Neth. J. Pl. Path. 98(1992) 343–360

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

G. TUITERT[1] and Y. HOFMEESTER[2]

[1] Sugar Beet Research Institute, P.O. Box 32, 4600 AA Bergen op Zoom, the Netherlands
[2] Research Station for Arable Farming and Field Production of Vegetables, P.O. Box 430, 8200 AK Lelystad, the Netherlands

Accepted 20 October 1992

Abstract

Using field plots where rhizomania had not previously been detected, different inoculum levels of beet necrotic yellow vein virus (BNYVV) were created by application of infested soil. A susceptible sugar beet cultivar (cv. Regina) was grown for two consecutive years (1988 and 1989), in the presence or absence of drip irrigation. In soil samples taken in spring 1989, the different initial inoculum levels of 1988 could be distinguished using a quantitative bioassay estimating most probable numbers (MPNs) of infective units per 100 g dry soil. The first sugar beet crop resulted in a ten-thousandfold multiplication of inoculum of BNYVV (viruliferous *Polymyxa betae*). Mean MPNs of BNYVV ranged from 0.6 and 7 per 100 g soil for the lowest inoculum level to 630 and 1100 per 100 g for the highest level, in plots without and with irrigation, respectively. In spring 1990, MPNs had again increased. In both years, the initial inoculum level of 1988 had a significant linear effect on log-transformed MPNs of BNYVV determined. Log-transformed MPNs for 1990 and 1989 showed a positive linear correlation, despite a decreasing multiplication ratio at higher inoculum levels. Drip irrigation during one or two years enhanced the increase in MPN of BNYVV, which was reflected by the enhancement of multiplication ratios at all inoculum levels. The total *P. betae* population was also higher after growing two irrigated crops than after growing two non-irrigated ones.

Additional keywords: *Polymyxa betae*, rhizomania, BNYVV, soil-borne virus, MPN, quantitative technique, bioassay, polyetic epidemic.

Introduction

Beet necrotic yellow vein virus (BNYVV), the causal agent of rhizomania in sugar beet, is transmitted by the fungus *Polymyxa betae* Keskin. The vector is an obligate parasite, which, after primary infection, develops zoosporangia releasing zoospores in a rapid multiplication cycle (Keskin, 1964), and thus has a potentially high multiplication rate. The formation of resting spores, and their longevity, assures its survival in soil between successive beet crops. The epidemiology of BNYVV is largely determined by the behaviour of its vector. Ecological aspects, including the influence of soil moisture and temperature on fungal development, have been studied (Abe, 1987; Asher and Blunt, 1987; Blunt et al., 1991; De Heij, 1991). However, quantitative epidemiology of the disease has received little attention. Studies on the dynamics of BNYVV in soil as influenced by a susceptible beet cultivar have not yet been published.

Field levels of BNYVV infestation have been characterized by: (a) the resulting disease incidence or severity or yield of susceptible cultivars as compared to yield of resistant/tolerant cultivars (Winner, 1988); (b) the number of BNYVV-infected bait plants in a bioassay on soil samples (Beemster and De Heij, 1987) and (c) the ELISA absorbance values for BNYVV in bioassay plants (Hillmann, 1984; Büttner and Bürcky, 1990). Quantitative methods used to assess the vector in soil did not include quantification of BNYVV (Goffart et al., 1987, 1989; Ciafardini and Marotta, 1989), and applications of these methods to study the dynamics of non-viruliferous *P. betae* were not reported. A bioassay procedure based on serial dilutions of BNYVV-infested soil permitted assessment of the inoculum potential of both vector and virus (Tuitert, 1990).

In 1988, a field experiment was set up to examine disease development at different initial inoculum levels of BNYVV, in the presence and absence of drip irrigation. Preliminary reports of the work were given (Hofmeester and Tuitert, 1989; Tuitert and Hofmeester, 1990). This paper presents the results of a study on the dynamics of BNYVV: the quantification of inoculum build-up during two successive beet crops.

Material and methods

Field plot design. In 1988, a field trial was laid out on a calcareous clay soil (pH-KCl 7.4; organic matter 4.2%), located at an isolated site in an urban area in the Noordoostpolder, the Netherlands. Sugar beet had never been grown on this field and BNYVV could not be detected in any of the plots by bioassay of soil samples taken in November 1987.

The experiment was arranged in a split-plot design, with irrigation levels as main plots and inoculum levels as subplots and with four blocks (Fig. 1). The five inoculum level subplots were not randomized within the main plots. In order to reduce the effect of infested soil being moved from one inoculum level to another, subplots were laid out in a fixed order of increasing inoculum level. Between and within blocks the irrigated and non-irrigated main plots were separated by grass strips 3 m wide. Drilling and soil cultivation always started in the uninfested control plot and, to clean the equipment, ended in an extra uninfested area behind the plot with the highest inoculum level. Tillage practices were performed when weather and soil were dry. Machines were cleaned before entering each main plot. As became apparent during the first year, one complete block suffered from water logging because of soil compaction. In view of the poor emergence and development of the plants in all three years the data from this block were not used.

Plot size was 6 x 10 m. Sugar beet cv. Regina was sown in three consecutive years in rows 50 cm apart with a sowing distance of 18.5 cm. Irrigation was applied along every row with a drip irrigation system with emitters spaced 30 cm apart. Soil moisture was recorded by tensiometers three to five times a week at 15 and 30 cm depth. During dry periods, when soil moisture tension (Hillel, 1982: soil water potential presented as a positive value) at 15 cm depth exceeded 20 cbar, drip irrigation was used to supply about 10 mm water during 2–3 hours.

Generating different inoculum levels. Infested soil from a field in the Noordoostpolder with a history of rhizomania was used as inoculum to create plots with different infestation levels. This soil was dried, ground and thoroughly mixed with sterilized river sand in

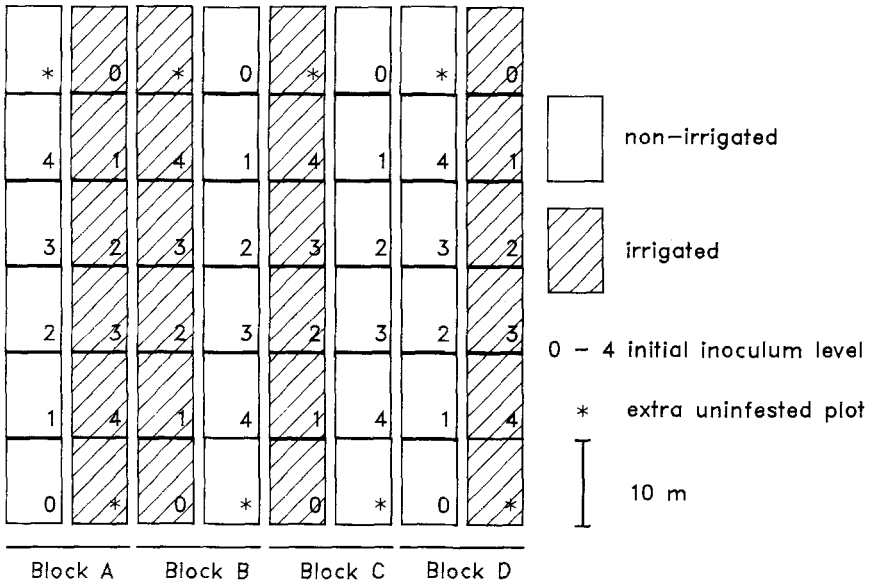

Fig. 1. Layout of the experiment. For explanation of initial inoculum levels see Table 1. Because of water logging, block A has not been used in the analysis.

different proportions. Two days before sowing, the mixtures were applied to the plots (3 kg per plot) by hand and were superficially raked into the soil. Volume ratios of infested soil at the five inoculum levels are given in Table 1.

