Quantitative studies on resistance to *Polymyxa betae* and beet necrotic yellow vein virus in beet

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Quantitative studies on resistance to *Polymyxa betae* and beet necrotic yellow vein virus in beet

Kwantitatief onderzoek naar resistentie tegen *Polymyxa betae* en het bieterhizomanievirus in de biet

Proefschrift

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in de landbouw- en milieuwetenschappen
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in het openbaar te verdedigen
op woensdag 8 december 1993
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van de Landbouwuniversiteit te Wageningen.

Aan mijn ouders
Aan Cora

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Stellingen

1. Bepaling van het resistentieniveau van bietezaailingen tegen het bieterhizomanievirus in kasproeven is een uitstekend hulpmiddel bij het schatten van de opbrengstderving van suikerbieten op percelen, die met dit virus besmet zijn.
   Dit proefschrift.

2. Het telen van vatbare bieterassen op percelen, die met het bieterhizomanievirus besmet zijn, impliceert het aanvaarden van het risico op grote opbrengstverliezen door rhizomanie.

3. Vanwege de zeldzaamheid van het symptoom 'necrotic yellow vein' verdient het de voorkeur de naam 'beet necrotic yellow vein virus' te vervangen door 'beet rhizomania virus'.

4. Het niet volledig zijn van de resistenties in bieten tegen het bieterhizomanievirus verhoogt de duurzaamheid van die resistenties.

5. Het voorstel van Johnson voor regionale toedeling van resistentiegenen is nog steeds aktueel. Voor resistentie tegen bodemgebonden pathogenen is verdeling in de tijd van belang.
   T. Johnson, 1958. Regional distribution of genes for rust resistance. Robigo 6: 16-17.

6. Het loslaten van het idee van specialisatie en bijbehorende kapitaalintensivering biedt mogelijkheden voor flexibele bedrijfsvoering op het individuele boerenbedrijf.

7. De opmerking in het boek van de Voorlopige Commissie Genetische Modificatie, dat door recombinant DNA techniek een nauwkeuriger effect verkregen wordt bij het veranderen van eigenschappen dan bij paring of kruising, is niet correct en is een oneigenlijk argument voor de promotie van genetische modificatie.
   VCOGEM, 1992. Erfelijke veranderingen bij bacteriën, planten en dieren, voordelen en risico's.

8. Het optreden van onvoorziene onderzoeksresultaten illustreert dat bescheidenheid nog steeds op z'n plaats is bij het uitoefenen van wetenschap.

9. Consensus over de bescherming van menselijk leven buiten het bijbelse gebod, om niet wederrechtelijk te doden, is levensbedreigend.

10. Wageningen....., ergens tussen Den Haag en Brussel.

Stellingen behorend bij het proefschrift van Hendrik Paul, getiteld "Quantitative studies on resistance to *Polymyxa betae* and beet necrotic yellow vein virus in beet", te verdedigen op 8 december 1993 te Wageningen.

Author's abstract

Paul, H., 1993. Quantitative studies on resistance to *Polymyxa betae* and beet necrotic yellow vein virus in beet. PhD Thesis, Wageningen Agricultural University, the Netherlands, 115 pages, 17 tables, 9 figures.

Beet necrotic yellow vein virus (BNYVV) causes rhizomania in sugar beet. The virus is transmitted by the soil-borne fungus *Polymyxa betae*. Rhizomania in sugar beet can cause serious losses in sugar yield. Breeding for resistance is the most promising way to control the disease. Several aspects of quantitative screening for resistance to rhizomania were investigated. A greenhouse test was established to screen and select beet seedlings for resistance to BNYVV. Plants were grown in a mixture of sand and infested soil. Virus concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Data of various fields were analysed using multiplicative models for cultivar by location interaction. Interactions for yield and quality parameters were described in terms of virus concentrations in beet plants grown in the greenhouse and in the field. After screening various beet material of the sections *Beta*, *Corollinae* and *Procumbentes*, it was concluded that resistance to *P. betae* could have some effect on the infection with BNYVV, but low numbers or even absence of resting spores did not always result in low virus concentrations. When viruliferous zoospores of *P. betae* were used to inoculate plants, resistant accessions often had virus concentrations similar to those of the susceptible control. Resistant and susceptible accessions could only be distinguished shortly after inoculation with zoospores or after transplanting the inoculated plants into sand. It is recommended to combine resistant accessions, which differ genetically and in their mechanism of resistance, in order to obtain sugar beet cultivars with a high level of resistance to rhizomania.

Voorwoord

De direkties van de voormalige Stichting voor Plantenveredeling (SVP) en het DLO-Centrum voor Plantenveredelings- en Reproduktieonderzoek (CPRO-DLO) zeg ik dank voor de mogelijkheid tot de uitvoering van het onderzoek en de afronding van het proefschrift. De promotor, professor dr. J.C. Zadoks, ben ik erkentelijk voor de discussies die gevoerd zijn over de onderzoeksresultaten en voor de correctie van de geschreven tekst. De co-promotor, dr. ir. D. Peters, wordt eveneens dank gezegd voor de correctie van de geschreven tekst en voor het beschikbaar stellen van gezuiverd virus aan het begin van het onderzoek, waardoor het mogelijk werd kwantitieve virusbepalingen uit te voeren. Wouter Lange zeg ik dank voor de aandacht voor het onderzoek en voor de nauwgezetheid waarmee de tekst van het proefschrift is doorgenomen. Belangstelling voor het onderzoek was er ook steeds van Coosje Hoogendoorn.

Veel collega's zijn betrokken geweest bij het onderzoek en de tot standkoming van het proefschrift. Marijke Alderlieste en Jannie de Vogel-Leeuwangh hebben een belangrijke bijdrage geleverd bij het begin van het onderzoek. Betty Henken zeg ik dank voor de goede samenwerking en voor de nauwgezette wijze waarop het werk werd verricht. Willem Heijbroek van het Instituut voor Rationele Suikerproduktie (IRS) te Bergen op Zoom wordt dank gezegd voor de goede contacten en het beschikbaar stellen van veldproefgegevens en Fred van Eeuwijk voor het analyseren van deze gegevens. Een deel van het werk uit hoofdstuk vier is uitgevoerd door de student Jochum Wiersma. Theo de Bock heeft een stimulerende rol gespeeld bij het gebruik van wilde bietesoorten. In Olga Scholten heb ik een goede en enthousiaste collega gehad. De hulp van Paul Keizer bij de statistische analyses was werkelijk onmisbaar. De medewerkers van de afdeling gewasverzorging, met name Gerrit Stunnenberg en Geert Scholten, bedank ik voor het werk dat verricht is in de kassen. Gea Jonker-Harskamp zeg ik dank voor de assistentie bij de tekstverwerking. De discussies met Ate de Heij van het DLO-Instituut voor Planteziektenkundig Onderzoek (IPO-DLO) heb ik erg op prijsgesteld. De deelnemers aan de studiegroep Rhizomanie wordt dank gezegd voor de onderlinge contacten.

The stimulating interest of the international sugar beet seed breeding companies for this research is appreciated. The contacts and discussions with my foreign colleagues, especially those with Dr M.J.C. Asher (Broom's Barn Experimental Station, Bury St. Edmunds, England), Dr H.H. Dahm (University Hannover, Germany), Dr R.T. Lewellen (United States Department of Agriculture, Salinas, California) and Dr R. Koenig (Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig, Germany) are gratefully acknowledged.

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Contents

The Chapters 2-6 of this thesis are based on the following papers:

Chapter 2. Paul, H., Henken, B. & Alderlieste, M.F.J., 1992. A greenhouse test for screening sugar-beet (*Beta vulgaris*) for resistance to beet necrotic yellow vein virus (BNYVV). Netherlands Journal of Plant Pathology 98: 65-75.

Chapter 3 Paul, H., Van Eeuwijk, F.A. & Heijbroek, W., 1993. Multiplicative models for cultivar by location interaction in testing sugar beets for resistance to beet necrotic yellow vein virus. Euphytica (in press).

Chapter 4 Paul, H., Henken, B., De Bock, Th.S.M. & Lange, W., 1992. Resistance to *Polymyxa betae* in *Beta* species of the section *Procumbentes*, in hybrids with *B. vulgaris* and in monosomic chromosome additions of *B. procumbens* in *B. vulgaris*. Plant Breeding 109: 265-273.

Chapter 5 Paul, H., Henken, B., Scholten, O.E., De Bock, Th.S.M. & Lange, W. Variation in the level of infection with *Polymyxa betae* and its effect on infection with beet necrotic yellow vein virus in beet accessions of the sections *Beta* and *Corollinae*. Published in abridged form in the Proceedings of the second Symposium of the International Working Group on Plant Viruses with Fungal Vectors, Montréal, 1993 (in press) and in the Journal of Sugar Beet Research (submitted).

Chapter 6 Paul, H., Henken, B., Scholten, O.E. & Lange, W., 1993. Use of zoospores of *Polymyxa betae* in screening beet seedlings for resistance to beet necrotic yellow vein virus. Netherlands Journal of Plant Pathology (in press).

Chapter 1

Introduction

Rhizomania in sugar beet

A new disease in sugar beet (*Beta vulgaris* L.) was reported in Italy in the fifties, giving abnormal root proliferation and therefore called "rizomania" (Canova, 1959). Following the identification of the fungus *Polymyxa betae* (Keskin et al., 1962) and of beet necrotic yellow vein virus (BNYVV) (Tamada, 1975), transmission studies revealed the host-parasite relationships involved with the disease (Abe and Tamada, 1986; Fujisawa and Sugimoto, 1976). BNYVV is classified as a member of the furoviruses, the fungus transmitted rod-shaped viruses (Brunt, 1992; Shirako and Brakke, 1984). *P. betae* itself might cause some damage at high soil temperatures, but apart of its role as a virus vector, its significance as a pathogen seems to be limited (Blunt et al., 1991). Other viruses have been mentioned to be associated with the rhizomania disease complex, such as beet soil-borne virus, but it is not clear whether this virus causes damage in the field (Henry et al., 1986), although in greenhouse experiments damage could be found (Kaufmann et al., 1993; Prillwitz and Schlösser, 1992).

Rhizomania can cause serious crop losses and the cultivation of sugar beet is endangered by the disease, also because of the persistance of the fungal spores in the soil. Various possibilities to control rhizomania in sugar beet have been suggested and investigated, including biological and chemical control of the fungus and agronomic measures. In their reviews, Asher (1993) and Schlösser (1988) concluded that the use of resistant cultivars is the most promising way to control rhizomania in sugar beet.

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Evaluation of resistance in sugar beet

In breeding programmes, screening for resistance to rhizomania in sugar beet can be carried out in the field or in the greenhouse. In field experiments, leaf and root symptoms can be scored during the growing season and at harvest time. Yield and quality data of the tested accessions provide the most important information for selection. In large scale field experiments on resistance, usually no observations on BNYVV and *P. betae* are made. The final yield of the accessions will be the result of genetically determined factors such as yield potential, the level of resistance to *P. betae* and BNYVV, the presence of tolerance, and by environmental factors.

Greenhouse tests allow direct observations on *P. betae* and BNYVV, and selection is based on the levels of resistance. Plants can be scored for symptom development and yield can be determined when grown for longer periods, but the rationale for greenhouse tests is the analysis of resistance levels to *P. betae* and BNYVV. Greenhouse tests allow better control of experimental conditions and infection pressure and take less time than field experiments. Especially to select individual plants with resistance to *P. betae* and/or BNYVV, greenhouse tests are useful. For the evaluation of the yield and quality performance of sugar beets with resistance to rhizomania, however, field experiments are indispensable.

In Table 1.1, the criteria for selection are given as used in greenhouse and field experiments, together with the factors that might determine the outcome of the evaluation. Yield potential of the accessions can be determined on non-infested fields and can be taken into account in the evaluation of sugar beets on infested fields.

In literature on rhizomania, cultivars that perform relatively better than the susceptible controls on infested fields are often called tolerant. Clarke (1986) described tolerance as "the ability of a plant to endure the effects of levels of disease, which, if they developed to equivalent levels in other plants of the same or similar species, would cause greater impairment of growth or yield". Resistance is defined as the ability of a plant to reduce the multiplication of a pathogen. Resistance might be complete or partial. The level of resistance in plants can be determined by measuring the amount of pathogen. For determining tolerance,

additional information on plant growth or yield is necessary. In many reports on tolerance to plant viruses, the amount of virus has often been assessed inaccurately so that the presence of some form of partial resistance should not be ruled out (Clarke, 1986; Fraser, 1990). In screening for resistance to rhizomania in sugar beet, reduced levels of BNYVV were detected in so-called tolerant material (Bürcky and Büttner, 1985; Giunchedi et al., 1987). The reduction in levels of BNYVV indicates that this material should be called resistant or partially resistant and, consequently, in recent literature more and more the word resistant is used to describe sugar beets in which reduced levels of BNYVV were found. As far as is known, no proof for the existence of tolerance to BNYVV in beet has been given.

Table 1.1. Selection criteria in sugar beet for resistance to rhizomania and factors that might determine the outcome of the evaluation.

	Greenhouse	Field
Selection criteria	-amount of vector	-symptoms
	-amount of virus	-yield parameters
		-quality parameters
Factors involved	-resistance to the vector	-resistance to the vector
	-resistance to the virus	-resistance to the virus
		-tolerance to the virus
		-yield potential
	-environmental conditions	-environmental conditions

Outline of the research

In the present study special attention has been given to quantitative aspects of screening for resistance to rhizomania. Accurate evaluation of resistance will increase efficiency in breeding and allows the development of strategies for optimal use of resources. Improvements of existing methods were made with respect to the development of a greenhouse test and inoculation with zoospores, and various novel aspects are included, such as statistical analysis of data from fields with various levels of infection, and studies on the effect of resistance to *P. betae* on the infection with BNYVV.

In Chapter 2 a greenhouse test for the screening of sugar beet seedlings for resistance to BNYVV is described. Special attention is given to the measurement of virus concentrations. In Chapter 3 results of field experiments are analysed using multiplicative models for cultivar by location interaction. With this type of analysis it was possible to include information of fields with various levels of infection in the same analysis. Furthermore, interactions for yield and quality parameters could be described in terms of virus concentrations determined in beet plants grown in the greenhouse and in the field. Studies on resistance to *P. betae* in *Beta* species of the section *Procumbentes* and on its effect on infection with BNYVV are described in Chapter 4. Monosomic chromosome additions of *B. procumbens* in *B. vulgaris* are used to locate the resistance to *P. betae* at the chromosome level. Further studies on the effect of the level of infection by *P. betae* on the infection with BNYVV, using various beet accessions of the sections *Beta* and *Corollinae*, are described in Chapter 5. In the experiments described in the Chapters 2-5 soil containing *P. betae* infested with BNYVV was used to inoculate the plants. In Chapter 6, a production system for zoospores of *P. betae* is described, together with several experiments in which viruliferous zoospores were used for inoculation of seedlings in screening for resistance to BNYVV. In Chapter 7, the results obtained in this study are evaluated and a strategy is outlined for breeding sugar beet with a high level of resistance to rhizomania.

Chapter 2

A greenhouse test for screening sugar beet (*Beta vulgaris*) for resistance to beet necrotic yellow vein virus (BNYVV)

Abstract

Small differences in activity between batches of purified beet necrotic yellow vein virus (BNYVV) were observed in ELISA. A four-parameter modelled dose-response curve of purified BNYVV was used for the conversion of ELISA values to virus concentrations. Seedlings of the susceptible cultivar Regina and the partially resistant cultivars Nymphe and Rima were tested for resistance to BNYVV in a mixture of sand and infested soil. Plants were grown in a greenhouse with low nutrient supply and at temperatures below the optimum of both the vector *Polymyxa betae* and BNYVV. Root systems were small and consisted mainly of lateral roots. Significant differences in average virus concentrations were found between cultivars, either using the complete root systems or the top or the bottom part of the root systems. Average virus concentrations in 'Regina' were always significantly higher than in 'Rima' and higher than in 'Nymphe' on all occasions except one ($P<0.05$). Differences between 'Nymphe' and 'Rima' were less evident. Variation between plants was greatest within 'Rima'. The test described in this chapter can be used for the discrimination of different cultivars and for the identification of individual plants with resistance to BNYVV.

Introduction

Rhizomania in beet, caused by beet necrotic yellow vein virus (BNYVV) (Tamada, 1975), has become a major threat to sugar beet (*Beta vulgaris* L.) crops in most beet growing areas of the world. The soil-borne fungus *Polymyxa betae* Keskin (*Plasmodiophoraceae*) acts as a vector of the virus. Symptoms of the disease consist of proliferation of lateral roots, constriction of the tap root and browning of the vascular system in the roots. Leaves of infected plants are light green in colour. Occasionally, when the virus becomes systemic, leaves are dark green with yellow veins. Rhizomania can cause serious losses both in root weight and sugar content. Cultural practices have limited effect on the level of infection (Müller and Gösswein, 1987) and the possibilities for controlling *P. betae* by chemicals are restricted (Schäufele, 1987). Breeding for resistance to rhizomania is regarded as promising to control the disease (Bolz and Koch, 1983). Since 1982, such breeding activities have increased considerably and test networks for evaluating cultivars have been set up in many countries (Richard-Molard, 1987). Because sugar beet is a cross-pollinating crop, differences in resistance to rhizomania between plants can occur within cultivars and breeding populations. In most breeding programmes for resistance, selection is usually done in field experiments using disease symptoms, root weight and sugar content as selection criteria. Greenhouse tests are also being used.

Giunchedi et al. (1985, 1987), using the enzyme-linked immunosorbent assay (ELISA) technique for virus determination in roots, reported a reduction in the virus concentration in so-called rhizomania-tolerant material. Differences in virus concentration could already be demonstrated in the seedling stage (Bürcky and Büttner, 1985). These results have stimulated the development and use of greenhouse screening tests, in which virus concentration in the roots is used as a selection parameter for resistance to rhizomania. ELISA readings were interpreted quantitatively either by converting the readings to absolute virus concentrations by means of a dose-response curve of purified virus, applying linear regression (Giunchedi et al., 1985; 1987), or by classifying the readings according to the response of different dilutions of purified virus and expressed as relative virus

concentrations (Bürcky and Büttner, 1988). Both tap roots and lateral roots could be used in preparing samples for virus assays. Virus concentrations were lower in tap roots than in lateral roots (Büttner and Bürcky, 1990; Giunchedi et al., 1985, 1987). Bürcky and Büttner (1988) recommended the use of lateral roots in experiments in which individual plants are selected for resistance, because sometimes in tap roots no virus could be detected. However, the difference in virus concentration of the lateral roots between a resistant and a susceptible cultivar decreased with time, which would mean that for the discrimination between a resistant and a susceptible cultivar, lateral roots could only be used during a limited testing period. Furthermore, the preparation of samples from lateral roots was more laborious than from tap roots (Bürcky and Büttner, 1985; 1988).

The aim of the present study was to develop a quantitative greenhouse screening test in which individual plants could be tested for resistance to rhizomania and in which lateral roots would be used for sampling. Different batches of purified virus were tested for their use in dose-response curves and special attention was given to the modelling and statistical analysis of ELISA data. Following the approach of Toxopeus and Lubberts (1979), who described a method for testing resistance to the beet cyst nematode *Heterodera schachtii* Schm. in seedlings, test plants were grown in sand with low nutrient supply, to obtain root systems consisting mostly of lateral roots. By growing the plants at temperatures below the optimum of both vector-fungus and virus it was aimed to get only a moderate development of the disease, so that the period in which resistant and susceptible cultivars could be discriminated, would not be restricted (Bürcky and Büttner, 1985). Furthermore, it was investigated whether subsamples of the roots could be used instead of the whole root system, which would facilitate the use of lateral roots.

Materials and methods

Purified virus

Three batches of purified BNYVV, named 90-1, 90-2 and 90-3, were received from Dr D. Peters (Department of Virology, Wageningen Agricultural University). Virus concentrations of the batches were spectrophotometrically estimated at 2.0, 0.8 and 1.0 mg ml^{-1} (E260,0.1% = 2.8). Batches were stored at 4 °C. Dilutions of 1600, 800, 400, 200, 100, 50, 25, 5 and 1 ng ml^{-1} were made in phosphate buffered saline (plus 0.05% Tween 20) (PBS-Tween), and tested with ELISA in three replicates.

Plant material and cultivation of test plants

Seeds of the sugar beet cultivars Regina (Hilleshög-Sweden, susceptible), Nymphe (Hilleshög-Sweden, partially resistant) and Rima (SES-Belgium, partially resistant) were sown in heat-sterilised (overnight, 105 °C) coarse sand. Seeds of 'Regina' and 'Nymphe' were disinfected with Dithane M45 (Duphar). For 'Rima' coated seeds were used. Soil was collected from a field in Nagele (Noord-Oost Polder), earlier described by Beemster and De Heij (1987) as containing high levels of *P. betae* infested with BNYVV. The soil was air-dried, mechanically ground and mixed thoroughly with heat-sterilised coarse sand in a ratio of 1:9 (v v^{-1}). Seedlings were transplanted to black PVC containers (40x40x150 mm, Kelder Plastibox) with folded bottom, filled with the soil-sand mixture. For the production of healthy plant material, seedlings were transplanted to containers with sand only. One seedling was planted per container. Each container was placed in an aluminium tray (80x80x29 mm, Ekco) (Fig. 2.1A). Twice a week the trays were supplemented to half height with a 0.1 dilution of a Steiner nutrient solution (pH 7) (Steiner, 1984). Tests were carried out in a climate-controlled greenhouse. Temperatures were 22 °C at day time (10 h) and 17 °C at night (14 h), which is below the optimum temperature of both the vector and the virus (Asher and Blunt, 1987; Horak, 1980). Natural day-length was extended to 16 h, with a minimum light intensity of 10 Watt m^{-2}.

