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Rhizomania in sugar beet. Survey for Beet Necrotic Yellow Vein Virus (BNYVV) and Beet Soil Borne Virus (BSBV) in Denmark 1988-1989 and host range of *Polymyxa betae*, BNYVV and BSBV

Rhizomania i sukkerroe. Undersøgelser over forekomsten af Beet Necrotic Yellow Vein Virus (BNYVV) og Beet Soil Borne Virus (BSBV) i Danmark 1988-1989 samt værtplantespektret for Polymyxa betae, *BNYVV og BSBV*

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Summary

Danish soil samples collected in 1988 and 1989 from sugar beet growing areas were screened for the presence of beet necrotic yellow vein virus (BNYVV), the causal agent of Rhizomania, and for the related virus, beet soil borne virus (BSBV). A total of 148 soil samples from 6 sugar factories were examined by means of ELISA and by sap inoculation of *Chenopodium quinoa* and *C. amaranticolor.*

BNYVV was not detected in any sample, while BSBV (serotype 86-109) was found in 64% of all samples. Little is known as yet about the agricultural significance of BSBV. The common vector for both viruses, the fungus *Polymyxa betae* Keskin, was found in all soil samples.

Virus detection by means of sap inoculation of *C. quinoa* and *C. amaranticolor* was inferior to the ELISA method. Only 46% of the BSBV infections were detected by sap inoculation compared to ELISA.

The ELISA method was found to be 8 times as sensitive as the ISEM method for detection of BNYVV.

For a host range experiment 13 plant species from the *Chenopodiaceae* and *Amaranthaceae* families were inoculated with resting spores of *P. betae* carrying both BNYVV and BSBV. 12 plant species were infected with *P. betae,* 4 species with BNYVV and 8 species with BSBV. Cross inoculation of sugar beet showed 8 cases of *P. betae* infection but the viruses were not detectable, probably due to the low amount of inoculum used for the cross infection.

Key words: Rhizomania, Beet Necrotic Yellow Vein Virus, BNYVV, Beet Soil Borne Virus, BSBV, *Polymyxa betae,* host range.

Resumé

Jordprøver samlet i 1988 og 1989 i Danmark fra områder med sukkerroedyrkning blev undersøgt for tilstedeværelsen af beet necrotic yellow vein virus (BNYVV), der forårsager sygdommen Rhizomania, og for beet soil borne virus (BSBV), der har mange fællestræk med BNYVV. I alt 148 jordprøver fra 6 forskellige sukkerfabrikker blev undersøgt ved hjælp af ELISA og saftinokulering af *Chenopodium quinoa* og *C. amaranticolor.*

BNYVV blev ikke fundet i nogen jordprøve, mens BSBV (serotype 86-109) blev fundet i 64% af alle prøverne. Der vides endnu kun lidt om betydningen af BSBV. Den jordbårne svamp, *Polymyxa betae,* der er vektor for begge vira, blev fundet i samtlige jordprøver.

Påvisning af BSBV-virus ved hjælp af saftinokulering af *C. quinoa* og *C. amaranticolor* var mindre effektiv end ELISA-metoden. Kun 46% af BSBV-infektionerne blev påvist ved saftinokulering sammenlignet med ELISA.

ELISA-metoden var 8 gange mere følsom end ISEM-metoden til påvisning af BNYVV.

Ved et værtplanteforsøg blev 13 plantearter fra 2 familier *(Chenopodiaceae* og *Amaranthaceae)* inokuleret med *P. betae*-hvilesporer, der var bærer af både BNYVV og BSBV. 12 af disse arter blev inficeret med *P. betae,* 4 arter med BNYVV og 8 arter med BSBV. Krydsinokulering af sukkerroe viste 8 tilfælde af *P. betae*-infektion, mens BNYVV og BSBV ikke kunne påvises. Dette skyldes formentlig den lille mængde inokulum, der blev brugt ved krydsinokuleringen.

Nøgleord: Rhizomania, Beet Necrotic Yellow Vein Virus, BNYVV, Beet Soil Borne Virus, BSBV, *Polymyxa betae,* værtspektrum.

Introduction

A monitoring program for Rhizomania in Denmark was initiated in 1985. Soil samples collected in 1985 and 1986 were screened for the presence of beet necrotic yellow vein virus (BNYVV) and for its vector *Polymyxa betae* Keskin. The fungus was present in 80% of the 152 samples, but BNYVV was not detected (7).

