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Epidemiology of beet necrotic yellow vein virus in sugar beet at different initial inoculum levels in the presence or absence of irrigation:
Disease incidence, yield and quality

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Abstract. A field experiment was set up in 1988 to study the development of rhizomania disease of sugar beet at different inoculum levels of beet necrotic yellow vein virus (BNYVV) in soil. Five, tenfold different, inoculum levels were created by addition of the approximate amounts of 0, 0.5, 5, 50 and 500 kg infested soil per ha (the latter corresponding to 0.01% v/v calculated to the tillage layer). A drip irrigation treatment was applied to study the influence of soil moisture on disease. Susceptible sugar beet, cv. Regina, was grown for three consecutive years.

In the first year, root symptoms were not observed, but BNYVV-infected plants were detected by ELISA in low numbers at all inoculum levels at harvest. After late drilling in 1989, high numbers of infected plants, up to 90–100% in plots with the highest inoculum level, were detected already in June. Root symptoms were also observed from June onwards. In both these years disease incidence increased in time and was significantly influenced by the initial inoculum level. In the third year, the whole field was heavily diseased, and only for the non-irrigated plots incidence differed for different initial inoculum levels. The expression of symptoms by BNYVV-infected plants was influenced by initial inoculum level, thus by the amount and timing of primary infection.

Root weight at harvest was not affected, but sugar content decreased with increasing inoculum level already in 1988, leading to a reduction in sugar yield of 10% at the highest inoculum level. In 1989, both root weight and sugar content decreased progressively with increasing inoculum level, resulting in sugar yield reductions of 11–66% (down to approximately 3000 kg ha^{-1}) for low to high inoculum levels compared to the control. As the control plots became contaminated, all yields were low in 1990, still showing a decrease with increasing inoculum level in the non-irrigated plots, but an overall mean sugar yield of 3323 kg ha^{-1} for the irrigated ones.

Sodium and α-amino nitrogen content of the root, additional quality parameters determining extractability of sucrose, showed an increase and decrease, respectively, with increasing initial inoculum level already in the first year. The relative differences in contents compared to those from the control were largest for Na content. A significant negative correlation was found between Na (mmol kg^{-1} root) and sugar content (% of fresh weight); linear for 1988, exponential for 1989 and 1990.

In spring 1989, the infestation of individual plots was assessed using a quantitative bioassay estimating most probable numbers (MPNs) of infective units of BNYVV per 100 g

dry soil. The relationship between the MPNs determined and root weight, sugar content and sugar yield at harvest could be described by Gompertz curves. The increase in disease incidence with increasing MPN in 1989 was adequately fitted with a logistic equation.

Introduction

A fundamental component of quantitative epidemiological investigations involving soil-borne pathogens is the relationship between inoculum density and disease incidence and severity. This paper presents the results of a study on this relationship for rhizomania disease of sugar beet, caused by beet necrotic yellow vein virus (BNYVV). Environmental factors, such as soil moisture and temperature, the susceptibility and sensitivity of the beet cultivar and the aggregation of inoculum can influence the inoculum density – disease relationship. As the fungal vector *Polymyxa betae* Keskin infects the roots by means of zoospores, moist conditions, e.g. through irrigation, will favour disease development. The inoculum density – disease relationship of this pathosystem has been studied under controlled conditions in pot experiments with dilutions of infested soil [Bürcky et al., 1986; Bürcky and Beiss, 1986]. It has not been studied under field conditions.

Therefore, a field experiment was set up in 1988 to study the development of disease at different inoculum levels of BNYVV. The influence of soil moisture conditions was investigated by applying drip irrigation. Inoculum levels were created by artificial infestation of a disease-free field with different amounts of infested soil.

In this paper, the effects of inoculum levels of BNYVV on disease incidence, sugar content, root weight and quality parameters of susceptible sugar beet in three successive years are reported. The quantification of inoculum build-up during two years was published previously [Tuitert and Hofmeester, 1992]. Part of the first-years' results were presented in preliminary reports [Hofmeester and Tuitert, 1989; Tuitert and Hofmeester, 1990].

Materials and methods

General description of the field trial

The field trial, for description and lay-out see Tuitert and Hofmeester [1992], was situated in the Noordoostpolder on a calcareous clay soil with a pH-KCl of 7.4 and 4.2% organic matter. The soil water retention curve is given in Fig. 1. The experiment was arranged in a split-plot design, with two irrigation levels as main plots, five inoculum level subplots and all in four blocks. Plot size was 6×10 m^2. Sugar beet cv. Regina was drilled in three consecutive years in 50 cm rows and a sowing distance of 18.5 cm

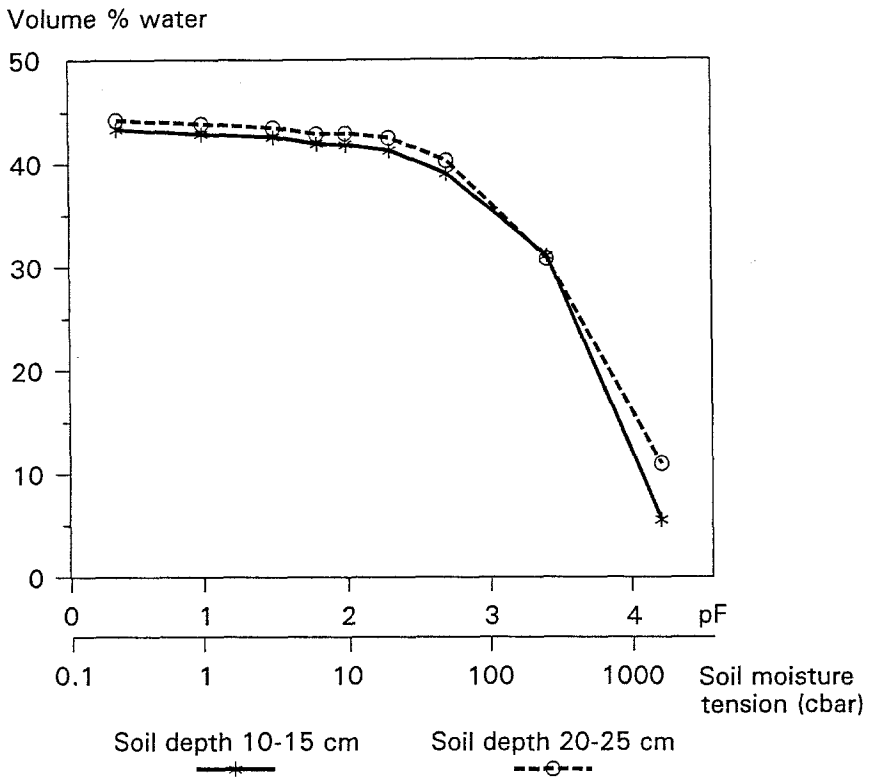

Fig. 1. Soil water retention curve for the trial field soil.

within the row. Sowing dates are given in Table 1. Irrigation was applied along every row with a drip irrigation system with emitters spaced 30 cm apart. Soil moisture tension was recorded by tensiometers three to five times a week at 15 cm and 30 cm depth. After dry periods, when soil moisture tension (soil water potential presented as a positive value; Hillel, 1982] at 15 cm depth exceeded *c.* 20 cbar (20 kPa), drip irrigation was used to supply around 10 mm water in 2 to 3 hours. The times of irrigation are indicated in Fig. 2. One of the four blocks was excluded from the analyses, because of the poor emergence and development of the plants in all three years caused by soil structure problems.

Fertilizer was applied prior to drilling at rates recommended on the basis of soil analysis. Pelleted seed was used, treated with thiram, hymexazole and furathiocarb. In the second and third year granular carbofuran was applied at drilling. Beet cyst nematodes were not detected in soil samples taken before the first, second and third beet crop. Generation of the inoculum levels, assessment of BNYVV in the soil by bioassay and calculation of most probable numbers (MPN 100 g^{-1} of soil) of infective

Table 1. Experimental details of the field trial in 1988, 1989 and 1990

	Year		
	1988	1989	1990
Sowing date	15 April	2 May	2 April
Harvest date	18 October	27 September	12 October
Length of growing season (days)	186	148	193
Date of 50% emergence	26 April	27 May	17 April
Periodical sampling date (weeks after sowing)	24 May (6)[a] 4 July (11) 23 August (19)	22 June (7)[a] 21 August (16)	18 June (11) 6 August (18)
Precipitation (> 1 mm)[b]			
– frequency (during growing season)	52	37	60
– amount (mm)	421	292	453

[a] Analysis of virus content only.
[b] Irrigation excluded.

units of BNYVV [Tuitert, 1990] were described before [Tuitert and Hofmeester, 1992].

Plant sampling and analysis

At harvest, all plants of the central 4 × 5 m^2 of each plot were taken for determination of root weight, sugar content, sodium and α-amino nitrogen contents of the root. Sugar content was determined by polarimetry [Anonymus, 1990], sodium by flame photometry and α-amino nitrogen by fluorimetry, all performed according to the standard procedures used in Dutch sugar industry [Anonymus, 1992]. The last two components, together with potassium, negatively influence the extractability of sugar and are referred to as (additional) quality parameters in this paper. Rhizomania affects the contents of Na, α-amino N and K in the root [Müller, 1983; Graf and Isak, 1986; Bürcky et al., 1986]. Potassium content is not presented because it is not a sensitive indicator of plant infection with BNYVV, as compared to the other parameters [Heijbroek, 1989]. Ten root tips were collected by 'every k^{th}' systematic sampling [Cochran, 1953] as described for application in virus surveys [Barnett, 1986]. The number of roots with symptoms of rhizomania was determined. Where the diameter of the root tip was approximately 1 cm, a small piece (1–2 cm) was cut from which sap was collected by handpress. Assessment of BNYVV was by means of double antibody sandwich ELISA [Clark and Adams, 1977], as described before [Tuitert, 1990].

In two adjacent sampling area's of 4 × 1 m^2 each (at either side of the central harvest area) samples were taken periodically, according to a

pre-determined scheme. Of ten plants, root tips were analyzed by ELISA and the remaining plant parts used for dry matter and mineral content assessments [Haverkort et al., unpublished]. Twenty plants were used for determination of root weight, sugar content and additional quality parameters. The 20-plant weights were converted to weights per ha. Sampling dates in the three years are shown in Table 1.