Experimental design of greenhouse experiments

In a first experiment, 120 plants of each of the cultivars Regina, Nymphe and Rima were tested in a completely randomised design. After 16, 22, 28, 34 and 40 days, 24 plants of each cultivar were sampled at random. The complete root systems of the plants were used. In a second experiment, 60 plants of each of the three cultivars were tested in a completely randomised design. After 22 and 29 days, 30 plants of each cultivar were sampled at random. Some 100 mg from the upper part of the root system and the remaining part were sampled separately. In each experiment, samples of all sampling times were stored and analysed simultaneously in ELISA at the end of the experiment. Non-infected plants were kept separately from the main experiments.

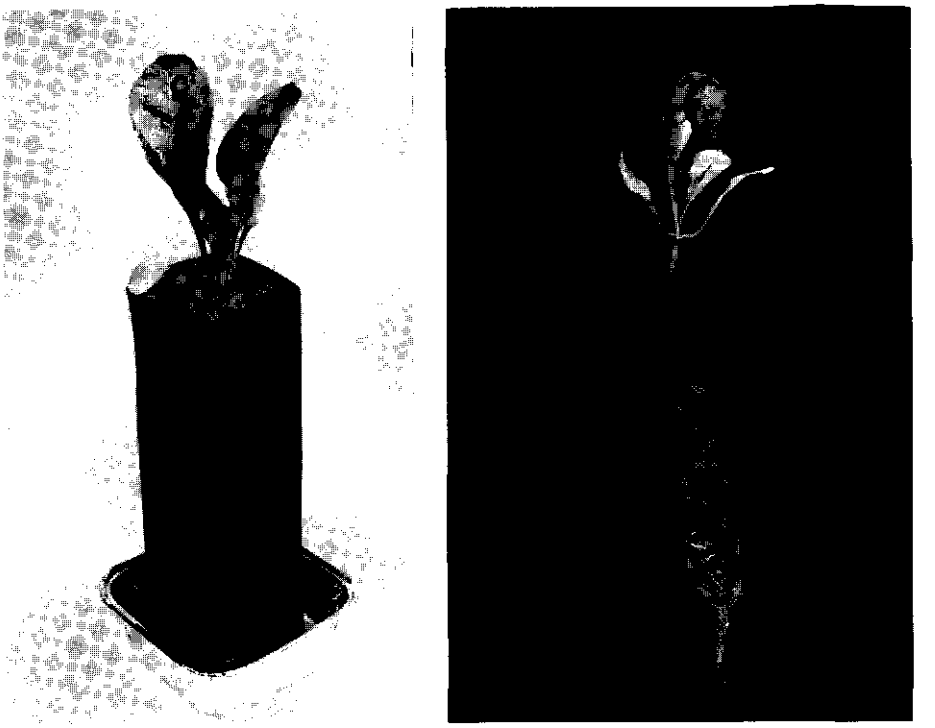

Fig. 2.1. A: Sugar beet seedling of 'Regina' in a container filled with a mixture of sand and infested soil, placed in a tray with nutrient solution (27 days after transplanting) (picture on the left); **B**: Idem, with roots washed free of sand and soil (picture on the right).

Root sampling

The containers were placed in a tray filled with water. Then, the soil and sand were washed away from the roots under a tap (Fig. 2.1B). The roots of each plant were checked for the presence of cystosori of *P. betae* by direct observation under an inverted microscope (Zeiss ID02). Roots were cut from the plants, dried carefully with tissue paper, weighted and crushed in Potter tubes with PBS-Tween in a ratio of 1:20 (w v^{-1}). Stock solutions of healthy plant sap were obtained by squeezing roots of non-infected plants in a garlic press. Samples and stock solutions were stored at -18 °C in Eppendorf tubes.

Virus assays

ELISA (Clark and Adams, 1977) was performed as described by Alderlieste and Van Eeuwijk (1992). Coating and conjugated antibodies (Sanofi commercial antiserum) were diluted 130 and 1000 times, respectively. Para-nitrophenyl-phosphate (Sigma, 1 mg ml^{-1}) was used as substrate. Incubation times and temperatures were according to Büttner and Bürcky (1987). Only the inner 60 wells of the microtiter plates (Costar 3590) were used. Outer wells were filled with PBS-Tween in all steps of ELISA, except for the last one, where these wells were filled with substrate.

Samples and stock solutions were thawed and centrifuged (2 min, Micro-spin 24, Sorvall Instruments). In the assays of the root samples, a dilution series of 1600, 800, 400, 200, 100, 50, 5 and 0 ng ml^{-1} of virus batch 90-2 was included as a standard on each microtiter plate. Since plant sap components influence ELISA (Alderlieste and Van Eeuwijk, 1992; Büttner and Bürcky, 1987; Koenig et al., 1984), virus standards were made up in a 0.05 (v v^{-1}) solution of healthy plant sap of 'Regina', diluted in PBS-Tween, to obtain a dilution comparable to that of the root samples. In the assays, dilutions of purified virus, and the virus standards and samples of the first greenhouse experiment were tested in duplicate. Virus standards and samples of the second greenhouse experiment were tested without duplication. All dilutions, standards and samples were randomised on the plates, with duplicates in adjacent wells. Reactions were stopped with 0.05 ml 5M NaOH per well, when the 800 ng ml^{-1} standard of batch 90-2 had reached a value of approximately 1.0. Readings were performed with an Anthos 2001 multiscan photospectrometer at

405 nm, with 620 nm as reference, in combination with the software package ELISA+ (Meddata Inc.), installed on a microcomputer.

Data analysis

Data were transferred to a main-frame computer (VAX, Digital). Readings of the front wells (B1-G1) were used for blanking the rows. When duplicates were used, the means of the two readings were used in the analysis. As is extensively described by Alderlieste and Van Eeuwijk (1992), a four-parameter logistic model (Dudley et al., 1985), which is characterised by the lower asymptote, the inflextion point, the slope parameter and the upper asymptote, fits well to the dose-response curve of purified BNYVV. The same model was used for the conversion of ELISA-readings to concentrations, using the virus standards for modelling the dose-response curve. The concentration of 0 ng ml^{-1} virus was read as 1 ng ml^{-1}. Readings equal to or below the estimated lower limit of the curve were replaced by that lower limit + 0.001. Curve-fittings and analyses of variance were carried out with the statistical package GENSTAT (GENSTAT 5, Rothamsted Experimental Station, U.K.). Pairwise comparisons between cultivars on the different sampling times were made with least significant differences (LSD).

Results

Purified virus

Fig. 2.2 shows the response in ELISA of dilutions of the three batches of purified BNYVV. Differences in response between the batches were observed. Slopes of the curves were similar. Before the means were used in analyses, the correlations between duplicate readings were computed. Correlations were higher than 0.99. After application of the four-parameter logistic model to the data presented in Fig. 2.2, curve fittings were 100.0, 99.9, and 99.8% for the batches 90-1, 90-2 and 90-3, respectively.

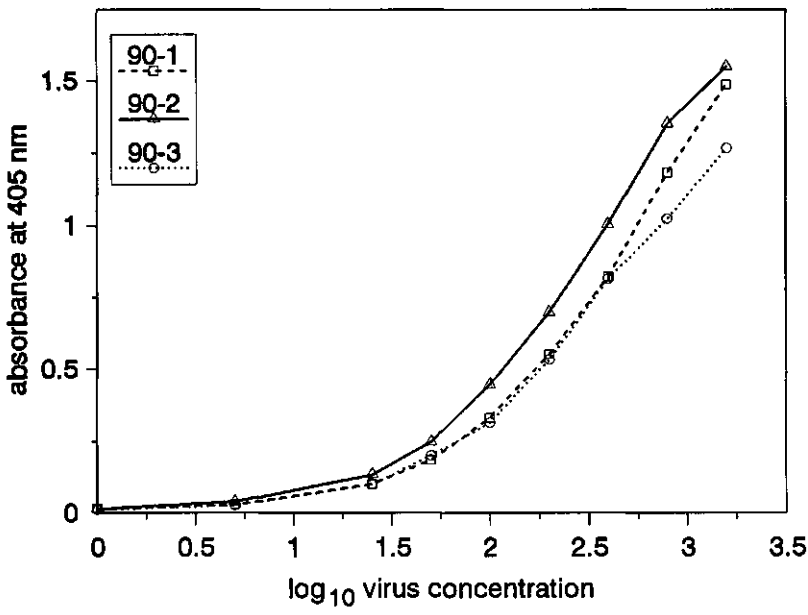

Fig. 2.2. Dose-response curves of three batches of purified BNYVV (means of three replicates, original data on virus concentration in ng ml^{-1}).

Greenhouse experiments

Observations on the roots. All three cultivars, Regina, Nymphe and Rima, were susceptible to *P. betae*. Cystosori, the resting spores of the fungus, were detected in the roots of all plants at all sampling times. Up till 22 days in both experiments many of the cystosori were still immature. Most cystosori were found in the first lateral roots, branching off the tap root.

Root weight increased with time (Table 2.1). Mainly lateral roots were formed, and the tap roots thickened only slightly during the experiments. In the first experiment, average root weight of 'Nymphe' was significantly higher than that of 'Regina' and 'Rima' at the first sampling time and higher than the average root weight of 'Regina' at the fourth sampling time ($P<0.05$).

Table 2.1. Average fresh root weight (mg) of sugar beet seedlings, with 95%-confidence intervals, at different sampling times during tests for resistance to BNYVV.

Cultivar	Experiment 1					Experiment 2	
	Days after transplanting					Days after transplanting	
	16	22	28	34	40	22	29
Regina	129 ± 10	258 ± 26	293 ± 32	338 ± 36	430 ± 56	304 ± 26	508 ± 41
Nymphe	145 ± 13	258 ± 22	328 ± 33	407 ± 37	487 ± 45	343 ± 23	573 ± 37
Rima	126 ± 11	263 ± 23	330 ± 28	389 ± 39	451 ± 43	347 ± 26	563 ± 35

Experiment 1: 24 plants per cv. per sampling time; Experiment 2: 30 plants per cv. after 22 days, and 28, 29 and 30 plants for cv. Regina, Nymphe and Rima, respectively, after 29 days.

In the second experiment, average root weights of 'Nymphe' and 'Rima' were significantly higher than that of 'Regina' at both sampling times ($P<0.05$). For root weight, no significant interaction between sampling times and cultivars was found in either experiment. Increased browning of the roots was observed in the course of the experiments, but no roots died.

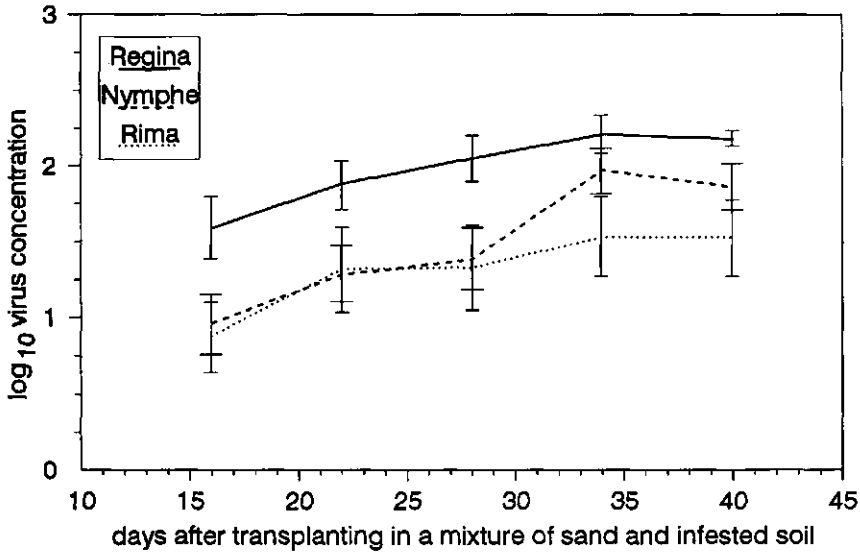

Fig. 2.3. Average virus concentrations in the root systems of sugar beet seedlings at different sampling times, with 95%-confidence intervals (original data on virus concentration in ng ml^{-1}).

Results of virus assays. Correlations between duplicate readings in the first experiment were higher than 0.99. In both experiments the dose-response curves of purified virus were adequately described by the four-parameter logistic model. Average curve fittings were 99.8% in both experiments. Standard concentrations

of 0 ng ml^{-1} were estimated from 0 to 2 ng ml^{-1} after curve fitting. Samples that gave experimental responses below 4 ng ml^{-1} were considered to be free of virus. All virus concentrations of the plant samples were within the range of the dose-response curves. For statistical analyses, the $\log_{10}$ of the concentrations was taken. Estimated values of 0 ng ml^{-1} were read as 1 ng ml^{-1}.

Figure 2.3 illustrates the development of BNYVV-concentrations with time in the different cultivars, tested in the first experiment. Average virus concentrations increased with time for all three cultivars from day 16 to day 34. Virus concentrations were similar at the last two sampling times. Virus was found in all samples of 'Regina', but not in all samples of 'Nymphe' and 'Rima'. Only at the fourth and fifth sampling time the virus could be detected in all plants. Average virus concentrations in 'Regina' were significantly higher than in 'Nymphe' and 'Rima' at all sampling times, except on day 34, when only a significant difference with 'Rima' was found. On days 34 and 40, 'Nymphe' and 'Rima' differed significantly ($P<0.05$) in virus concentration. No significant interaction between the sampling times and cultivars could be detected. Variation, as expressed by the width of the confidence interval, was higher in 'Rima' than in the other cultivars.

Results of the virus assays on the samples of the second greenhouse experiment are presented in Figs 2.4A and 2.4B. Histograms were used to depict the variation in virus concentration between plants within cultivars. Increases in virus concentration between the sampling times were observed for all cultivars and in both parts of the root system. As in the first experiment, all samples of 'Regina' contained virus, but no virus could be detected in several samples of 'Nymphe' and 'Rima'. Average virus concentrations in 'Regina' were significantly higher than in 'Nymphe' and 'Rima', at both sampling times and in both parts of the root system. Although average virus concentrations in 'Nymphe' were higher than in 'Rima' in both parts of the roots, significant differences were only found in the lower part of the roots, at both sampling times. No interaction was found between sampling times and cultivars or between sample types and cultivars. Distribution of the data of 'Rima' was less regular than for 'Regina' and 'Nymphe', and the data of the lower part of the root system even suggested a segregation between resistant and susceptible plants.

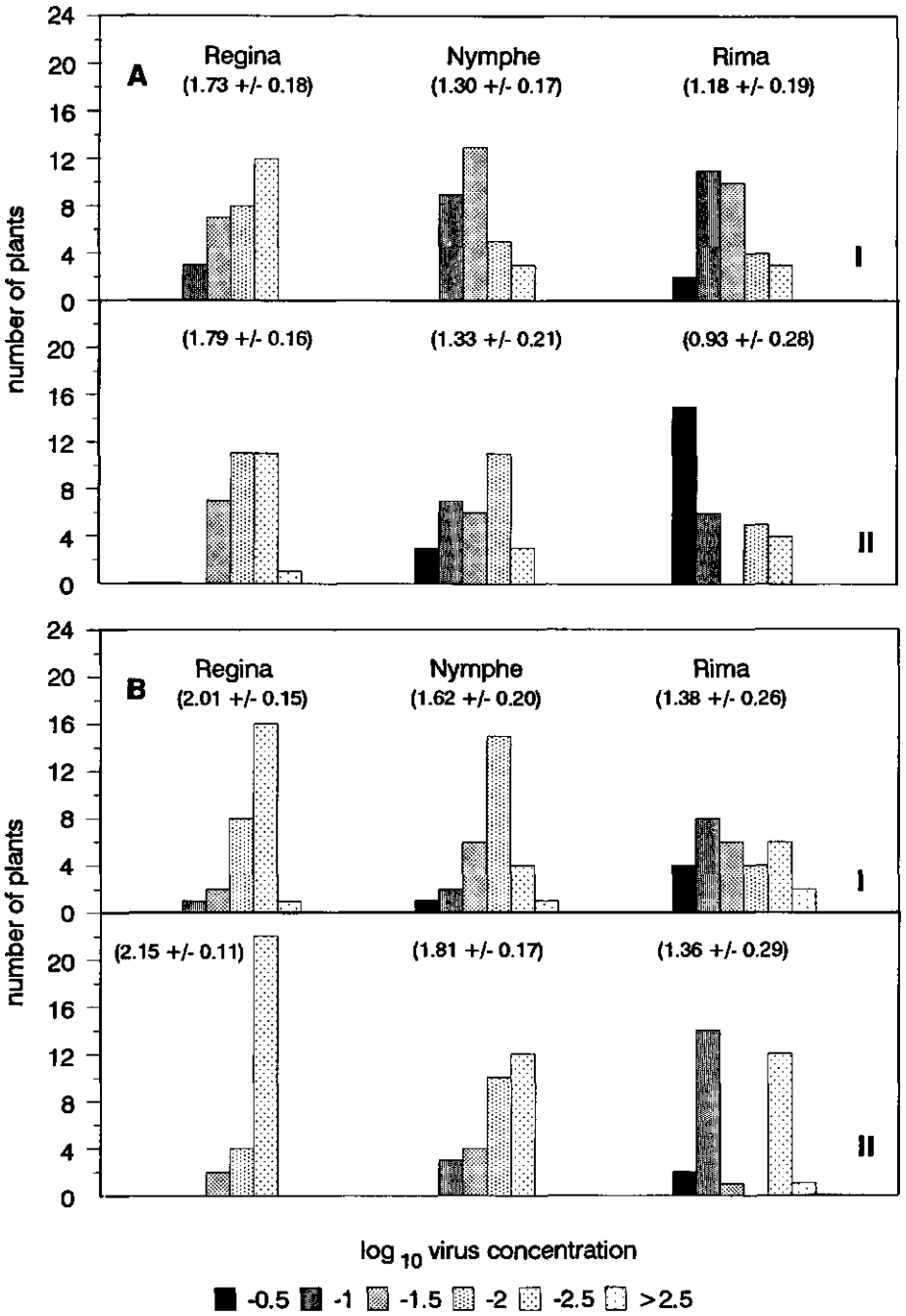

Fig. 2.4. A: Average virus concentrations (original data on virus concentration in ng ml^{-1}), with 95%-confidence intervals, and frequency distribution of concentrations of BNYVV, in 100 mg of the upper part (I) and in the remaining part (II) of the root systems of sugar beet seedlings on day 22 (n=30 plants per cultivar); **B**: Idem on day 29 (n=28, 29 and 30 plants for 'Regina', 'Nymphe' and 'Rima', respectively).

Discussion

Differences in response were detected between batches of purified virus. Estimates of virus concentrations of the batches were made with the help of a spectrophotometer, while activities were compared in ELISA. These two methods could respond differently, which might be due to damage of virus particles (Korpraditskul et al., 1980) or to aggregating of virus particles. When used as standard in dose-response curves, the use of different batches will result in different outcomes of estimated virus concentrations in plant samples. However, the differences between batches were small, so that the error due to the use of different batches is not considered to be a problem in quantitative assays of BNYVV. Another error in the virus assays might be a decline in activity of the virus in the batches during storage. No decline in activity of batch 90-2, estimated by the response of different dilutions, was observed within the time span of half a year in which the experiments in this study were carried out. The storage of samples therefore is thought to be of little influence on the outcome of the virus assays.

The four-parameter model proved to be appropriate for estimating virus concentrations. High percentages of curve fitting were obtained in all virus assays. In modelling dose-response curves the four-parameter model should be preferred to the linear regression model (Giunchedi et al., 1985; 1987), because of the non-linear relationship between absorbance values and virus concentrations. The use of the four-parameter model could also be used for relative virus concentrations (Bürcky and Büttner, 1988). High correlations were found between duplicate samples. Therefore single samples seem to be sufficient, although an internal check within the assay is no longer possible then.

The method of Toxopeus and Lubberts (1979), growing seedlings under low nutrient supply, was successful in obtaining root systems with mainly lateral roots. Significant differences in average root weights between cultivars were observed. However, no significant interactions were found between sampling times and cultivars, indicating that the growth rate of the cultivars did not differ, despite the differences in virus concentration. The roots in the second experiment were bigger than those in the first experiment. This could be explained by differences in nutrient

supply. During the second experiment the relative humidity in the greenhouse was lower than in the first experiment and more nutrient solution was used to fill the trays. Virus concentrations were similar in both experiments.

Due to temperatures below the optimum of both the fungus and the virus, only moderate levels of virus were obtained. Concentrations of all plant samples were within the range of the dose-response curves. The virus could be detected in all plants of the susceptible cultivar Regina, but especially at early sampling times, not in all plants of 'Nymphe' and 'Rima'. Differences in virus concentrations between the susceptible cultivar Regina and the partially resistant cultivars Nymhe and Rima were significant at all times, except the fourth sampling time in the first experiment. Differences between 'Nymphe' and 'Rima' were less evident. The absence of interaction between sampling time and cultivar indicated that the choice of sampling time is not critical for the present greenhouse test. In view of the first experiment, however, it is suggested that sampling should not take place early after transplanting. At early sampling times, virus concentrations might be too low for discrimination between partially resistant cultivars or between susceptible and resistant plants, and the same problem might occur as with the use of tap roots (Bürcky and Büttner, 1988).

The second experiment showed that a subsample of lateral roots could be used instead of the whole root system. No interactions between the sampling times and the two sample types, the upper and lower part of the roots, were observed and 'Regina' could be distinguished from the other cultivars with either sample type. Because a better discrimination between 'Nymphe' and 'Rima' was found with the lower part of the roots, it is suggested to sample the lateral roots in the lower part of the root system. This would also facilitate the maintenance of selected plants. It was not possible to differentiate between 'Rima' and 'Nymphe' under all circumstances, using average virus concentration as a criterium. Variation, as expressed by the width of the confidence interval, was always higher in 'Rima' than in 'Nymphe'. The two peaks in the distribution of the data of 'Rima' suggested the presence of both resistant and susceptible plants in the same population. It is suggested, that in evaluation tests, next to the average virus concentrations of the cultivars, also the variation in virus concentration between plants within a cultivar

should be taken into account. Low virus concentration may be caused either by partial resistance to the virus or to the vector *P. betae*. Since cystosori of the fungus could be detected in the roots of all cultivars, it is assumed that resistance to rhizomania in 'Nymphe' and 'Rima' is based on resistance to the virus.