Soil sampling. Soil samples were composed of 1.3 cm diameter cores taken to a depth of 25 cm in a 1 x 1.4 m grid in every plot (42 cores per sample). Soil was air-dried and ground before use. Mean bulk density of the soil was 1.3 g • ml^{-1}. Samples were collected before (1987) and shortly after introduction of the infested soil to the plots (April 1988) and immediately after harvest (October 1988). In 1989 and 1990 samples were taken shortly after drilling (May).

Assessment of BNYVV and P. betae in soil. Samples collected in 1987 and 1988 were analysed by bioassay for the presence of BNYVV and *P. betae*. In 1987, five pots of 200

Table 1. Amount of BNYVV-infested soil applied per inoculum level in April 1987. The volume ratio was based on a tillage layer of 30 cm.

Initial inoculum level	Amount of BNYVV-infested soil applied	
	Volume ratio (%)	Average amount (g • m^2)
0	0	0
1	0.00001	0.05
2	0.0001	0.48
3	0.001	4.80
4	0.01	48.00

ml were filled with undiluted soil per sample. The dried soil samples of 1988 were mixed with 50% (v/v) of sterile coarse sand. Ten pots of 200 ml were filled with this mixture per sample. In each pot a sugar beet seedling (cv. Regina, 2–3 weeks old) was planted. For this bioassay the detection limit was estimated as 0.08 infective units per 100 g of soil. Samples collected in 1989 and 1990 were serially diluted. Assessments of the inoculum potential of BNYVV in 1989 and of *P. betae* and BNYVV in 1990 were made by the most probable number method (Tuitert, 1990). The soil used to infest the field in 1988 was analysed in the same way, both for *P. betae* and the virus. A dilution ratio of 10 with six replicates per dilution was used. Greenhouse conditions and plant analysis for BNYVV and *P. betae* for both types of assays were as described by Tuitert (1990).

The inoculum potential of BNYVV and *P. betae* in soil was estimated as the most probable number (MPN) of infective units per 100 g of soil. The detection limit of the dilution method was a MPN of 0.6 units per 100 g of soil. Multiplication ratios of BNYVV in 1988 were estimated by dividing the measured MPNs in spring of 1989 by the calculated MPNs for the initial situation (spring 1988). Multiplication after two beet crops was estimated by dividing MPNs of 1990 by MPNs of 1988, both for BNYVV and *P. betae*. For 1989, multiplication ratios were calculated by dividing MPNs determined in the spring of 1990 by those determined in 1989. The viruliferous proportion of the population was estimated in 1990, by dividing MPNs determined for BNYVV by MPNs for *P. betae*.

Statistical analysis. Statistical computations were made using GENSTAT 5 (Payne et al., 1988). The analysis was based on the split-plot design. Prior to analysis, angular transformation was performed on percentages of infected bait plants in the bioassay on soil samples from October 1988, in order to improve homogeneity of error variances (Mead and Curnow, 1983). Analysis of variance (ANOVA) was performed on log-transformed MPNs ($\log_{10}$(MPN+1)), multiplication ratios ($\log_{10}$) and percentage viruliferous *P. betae* ($\log_{10}$(%+1)), because of the increase in variance with an increase in magnitude of the untransformed data. Means of log values were back transformed and presented as geometric means of the original data (Steel and Torrie, 1980).

Orthogonal polynomial contrasts were used to examine linear and quadratic trends in data. Although not meant to be used for responses to a quantitative factor (Baker, 1980; Dawkins, 1981), a LSD was given and Duncan's new multiple range test was applied (Duncan, 1955) for discriminating between means, only when interaction between factors was absent. This was done to see whether the (quantitative) bioassay methods used could distinguish between the plots with different initial inoculum levels, after growing one or two beet crops. As no inoculum had been applied to the control plots, they were omitted from the analysis of the multiplication ratio.

The log-transformed MPNs for BNYVV determined in 1990 and the multiplication ratio in 1989 were regressed against the log-transformed MPNs in 1989. Two plots in which BNYVV was not detected in 1989 were excluded from the analysis. Stepwise regression was performed with pooled data of non-irrigated and irrigated plots. In order to consider the split-plot design in the regression, first the factors block and block times irrigation (representing non-irrigated and irrigated main plots or strips) were fitted. Then the factors irrigation, $\log_{10}$ MPN and their interaction were added to the model. The significance of the regression on $\log_{10}$ MPN was determined with F tests. The significance of the irrigation effect was tested by comparison of fitted lines for each strip. The percentage of

variance accounted for by the regression in the interactive model was calculated considering the split-plot structure and is presented as a partial R^2 adjusted. To justify the use of $\log_{10}$ MPN 1989 for explanation of parameters measured in 1990, the absence of significant contributions of the factor 'initial inoculum level' and its interaction with irrigation to the regression model were checked. Fit of the linear model was assessed by examination of the significance of the regression, the percentage of variance accounted for and the plots of standardized residuals versus expected values for the regressions. Non-linear models, polynomial with a quadratic term and exponential, were fitted to the data and results compared with those of the linear model.

The correlation between (angular transformed) percentages of BNYVV-infected plants in the bioassay of October 1988 and the MPN of spring 1989 was determined.

Results

Field observations. In 1988, the beet crop developed well, only a slight yellowing of the leaves was observed in plots of the highest inoculum level and low numbers of BNYVV-infected plants were detected. In 1989, sowing was late and emergence was irregular because of drought. Plants with root symptoms of rhizomania were observed from the end of June onwards. Disease incidence was high.

Soil temperatures above 15 °C were recorded soon after sowing in both years. The frequency of rainfall events (≥ 1 mm per day, or occasionally per 2 days) and of irrigations after emergence of the plants till harvest was, respectively, 52 (421 mm accumulated) and 11 in 1988, and 37 (292 mm accumulated) and 7 in 1989. In 1988, long dry periods did not occur and the soil moisture tension did not attain extreme values. Irrigation kept the soil moisture tension below 20–30 cbar at 15 cm depth at times when it would have exceeded these values without irrigation. In 1989, there were three very dry periods and, since timing and execution of irrigation were not always as recommended, also the irrigated plots were from time to time subjected to high soil moisture tensions.