Statistical analysis

Results at each sampling date were analyzed by analysis of variance (ANOVA) of a split-plot design, using GENSTAT 5 [Payne et al., 1988]. Disease incidence (percentage of infected plants) was angular transformed [Mead and Curnow, 1983] before analysis. ANOVA of disease incidence was performed including time as an experimental factor; the repeated-measures design within each year [Campbell and Madden, 1990] was taken into consideration. There was no need to consider temporal autocorrelation of the error [Madden and Campbell, 1990], because successive samplings within a year were destructive [Zadoks, 1978] and plants adjacent to empty positions from the previous sampling were not taken. The number of sampling times was not adequate for disease progress curve analysis within a year [Campbell, 1986]. Regression was applied for analysis of the relationship between inoculum potential ($\log_{10}$ MPN + 1) and yield parameters in 1989 and 1990. The results of linear and nonlinear regression (polynomial with a quadratic term, exponential, line + exponential, logistic and Gompertz curves) were evaluated by means of their significance (F-test), the percentage of variance accounted for (R^2 adjusted) and plots of standardized residuals versus fitted values. Because of the split-plot design, effects of irrigation were investigated by fitting curves for each strip separately, and comparing the parameters by ANOVA [Campbell and Madden, 1990]. However, the comparison of the parameters of the nonlinear models will still be approximate, as the X-values (log MPNs) of the non-irrigated and irrigated sets have different positions (on the X-axis) and therefore the different parameters are not all estimated with the same precision. This should be taken into consideration, especially for the 'extrapolated' asymptotes. The nonlinear relationship between disease incidence and inoculum potential ($\log_{10}$ MPN + 1) was investigated by fitting different disease progress models to the untransformed data (using the 'Fitnonlinear' directive of GENSTAT 5): the monomolecular or negative exponential model, the logistic and the Gompertz model were compared. For the logistic and Gompertz model, the lower and upper asymptote were set at 0 and 1 (limits of disease incidence are 0 and 100%). The statistical fit of the models to the data was evaluated as described before.

Results

Environmental conditions

Moisture conditions, precipitation (≥ 1 mm) and soil moisture tensions at 15 and 30 cm depth, are presented in Fig. 2. The frequency and amounts of precipitation (irrigation not included) between emergence and harvest are given in Table 1. In 1988, soil moisture tensions did not attain extreme values and in irrigated plots tensions were reduced to below 20–30 cbar at 15 cm depth at times when in non-irrigated ones these values were amply exceeded (Fig. 2A). In the summer of 1989, temperatures were relatively high and since irrigation was not always adequately applied in this year, moisture tensions were high also in the irrigated plots. Therefore the difference between irrigated and non-irrigated conditions was not very pronounced (at 15 cm depth) in 1989, although at 30 cm depth the effect of irrigation could be noticed (Fig. 2B). In 1990, irrigation was applied more adequately and differences between irrigated and non-irrigated soil moisture conditions were present almost throughout the season (Fig. 2C). Soil temperatures above 15 °C, at 15 cm depth, were recorded in the beginning of May in 1988 (directly after emergence) and 1990 (two weeks after emergence), and around 13 May (before emergence) in 1989.

Field observations

In 1988, 50% emergence was approximately 11 days after sowing and was regular. The crop developed well. From the end of August, leaves of the plants in plots with the highest inoculum level had a slightly lighter green colour than in the other plots. Root symptoms, such as vascular browning and bearding, were not observed.

Because of weather conditions, sowing was late in 1989, and emergence was delayed and irregular because of the dry top layer of the soil and the absence of precipitation in the first weeks after sowing. Vascular browning in roots was observed already at the first sampling date in June. Yellowing of the leaves started to show around mid July, and more so at higher inoculum levels. In August, most plots had an overall yellow appearance, only those with inoculum levels 0 and 1 had just a few yellow plants. One plant with systemic leaf symptoms was observed in August. Root symptoms were severe in August and September; all types of symptoms were observed, from brown vessels with a yellow to brown discoloration of the surrounding tissue to strong bearding and sometimes rot of the root tip. The early harvest in 1989, resulting in a short growing season, was unintentional but due to the allocation of labour for the manual harvest.

In June 1990, in the non-irrigated strips leaf yellowing was observed, increasing in intensity from the originally non-infested plots towards the plots with the highest inoculum level. Plants in the irrigated strips showed

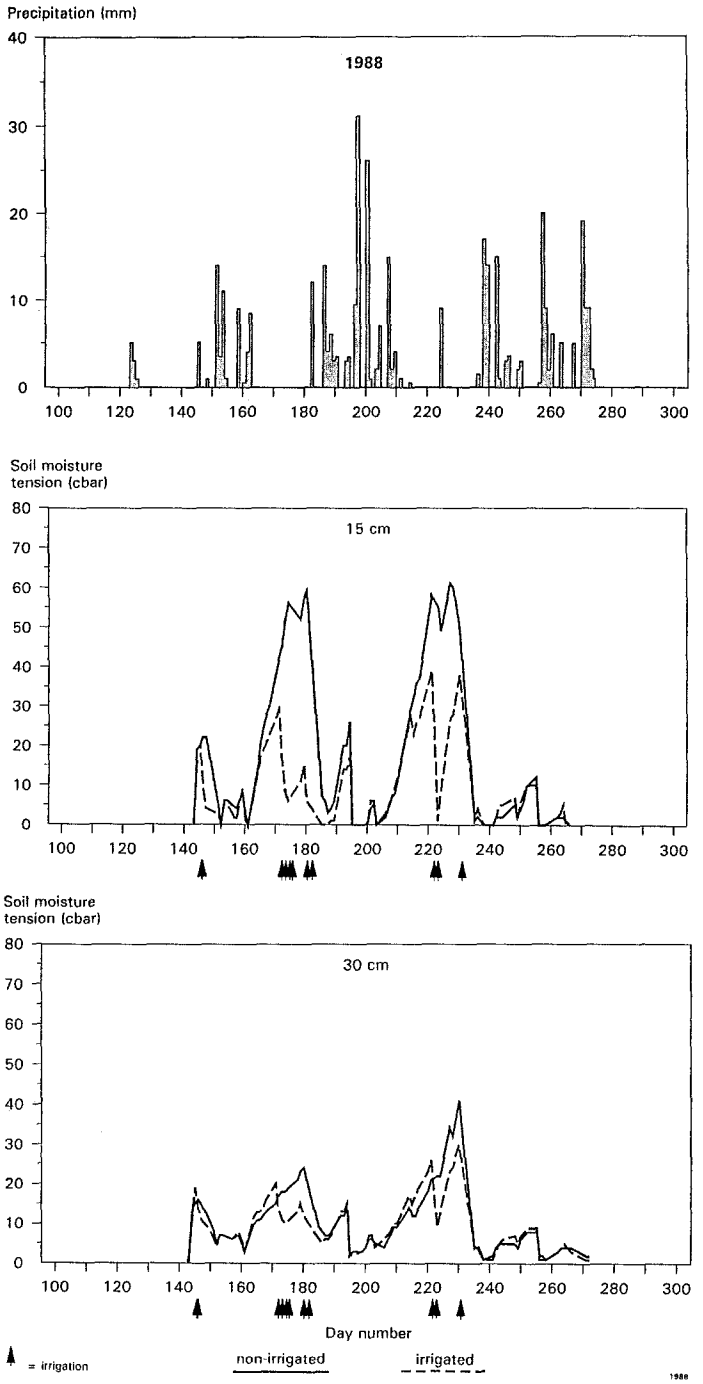

Fig. 2. Amount of precipitation (≥1 mm) per one, occasionally two, days and soil moisture tension (cbar) at 15 and 30 cm depth for the non-irrigated and irrigated plots. Applications of drip irrigation (c. 10 mm each time) are indicated by arrows. A) 1988.

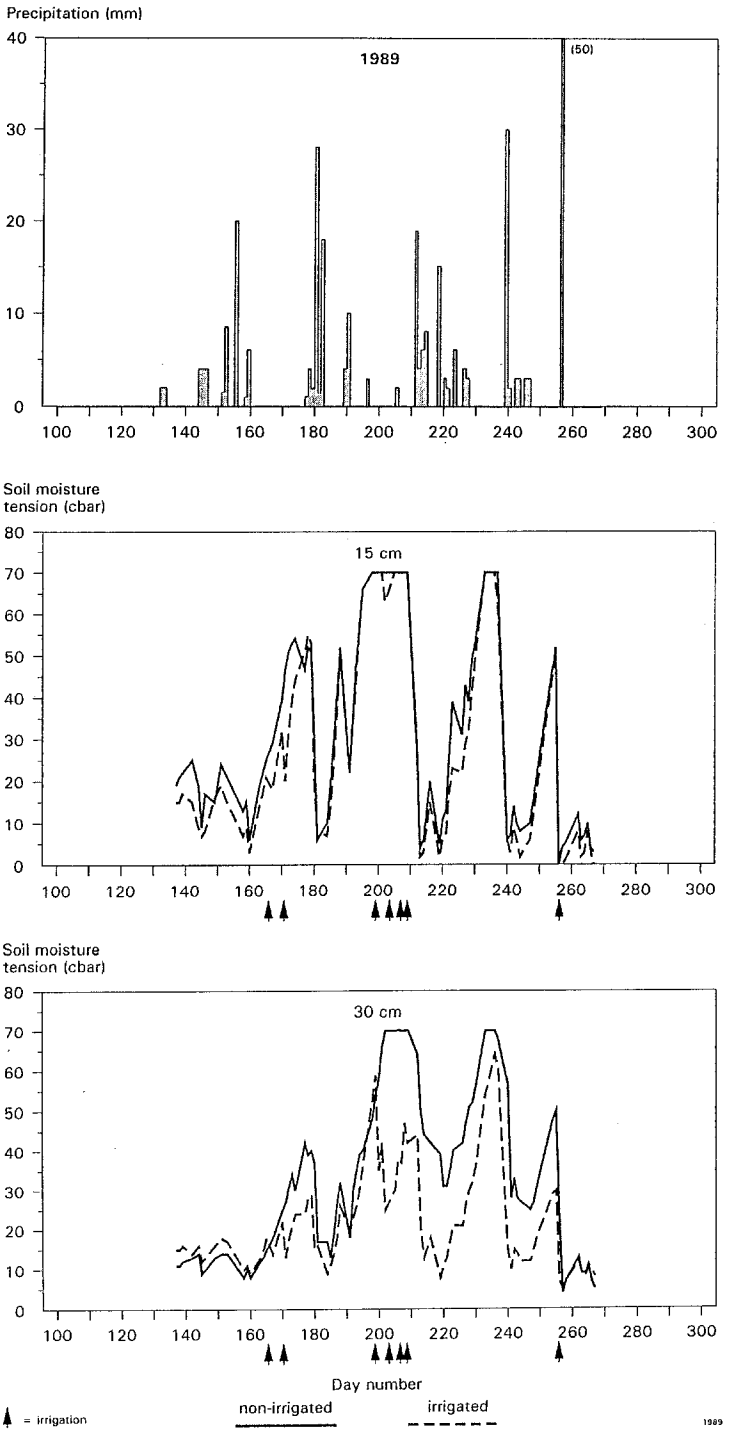

Fig. 2. (Continued) (B) 1989.