The present method, in which small root systems, consisting mainly of lateral roots, are used for sampling, adequately discriminates between susceptible and resistant cultivars and can be used for the selection of individual plants. With the chosen culturing conditions, the period in which different cultivars could be discriminated, did not seem to be very much restricted, and a subsample of the roots could very well be used instead of the complete root system.

Virus concentrations will not only be influenced by cultural conditions, but also by the level of inoculum. Although little is known about the number of spores present in the soil, the present method seemed to provide sufficient inoculum to obtain infection in all plants tested.

Chapter 3

Multiplicative models for cultivar by location interaction in testing sugar beets for resistance to beet necrotic yellow vein virus

Abstract

Sugar beet cultivars were evaluated for resistance to beet necrotic yellow vein virus (BNYVV) on various locations in two consecutive years. Resistance levels of cultivars were measured by virus assays of plants from the field and the greenhouse. Infection levels in the fields were characterised by sampling plants of a susceptible indicator cultivar. For each year, statistical analyses were performed on two-way tables of cultivar by location for yield and quality parameters. In analysis of variance (ANOVA) significant main effects and significant cultivar by location interaction were found for all parameters ($P<0.05$). Interactions were further investigated by multiplicative models. In the Additive Main effects and Multiplicative Interaction effects (AMMI) model, interaction was written as the product of a cultivar score and a location score. Cultivar interaction scores were highly correlated to virus concentrations of the cultivars, and location interaction scores to virus concentrations of the susceptible indicator cultivar. Main effects of cultivars and locations were less clearly related to virus concentrations than interaction effects. In general, virus concentrations of plants from a greenhouse test gave higher correlations than virus concentrations of plants from the field. In the factorial regression model, virus concentrations were incorporated in the model. The model can be understood as a two-way ANOVA, with greenhouse virus concentrations and virus concentration of the indicator cultivar as concomitant variables on the cultivar

and location factor. Results of analyses with both multiplicative interaction models showed that interactions of all yield and quality parameters can be described in terms of virus concentrations. Therefore, the relative performance of susceptible and partially resistant cultivars in infested fields can be estimated by means of three independant parameters, (i) the level of resistance determined in a greenhouse experiment, (ii) the yield and quality in non-infested fields, and (iii) the level of infection in the field.

Introduction

The relationship between the performance of sugar beets (*Beta vulgaris* L.) in fields infested with beet necrotic yellow vein virus (BNYVV) and the virus concentration in the roots has been investigated in several studies (Ahrens, 1987; Bürcky and Büttner, 1989b, 1991; Giunchedi et al., 1987; Hillmann, 1984; Shimada et al., 1989). Sugar beet cultivars with various levels of resistance to BNYVV were incorporated in all studies. Negative correlations were found between virus concentrations and the parameters root yield, sugar content and sugar yield. Giunchedi et al. (1987) and Hillmann (1984) included quality parameters in their study and found a positive correlation between virus concentrations and sodium content, whereas a negative correlation was found between virus concentration and α-amino-nitrogen content. Correlation with potassium content was not always clear. In these studies, the plant material for the virus assays either came from infested fields, or from greenhouse experiments, which led to similar results (Bürcky and Büttner, 1991). The results from a single field were used (Ahrens, 1987; Bürcky and Büttner, 1991; Hillmann, 1984) or the average data of several fields (Bürcky and Büttner, 1989b; Giunchedi et al., 1987; Hillmann, 1984). Shimada et al. (1989) used multiple regression analysis to study the data from various fields.

In non-infested fields, susceptible sugar beet cultivars generally perform better than partially resistant cultivars. When the level of infestation with BNYVV increases, resulting in higher levels of infection in the beets, the ranking of the cultivars changes and in severely infested fields highest yields are obtained by partially resistant cultivars (Fig. 3.1). Thus, an adequate statistical model for the analysis of

data from cultivars with various levels of resistance in fields with varying levels of infestation has to include terms for the description of interaction.

In the present study, special attention is given to the explanation of cultivar by location interaction by means of virus concentrations. Virus concentrations are used to determine the resistance level of the cultivars as well as the infection level in the trial fields. In order to describe the interaction, three statistical models are considered in this chapter. Firstly, a two-way analysis of variance (ANOVA) model with interaction is used, the factors being cultivars and locations. In this model, each cell of the cultivar by location table has its own interaction parameter, so that the model uses a relatively large number of degrees of freedom for the interaction, and results of the description of the interaction are usually difficult to interpret. Secondly, a model with additive main effects and multiplicative scores for cultivars and locations is used, the so-called Additive Main effects and Multiplicative Interaction effects (AMMI) model (Gauch, 1988; Perkins, 1972, Zobel et al., 1988).

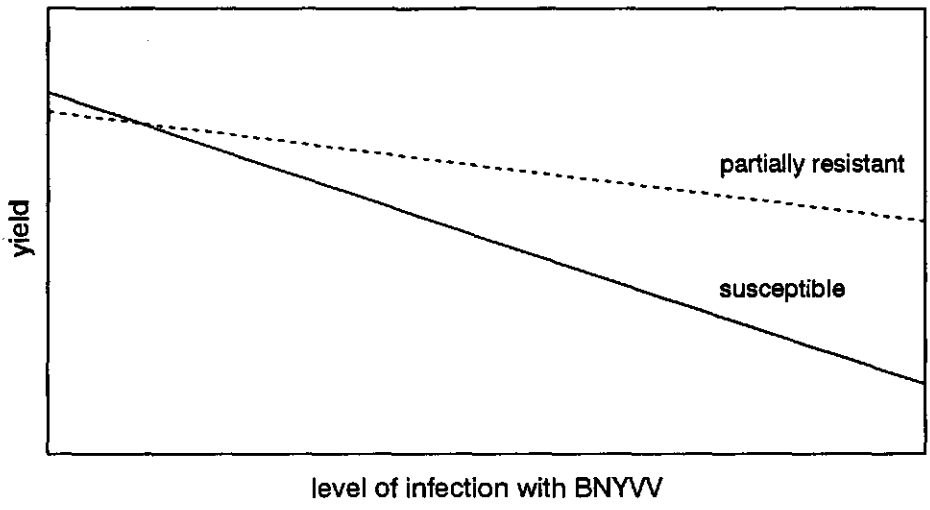

Fig. 3.1. Example of the occurrence of cultivar by location interaction in field trials for resistance to BNYVV with a susceptible and a partially resistant sugar beet cultivar (after Richard-Molard, 1987); yield in ton ha^{-1}, and infection expressed as virus concentration in ng ml^{-1} in samples of a susceptible indicator cultivar.

Description of the interaction in this model requires a smaller number of degrees of freedom and is more accurate than in the first model. Cultivar scores represent sensitivities and location scores can be interpreted as valuations of the environmental circumstances. After the analysis, scores of cultivars and locations can be related to additional information, in the present study the virus concentrations, to improve interpretation. The third model is a factorial regression model, a two-way ANOVA model with concomitant variables on both the cultivar and location factor (Denis, 1980, 1988; Snedecor and Cochran, 1980). Virus concentrations were directly incorporated in this model.

Results of these studies were expected to provide insight in the use of statistical models for the analysis of field experiments for resistance to BNYVV. Furthermore, explanation of cultivar by location interaction by means of virus concentrations would indicate that the relative performance of susceptible and partially resistant sugar beet cultivars in BNYVV-infested fields could be estimated using information on the resistance levels of the cultivars, the performance in non-infested fields, and the infection levels in the fields (Fig. 3.1).

Materials and methods

Field experiments

In two consecutive years, cultivar trials for resistance to BNYVV, each carried out in a completely randomised block design, were carried out at various locations. Data were obtained of the yield parameters root yield (ton ha^{-1}), sugar content (%), sugar yield (ton ha^{-1}), and the quality parameters sodium (Na), potassium (K) and α-amino nitrogen (α-amino N) (mmol/100 g beet) (De Nie and Van de Poel, 1989).

In 1989, the field trials were located at Colijnsplaat, Nagele, Veere and St. Maartensdijk, and included the susceptible cultivars Accord and Univers and the partially resistant cultivars Rima, Rizofort, Rizo 91, M 8906, HM 5682, Donna, Roxane and Samba. The trial at Colijnsplaat was located on a field considered to be free of infestation with BNYVV, and was carried out in four replicates with a plot size of 7 x 3 m. The complete plots were harvested. The other trials were located on fields infested with BNYVV and were performed in five replicates. Gross size of

the plots on the infested fields varied from 25 to 27 x 3 m. Net plot size, used for harvesting, varied from 18 to 20 x 3 m.

The field trials in 1990 were located at Ovezande, Wieringerwerf, Lage Zwaluwe, Nagele (two fields, coded I and II) and Arnemuiden, and included the susceptible cultivars Accord, Univers and Regina and the partially resistant cultivars Rima, Rizofort, Rizo 92, M 8917, Donna, Roxane and Samba 2. The fields at Ovezande and Wieringerwerf were considered to be disease free, whereas the other trials were located in fields infested with BNYVV. All trials were performed in four replicates with a plot size of 7 x 3 m. The complete plots were harvested.

Sampling for virus assays

At all fields in both years, a susceptible cultivar was sampled to characterise the level of infection with BNYVV. The cultivar Accord was sampled in most fields, but the cultivar Lucy was taken at Colijnsplaat in 1989 and the cultivar Univers at Wieringerwerf in 1990. At fields that were considered to be free of infestation with BNYVV, 20 plants were sampled, whereas at the other fields 10 plants were taken per replicate. At St. Maartensdijk in 1989 and at Nagele II in 1990 the other cultivars were also sampled, taking 10 plants per replicate, to assess the level of resistance. In 1989, samples were taken from the gross strips of the plots, or from the border rows of the field. These samples were taken in July and August. In 1990, samples were taken from special sample plots that were located next to the harvest plots, or from border rows of the field. Samples were taken in June and July. Additional samples from all cultivars were taken at Nagele II in August.

To assay the virus content, sap was extracted from the main root of the plants near the tip of the beets, using a garlic press. The sap from 10 plants of each replicate was combined into one sample. The 20 plants, taken from fields considered disease free, were analysed individually. The sap was diluted with phosphate buffered saline, containing 0.05% Tween 20 (PBS-Tween) in a ratio 1:9 (v v^{-1}).

Greenhouse experiments

In both years, the cultivars used were also tested for resistance to BNYVV in the greenhouse, using the same seed lot as was used in the field. Thirty seedlings of each cultivar were grown in a mixture of sand and soil infested with BNYVV (ratio 9:1 (v v^{-1})) for a period of five weeks at a temperature of 22/17 °C (10 h/ 14 h). A sample of 100 mg lateral root material from each plant was crushed with PBS-Tween in a ratio 1:20 (w v^{-1}) (Chapter 2).

Virus assays

Enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977) was used for virus detection. ELISA was performed as described by Alderlieste and Van Eeuwijk (1992) and in Chapter 2, with incubation times and temperatures as described by Büttner and Bürcky (1987), and using a dose response curve of purified virus, modelled by a four parameter logistic model, for the conversion of ELISA readings to virus concentrations. Virus standards were diluted in a 0.05 (v v^{-1}) or 0.1 (v v^{-1}) solution of healthy plant sap of cultivar Regina with PBS-Tween. Samples with a value below 4 ng ml^{-1} were considered to be free of virus. Results of virus assays were presented as $\log_{10}$ of virus concentrations in ng ml^{-1}. Zero concentrations were given the value of 1 ng ml^{-1} before the $\log_{10}$ conversion.

Statistical analysis

For each yield and quality parameter, data were arranged in a cultivar by location table of means over replicates. Because the cultivars were not the same and different seed lots were used in the two years, results of the two years were analysed separately. Firstly, as a kind of base-line model, a two-way ANOVA model with interaction was fitted to each table:

(1) $$\mathcal{E}(Y_{ij}) = \mu + G_i + E_j + GE_{ij}$$

In (1) $\mathcal{E}(Y_{ij})$ stands for the expectation of the variable Y for the i-th cultivar at the j-th location. The term μ denotes the general mean, G_i (i=1...I) the cultivar main effect, E_j (j=1...J) the location main effect and GE_{ij} the cultivar by location interaction having (I-1)(J-1) degrees of freedom. The mean within block error, that is the mean of the errors obtained from the complete block analyses per location, divided by the number of replicates per location, was used to test the main effects of cultivar and location and their interaction. This error was also used for the computation of LSDs for cultivars and locations.

Secondly, AMMI-models were fitted:

(2) $$\mathcal{E}(Y_{ij}) = \mu + G_i + E_j + \underbrace{\sum_{n=1}^{N} \sigma_n u_{ni} v_{nj}}_{GE_{ij}} + \delta_{ij}$$

Here, μ, G_i and E_j have the same meaning as in (1). The term GE_{ij} of (1) is now split into a model part, $\sum_{n=1}^{N} \sigma_n u_{ni} v_{nj}$ and a residual, δ_{ij}. For orthogonal tables, with no cells missing, the least squares estimates for μ, G_i and E_j are the usual two-way analysis of variance estimates, whereas the multiplicative terms for cultivars, u_{ni}, and for locations, v_{nj}, also called scores, and the scaling constant σ_n are obtained from the singular value decomposition (Gabriel, 1978) of the two-way table of means corrected for the cultivar and location effect, containing the so-called residuals from additivity. The scalar constant σ_n, the singular value for the n-th set of product terms, indicates the importance of that set for the description of the interaction. Its square is equal to the sum of squares explained by the set. A corresponding mean square can be obtained by dividing this sum of squares by (I-1) + (J-1) + 1 - 2n, its degrees of freedom (Gollob, 1968). N indicates the number of sets necessary for an adequate description of the interaction and was assessed by testing the mean squares for successive terms against the mean within block error. After the analysis, main effects and interaction scores for cultivars and

locations of the AMMI model were correlated with virus concentrations of the ten cultivars and the susceptible indicator cultivar to facilitate interpretation.

Thirdly, direct modelling of the additional information took place in factorial regression models. Virus concentrations of the cultivars that were determined in the greenhouse were used as concomitant variable on the cultivar factor, and field virus concentrations estimated as the mean virus concentration of the two samples from the susceptible indicator cultivar were used as concomitant variable on the location factor. The model used in the present study was:

(3)
$$\mathcal{E}(Y_{ij}) = \mu + \underbrace{\alpha_i + \xi x_i}_{G_i} + \underbrace{\beta_j + \zeta z_j}_{E_j} + \underbrace{\eta x_i z_j + \rho_i z_j + x_i \tau_j}_{GE_{ij}} + \delta_{ij}$$

The term μ again stands for the general mean. For the cultivars the concomitant greenhouse virus concentrations are denoted by x_i, for the locations the concomitant field virus concentrations are denoted by z_j. Both variables were centred. G_i from (1) and (2) is replaced by a regression through the origin of the cultivar main effect on x_i , ξ represents the slope and the α_i values reflect deviations from this regression. E_j is likewise replaced by ζ and β_j values. The interaction is decomposed into three parts. The first part, $\eta x_i z_j$, can be thought of as a regression through the origin of the residuals from additivity on an explanatory variable consisting of the product of x_i and z_j , with slope η , with one degree of freedom only. The second and third part of the interaction represent additional regressions per cultivar on z_j , giving slopes ρ_i and with *I*-2 degrees of freedom, and per location on x_i, giving slopes τ_j and with *J*-2 degrees of freedom. The term δ_{ij} again denotes a residual.

Table 3.1. Yield and quality data of ten sugar beet cultivars, averaged over locations, in 1989 and 1990, and virus concentrations in roots of beet plants on a heavily infested field and in the greenhouse.

Cultivars	Root yield (ton ha^{-1})	Sugar content (%)	Sugar yield (ton ha^{-1})	K (mmol/100 g beet)	Na	α-amino N	Virus concentrations (log_{10}, ng ml^{-1})			
1989							St. Maartensdijk			Greenhouse
							July	August		
Accord	44.5	12.2	5.93	5.19	1.17	1.02	2.44	1.99		2.37
Univers	50.6	12.9	6.79	4.85	1.04	1.19	2.07	1.85		2.31
Rima	55.1	14.8	8.25	4.89	0.53	1.61	2.00	1.24		1.53
Rizofort	58.6	14.9	8.74	5.01	0.50	1.69	1.54	0.95		1.69
Rizo 91	52.1	15.4	8.07	4.92	0.42	1.56	1.59	1.58		1.56
M 8906	55.0	13.3	7.47	4.77	0.83	1.37	1.82	1.69		2.10
HM 5682	49.1	14.1	7.06	4.97	0.70	1.34	2.32	1.89		2.07
Donna	53.2	14.7	7.88	4.07	0.54	1.13	1.22	1.50		1.99
Roxane	53.0	14.2	7.61	5.01	0.72	1.35	2.25	1.71		2.02
Samba	54.7	14.6	8.01	4.97	0.55	1.58	2.00	1.33		1.57
LSD(95%)	2.1	0.2	0.33	0.16	0.07	0.10	0.62	0.46		0.27
1990							Nagele II			Greenhouse
							June	July	August	
Accord	65.1	15.1	9.96	4.84	0.84	1.51	1.55	1.46	1.82	2.51
Univers	69.1	14.8	10.29	4.77	0.85	1.89	1.02	1.96	2.08	2.40
Regina	66.5	14.7	9.84	4.62	0.87	1.63	0.82	1.96	1.88	2.49
Rima	69.4	16.1	11.14	4.98	0.39	2.37	0.51	0.33	1.08	1.87
Rizofort	68.6	16.0	10.95	5.03	0.41	2.24	1.08	1.13	0.95	1.91
Rizo 92	65.4	15.8	10.31	4.85	0.35	2.18	0.08	0.78	0.71	1.72
M 8917	74.5	14.9	11.07	4.73	0.79	1.82	0.41	1.44	1.78	2.31
Donna	62.0	16.3	10.06	4.14	0.42	1.61	1.23	0.91	1.47	2.17
Roxane	69.9	16.6	11.56	5.05	0.44	2.10	1.39	1.29	1.24	1.68
Samba 2	67.6	15.8	10.63	4.96	0.40	2.19	0.52	0.93	1.13	1.71
LSD(95%)	2.7	0.2	0.47	0.11	0.06	0.15	1.16	1.09	0.68	0.22

Table 3.2. Yield and quality data on four locations in 1989 and six locations in 1990, averaged over cultivars, and virus concentrations in the roots of a susceptible indicator cultivar.

Locations	Root yield (ton ha^{-1})	Sugar content (%)	Sugar yield (ton ha^{-1})	K (mmol/100 g beet)	Na (mmol/100 g beet)	α-amino N (mmol/100 g beet)	Virus concentrations (log_{10}, ng ml^{-1})	
1989							July	August
Colijnsplaat	66.1	15.7	10.38	3.77	0.37	1.84	0.28	0.23
Nagele	73.8	14.1	10.39	4.47	0.65	1.28	0.89	0.63
Veere	32.4	14.1	4.71	5.72	0.99	1.57	-	2.02
St.Maartensdijk	38.1	12.5	4.85	5.51	0.78	0.85	2.44	1.99
LSD(95%)	3.3	0.4	0.52	0.26	0.11	0.16		
1990							June	July
Ovezande	58.2	16.1	9.41	4.36	0.34	3.30	0.02	0.00
Wieringerwerf	71.3	17.5	12.49	5.97	0.30	2.07	0.00	0.00
Lage Zwaluwe	66.7	16.2	10.77	4.15	0.55	1.63	0.12	0.62
Nagele I	82.5	14.2	11.84	4.48	0.84	1.72	0.61	0.80
Nagele II	69.1	14.5	10.02	5.03	0.83	1.56	1.55	1.46
Arnemuiden	59.1	15.1	8.96	4.78	0.58	1.45	1.94	2.25
LSD(95%)	3.5	0.3	0.60	0.14	0.08	0.19		

Results

Field trials and virus assays

Average yield and quality data per cultivar and per location are given in Table 3.1 and 3.2, together with the results of the virus assays. In 1989, virus concentrations in plants from the field were lower at the second than at the first sampling date. In 1990, variations between sampling dates were found for the virus concentrations in field material, but in general, virus concentrations increased with time. Experimental errors were greater for the results of virus assays of plants from the field than of plants from the greenhouse (Table 3.1). Fields were arranged according to increasing levels of infection with BNYVV in the susceptible cultivar (Table 3.2). In 1989, a few positive samples were found at Colijnsplaat, however, the average virus concentration was below the level indicating for the presence of the virus. In 1990, no virus was detected at Ovezande and Wieringerwerf. Virus could be detected in all other fields, except for Lage Zwaluwe at the first sampling date. Average virus concentrations of the samples from the different fields were higher in 1989 than in 1990.

It may be noticed, that highest yields were not necessarily found on fields without infection.

ANOVA

Table 3.3 shows the results of the ANOVA. Main effects and interaction were significant for all yield and quality parameters in both years ($P<0.05$).

Table 3.3. Analysis of variance of yield and quality data in 1989 and 1990.

Source of variation	Degrees of freedom	Root yield		Sugar content		Sugar yield		K		Na		α-amino N	
		SS[1]	MS[1]	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS
1989													
Cultivar	9	540.7	60.08	37.43	4.16	23.69	2.63	3.29	0.37	2.23	0.25	1.81	0.20
Location	3	12520.0	4173.32	51.35	17.12	314.19	104.73	24.94	8.31	2.00	0.67	5.37	1.79
Cultivar x location	27	949.5	35.17	14.96	0.55	23.92	0.89	3.72	0.14	1.18	0.04	0.92	0.03
Mean within block error	135		5.50		0.07		0.14		0.03		0.006		0.01
1990													
Cultivar	9	617.5	68.61	25.90	2.88	17.97	2.00	3.87	0.43	2.77	0.31	4.91	0.55
Location	5	3996.4	799.29	78.96	15.79	95.50	19.10	21.31	4.26	2.67	0.53	23.88	4.78
Cultivar x location	45	1410.9	31.35	12.41	0.28	54.15	1.20	1.62	0.04	0.93	0.02	3.18	0.07
Mean within block error	182		9.41		0.07		0.28		0.02		0.005		0.03

[1] SS=Sum of squares; MS=Mean squares. All effects were significant at the 5% level.

