colmar (France), or from Boehringer, Mannheim (Federal Republic of Germany). In all ELISA tests standard positive and standard negative samples were incorporated. Extinction values of more than 0.060 at 405 nm (E_{405} were considered to be positive.

Dissemination by soil. The trial to measure the effect of inoculation of infested soil was laid out in four replicates (6 × 15 m each) at 's-Gravenpolder. Infested soil in a quantity of 20 ng dm^{-3}, calculated for the total furrow (20 cm of depth), was spread over the surface and lightly incorporated. Every two weeks samples of five roots were taken and analysed by ELIZA; at harvest the plants showing root symptoms were counted. Root weight, sucrose content and Na-content were determined according to standard procedures (De Nie and Van der Poel, 1986).

Transmission by seeds. Healthy sugar-beet stecklings were grown in infested soil and when BNYVV was detected in the leaves by ELISA, the seeds were harvested and sown in a cylinder, filled with 900 ml of silver sand and a nutrient solution (Steiner, 1968).

After rubbing, grading and cleaning a very dirty lot of seeds, originating from infested fields in Italy, the processing waste was separated in different fractions, containing pericarp and small seeds, pericarp and fine soil and larger soil particles. These were analysed in a bio-assay by mixing with silver sand and a nutrient solution (Steiner, 1968), into which sugar-beet seedlings were planted.

Raw seeds from lots which had been produced on healthy fields were divided in two groups, one of which was mixed with infested soil and the second remained untreated. Both groups were put through the processing machinery. The cleaning waste was mixed with silver sand in a dilution of 1 : 6 (v/v) and put in forementioned bio-assay to simulate contamination of seeds from plants grown on diseased soil, checking the suitability of the bio-assay for this purpose.

Detection in stable manure. The passage of cystosores and BNYVV through the intestine was investigated by feeding two sheep circa 60 kg of contaminated sugar-beet tails (containing a high level of cystosores), after they had grown accustomed to this diet over a period of two weeks. Fresh excrement was collected and mixed with silver

sand at a ratio of 1 : 5 (v/v). This was based upon preliminary experiments using different proportions of sand and manure in order to find the maximal manure dosage which did not damage the test plants. After 8 and 13 weeks, respectively, the roots were analysed for *P. betae* and BNYVV. Parts of the manure, particularly straw components and dried excrement, were tested separately.

Results

Dissemination by soil. In a trial field at 's-Gravenpolder 20 ng dm^{-3} of infested soil (result of bio-assay: E_{405} = 0.836) was added to the furrow in February 1985. Sugar-beets (cv. Regina) were drilled on the 6th of May and from the 13th of August onwards ELISA was applied to sap of pressed root tip samples.

As can be seen in Table 1, plants in infected plots were positive on the 24th of September, but not on the 13th of August. The first weak symptoms were detected by mid August but from these beets no extracts with positive ELISA values were obtained. At harvest nearly 5% of the beets showed proliferation of lateral roots and browning of the vascular bundles. At the same time significant differences in the contents of sucrose and Na were detected.

Although root weight was somewhat lower in the infected plots, statistically significant differences could not be found. In 1986 a second crop of sugar-beets was grown and monitored for the presence of symptoms, which first appeared at the beginning of August. At the same time positive ELISA readings were obtained. Although larger differences in root weight were obtained as compared to 1985, a large number of plants in the untreated plots (26%) showed root symptoms, probably caused by a rapid spread of the disease. As a comparison the tolerant cultivar Rizor was grown, which showed

Table 1. The effect of inoculation with rhizomania on sugar-beet yield and quality, by adding diseased soil (20 ng dm^{-3}) on February 1985. Sugar-beets were grown for two consecutive years. Cultivars: Regina in 1985 and Regina and Rizor in 1986 ('s-Gravenpolder).

	BNYVV extiction at 405 nm			Percentage of plants showing root symptoms at harvest date	Root weight (t/ha)	Sucrose content (%)	Na (mmol/kg of beets)
1985	*13/08*	*24/09*	*31/10*				
Infected	0.022	0.174	0.180	4.9	62.4	15.8*	13.9*
Untreated	0.026	0.012	0.040	0.0	65.1	17.5	8.3
1986	*10/08*	*12/09*	*20/10*				
Infected	0.334	0.261	0.231	47	51.2*	14.9*	4.6*
Untreated 'Regina'	0.009	0.008	0.131	26	64.6	16.0	3.2
Untreated + 'Rizor'	—	—	—	7	61.4	16.6	2.1

* Significant differences at P = 0.05.

Table 2. Infestation of processing waste from seed produced on rhizomania infested fields in Italy. The test plants remained at 25 °C for 8 weeks.

Fractions	Number of bio-assay	Cystosores per mg of roots × 10^3	BNYVV Extinction at 405 nm	
pericarp + small seeds	4	37	—	0.036
fine soil + pericarp	3	86	—	0.028
larger soil particles	3	64	+ (2×)	0.078

far less root proliferation and a higher sugar content. The Na-content was low, but this is a general feature of this cultivar.

Possible transmission by beet seeds and adhering soil. If BNYVV could be transported systemically through the plant in the second year, the newly formed seeds might be infected. Since infected stecklings after vernalization nearly always died before producing seeds, we tried to grow healthy stecklings in diseased soil or to inoculate with sap containing BNYVV. From 20 plants treated in each of these two ways, only two were found to contain BNYVV in the leaves, after having grown for two months. Both plants produced viable seeds, but in a bio-assay of 20 replicates no BNYVV could be detected after 8 weeks at 25 °C.

