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Horizontal spread of beet necrotic yellow vein virus in soil

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Abstract

Horizontal dispersal of beet necrotic yellow vein virus (BNYVV) by means of viruliferous zoospores of *Polymyxa betae* was studied in greenhouse experiments. BNYVV was not detected in roots of sugar beet plants grown in silver sand for 4 weeks at a root-free distance of 5 cm from either *P. betae*- and BNYVV-infected plants or BNYVV-infested soil. Spread of BNYVV from inoculum sources in the field was studied in the absence and presence of tillage practices. Active dispersal in combination with root growth from and towards point sources of inoculum contributed only little to horizontal dispersal of viruliferous inoculum and spread of disease during the season, as determined for one soil type, two different years and in the absence of tillage and tread. In the second beet crop after application of inoculum to whole field plots, more BNYVV-infected plants were detected at 2 m than at 8 m distance from the infested plots in the tillage direction. In the third year, disease incidence at 8 m was high and equivalent to that at 2 m.

Additional keywords: BNYVV, *Polymyxa betae*, epidemiology, dispersal, tillage

Introduction

Epidemiological studies on rhizomania of sugar beet require knowledge of distances of spread of the disease. The causal agent, beet necrotic yellow vein virus (BNYVV), is transmitted by the fungus *Polymyxa betae*. Spread of the virus thus mainly depends on the behaviour of the vector and on root development of the plant. By definition a pathogen spreads where it goes and infects, and dispersal is the movement of propagules from infected tissue or plants to healthy susceptible tissue or plants (Van der Plank, 1967). Different modes of dispersal of viruliferous propagules of *P. betae* through or with soil can be distinguished. First, there is 'active dispersal' by zoospores moving autonomously through soil. Second, there are various ways in which propagules of *P. betae* are 'passively dispersed', i.e. without having to spend their energy. Zoospores can be passively displaced by water currents over the soil surface or through soil pores. The persistent resting spores can be dispersed by means of movement of contaminated soil and manure and by water (Hillmann, 1984; Heijbroek, 1987).

In a greenhouse experiment, distances covered by active dispersal were investigated. Field trials were set up in 1988 and 1989 in order to estimate distances of spread of rhizomania from point sources of inoculum in a sugar beet crop under field conditions, in the absence of soil tillage. In addition, in plots of the 1988 trial, it was studied how the extension of the different original inoculum sources manifested itself in a second beet crop. Spread of rhizomania by displacement of infested soil was investigated in another field trial.

Material and methods

I. Experiments with inoculum sources

Greenhouse experiment

Dispersal of viruliferous zoospores was assessed by growing target plants at various root-free distances from different inoculum sources. In wide shallow trays, 30 × 30 × 8 cm, 2 cm wide and 30 cm long pockets of Monodur gauze (50 μm) were placed, filled with either a mixture (50/50) of rhizomania-infested soil and silver sand or silver sand only and planted with sugar beet seedlings cv. Regina infected by a short incubation in infested soil (according to Beemster and De Heij, 1987). Trays were filled with sterilized fine silver sand to which 0.25 g NPK (14–16–18) and 0.30 g lime fertilizer per l were added, the pH was thus adjusted to 7.0. Target plants, sugar beet cv. Regina, were sown at distances of 5 and 10 cm from the two types of inoculum sources. Their root growth towards the inoculum sources was prevented by Monodur gauze (50 μm). Two controls were included: trays without an inoculum source and trays of which the upper layer of 1–2 cm was infested with rhizomania-infested soil. Each treatment was replicated three times. Water was given regularly from below (soil water potential fluctuated between approximately –3 and –6 cbar). Temperature in the greenhouse was about 23 °C during daytime (16 h) and 15 °C at night. Two and four weeks after emergence (emergence about 6 days after sowing), the target plants were sampled and tested by ELISA for the presence of BNYVV, either directly or after 5 weeks growing in sterile silver sand.

Field experiments

In 1988 and 1989, two identical field trials were laid out on a calcareous clay soil at an isolated site in an urban area in the Noordoostpolder, the Netherlands. The trials were situated at adjacent parts of the field. Experimental details are given in Table 1. Sugar beet had never been grown in this field. Circular inoculum sources (described below) were created, target plants were grown at different distances from the sources and the area between target and source was devoid of plants (Fig. 1). Control plots were either non-in-

Table 1. Experimental details of the field trials in 1988 and 1989 on adjacent parts of a field.