Table 3.4. Analysis of variance for sugar yield in 1989 and 1990, with subdivision of sum of squares by two multiplicative models for cultivar by location interaction.

Source of variation	Degrees of freedom	Sum of squares	Percentage explained	Mean square	Degrees of freedom	Sum of squares	Percentage explained	Mean square
1989					1990			
Cultivar	9	23.69		2.63	9	17.97		2.00
[2] ξ	1	17.71	75	17.71	1	7.02	40	7.02
deviations (α_i s)	8	5.98	25	0.75	8	10.95	60	1.37
Location	3	314.19		104.73	5	95.50		19.10
[2] ζ	1	296.34	94	296.34	1	29.71	31	29.71
deviations (β_j s)	2	17.86	6	8.93	4	65.79	69	16.45
Cultivar x Location	27	23.92		0.89	45	54.15		1.20
[1] $\sigma_1 u_{1i} v_{1j}$	11	21.39	89	1.94	13	48.40	89	3.72
deviations (δ_{ij})	16	2.53	11	0.16	32	5.75	11	0.18
[2] η	1	11.85	50	11.85	1	35.71	66	35.71
ρ_i	8	6.05	25	0.76	8	7.57	14	0.95
τ_j	2	3.41	14	1.71	4	5.89	11	1.47
deviations (δ_{ij})	16	2.60	11	0.16	32	4.98	9	0.16
Mean within block error	135			0.14	182			0.28

[1] Subdivision by singular value decomposition of residuals from additivity (AMMI model); [2] Subdivision by factorial regression.

AMMI analysis

In the Tables 3.4 and 3.5, the results of AMMI analyses are given for the parameter sugar yield in 1989 and 1990. In both years, mean squares corresponding to the first multiplicative term were highly significant when tested against the mean within block error. One set of interaction parameters (σ_1, u_{1i}, v_{1j}) sufficed for an adequate description, as can be seen from the high percentage of explained interaction and the non-significant residual (Table 3.4). In Table 3.6 results of the AMMI analysis with one set of interaction parameters are presented for all parameters, together with the results of the factorial regression. High percentages of explained interaction were found for all yield and quality parameters.

Table 3.5. AMMI-interaction scores for the cultivars and locations for sugar yield in 1989 and 1990.

Cultivar	AMMI score (u_{1i})	Location	AMMI score (v_{1j})
1989			
Accord	-0.59	Colijnsplaat	-0.77
Univers	-0.46	Nagele	-0.08
Rima	0.10	Veere	0.58
Rizofort	0.35	St. Maartensdijk	0.27
Rizo 91	0.19		
M 8906	-0.27		
HM 5682	-0.04		
Donna	0.21		
Roxane	0.13		
Samba	0.37		
1990			
Accord	-0.52	Ovezande	-0.39
Univers	-0.41	Wieringerwerf	-0.51
Regina	-0.44	Lage Zwaluwe	-0.28
Rima	0.23	Nagele I	0.25
Rizofort	0.15	Nagele II	0.35
Rizo 92	0.24	Arnemuiden	0.57
M 8917	-0.06		
Donna	0.19		
Roxane	0.37		
Samba 2	0.26		

Table 3.6. Percentages of explained sum of squares of interaction for yield and quality parameters in 1989 and 1990 by two multiplicative models for cultivar by location interaction.

Model	Root yield	Sugar content	Sugar yield	K	Na	α-amino N
1989						
AMMI-1[1]	94	85	89	94	92	81
Fact. Regr.	94	84	89	88	88	66
1990						
AMMI-1[1]	85	88	89	61	87	77
Fact. Regr.	86	88	91	63	81	60

[1] AMMI analysis with one set of multiplicative scores for cultivars and locations

In Table 3.7, correlation coefficients of virus concentrations of plants from the field and the greenhouse with cultivar main effects and cultivar interaction scores of the different parameters are presented. Virus concentrations of the plants sampled in August gave higher correlations with both cultivar main effects and scores for interaction than virus concentrations of plants sampled in June or July. In general, virus concentrations of plants from the greenhouse gave higher correlations than virus concentrations of plants from the field. Correlation coefficients of virus concentrations of a susceptible indicator cultivar with location main effects and location interaction scores are presented in Table 3.8. For the sampling dates in each year, correlations of the virus concentrations of the susceptible indicator cultivar with the location main effects and interaction scores were similar.

For the parameters root yield, sugar content, sugar yield and α-amino nitrogen, cultivar main effects were negatively correlated to virus concentration, although results were not clear for root yield in 1990 (Table 3.7). Correlations with the main effect were low for potassium, and tended to be contradictory between both years. For sodium, main effects were positively correlated to virus concentrations.

Table 3.7. Correlation coefficients between virus concentrations of sugar beets and cultivar main effects (M) and scores for interaction (I) in 1989 and 1990.

Plant material		Root yield		Sugar content		Sugar yield		K		Na		α-amino N	
		M	I	M	I	M	I	M	I	M	I	M	I
1989[1]													
Field	July	-0.61	-0.52	-0.59	-0.59	-0.67	-0.54	0.73	-0.80	0.64	-0.57	-0.27	-0.47
	August	-0.87	-0.70	-0.70	-0.85	-0.91	-0.78	0.12	-0.77	0.74	-0.76	-0.80	-0.64
Greenhouse		-0.67	-0.82	-0.89	-0.91	-0.87	-0.84	0.00	-0.79	0.90	-0.88	-0.89	-0.85
1990[1]													
Field	June	-0.29	-0.36	0.11	-0.25	-0.11	-0.30	-0.13	-0.19	0.26	-0.22	-0.49	-0.16
	July	0.15	-0.80	-0.74	-0.71	-0.38	-0.77	-0.15	-0.87	0.85	-0.78	-0.62	-0.92
	August	0.13	-0.85	-0.76	-0.82	-0.42	-0.84	-0.39	-0.90	0.93	-0.89	-0.77	-0.86
Greenhouse		-0.06	-0.92	-0.81	-0.90	-0.63	-0.93	-0.48	-0.84	0.91	-0.89	-0.84	-0.79

[1] Absolute values higher than 0.63 are significant at the 5% level

Table 3.8. Correlation coefficients between virus concentrations of a susceptible indicator cultivar and location main effects (M) and scores for interaction (I) in 1989 and 1990.

Plant material		Root yield		Sugar content		Sugar yield		K		Na		α-amino N	
		M	I	M	I	M	I	M	I	M	I	M	I
1989													
Field	July[1]	-0.89	1.00	-0.97	0.84	-0.96	0.91	0.99	-0.88	0.90	-0.87	-0.95	0.24
	August[2]	-0.94	0.99	-0.78	0.86	-0.98	0.92	0.99	-0.92	0.90	-0.83	-0.56	0.42
	Mean[2]	-0.91	0.97	-0.84	0.83	-0.97	0.91	0.98	-0.88	0.88	-0.79	-0.64	0.41
1990													
Field	June[3]	-0.17	0.93	-0.68	0.80	-0.56	0.94	0.02	0.20	0.58	-0.67	-0.60	0.67
	July[3]	-0.17	0.92	-0.66	0.83	-0.55	0.93	-0.12	0.19	0.58	-0.70	-0.70	0.73
	Mean[3]	-0.17	0.93	-0.67	0.82	-0.56	0.94	-0.05	0.20	0.59	-0.69	-0.66	0.71

[1,2,3] Absolute values higher than 1.00, 0.95 and 0.81 are significant at the 5% level, respectively

Cultivar scores for interaction were negatively correlated with virus concentrations for all parameters. Results of correlation studies between virus concentrations of a susceptible indicator cultivar and location main effects (Table 3.8) generally corresponded with correlation studies for the cultivar main effect. A strong positive correlation was found for potassium in 1989, while no correlation was found in 1990. Results of the correlation studies for the location interaction scores were different from those of the cultivar interaction scores. Location interaction scores were positively correlated to virus concentrations for the parameters root yield, sugar content, sugar yield and α-amino nitrogen. For potassium negative correlations were found in 1989, while correlations in 1990 were low. Correlations with interaction scores of sodium were negative.

Within the AMMI model, adequate descriptions for cultivar by location interaction were given by the product $\sigma_1 u_{1i} v_{1j}$. Susceptible cultivars were corrected downward in fields with high levels of infection, and upward in fields without or with a light infection for the parameters root yield, sugar content, sugar yield and α-amino nitrogen (see example for sugar yield in Table 3.5). For sodium, opposite corrections were made. Only in 1989, potassium gave results similar to sodium. The partially resistant cultivars exhibited smaller fluctuations than the susceptible cultivars. The high correlations between virus concentrations and cultivar and location scores indicate that interactions can be described in terms of virus concentrations of the cultivars and the susceptible indicator cultivar.

Factorial regression analysis

Results of the factorial regression analysis for the parameter sugar yield are presented in Table 3.4. The percentage of the main effects' sums of squares explained by the concomitant variables can be obtained by squaring the correlation coefficients of the Tables 3.7 and 3.8. For the factorial regressions, greenhouse virus concentrations were used as concomitant variable for the cultivars and the means over both assessments of the field infection for the locations. For sugar yield, the interaction could be described for 50% in 1989 and 66% in 1990 by the regression of the residuals from additivity on the variable formed by the product of the cultivar greenhouse virus concentrations and field virus concentrations, with slope η . This

regression can be interpreted as reflecting a downward correction for sugar yield for susceptible cultivars in fields with a high level of infection and an upward correction in fields without or with a low level of infection, which corresponds to the AMMI analysis. Additional regressions for each cultivar on the field virus concentrations, with slopes ρ_i, and regressions for each location on the cultivar greenhouse virus concentrations, with slopes τ_j, also accounted for a significant part of the interactions. Deviations from these regressions were not significant. The three regressions, together constituting the model for interaction in the factorial regression model, accounted for 89% of the interaction in sugar yield in 1989 and 91% in 1990. These percentages are about the same as those for the AMMI analysis (Table 3.6). For the other parameters AMMI analysis and factorial regression analysis gave similar results too. Furthermore, the number of degrees of freedom (n=11 in 1989 and n=13 in 1990) for the factorial regression description of interaction was equal to that of the AMMI model. The two models provided more or less equivalent descriptions of the interaction and, as for the AMMI analysis and correlation studies, results of the factorial regression analysis support the conclusion that measured virus concentrations of the cultivars and the susceptible indicator cultivar can be used to describe the observed interactions.

Discussion

Main effects

In the present study, a decrease in root yield, sugar content, sugar yield and α-amino nitrogen and an increase in sodium was found with increasing susceptibility of the cultivars and increasing levels of infection in the fields, while trends for potassium were not clear. Similar results for the cultivars were described earlier (Ahrens, 1987; Bürcky and Büttner, 1989b, 1991; Giunchedi et al., 1987; Hillmann, 1984, Shimada, 1989). The results also correspond with the numerous reports on cultivar trials that were performed in various countries. At a low infection level, as in 1990, correlations between virus concentrations and yield parameters were less evident than at a high infection level as in 1989. A similar finding was reported by Ahrens (1987). However, for sugar content, sodium and α-amino nitrogen high

correlations with virus concentrations were found in 1990, indicating that these parameters were more sensitive to BNYVV than the other parameters. Similar observations were made by Heijbroek (1989), Pollach (1984) and Takeda et al.(1988) for susceptible cultivars, while findings in the present study on the reaction of yield and quality parameters in resistant and susceptible cultivars to infection with BNYVV confirm the results of Bürcky and Büttner (1989a), Casarini-Camangi and Canova (1987) and Rosso et al. (1988, 1989).

Interaction effects

In both years, cultivar by location interactions were significant for all yield and quality parameters. AMMI analyses and factorial regressions demonstrated the feasibility of a description of the interaction in terms of virus concentrations, representing resistance level of the cultivars and infection level in the fields. Giunchedi et al. (1987) did not find interaction for most parameters, indicating that their fields had identical levels of infection. However, in most studies which include fields with various infection levels, interactions will occur and the use of models with terms for interaction is inevitable.

Statistical models and additional information

Virus concentration of the cultivars measured in the greenhouse was a better explanatory variable than virus concentration measured in the field. Greenhouse experiments have lower residual variance than field experiments. For the evaluation of the level of resistance, greenhouse tests provide the most accurate information. Variation in virus concentrations of plants from the field was also reported by Casarini-Camangi and Canova (1988). For the assessment of the infection level in the field, reflecting the level of infestation, a susceptible indicator cultivar was used. As an alternative, infestation levels can de determined by estimates of the number of infectious units, assessed by the most probable number technique as done by Tuitert (1990).

An AMMI model can be used even in the absence of additional information on cultivars and locations. Extra information, such as results of greenhouse tests and characterisation of the level of infection in the fields by sampling of susceptible

cultivars, allows a better interpretation of AMMI results. Alternatively, factorial regression models use extra information directly. For the experiments described here, virus measurements greatly improved the quality of the descriptions of cultivar by location interaction.

Cultivar evaluation

In breeding programmes and cultivar trials, field experiments for resistance to BNYVV are carried out on several locations with various levels of infection, including non-infested fields (Richard-Molard, 1987). The application of statistical models that include terms for cultivar by location interaction helps to interpret the results of such studies. In the present study, virus concentrations of the cultivars in a greenhouse test, and of a susceptible cultivar in the field, gave a satisfactory explanation for cultivar by location interactions in field trials for resistance. This leads to the conclusion, that the relative performance of susceptible and partially resistant cultivars, in infested fields, can be estimated by means of three independant parameters, (i) the level of resistance determined in a greenhouse experiment, (ii) the yield and quality in non-infested fields, and (iii) the level of infection in the field. This approach will reduce the need for testing cultivars and breeding accessions on a large number of infested fields, and thus will increase the efficiency of sugar beet breeding and cultivar evaluation.

Chapter 4

Resistance to *Polymyxa betae* in *Beta* species of the section *Procumbentes*, in hybrids with *B. vulgaris* and in monosomic chromosome additions of *B. procumbens* in *B. vulgaris*

Abstract

Resistance to *Polymyxa betae* was studied in *Beta* species of the section *Procumbentes*, in hybrids of *B. vulgaris* with *B. procumbens* or *B. patellaris*, and in monosomic additions of chromosomes of *B. procumbens* in *B. vulgaris*. In all experiments *P. betae* infested with beet necrotic yellow vein virus (BNYVV) was used. This virus causes rhizomania in sugar beet, and the effect of vector resistance was studied by measuring virus concentrations. Cystosori of *P. betae* were not found in the wild species and the hybrids. Virus concentrations in these plants were low, and in half the number of plants the virus could not be detected. Results of experiments with the monosomic additions indicate, that resistance to *P. betae* in *B. procumbens* is located on chromosomes 4 and 8. Some cystosori were present in plants of these addition types, while cystosori were abundantly present in plants of other addition types and all sib-plants. Virus concentrations in the addition types 4 and 8 were lower than in their sib-plants, but in almost all plants the virus could be detected. A significant correlation ($r=0.91$; $P<0.05$) between average numbers of cystosori and average virus concentrations was found when addition families of type 8 were tested together with *B. procumbens* and *B. vulgaris* cultivar Regina.

Introduction

Beta species of the section *Procumbentes* (formerly *Patellares*), including *B. procumbens* Chr. Sm., *B. webbiana* Moq. and *B. patellaris* Moq., are resistant to many pathogens and pests of sugar beet (*Beta vulgaris* L.). Resistance to *Polymyxa betae* Keskin in *Procumbentes* species has been reported by Fujisawa and Sugimoto (1979), Abe and Ui (1986), and Asher and Barr (1990). *P. betae* is the vector of beet necrotic yellow vein virus (BNYVV) (Fujisawa and Sugimoto, 1976), which causes rhizomania in sugar beet. Fujisawa and Sugimoto (1979) observed local lesions after mechanical inoculation of the leaves of *Procumbentes* species with BNYVV, indicating that these species are susceptible to the virus.

Resistance to *P. betae* might be useful to improve the level of resistance to rhizomania in sugar beet. Transfer of resistance to *P. betae* from *Procumbentes* species to *B. vulgaris* is hampered by crossing barriers between the species, as has been experienced by Savitsky (1975) when trying to transfer resistance to the beet cyst nematode *Heterodera schachtii* Schm. Monosomic additions in beet, carrying an extra chromosome of the wild species, were produced as a means to transfer the resistance to *B. vulgaris*. Sugar beets, homozygous for resistance to the beet cyst nematode, could finally be selected (Savitsky, 1978; Yu, 1981; Heijbroek et al., 1988; Jung and Wricke, 1987; Lange et al., 1990). Using isozymes, Van Geyt et al. (1988) were able to identify all nine possible monosomic addition types of *B. procumbens* in *B. vulgaris*.

In earlier studies on resistance to rhizomania, Paul et al. (1991) did not detect cystosori of *P. betae* in the roots of any of the *Procumbentes* species or hybrids between *B. vulgaris* and *B. procumbens*. The absence of *P. betae* in the hybrids indicated that resistance to *P. betae* behaved as a dominant factor when combined with the genome of *B. vulgaris*. Low concentrations of virus were detected in these wild species and hybrids.

In the present study, the species of the section *Procumbentes*, hybrids with *B. vulgaris* and the nine different monosomic addition types of *B. procumbens* in *B. vulgaris* were tested for resistance to *P. betae*. By screening the different addition types, resistance to *P. betae* could be located at chromosome level. Viruliferous

P. betae was used in all experiments, and the effect of resistance to the vector on the level of BNYVV infection was studied by measuring virus concentrations.

Materials and methods

Plant material

Plant material consisted of *B. vulgaris* cultivar Regina, the wild species *B. procumbens* (2n=18), *B. webbiana* (2n=18) and *B. patellaris* (2n=36), several hybrid populations of tetraploid *B. vulgaris* (2n=36) with *B. procumbens* or *B. patellaris*, and addition families representing the complete set of nine different monosomic additions (2n=19) of *B. procumbens* in diploid *B. vulgaris*. Hybrids and monosomic additions were made at the former Foundation for Agricultural Plant Breeding, SVP (now: CPRO-DLO), at Wageningen and are described here as they were described by Speckmann and De Bock (1982) and by Lange et al. (1988).

Seeds were disinfected with Dithane M45 (Duphar) and sown in heat-sterilised peat soil. Seedlings were transplanted to trays, filled with peat soil. Populations obtained after crossing *B. vulgaris* with *B. procumbens* or *B. patellaris* contained individuals which resulted from self-pollination of the *B. vulgaris* parents, but it was possible to identify and select them morphologically. As observed by Speckmann and De Bock (1982), many hybrids showed lethality. Addition families consisted for a major part of sib-plants without the additional chromosome. Morphological characteristics of the additions plants (Lange et al., 1988) were used for pre-selection and further identification was performed by counting the number of chromosomes (Speckmann et al., 1985). As observed by Lange et al. (1988), most plants of addition type 4 showed lethality. Curling of the leaves was successfully used as an additional characteristic for the preselection of addition type 8. Plant material had been characterised within five weeks after transplanting.

Tests for resistance and experimental design

Tests for resistance to rhizomania were carried out in the greenhouse in a mixture of sand and soil, containing *P. betae* infested with BNYVV. Conditions in the

greenhouse and nutrient supply were as described in Chapter 2. Before transplantation to the test containers, the roots of the plants were trimmed to a length of approximately 3 cm and some of the leaves were removed to reduce evaporation. At the end of the incubation period, roots were washed. Resistance to *P. betae* was investigated by identifying cystosori of *P. betae* in the roots by direct observation under an inverted microscope (Zeiss ID02). For the virus assay some 100 mg of the lateral roots was crushed in Potter tubes with PBS-Tween in a ratio 1:20 (w v^{-1}). When testing the hybrids, a ratio of 1:10 (w v^{-1}) was used. Only plants with viable root systems were analysed. In one experiment, in which the number of cystosori was determined, a different procedure was used. All lateral roots were removed and homogenised with PBS-buffer without Tween in a ratio 1:20 (w v^{-1}), using a mixer (Polytron, Kinematica) (3x30 sec). Samples were centrifuged for 3 min at 3000 rpm (Centaur 2, MSE). Supernatants were used for the virus assay. Pellets were resuspended and cystosori were counted, using a nematode counting dish (Notra 905).

The performance of *B. procumbens*, *B. webbiana* and *B. patellaris* was tested in an experiment together with 'Regina'. Plants in sand were used as controls. For each treatment twelve plants per accession were tested. A completely randomised design was used. The first half of the plants were sampled after 34 days and the remaining plants after 45 days. Hybrids of *B. vulgaris* with *B. procumbens* and *B. patellaris* were tested together with *B. vulgaris* plants from the same population, and with the three wild species and 'Regina'. Plants were sampled after four weeks. Monosomic additions, with their sibs as controls, were tested in a series of experiments. Each experiment consisted of a small number of addition types. If possible, two families with the same additional chromosome, but in a different *B. vulgaris* background, and for each family eight addition plants and eight sib-plants, were tested. Three to four plants of 'Regina' were added to each experiment as controls. Plants were sampled after approximately five weeks. In the experiment, in which cystosori were counted, two addition families of type 8 and their sib-plants were compared with *B. procumbens* and 'Regina', using ten plants per accession. Plants were sampled after four weeks.

Virus assays

Enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977) was used for measurement of virus concentrations. Virus assays and conversion of the ELISA-readings to concentrations, using standards of purified virus together with a four-parameter logistic model, were performed as described by Alderlieste and Van Eeuwijk (1992) and in Chapter 2. Standard concentrations of virus in the assays varied from 0-1200 or 0-1600 ng ml^{-1}, diluted in a 0.05 (v v^{-1}) or 0.1 (v v^{-1}) solution of healthy plant sap of 'Regina' with PBS-Tween. Different batches of purified virus were used, but only one batch was used within an experiment.

