Beet mosaic virus: epidemiology and damage

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Propositions

1. The use of multiple regression analysis to relate aphid species and potyvirus spread can result in biologically meaningless negative coefficients, depending on the combination of aphid species used.

   This thesis

   Garrett, G. (1988). Epidemiology - its scope and value. Acta Horticulturae 234:345-357

2. *Beet mosaic virus* (BtMV) has no economic importance above parallel 45N.

   This thesis

3. The spread of BtMV in sugar beet can be related to the total migrating aphid population rather than to a single aphid species.

   This thesis

4. So far, too little attention has been given to the description of the spatial pattern of vectored plant virus diseases, which is essential for modeling and simulation activities, and for designing experiments and sampling programs for disease epidemiology and management studies.

   Campbell, C. L. and Madden, L. V. (1990). Introduction to plant disease epidemiology. John Wiley & Sons.; New York; USA.

5. The relative efficiency factors used to estimate the contribution of a single aphid species in the spread of *Potato virus Y* need to be re-evaluated.

   De Bokx, J. A. and Piron, P. G. M. (1990). Relative efficiency of a number of aphid species in the transmission of potato virus Y^N in The Netherlands. Netherlands Journal of Plant Pathology 96:237-246.

   Sigvald, R. (1984). The relative efficiency of some aphid species as vectors of potato virus Y^O (PVY^O). Potato Research 27:285-290.

   Sigvald, R. (1992). Progress in aphid forecasting systems. Netherlands Journal of Plant Pathology 98 (Supplement 2):55-62.

6. The use of properly developed models, which incorporate experimentally collected parameters, to predict the likely effects of certain control measures, can improve decision making.

   Jeger, M. J. and Chan, M. S. (1995). Theoretical aspects of epidemics: uses of analytical models to make strategic management decisions. Canadian Journal of Plant Pathology 17:109-114.

7. The conclusion that a given plant species might be a natural reservoir of a virus is premature as long as no transmission tests using the natural vector have been done.

   Höfer, P.; Engel, M.; Jesle, H. and Frischmuth, T. (1997), Nucleotide sequence of a new bipartite geminivirus isolated from common weed *Sida rhombifolia* in Costa Rica. Journal of General Virology 78:1785-1790.

8. The knowledge on the spread of aphid transmitted plant viruses in temperate regions can not be directly transposed to the tropics.

9. Music has magical powers. It even makes Dutch sound beautiful.

10. Those who are highly specialized in agricultural research must not forget that the solution for increasing food production might be in the extension of the size of the hoe's handle.

11. The unforgettable mark that the Dutch left in Brazil, during their stay in the XVII century, is their gene pool.

12. Rabbits share with human beings the taste for good food.

13. Everyone we meet in our lives has a purpose.

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Wageningen, 22 juni, 1999 André Dusi

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André Dusi

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Chapter 1

Introduction

1.1. The Potyviruses:

The genus *Potyvirus*, containing 82 definitive and over 100 possible members, is the largest taxon within the family *Potyviridae* (Murphy *et al.*, 1995). In addition, this family comprises five other genera: *Bymovirus*, *Ipomovirus*, *Macluravirus*, *Rymovirus* and *Tritimovirus* (http://www.scri.sari.ac.uk/vir/ictvhome.html).

Potyvirus particles are flexuous filaments, 720 to 770 nm long. The virus genome consists of a single stranded, positive sense RNA (~10 kb) that contains a single open reading frame coding for a large polyprotein, which is cleaved to produce at least nine functional proteins. The coat protein varies in size between 30 and 35 kDa depending on the species.

Most potyvirus species have a relatively narrow host range but their cumulative host range embraces a large number of plants species, including species of economic importance from several families such as *Chenopodiaceae, Cucurbitaceae, Fabaceae, Poaceae* and *Solanaceae*. The symptoms in potyvirus-infected plants depend on the virus and host species, and can be observed on the leaves, flowers, fruits, seeds, tubers and bulbs. The growth of the infected plant is often retarded.

Viruses belonging to the genus *Potyvirus* are, under natural conditions, exclusively transmitted by aphids (*Homoptera: Aphididae*) in a non-persistent manner. This mode of transmission is characterized by acquisition and transmission access periods which last seconds or minutes, and in which the epidermis and occasionally the parenchyma is penetrated by the stylets. The absence of a latent or incubation period in the vector is an additional characteristic of this type of transmission. The aphid usually looses the ability to transmit the virus after the first or second probe or penetration (Berger *et al.*, 1989; Chapter 2). Transmission is dependent upon two virus-encoded proteins, the helper component protein and the coat protein. The role of these two proteins in the acquisition and transmission of the potyviruses is not yet fully understood (Gray, 1996; Nault, 1997). Apparently, they are involved in the binding of the virions to the food canal and upper alimentary track (Berger and Pirone, 1986), and in their release from the lining of the food canal. The efficiency with which potyviruses are acquired or transmitted depends on aphid species, virus species and its strain. High vector abundance, however, may compensate for low transmission efficiency for some aphid species (Sigvald, 1992). The virus is usually retained by the vector for a few hours, but, under certain conditions, a small proportion of the aphids can retain the virus for 30 to 40 h. (Berger *et al.*, 1987). These long retention periods can explain the spread of these viruses over long distances, as has been demonstrated for *Maize dwarf mosaic virus* (Zeyen *et al.*, 1987; Zeyen and Berger, 1990).