Detection of BNYVV in soil in 1988. In the experimental field, BNYVV could not be detected in samples taken before the start of the trial. After application of the infested soil to the field, the inoculum could only be traced in soil samples from two plots of inoculum level 3 and two plots of level 4 (Table 2). Immediately after harvesting the first beet crop, BNYVV was detected by bioassay at all inoculum levels. The average percentage of infected bait plants increased with increasing inoculum levels. Irrigation significantly increased these percentages (no significant interaction between inoculum level and irrigation). Three levels of infestation were distinguished by means of this bioassay (Table 2).

As at this time the most probable number method for estimation of infestation with rhizomania had not yet been developed, no quantitative assessments of BNYVV were made.

Quantitative assessment of BNYVV in soil in 1989 and 1990. For a quantitative assessment of the inoculum potential after growing one beet crop, soil samples were taken in spring 1989. Two plots in which no virus was detected in October 1988 (at inoculum level 0 and 2), were BNYVV-positive in May 1989, for one plot (inoculum level 0) the situation was the reverse. The MPN determined in May 1989 showed a positive correlation with the percentage of infected bait plants in the bioassay of October 1988, either trans-

Table 2. Detection of BNYVV by bioassay in soil samples from five inoculum levels, taken at the beginning and the end of the growing season in 1988. Six plots per inoculum level: three non-irrigated (NI) and three irrigated (IR). One sample per plot, ten plants per sample. Data are numbers of BNYVV-positive plots and arithmetic means of percentages and angular transformed percentages of BNYVV-infected plants.

Inoculum level[a] or irrigation	Number of BNYVV-positive plots		Means of percentages of BNYVV-infected bait plants				
			April[b]		October		October
	April[b]	October	NI	IR[c]	NI	IR	Mean angular[d]
0	–	2	–	–	0	7	6 a
1	0	4	0	0	3	40	24 ab
2	0	5	0	0	7	54	30 b
3	2	6	13	0	67	93	69 c
4	2	6	13	0	83	100	83 c
Significance (P-value)[e]							<0.001
NI							30
IR							55
Significance (P-value)[e]							0.03

[a] For explanation see Table 1.
[b] Due to an incident in the greenhouse all bioassay plants of level 0 (–), and 10–40% of those from levels 1, 2 and 4 were lost.
[c] Irrigation had not yet been applied prior to this sampling date.
[d] Mean values for the levels of each factor are given, because interaction between factors was not significant. Means with the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$). LSD (0.05) = 21.
[e] Significance = F probability of main effects in ANOVA of angular transformed percentages. Interaction between irrigation and inoculum level was not significant at P=0.05. Analysis of orthogonal polynomial contrasts showed a significant linear (P<0.001), but no quadratic effect of initial inoculum level.

formed or untransformed (R^2 adjusted = 74%, $P < 0.01$).

Table 3 shows the geometric mean inoculum potential of BNYVV per inoculum level as determined in May 1989 and 1990. After one year, increasing MPNs were found with increasing initial inoculum level. In 1989, the MPNs were significantly different for the various initial inoculum levels. Irrigation in 1988 significantly influenced the MPN determined in the following year (Table 3, Fig. 2). Significant differences between plots that had different initial inoculum levels were also recorded in 1990, and two years of irrigation still resulted in higher MPNs as compared to the non-irrigated plots (Table 3, Fig. 2). The size of the effect of one or two years of irrigation did not depend on the initial inoculum level in either year; apparent interaction of inoculum level and irrigation was not significant. One of the irrigated control plots showed a severe contamination of the front part of the plot in 1990, therefore it was excluded from the calculations.

The effect of two years of extra moist conditions on the inoculum potential in 1990, as demonstrated by the analysis of variance, could be the result of the effect of irrigation in 1988 only or in combination with an effect in 1989; therefore regression analysis was carried out. The linear model gave the best fit for the range of values available (Fig. 3). The

Table 3. Inoculum potential of BNYVV in soil after growing one or two beet crops in an artificially infested field (May 1989 and May 1990). Assessment of the most probable numbers (MPN) of infective units by means of the soil dilution method, dilution ratio 10, six replicates per dilution. Data are geometric means of three MPNs per inoculum level and arithmetic means of log-transformed MPNs. *P*-values refer to main effects in ANOVA.

Inoculum level or irrigation[a]	May 1989			May 1990		
	MPN BNYVV/100 g soil[b]		Mean $\log_{10}$ MPN BNYVV	MPN BNYVV/100 g soil[b]		Mean $\log_{10}$ MPN BNYVV
	Non-irrigated	Irrigated		Non-irrigated	Irrigated	
0	0.2	0.3	0.10 a[c]	15	150	1.69 a[c]
1	0.6	7	0.53 b	32	450	2.09 a
2	12	44	1.38 c	200	840	2.61 b
3	80	280	2.17 d	340	1200	2.81 b
4	630	1100	2.91 e	1700	3700	3.40 c
Significance (*P*-value)[d]			<0.001			<0.001
NI			1.21			2.56
IR			1.63			2.97
Significance (*P*-value)[d]			<0.01			0.04

[a] For explanation see Table 1. NI = non-irrigated, IR = irrigated.
[b] Geometric means: back transformed mean $\log_{10}$ MPNs. For plots in which BNYVV was not detected (four and two respectively, for inoculum levels 0 and 1 in 1989) calculations were made using MPN = 0.
[c] Means with the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$). LSD (0.05) was 0.33 for 1989 and 0.46 for 1990.
[d] Significance = *F* probability of main effects in ANOVA of $\log_{10}$ (MPN+1). Interaction between irrigation and inoculum level was not significant at $P = 0.05$ in either year. Analysis of orthogonal polynomial contrasts showed a significant linear ($P < 0.001$), but no quadratic effect of initial inoculum level.

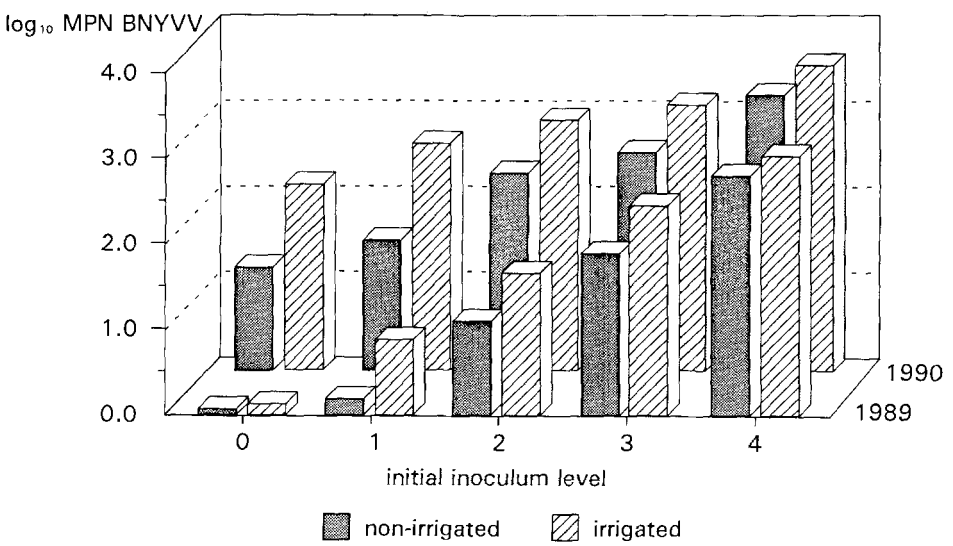

Fig. 2. The inoculum potential of BNYVV in soil in May 1989 and May 1990, after growing one or two beet crops, respectively, in an artificially infested field. Five inoculum levels, with or without irrigation. Inoculum potential given as arithmetic means of $\log_{10}$(MPN + 1); MPN is the most probable number of infective units per 100 g soil.