Data analysis

Samples that gave experimental responses in the virus assays below 4 ng ml^{-1} were considered to be free of virus. Virus concentrations exceeding the range of the virus standards were equalled to the highest concentration. Log_{10} values of the data were used in the statistical analyses. Data smaller than 1.0 were replaced by 1.

Results

Cystosori of *P. betae* were abundantly present in the roots of 'Regina' plants that had been grown in the mixture of sand and infested soil, but were absent from the roots of the plants that had been grown in sand. No cystosori were detected in the roots of the wild species in either treatment. All experimental responses in the virus assays of the plants of both 'Regina' and the wild species that had been grown in sand were below the threshold level that is taken as indicative for the presence of the virus (4 ng ml^{-1}) (Fig. 4.1A/B). Responses were also low for the wild species that had been grown in the mixture of sand and infested soil, but approximately half the number of samples of these plants had responses higher than the threshold level, indicating that BNYVV was present. In all 'Regina' plants grown in the mixture the virus could readily be detected. No significant difference in virus concentrations was observed between the two sampling days.

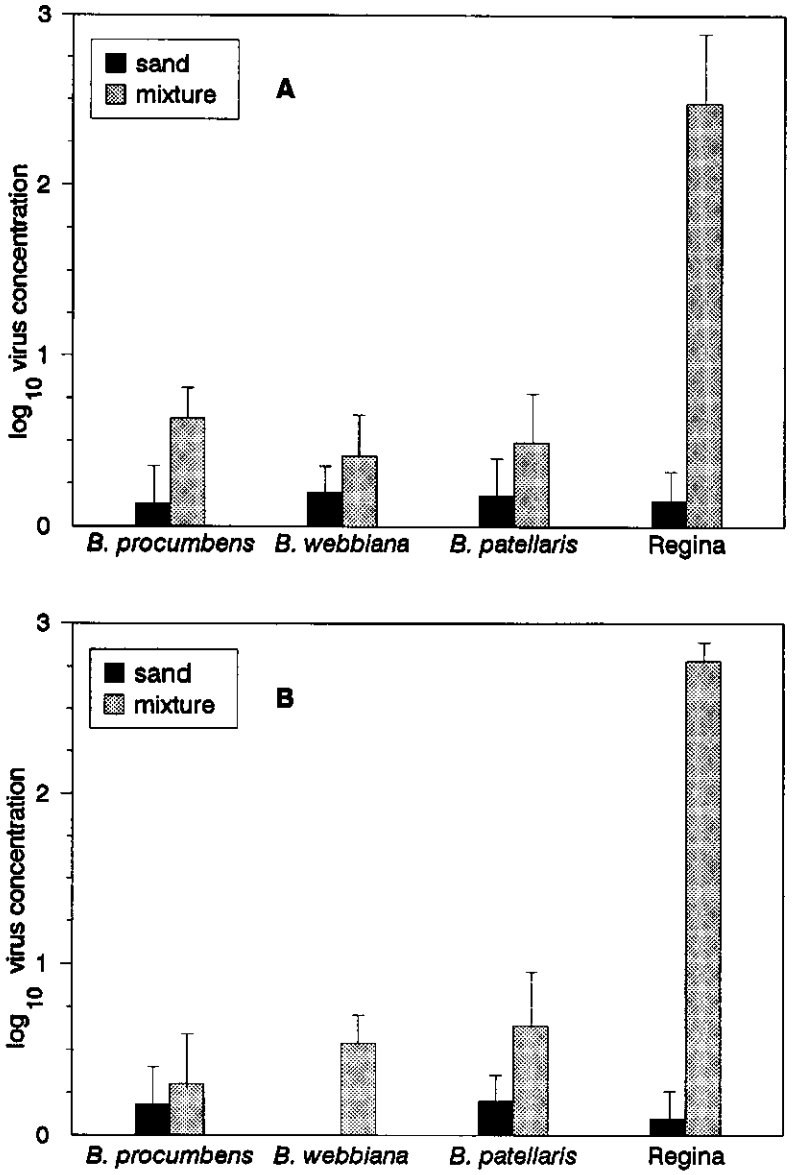

Figure 4.1. Responses in virus assays (average virus concentrations and 95%-confidence intervals) of wild *Procumbentes* species and *B. vulgaris* cultivar Regina, when grown in sand and in a mixture of sand and infested soil, for a period of 34 **(A)** and 45 **(B)** days (means of six plants; original data in ng ml^{-1}).

Table 4.1. Evaluation of resistance to *P. betae* in hybrids, *B.vulgaris* selfings, and wild *Procumbentes* species, using viruliferous *P. betae* (entries are $\log_{10}$ of virus concentrations, presented as means with 95%-confidence intervals; original data in ng ml^{-1}).

Accessions	Hybrids			*B. vulgaris* selfings		
	Number of plants	*P. betae*[1]	Virus conc.	Number of plants	*P. betae*[1]	Virus conc.
Cross: *B. vulgaris* (4x) x *B. procumbens* (2x)						
B. vulgaris parent:						
cv. Civarres	10	-	0.66 ± 0.38	17	+	2.73 ± 0.10
cv. Rozekraag Beroka	4	-	0.75 ± 0.39	7	+	2.73 ± 0.15
subsp. maritima Gr5622	2	-	1.03 ± 1.01	4	+	2.87 ± 0.36
Cross: *B. vulgaris* (4x) x *B. patellaris* (4x)						
B. vulgaris parent						
subsp. maritima Gr5622	13	-	0.35 ± 0.16	24	+	2.72 ± 0.14
VD	8	-	0.44 ± 0.17	22	+	2.81 ± 0.07

	Wild species and control		
	Number of plants	*P. betae*[1]	Virus conc.
B .procumbens	8	-	0.68 ± 0.25
B. webbiana	8	-	0.73 ± 0.17
B. patellaris	8	-	0.52 ± 0.09
B. vulgaris cv. Regina	8	+	2.82 ± 0.12

[1] - = no cystosori observed; + = cystosori present

In the experiment in which hybrids of *B. vulgaris* with *B. procumbens* or *B. patellaris* were compared with *B. vulgaris* plants from the same population, and with the three wild species and 'Regina', no cystosori of *P. betae* were detected in the wild species. Cystosori could also not be found in the hybrids of *B. vulgaris* with *B. procumbens* or *B. patellaris*. Cystosori were abundantly present in the *B. vulgaris* plants and in 'Regina' (Table 4.1). Responses in the virus assays of the hybrids and the wild species were low. In approximately half the number of samples of the hybrids and the wild species responses were above the threshold level, and it can be concluded that some virus was present in these plants. Virus concentrations were high in the corresponding *B. vulgaris* plants and in 'Regina'.

Cystosori of *P. betae* were not completely absent in any of the addition types. In plants of addition type 4, of which only two plants with a viable root system were obtained in two experiments, and in addition type 8, cystosori could only be detected in a few lateral roots (Table 4.2). In the other monosomic addition types and in the sib-plants of all nine types, cystosori of *P. betae* were abundantly present. It must be noted, that cystosori were always strongly clustered, irrespective of the number of lateral roots affected. Virus concentrations in the plants of addition types 4 and 8 were lower than in the corresponding sib-plants, but the virus could be detected in almost all addition plants. Virus concentrations in the addition plants and in the sib-plants of the other addition types were similar. All 'Regina' controls contained cystosori of *P. betae* and virus (data not included in Table 4.2).

In the experiment in which the two addition families of chromosome number 8 were tested together with *B. procumbens* and 'Regina', cystosori were not found in *B. procumbens*. As was expected from the results of the previous tests, lower numbers of cystosori were found in the plants of addition type 8 than in the corresponding sib-plants and 'Regina' (Table 4.3). The virus could be detected in almost all addition plants of type 8, but virus concentrations were lower than in the sib-plants and 'Regina'. The virus could only be detected in one plant of *B. procumbens*. The data in Table 4.3 suggest that the number of cystosori might be directly related to the virus concentration.

Table 4.2. Evaluation of resistance to *P. betae* in monosomic additions of *B. procumbens* in *B. vulgaris*, using viruliferous *P. betae* (entries are $\log_{10}$ of virus concentrations, presented as means with 95%-confidence intervals; original data in ng ml^{-1}).

Chromosome number	Accession	Additions			Sibs		
		Number of plants	*P. betae*[1]	virus conc.	Number of plants	*P. betae*[1]	virus conc.
1	AU 4-1-1	8	+	2.07 ± 0.42	8	+	2.32 ± 0.18
	D 1-2-13	8	+	2.65 ± 0.28	7	+	2.70 ± 0.17
2	D 2-2-27a	7	+	2.47 ± 0.18	8	+	2.65 ± 0.15
	J 5-1-1	7	+	2.51 ± 0.49	8	+	2.44 ± 0.30
3	D 3-2-17a	8	+	2.07 ± 0.66	8	+	2.31 ± 0.22
	AI 2-2-61	8	+	2.85 ± 0.21	8	+	2.37 ± 0.50
4	I 3-2-1c	1	(+)	1.56	8	+	2.55 ± 0.14
	I 3-2-1c	1	(+)	0.48	8	+	2.71 ± 0.30
5	I 3-2-24a	8	+	2.73 ± 0.22	8	+	2.66 ± 0.33
	AU 6-1-5c	8	+	2.77 ± 0.10	8	+	2.82 ± 0.17
6	D 3-2-35a	7	+	2.89 ± 0.21	7	+	2.82 ± 0.12
	K 3-1-17b	7	+	2.46 ± 0.31	8	+	2.10 ± 0.23
7	K 3-1-1	8	+	2.38 ± 0.29	8	+	2.24 ± 0.48
	A 6-2-6	7	+	2.00 ± 0.35	8	+	2.23 ± 0.22
8	D 3-2-13a	8	(+)	1.25 ± 0.41	8	+	2.59 ± 0.29
	D 3-2-13b	8	(+)	1.60 ± 0.56	8	+	2.54 ± 0.38
9	C 6-1-3a	8	+	2.39 ± 0.32	8	+	2.58 ± 0.37
	J 4-1-1	6	+	2.50 ± 0.21	8	+	1.85 ± 0.65

[1] + = cystosori present; (+) = cystosori only present in a few lateral roots

Using the means of the data of the different accessions, a significant correlation ($r=0.91$; $P<0.05$) was found between numbers of cystosori and virus concentrations. Correlations within accessions were not significant.

Table 4.3. Average numbers of cystosori of *P. betae* and virus concentrations, with 95%-confidence intervals, in monosomic additions type 8, compared with sib-plants, *B. procumbens* and 'Regina' (entries are $\log_{10}$ of the data; original data in cystosori mg^{-1} root and ng ml^{-1}).

Accession	Number of plants	Number of cystosori	Virus concentration
B. procumbens	10	0.00	0.06 ± 0.14
D 3-2-13a (additions)	10	0.94 ± 0.38	1.91 ± 0.48
D 3-2-13b (additions)	10	0.27 ± 0.26	1.32 ± 0.58
D 3-2-13a (sibs)	9	2.35 ± 0.11	2.56 ± 0.19
D 3-2-13b (sibs)	10	2.07 ± 0.34	2.44 ± 0.19
B. vulgaris cv. Regina	10	2.34 ± 0.14	2.44 ± 0.22

Discussion

Cystosori of *P. betae* could not be detected in the roots of the wild *Beta* species of the section *Procumbentes*. These results correspond with the findings of Fujisawa and Sugimoto (1979) and Asher and Barr (1990). Abe and Ui (1986), investigating the host range of *P. betae* with different infested soils, observed traces of cystosori in *B. procumbens* once. Dahm (University of Hannover, personal communication) found zoosporangia of *P. betae* in *B. procumbens* and occasionally also in *B. patellaris*, but no cystosori. Asher and Barr (1990) were not able to detect early infection structures in *B. patellaris*.

Results for the hybrids of *B. vulgaris* with *B. procumbens* or *B. patellaris* were similar to those for the wild species. Cystosori of *P. betae* could not be found in the

roots of the hybrids, showing that resistance to *P. betae* was dominant when combined with the genome of *B. vulgaris*. Results of the experiments with the addition types indicated that resistance to *P. betae* in *B. procumbens* is located on chromosomes 4 and 8. In contrast to the wild species and the hybrids, cystosori were not completely absent from these addition types. Either the genes on the two chromosomes are complementary, or other genes in the wild species have an effect on the level of resistance.

Virus could be detected in several samples of the wild species that had been grown in a mixture of sand and infested soil, but not in samples of plants that had been grown in sand. Transmission of the virus does not occur without the help of a vector (Ivanović, 1985) and the virus is thought to be located within the spores (Abe and Tamada, 1986). Therefore, the presence of some virus in the wild plants indicates that *P. betae* had penetrated the roots and had transmitted the virus. De Heij (IPO-DLO, Wageningen, personal communication) could also detect BNYVV in seedlings of *Procumbentes* species. The presence of some virus in the plants of the wild species supports the assumption based on results of mechanical inoculation (Fujisawa and Sugimoto, 1979), that these species are susceptible to the virus. Mechanical inoculation of the roots with BNYVV, as developed by Koenig and Stein (1990), should provide a further proof for this assumption.

Virus transmission in the presence of vector resistance has also been reported in peanut. Thouvenel and Fauquet (1981) could not find cystosori of *P. graminis* in the roots of peanut plants, though plants became infected with peanut clump virus. In later studies, Ratna et al. (1991) found small amounts of resting spores in young peanut plants. Mowat (1970) found that *Olpidium brassicae* transmitted tobacco necrosis virus to tulip, but no resting spores were observed in the roots. However, some zoospores were found in root washings.

Virus concentrations were low in the hybrids, as was found before (Paul et al., 1991). Resistance to *P. betae* in addition types 4 and 8 coincided with reduction in the levels of BNYVV as compared to the corresponding sib-plants. In the experiment, in which cystosori were counted, a positive correlation ($r=0.91$; $P<0.05$) was found between average numbers of cystosori and average virus concentrations, indicating that resistance to *P. betae* gave protection against infection with BNYVV,

also when resistance was not complete, as was the case in the addition families. Such a correlation was not found within accessions, although variation for both parameters was present. The absence of a correlation within accessions could be due to the uneven distribution of the cystosori in the roots and the poor transport of the virus (Hillmann, 1984), so that infection would remain localised, even if high numbers of cystosori were found at infection sites. The fact that not all *P. betae* in soil is infested with BNYVV (Beemster and De Heij, 1987) and some *P. betae* at infection sites do not carry virus, may further reduce the correlation. The virus can also be transmitted, while no cystosori develop, as was seen for the wild species and the hybrids.

Resistance to *P. betae* from *Procumbentes* probably is simply inherited, which might endanger its durability. The main problem in exploiting the resistance to the vector will be the transfer of resistance from *Procumbentes* species to the cultivated beet. Partial resistance to *P. betae* is found in *B. vulgaris* subsp. *maritima* (L.) Arcang. (Fujisawa and Sugimoto, 1979; Asher and Barr, 1990), which can easily be crossed with *B. vulgaris*. It has been assumed that this partial resistance was quantitatively inherited. Although a more complex inheritance of resistance might be more durable, a quantitatively inherited resistance is difficult to handle in breeding programmes. It is not yet known whether such a partial resistance would provide useful protection against rhizomania.

In the present study, resistance to *P. betae* in *B. procumbens* was located at chromosome level and was associated with a reduction in the level of BNYVV. Resistance to *P. betae* was investigated by looking for the presence of cystosori and by counting cystosori after homogenising the roots. Detailed observations on the presence of *P. betae* and its distribution in the roots might provide further insight in the relationship between resistance to the vector and its effect on infection of BNYVV. All *Procumbentes* species and hybrids reacted similarly in tests for resistance to *P. betae*. Reamon-Ramos and Wricke (1992) identified the nine different addition types of *B. webbiana*. It would be interesting to know whether resistance to *P. betae* in *B. webbiana*, which might be the same species as *B. procumbens* (Wagner et al., 1989), can be found on the same chromosomes as in *B. procumbens*.

Chapter 5

Variation in the level of infection with *Polymyxa betae* and its effect on infection with beet necrotic yellow vein virus in beet accessions of the sections *Beta* and *Corollinae*

Abstract

High levels of resistance to *Polymyxa betae* were not found after screening of accessions of *Beta vulgaris* and its subspecies *maritima* of the section *Beta*. Resistance to beet necrotic yellow vein virus (BNYVV) could be demonstrated in a few accessions. Large variation in the level of infection by *P. betae* and BNYVV was found in accessions of the section *Corollinae*. Plants without resting spores (cystosori) of *P. betae* were found in *B. corolliflora*, *B. macrorhiza* and *B. lomatogona*, whereas plants without virus were found in *B. corolliflora*, *B. intermedia*, *B. lomatogona* and in some hybrids between *B. vulgaris* and *B. lomatogona*. There was only little evidence that resistance to *P. betae* had an effect on the concentration of BNYVV. Low numbers or even the absence of cystosori of *P. betae* often did not result in low virus concentrations. In screening sugar beets for resistance to rhizomania with virus concentration as the selection parameter, no interfering effect of resistance to *P. betae* is expected. Perspectives of the use of resistance to *P. betae* in breeding for resistance to rhizomania seem to be limited.

Introduction

The concentration of beet necrotic yellow vein virus (BNYVV) in the roots of sugar beet plants is used as a selection criterium in greenhouse tests for resistance to rhizomania. It is generally assumed, that low virus concentrations in the resistant sugar beet selections and cultivars are caused by resistance to BNYVV and not by resistance to the vector *Polymyxa betae* Keskin. Some variation in infection by *P. betae* in beet accessions was found by Habibi (1969). *Beta vulgaris* L. cultivars and breeding material (Bolz and Koch, 1983) and a number of cultivars and accessions with various levels of resistance to rhizomania (Asher and Barr, 1990) proved to be susceptible to *P. betae*, although some variation was found. In *B. vulgaris* subsp. *maritima* (L.) Arcang. resistance to both BNYVV (Whitney, 1989) and *P. betae* (Fujisawa and Sugimoto, 1979; Asher and Barr, 1990) was reported. Resistance to *P. betae* in *Beta* species of the sections *Procumbentes* and *Corollinae* was demonstrated too (Fujisawa and Sugimoto, 1979).

Using *B. procumbens* Chr. Sm. and monosomic chromosome additions of *B. procumbens* in *B. vulgaris* with high levels of resistance to *P. betae*, a significant correlation was found between the average numbers of cystosori in the roots and the average virus concentrations (Chapter 4). Resistance to *P. betae* could have an limiting effect on the infection by BNYVV in the roots and thus interfere with the interpretation of screening results with low virus concentration as selection criterium for resistance.

In this chapter, the relationship between the level of infection with *P. betae* and the level of infection with BNYVV is further investigated, using beet accessions from the sections *Beta* and *Corollinae*. The results may provide further information on the reliability of virus concentration as the selection criterium in screening experiments and on the perspectives of the use of resistance to *P. betae* in breeding for resistance to rhizomania.

Materials and Methods

Plant material

Experiment 1. Plant material consisted of the susceptible *B. vulgaris* cultivar Regina, the cultivar Rima with partial resistance to BNYVV, and 18 accessions of *B. vulgaris* subsp. *vulgaris* and *B. vulgaris* subsp. *maritima* from the collection at CPRO-DLO. Holly-1-4 is a homozygous BNYVV resistant selection from the Holly material (Lewellen et al., 1987). WB42 and WB52 have dominantly, simply inherited resistance to BNYVV (Whitney, 1989). Per accession 16 plants were tested.

Experiment 2. The 21 accessions of the section *Corollinae*, that were included in this experiment, represented both the species and the geographical distribution of the section. Dr Lothar Frese from the German-Dutch Beta Genebank is acknowledged for providing most of the seed samples of the *Corollinae* species. Some interspecific hybrids of *B. vulgaris* with *B. lomatogona* Fisch. and Mey. or *B. intermedia* Bunge from the CPRO-DLO collection were also included in this experiment. Hybrids were produced by Cleij et al. (1968, 1976). In the description of the hybrids, V,I and L stand for the genomes of *B. vulgaris*, *B. intermedia* and *B. lomatogona*, respectively. Cultivar Regina was included in this experiment twice. If available, 6 plants per accession were tested.

The seed coat of the seeds of the *Corollinae* species was removed mechanically before sowing. Thiram (TMTD, Luxan) was used for disinfection of the seeds. Seedlings of the first experiment were transplanted for testing directly after emergence. Seedlings of the second experiment were transplanted to peat soil first and retransplanted for testing approximately four weeks later.

The level of infection with *P. betae* and BNYVV

Seedlings were inoculated in a mixture of sand and soil containing *P. betae* infested with BNYVV, as described in Chapter 2. In the first experiment, half the number of plants were sampled after four weeks and the other half after five weeks. Plants of

the second experiment were sampled after four weeks. Sand and soil was removed from the roots by washing. Roots were homogenised in phosphate buffered saline (PBS) in a ratio 1:20 (w v^{-1}), using a Polytron mixer. Resting spores (cystosori) were counted to estimate the level of infection with *P. betae* and ELISA was applied to determine the concentration of BNYVV in the roots. Preparation of samples and counting of spores was done as described in Chapter 4, whereas ELISA was applied as described in Chapter 2. Data are presented as $\log_{10}$ of the number of cystosori mg^{-1} of root and $\log_{10}$ of the virus concentration in ng ml^{-1}. Zero values were read as one.

Results

Experiment 1. Three plants of accession BMA-P9-983 had a dwarf-like appearance and were omitted from the analysis. Accessions were arranged per subspecies and according to increasing numbers of cystosori in the roots after four weeks. Significant differences in the average numbers of cystosori and average virus concentrations between accessions were found at both sampling times ($P<0.05$; Table 5.1), but differences between numbers of cystosori were small. After five weeks, the average numbers of cystosori were significantly higher than after four weeks, but no differences in virus concentrations were found between the two sampling times ($P<0.05$). Interactions between sampling times and accessions were not significant ($P<0.05$).