Parameter	1988	1989
Soil		
pH-KCl	7.1	7.1
organic matter %	6.7	6.7
Layout		
plot size (m^2)	3 × 4	3 × 3
Sowing		
date	15 April	2 May
distance within row (cm)	5[a]	10
distance between rows (cm)	12.5	25
number of target plants at 25 cm, per plot	12	6
number of target plants at 50 cm, per plot	24	10
Inoculum		
MPNs[b] of infective units BNYVV	500	6

[a] Plants were thinned to a distance of approximately 10 cm.
[b] Most probable numbers per 100 g dry soil.

Fig. 1. Inoculum source in a field trial with target plants at a distance of 25 cm.

fested or infested by application of infested soil to the top layer. The five treatments (Table 2) with four replications were laid out in a randomized block design. Plots were separated by 3 m wide grass borders. In the available free space at the experimental location some extra plots with inoculum sources were arranged as described in Table 3.

Table 2. Treatments in the field trials of 1988 and 1989 and details of plant sampling at the end of the growing season.

Treatment	Type of inoculum source[a]	Approximate distance of target plants[b]	Number of target plants sampled			
			1988		1989	
			25 cm	50 cm	25 cm	50 cm
1	Soil without plants	25 cm	16	–	24	–
2	Soil with plants	25 cm	16	–	24	–
3	Soil with plants	50 cm	–	24	–	24
4[c]	Non-infested	–	8	12	12	12
5[c]	Superficially infested	–	8	12	12	12

[a] The circular inoculum sources in Treatments 1, 2 and 3 had a diameter of 20 cm and a depth of 12 cm. In Treatment 5, infested soil was spread evenly over the surface of the whole plot.
[b] Actual distances between target plants and inoculum sources varied between 20–30 cm and 45–55 cm for the distances presented as 25 and 50 cm.
[c] Plant samples were taken at positions corresponding to the sampling positions in Treatments 1–3.

Table 3. Treatments and assessments performed in single plots to determine BNYVV at different distances from inoculum sources, either in target plants by ELISA or in soil by bioassay.

Year	Treatment: inoculum source[a]	Assessments	BNYVV-infected plants		
			Target[b]	Bioassay[c]	
				20,25 cm	50,60 cm
1988	Circular source of soil in between two rows of beet; sowing distance 18.5 cm, 50 cm between the rows	All plants in three rows of 2 m length on either side of the source tested for BNYVV	0/59		
		Bioassay of soil samples at 20 and 60 cm distance		7/10	0/10
	Circular source of soil in fallow plot	Bioassay of soil sample at 20 cm distance		0/10	
1989	Circular source of soil in fallow plot	Bioassay of soil samples at 25 and 50 cm distance		0/10	0/10
	Circular source of soil with plants in a plot left fallow	Bioassay of soil samples at 25 and 50 cm distance		0/10	0/10

[a] Circular inoculum sources had a diameter of 20 cm and a depth of 12 cm.
[b] Distances between inoculum source and nine target plants in the adjoining row on either side of the source varied between 15 and 45 cm. The other 50 target plants grew at larger distances.
[c] Soil samples consisted of 40 cores, taken at the described distances. Bioassays were performed with 10 plants per soil sample, as indicated (/10).

Sowing. In both years sugar beet cv. Regina was sown. To ensure sufficient plants at the desired distances from the inoculum sources, sowing was dense (Table 1). The numbers of target plants per plot at a distance of 25 and 50 cm from the source are given in Table 1. Seedlings and weeds in the zone between inoculum source and target plants were removed directly after emergence.