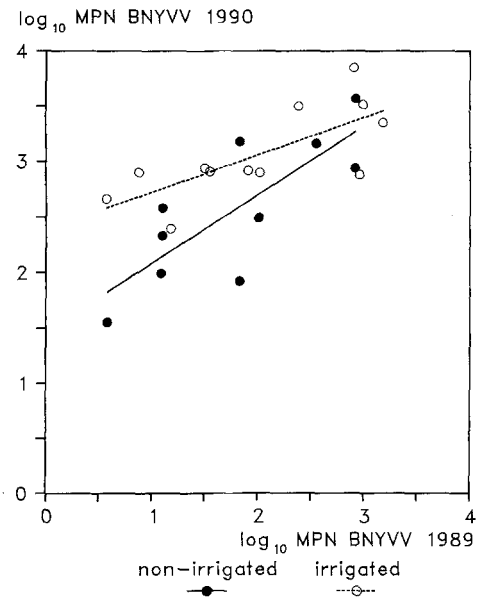

Fig. 3. Relationship between the inoculum potentials of BNYVV in 1990 (Y) and in 1989 (X). Regression was based on individual assessments of all initially infested plots, except for two plots that had an inoculum level in 1989 below the detection level of the bioassay (X = 0.20). Analysis of $\log_{10}$ (Most Probable Number/100 g soil + 1). Regression was significant ($P < 0.001$) with partial R^2 adjusted = 73%. The effect of irrigation was not significant at $P = 0.05$. Equations for the non-irrigated and irrigated data-set were, respectively: $Y = 1.46 + 0.62X$ and $Y = 2.38 + 0.34X$.

apparent effect of irrigation on individual MPNs was not statistically significant ($P = 0.05$) in an analysis of the effect with strips as experimental units because of the split-plot structure.

Multiplication ratio of BNYVV (viruliferous P. betae). For the severely infested soil that was used to contaminate the field in 1988 the MPN of BNYVV was 710 per 100 ml soil, the viruliferous population comprising 15% of the total *P. betae* population. Using this MPN, the volumes of infested soil added to the plots in 1988 and the bulk density of the field soil, the initial inoculum levels could be estimated as $6.6 \cdot 10^{-5}$ per 100 g soil for level 1 to 0.066 for level 4. Division of the MPNs assessed for 1989 by the ones calculated for 1988 indicated a ten-thousandfold multiplication of BNYVV in the first year (Table 4). The multiplication ratio decreased with increasing initial inoculum level and irrigation enhanced multiplication at all levels.

With the MPNs determined in 1990, the overall multiplication after two beet crops was assessed in the same way as that after one (Table 4). These two-year ratios also showed a decrease with increasing initial inoculum levels. The difference in two-year ratios due to two years of irrigation was not significant at $P = 0.05$.

Actual multiplication during the second year was estimated by dividing MPNs determined in 1990 by those of 1989. Geometric mean values for multiplication ratios ranged from 70 for the lowest inoculum level in irrigated plots to around 3 for the highest level either irrigated or non-irrigated (Table 4). Two years of irrigation had no effect on the multiplication ratio in the second year. Because irrigation had caused a significant increase of MPNs in the first year, initial inoculum levels in 1989 were not comparable. Thus, stepwise regression was performed to analyse effects in the second year (Fig. 4). Log_{10} transformed multiplication ratios decreased linearly with increasing $\log_{10}$ MPNs ($P < 0.001$). The apparent effect of irrigation was not significant ($P = 0.05$).

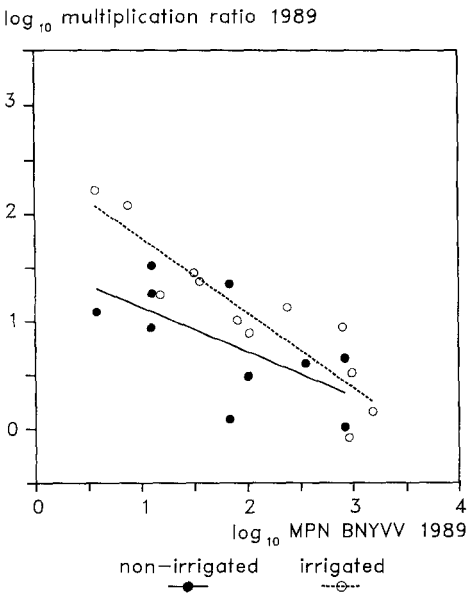

Fig. 4. Relationship between $\log_{10}$ multiplication ratio in 1989 (Y) and the inoculum potential of BNYVV in May 1989 (X). Regression was based on individual assessments of all initially infested plots, except for two plots that had an inoculum level in 1989 below the detection level of the bioassay ($X = 0.20$). Regression was significant ($P < 0.001$) with partial R^2 adjusted = 81%. The effect of irrigation was not significant at $P = 0.05$. Equations for the non-irrigated and irrigated data-set were, respectively: $Y = 1.55 - 0.41X$ and $Y = 2.47 - 0.70X$.

Quantitative assessment of P. betae. In the bioassays on soil samples taken in 1987 only 0, 1 or 2 bait plants were infected by *P. betae*. The low incidence of *P. betae* permitted an estimation of numbers of infective units of *P. betae* in soil by means of this 'dilution'. In 1987, inoculum potential of *P. betae* in the field prior to infestation was 0.19 infective units per 100 ml soil or 0.14 per 100 g soil. For the infested soil applied to the field in 1988 the MPN of *P. betae* was 4800 per 100 ml soil. By means of this MPN and the volumes of soil applied to the plots, the numbers of infective units added to the resident population were calculated. In this way, the initial inoculum levels could be estimated; they ranged from 0.14 to 0.58 infective units of *P. betae* per 100 g soil for level 0 and level 4, respectively. Because of the presence of the resident population, the initial levels of *P. betae* did not differ by a factor of 10. The initial levels of *P. betae* for the inoculum levels of BNYVV from 0 to 4 given in proportion to that at BNYVV level 4 were 0.25, 0.25, 0.25, 0.32 and 1.0.

Bait plants of the quantitative bioassays in 1990 were examined for the presence of *P. betae* (Fig. 5). The MPNs were high and only that for inoculum level 4 was significantly different from the rest (Table 5). Two years of irrigation had caused an overall increase of the population. Two-year multiplication ratios were calculated using the MPNs in 1990 and the estimated MPNs in 1988. Multiplication was the same for all inoculum levels (ANOVA of $\log_{10}$ multiplication ratio, $P = 0.05$), but had been enhanced by irrigation ($P < 0.01$), as was already apparent from the MPNs themselves. Mean $\log_{10}$ transformed multiplication ratios were 4.3 and 4.8 for non-irrigated and irrigated plots, respectively; back transformed means were 2.2×10^4 and 6.3×10^4.