Variation for the number of cystosori was found within accessions, but none of the plants was free of cystosori and most plants were highly infected with *P. betae*. The lowest number of cystosori was found in the accession BM-8-80-209. More than one plant with a virus concentration below the detection level (4 ng ml^{-1}) was found for 'Rima', Holly-1-4, WB42 and WB52. In one plant of BMA-P9-983 no virus was detected.

Investigations into the effect of variation in the level of infection with *P. betae* on the infection by BNYVV were difficult, because of the presence of resistance to BNYVV in some accessions. Therefore, no correlation of the average number of cystosori with average virus concentrations over accessions was calculated.

Table 5.1. Average numbers of cystosori of *P. betae* and virus concentrations, with 95% confidence intervals, in accessions of the section *Beta*, after a test period of four and five weeks (entries are $\log_{10}$ of the data; original data in cystosori mg^{-1} root and ng ml^{-1})(n=8).

Accession	Four weeks		Five weeks	
	Number of cystosori	Virus concentration	Number of cystosori	Virus concentration
B. vulgaris subsp. *vulgaris*				
MS-2	2.17 ± 0.19	2.62 ± 0.18	2.54 ± 0.13	2.52 ± 0.25
Regina	2.25 ± 0.33	2.49 ± 0.22	2.43 ± 0.08	2.64 ± 0.25
Rima	2.30 ± 0.31	1.65 ± 0.46	2.51 ± 0.15	1.43 ± 0.73
Holly-1-4	2.34 ± 0.17	1.18 ± 0.47	2.46 ± 0.16	1.01 ± 0.49
R39-2-4	2.38 ± 0.20	2.48 ± 0.20	2.41 ± 0.16	2.57 ± 0.24
subsp. *maritima*				
BM-8-80-209	1.75 ± 0.33	2.57 ± 0.13	2.03 ± 0.38	2.68 ± 0.23
BMH-CHL1	2.04 ± 0.35	2.60 ± 0.21	2.34 ± 0.19	2.62 ± 0.18
BM-759	2.23 ± 0.20	2.69 ± 0.25	2.44 ± 0.15	2.72 ± 0.25
BMtZwin	2.25 ± 0.26	2.50 ± 0.37	2.40 ± 0.20	2.55 ± 0.36
BM-753	2.26 ± 0.14	2.25 ± 0.53	2.52 ± 0.19	2.26 ± 0.46
BM-758	2.27 ± 0.13	2.56 ± 0.55	2.44 ± 0.25	2.49 ± 0.57
WB42	2.31 ± 0.21	0.40 ± 0.28	2.65 ± 0.14	0.44 ± 0.41
BMA-P9-983	2.35 ± 0.24[1]	2.31 ± 0.51[1]	2.35 ± 0.20	1.85 ± 0.67
BMF-K4	2.35 ± 0.20	2.61 ± 0.13	2.53 ± 0.13	2.55 ± 0.15
BMIII-839-3	2.36 ± 0.14	2.57 ± 0.15	2.52 ± 0.12	2.62 ± 0.21
WB52	2.39 ± 0.14	0.33 ± 0.32	2.61 ± 0.13	0.47 ± 0.31
BMF-T8	2.40 ± 0.19	2.46 ± 0.29	2.45 ± 0.13	2.54 ± 0.13
BM-275-746	2.45 ± 0.10	2.69 ± 0.20	2.55 ± 0.10	2.79 ± 0.19
BMF-R9	2.52 ± 0.10	2.30 ± 0.41	2.59 ± 0.07	2.69 ± 0.11
BMIV-840-1	2.55 ± 0.15	2.18 ± 0.52	2.58 ± 0.08	2.47 ± 0.17

[1] n=5

Only within accessions such correlations were taken into consideration. Correlation coefficients between numbers of cystosori and virus concentrations were not significant for any of the accessions at either sampling time, nor when data of the sampling times were combined, except for BMIII-839-3. For this accession, a significant negative correlation coefficient was found after four weeks ($r=-0.75$) and for the combined data ($r=-0.50$) ($P<0.05$).

Experiment 2. Accessions were arranged per species and according to increasing numbers of cystosori (Table 5.2). Significant differences in the average numbers of cystosori and average virus concentrations were found between accessions ($P<0.05$). Large variation was found within most accessions. In several accessions of various species plants were found without or with few cystosori. Some accessions of various species had plants in which low amounts of virus or no virus could be detected. The low numbers of accessions and the low numbers of plants per accession do not allow to draw pertinent conclusions on the resistance to *P. betae* and/or BNYVV in the different species. Plants without cystosori were found in the accessions *B. corolliflora* Zos. 18253, 17822 and 58248, *B. lomatogona* 61241 and *B. macrorhiza* Stev. WB65. All hybrid plants had high numbers of cystosori. Plants with virus concentrations below the detection limit (4 ng ml^{-1}) were found in the *B. corolliflora* accessions 18253 and 61227, the *B. intermedia* accessions 61218, 17913 and 17967, and the *B. lomatogona* accessions WB23, 61191 and WB5. In some plants of the hybrids VLL and VVLL no virus was detected. Resistance to BNYVV in VLL and VVLL appears to behave as a dominant trait with the genome of *B. vulgaris*.

As in Experiment 1, the presence of resistance to BNYVV made it difficult to investigate the relationship between the levels of infection with *P. betae* and with the virus. In Fig. 5.1, the data of the individual plants of four accessions are given as an illustation of the various situations that occurred. In plants of 'Regina' high numbers of cystosori and high virus concentrations were found. Plants of *B. intermedia* accession 17967 had high numbers of cystosori and low virus concentrations. Accession *B. corolliflora* 18253 had resistance to *P. betae*, but both plants with high and low virus concentrations were found. In accession

Table 5.2. Average numbers of cystosori of *P. betae* and virus concentrations, with 95% confidence intervals, in beet accessions of the section *Corolinae* and in some hybrids with *B. vulgaris*, after a test period of four weeks (entries are $\log_{10}$ of the data; original data in cystosori mg^{-1} root and ng ml^{-1}) (n=6).

Accession	Number of cystosori	Virus concentration
B. vulgaris subsp. *vulgaris*		
Regina	2.35 ± 0.20	2.42 ± 0.18
B. corolliflora		
BGRC 18253	0.44 ± 0.66	1.52 ± 0.96
BGRC 61227	1.10 ± 0.58	1.12 ± 1.12
BGRC 35314	1.32 ± 0.83	2.62 ± 0.22
BGRC 17822	1.42 ± 0.95	2.56 ± 0.29
BGRC 58248	1.43 ± 0.85	2.15 ± 0.48
Ames 4527	1.57 ± 0.86	2.05 ± 0.40
B. intermedia		
BGRC 61218	1.53 ± 0.61	2.21 ± 1.30
WB32[3]	1.82 ± 0.39	2.97 ± 0.21
BGRC 17913	1.86 ± 0.34	0.37 ± 0.33
BGRC 17990	2.28 ± 0.23	2.72 ± 0.11
BGRC 61233	2.32 ± 0.24	1.55 ± 0.90
BGRC 17967	2.46 ± 0.17	0.42 ± 0.53
B. lomatogona		
BGRC 61241[3]	1.63 ± 1.30	2.04 ± 1.40
BGRC 61238[1]	1.87 ± 1.64	2.23 ± 3.50
WB23	2.17 ± 0.89	0.67 ± 0.47
BGRC 61191[1]	2.35 ± 0.27	0.91 ± 0.94
WB5[2]	2.41 ± 0.29	0.70 ± 0.33
B. macrorhiza		
WB65	1.06 ± 1.01	2.22 ± 0.48
BGRC 18242	1.71 ± 0.87	2.17 ± 0.36
B. trigyna		
BGRC 35313	1.33 ± 0.37	2.98 ± 0.27
PI 264352	1.90 ± 0.41	2.13 ± 0.44
B. vulgaris subsp. *vulgaris*		
Regina	2.43 ± 0.20	2.74 ± 0.22
Interspecific hybrids		
VVLL	2.00 ± 0.36	1.41 ± 0.94
VL	2.15 ± 0.24	3.04 ± 0.13
VLL	2.45 ± 0.24	0.37 ± 0.50
VVVI-1[3]	1.97 ± 0.28	2.93 ± 0.19
VVVI	2.19 ± 0.34	2.84 ± 0.20

[1] n=3; [2] n=4; [3] n=5

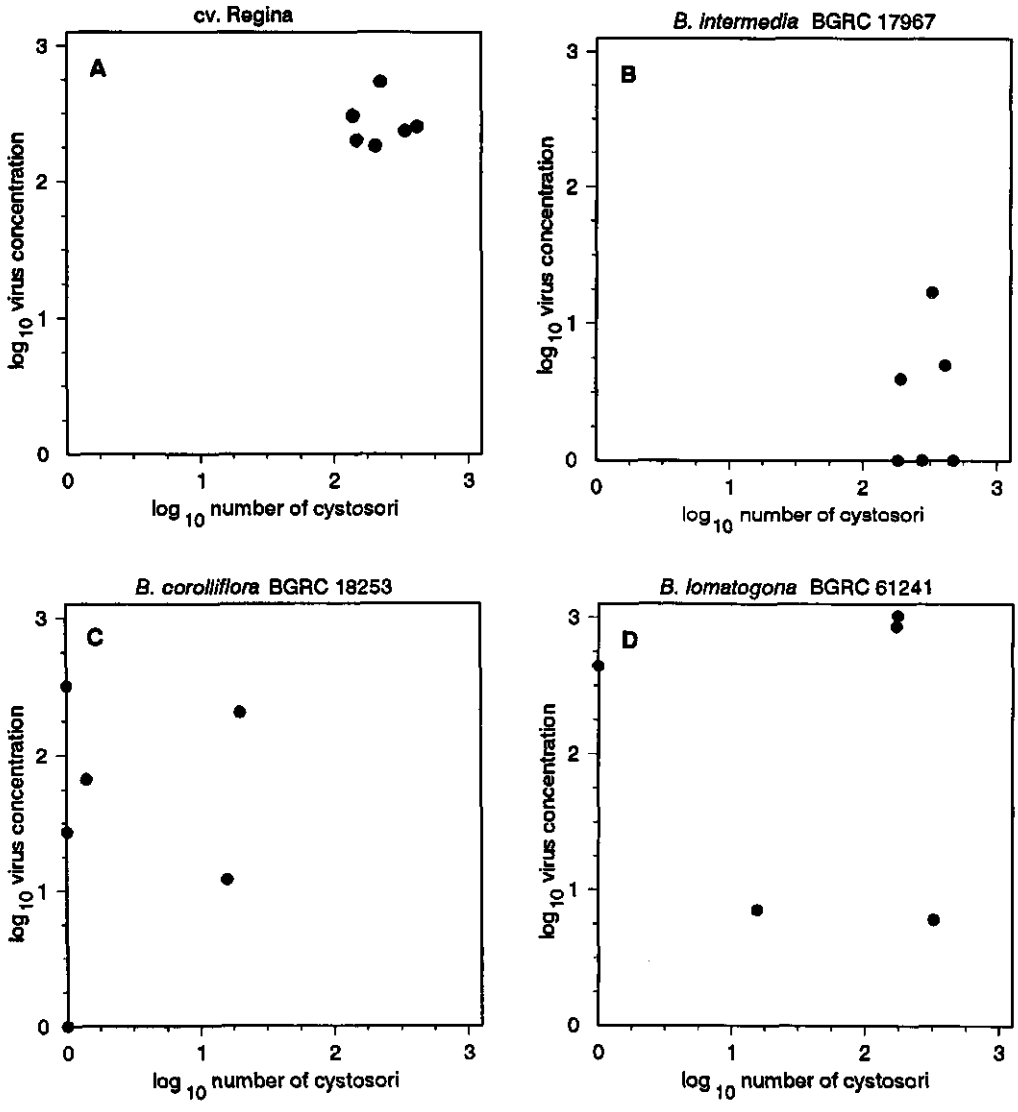

Fig. 5.1. Variation in the levels of infection by *P. betae* and BNYVV. **A.** Cultivar Regina, with high numbers of cystosori and high virus concentrations; **B.** *B. intermedia* 17967, with high numbers of cystosori and low virus concentrations; **C.** *B. corolliflora* 18253, with low numbers of cystosori and low and high virus concentrations; **D.** *B. lomatogona* 61241, with low and high numbers of cystosori and low and high virus concentrations.

B. lomatogona 61241 both plants were found with high and low numbers of cystosori and with high and low virus concentrations. Low numbers or even the absence of cystosori often did not result in low virus concentrations and the presence of resistance to BNYVV as the cause of the low virus concentrations could not be ruled out. A significant positive correlation between the number of cystosori and virus concentration was only found for *B. macrorhiza* WB65 ($r=0.85$; $P<0.05$). Since virus could be detected in all plants of this accession, probably no resistance to BNYVV was present. A significant negative correlation between the number of cystosori and the virus concentrations was found for the *B. intermedia* accession 61218 ($r=-0.80$) and the hybrid VVVI ($r=-0.95$) ($P<0.05$).

Discussion

The accessions of the section *Beta* had no high levels of resistance to *P. betae* and no effect of variation in the number of cystosori on the level of infection with BNYVV could be demonstrated. The occurrence of plants with low virus concentrations and high numbers of cystosori in the accessions 'Rima', Holly-1-4, WB42, WB52 and BMA-P9-983 show that they are resistant to BNYVV.

Resistance to *P. betae* was present in *Corollinae* species, thus confirming the results of Fujisawa and Sugimoto (1979). The results of these investigators suggested an absolute resistance to *P. betae* in these species, whereas the results of the present study, in which only a few plants without cystosori were found, indicate high levels of partial resistance. Within the *Corollinae* species resistance to BNYVV was found, which was not reported before. Fujisawa and Sugimoto (1979) found chlorotic lesions on the leaves of some *Corollinae* species after mechanical inoculation, indicating susceptibility to BNYVV. In the present study, only in a few plants no virus was detected. Therefore, resistance to BNYVV in *Corollinae* species does not seem to be complete, but seems to be high levels of partial resistance.

Resistance to *P. betae* was found in *B. corolliflora*, *B. macrorhiza* and *B. lomatogona*, while resistance to BNYVV was detected in *B. corolliflora*, *B. intermedia* and *B. lomatogona*. The numbers of accessions and the numbers of plants per accession that were tested were small, and the presence of resistance to

P. betae and BNYVV in other species should not be ruled out. Fugisawa and Sugimoto (1979) could not find *P. betae* in *B. corolliflora*, *B. intermedia* and *B. trygina* Wald. and Kit.

Only for one accession a positive correlation was found between the numbers of cystosori and virus concentrations. Low virus concentrations could be caused by resistance to BNYVV, however, since plants with low numbers or even without cystosori were found with high virus concentrations, it should be concluded that there is only little evidence for an effect of resistance to *P. betae* on the level of infection with BNYVV. Such an effect has been reported from a study with chromosome addition plants of *B. procumbens* in *B. vulgaris*, in which average numbers of cystosori were significantly correlated with average virus concentrations, though correlations within accessions were not significant (Chapter 4). The *Procumbentes* species and their hybrids with *B. vulgaris* had no cystosori and virus concentrations were low or the virus was absent, while the results reported here showed that several *Corollinae* accessions had plants without cystosori, but with high virus concentrations. It remains to be investigated whether different mechanisms of resistance to *P. betae* are involved in the different *Beta* sections. Also the effect of the mechanisms on the transmission of BNYVV has to be studied in more detail. Dahm (1993) reported that zoospores of *P. betae* encysted less frequently on the roots of *B. patellaris* than on the roots of susceptible sugar beet. It will be interesting to conduct a similar study with the *Corollinae* species.

The negative correlations between the numbers of cystosori and the virus concentrations that were found in three accessions in both experiments might be explained as a negative effect of the virus on the development of *P. betae*, as was reported by Schlösser (1990).

Since resistance to *P. betae* is rare in accessions of the section *Beta*, and small differences in susceptibility do not have any effect on the infection with BNYVV, these phenomena do not interfere with the use of low virus concentration as the selection criterium in tests with sugar beets for resistance to BNYVV.

Corollinae and *Procumbentes* species have high levels of resistance to *P. betae*, but the use of these species in breeding programmes is hampered by crossing barriers (Van Geyt et al., 1990), while also the effect of the resistance on the

infection by BNYVV appears to be limited. It is expected that quantitatively inherited resistances to *P. betae*, as found in *B. vulgaris* subsp. *maritima* (Asher and Barr, 1990) have only little effect on the infection with BNYVV. Cultivars with resistance to rhizomania could be obtained using resistance to BNYVV, without resistance to the vector. However, resistance to *P. betae* could help to reduce the inoculum level in the soil and retard the spread of the disease.

Chapter 6

Use of zoospores of *Polymyxa betae* in screening beet seedlings for resistance to beet necrotic yellow vein virus

Abstract

A system to culture viruliferous *Polymyxa betae* and to produce zoospores is described. The zoospores were used for inoculation of beet seedlings, grown in nutrient solution, in tests for resistance to beet necrotic yellow vein virus (BNYVV). On most occasions in a time course experiment, and with various zoospore cultures, the partially resistant cultivar Rima and accession Holly-1-4 had virus concentrations similar to the susceptible cultivar Regina, but virus concentration in *Beta vulgaris* subsp. *maritima* accession WB42 was significantly lower ($P<0.05$). 'Regina' could be distinguished from various resistant accessions by a significantly higher virus concentration ($P<0.05$) shortly after inoculation, or after transplanting the seedlings from the nutrient solution into sand. Results of screening for resistance to BNYVV, using zoospores for inoculation, did not correspond with results of a test in which infested soil was used. Tests in which seedlings are grown in nutrient solution and inoculated with zoospores are suitable for the detection of accessions with a high level of resistance to BNYVV. To obtain virus infection in all plants, the optimal density of the zoospore suspension should first be determined and plants should be assayed shortly after inoculation.

Introduction

The life cycle of *Polymyxa betae*, a soil-borne fungus belonging to the *Plasmodiophoraceae*, was described by Keskin et al. (1962) and Keskin (1964). Resting spores release zoospores, which penetrate into the root system of the host plant. After infection plasmodia are formed, that develop either into zoosporangia, from which new zoospores are released, or into clusters of resting spores (cystosori). *P. betae* is the vector of beet necrotic yellow vein virus (BNYVV), which causes rhizomania in sugar beet (Tamada, 1975). Both resting spores (Abe and Tamada, 1986; Fujisawa and Sugimoto, 1976) and zoospores (Abe and Tamada, 1986; Giunchedi and Langenberg, 1982; Ivanović, 1985) of *P. betae* have been used in transmission experiments with BNYVV to demonstrate the role of the fungus as a vector of the virus.

The use of viruliferous zoospores in stead of soil in seedling tests for resistance to BNYVV could improve the quantification of the inoculum and the standardisation of the inoculation. Rössner and Grösz (1987) found variable results in screening for resistance to BNYVV with zoospore suspensions, and concluded that the experimental conditions had to be standardised. After inoculation of seedlings, grown in sand, with a viruliferous zoospore suspension, differences in virus concentrations between susceptible and resistant cultivars could be found, but when seedlings were grown in nutrient solution, differences were not so clear (Dahm, 1993). Peters and Godfrey-Veltman (1990), who also used inoculated seedlings in nutrient solution with a zoospore suspension, only found a difference between resistant and susceptible accessions a few days after inoculation.

Several systems have been described to maintain cultures of viruliferous *P. betae* and to produce zoospores. Dahm (1993), Dahm and Buchenauer (1993), Peters and Godfrey-Veltman (1989) and Rössner and Grösz (1987) raised infected beet seedlings in a nutrient solution. Because of the continuous release of zoospores, these solutions can directly be used for the inoculation of test plants. In other systems, infected plants were grown in sand and watered with a nutrient solution. Zoospores of *P. betae* were obtained by collecting drench water (Abe and Tamada, 1987). After washing away the sand from the roots, zoospores were collected with

a micromanipulator immediately after their release from the roots (Giunchedi and Langenberg, 1982). Ivanović (1985) placed roots in water, in which the zoospores were released. Adams et al. (1986) described a similar system for the production of zoospores of viruliferous *P. graminis* in studies on the resistance to the fungus and to barley yellow mosaic virus (BaYMV) in barley. Infected plants were maintained in sand and zoospores were obtained after the roots were placed in a diluted nutrient solution.

In the present chapter, a system to start and maintain cultures of viruliferous *P. betae* and to produce zoospores is described. The system combines growing of plants in a nutrient solution and in sand. Zoospores were used to screen beet seedlings for resistance to BNYVV, and testing methods were compared.

Materials and methods

Zoospore production

Plant material consisted of seedlings of the susceptible sugar beet (*Beta vulgaris* L.) cultivar Regina. Seeds were disinfected in water at 55-60 °C for 15 min and sown in heat-sterilised sand. Seedlings were grown in the greenhouse in a mixture of sand and soil, containing *P. betae* with BNYVV (Chapter 2). Approximately five weeks later, after washing away the sand and the soil, roots were homogenised in water, using a Waring blender. In Fig. 6.1 the system to cultivate *P. betae* and to produce zoospores is shown. The cultures were started with single cystosori (1) (Dahm and Buchenauer, 1993; Keskin, 1964). Cystosori were isolated with a micromanipulator under an inverted microscope (Zeiss ID02) and transferred into a 4.5 ml polyethylene tube, filled with a 0.1-1.0 strength Steiner nutrient solution (Steiner, 1984). One seedling was placed in each tube, containing one cystosorus (2). After three to four weeks, seedlings were checked for the presence of *P. betae* under an inverted microscope. Infected plants were transferred to Erlenmeyer flasks, containing 150 ml 0.5 strength Steiner nutrient solution, together with a few young, uninfected seedlings (3). Twice a month, half the number of plants was replaced by new seedlings. Once a month, the nutrient solution was renewed. The roots were regularly assayed by ELISA for the presence of BNYVV.