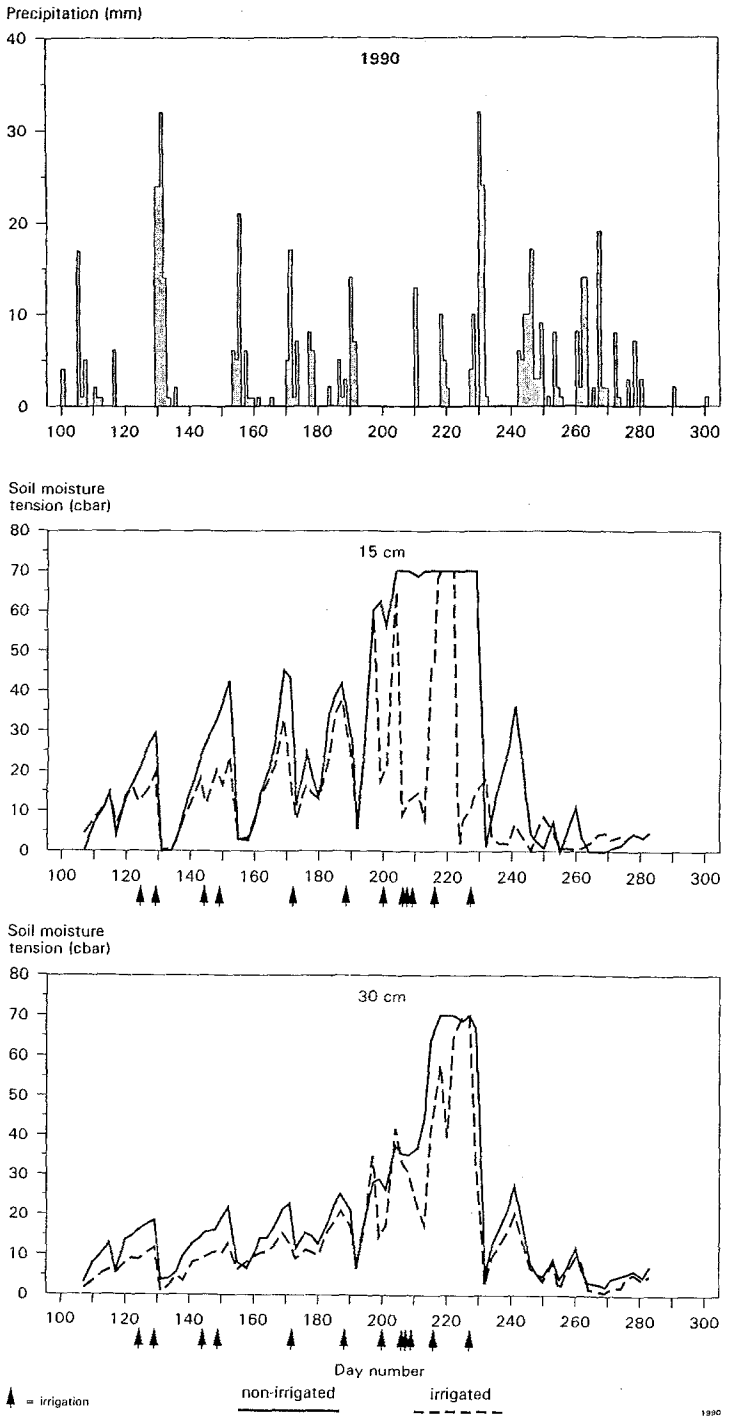

Fig. 2. (Continued) (C) 1990.

a general discoloration of leaves. Other a-typical leaf symptoms, such as a tapering of leaves and upright growth, were observed, as well as several plants with systemic leaf symptoms. Vascular browning in roots occurred in almost all plants of the two highest inoculum levels and in a lower number in the other plots. Bearding of roots was observed on some of these plants in June already. In August and September, high numbers of plants showed both vascular browning and heavy bearding. Plots were 'completely diseased'. From August onwards, leaf infection with *Phoma betae* and *Ramularia beticola* occurred with increasing severity.

Plant development between blocks differed because of differences in soil structure. At the beginning of July 1988, in one complete block (A) soil compaction became visible through water logging after heavy rainfall. In a second block, the same occurred to a minor extent and only in the irrigated strip. In 1989, plants in block A showed more leaf yellowing than plants in the other strips. After a large shower in the beginning of 1990, the whole block was flooded again and plants hardly formed tap roots because of the compact soil. In this year, plants in the irrigated plots of block B were also hampered in their development. Block A was omitted from the analyses of data in all three years.

Contamination of some originally non-infested plots was observed from the end of the first season onwards, the number of contaminated plots and their level of infestation increased during the years [Tuitert and Hofmeester, 1992].

Inoculum of BNYVV in soil increased after one and two beet crops; differences between the originally applied levels diminished. Inoculum levels in 1989 and 1990 differed between non-irrigated and irrigated plots [Tuitert and Hofmeester, 1992]; the effect of irrigation on parameters measured in these years should be considered in combination with the effect of irrigation on inoculum levels in the preceding year(s).

Disease incidence

1988. The first BNYVV-infected plants were detected in July in plots with the highest inoculum level (Fig. 3A). In August, infection was also detected in the irrigated plots of inoculum levels 2 and 3. At harvest, infected plants were found at all levels. The effect of inoculum level on disease incidence was significant ($P < 0.05$). Disease incidence increased in time ($P < 0.001$), with the increase depending on the inoculum level (interaction $P < 0.05$) and on the application of drip irrigation (interaction $P < 0.05$). Disease incidence did not exceed 20%. None of the infected plants showed root symptoms. The few infected plants detected in the control at harvest time (3% in non-irrigated plots, 10% in irrigated plots) were found in the three plots in which BNYVV was also detected in soil samples taken after harvest in October 1988 [Tuitert and Hofmeester, 1992].

1989. Disease incidence was considerably higher in the second year (Fig. 3A, B) than in the first. Already around mid June (six weeks after sowing) high numbers of infected plants were detected (Fig. 3A), up to 90–100% in plots with the highest inoculum level. Disease incidence was higher at higher inoculum levels ($P < 0.001$), was enhanced in the two-year irrigated plots ($P < 0.01$) (Fig. 3B) and increased during the season ($P < 0.01$). The incidence of root symptoms in September showed significant linear correlation (R^2 adjusted 76% with angular transformed data) with the incidence determined by ELISA. In ANOVA of symptom-showing infected plants, a linear effect of initial inoculum level was apparent ($P = 0.055$). The percentage of BNYVV-infected plants (back-transformed angular means) that exhibited root symptoms increased from 50–80% at the lowest inoculum levels to 100% at inoculum level 4. Infected plants were detected in the three control plots already found to be contaminated in 1988, and in one additional plot.

1990. In the third year, the whole field was heavily diseased; high incidences, accompagnied by root symptoms, were recorded by mid June (Fig. 3A). Disease incidence was still related to the initial inoculum level applied ($P < 0.001$), but, as was confirmed by the significant interaction with irrigation ($P < 0.001$), incidences between inoculum levels were only different for the non-irrigated plots (Fig. 3B). There was still an increase in incidence during the season ($P < 0.001$). In all control plots, infected plants were detected. At the three sampling times, there was a significant linear correlation between root symptom incidence and virus infection detected by ELISA; R^2 adjusted was 61, 51 and 71% for the first, second and third sampling, respectively. Addition of the factor initial inoculum level in stepwize regression resulted in a significant increase in the percentage of variance accounted for. The explanation for this and for the deviation from linearity was the finding that, especially at the first sampling, the percentage of BNYVV-infected plants showing root symptoms was higher at higher inoculum levels. In ANOVA of angular transformed percentages of virus-infected plants showing root symptoms, there was a significant linear effect of inoculum level ($P < 0.001$); back-transformed mean percentages increased from 16% for inoculum levels 0 and 1 to 100% for inoculum level 4 at the first sampling date. At harvest these figures ranged from 53% to 100% for the non-irrigated plots of inoculum level 0 and 4, with a mean of 98% for all the irrigated plots.

Root weight, sugar content and sugar yield

1988. In the first year, sugar content was reduced as a result of the applied infestation, but root weight was not significantly influenced (Table 2, Fig. 4A, B). The reduction in sugar content was significant from the first sampling time onwards (Fig. 5B). The mean sugar content at inoculum

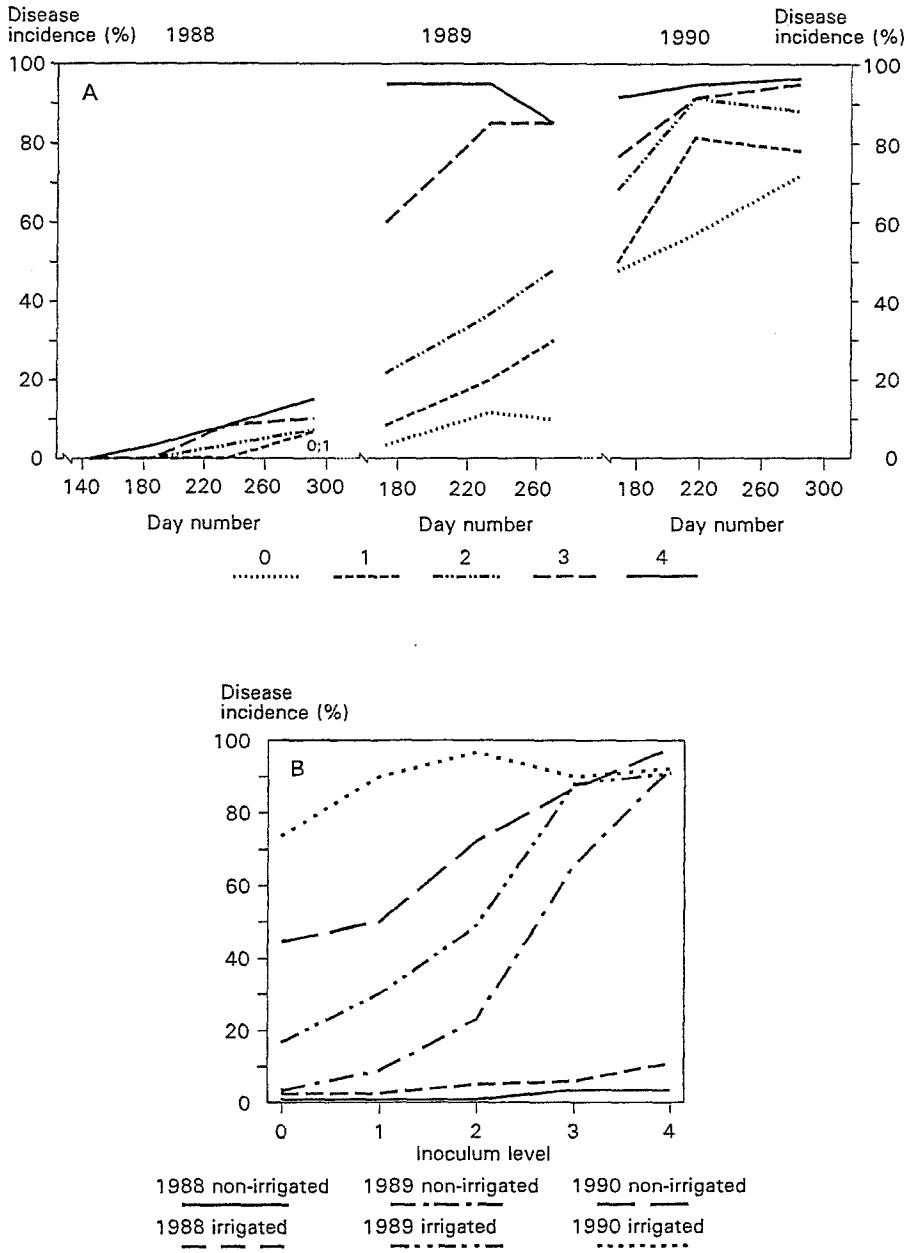

Fig. 3. A) Disease progress curves for the five initial inoculum levels (0–4) in 1988, 1989 and 1990. Arithmetic mean disease incidence (percentage of BNYVV-infected plants detected by ELISA) is plotted. B) Disease incidence, the arithmetic mean of all sampling times, for non-irrigated and irrigated treatments separately.