Inoculum. Soil of the same rhizomania-infested field in the Noordoostpolder was used as inoculum in both years. However, quantitative assessments of the inoculum (Tuitert, 1990) showed a big difference in level of infestation between the two years (Table 1). Inoculum was applied within one week after sowing. In order to create the inoculum sources (Table 2, Treatments 1–3) a round hole was dug in the middle of the plot, with a diameter of 20 cm and a depth of 12 cm. The hole was carefully filled with a mixture of 2 l infested soil and 1 l non-infested soil from the trial field. The soil mixture was compressed to enable addition of a 2 cm thick top layer of non-infested soil. For Treatments 2 and 3 three seeds were sown in this site. Treatment 5 was infested with approximately 48 g soil per m^2 in 1988 and 3 kg soil per m^2 in 1989, equally spread over the top layer. The higher amount of soil applied in 1989 was based on a preliminary bioassay indicating a lower level of infestation of this soil. After the MPNs had been determined (Table 1), the amount used in the field appeared to be adequate, compared to 1988. To avoid spread by treading the plots, a movable bridge spanning the plots was used from which all activities were performed. Machinery was not used on the plots after inoculum had been applied.

Determination of BNYVV in target plants. Target plants at the nearest distances were sampled on 11 October 1988 and 15 September 1989 (Table 2). In 1988, plants were also periodically sampled during the season. From each plant, sap from the root tip was analysed by double antibody sandwich ELISA (Clark and Adams, 1977; Tuitert, 1990) for the presence of BNYVV. The plants growing in the sources were analysed too.

Determination of BNYVV in soil. Before establishing the trials, all plots were sampled to a depth of 25 cm with 42 cores of 1.3 cm diameter, taken in a regular grid (0.5 × 0.4–0.6 m). To register the extent of spread of BNYVV from the sources in soil, soil samples were taken in October 1988 and September 1989. After careful removal of the plants, preventing displacement of adhering soil, soil samples consisting of 40 cores were collected along a circle around the sources. For Treatments 1 and 2 sampling distances from the sources were 25 cm and 50 cm. In Treatment 3 the cores were taken at 50 cm in 1988 and at 25 and 50 cm in 1989. From the non-infested and infested control plots 40 cores were taken by sampling the whole area of the plots. Soil samples were air-dried, ground and mixed with 50 % (v/v) of sterile river sand. Per soil sample ten (1988) or six (1989) pots were filled with 200 ml of this mixture. In each pot one sugar beet seedling (cv. Regina) was planted. After a 6-week growing period in the greenhouse, sap was collected from the roots and analysed by ELISA.

Extension of inoculum sources measured in the second year. From the plots of the 1988 trial all plants were removed by hand in October. In November 1988, plots were cultivated to a depth of 20–25 cm with a spading machine used in horticultural crops (width 1.50 m), to avoid the sideward displacement of soil by ploughing. The seedbed was prepared using a power harrow and beet cv. Regina was sown again in 1989. Sowing distance was 18.5 cm with 50 cm between the rows. In September, all plants in 1 m^2 around the 1988 inoculum sources were analysed for the presence of BNYVV by ELISA. At a distance of 1 m from this square meter eight plants were taken and also analysed for BNYVV (distance to original source approximately 1.4 m).

II. Dispersal of inoculum by soil displacement.

Assessments in a field experiment

A field trial with different levels of inoculum, with or without irrigation, was laid out in 1988 (Tuitert and Hofmeester, 1992). Spread of BNYVV by means of infested soil was monitored yearly by analysing target plants at two distances from an infested area. As described for the complete trial, three of the four blocks were used for assessments. Behind the plots with the highest inoculum levels there were buffer zones (extra non-infested plots) in which mechanical operations ended to free the implements from infested soil (Figure 1 in Tuitert and Hofmeester, 1992). These plots were sown with beet similar to the other plots.

Plots were infested just before sowing in 1988. A tractor and a seed drill were the only machinery used, under dry conditions, after the inoculum was applied. In October 1988, target plants were sampled in the buffer zones at 2 and 8 m distance from the infested plot. The remaining plants were harvested by hand. In November 1988, plots were spaded as described before. Under dry conditions a power harrow was used to prepare the seedbed before sowing in 1989. In September 1989, plants were sampled again at 2 and 8 m from the infested plots. After spading in December 1989, seedbed preparation and sowing in April 1990, a third sampling was performed in October 1990.

Target plants were analysed for infection by BNYVV by means of ELISA using sap from the tips of the tap roots. The total number of plants examined per distance was 24 in all 3 years.