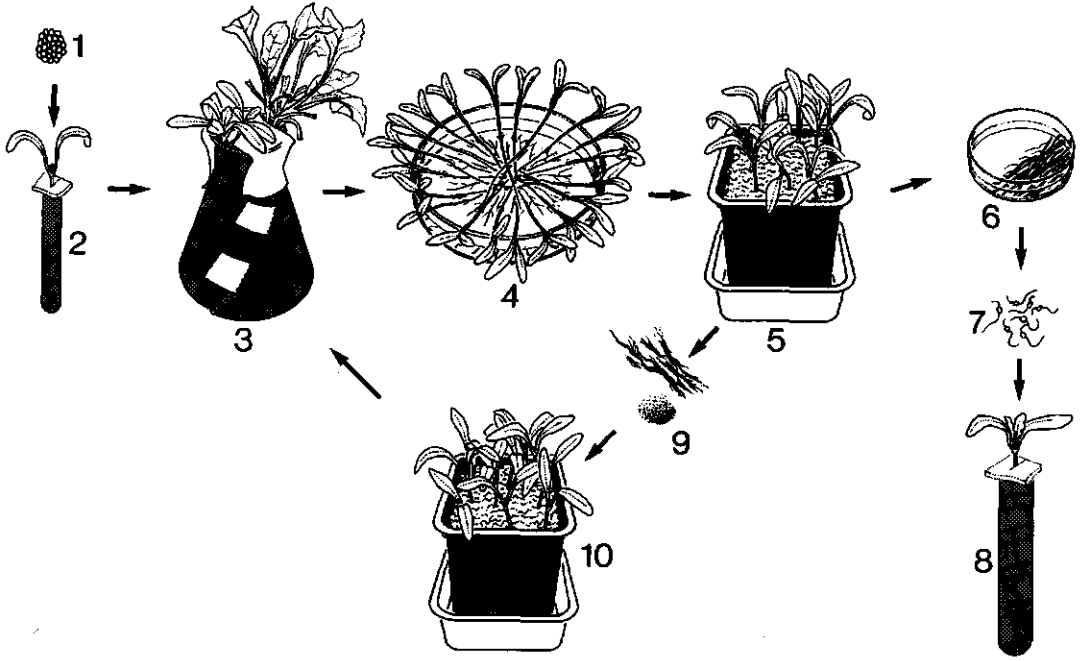

Fig. 6.1. Schematic presentation of the system to start, maintain and store cultures of *P. betae*, and to produce zoospores. For explanation, see text.

Cultures of *P. betae* with and without BNYVV were produced. Two weeks before zoospores were needed for inoculation experiments, 10-30 uninfected seedlings were incubated with 50 ml of the culture suspension in a Petri dish for 4 h in the dark (4). After incubation, groups of 10 seedlings were transplanted into 150 ml pots filled with sand, and regularly watered with 0.5 strength Steiner nutrient solution (5). After one and a half week the pots were left to dry out. Approximately three days later, when zoospores were needed, the sand was washed away and the roots were placed into a small Petri dish, containing a few ml 0.5 strength Steiner nutrient solution (6). The Petri dish was placed in the dark. After 45 min the density of zoospores was determined under a phase contrast microscope, using a hemocytometer (7). The zoospores could be used directly for inoculation of the test plants (8) (see below). Zoospore production varied from 10^6-10^7 per g roots, with root weights of 0.2-0.4 g per pot (fresh weight). The roots, from which the zoospores had been released, were assayed for BNYVV by ELISA. For storage of the infected material, infected seedlings were grown in sand for at least four weeks (5), the roots were then air-dried and stored at 6 °C. To restart the zoospore production, the dried roots were cut into small pieces, and the roots of uninfected seedlings were dipped in the root fragments (Adams et al., 1988) (9). The inoculated seedlings were transplanted into sand (10) and two weeks later transferred to Erlenmeyer flasks (3). Tubes and Erlenmeyer flasks in which the seedlings were grown were painted black or wrapped in black plastic to prevent the growth of algae. The whole zoospore production system was carried out in a climate-controlled room at 25 °C.

Inoculation experiments

Seeds of test plants were disinfected with thiram (TMTD, Luxan) and sown in heat-sterilised sand. After emergence, seedlings were washed in tap water and placed in small tubes containing 13 ml 0.5 strength Steiner nutrient solution. In each tube one seedling was placed. After 6-7 days, when new lateral roots had been formed, 1 ml of a zoospore suspension was added to each tube. Twice a week tubes were watered, using alternately demineralised water and 0.5 strength Steiner nutrient

solution. Cotyledons were trimmed to reduce evaporation. Test plants were grown and incubated in a climate-controlled greenhouse at 22/17 °C (day/night).

Experiment 1. The sugar beet cultivar Regina, which is susceptible to BNYVV, the partially resistant cultivar Rima, the accession Holly-1-4, which is a homozygous resistant selection from the Holly material (Lewellen et al., 1987) and *B. vulgaris* subsp. *maritima* (L.) Arcang. WB42 were tested for resistance to BNYVV. The last two accessions carry a monogenic resistance to BNYVV (Lewellen et al., 1987; Whitney, 1989). All four accessions are susceptible to *P. betae*. Individual seedlings were inoculated with 200 or 2000 zoospores of the viruliferous isolate 91-6 of *P. betae*. Per zoospore density 32 seedlings of each accession were used. Plants were tested in a completely randomised design. After 3, 7, 10 and 14 days, eight plants per accession and per zoospore density were taken from the tubes and assayed for BNYVV in ELISA. The plants assayed at day 14 were checked for the presence of *P. betae*.

Experiment 2. The same accessions as in experiment 1 were used. Three different isolates of *P. betae*, that were found to be infested with BNYVV, named 91-1, 91-3 and 91-6, were used. All isolates originated from plants grown in the same soil. For each isolate, eight plants per accession were grown in nutrient solution and inoculated with 2000 zoospores per plant. Plants were randomised per isolate and assayed after 11 days. All plants were checked for the presence of *P. betae*.

Experiment 3. Twenty-four seedlings of 'Regina', and of 'Rima', 'R39', 'D881', 'Roxane', Holly-1-4 and WB42, with various levels of resistance to BNYVV, were grown in nutrient solution and inoculated with 1000 spores per plant of isolate 91-6. After 4 h, eight seedlings per accession were transplanted into 240 ml plastic containers filled with sand. For each accession, eight non-transplanted plants were assayed after 4 days and the remaining non-transplanted plants, as well as the plants in sand, were assayed after 7 days. Separately, 24 uninfected seedlings of each accession were transplanted into 240 ml plastic containers filled with a mixture

of sand and infested soil in a ratio of 9:1 (v v^{-1}) and assayed for BNYVV after 34 days (Chapter 2). All plants were checked for the presence of *P. betae*.

Virus assays

The complete root systems of the test plants were dried with tissue paper, weighed and crushed in phosphate buffered saline with 0.05% Tween (PBS-Tween) in a ratio 1:20 (w v^{-1}). Virus concentrations in the roots were estimated with ELISA (Clark and Adams, 1977), as described by Alderlieste and Van Eeuwijk (1992) and in Chapter 2, using incubation times and temperatures as described by Büttner and Bürcky (1987). Samples with estimated virus concentrations below 4 ng ml^{-1} were considered to be free of virus. In analyses of variance (ANOVA), the log_{10} of the virus concentrations was taken. Zero values were read as 1 ng ml^{-1}. Pairwise comparison of the data were made using least significant differences (LSD) between cultivars. In two-factorial experiments the interaction standard error of difference was taken for computing the LSD.

Results

Experiment 1. Average concentrations of BNYVV in 'Regina', 'Rima', Holly-1-4 and WB42 after inoculation with 200 and 2000 zoospores, are presented in Fig. 6.2. After inoculation with 200 zoospores, plants with virus concentrations below the threshold level of 4 ng ml^{-1} were found in all accessions and at all sampling times. The highest numbers of such plants were found in WB42 and at early sampling times. After inoculation with 2000 zoospores, virus concentrations below the threshold level were only found in WB42. The occurrence of plants with virus concentrations below the threshold level indicated, that not all plants were infected with BNYVV. Especially at early sampling times, it was not possible to discriminate between plants which escaped from virus infection and plants that were infected, but of which the virus concentration was too low to be detected. Low virus concentrations may be due to either the short time after infection or to resistance to BNYVV, as was thought to be the case for WB42. All data were included in the analysis. Virus concentrations increased with time. In analysis of variance (ANOVA)

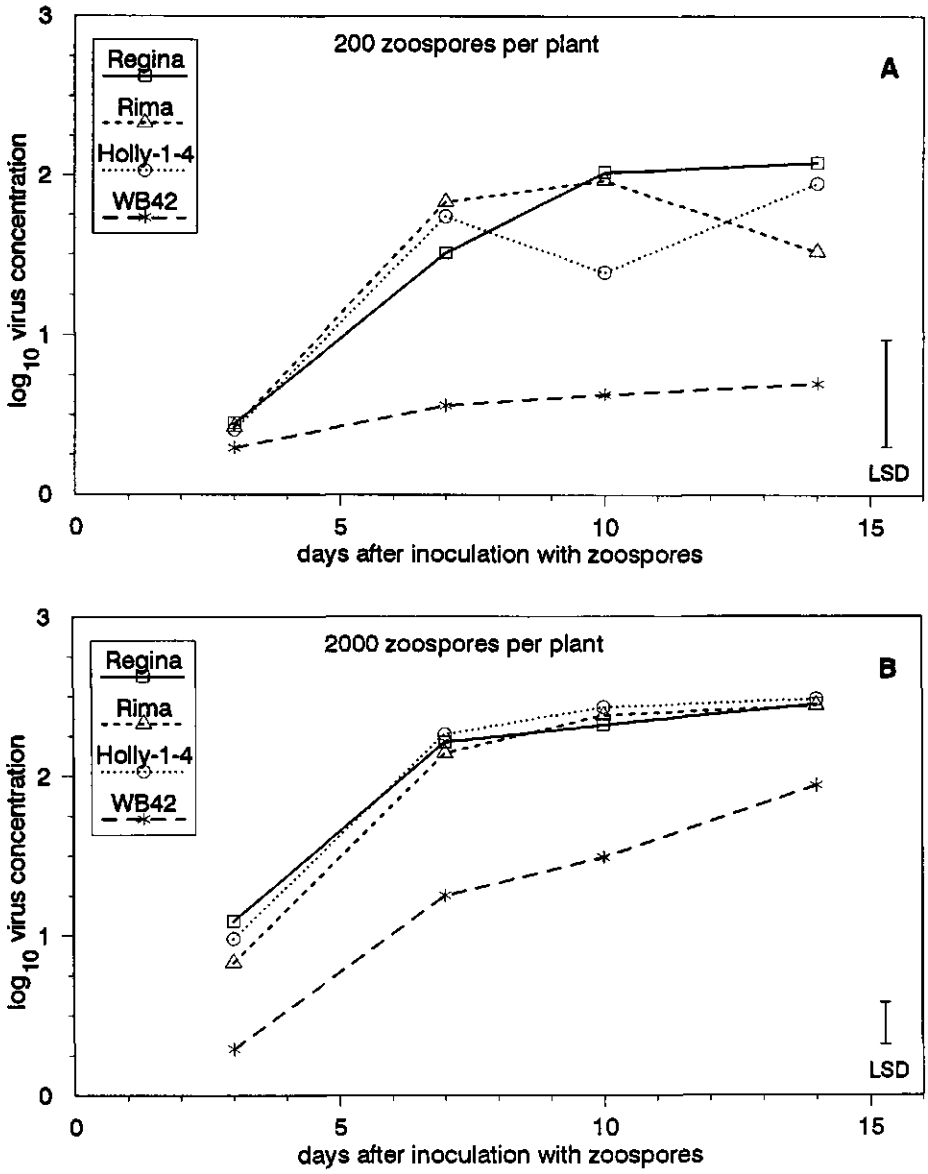

Fig. 6.2. Experiment 1. Average concentrations of BNYVV (entries are $\log_{10}$ of the virus concentrations; original data in ng ml^{-1}), with LSD (95%), in the roots of beet seedlings at four sampling times, after inoculation with 200 (A) and 2000 (B) zoospores of a viruliferous isolate of *P. betae* (n=8).

per inoculum density, differences in virus concentrations between accessions and between sampling times were highly significant at both inoculum densities ($P<0.001$), while the interaction between accessions and sampling times was not. Virus concentrations of WB42 and of the other accessions were significantly different on all occasions, except at three days after inoculation with 200 zoospores. A significant difference in virus concentration between 'Regina' and 'Rima' was only found at three days after inoculation with 2000 zoospores. On all further occasions, differences between 'Regina', 'Rima' and Holly-1-4 were not significant. At day 14, when the individual plants were checked for the presence of *P. betae*, fungal structures were detected in all plants.

Table 6.1. Experiment 2. Average concentrations of BNYVV (entries are $\log_{10}$ of the virus concentrations; original data in ng ml^{-1}), with LSD (95%), in the roots of beet seedlings, 11 days after inoculation with three viruliferous isolates of *P. betae* (2000 zoospores per isolate, n=8).

Accession	Isolate		
	91-1	91-3	91-6
Regina	2.16	1.01	2.14
Rima	2.19	0.71	2.22
Holly-1-4	2.13	0.43	2.22
WB42	0.50	0.23	1.41
LSD	0.19	0.59	0.35

Experiment 2. Average concentrations of BNYVV, obtained 11 days after inoculation with 2000 zoospores of three different viruliferous isolates, are presented in Table 6.1. Differences were found between isolates. Less than one third of the plants was infected with BNYVV after inoculation with isolate 91-3. In the roots from which the zoospores of isolate 91-3 were released, the virus concentration was just above the detection level, while in the roots, used to obtain zoospores of the isolates 91-1 and 91-6, the virus concentration was much higher. All plants contained virus after inoculation with the isolates 91-1 and 91-6, except a few WB42 plants inoculated with isolate 91-1. Results were analysed per isolate. Only differences in virus concentration between WB42 and the other accessions were significant, except for Rima and Holly-1-4 after inoculation with isolate 91-3 ($P<0.05$). *P. betae* was observed in all plants.

Experiment 3. Results of the treatments in which plants were inoculated with zoospores, and the results of the treatment in which plants were grown in a mixture of sand and infested soil were analysed separately (Table 6.2).

Average concentrations of BNYVV in the plants that were inoculated with viruliferous zoospores were low, but except for some plants of 'Roxane' and WB42, all plants had virus. Differences in virus concentrations between accessions and between treatments were highly significant, as was the interaction between treatments and accessions ($P<0.001$). The occurrence of interaction indicated that variation was found in the reaction of the accessions among treatments, however, the contribution to the variance by the term for interaction was small compared to the contributions by the main effects. After four days in nutrient solution, and when plants were transplanted to sand and assayed after seven days, all remaining accessions had lower concentrations than 'Regina' ($P<0.05$). After seven days in nutrient solution, only virus concentrations of 'Roxane' and WB42 were significantly lower than in 'Regina' ($P<0.05$). The lowest virus concentrations were found in WB42 in all treatments.

When seedlings were grown for 34 days in a mixture of sand and infested soil, virus was not detected in some plants of the accessions 'Rima', 'Roxane', Holly-1-4 and WB42. All remaining accessions had significantly lower virus concentrations

than 'Regina', except 'R39' ($P<0.05$). Again, the lowest virus concentration was found in WB42, but the concentrations in Holly-1-4 were also low. High virus concentrations were found in two plants of WB42, which indicates, that the population of this accession contains a low percentage of susceptible plants.

Linear correlation coefficients between the average virus concentrations obtained with the treatments in which plants were inoculated with zoospores, and average virus concentrations obtained after the plants had grown in infested soil, were not significant. *P. betae* was found in all plants, but less fungal structures in the transplanted plants than in the plants that had been grown in the nutrient solution.

Table 6.2. Experiment 3. Average concentrations of BNYVV (entries are $\log_{10}$ of the virus concentrations; original data on virus concentration in ng ml^{-1}), with LSD (95%), in the roots of beet seedlings, inoculated and grown under various conditions.

Accession	Zoospores (1000 per plant)			Infested soil
	4 days (n=8)	7 days (n=8)	7 days(sand) (n=8)	(n=24)
Regina	1.84	2.12	1.52	1.87
Rima	1.48	1.97	1.21	0.96
R39	1.51	2.06	1.28	1.72
D881	1.58	1.90	0.98	1.62
Roxane	1.19	1.80	0.71	1.07
Holly-1-4	1.59	2.01	1.00	0.54
WB42	0.54	0.76	0.48	0.38
LSD	0.24			0.22

Discussion

A system for culturing viruliferous *P. betae* and for the production of zoospores is described. Single cystosori were used to start the cultures as was done by Dahm (1993) and Keskin (1964). This system makes it possible to keep cultures for months and, when necessary, large quantities of zoospores can be produced. Cultures could be stopped and restarted when needed. Release of zoospores was synchronised, by placing the roots of infected seedlings in a diluted nutrient solution for a defined period of time. This system should be preferred above the use of zoospore suspensions directly taken from the nutrient cultures, because then the age of the zoospores will vary. Zoospore counts can easily be made in the concentrated zoospore suspensions, as was done in the present study. By counting the number of zoospores, the inoculum can be standardised, provided the fungus remains infested with the virus at the same level during cultivation. In all experiments described here isolate 91-6 was used, which gave constant results.

Probably only a few zoospores contained virus, as could be concluded from the occurrence of escapes from virus infection after inoculation with 200 zoospores per plant. Alternatively, multiple infection with viruliferous zoospores might be needed to get virus concentrations above the detection level. Histological studies with immunogold-silver labelling on zoospores of viruliferous *P. betae* and *P. graminis* showed, that virus could only be detected in some of the zoospores (Jianping et al., 1991; Rysanek et al., 1992). If the fraction of viruliferous zoospores is small, the virus may be lost during the production of zoospores, due to the low probability of infection. This might explain the low infectivity of isolate 91-3. Also, genetic variation for the ability of *P. betae* to acquire and to transmit the virus might cause variation between isolates (Gerik and Duffus, 1988). Rössner and Grösz (1987) suggested that the virus might be lost by applying short intervals between transfers.

In inoculation experiments with zoospores, virus concentrations in several of the resistant accessions often did not differ from the virus concentration in the susceptible cultivar Regina. Similar results were found with different isolates. After inoculation of seedlings, grown in nutrient solution, with zoospores, 'Regina' could best be distinguished from the resistant accessions shortly after inoculation. This

result corresponds with the findings of Peters and Godfrey-Veltman (1990). When seedlings were transplanted into sand, discrimination between accessions could be obtained after seven days. *P. betae* could be detected less easily in the transplanted plants. The physical barrier of the sand may prevent the zoospores from reaching all parts of the root system, and thus reducing the infection pressure on the root system, simulating the natural conditions in the field.

The accessions Holly-1-4 and WB42 had the lowest virus concentrations in the soil test. These two accessions, which carry a monogenic resistance to BNYVV, behaved differently in inoculation experiments with zoospores. WB42 had significantly lower virus concentrations than 'Regina', while Holly-1-4 did not differ from 'Regina'. Either the gene for resistance to BNYVV in WB42 is stronger than in Holly-1-4, or the presence or absence of minor genes for resistance to BNYVV plays an important role. Currently, mechanisms of resistance to BNYVV in WB42 and Holly-1-4 are being studied using the immunogold-silver labelling technique (Scholten et al., in preparation). The presence of some susceptible plants in WB42 might have contributed to the increase of the average virus concentration in time.

Correlation coefficients in Experiment 3, between the results obtained with various treatments after inoculation with zoospores and results of the soil test were not significant. Best differentiation between accessions was obtained with the use of infested soil. Results of this test are in agreement with earlier results obtained with some of these accessions, both in greenhouse and field experiments (Lewellen et al., 1987; Whitney, 1989; Chapter 3). Although after transplanting the different accessions could better be distinguished than when plants were kept in nutrient solution, differences between accessions were not so evident as when the plants were inoculated with and grown in infested soil.

Dahm (1993) added zoospores to plants, grown in sand, whereas Adams et al. (1986), in a test for resistance to *P. graminis* and BaYMV, transplanted seedlings after inoculation. Bos and Huijberts (1990), in a test for resistance to lettuce big vein virus, added zoospores of *Olpidium brassicae* directly into the pots with soil. In all situations, susceptible and resistant cultivars could be distinguished.

Zoospore suspensions can be used for inoculation in screening for resistance to BNYVV. A high level of standardisation can be obtained, as was suggested by

Rössner and Grösz (1987). Before large scale inoculation experiments with zoospore suspensions are carried out, the optimum density for inoculation should be determined in dilution experiments to ensure that all plants will be infected with virus. Furthermore, when zoospore suspensions are used to inoculate plants, the length of the test period should be carefully investigated, because only at a few days after inoculation a difference between the susceptible control and accessions with resistance to BNYVV was detected and valuable sources of partial resistance might easily be overlooked. To overcome this problem, seedlings can be transplanted into sand or zoospores can be added to plants grown in sand. However, then the test does not largely differ from the soil test (Chapter 2), which does not require the zoospore production system. Despite the poor standardisation of the inoculum and the infection process, the soil test gave the best discrimination between susceptible and resistant cultivars.

Inoculation of seedlings in nutrient solution with zoospores can be useful to detect accessions with a high level of resistance and for studies on mechanisms of resistance, in which standardisation of the infection process is important.