Table 2. Results of the analysis of variance of yield and quality parameters of sugar beet at different sampling times during three successive years. The trial had a split-plot design, with irrigation levels (two) as main plots and inoculum levels of rhizomania (five) as subplots

Parameters[a]	Significance[b] of factors [c] in ANOVA									Orthogonal polynomial contrasts[d]					
	Early sampling			Intermediate sampling			Harvest time			Harvest time					
	I	R	IxR	I	R	IxR	I	R	IxR	R-lin	R-quad	R-dev	IxR-lin	IxR-quad	IxR-dev
1988	4 July (80)[e]			23 August (130)			18 October (186)								
root weight	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
sugar content	ns	***	ns	ns	***	***	ns	***	*	***	***	ns	***	ns	ns
sugar yield	ns	ns	ns	ns	*	ns	ns	**	ns	**	**	ns	ns	ns	ns
Na	ns	ns	*	ns	ns	ns	ns	**	ns	***	ns	ns	ns	ns	ns
α-amino N	ns	ns	*	ns	ns	ns	ns	***	ns	***	***	ns	ns	ns	*
1989	22 June (68)			21 August (111)			27 September (148)								
root weight		n.d.		***	*	ns	ns	***	*	***	ns	ns	ns	**	ns
sugar content				ns	***	ns	ns	***	ns	***	ns	ns	*	ns	ns
sugar yield				***	**	ns	**	***	*	***	ns	*	*	*	ns
Na				ns	***	ns	ns	***	ns	***	ns	ns	ns	ns	ns
α-amino N				*	*	ns	*	***	***	***	ns	ns	***	ns	ns
1990	18 June (77)			6 August (126)			12 October (193)								
root weight	*	***	*	ns	ns	*	ns	***	***	***	ns	ns	***	ns	ns
sugar content	*	*	ns	ns	*	ns	ns	*	ns	***	ns	ns	**	ns	ns
sugar yield	**	***	*	*	*	*	*	***	***	***	ns	ns	***	ns	ns
Na	**	ns	ns	ns	**	ns	ns	*	ns	**	ns	ns	ns	ns	ns
α-amino N	ns	ns	ns	ns	ns	ns	ns	ns	**	*	ns	ns	***	ns	ns

[a] Units of measurements: fresh weight of root and sugar yield in kg ha^{-1}, sugar content as a percentage of fresh weight of root; contents of Na and α–amino N in mmol kg^{-1} fresh weight of root. At harvest all plants from an area of 20 m^2 were taken for the assessments. At the periodical sampling dates 20 plants were sampled, root weights were converted to weights per ha by means of the plant density of each plot. [b] Significances: *** = $P \leq 0.001$; ** = $P \leq 0.01$; * = $P \leq 0.05$; ns = not significant. [c] Factors: I = Irrigation; R = Rhizomania inoculum level; IxR = interaction between irrigation and inoculum level for one (1988), two (1989) or three (1990) years. [d] Orthogonal polynomial contrasts were used to examine trends in data: linear (lin) and quadratic (quad) effects of inoculum level (R) were examined, variance not accounted for by these effects was assigned to deviations (dev). [e] ( ) number of days after sowing. n.d.: not determined.

level 4 was reduced by 5% in June and 11% in October, compared to the control. Irrigation significantly influenced the linear effect of inoculum level; in the control and the lowest inoculum level sugar content was not affected or slightly increased, from level 2 onwards it was decreased by irrigation (Fig. 4B). The resulting sugar yield was significantly influenced by inoculum level, the reduction by and interaction with irrigation was not significant. Only at the highest inoculum level sugar yield was reduced (by 10%) compared to the control (Fig. 4C and 6), in October.

1989. Root weight decreased progressively with increasing inoculum level in 1989, both in August and September (Tables 2 and 3, Fig. 5A). The effect of two years of irrigation was detected in August as an overall reduction, in September the effect of irrigation depended on the inoculum level. Root weights ranged from 70 and 63 ton ha^{-1} for the non-irrigated and irrigated control, to 32 and 26 ton ha^{-1} for both highest inoculum levels. Sugar content showed a progressive decrease with increasing inoculum level, which was found both in August and September (Fig. 4B). Between the two sampling times, mean sugar content of the highest inoculum level increased with only 0.4%, that of the control with 1.5% (Fig. 5B). Two years of irrigation affected the linear relationship of sugar content with inoculum level (Table 2). The effect of rhizomania on sugar yield was disastrous in this year, where the highest inoculum levels resulted in only about 3000 kg ha^{-1}, a mean reduction of 66% compared to the control plots (Fig. 6). The strong decrease in sugar yield with increasing inoculum level (linear + nonlinear) was enhanced, to a degree depending on the inoculum level, by two-year irrigated conditions (Table 2, Fig. 4C).

1990. The build-up of inoculum at all plots was such that yields obtained in this year were extremely low. Early in the year, root weight still showed a decrease with increasing inoculum level, both for the non-irrigated and irrigated plots (Table 2). At harvest, an effect of inoculum level was detected only for the non-irrigated plots (significant interaction) (Table 2, Fig. 4B), the irrigated plots attained a mean yield level of only 33 ton ha^{-1} (Table 3). In the two periodical samplings, sugar content decreased as inoculum level increased, with an extra decrease in the three-year irrigated plots in June. Sugar content hardly increased between August and October (Fig. 5B). At harvest, there was still a significantly linear effect of inoculum level (Table 2). Effects of inoculum levels and three years of irrigation on root yield were reflected in the effects on sugar yield (Table 2). The increase in sugar yield during the season is presented in Fig. 5C. At harvest, sugar yields ranged from 6587 to 4133 kg ha^{-1} for the non-irrigated plots (Fig. 4C), with an overall mean of 3323 kg ha^{-1} for the irrigated plots (Table 3).

Table 3. Final root weight, sugar content and sugar yield of susceptible sugar beet at different levels of inoculum of BNYVV, without or with irrigation in three successive years. Means of factors are presented for all three parameters, irrespective of the significance of main effects and interactions, as given in Table 2

Year	Inoculum level[a]					Irrigation[b]		Overall mean
	0	1	2	3	4	–	+	
Root weight (× 1000 kg ha^{-1})								
1988	66.17	71.32	67.35	68.90	66.91	73.03	68.13	68.13
1989	66.62	61.32	46.88	35.97	29.41	53.34	42.73	48.04
1990	43.28	43.47	40.49	38.69	35.94	47.73	33.02	40.37
Sugar content (%)								
1988	16.63	16.56	16.29	15.77	14.71	16.18	15.80	16.00
1989	13.38	12.92	11.90	10.79	10.41	12.20	11.56	11.88
1990	11.28	10.83	10.48	10.27	10.28	11.16	10.10	10.63
Sugar yield (kg ha^{-1})								
1988	11002	11809	11005	10899	9871	11813	10022	10917
1989	8932	7952	5624	3873	3051	6722	5050	5886
1990	4959	4775	4305	3964	3696	5356	3323	4340

[a] Initial inoculum levels: 0 = non-infested; 1 to 4 = levels increasing with tenfold steps.
[b] Drip irrigation applied (+) or not (–) during one, two or three successive years, for 1988, 1989 and 1990, respectively.

Quality parameters

1988. Already in the first year, Na content of the roots at harvest increased with increasing inoculum level (Tables 2 and 4), although values were low. At the first sampling in July, the increase was already noticeable in the irrigated plots, but not in the non-irrigated ones, as confirmed by the significant interaction of inoculum level and irrigation. In August, differences were not significant. Mean Na-content at the three sampling dates decreased from 3.95 to 2.11 to 1.66 mmol kg^{-1} root. Alpha-amino N content showed a significant decrease with increasing inoculum level at harvest (Tables 2 and 4). Contents in the irrigated plots were lower than in the non-irrigated ones, but the interaction was not significant ($P = 0.09$). In July an interaction was seen, which did not reflect any clear trend, in August no differences were found. Mean values at the three sampling times were 11.80, 7.77 and 10.98 mmol kg^{-1} root.

1989. Both in August and September, Na content showed a large and significant increase with increasing inoculum level (Table 4, Fig. 7). Values for inoculum level 0 to 4 ranged from 3.80 to 7.58 mmol kg^{-1} root in August and 2.98 to 6.80 mmol kg^{-1} root in September, thus showing up to a 128% increase at the highest inoculum level. Alpha-amino N content

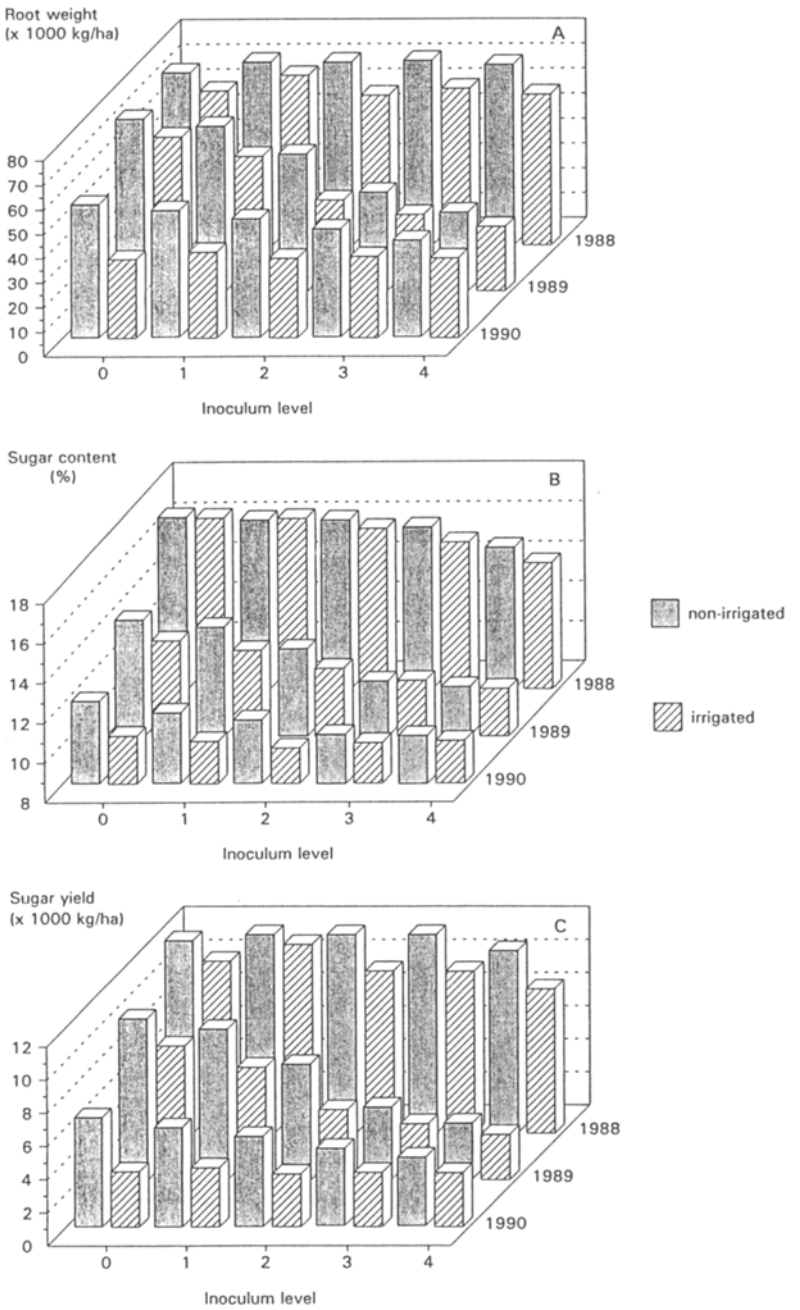

Fig. 4. Yield parameters of sugar beet cv. Regina at five inoculum levels of BNYVV in three successive years, with or without application of drip irrigation. Approximate amounts of BNYVV-infested soil used to create the different inoculum levels in 1988 were 0, 0.5, 5, 50 and 500 kg ha^{-1} for inoculum levels 0–4. See Table 2 for significances of factors and interactions. A. Root weight; B) Sugar content; C) Sugar yield.