A Swedish serotype of beet soil borne virus (BSBV) has been found associated with beet roots in Sweden and in German soil has been found infested with BNYVV. BSBV does not react with BNYVV-antiserum, but is transmitted by *P. betae* and causes symptoms resembling those of BNYVV when inoculated to *Chenopodium quinoa.* It is an unstable virus, difficult to detect by immunosorbent electron microscope (ISEM). Little is known about its effect on beet plants (13).

The results reported here represent a continuation of the BNYVV monitoring program in Denmark, which has been extended to include screening for the 86-109 serotype of BSBV (7).

Like BNYVV, BSBV is reported to infect various species of the *Chenopodiaceae* family when inoculated on leaves (6). As the spread of BNYVV and BSBV under natural conditions depends on the presence of *Polymyxa betae,* the survival of *P. betae,* BNYVV and BSBV on other host plants may play a role in the survival and spread of the 2 viruses, although the very persistent resting spores of *P. betae* are considered to be the main source of infection (14). According to the experiments of *Barr & Asher* (3) and *Abe & Ui* (2), *P. betae* is able to infect most *Beta*-species, other *Chenopodiaceae*-species and various *Amaranthaceae*- and *Portulacaceae*-species and a few other species, e.g. *Silene alba.* Cross infection tests show that there seems to be a physiological specialization within *P. betae.* The host range of each *P. betae* strain is limited to the family from which it is isolated (1). Not all *P. betae* strains are able to transmit BNYVV, e.g. viruliferous *P. betae* from sugar beet can infect *Chenopodium ficifolium,* but the virus is lost when *P. betae* from *C. ficifolium* cross infects sugar beet.

Beyond the survey program for Rhizomania, a host range experiment was carried out to determine the susceptibility of some common weeds and other species to *P. betae,* BNYVV and BSBV under Danish conditions.

Materials & methods

Soil samples were collected by 6 Danish sugar factories in October-November 1988 and 1989 as scrape from the trucks which transported beets to the factories. Of a total of 148 samples, 84 samples were collected in 1988 and 64 in 1989. The factories were: Stege, Gørlev, Nakskov, Nykøbing Falster, Assens and Sakskøbing, located in the supposedly most BNYVV-threatened regions of Denmark, i.e. Lolland and Fyn.

The baiting method of *Beemster & de Heij* (4) was used as modified by *Kloster* et al. (8). The sugar beet variety "Magnamono" susceptible to Rhizomania was used. Briefly, the method was as follows:

Seeds were germinated at 20°C under soilless humid conditions for 7-8 days. 50 grammes of soil from each sample were heated at 40°C for 30 minutes and 30-40 ml distilled water was added. 32-40 seedlings were placed in the soil as bait plants and incubated for 3-4 days. Then, the seedlings were planted in vermiculite (using 4 plastic pots with 8-10 seedlings in each one) and grown in a greenhouse at 22-25°C with a 16 hour photoperiod for 7 weeks. Water was added regularly to keep the vermiculite wet, and fertilizer was added when necessary.

For the virus detection, sap inoculation and enzyme-linked immunosorbent assay (ELISA) were used.

Sap inoculation: Side roots of 8-10 beet plants from one pot were ground with 3-4 ml distilled water and 400-mesh carborundum in a mortar and inoculated mechanically to *C. quinoa* and *C. amaranticolor.* Plants which showed symptoms after 2-3 weeks were tested by ELISA.

ELISA: The ELISA test was performed as described by *Clark & Adams* (5). Side roots of 8-10 beet plants from one pot or leaves of *C. quinoa* or *C. amaranticolor* which showed any symptoms were ground in a mortar with PSB-T extraction buffer (5) containing 2 g polyvinylpyrrolidone per 100 ml (pH = 7.4). BNYVV-antiserum and BSBV-antiserum (serotype 86-109) were kindly provided by prof. *K. Lindsten,* Uppsala, Sweden. The absorbance at 405 nm was determined by means of a Titertek Multiscan 1 hour after addition of enzyme substrate. A_{405} value higher than 0.100 was considered positive. All tests were performed as double determinations. As a positive control for both sap inoculation and ELISA, freeze-dried leaves of BNYVV-infested *C. quinoa* were used. The leaves had been stored at –20°C since 1986. The positive control for BNYVV served as negative control for BSBV.

The side roots of the bait plants were examined by light microscopy to detect the resting spores of *P. betae.* The degree of infection was not estimated.