Chapter 7

General discussion

Screening for resistance

In breeding programmes for resistance to rhizomania, it is important to have reliable screening methods. In the present study various methods were investigated to determine resistance to rhizomania. Satisfactory results were obtained with a greenhouse test for screening sugar beet seedlings for resistance to beet necrotic yellow vein virus (BNYVV), which is described in Chapter 2. In accordance with the results of Bürcky and Büttner (1985) and Giunchedi et al., (1987), virus concentration in the roots of beet plants proved to be a suitable criterion for the evaluation of resistance to BNYVV. The use of lateral roots for sampling and the quantitiative virus measurements (Alderlieste and Van Eeuwijk, 1992) resulted in accurate assessment of the level of resistance in beet seedlings. Quantitative virus measurements are inevitable in screening seedlings for resistance to BNYVV, in contrast to screening for resistance to other viruses that are transmitted by soil-borne fungi, such as lettuce big-vein virus (Bos and Huijberts, 1990), where symptoms could be used for the evaluation of test plants, or barley yellow mosaic virus (Adams et al., 1986), for which immunity has been found. The use of absolute virus concentrations in the presentation of the results permits comparison of the results from various experiments reported in this thesis. Further standardisation of the test might be obtained by determining the level of infestation of the soil, used for inoculation, with the most probable number technique (Tuitert, 1990). Since no escapes from virus infection occurred with the method described in Chapter 2, there seems to be little need for such standardisation. The data from different fields with varying levels of infestation could successfully be analysed using multiplicative

models for cultivar by location interaction, as described in Chapter 3. These results showed, that the relative performance of cultivars on infested fields can be estimated by means of three independant parameters, (i) the level of resistance, expressed in virus concentration in the roots of plants grown in the greenhouse, (ii) the yield and quality on non-infested fields and (iii) the level of infection in the field. Results can be used to optimise the number of trial fields that is needed to evaluate cultivars with resistance to rhizomania. The explanation of cultivar by location interaction by virus concentration gave further evidence that better relative performance of the resistant cultivars on severly infested fields, compared to the susceptible cultivars, is based on virus resistance and not on tolerance (Clarke, 1986; Fraser,1990).

Investigations on the level of infection by *Polymyxa betae* Keskin in various beet material and its effect on infection with BNYVV were described in the Chapters 4 and 5. Although the presence of resistance to *P. betae* in *Beta* species has been reported (Abe and Ui, 1986; Asher and Barr, 1990; Fujisawa and Sugimoto, 1979), there is no information thus far on the quantitative relationship between *P. betae* and BNYVV. In the species of the section *Procumbentes* and their hybrids with *B. vulgaris* L., cystosori could not be detected in the plants and virus concentrations were always around the detection limit. Resistance to *P. betae* in monosomic chromosome additions of *B. procumbens* Chr. Sm. in *B. vulgaris* had some effect on the level of infection with BNYVV. Since local lesions were obtained after mechanical inoculation of the leaves (Fujisawa and Sugimoto, 1979) and some virus was found in the wild species, it was assumed that the *Procumbentes* species were susceptible to BNYVV. However, Dahm (1993) could not find BNYVV infection in *B. patellaris* after mechanical inoculation of the leaves and the roots.

In *Beta* species of the section *Corollinae*, only some plants were without cystosori. However, such plants often had high levels of BNYVV. It is suggested, that resistances to *P. betae* in the sections *Procumbentes* and *Corollinae* are based on different mechanisms.

Viruliferous zoospore suspensions of *P. betae* could be used for the inoculation of the test plants instead of infested soil (Chapter 6). A high level of standardisation could be obtained, as suggested by Rössner and Grösz (1987). Results of Peters and

Godfrey-Veltman (1990), who discriminated between accessions shortly after inoculation, could be confirmed and further quantified. With the use of zoospore suspensions valuable partial resistances might be overlooked because of the high level of the infection pressure. For routine screening programmes infested soil is still recommended, although the zoospore test could be used for the detection of accessions with high levels of resistance to BNYVV. With the use of zoospores, resistant and susceptible accessions could be distinguished a few days after inoculation. It would be interesting to perform further studies on the optimum testing period.

The results described in this thesis show that screening for resistance to *P. betae* and BNYVV could be performed adequately with the methods developed. Results of the experiments could be repeated, showing the reliability of the methods.

Future strategies in breeding resistant sugar beet

Screening techniques are important in breeding resistant crops, but most important is the plant material to start with. Within the scope of this thesis plant material from different sources was evaluated with respect to resistance to *P. betae* or BNYVV. Using the present results and those described by others, perspectives for the application of the various resistances are evaluated and a strategy is outlined for breeding sugar beets with high levels of resistance to rhizomania. The perspectives for genetically modified sugar beets, in which the viral coat protein gene is incorporated to obtain resistance to BNYVV, are not included in this review.

First, the perspectives for the exploitation of resistance to *P. betae* will be considered. The main objective for resistance to *P. betae* in sugar beet is to prevent or reduce the infection with BNYVV. Some effect of the resistance to *P. betae* on the infection with BNYVV could be found in material from *B. procumbens* of the section *Procumbentes*. The resistance in *B. procumbens* is dominant and probably simply inherited. The exploitation of the resistance from *Procumbentes* species is hampered by crossing barriers with *B. vulgaris*, as was experienced in attempts to transfer resistance to *Heterodera schachtii* Schm. (Lange et al., 1990). Resistance to *P. betae* in *Corollinae* often did not result in low virus concentrations. Nothing is

known about the inheritance of the resistance to *P. betae* in *Corollinae* species. Crossing barriers with *B. vulgaris* also exist in the *Corollinae* species (Van Geyt et al., 1990).

Asher and Barr (1990) found high levels of resistance to *P. betae* in *B. vulgaris* subsp. *maritima* (L.) Arcang., which plant material can easily be crossed with *B. vulgaris*. This resistance seemed to be polygenic, thus making its use rather difficult. Results of the present study showed that the variation in the level of resistance to *P. betae*, which was found in accessions of the section *Beta*, had no effect on the infection with BNYVV. When the resistant *maritima* material is hybridised with susceptible sugar beets, no effect of the resistance to *P. betae* on the infection with BNYVV is expected. It can be concluded, that perspectives of the use of the various resistances to *P. betae* in breeding sugar beet with resistance to rhizomania are limited.

Resistance to BNYVV gives better perspectives for the development of rhizomania resistant sugar beet cultivars. In *B. vulgaris*, both quantitatively inherited and monogenic resistances to BNYVV were found (Lewellen et al., 1987; Lewellen and Biancardi, 1990). Selection within *B. vulgaris* accessions resulted in levels of resistance to rhizomania in the cultivars as currently available. Although the partially resistant cultivars perform much better on severely infested soil than the standard susceptible cultivars, considerable losses may occur nevertheless (Chapter 3). Within the subspecies *maritima* a number of accessions were found with a proposed monogenic resistance to BNYVV (Whitney, 1989). The use of resistances with a simple, probably monogenic, inheritance from *B. vulgaris* and its subspecies *maritima*, using a backcrossing programme, appears to be the most efficient strategy, both because of the high levels of resistance to rhizomania and the ease of handling them in breeding programmes. Simply inherited resistance to BNYVV might also be present in *Corollinae* species, but the exploitation is hampered by crossing barriers.

It was suggested that genes for resistance from different accessions should be combined to obtain higher levels of resistance. A quantitatively inherited resistance might be used for increasing the level of resistance in the genetic background in case of the use of a monogenic resistance (Lewellen and Biancardi, 1990).

Combination of resistance genes that generate different mechanisms of resistance will not only increase the level of resistance, but will also have a negative effect on the selection of virus strains. Therefore, it is necessary to know whether the resistance genes in the various accessions are different and result in different mechanisms. Studies on mechanisms of resistance were performed, in which susceptible and resistant beets were compared (Giunchedi and Poggi Pollini, 1988; Scholten et al., in preparation). The use of genetic markers in sugar beet (Barzen et al., 1992; Pillen et al., 1992; Uphoff and Wricke, 1992) and allelic studies will give further information. Barzen et al. (1992) found a genetic marker which was linked to resistance to BNYVV. Histological and genetical studies, including the use of molecular markers, on a series of accessions, obtained from Dr R.T. Lewellen, with probably monogenic resistance to BNYVV are currently carried out at CPRO-DLO.

The presence of accessions with resistance to *P. betae* and BNYVV in *Beta* species shows the importance of conservation of genetic variation, as was advocated by Doney and Whitney (1990). Although rhizomania is a relatively new disease, considerable progress has been made in breeding resistant sugar beet, due to the resistant accessions found. Using the techniques described in this thesis, and following the strategy outlined here, the resistant accessions could efficiently be used in breeding programmes and give rise to sugar beet cultivars with high levels of resistance to rhizomania.

Epidemiology

Special attention was given to screening methods for resistance to *P. betae* and BNYVV in beet. Most experiments were carried out with single plants in the greenhouse using identical inoculation levels. Another topic of study would be the use of resistant sugar beet cultivars in the field. The first step to translate the results of the greenhouse experiments to the situation in the field was made in Chapter 3. Various techniques are described to perform epidemiological studies (Tuitert, 1990; Zadoks and Schein, 1979). Besides studies on the relationship between the level of infection by BNYVV and crop damage, and the effect of the resistant crop on the infestation in the soil, such studies might enclose investigations on the use of

various resistances at different soil infestation levels and studies on the durability of resistances. Resistance to *P. betae* might have little effect on the level of infection with BNYVV in in a beet crop, but will help to reduce the multiplication of the inoculum in the soil, which may retard its spread and lead to a gradual elimination of the virus over the years.

Summary

Chapter 1. Beet necrotic yellow vein virus (BNYVV) causes a disease in sugar beet called rhizomania. The virus is transmitted by the soil-borne fungus *Polymyxa betae*. Infection with BNYVV can lead to severe losses in sugar beet crops. Breeding for resistance to rhizomania is considered to be the best way to control the disease. Screening experiments for resistance can be carried out in infested fields and in greenhouse experiments. In field experiments, selection criteria are based on the severity of plant symptoms, yield and beet quality. Differences between cultivars measured in field experiments are not only caused by differences in resistance, but also by other factors, such as differences in yield potential. The use of greenhouse tests enables direct measurements on the level of infection with *P. betae* and on concentration of BNYVV in the roots, as an indication of the level of resistance of the beet plants to either the fungus or the virus.

Chapter 2. A quantitative test to screen sugar beet seedlings for resistance to BNYVV was developed. A mixture of sand and soil containing *P. betae* infested with BNYVV was used to inoculate plants. Virus assays were performed by enzyme-linked immunosorbent assay (ELISA). A dose-response curve of purified virus, fitted by a four-parameter logistic model, was used for the conversion of the ELISA readings to virus concentrations. The results obtained show that the test can be used to distinguish cultivars with various levels of resistance and to identify individual plants with resistance to BNYVV.

Chapter 3. Sugar beet cultivars which varied in their resistance to BNYVV were tested on locations with various levels of infestation in two consecutive years. Virus concentrations were determined in roots from plants in an accompaning greenhouse experiment and from plants in the field. Yield and quality data were analysed with two multiplicative models for cultivar by location interaction. Virus concentrations were correlated to the interaction scores for cultivars and locations from the Additive Main effects and Multiplicative Interaction effects model (AMMI), or incorporated in a statistical model as done in factorial regression analysis. Results

of both analyses showed that cultivar by location interaction of yield and quality parameters could be described in terms of virus concentrations, measured in plants grown in the greenhouse and in the field. Therefore, the relative performance of cultivars on infested fields can be estimated by means of three independant parameters, (i) the level of resistance of the beets, determined in a greenhouse experiment, (ii) the yield and quality of the beets on non-infested fields, and (iii) the level of infection in the field.

Chapter 4. Resistance to *P. betae* was studied in *Beta* species of the section *Procumbentes,* in hybrids of *B. vulgaris* with *B. procumbens* or *B. patellaris,* and in the complete series of monosomic chromosome additions of *B. procumbens* in *B. vulgaris.* Resting spores (cystosori) of *P. betae* were not found in the wild species and their hybrids. Virus concentrations in these plants were low, and in half the number of plants no virus could be detected. Results with the monosomic addition plants indicated that resistance to *P. betae* in *B. procumbens* is located on chromosome 4 and 8. Both the average number of cystosori and average virus concentrations in plants of these addition types were lower than in plants of the same population without these extra chromosomes.

Chapter 5. The relationship between the level of infection by *P. betae* and the concentration of BNYVV was further investigated, using beet accessions of the sections *Beta* and *Corollinae.* High levels of resistance to *P. betae* were not found in accessions of *Beta vulgaris* and its subspecies *maritima* of the section *Beta.* Within these accesions, no effect of variation in the level of infection by *P. betae* on the virus concentration was found. High levels of resistance to *P. betae* were detected in *B. corolliflora, B. macrorhiza* and *B. lomatogona* of the section *Corollinae,* whereas resistance to BNYVV was found in *B. corolliflora, B. intermedia* and *B. lomatogona.* Low numbers or even the absence of cystosori of *P. betae* did not always result in low virus concentrations. It may be concluded that resistance to *P. betae* seemed to have little effect on the level of infection with BNYVV. In screening sugar beets for resistance to BNYVV, no interference by resistance to *P. betae* is expected.

Chapter 6. Attempts were made to replace the infested soil in the test for resistance to BNYVV by a quantified suspension of viruliferous zoospores of

P. betae. A system to initiate and maintain cultures of viruliferous *P. betae*, and to produce zoospores was developed. The obtained zoospore suspensions were used to inoculate seedlings, grown in nutrient solution. Virus concentrations in resistant accessions were often similar to those in the susceptible control. Results of screening experiments in which zoospores were used for inoculation showed little agreement with results of a test in which infested soil was used, because of the high virus concentrations in the resistant accessions in the zoospores test. The best discrimination between accessions was obtained shortly after inoculation of the plants, or, alternatively, after transplanting the inoculated plants to sand. Zoospore suspensions could be used in screening for resistance, especially for the detection of accessions with a high level of resistance to BNYVV. For routine screening, however, the use of soil is recommended.

Chapter 7. The results described in this thesis indicate that screening for resistance to *P. betae* and BNYVV could be performed adequately. The experimental results show, that perspectives of the use of resistance to *P. betae* in breeding sugar beets with resistance to rhizomania are limited because of the difficulty of handling it in breeding programmes and the limited effect on the concentration of BNYVV. Admittedly, resistance to P. *betae* reduces the multiplication of the inoculum in the soil and retards the spread of the disease. The use of monogenic resistances to BNYVV, which have been found in *B. vulgaris* and the subspecies *maritima*, has become inevitable, both because of their high levels of partial resistance to rhizomania and the ease to handle them in breeding programmes. Different sources of resistance should be combined to obtain sufficiently high levels of resistance to rhizomania to grow sugar beet in severely infested fields. Therefore, it is necessary to know whether the various resistant accessions are genetically different and whether their resistances are based on different mechanisms.

Samenvatting

Hoofdstuk 1. Het bieterhizomanievirus (BNYVV) veroorzaakt een ziekte in de suikerbiet, genaamd rhizomanie. Het virus wordt overgedragen door de bodemschimmel *Polymyxa betae*. Infectie met BNYVV kan grote verliezen bij de suikerbiet tot gevolg hebben. Veredeling op resistentie tegen rhizomanie wordt beschouwd als de beste manier om de ziekte tegen te gaan. Toetsing op resistentie kan plaatsvinden op besmette velden en in kasexperimenten. Selectiecriteria die in het veld gebruikt worden zijn gebaseerd op de hevigheid van symptomen aan de plant, de opbrengst en de kwaliteitsparameters van de biet. Verschillen tussen rassen, zoals die gemeten worden in het veld, worden echter niet alleen veroorzaakt door verschillen in resistentie, maar ook door andere factoren zoals verschillen in het opbrengstpotentieel. Het gebruik van kastoetsen maakt het mogelijk om directe waarnemingen te verrichten met betrekking tot de mate van infectie met *P. betae* en de concentratie BNYVV in de wortels, als aanwijzing voor het niveau van resistentie die de bieteplanten vertonen tegen hetzij de schimmel of het virus.

Hoofdstuk 2. Een kwantitatieve methode werd ontwikkeld om bietezaailingen te toetsen op resistentie tegen BNYVV. Planten werden geïnoculeerd met behulp van een mengsel van zand en grond met *P. betae*, besmet met het virus. De 'enzyme-linked immunosorbent assay' (ELISA) werd gebruikt bij de virusbepalingen. De gevonden ELISA-waarden werden omgerekend naar virusconcentraties met behulp van een stimulus-response curve, verkregen met gezuiverd virus, die berekend was met een vier-parameter logistisch model. De verkregen resultaten wezen uit, dat de toets gebruikt kan worden ter onderscheiding van de verschillende rassen met een verschillend resistentieniveau en voor het opsporen van individuele planten met resistentie tegen BNYVV.

Hoofdstuk 3. In twee opeenvolgende jaren werden suikerbieten met verschillende niveaus van resistentie tegen BNYVV getoetst op proefvelden met verschillende besmettingsniveaus. Zowel van planten uit het veld als van planten uit een bijbehorende kastoets werd de virusconcentratie in de wortel bepaald. Opbrengst-

en kwaliteitsgegevens werden geanalyseerd met behulp van twee multiplicatieve modellen voor ras x locatie interactie. De virusconcentraties werden gecorreleerd met de interactie-scores voor de rassen en de locaties uit het 'Additive Main effects and Multiplicative Interaction effects' (AMMI) model, of opgenomen in het statistische model met behulp van factoriële regressie. De resultaten van beide analyses wezen uit, dat ras x locatie interactie voor zowel de opbrengst- als de kwaliteitsgegevens kon worden beschreven met behulp van de virusconcentraties, gemeten in planten uit de kas of uit het veld. Uit de resultaten kan men concluderen, dat de relatieve opbrengst van rassen op besmette velden vastgesteld kan worden met behulp van drie onafhankelijke parameters, (i) het resistentieniveau van de bieten, bepaald in een kastoets, (ii) de opbrengst en kwaliteit van de bieten op niet besmette velden en (iii) het infectieniveau op het veld.

Hoofdstuk 4. Resistentie tegen *P. betae* werd bestudeerd in *Beta* soorten uit de sectie *Procumbentes*, in hybriden tussen *B. vulgaris* en *B. procumbens* of *B. patellaris*, en in de volledige set van monosome chromosoom addities van *B. procumbens* in *B. vulgaris*. In de wilde soorten en hun hybriden konden geen rustsporen (cystosori) van *P. betae* worden gevonden. De virusconcentraties in deze planten waren laag, en in de helft van de planten kon geen virus worden aangetoond. De resultaten met de monosome additieplanten toonden aan, dat resistentie tegen *P. betae* in *B. procumbens* is gelegen op de chromosomen 4 en 8. Zowel het gemiddelde aantal cystosori als ook de gemiddelde virusconcentratie in de planten van deze additietypen waren lager dan in de planten uit dezelfde populaties, maar dan zonder extra chromosoom.

Hoofdstuk 5. Het verband tussen het infectieniveau met *P. betae* en de concentratie BNYVV werd verder onderzocht met behulp van bieten uit de secties *Beta* en *Corollinae*. In herkomsten van *B. vulgaris* en de ondersoort *maritima* uit de sectie *Beta* werden geen hoge niveaus van resistentie tegen *P. betae* aangetroffen. Binnen deze herkomsten werd geen effect van de variatie in het infectieniveau met *P. betae* op de virusconcentratie gevonden. Hoge niveaus van resistentie tegen *P. betae* werden gevonden in *B. corolliflora*, *B. macrorhiza* en *B. lomatogona* uit de sectie *Corollinae*, terwijl resistentie tegen BNYVV werd aangetroffen in *B. corolliflora*, *B. intermedia* en *B. lomatogona*. Lage aantallen, of ook de

afwezigheid van rustsporen van *P. betae* hadden niet altijd lage virusconcentraties tot gevolg. Uit deze waarneming kan men concluderen, dat resistentie tegen *P. betae* weinig effect lijkt te hebben op het besmettingsniveau met BNYVV. In het toetsen van suikerbieten op resistentie tegen BNYVV wordt geen verstoring verwacht door resistentie tegen *P. betae*.

Hoofdstuk 6. Pogingen werden ondernomen om het gebruik van besmette grond in de toets op resistentie tegen BNYVV te vervangen door gebruik van een gekwantificeerde suspensie van virusbesmette zoösporen van *P. betae*. Een methode werd ontwikkeld voor het kweken van virusbesmette cultures van *P. betae* en voor de productie van zoösporen. De verkregen zoösporensuspensies werden gebruikt om zaailingen te inoculeren, die in een voedingsoplossing groeiden. De virusconcentraties in de planten van resistente herkomsten waren vaak gelijk aan die in de vatbare planten, die als controle dienden. De resultaten van de experimenten, waarbij zoösporen gebruikt waren voor de inoculatie toonden nauwelijks overeenkomst met de resultaten van een toets waarin besmette grond gebruikt was. Dit kwam met name door de hoge virusconcentraties in de resistente herkomsten in de toets met zoösporen. Het beste onderscheid tussen herkomsten bij het gebruik van zoösporen werd verkregen bij een korte toetsduur, of nadat de planten overgepoot waren naar zand. Zoösporensuspensies kunnen gebruikt worden in resistentietoetsing voor het opsporen van herkomsten met een hoog niveau van resistentie tegen BNYVV. Echter, voor routinematige toetsen wordt het gebruik van besmette grond aanbevolen.

Hoofdstuk 7. De resultaten, die in dit proefschrift beschreven worden, laten zien dat toetsingen op resistentie tegen *P. betae* en BNYVV op betrouwbare wijze kunnen worden uitgevoerd. Uit de resultaten kan men concluderen, dat de perspectieven van het gebruik van resistentie tegen *P. betae* in de veredeling van de suikerbiet op resistentie tegen rhizomanie beperkt zijn, vanwege de moeilijkheid in veredelingsprogramma's en het geringe effect op de concentratie van het BNYVV. Anderzijds vermindert resistentie tegen *P. betae* de vermeerdering van het inoculum in de bodem, waardoor de verspreiding van de ziekte vertraagd wordt. Het gebruik van monogene resistenties tegen BNYVV, die gevonden zijn in *B. vulgaris* en de ondersoort *maritima*, is noodzakelijk, zowel vanwege de hoge

niveaus van partiële resistentie die ze bezitten, alsook vanwege het gemak waarmee ze in veredelingsprogramma's gebruikt kunnen worden. Verschillende bronnen van resistentie dienen gecombineerd te worden ter verkrijging van voldoend hoge niveaus van resistentie tegen rhizomanie om suikerbieten te kunnen telen op zwaar besmette velden. Daarvoor is het nodig te weten, of de resistente accessies genetisch verschillend zijn en of de resistenties gebaseerd zijn op verschillende mechanismen.

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Curriculum vitae

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