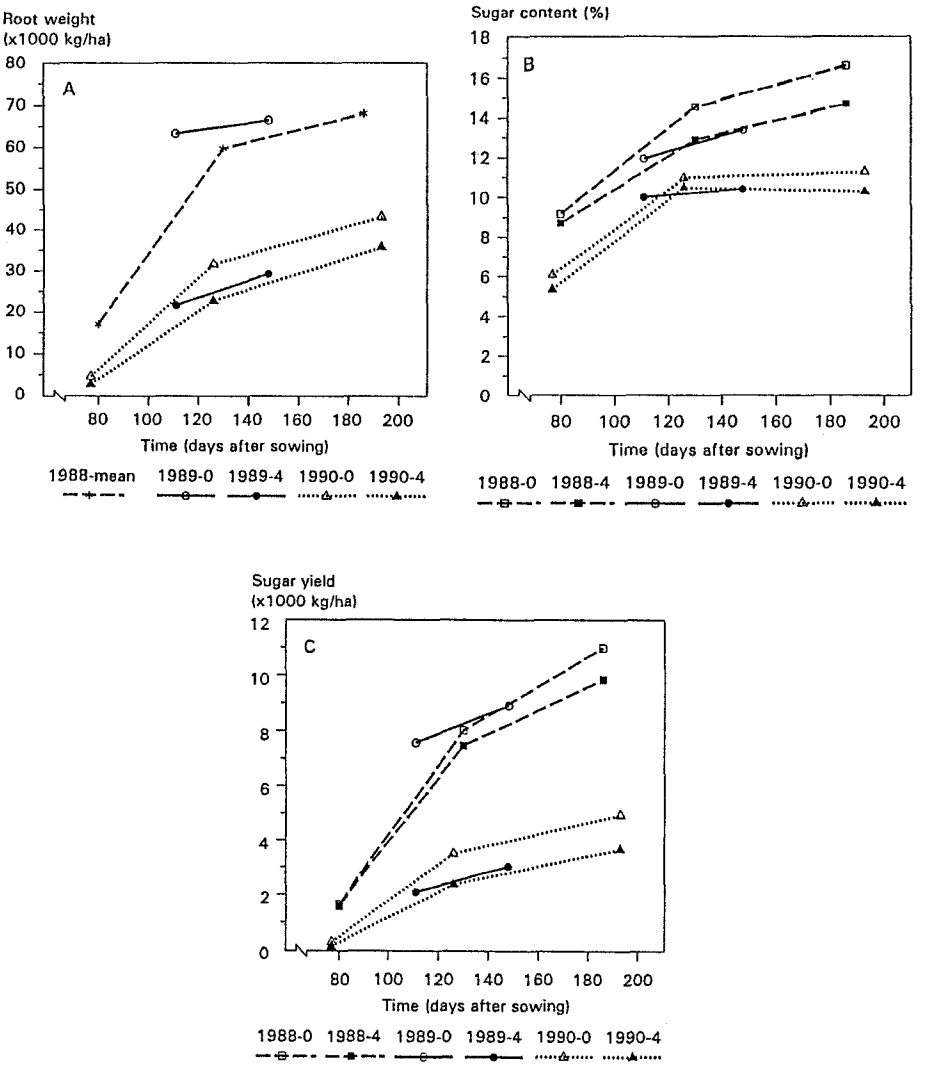

Fig. 5. The increase in yield parameters during the season for the originally non-infested plots (inoculum level 0) and plots with the highest infestation of BNYVV (inoculum level 4) in three successive years. Data are means of non-irrigated and irrigated plots, plotted against number of days after sowing. A) Root weight; B) Sugar content; C) Sugar yield.

reacted distinctly to the different lower inoculum levels, discriminating the two-year irrigated plots from the non-irrigated ones, but at higher inoculum levels the contents leveled off (Fig. 8). In August these effects were also observed, but less pronounced. In August, values ranged from 9.97 to 5.83 mmol kg^{-1}, in September from 7.80 to 3.27 mmol kg^{-1} for inoculum levels 0 to 4, non-irrigated.

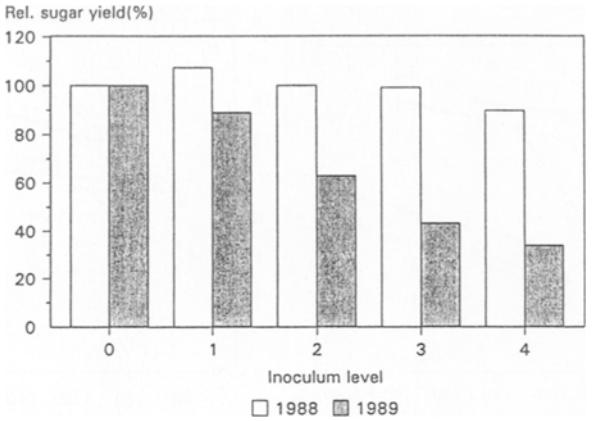

Fig. 6. Mean sugar yield of beet cv. Regina at different inoculum levels of BNYVV in soil, relative to the sugar yield obtained on non-infested control plots, in 1988 and 1989.

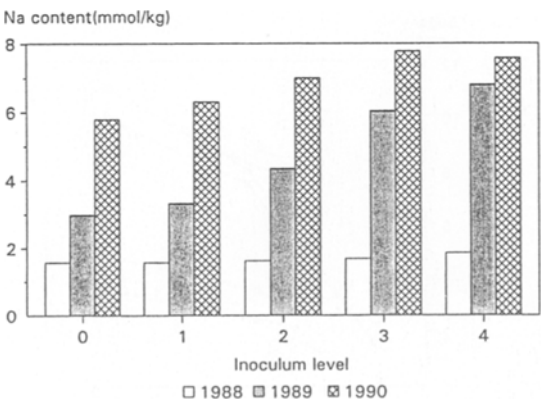

Fig. 7. Mean sodium content in fresh beet root, cv. Regina, at five inoculum levels of BNYVV, in three successive years. The effect of initial inoculum level on Na content was significant in all three years (Table 2).

1990. Early in 1990, Na content was increased by irrigation. Later, in August and October, Na content increased with increasing inoculum level. Mean values at the three sampling times were 15.18, 5.65 and 6.89 mmol kg^{-1}. For α-amino N content, the interaction between inoculum level and irrigation was significant at harvest; a linear trend was present and more so under non-irrigated conditions. Mean values during the year were 15.82, 7.23 and 5.45 mmol kg^{-1}.

In Fig. 9 the relative contents of Na and α-amino N of the four infested treatments as compared to the originally non-infested control at harvest are shown. Na content showes a stronger reaction to increasing inoculum level than α-amino N.

Table 4. Contents of Na and α–amino N in the roots of susceptible sugar beet at harvest, at different levels of inoculum of BNYVV, without or with irrigation in three successive years. Means of factors are presented for both parameters, irrespective of the significance of main effects and interactions, as given in Table 2

Year	Inoculum level[a]					Irrigation[b]		Overall mean
	0	1	2	3	4	–	+	
Na (mmol kg^{-1} root)								
1988	1.57	1.57	1.62	1.68	1.85	1.57	1.74	1.66
1989	2.98	3.33	4.35	6.03	6.80	4.16	5.24	4.70
1990	5.79	6.30	7.00	7.78	7.58	5.78	8.00	6.89
α-*amino N* (mmol kg^{-1} root)								
1988	11.92	11.93	11.70	10.72	8.65	12.17	9.79	10.98
1989	6.07	5.32	4.03	3.53	3.38	5.27	3.66	4.47
1990	6.12	5.72	5.33	5.08	5.00	6.30	4.61	5.45

[a] Initial inoculum levels: 0 = non-infested; 1 to 4 = levels increasing with tenfold steps.
[b] Drip irrigation applied (+) or not (–) during one, two or three successive years, for 1988, 1989 and 1990, respectively.

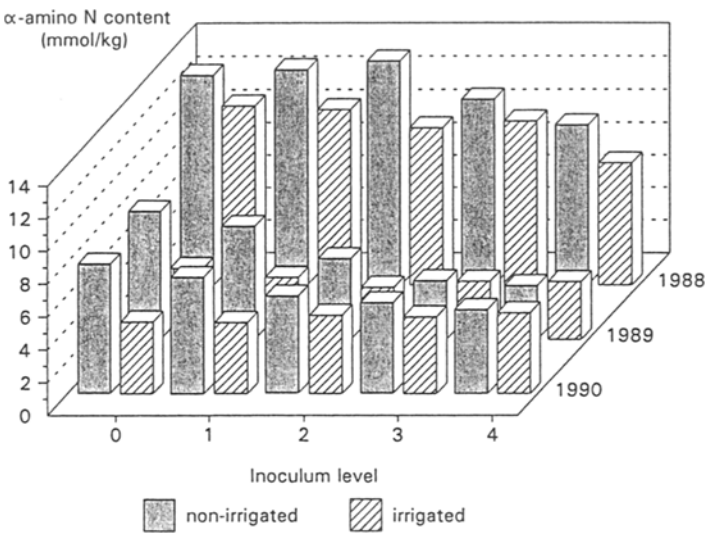

Fig. 8. α-Amino nitrogen content in fresh beet root, cv Regina, at five inoculum levels of BNYVV, in three successive years, with or without application of drip irrigation. See Table 2 for significance of factors and interactions.