However, if the seeds were not processed, contaminated soil particles adhering to the pericarp could transmit rhizomania. In Table 2 the results of a bio-assay carried out on the processing waste from seeds produced on infested soil are summarized. Cystosores could be detected in all fractions, but only the one containing larger soil particles in two out of three replicates, showed a positive reaction in the ELISA test. Since only a very small quantity of material was available and contamination from outside could not be completely excluded, a simulation trial was set up. Seeds harvested from healthy fields were artificially contaminated with rhizomania infested soil and afterwards processed. In a bio-assay of the contaminated waste an average ELISA extinction was obtained of 0.307 in 3 replicates (all positive), whereas for the untreated cleaning waste this value was 0.022 (no positives).

Detection in manure from sheep. Beet tails to be fed to sheep were selected on the presence of high numbers of cystosores of *Polymyxa betae* and BNYVV (E_{405} = 0.432). The manure mixed with silver sand was put in a bio-assay. A part of the manure was dried before mixing, in order to investigate the effect of drying on the viability of the cystosores. In the first trial cystosores were found in all replicates, but BNYVV was not detected (Table 3, A). In a second trial the bio-assay was prolonged to a period of 13 weeks and this time BNYVV was detected in all replicates (Table 3, B).
Dried manure tested for 8 weeks at 25 °C, contained cystosores and BNYVV was found only occasionally. The coarse fraction consisted mainly of not completely degradated plant material and straw. Longer incubation periods were not tested.

Table 3. Detection of cystosores of *Polymyxa betae* and beet necrotic yellow vein virus in manure from sheep fed with diseased root tails.

Manure type		Number of replicates	Cystosores per mg of roots	BNYVV content ELISA-reading at 405 nm	Percentage of positive
fresh manure	A[1]	27	1.380	0.014	0
	B[2]	6	7.417	0.143	100
dried manure	A	3	1.250	0.015	0
coarse fraction of manure	A	8	0	0.029	12.5

A = bio-assay for 8 weeks at 25 °C, B = bio-assay for 13 weeks at 25 °C.

Discussion

Since cystosores of *P. betae*, the vector of BNYVV, are very persistent (Asher and Blunt, 1987), rhizomania infestations can survive in the soil for a long time, even if no host crops are grown. This implies also that there is a major risk of disseminating the disease by soil tillage or mechanical weed control within one field, and by sugar-beet or potato harvesting machines and transport vehicles throughout a whole area, because much of this work is done by contractors or co-operatives. Apart from this, infested soil adhering to plant material such as seed potatoes, bulbs and different rootstocks, constitutes a risk for further spread, causing a more or less uniform distribution of the inoculum in the field. It is not worthwhile investigating the risks of all the different kinds of transportation, because much will depend on the quantity of adhering soil and the possibilities for multiplication in the infected field.

Therefore the potential danger of soil transmission was investigated in one trial field, which will not be used for agricultural purposes in the future. From the results it can be deduced that dissemination by soil is a serious danger and the infestation will increase and extend very rapidly if conditions for *P. betae* are favourable, wet soil and temperatures of more than 15 °C. Depending on these two factors and the quantity of soil transferred, the disease will be established more or less rapidly.

Transmission of rhizomania by sugar-beet seeds could have an enormous impact on the dissemination. In this research no evidence was obtained of direct transfer of BNYVV to the seeds even by systemically infected seed-bearing plants.

Infection, through contamination of harvested seeds by soil, was established in some cases, but after processing and cleaning no cystosores nor BNYVV could be detected. Some fractions of the cleaning waste were found to be contaminated and therefore it should be advised that this waste is destroyed by the seed companies. These findings will not guarantee that processed seeds are completely free from cystosores and BNYVV. Since we are dependent on a bio-assay the detection in a small volume is difficult; the quantity of dust within a package of 100 000 seeds is too low for application of this test. Previous investigations, carried out by Hess et al. (1984) and Putz (quoted by

Schlösser, 1987), have failed to demonstrate transmission by seeds.

Cystosores of *P. betae* survived the passage of the sheep intestine, but BNYVV could be detected only after a prolonged bio-assay. However, transmission by manure seems likely, since Hillmann (1984) obtained similar results. Apart from this, during fodder uptake a part of the material is spilled directly in the manure, where it can remain viable for at least two weeks (Hillmann, 1984). The major risk occurs where cattle is fed with sugar-beet tails from beets grown on contaminated soil. Tops and leaves constitute less risk, since no cystosores are present in these parts, unless they are contaminated with infested soil.

Contamination of sugar-beet tails with viable cystosores can be prevented by including them in the factory process, heating and drying them in a pulp drier or applying disinfectants such as formaldehyde.

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Samenvatting

Verspreiding van rhizomanie met grond, bietezaad en stalmest

De vector van het bieterhizomanievirus, *Polymyxa betae*, vormt zeer persistente ruststructuren (cystosoren) die verspreid worden met grond, bietezaad, plantmateriaal en stalmest.

Onderzoek is uitgevoerd naar de risico's van verspreiding, maar door het ontbreken van kwantitatieve methoden kon de relatieve belangrijkheid niet worden vastgesteld.

Wanneer een gezond perceel werd besmet met geïnfecteerde grond (20 ng dm^{-3} op bouwvoor), werd binnen één jaar aantasting door rhizomanie waargenomen. Dit wijst erop dat geringe hoeveelheden grond, die meekomen met wortelmateriaal van plantgoed, een potentieel gevaar vormen.

Directe overdracht van het bieterhizomanievirus door bietezaad kon niet worden aangetoond, maar na het schonen en bewerken van zaadpartijen afkomstig van zieke percelen bleek dat schoningsafval en in het bijzonder de grondfractie daarvan, wel was besmet.

Cystosoren van *P. betae* en in mindere mate het bieterhizomanievirus konden het maag-darmkanaal van schapen passeren, hetgeen werd aangetoond in enkele voederproeven, uitgevoerd met zwaar besmette suikerbietestaartjes.

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