The viruliferous part of the population of *P. betae* was estimated by dividing the MPN of the virus by that of the vector. The initial viruliferous percentages in 1988 were estimated as 0, 0.04, 0.4, 3.4 and 11.0% of the total population for inoculum levels 0 to 4, respectively. By 1990 they had increased to 1, 2, 8, 12 and 12% of the total population (Table 5).

Table 4. Estimated multiplication ratios of BNYVV (viruliferous *Polymyxa betae*) at different initial inoculum levels, calculated after growing the first (1988) and the second (1989) beet crop. Data are geometric means of multiplication ratios and arithmetic means of log-transformed multiplication ratios. *P*-values refer to main effects in ANOVA.

Level[a]	One beet crop (1988)			Two beet crops (1988 + 1989)			The second beet crop (1989)		
	Multiplication ratio (x10000)[b]		Mean $\log_{10}$ ratio	Multiplication ratio (x10000)[b]		Mean $\log_{10}$ ratio	Multiplication ratio		Mean $\log_{10}$ ratio
	NI	IR		NI	IR		NI	IR	
1	4[c]	10	4.8	50	740	6.3	33[c]	71	1.7
2	2	7	4.6	31	130	5.8	17	19	1.3
3	1	4	4.4	5	19	5.0	4.4	4.4	0.7
4	1	2	4.1	3	6	4.6	2.7	3.5	0.5
Significance (*P*-value)[d]			0.01			<0.001			<0.01
NI			4.2			5.1			1.0
IR			4.7			5.8			1.1
Significance (*P*-value)[d]			<0.01			0.08			0.71

[a] Inoculum level; for explanation see Table 1. NI = non-irrigated, IR = irrigated.
[b] Values presented should be multiplied by 10 000.
[c] For two plots of this treatment BNYVV was not detected in 1989, whereby multiplication ratios could not be determined. Calculations were made using missing values estimated by an iterative approach in GENSTAT5.
[d] Significance = *F* probability of main effects in ANOVA of log-transformed multiplication ratios. Interaction between irrigation and inoculum level was not significant at $P = 0.05$ in either year. Analysis of orthogonal polynomial contrasts showed a significant linear effect in both years ($P < 0.01$, $P < 0.001$ and $P < 0.01$ for 1988, 1988+1989 and 1989, respectively), but no quadratic effects of initial inoculum level. LSD (0.05) was 0.38 for 1988, 0.42 for 1988+1989 and 0.61 for 1989.

Discussion

Qualitative detection of BNYVV in 1988. BNYVV was not detected prior to the introduction of infested soil to the experimental field. The different amounts of BNYVV-infested soil added to the plots created initial inoculum levels that could hardly be traced by intensive soil sampling followed by bioassay. It may be assumed that the infective units applied to the plots were randomly dispersed and that samples obtained by means of the regular sampling grid were representative of the plots. Thus, comparison of the detection limit of the bioassay used (0.08 infective units per 100 g soil) with the highest initial inoculum level (approximately 0.06 infective units per 100 g soil) explains why BNYVV was only detected in a few plots.

Growing a susceptible beet crop resulted in a remarkable increase in the inoculum levels after only one year. Even at the lowest initial inoculum levels plant roots encounter

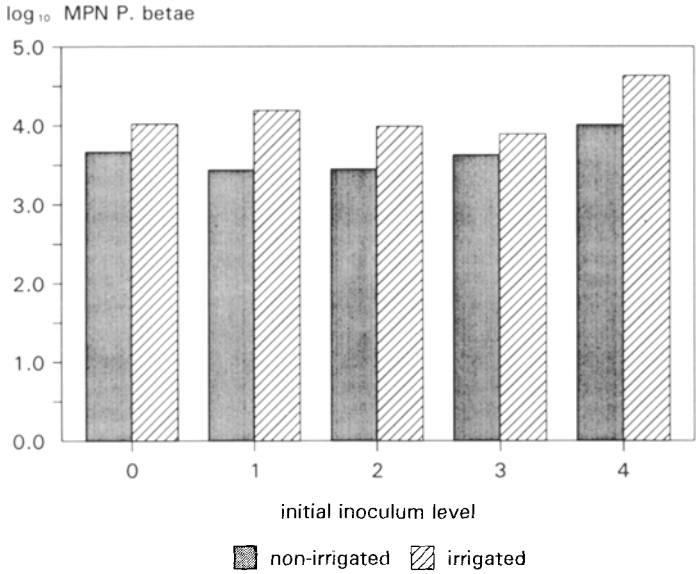

Fig. 5. The inoculum potential of *Polymyxa betae* in soil in May 1990, after growing two successive beet crops in an artificially infested field. Five inoculum levels, with or without irrigation. Inoculum potential presented as $\log_{10}$(MPN + 1); MPN is the most probable number of infective units per 100 g soil.

infective propagules, because during the season the root mass extends and root density increases, so that a large area will be explored by roots, to varying depths and resulting in various root densities (Brown and Biscoe, 1985). The total number of plants that became infected and the time of primary infection of plants differed for the different low inoculum levels of 1988, resulting in different amounts of infected root tissue. Soil samples taken in October of 1988 and analysed by bioassay showed the presence of BNYVV at all inoculum levels. Apparently, an abundance of resting spores formed during the season was already able to germinate, in the bioassay, immediately after harvest of the crop and did not need a long maturation period after being liberated from infected root debris. The average percentage of infected bait plants increased with increasing inoculum level, indicating that differences between these levels were still present and could be detected even though the initial random pattern of propagules had probably changed to randomly dispersed clusters of propagules.

In spite of phytosanitary precautions taken, after one year three of the control plots had become contaminated; two of these contaminations were detected in October of the first year, the additional one in May of the second year. The contamination of the control plots could have been caused by a number of factors, including dispersal by wind (at the time of application of infested soil), water, animals and man.

Quantification of BNYVV in 1989 and 1990. Soil samples taken after winter should be more representative of the plots as a whole than those taken at an earlier time after harvest. Cultivation of the soil should have distributed the root remnants more evenly through the

Table 5. Analysis of the inoculum potential of *Polymyxa betae* in soil and of the percentage of the viruliferous population in May 1990, after two successive beet crops in an artificially infested field. Assessment of the most probable numbers (MPN) with the soil dilution method, dilution ratio 10, six replicates per dilution. ANOVA of $\log_{10}$(MPN + 1) and $\log_{10}$ (% viruliferous population + 1). Data are geometric means of MPNs and percentages and arithmetic means of their log-transformed values. *P*-values refer to main effects in ANOVA.