Correlation of sugar content and Na content of the root

The negative correlation between sugar and Na content of the root has been recognized as a feature of rhizomania disease [Heijbroek, 1989]. Therefore

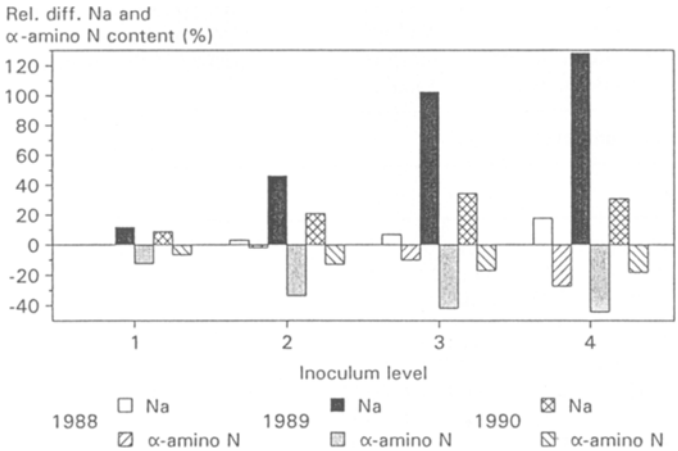

Fig. 9. Relative differences in sodium and α-amino nitrogen content in fresh beet root in plants at different inoculum levels of BNYVV, compared to the contents in plants of the originally non-infested control plots in each year.

the relationship between these parameters was investigated with regression analysis, comparing a linear, a polynomial (linear + quadratic) and an exponential model. Although in 1988 disease severity was low, a linear relationship between the parameters was present (R^2 adjusted 64%, $P < 0.001$). A wide range of inoculum and disease levels was present in 1989, the two quality parameters were highly correlated in this year (R^2 adjusted 94%, $P < 0.001$, for an exponential relationship). In 1990, severity was high for all plots; for the small range of sugar contents in this year the relationship was less close, and best described by an exponential curve (R^2 adjusted 69%, $P < 0.001$). Coefficients of determination from the linear correlation matrix of parameters were –0.81, –0.95 and –0.81 for 1988, 1989 and 1990, respectively. Data of the three years showed a continuous decrease (Fig. 10). For α-amino N the coefficients of determination for linear correlation with sugar content were 0.64, 0.72 and 0.37 for 1988, 1989 and 1990, respectively.

Relationship between MPNs of BNYVV in soil in spring and yield parameters of sugar beet at harvest

1989. The relationship between $\log_{10}$ MPNs of BNYVV and root weight, sugar content and sugar yield was best decribed by Gompertz curves ($P < 0.001$). The effect of irrigation was determined by strips (main plots) and not by individual plots, because of the split-plot design. In ANOVA of regression parameters per strip, for sugar yield only the difference in upper asymptote (A) was significant ($P < 0.05$), whereas for root weight and sugar content differences between parameters for the non-irrigated and

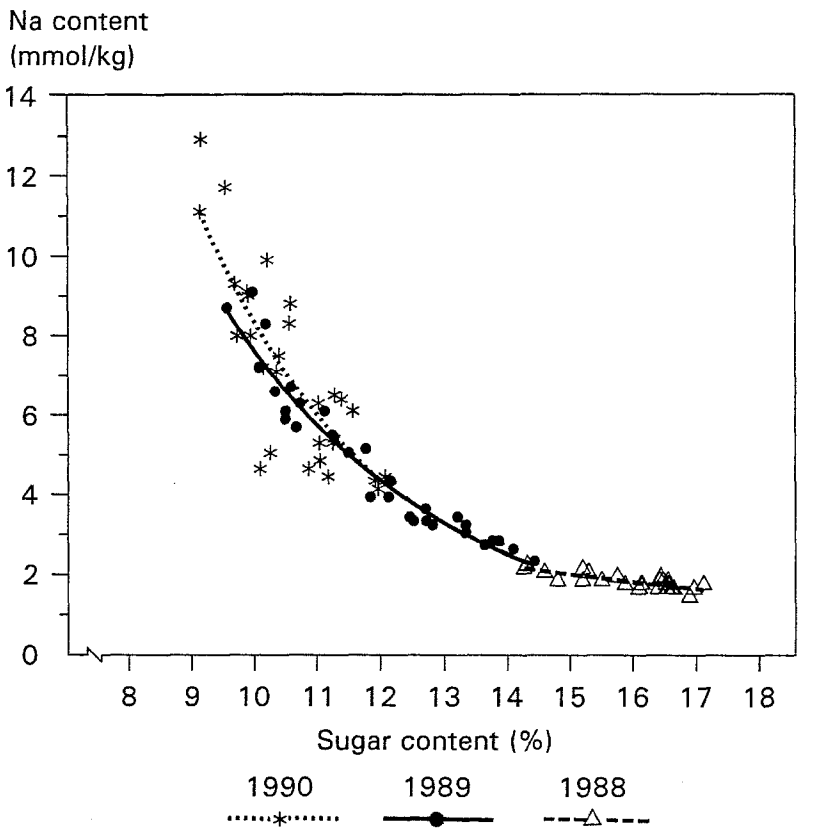

Fig. 10. The relationship between sodium content (mmol kg^{-1} fresh root) and sugar content (% of fresh root) in sugar beet cv. Regina grown on BNYVV-infested plots in three successive years. Regression analysis was performed per year. Regression equations were:
1988: $Y = 4.62 - 0.185\ X$, R^2 adjusted = 64%, $P < 0.001$;
1989: $Y = 1.24 + 401 * 0.66^X$, R^2 adjusted = 94%, $P < 0.001$;
1990: $Y = 3.58 + 7377 * 0.48^X$, R^2 adjusted = 69%, $P < 0.001$.

irrigated set were not significant. For root weight and sugar content the best curve for the pooled data is presented (Fig. 11A, B). For sugar content the logistic model gave the same fit as the Gompertz model. In Fig. 11C the best fitted lines for sugar yield (non-irrigated and irrigated separately) are presented. The considerations with respect to the estimation of the asymptotes and the use of MPN = 0 for inoculum levels below the detection level in bioassay should be kept in mind.

1990. For the three-year irrigated plots, there was no significant relationship between the inoculum potential in soil and yield parameters. For the non-irrigated plots the logistic and Gompertz equations were not appropriate. At the high inoculum levels occurring in this year (log_{10} MPNs

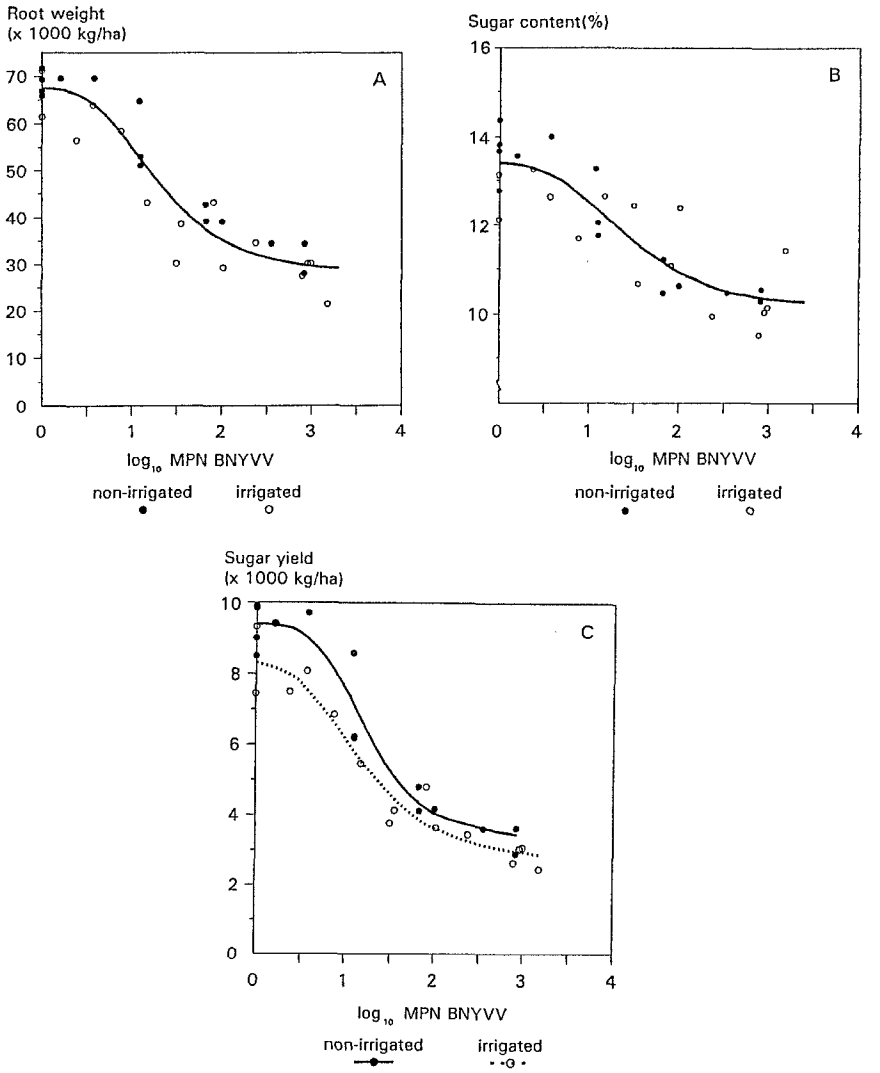

Fig. 11. Yield parameters of sugar beet cv. Regina in relation to the inoculum potential of BNYVV in soil in 1989. Data of all individual plots were used for regression analysis. The inoculum potential is the log-transformed most probable number of infective units per 100 g soil. The Gompertz equation, $y = A + C*\exp(-\exp(-r_g*(x - M)))$, fitted the data best. The effect of irrigation was tested by ANOVA of the equation parameters for each strip separately. For root weight and sugar content the effect of irrigation was not significant (at $P = 0.05$) and one line for the pooled data is drawn, for sugar yield the difference in upper asymptote A was significant ($P < 0.05$), best lines for the non-irrigated and irrigated data set are drawn.

A) Root weight: A = 67.5, r_g = 1.77, C = –39.0, M = 1.08, R^2 adjusted = 88%, $P < 0.001$.

B) Sugar content: A = 13.36, r_g = 1.55, C = –3.22, M=1.19, R^2 adjusted = 73%, $P < 0.001$.

C) Sugar yield: non-irrigated A = 9443, r_g = 2.38, C = –6078, M = 1.08, R^2 adjusted = 92%, $P < 0.001$; irrigated A = 8376, r_g = 1.77, C = –5599, M = 0.96, R^2 adjusted = 92%, $P < 0.001$.

between 1 and 4), root weight and sugar yield showed a linear decrease with increasing soil inoculum (R^2 adjusted 81 and 84%, respectively). The equations for root weight (tons ha^{-1}) and sugar yield (kg ha^{-1}) were: Y = 61.88 – 6.60 * log (MPN BNYVV) and Y = 7582 – 1045 * log (MPN BNYVV), respectively. A slightly higher R^2 adjusted was obtained with the exponential equation (Y = A – B * exp (R * X)), but then standard errors of parameter estimates were very high, amounting up to 1.5 times the estimate itself for B. This was also found for sugar content, were the linear equation, Y = 12.64 – 0.70 * log (MPN BNYVV), explained 62% of the variance.

Relationship between MPNs of BNYVV in soil and disease incidence

1989. Disease incidence recorded as the proportion of infected plants determined by ELISA showed a logistic increase with increasing inoculum potential of BNYVV in the soil for all three sampling times (Table 5, Fig. 12A). The logistic rate of increase and the log MPN value at the point of inflection (Y = 0.5) both were highest in June and decreased in the later samplings. Incidence of plants with root symptoms at harvest could also be described by the logistic equation (Fig. 12B).

1990. Only for the very early infected plants, showing root symptoms already in June, a logistic increase (R^2 adjusted 75%) was found. For all other assessments an increasing trend was present, but the different equations could not explain more than 60% of the variance (Table 5, Fig. 12C, D).