The above mentioned positive control for BNYVV was also used for a sensitivity test comparing the 2 serological methods, ELISA and Immunosorbent Electron Microscopy (ISEM). About 0.5 gramme of the infested leaves was ground in a mortar with 2 ml distilled water. This suspension was the starting point for a dilution series, the final dilution being 1:512. The ISEM analysis was carried out as described by *Lesemann* et al. (10).

For the host range experiment Rhizomania infested soil was kindly provided from Germany by Dr. *E. Schlösser,* Giessen. The soil had been stored at 5°C for 4 years, and to check the infection capacity a susceptible sugar beet variety (Matador) was sown into the soil. The sugar beet was strongly infested by both *P. betae,* BNYVV and BSBV. The BSBV isolate from this soil reacted positively with the 86-109 antiserum derived from Sweden, i.e. the BSBV isolate belongs to the Ahlum serotype (11). The Rhizomania-soil was diluted by vermiculite at a proportion of 20:1 (v/v), and the seeds of 13 plant species from 2 families, i.e. *Chenopodiaceae* and *Amaranthaceae* including sugar beet as control were sown directly into the soil/vermiculite mixture, one pot (10×10 cm) per species. The test plants are listed in table 4. To ensure a uniform plant height within each pot, the test plants were thinned out during the bait period. The test plants were grown in a green house for 6-7 weeks at a temperature varying from 15 to 40°C.

After the bait period the roots were examined by light microscopy for the presence of *P. betae* resting spores.

The roots were tested for the presence of BNYVV and BSBV by the ELISA method as described previously.

The *P. betae* infested roots from the above mentioned host range experiment served as inoculum for cross infection. 8 seed plants of the Rhizomania-susceptible sugar beet variety, Matador, were sown in a pot containing vermiculite. The roots of the *P. betae*-donor plant were thoroughly

Table 1. Number and percentage of samples infested with BSBV tested with ELISA in 1988 and 1989.
Antal og procent prøver inficeret med BSBV bestemt ved ELISA i 1988 og 1989.

	1988			1989			Total *I alt*		
Factory *Fabrik*	Number of samples *Antal prøver*	Number infested *Antal inficeret*	% infested *% inficeret*	Number of samples *Antal prøver*	Number infested *Antal inficeret*	% infested *% inficeret*	Number of samples *Antal prøver*	Number infested *Antal inficeret*	% infested *% inficeret*
Assens	15	7	47	23	10	44	38	17	45
Gørlev	15	10	67	0	0	0	15	10	67
Nakskov	20	17	85	22	6	27	42	23	55
Nykøbing	14	9	64	0	0	0	14	9	64
Stege	15	13	87	0	0	0	15	13	87
Sakskøbing	5	5	100	19	18	95	24	23	96
Total	84	61	73	64	34	53	148	95	64

washed in water, cut into small pieces and placed on top of the 8 sugar beet seeds before being covered with vermiculite. After another bait period of 5-6 weeks the sugar beet rootlets were examined for the presence of *P. betae,* BNYVV and BSBV as described previously.

Results

BNYVV was not found in any soil sample, either by sap inoculation or by ELISA.

Soil samples collected by Stege and Sakskøbing factories were found to be heavily infested with BSBV while samples from the other factories were moderately infested. The results of the virus detection are listed in Table 1.

All the soil samples were found to be infested with *P. betae,* but the degree of infection was not estimated.

Symptoms caused by mechanical inoculation of BNYVV and BSBV on leaves of *C. quinoa* and *C. amaranticolor* were compared. BNYVV caused local diffuse chlorotic lesions in both plants 10-14 days after inoculation. The lesions spread along the leaf veins, enlarged and turned into a red-yellow necrotic patch. BSBV caused similar symptoms on *C. quinoa* leaves initially but after 7-10 days these lesions enlarged and turned into a large brown necrotic patch. *C. amaranticolor* developed local lesions after 10-14 days. These lesions were large and round with a yellow colour and necrotised in the middle, but did not coalesce.

The sensitivities of the 2 detection methods: sap inoculation and ELISA, for detection of BSBV were compared (see Table 2). The 86-109 serotype of BSBV was found in 64% of the soil samples using ELISA, but only in 30% of the samples using sap inoculation.