Results

I. Experiments with inoculum sources

Active dispersal in a greenhouse experiment
At 2 and 4 weeks after emergence of bait plants, none was infected by BNYVV at 5 and 10 cm from the inoculum sources. In the superficially infested control, all plants were infected after 4 weeks. Infection was absent in the non-infested control. The zoospore-emitting plants in the inoculum sources were all BNYVV-positive.

Spread of BNYVV from inoculum sources in the field
In none of the plots BNYVV was detected by bioassay in soil samples taken before the experiment started. In the single plot with a source of infested soil in between two rows, BNYVV-infected plants were not detected (Table 3). Distances between the inoculum source and the heart of the nine nearest plants in adjoining rows varied between 15 and 45 cm. In soil, BNYVV was detected at a distance of 20 cm from the source. BNYVV was not detected in soil around inoculum sources when there had been no target plants during the season (Table 3).

Detection of BNYVV-infected plants in the field. At the end of the growing season of 1988, two out of sixteen plants at 25 cm from an inoculum source were found to be infected by BNYVV, with low absorbance values in ELISA (Treatment 1, Table 2). In the other treatments, including the non-infested and the superficially infested plots, BNYVV-infected plants were not detected. Most plants growing in the inoculum sources showed rhizomania symptoms; all were highly positive in ELISA. In 1989, in none of the treatments BNYVV-infected plants were found. The only plants infected were those growing in the inoculum sources.

Detection of BNYVV in soil. Dispersal of BNYVV in soil had occurred at least over a distance of 25 cm in 1988 (Table 4), and perhaps over 50 cm. When the plant-free zone was 50 cm (Treatment 3), BNYVV was not detected at that distance. In 1989, dispersal had occurred over a distance of 25 cm (Table 4), but not when the inoculum source was without plants (Treatment 1). In Treatment 3 an additional assessment was made halfway between the plant-free zone and BNYVV was detected.

Extension of inoculum sources measured in the second year. The mean incidences of BNYVV-infected plants in 1989 around the inoculum sources of 1988 are presented in Table 5. Treatment effects on incidences were significant ($P < 0.001$) in the analysis of variance of angular transformed percentages. Treatment 1 was not different from the originally non-infested control (Treatment 4), but Treatments 2 and 3 were. In none of these treatments, the incidence of the completely infested plots (Treatment 5) was attained. At 1.4 m distance of the sources an occasional infected plant was detected (Table 5). Treatments 1 to 4 were not significantly different, only the superficially infested plots (Treatment 5) had a significantly higher incidence.

Table 4. Detection of BNYVV in soil at different distances from inoculum sources in the field. Experiments performed in 1988 and 1989. Four plots per treatment per year. One soil sample per sampling distance per plot. Bioassays with ten (1988) or six (1989) plants per soil sample.

Treatment[a]	Presence (+) or absence (–) of BNYVV-infected plants in bioassay[b]					
	1988			1989		
	25 cm	50 cm	plot area	25 cm	50 cm	plot area
1	+ (3/40)	+ (1/40)		– (0/24)	– (0/24)	
2	+ (3/40)	– (0/40)		+ (4/24)	– (0/24)	
3		– (0/40)		+ (1/24)	– (0/24)	
4			– (0/40)			– (0/24)
5			+ (16/40)			+ (4/24)

[a] Circular inoculum source for Treatment 1 was soil and for Treatments 2 and 3 soil with plants. Distances to target plants were 25, 25 and 50 cm, for Treatments 1, 2 and 3, respectively. Treatments 4 and 5 were non-infested or superficially infested with soil, respectively.
[b] Proportion of infected plants in bioassay given in parentheses.

II. Dispersal of inoculum by soil displacement

The effects of displacement of BNYVV-infested soil as shown by the incidences of infected plants are given in Table 6. In 1988, infection by BNYVV was not detected. In 1989, many plants were infected. Analysis of variance was performed on angular transformed percentages of infected plants, considering the split-plot design of the trial. The difference between the incidence of infected plants at 2 and at 8 m was significant ($P < 0.05$). In 1990, the plots were totally diseased. The apparent effect of irrigation on the expression of the displaced infested soil in 1989 was not significant ($P = 0.09$) at the confidence level applied.