Inoculum level or irrigation[a]	Inoculum potential of *P. betae*		Viruliferous *P. betae* population	
	MPN/100 g	$\log_{10}$(MPN+1)	%	$\log_{10}$(% virul.+1)
0	7000	3.85 a[b]	1	0.34 a[b]
1	6500	3.81 a	2	0.49 a
2	5200	3.72 a	8	0.95 b
3	5700	3.76 a	12	1.10 b
4	20700	4.32 b	12	1.13 b
Significance (*P*-value)[c]		<0.01		<0.001
NI		3.64		0.73
IR		4.14		0.87
Significance (*P*-value)[c]		<0.01		0.29

[a] Inoculum level; for explanation see Table 1. NI = non-irrigated, IR = irrigated.
[b] Means with the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$). LSD (0.05) for log (MPN + 1) was 0.28 and for log (% viruliferous+1) 0.33.
[c] Significance (*P*-value) = *F* probability of main effects in ANOVA. Interaction between irrigation and inoculum level was not significant at $P = 0.05$ for either parameter. Analysis of orthogonal polynomial contrasts showed a significant linear ($P < 0.01$) and quadratic ($P < 0.01$) effect of initial inoculum level on $\log_{10}$(MPN + 1). For $\log_{10}$(percentage viruliferous population + 1) only a linear effect was significant ($P < 0.001$).

tillage layer, roots should be more disintegrated and resting spores may have matured. Therefore, in spring 1989 soil samples were taken for quantitative assessment of the inoculum. In the only inoculated plot, of inoculum level 2, where BNYVV was not detected in October 1988, it was detected in the following spring. In two plots of level 1, non-irrigated, BNYVV was detected neither in spring 1989 nor in October 1988. Here, the mean inoculum level was still below the detection level of the quantitative bioassay (calculated as MPN = 0.6 infective units per 100 g soil). Assuming that all infective units introduced in 1988 resulted in clusters of infective propagules with a radius of 30 cm, based on spread from an inoculum source (Tuitert, 1993) the (Poisson) probability of obtaining one infested soil core would be approximately 0.05. Although soil cultivation in autumn 1988 and spring 1989 would enlarge but also dilute the infested patches, the chance of missing infested areas during the sampling procedure cannot be disregarded.

MPNs of BNYVV determined at successive inoculum levels in 1989 did not differ by a factor of 10, but differences between the initially applied densities were reflected (Table 3). For *Phytophthora parasitica*, another fungus with a secondary multiplication cycle, Neher and Duniway (1991) also found that population densities during the season ranked according to volumes of inoculum added to the soil. Mean log MPNs enabled a distinc-

tion between the five original inoculum levels (Table 3). The bioassay results of October 1988 permitted a division into three groups of infestation levels only (Table 2), despite the significant correlation of the (angular transformed) bioassay results with log MPNs in 1989. It is noticeable that after one year mean MPNs of inoculum level 4 attained values corresponding to the MPN of the soil used as inoculum in 1988.

In 1990, MPNs had increased as compared to 1989. The significance of main effects in ANOVA showed that after two successive beet crops inoculum levels were still determined by the initial levels of inoculum applied and that two years of irrigation resulted in higher MPNs. The MPNs increased with increasing initial inoculum level, and three groups of levels could be distinguished in 1990 (Table 3). In all control plots contamination had increased to detectable levels. Because irrigation had caused an increase in inoculum in the first year, the initial levels in 1989 for non-irrigated and irrigated plots were no longer identical. By regression analysis a significant linear relation was shown between log-transformed MPNs of both years.

The increase in BNYVV per year. The estimated ten-thousandfold increase in inoculum levels of BNYVV during 1988 reflects the high reproductive potential of *P. betae* by means of its secondary cycle. Multiple infection of plants might have been responsible for the observed decrease in multiplication ratio with increasing inoculum level in the first year. Multiplication ratios in 1989 were small compared to those in 1988; mean ratios for low to high inoculum levels ranged from approximately 70 to 3.

Assessments of changes in the population of *P. betae* and/or BNYVV in soil have not been made before, only reports on the colonization of roots by *P. betae* are available. In the early work of Kanzawa (1974) a linear correlation was found between the numbers of resting spore clusters (seven concentrations each differing by a factor of 10) added to the soil and the percentage of rootlets infected by *P. betae* after 40 days. Bürcky et al. (1986) reported an increase in the number of *P. betae*-infected root pieces and intensity of infection with resting spores with increasing inoculum level (0.1–100% dilutions of infested soil). Unfortunately, their assessments were neither really quantitative nor statistically tested. With values read from figure 3 of Goffart et al. (1987), a logistic increase of mean numbers of resting spores of *P. betae* per g root of bait plants with increasing concentration of spores in the soil can be demonstrated. The estimated multiplication ratio of the fungus in the 5-week period was lowest at the highest density of spores in soil (dilutions of infested soil tested: 0.006–100%). Since the amount of spores was given per g of root and total root weights of plants at the different soil dilutions were not presented, these calculations cannot be translated to soil populations.

Rintelen and Walla (1985), on the other hand, did not detect differences in percentages of infected root pieces in roots of sugar beet grown for 5 weeks in dilutions of infested soil (1–33%). These assessments did not take into account the degree of infection of the root pieces. Very likely, differences were not observed because the soil used had a high inoculum level and the dilution range was too limited.

Results may be different when *P. betae* is examined, which is the case with the roots, as compared to BNYVV only, done in our experiment. Nevertheless, the conclusion of Rintelen and Walla (1985) that the amount of infection in the roots is not dependent on the degree of contamination of the soil appears to be contradicted by the results presented here.

Decreasing multiplication ratio of BNYVV in 1989. Regression analysis revealed that log-transformed multiplication ratios decreased linearly with increasing log MPN. The reason for the lower multiplication at higher inoculum levels may be found in the availability of infection sites. At high levels of inoculum, sites on roots will quickly be occupied through primary infection, so sites for secondary infection will sooner become limiting at these levels compared to lower inoculum levels, where primary infection is less frequent and larger zoosporangia might be formed (Keskin, 1964).

There is no reason to suppose that there would be a decrease in the proportion of resting spores germinating at higher inoculum levels. But it is not known what proportion of a population will germinate during the season, and if this proportion is influenced by virus content of the resting spores. A complicating factor with *P. betae* is that perhaps only a fraction of the resting spores contained in one cluster will germinate (Habibi, 1969); so the germinated 'cluster' continues to be an infective unit.

The trigger in the infection process determining if a plasmodium follows the pathway to zoosporangium or resting spore is unknown. At some stage there may be a general change from zoospore production to the formation of resting spores (Asher and Blunt, 1987). Possibly the onset and impact of that trigger is influenced during the infection process, e.g. by the abundance of initial infection.

An interacting factor could be the influence of the transmitted virus on root growth. BNYVV may inhibit root growth and cause roots to die. To compensate for the loss of roots and the effects of blocked vessels, plants will form extra roots; a proliferation well-known as a rhizomania symptom. The net effect of these phenomena on multiplication of *P. betae* is not known. Non-viruliferous *P. betae* can acquire BNYVV from systemically infected roots (Abe and Tamada, 1986). There are, however, records of inhibition of infection of roots regenerated from BNYVV-infected tap roots by non-viruliferous *P. betae* (Schlösser, 1990). The efficiency of acquisition of virus from infected roots by *P. betae* will of course play a role in the increase in BNYVV. Alteration of epidemic components of a fungal (leaf) disease by simultaneous virus infection of the plant has been observed by Nelson and Campbell (1991).