The sensitivity of the ELISA method was com-

Table 2. Detection of Beet Soil Borne Virus in the side roots of bait plants by ELISA and by sap inoculation on *Chenopodium quinoa* and *C. amaranticolor.*
Påvisning af Beet Soil Borne Virus i siderødderne på fangplanter ved hjælp af ELISA og ved saftinokulering af Chenopodium quinoa *og* C. amaranticolor.

	Number of samples *Antal prøver*	Number of positive reactions by *Antal positive reaktioner ved*	
		Inoculation *Inokulering*	ELISA
BSBV	148	44(30%)	95(64%)

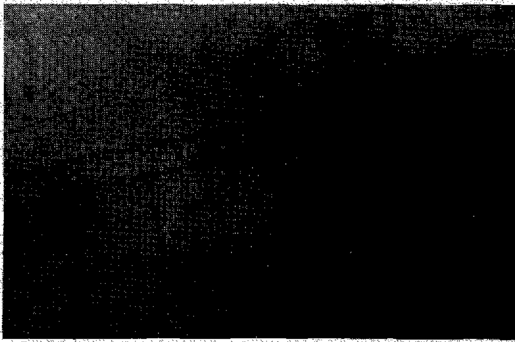

Figure 1. Resting spores of *Polymyxa betae* in the side roots of red beet. × 400.
(Foto: Solveig Danielsen)
Fig. 1. Polymyxa betae-*hvilesporer i siderødderne af rødbede. 400×forstørrelse.*

Table 3. Comparison of dilution end points for BNYVV by ELISA and ISEM tests.
Sammenligning af fortyndingsendepunkter for BNYVV målt ved ELISA og ISEM.

Dilution *Fortynding*	ELISA A_{405} [a]	ISEM +/–
1/1	0.744	+
1/2	0.622	+
1/4	0.717	+
1/8	0.790	+
1/16	0.555	+
1/32	0.247	–
1/64	0.138	–
1/128	0.109	–
1/256	0.068	–
1/512	0.017	–

[a] Mean value of 2 determinations; A_{405} value higher than 0.100 is considered as positive
Gennemsnit af 2 bestemmelser; A_{405}-værdier større end 0,100 anses for at være positive

pared to ISEM for detection of BNYVV (see Table 3). ISEM detected virus particles up to a 1:16 dilution while the virus was detectable up to a 1:128 dilution by ELISA.

The results of the host range experiment are shown in table 4 and 5. 12 out of 13 test plants were infested with *P. betae.* Figure 1 shows *P. betae* resting spores in the roots of red beet. The *P. betae* infected species can be roughly divided into 2 groups depending on the amount of resting spores in the roots: Group 1 (very few resting spores) and Group 2 (a large number of resting spores) as indicated in table 4. The roots of *Spinacia oleracea* were extensively damaged due to the very high temperature in the green house (40°C). The P. betae structures found in the roots of *Amaranthus retroflexus* appeared to be very weak and diffuse and are more likely to be zoosporangia or plasmodia rather than resting spores.

4 species were found to be susceptible to BNYVV, namely sugar beet (control), *Spinacia oleracea* (spinach), *C. murale,* and red beet, while BSBV infested a total of 8 species (see Table 4). None of the virus infested plants showed any root or leaf symptoms. Figures 2 and 3 show BNYVV and BSBV particles, respectively, isolated from the roots of *C. murale.*

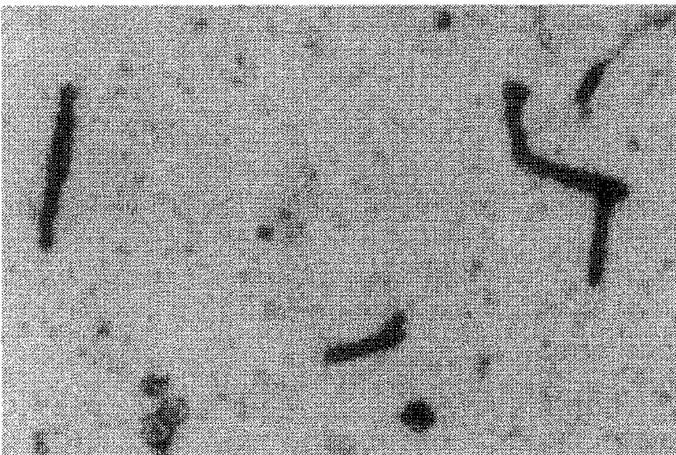

Figure 2. Antiserum decorated BNYVV particles from the roots of *Chenopodium murale.* ISEM test. × 93.000.
(Foto: A.M. Ravnkilde).
Fig. 2. Antiserumdekorerede BNYVV-partikler fra rødderne på Chenopodium murale. *ISEM-test. 93.000×forstørrelse.*