Discussion

Yield of sugar beet at different inoculum levels of BNYVV in soil

Inoculum levels that could hardly be detected in soil by bioassay directly after application of the inoculum to the field [Tuitert and Hofmeester, 1992] caused a reduction of sugar content already in the first year, but had no effect on root weight. In the absence or at the lowest inoculum level of BNYVV, irrigation did not affect sugar content, but at the higher inoculum levels it caused an extra reduction. At the highest inoculum level (0.01% of infested soil calculated to the upper 30 cm of the soil), sugar yield was reduced by 10% compared to the non-infested control. The effect of irrigation, periodically decreasing (positive) soil moisture tensions, will primarily be due to an effect on the vector *P. betae*, enabling more primary infections and more secondary infection cycles, resulting in an enhanced virus content of and spread in the roots. The higher inoculum potentials in the irrigated plots than in the non-irrigated plots after one year [Tuitert and

Table 5. Summary of nonlinear regression parameters and statistics of different models fitted to describe disease incidence (proportion) with increasing inoculum potential ($\log_{10}$ (MPN BNYVV/100 g + 1)) at different sampling times

Model[a]	BNYVV-infected plants, determined by ELISA				Plants showing root symptoms			
	R^2 adj. (%)[b]	Rate ± s.d.[c]		M or B[d]	R^2 adj. (%)[b]	Rate ± s.d.[c]		M or B[d]
1989: June								
Logistic	87	2.78 ± 0.56		1.96	n.d.			
Gompertz	84	1.88 ± 0.39		1.73				
Monomolecular	68	0.64 ± 0.05	(0.45)	1.08				
1989: August								
Logistic	79	2.00 ± 0.39		1.44	n.d.			
Gompertz	77	1.27 ± 0.25		1.09				
Monomolecular	71	0.55 ± 0.05	(0.59)	0.99				
1989: September								
Logistic	81	1.84 ± 0.30		1.29	89	1.98 ± 0.28		1.52
Gompertz	80	1.20 ± 0.19		0.93	89	1.41 ± 0.20		1.21
Monomolecular	76	0.55 ± 0.05	(0.60)	0.95	80	0.57 ± 0.04	(0.56)	1.02
1990: June								
Logistic	60	1.61 ± 0.36		2.00	75	2.81 ± 0.61		2.54
Gompertz	58	1.14 ± 0.25		1.60	74	1.88 ± 0.39		2.30
Monomolecular	54	0.50 ± 0.07	(0.70)	1.71	59	0.54 ± 0.06	(0.61)	2.07
1990: August								
Logistic	42	1.25 ± 0.33		0.97	56	1.31 ± 0.28		1.35
Gompertz	42	1.08 ± 0.28		0.65	57	1.08 ± 0.21		1.00
Monomolecular	42	0.40 ± 0.09	(0.92)	1.27	57	0.42 ± 0.06	(0.87)	1.55

Table 5. (Continued)

Model[a]	BNYVV-infected plants, determined by ELISA				Plants showing root symptoms		
	R^2 adj. (%)[b]	Rate ± s.d.[c]		M or B[d]	R^2 adj. (%)[b]	Rate ± s.d.[c]	M or B[d]
1990: October							
Logistic	50	1.19 ± 0.28		0.72	58	1.44 ± 0.31	1.47
Gompertz	49	1.04 ± 0.24		0.40	56	1.11 ± 0.23	1.07
Monomolecular	48	0.41 ± 0.08	(0.90)	1.02	54	0.43 ± 0.07	1.50

[a] The models were fitted using the FITNONLINEAR directive of GENSTAT 5 [Payne et al., 1988]. The following equations were used: $y = (1 - B*R^x)$ for the monomolecular model, with B the integration constant and the general form of the monomolecular rate r_m equivalent to –log(Rate) and presented in brackets. The logistic equation used was $y = A + C/(1 + \exp(-r_l(x - M)))$, with the upper and lower asymptote set to 0 and 1 by setting A = 0 and C = 1, r_l the logistic rate of increase and M the point of inflection for the explanatory variable ($\log_{10}$ MPN BNYVV). For the Gompertz equation, $y = A + C * \exp(-\exp(-r_g(x - M)))$, A and C were also set to 0 and 1, respectively, r_g was the rate of increase and M the point of inflection. All regressions were significant ($P < 0.001$), there were no differences between non-irrigated and irrigated plots.

[b] The percentage of variance accounted for by the model.

[c] The rate parameters of the different models are presented with their standard deviations. For the monomolecular model rate (r_m), in the equation $y = 1 - B * \exp(-r_m * x)$, is given in brackets.

[d] For logistic and Gompertz equations the points of inflection (M) are given, for the monomolecular equations the integration constant (B).

n.d.: not determined.

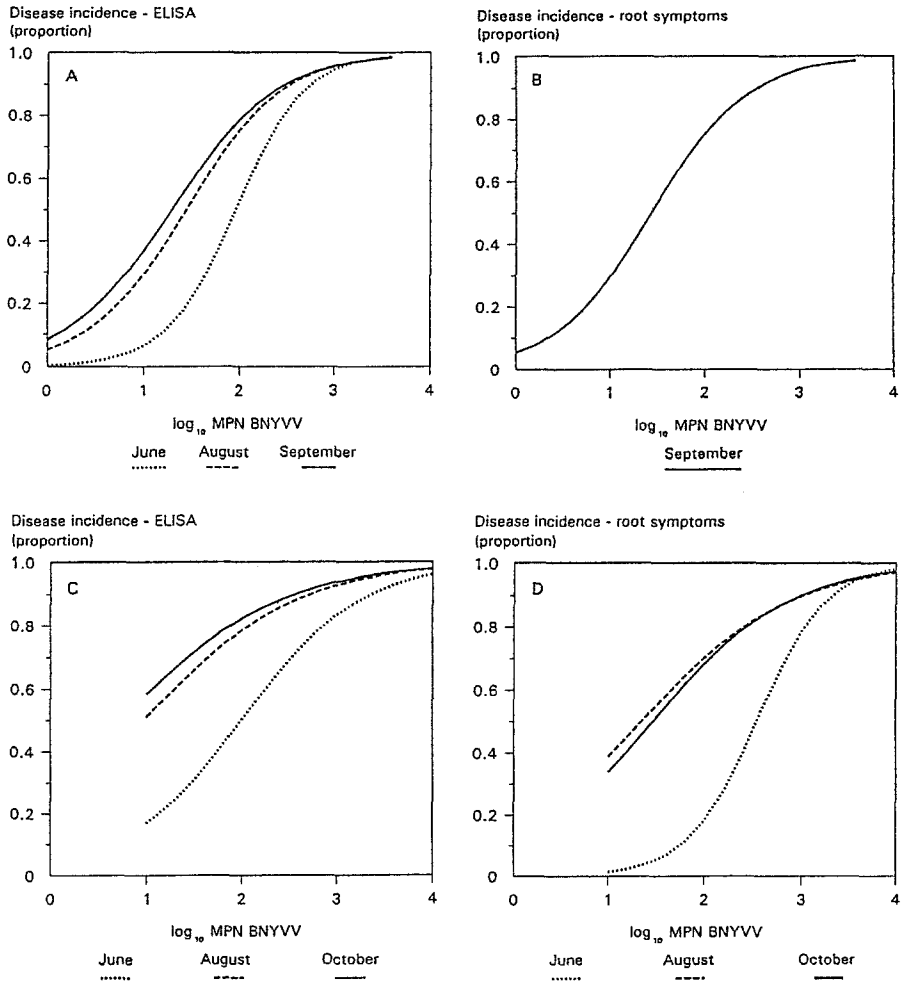

Fig. 12. The logistic relationship between the inoculum potential of BNYVV in soil (log most probable number of infective units per 100 g soil) in spring and disease incidence at different sampling times. Disease incidence (proportion) was determined by ELISA of root tips (A and C), or by visual rating of tap roots for the presence of symptoms (B and D). Parameters of the equations and some statistics are given in Table 5. A) 1989-ELISA; B) 1989-root symptoms; C) 1990-ELISA; D) 1990-root symptoms.

Hofmeester, 1992] support the statement on the irrigation effect. Other effects of irrigation relevant to the infection of roots were discussed earlier [Tuitert and Hofmeester, 1992].

In the first year, multiplication of inoculum in soil [Tuitert and Hofmeester, 1992] was such that in the next year, 1989, both sugar content and root yield decreased progressively with increasing inoculum level. Sugar yield reductions of 11% up to 66% at the highest inoculum level occurred. Two years of extra moist conditions aggravated the reduction of

sugar yield in the second year, especially at the lower inoculum levels. The effect of irrigation should mainly be ascribed to the higher infestation of irrigated plots of the same initial inoculum level, caused by irrigation in 1988 [Tuitert and Hofmeester, 1992]. An additional reduction by irrigation in 1989 apparently occurred at the lower inoculum levels (Fig. 11), remarks on the statistical analysis were made before. Since timing and frequency of irrigation during the summer of this year were not adequate to maintain a higher moisture content in the irrigated plots compared to non-irrigated ones for longer periods of time, effects of irrigation could have been stronger.

After two successive years, BNYVV-infestation had increased to high levels and all control plots had become contaminated. For the non-irrigated plots, root weight and sugar yield still showed a decrease with increasing inoculum level, for the three-year irrigated plots the mean sugar yield was 3300 kg ha^{-1}. At the highest inoculum level this low yield level (around 30% of the disease-free yield in 1988) had already been recorded in the second beet crop after infestation of the plots.

In climate room experiments, BNYVV affected root weight at lower inoculum levels than in the field. In an 18-week bioassay using small pots, tap root weights of plants growing in soil dilution 10^{-4}, comparable to inoculum level 4 in the field in 1988, were reduced by 50% [Tuitert and Bollen, 1993]. In the field, only sugar content was reduced, by 9–14%, at this infestation level. Results of greenhouse and climate room experiments may be more pronounced than those of field experiments for several reasons: optimal temperatures are maintained, water is supplied regularly, inoculum will be uniformly distributed in the soil, the pot volume limits the extension of the root system, and the soil volume will be more densely explored by the roots. In greenhouse experiments with large pots and a three-month growing period, the effects of a lowest soil dilution of 10^{-3} on sugar content and root yield [Bürcky et al., 1986] corresponded with those of our field experiment.

Müller and Gößwein [1987] studied the influence of irrigation on rhizomania in naturally infested fields. Twice they found that irrigation increased sugar content, twice a decrease was observed. Root weight was increased by irrigation in a relatively dry year, but was not affected by irrigation in a year with sufficient precipitation. No information on the degrees of infestation of the experimental fields was given and disease incidence recordings were not presented. Sugar beet is moderately tolerant to low soil moisture content [Van der Schans and Drenth, 1989], the severity of effects of drought depending on the growth stage at which drought is experienced [Brown et al., 1987]. Under drought stress conditions, in the absence of rhizomania, irrigation treatments increase root weight, but have little effect on sugar content [Winter, 1988; Davidoff and Hanks, 1989; Van der Schans and Drenth, 1989]. When irrigation is applied shortly before harvest, hydration effects may cause a lower sugar content on fresh weight basis.

In 1988, with sufficient precipitation, irrigation did not significantly affect root weight; neither in the control, nor in the infested plots. In the relatively dry second year, an increase in root yield in the absence of disease could have been expected. However, the slight contamination of the control plots in 1989 was probably responsible for the negative effect of irrigation on root weight also in these plots.