Table 5. Incidences of BNYVV-infected plants in 1989 in 1 m^2 around the original inoculum sources of 1988 (diameter 20 cm, depth 12 cm) and at a distance of 1.4 m thereof.

Treatment	Type of inoculum source in 1988[a]	Distance of target plants in 1988	Proportion of BNYVV-infected plants in 1989[b]	
			Area 1 m^2	Distance 1.4 m
1	Soil without plants	25 cm	3/33 (9%) a	2/32 (6%) a
2	Soil with plants	25 cm	9/35 (26%) b	1/32 (3%) a
3	Soil with plants	50 cm	13/37 (35%) b	4/32 (13%) a
4	Non-infested	–	1/30 (3%) a	0/32 (0%) a
5	Superficially infested	–	29/38 (76%) c	20/32 (63%) b

[a] Treatments 1, 2 and 3 had circular inoculum sources, in Treatment 5 infested soil was spread evenly over the surface of the whole plots.
[b] In parentheses: arithmetic mean percentage of BNYVV-infected plants, determined by ELISA. Treatments were significantly different in ANOVA of angular transformed percentages ($P < 0.001$, for both area and distance assessments). Treatment means with the same letter are not significantly different ($P < 0.05$) according to Duncan's new multiple range test (Duncan, 1955).

Table 6. The incidence of BNYVV-infected plants at two distances from infested plots in the direction of the mechanical tillage operations. Assessments in three consecutive beet crops following artificial infestation of the plots in 1988[a].

Year	Number of BNYVV-infected plants[b]	
	2 m	8 m
1988	0 (0%)	0 (0%)
1989[c]	17 (71%)	10 (42%)
1990	23 (96%)	23 (96%)

[a] The trial was arranged in a split-plot design with drip irrigation levels (two) as main plots (Tuitert & Hofmeester, 1992).
[b] Number of plants tested per distance per year was 24. In parentheses: arithmetic mean percentage of BNYVV-infected plants.
[c] The difference between the two distances was significant ($P < 0.05$), the effect of irrigation was not significant ($P = 0.09$), and there was no interaction according to ANOVA of angular transformed percentages. In non-irrigated and irrigated plots the mean numbers of infected plants out of 24 (averaged over the two distances) were 10 and 17, respectively.

Discussion

Active dispersal of viruliferous P. betae. Spread of BNYVV in soil by active dispersal of vector propagules appears to be restricted by the limited distance covered by zoospores of *P. betae*. Relatively large distances, compared to the dimensions of a zoospore, were investigated. When the distance between infected 'source' roots and target roots was 5 cm, transmission of BNYVV to target roots was not detected. Under the prevailing conditions with fine sand, a fluctuating soil water potential and no watering from above, the distance of migration of viruliferous zoospores, resulting in infection, apparently was less than 5 cm. The presence of BNYVV in the roots of the infected source plants was verified, but the efficiency of the source plants as zoospore 'emitters' was not tested. In order to check the zoospore production and infection potential of the source plants during the experiment, a treatment could have been added with source plants surrounded by healthy target plants planted at different times. Application of a zoospore suspension to soil, instead of using infected plants as zoospore sources, would perhaps have resulted in migration of the zoospores over larger distances because of the absence of nearby source roots to re-infect. Soil water potential fluctuated between –3 and –6 cbar but fulfilled the requirements for infection of *P. betae* as determined in pot experiments (Gerik et al., 1990; De Heij, 1991). Literature on autonomous zoospore movement of other fungi shows that a few centimeters can be covered, 2–4 cm for *Phytophthora cryptogea* (Duniway, 1976), 4 cm for *Olpidium brassicae* (Westerlund et al., 1978), and that soil texture and soil moisture influence the distance.

At 5 and 10 cm distance from an inoculum source consisting of infested soil, BNYVV-infected plants were not detected. From this observation it may not be deduced that resting spores in soil were not induced to germinate by exudates of target roots at these distances, because zoospores, if released, could not overcome a distance of 5 cm. In general, the effect of root exudates on fungal propagules is limited to small distances from the root surface; from 1.5 and 3 mm for *Pythium ultimum* (Johnson and Arroyo, 1983) and *Phytophthora cinnamomi* (Zentmeyer, 1961), respectively, up to 10 mm for *Sclerotium cepivorum* (Coley-Smith, 1960) and 12–16 mm for *Gaeumannomyces graminis* (Gilligan and Simons, 1987).