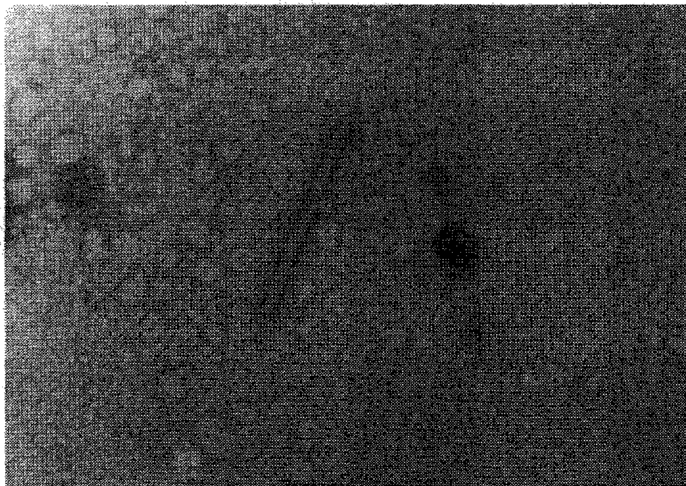

Figure 3. Antiserum decorated BSBV particles from the roots of *Chenopodium murale.* ISEM test.×250.000. (Foto: A.M. Ravnkilde).

Fig. 3. Antiserumdekorerede BSBV-partikler fra rødderne på Chenopodium murale. *ISEM-test. 250.000×forstørrelse.*

Table 4. Susceptibility of test plants to *Polymyxa betae,* Beet Necrotic Yellow Vein Virus and Beet Soil Borne Virus (Ahlum-serotype). The numbers below the *P. betae* column indicate the number of infested plants and the total number of plants, respectively.

Testplanters følsomhed overfor Polymyxa betae, *Beet Necrotic Yellow Vein Virus og Beet Soil Borne Virus (Ahlum-serotype). Tallene i P. betae-søjlen angiver henholdsvis antallet af inficerede planter og det totale antal planter.*

Test plant *Testplante*	*Polymyxa betae*		BNYVV	BSBV
Amaranthus retroflexus L.	11/17	1)	–	+
Chenopodium ficifolium Sm.	5/6	1)	–	–
C. glaucum L.	15/17	1)	–	+
C. polyspermum L.	15/16	1)	–	–
C. rubrum L.	0/26		–	–
Atriplex patula L.	10/10	2)	–	+
Beta vulgaris L. (red beet/*rødbede)*	9/9	2)	+	+
B. vulgaris L. (sugar beet/*sukkerroe)*	8/8	2)	+	+
C. album L.	15/15	2)	–	–
C. amaranticolor C. et R.	10/10	2)	–	–
C. murale L.	23/23	2)	+	+
C. quinoa Willd.	7/7	2)	–	+
Spinacia oleracea L.	6/8	2)	+	+

1) Very few resting spores in the side roots
Meget få hvilesporer i siderødderne

2) A large number of resting spores in the side roots
Et stort antal hvilesporer i siderødderne

\+ positive reaction by ELISA
positiv reaktion ved ELISA

– negative reaction by ELISA
negativ reaktion ved ELISA

The cross inoculation of sugar beet with *P. betae* from various donor plants gave infection in 8 cases (see Table 5) although the number of resting spores was very small.

The ELISA analysis for BNYVV and BSBV in the sugar beet rootlets showed no positive reaction for any of the donor plants.

Discussion

BNYVV was not detected in any of the soil samples. The presence of *P. betae* in all the soil samples shows, however, that there is a great potential risk of the disease being spread if the virus is brought to Denmark.

The BSBV, serotype 86-109, was detected in 64% of the soil samples. This level of infection has been found in other European countries, e.g. Sweden and Belgium (*Månsson,* unpublished results, 15). The percentage of samples infected with BSBV varied between factories. In the case of one factory (Nakskov) the percentage was also widely different in 1989 compared to 1988. However, the material is too limited to allow firm conclusions concerning the geographical and temporal variation of BSBV infection. All samples were simply classified as either positive or negative, so the degree of infection has not been estimated.