In the soil used to infest the plots no other pathogens were observed. The correlation of yield and damage with the amount of infested soil added can be ascribed to BNYVV, as was apparent also from the correlation of infestation levels with disease incidence. Effects of the vector itself on yield under field conditions have not been reported. Initial inoculum levels, created by application of tenfold different amounts of infested soil, did not result in tenfold differences in *P. betae* inoculum, because of the presence of a relatively high resident *P. betae* population [Tuitert and Hofmeester, 1992]. Therefore, vector populations could not be responsible for the described effects. The presence of other soil-borne viruses of sugar beet can not be excluded, however, these viruses have not been shown to cause rhizomania symptoms or yield reductions in the field [Henry et al., 1986; Lesemann et al., 1989; Büttner, 1992]. The occurrence of beet soil-borne virus (BSBV) in the Netherlands has not been investigated. Beet cyst nematodes were detected neither in the infested soil added, nor in soil samples taken from the plots before and after the first and second beet crop. In the absence of beet cyst nematodes (or BNYVV), sugar beet has been grown in monoculture for 3 [Heijbroek and Van de Bund, 1982] up to 6 [Lamers, 1981] years without a reduction in yield level. The lower yield in the control plots in 1989 and 1990 compared to 1988 can mainly be ascribed to a short growing season (1989) and to contamination of these plots by BNYVV (1989 and 1990).

Yield parameters measured were all fresh weights. The effects of BNYVV on the conversion efficiency between intercepted solar radiation and dry matter production, and the effects on dry matter distribution will be published separately [Haverkort et al., unpublished].

The effect of infection with BNYVV on quality parameters of beet

Rhizomania affects the concentrations of sodium, potassium and α-amino nitrogen in the root and equations were described, using these parameters and sugar content, for establishing rhizomania signals [Wieninger and Rösner, 1983; Pollach, 1984]. High concentrations of these compounds negatively influence the extractability of sugar [Van Geijn et al., 1983].

In this field experiment, we found that both Na and α-amino N content were sensitive indicators of rhizomania, showing slight but significant changes at the low infestation levels of the first year. At the higher disease levels in 1989, Na showed the highest relative differences compared to the control with distinguishable values over the whole range of inoculum

levels, whereas α-amino N distinguished between the non-irrigated and irrigated plots at lower levels. As in 1990 all control plots were infested, with a geometric mean inoculum potential corresponding to a value between those of inoculum level 2 and 3 in 1989, the relative differences compared to these plots were smaller than they would have been with disease-free controls. Actually, Na content of inoculum level 0 in 1990 corresponded to a value between the contents at inoculum levels 2 and 3 in 1989.

Na content was highly correlated with sugar content, especially in 1989, and although the relationship was nonlinear, the results for Na confirmed the observations of Heijbroek [1989] for a series of infested fields. For the underlying physiological causes of the increased Na content there are only speculations, as described in a review by Bürcky [1987].

Heijbroek [1989] chose the quotient of Na (mmol kg^{-1} root) and sugar (sucrose, % of fresh weight) as an indicator of rhizomania, mentioning that if this exceeded 0.5 (mmol 10 g^{-1} sugar), rhizomania infestation was present. In our trial, the value of 0.5 was exceeded only in inoculum level 3 and 4 in 1989, in non-irrigated inoculum levels 2, 3 and 4 and in all irrigated levels of 1990; whereas this was not the case in the lower, but diseased, levels in 1989 and 1990, and at the low infestations in 1988. A threshold value of 0.5 was too conservative for this field and also for two naturally infested trial fields at other locations [Tuitert, unpublished]. For non-infested trial fields, ratios of 0.24 and 0.27 were found [Tuitert, unpublished], which exceeded the values for inoculum level 4 in 1988. As for a range of inoculum levels α-amino N content was significantly affected, inclusion of the content of this compound in the formula might add to its indicative possibilities. Division of Na content by the product of α-amino N and sugar content followed by multiplication with a factor of 100, could be applied. In this experiment, treatments with yield reductions had values ≥ 2. Whatever formula is used, determination of a threshold value is difficult; with the formulas from Wieninger and Rösner [1983] and Pollach [1984] (both included K content) all inoculum levels of 1988, and two non-infested trial fields referred to before, exceeded the threshold for rhizomania using the formula from the first-mentioned authors, but were in the range of non-infested fields when using the second one.

Data of potassium contents were not presented, because this parameter is less sensitive to rhizomania than the ones treated before. In 1989 and 1990, however, a general increase in K content was found with increasing inoculum level, but the ranking order of inoculum levels was not always reflected in the order of K contents found. In 1988, K content was lowest in plots with the highest inoculum level. This reverse effect might be explained by the relative insensitivity of K to the very low rhizomania disease levels in the first year, whereby K behaves as in the absence of disease. K and Na are partly exchangeable cations for the sugar beet plant [Kirby et al., 1987]; in fertilization experiments a negative correlation

between K and Na content in the root was observed for Na contents up to approximately 4 mmol kg^{-1} root [Van der Beek and Withagen, 1988].

Modelling yield in relation to inoculum potential of BNYVV in soil

Within the range of MPNs of BNYVV occurring in 1989, a nonlinear relationship was demonstrated between soil inoculum and yield parameters of sugar beet. Gompertz (or logistic) equations were appropriate for describing the relationship between inoculum potential (log_{10} transformed) and root weight, sugar yield and sugar content in this year on this particular field. The finding underlines the importance of initial soil inoculum, and thus primary infection, in determining disease severity, caused by a pathogen whose vector has several secondary infection cycles during the season. The upper sugar yield level where little or no yield loss occurred, partly because of compensation [Zadoks and Schein, 1979], continued to an inoculum level of approximately 3–5 infective units of BNYVV per 100 g of dry soil. The sigmoidal transition phase led to the residual yield level of 3 tons ha^{-1} already referred to, beyond which an increase in inoculum did not have an additional effect. The parameters of the curve will not only depend on the MPNs determined, and environmental influences, but also on the pattern of dispersion of inoculum in the field and the age of the plants at primary infection. Inoculum levels below the detection level of the bioassay (at X = 0.2 in Fig. 11) were considered 0 in the analyses. As even some control plots for which a MPN = 0 was determined were probably contaminated, this may have led to an underestimation of the upper asymptote of the curves, especially for the irrigated plots.

In 1990, inoculum potentials had further increased, all control plots were highly infested and MPNs below 10 infective units per 100 g of soil were not available. The range of inoculum levels was too narrow and too much restricted to high MPNs for investigation of the inoculum – yield relationship. For the non-irrigated plots a significant (linear) decrease in yield parameters with increasing inoculum potential was still present. In the irrigated plots, with even higher MPNs, no significant relationship was present, due in part to the distinct data of block B, which were strongly influenced by bad soil structure in this year.

Disease incidence

Disease incidence was measured by analyzing plants for the presence of BNYVV and by observation of root symptoms on these plants. At the low infestations in 1988, disease incidence – the number of BNYVV-infected plants determined by ELISA – was low and gradually increased in time. The measured increase in disease incidence during the season will have been the result of a) an increased number of infected plants because of an

increase in primary infection of the growing root system of plants exploring a larger volume of soil with increasing root density, and b) the time occurring between primary infection of rootlets and occurrence of BNYVV in the tap root in a concentration that can be detected by ELISA. Irrigation caused an earlier increase in infected plants and two- to threefold higher numbers of infected plants at harvest. The latter effect is in agreement with a mean threefold higher multiplication ratio of BNYVV in soil in the irrigated plots in 1988 [Tuitert and Hofmeester, 1992].

In 1989, disease incidence was determined by the inoculum level in soil at the three sampling times. At harvest, the proportion of BNYVV-infected plants that showed root symptoms was also influenced by the initial inoculum level. The higher proportion of symptomatic plants at high inoculum levels may have been the result of a more extensive, early, primary infection at these levels, whereas an earlier time of infection may also have contributed by allowing a longer period of symptom development. The two-year effect of irrigation on disease incidence was probably mainly due to higher soil infestation (MPNs) caused by irrigation in the first year, as in regression analysis, using the MPNs determined for each plot in 1989, irrigation was not a significant factor.

Disease incidence was very high in all plots in 1990; at inoculum levels (expressed in MPNs) similar to those in 1989, disease incidence was higher in 1990. The latter might partly be due to a more regular dispersion of inoculum after two years of beet growing, whereas the few infected plants in 1988 might have caused an aggregated pattern (randomly dispersed infested patches) of inoculum in 1989 at the same measured inoculum potential. The final level of disease may be lower if soil-borne inoculum is highly clustered than when it is evenly distributed [Campbell, 1986]. Especially at the early sampling date, the number of BNYVV-infected plants that showed symptoms increased with increasing initial inoculum level, as was observed in 1989.

Modelling the relationship between inoculum potential and disease incidence of BNYVV

The relationship between inoculum potential (log MPN) and disease incidence in 1989 fitted a logistic model. The fitted lines shifted to the left during the season, whereby the delay parameter, the X-value where the inflection point of the curve occurs, shifted to lower MPNs. Because the Y-max (upper asymptote) cannot increase above 1 and incidences do increase at low MPNs, the rates of increase in disease incidence with increasing log MPN decrease during the season. The increase in disease incidence can again mainly be ascribed to the different times and intensities of primary infection of the growing root system and to either the time necessary for multiplication of virus in the root to detectable levels or the incubation period to symptom development. Periods for virus multiplication

and symptom development are likely to be variable, because both will depend on primary infection and environmental conditions such as temperature and soil moisture (determining the number of secondary infection cycles). All these factors may have contributed to the fit of a logistic equation for describing the increase of disease incidence with increasing log MPN.

It was hypothesized that, although multiplication of inoculum during the season and the increase of root infection of a single plant are polycyclic processes, development of rhizomania incidence in the field during one season is a monocyclic process [Tuitert, 1993]. The finding that disease incidence at different inoculum levels could better be fitted with a logistic than a monomolecular equation, neither contradicts nor proves the correctness of the hypothesis. Deterministic models (descriptions) such as the logistic, Gompertz or monomolecular models can be used to characterize an epidemic and provide a basis for comparison between epidemics, but no conclusions on the nature of the disease cycle should be inferred from the statistical appropriateness of one of these models [Pfender, 1982].

Disease progress in time could not be modelled because of the limited number of samplings. The relationship found between inoculum density and disease incidence indicates that when disease progress curves in time would be constructed, the inoculum potential of the field has to be taken into account. Maximum disease level cannot be set to 1 (100%) at all inoculum potentials, as this might lead to the underestimation of the rate parameter [Neher and Campbell, 1992].

As the logistic model gave the best fit, an alternative for calculation of curve parameters would have been by means of generalized linear modelling with a logistic link function and declaration of a binomial error distribution of the disease incidence data. Rate parameters thus calculated did hardly differ from the ones presented (Table 5).

Risk of soil displacement

Displacement of infested soil is an important way of spreading disease. The risks attached to small amounts of infested soil are illustrated by the results of the described field experiment. One year of sugar beet may be sufficient to increase hardly detectable inoculum levels to harmful levels. To plots of inoculum levels 2 and 3 about 5 and 50 kg infested soil ha^{-1} was added, which resulted in mean yield reductions of 37 and 57%, respectively, in the second beet crop after infestation of the plots. As the average amount of soil allowed on seed potatoes for 1 ha of land is approximately 20 kg, the potential risk of using planting material from infested fields is obvious.

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