Spread of BNYVV from inoculum sources in the field. For one soil type, two different years and in the absence of tillage and tread, it was shown that active dispersal, in combination with root growth, contributed only little to the horizontal dispersal of inoculum and spread of disease during the season. At 25 cm from an inoculum source, either infested soil or infested soil with plants, BNYVV was detected in only 2.5% of all plants sampled at that distance. At 50 cm, BNYVV-infected plants were not detected at all. In a single plot with a 20-cm-diameter source of infested soil between two rows at 50 cm distance, infected plants were not even detected in the adjoining rows (Table 3).

As it was surmised that roots could have become infected too late in the season to detect the virus in the tap root of the plant, soil samples were taken at the target distances after plants were removed, and assayed for viruliferous inoculum. The observations on root growth patterns by Brown and Biscoe (1985) supported the assumption that root contact of target roots with infested soil or the intertwining of infected and target roots can cause spread of the disease later in the season. Root density mid-way between rows (at 50 cm distance) was shown to be equal to root density close to the plant by mid-June (Brown and Biscoe, 1985). Nagata (1970) also observed an extension of roots till mid-way between the rows from July onwards. During the first two months after sowing, lateral roots extended only 5 cm horizontally (Nagata, 1970; Brown and Biscoe, 1985).

A bioassay on the soil samples revealed the presence of viruliferous *P. betae* at a distance of 25 cm from the inoculum sources (Table 4). When an area of 50 cm around the source had been kept free of plants, BNYVV was also detected at 25 cm distance (in 1989). This observation might suggest a role for root growth from the inoculum source in dispersal of inoculum, a suggestion in line with the failure to detect BNYVV at that distance around plant-free inoculum sources in fallow plots (Table 3). The role of root growth in the build-up and spread of inoculum was also evident from the disease incidence recordings in the second year after creating the inoculum sources. Disease incidence in 1 m^2 around the initial sources in which plants had been growing was significantly higher than in the absence of plants in the source (Table 5). Still, the incidences around sources were lower than those recorded in plots where inoculum had been applied to the whole surface. A trace of infestation, one out of 40 bioassay plants was BNYVV-positive with a low absorbance value in ELISA, was found at 50 cm distance when only infested soil was the inoculum source (Table 4). It is doubted whether this single observation should be ascribed to dispersal through the soil, because of the low incidence of disease in this treatment in the second year (Table 5). Besides, in 1989 BNYVV was not even detected at 25 cm in this treatment, although here the lower infestation level of the point source might have played a role. The latter may also explain why BNYVV was not found at 25 cm distance in the single fallow plot where plants grew in the inoculum source (Table 3). It should be added that environmental circumstances differed in both years. The warm summer of 1989, combined with a lower frequency and amount of precipitation compared to 1988, led to relatively dry soil moisture conditions, not favouring development of *P. betae*.

Epidemiologic implications. The results obtained indicate that secondary infection of a plant is not likely to occur from one row to another, current row spacing being 50 cm. The usual sowing distance within the row is 18.5 cm. A treatment with an infected plant within the row was not included and distances smaller than 20–30 cm were not investigated. And although viruliferous inoculum could be detected at 20–25 cm distance from a point source, detectable infection (BNYVV in tap root, occurrence of symptoms) of a plant by adjacent plants is not expected to play an important role in the increase of disease during the season. Therefore, it is suggested that the increase in disease incidence in the plant

population will depend on infection by inoculum (resting spores) present in the soil rather than on plant-to-plant spread of secondary zoospores.

Thus, although inoculum multiplies during the season by means of several secondary infection cycles, whereby the increase in number of infections on roots of individual plants is a polycyclic process, the hypothesis is put forward that epidemic development of rhizomania in the field will generally be a monocyclic process ('simple interest disease'; Van der Plank, 1963).

The situation may be different under very wet conditions (heavy showers, irrigation), depending on the soil type and the slope of the field (Shew, 1987), when transport of zoospores by runoff water over the surface or through soil pores (Wilkinson et al., 1981) will be more frequent. Besides having a direct moisture effect as just mentioned, irrigation may increase secondary spread of the disease because it favours root growth in the upper soil layers (Brown et al., 1987; Wild and Russell, 1988). Plant density will influence the frequency of plant-to-plant spread by changing the moment and intensity of root contact.