C. amaranticolor was better than *C. quinoa* for distinguishing between BNYVV and BSBV. For routine tests, however, the sap inoculation was found to be very inferior to ELISA. Not even half of the BSBV infections were identified by sap inoculation compared to ELISA. This is supported by the findings of *Lindsten* (12) who showed that sap inoculation is unreliable for BNYVV detection. Therefore, the sap inoculation method should be used only for the production of inocula and not for virus detection in routine tests.

The ELISA method was found to be superior to ISEM for detecting BNYVV. The sensitivity was considerably higher (8 times), and the ELISA method is much less time consuming when a large number of samples have to be tested. *Koenig* et al. (9) found the ELISA method to be 10 times as sensitive as ISEM for the detection of BNYVV.

The *P. betae* isolate used for the host range experiment must be considered to originate from sugar beet, as the soil sample from Germany was collected from a Rhizomania infested sugar beet growing area. 4 plant species were lightly infested by this *P. betae* isolate as they produced very few resting spores (group 1). 2 of these species *(C. glaucum* and *A. retroflexus)* were furthermore infested by BSBV but not by BNYVV. From this group the cross inoculation of sugar beet only gave one *P. betae* infection, namely with *C. polyspermum* as donor plant. None of the viruses was found in *C. polyspermum* by this experiment.

Table 5. Cross inoculation of *Beta vulgaris* (sugar beet) with *Polymyxa betae* derived from the roots of donor plants. Root pieces containing resting spores of P. betae. were used as inoculum.

Krydsinokulering af Beta vulgaris *(sukkerroe) med* Polymyxa betae *fra rødderne af donorplanter. Rodstykker med hvilesporer blev anvendt som inokulum.*

Donor plant *Donorplante*	P. betae infection on sugar beet *P. betae-infektion i sukkerroe*
Amaranthus retroflexus	–
Chenopodium ficifolium	–
C. glaucum	–
C. polyspermum	+
Atriplex patula	+
Beta vulgaris (red beet/*rødbede)*	+
B. vulgaris (sugar beet/*sukkerroe)*	+
C. album	+
C. amaranticolor	+
C. murale	+
C. quinoa	+

– No P. betae infection
Ingen P. betae-infektion
\+ P. betae infection
P. betae-infektion

The 8 plant species that were heavily infested by *P. betae* (group 2) also showed a higher degree of virus propagation, especially for BSBV, than the test plants from group 1. The *P. betae* cross inoculation of sugar beet was successful in all cases, although the number of resting spores found in the rootlets of sugar beet was very low. *Abe & Tamada* (1) found that at least 50 resting spore clusters, each containing 35 resting spores, are needed for *P. betae* infection of one plant. The same number of viruliferous resting spores forms the lower limit of BNYVV detection by ELISA. Although this value may differ between P. betae strains, the lack of *P. betae* cross infection by 3 species from group 1 and the lack of BNYVV and

BSBV cross infection in all cases might be due to an insufficient amount of inoculum used for the cross infection of sugar beet. Therefore, whether BSBV and BNYVV are able to cross infect sugar beet from another host can not be established by this experiment.

Compared to other experiments (1, 2) this *P. betae* isolate has a broad host range, even though the fungus showed a poor development in some species.

Among the test plants, *C. album, Atriplex patula, C. glaucum, C. rubrum* and *C. polyspermum* are common weeds in sugar beet growing areas in Denmark. Although all of these species, except for *C. rubrum,* are able to propagate the virus vector, they do not seem to increase the spread of Rhizomania. BNYVV is apparently not able to reproduce in these host plants and is lost.

On the other hand, BSBV is less specific than BNYVV and seems to be able to propagate on a broader range of host plants. But whether BSBV is able to maintain its infectivity after several cross infections can not be concluded from this experiment. Further investigation is needed to establish the significance of BSBV.

Conclusion

The Rhizomania virus was not found in any of the 148 soil samples. The virus vector, *P. betae,* was found in all soil samples, i.e. there is a potential risk of a rapid spread of the disease if the virus is introduced into the country.

A Swedish serotype (86-109) of beet soil borne virus (BSBV) was found widely spread in the sugar beet growing areas. 64% of the soil samples contained this virus. BSBV does not seem to have any impact on the yield of sugar beet.

Although viruliferous *P. betae* is able to attack various plant species from the *Chenopodiaceae* and *Amaranthaceae* families, the spread of Rhizomania by alternative host plants seems to be of limited importance as none of the most common weed species were infested with BNYVV.

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