The observation of decreasing multiplication with increasing inoculum level indicates that when effects of different factors (e.g. resistant cultivars) on BNYVV in soil are studied, multiplication ratios may not be directly comparable unless initial inoculum is more or less uniform, or a range of inoculum levels is used. Effects of sanitation (the reduction of initial inoculum, c.f. Van der Plank, 1963), on the inoculum levels in soil are likely to be quickly diminished when a beet crop is grown, because of the accompanying increase in multiplication of BNYVV at lower inoculum levels.

Two-year multiplication ratios of BNYVV and P. betae. Two consecutive beet crops resulted in a higher multiplication ratio for BNYVV at the lowest inoculum levels. The *P. betae* population already present at the experimental site was high relative to the small amounts of inoculum added in 1988. Only the *P. betae* population at inoculum level 4 was increased (threefold) by the addition of inoculum. Two-year multiplication ratios for *P. betae* were equal for all levels, thus resulting in similar MPNs for levels 0 to 3, and a higher MPN at level 4. Apparently, the initial differences in virus content of the *P. betae* populations did not affect the multiplication of *P. betae*. The higher two-year multiplication ratio for BNYVV than for *P. betae* at low inoculum levels (even when BNYVV levels

are corrected for the MPNs found at the uninfested control) is striking. It should be emphasized that initial inoculum levels for BNYVV each differed by a factor of 10, but that this was not the case for *P. betae*, because of the presence of the resident population. This circumstance interferes with the comparison of multiplication ratios for vector and virus at equal initial inoculum levels of BNYVV. Even so, it appears that virus acquisition by *P. betae* was more efficient at low initial inoculum levels. Once BNYVV has multiplied in a root, at low inoculum levels a majority of non-viruliferous zoospores infecting the root will acquire the virus during plasmodium formation. At high inoculum levels, many zoospores infecting the BNYVV-infected root will already be viruliferous, and thus the net increase in viruliferous propagules will be less. Furthermore, there may be more secondary infection cycles at the lower inoculum levels, causing an increasing virus content and spread in the roots, compared to the high inoculum levels, where primary infection will be more prominent.

By dividing the MPNs of BNYVV and *P. betae* an estimation of the viruliferous proportion of the population of *P. betae* can be obtained, on the assumption that the rate of germination and infection is the same for viruliferous and non-viruliferous *P. betae* (Tuitert, 1990). Implicitly it is also assumed that for successful infection of bait plants by *P. betae* or BNYVV the same number of non-viruliferous or viruliferous propagules is required. Because of these assumptions the real viruliferous percentage of the population may be over- or underestimated; however, the figure enables comparison of different soil samples. The geometric mean viruliferous percentages ranged from 1 to 12%. At the three highest inoculum levels the viruliferous percentage of the population of *P. betae* after two beet crops corresponded with the percentage of that in the soil used as inoculum in 1988. In comparison, 1–2% of secondary zoospores of an efficient viruliferous isolate of *P. graminis* appeared to be carrying particles of barley mild mosaic virus (Jianping et al., 1991).

Influence of soil moisture conditions. Drip irrigation enhanced the increase in MPN of BNYVV in both years (Table 3). The *P. betae* population was also higher following two irrigated crops compared to the non-irrigated situation (Table 5). Irrigation may have stimulated multiplication by the formation of extra primary zoospores, by extra secondary cycles, and both possibly combined with a greater 'efficiency' of infection by the zoospores released (e.g. by swimming further). All these effects would result in a multiplicative effect compared to the non-irrigated treatments, which is additive when transformed to log values. Since irrigation may cause an increase in root density, especially in the tillage layer (Brown et al., 1987; Wild and Russell, 1988), extra availability of infection sites may be an important factor in the increase in inoculum caused by irrigation, especially at already high initial inoculum levels.

Soil moisture conditions in 1988 appeared to have favoured fungal development. In June a dry period may have inhibited the life cycle of *P. betae*, and irrigation may then have exerted its influence on multiplication. Irrigation was responsible for a threefold higher multiplication ratio of BNYVV in 1988 (Table 4; antilog value of 4.7 minus 4.2). The objective of maintaining a threshold soil moisture tension of approximately 20 cbar at 15 cm depth in the irrigated plots was not always achieved. De Heij (1991) reported that even lower tensions (10–12 cbar) were required in pot experiments for primary and secondary infection to occur. It is difficult to translate these precisely measured threshold tensions from pot experiments using compact sieved soil to field conditions where soil is not

homogeneous and where tensions are measured less precisely. Soil moisture tensions at depths greater than 15 cm were lower but, especially in the first year, infection by the superficially raked-in inoculum will mostly have taken place in the upper soil layers. Preliminary results of Gerik et al. (1990) indicated that infection could take place at tensions up to 40 cbar. For *Plasmodiophora brassicae*, germination of resting spores and primary infection was recorded at tensions up to 20 cbar (Dobson et al., 1982). Soil moisture requirements for zoospore movement will also depend on the type (texture) of soil (Duniway, 1976; Dobson et al., 1982).

In 1989, there were three very dry periods and from time to time high tensions were recorded at 15 cm depth, and to a lesser extent at 30 cm depth. Nevertheless, differences in soil moisture conditions between irrigated and non-irrigated plots were less conspicuous than in 1988. Regression analysis revealed that irrigation in the second year had no significant effect on log MPN BNYVV in addition to irrigation in the first year.

The combined effect of two years of irrigation on log MPNs of BNYVV was just significant (Table 3). Both one and two years of irrigation caused an approximately threefold increase of MPNs of BNYVV (Table 3; antilog values of 0.42 and 0.41, respectively). Because higher MPNs caused by irrigation in the first year have lower multiplication ratios, a possible increase in MPNs by irrigation in the second year would be partly hidden by this reverse effect of inoculum level. The apparent smaller increase by irrigation at high inoculum levels (Fig. 2), probably approaching 'saturation' level, was not significant because interaction of inoculum level and irrigation was not significant. When inoculum level 0 was excluded from the analysis of MPNs in 1990, the effect of irrigation was not large enough to be detected significantly (P=0.08). The effect of irrigation on the two-year multiplication ratios of BNYVV was therefore also not significant at the confidence level applied (Table 4). Two-year multiplication ratios of *P. betae* were significantly increased by two years under irrigated conditions.

Conclusion. The research described in this paper is the first report on the population dynamics of viruliferous *P. betae*. The results indicate the potential hazards of introduction of small amounts of rhizomania-infested soil to a field. With an evenly distributed infestation and favourable conditions for development of *P. betae* and BNYVV the build-up of inoculum can be very fast.