An increase in disease incidence in the field, resulting from conditions favouring the secondary infection cycle of *P. betae* and virus multiplication in the plant, should not be ascribed to spread, but primarily to recurrent infection of the same plant, an increasing virus content of the plant and/or an increased frequency of primary infections of the growing root mass exploring a larger volume of the infested soil.

Literature data on field experiments that confirm or negate these results and hypotheses stated are not available. The statement of Thresh (1986) on spread by soil-borne fungal vectors still applies: 'there is little information on the pattern and sequence of spread in crops, or on the relative amounts of spread over long and short distances'.

Dispersal of inoculum by soil displacement. In plots where circular inoculum sources were created in 1988, only few infected plants were detected at 1.4 m distance from the sources in 1989 (Table 5). Incidences of infected plants in these plots were not significantly different from those in the originally non-infested, and still disease-free, plots. Without tillage, viruliferous inoculum was detected at 25 cm from the sources in 1988. Tillage practices performed, spading, harrowing and drilling, were most likely responsible for displacement of infested soil resulting in diseased plants at further distances in 1989.

With whole-plot infestations, soil displacement can have more remarkable effects than with relatively small sources, as is apparent from the high incidences of infected plants at 2 and 8 m distance from infested plots in 1989 (Table 6). In 1988, BNYVV-infected plants were not detected at 2 and 8 m behind infested plots in the field trial with different inoculum levels. Nevertheless, some inoculum might have been present, presumably due to dispersal by wind, at the time of application of the infested soil to the plots, and possibly through displacement by tractor and seed drill and by animals. In 1989, infected plants were detected at both distances, showing that high inoculum levels were present. This infection will have been the result of multiplication in 1988 of the inoculum dispersed in 1988 as mentioned before, and of inoculum transported from the infested plots by tractor and spading machine in October 1988. Harrowing and sowing in spring 1989 were performed under dry conditions, limiting the displacement of soil. Plots were harvested by hand, and inside the buffer zone plots inoculum may have been dispersed by treading. An indication of the role of displacement of soil, most probably by the tillage in October, is given by the marked decrease in disease incidence with distance from the infested plots. Samples at larger distances were not included but, in view of the already strongly manifest level of infestation at 8 m, one can surmise that infested soil was distributed even further,

in spite of the limited tillage practices performed (e.g. no mechanical harvest).

Schäufele et al. (1985) recorded spread of inoculum of BNYVV in a particular field in the cultivation direction. Within 5 years, during which two sugar beet crops were grown and an intensive soil tillage was performed, BNYVV inoculum was detectable in soil samples throughout a 0.5-ha field, having overcome 60 m distance from the edges of the field to the centre. This observation will not have been the result of spread only. Multiplication of inoculum might also have been involved. It has been demonstrated in an artificially infested field that inoculum levels below the detection level of a bioassay and causing very low disease incidences in the field can increase to detectable and harmful levels after one or two beet crops (Tuitert and Hofmeester, 1992; Tuitert and Hofmeester, in preparation). In order to study the extent of spread of soil adhering to machinery, Hofmeester and Van Dullemen (1989) simulated a pathogen-infested area in a field crop by adding cress seeds to 1 m^2 just before the harvest of beets or potatoes. By observing the cress seedlings they assessed spread of 'infested' soil by harvesting machines up to 8 and 16 m distance and in small amounts even at 64 m distance from the original source.

The effect of irrigation on disease incidence in 1989 was too small to be significant, but should not be completely ignored (P=0.09). Irrigation may have influenced disease incidence in two ways. First, irrigation caused an increase in inoculum levels during 1988 (Tuitert and Hofmeester, 1992) and, therefore, soil transported from the irrigated plots had a higher inoculum level. Second, irrigation may have stimulated the development of disease during 1989.

After three beet crops, the area up to 8 m from the infested plots was heavily diseased, demonstrating once more the risks of spread of infested soil and the rapid multiplication of inoculum (Tuitert and Hofmeester, 1992).

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