Acknowledgements

The authors wish to thank the Plant Protection Service, Wageningen, for providing the trial site, and the personnel at Emmeloord for their kind co-operation and trial maintenance. Thanks are due to J.P.C. Hartveld and P.M.S. Musters-van Oorschot for technical assistance. We are grateful to Drs A. Otten (Department of Mathematics, Agricultural University Wageningen) for advice on the statistical analyses and to Dr J.A. Walsh (Horticulture Research International, Wellesbourne) for linguistic corrections. The critical reading of the manuscript by Prof. Dr J.C. Zadoks, Drs G.J. Bollen (Department of Phytopathology, Agricultural University Wageningen) and Drs W. Heijbroek is gratefully acknowledged.

References

Abe, H., 1987. Studies on the ecology and control of *Polymyxa betae* Keskin, as a fungal vector of the causal virus (beet necrotic yellow vein virus) of rhizomania disease of sugar beet. Report of the Hokkaido Prefectural Agricultural Experimental Station No. 60, 90 pp.

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.

Asher, M.J.C. & Blunt, S.J., 1987. The ecological requirements of *Polymyxa betae*. Proceedings 50th Winter Congress IIRB, Brussels. p. 45–55.

Baker, R.J., 1980. Multiple comparison tests. Canadian Journal of Plant Science 60: 325–327.

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.

Blunt, S.J., Asher, M.J.C. & Gilligan, C.A., 1991. Infection of sugar beet by *Polymyxa betae* in relation to soil temperature. Plant Pathology 40: 257–267.

Brown, K.F. & Biscoe, P.V., 1985. Fibrous root growth and water use of sugar beet. Journal of Agricultural Science, Cambridge, 105: 679–691.

Brown, K.F., Messem, A.B., Dunham, R.J. & Biscoe, P.V., 1987. Effect of drought on growth and water use of sugar beet. Journal of Agricultural Science, Cambridge 109: 421–435.

Bürcky, K., Büttner, G. & Winner, C., 1986. Schädigung der Zuckerrübe durch das Aderngelbfleckigkeitsvirus (BNYVV) in Abhängigkeit vom Verseuchungsgrad des Bodens. I. Gewicht, Rübenqualität und Virustiter. Zuckerindustrie 111: 216–224.

Büttner, G. & Bürcky, K., 1990. Versuche und Überlegungen zum Nachweis des BNYVV im Boden mittels Fangpflanzen. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 97: 56–64.

Ciafardini, G. & Marotta, B., 1989. Use of the most probable number technique to detect *Polymyxa betae* (Plasmodiophoromycetes) in soil. Applied and Environmental Microbiology 55: 1273–1278.

Dawkins, H.C., 1981. The misuse of t-tests, LSD and multiple-range tests. Bulletin of the British Ecological Society 12: 112–115.

De Heij, A., 1991. The influence of water and temperature on the multiplication of *Polymyxa betae*, vector of beet necrotic yellow vein virus. In: Beemster, A.B.R., Bollen, G.J., Gerlagh, M., Ruissen, M.A., Schippers, B. & Tempel, A. (Eds), Biotic interactions and soil-borne diseases. Elsevier, Amsterdam. p. 83–90.

Dobson, R., Gabrielson, R.L. & Baker, A.S., 1982. Soil water potential requirements for root-hair and cortical infection of chinese cabbage by *Plasmodiophora brassicae*. Phytopathology 72: 1598–1600.

Duncan, M.B., 1955. Multiple range and multiple F tests. Biometrics 11: 1–42.

Duniway, J.M., 1976. Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. Phytopathology 66: 877–882.

Gerik, J.S., Hubbard, J.C. & Duffus, J.E., 1990. Soil matric potential effects on infection by *Polymyxa betae* and BNYVV. Proceedings of the First Symposium of the International Working Group on Plant Viruses with Fungal Vectors, Braunschweig, Germany, August 21-24 1990. Schriftenreihe der Deutschen Phytomedizinischen Gesellschaft, Band 1: 75–78.

Goffart, J.P., Van Bol, V. & Maraite, H., 1987. Quantification du potentiel d'inoculum de *Polymyxa betae* Keskin dans les sols. Proceedings 50th Winter Congress IIRB, Brussels. p. 295–306.

Goffart, J.P., Horta, V. & Maraite, H., 1989. Inoculum potential and host range of *Polymyxa betae* and beet necrotic yellow vein furovirus. EPPO Bulletin 19: 517–525.

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

Hillel, D., 1982. Introduction to soil physics. Academic Press, New York. 364 pp.

Hillmann, U., 1984. Neue Erkenntnisse über die Rizomania an Zuckerrüben mit besonderer Berücksichtigung Bayerischer Anbaugebiete. Dissertation Giessen. 226 pp.

Hofmeester, Y. & Tuitert, G., 1989. Development of rhizomania in an artificially infested field. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 51: 827–834.

Jianping, C., Swaby, A.G. & Adams, M.J., 1991. Barley mild mosaic virus inside its fungal vector,

Polymyxa graminis. Annals of Applied Biology 118: 615–621.
Kanzawa, K., 1974. Studies on rhizomania. 7. Influence of density of *Polymyxa betae* in soil prepared for raising the paperpot on rhizomania. Proceedings of the Sugar Beet Research Association 16: 37–43.
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.
Mead, R. & Curnow, R.N., 1983. Statistical methods in agriculture and experimental biology. Chapman and Hall, London, New York. 335 pp.
Neher, D. & Duniway, J.M., 1991. Relationship between amount of *Phytophthora parasitica* added to field soil and the development of root rot in processing tomatoes. Phytopathology 81: 1124–1129.
Nelson, S.C. & Campbell, C.L., 1991. Infection by clover yellow vein virus alters epidemic components of *Cercospora* leaf spot on white clover. Phytopathology 81: 989–994.
Payne, R.W., Lane, P.W., Ainsley, 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.
Rintelen, J. & Walla, J., 1985. Zum Einfluss von Vorfrüchten auf den Befall von Rüben mit *Polymyxa betae*. Bayerishes Landwirtschaftliches Jahrbuch 62: 565–568.
Schlösser, E., 1990. Rizomania. XII. Effect of BNYVV and BSBV on the development of *Polymyxa betae*. Mededelingen van de Faculteit Landbouwwetenschappen van de Rijksuniversiteit Gent 55: 1069–1071.
Steel, R.G.D. & Torrie, J.H., 1980. Principles and procedures of statistics. A biometrical approach, 2nd edition. McGraw-Hill Kogakusha, Tokyo. 663 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., 1993. Horizontal spread of beet necrotic yellow vein virus in soil. Netherlands Journal of Plant Pathology 99 (in press).
Tuitert, G. & Hofmeester, Y., 1990. Development of rhizomania at different initial inoculum levels of the soil. Proceedings of the First Symposium of the International Working Group on Plant Viruses with Fungal Vectors, Braunschweig, Germany, August 21-24 1990. Schriftenreihe der Deutschen Phytomedizinischen Gesellschaft, Band 1: 73.
Van der Plank, J.E., 1963. Plant diseases: epidemics and control. Academic Press, New York and London. 349 pp.
Wild, A. & Russell, E.W., 1988. Russell's soil conditions and plant growth, 11th edition. Longman, London. 991 pp.
Winner, C., 1988. Terminologische Fragen in der Rizomaniaforschung. Zuckerindustrie 113: 597–600.