For some plant and potyvirus species, pollen and seed transmission have been described as alternative mechanisms by which these viruses are spread (Shukla *et al.*,

1994). Infected seeds give rise to primarily infected plants in a crop. These plants will form the initial inoculum source from which the virus can be spread. Pollen transmission occurs less frequently, affecting mainly plants in the next year generation by infecting the embryo in the newly produced seeds.

Viruses, as well as other plant pathogens, can cause injuries, damages and losses in crops. Injury refers to the visible and measurable symptoms caused by harmful organism; damage is defined as the reduction in quantity or quality of the yield; and loss as the reduction in financial return due to damage (Zadoks, 1987). *Tomato spotted wilt virus* resistant lines of *Capsicum chinense* usually react with local lesions to virus inoculation, while a systemic infection does not develop. This is an example of injury which does not result in damage. Breeding lines resulting from this genotype, segregate with respect to this character and formation of the local lesions are used as selection tool (Black *et al.*, 1991; Boiteux and Avila, 1994). As an example of damage, infection of propagative material can be mentioned. Although infection of the virus in a current growing season will not affect yield, the quality of the seed stock such as tubers, stems, bulbs or seeds can be reduced in potato, sweet potato, and garlic, respectively (Fajardo *et al.*, 1997; Pozzer *et al.*, 1995; Salazar, 1995). Loss can be primarily due to a reduction of yield as in production of potatoes infected by *Potato virus Y* and melon infected by *Papaya ringspot virus* (Beukema and van der Zaag, 1979; Dusi, 1992). Loss can also result from cosmetic damages decreasing crop value such as fruit distortion in cucumber by *Zucchini yellow mosaic virus* (Lisa and Lecoq, 1984). The effects of potyvirus infections on crops may vary from absence of any discernible injury or damage to complete crop failure. The extent of loss depends on the proportion of infected plants, the moment at which the crop becomes infected, virus virulence, variety tolerance or resistance and climatic conditions, among others.

1.2. The aphids vectors and the spread of potyviruses in annual crops:

The holocyclic life cycle of the aphids is presented here to provide a background for understanding the role of various morphs in the spread of potyviruses. The aphids of most species in the temperate climatic regions overwinter as eggs that hatch in early spring to produce parthenogenic females which are designated fundatrices. These fundatrices establish the first colonies on their winter or primary hosts. Depending on the host, environmental conditions and species, these early colonies produce after some generations winged (alate) parthenogenic females, which move to the secondary or summer hosts on which wingless (apterous) females are produced. The populations, established on these hosts, will produce again alatae, when the condition of the plant deteriorates. These alatae

may form new colonies of apterae on summer hosts. When the temperature drops and the day length becomes shorter, alate males and females are formed, which migrate to the winter host. The females parthenogenically produce oviparae which mate with the males and lay eggs. For most aphid species, the winter and summer hosts are usually the same plant species. However, some well known plant virus vectors like *Myzus persicae*, *Aphis fabae* and *Rhopalosiphum padi* have different winter and summer hosts. Complementary information on life cycle of aphids, hosts and population dynamics can be found in Dixon (1985), Eastop (1983), and Maramorosch and Harris (1981).

For epidemiological purposes, a distinction between colonizing and non-colonizing aphid species is made. Potyviruses are transmitted in a non-persistent manner by both colonizing and non-colonizing alatae in a process in which these aphids search for a suitable host by sequentially probing on infected and healthy plants. Contrary to non-persistently transmitted viruses, semi-persistently and persistently transmitted viruses are transmitted in a process where the aphids feed on the host to acquire the virus and transmit. Thus, only colonizing species are able to feed long enough on an infected host plant to become infective.

Potyvirus diseases spread differently in annual, semi-perennial and perennial crops. In annual crops, the spread of a potyvirus often follows the flight of transient aphids (this point will be further discussed). Several cycles of transmission can occur in a growing season (polycyclic epidemic) as the latent and incubation periods are short (1 to 3 weeks). Annual crops consist of herbaceous plant species. Rapid multiplication of the virus converts the infected plant into a virus source in one or two weeks following inoculation, so that the virus may be transmitted in several cycles of infection during the season, provided the presence of vectors. Such a polycyclic pattern, in the sense as defined by Van der Plank (1963), is common for the spread of potyviruses in annual crops. Once a potyvirus infection source is established, the virus can be spread by alatae resulting in a clustered pattern of infected plants around the primary infected plant (Eckel and Lampert, 1993; Nemecek, 1993; Scott, 1985). These clustered patterns of infections in high density crops (5 to 50 plants/m^2) are, thus, related to the behavior of the aphids. Patch size and rate of development depend on the crop in which the spread occurs.

Potyviruses spread more slowly in semi-perennial and perennial crops, which are mostly wooden plant species with long life cycles. Latency and/or incubation periods usually are in the order of weeks or months (Maison, 1975; Minoiu, 1971). The role of the colonizing (non-transient) aphid species may be different from what is observed in annual crops. Colonizing species will have time to build up a population of alatae in these crops and contribute to the spread of the virus within the field when the populations reach high density and start to disperse.

Several studies have been made to correlate the development of aphid populations with the spread of certain potyviruses (Atiri, 1992; Eckel and Lampert, 1993; Garrett, 1988; van Hoof, 1977; Karl *et al.*, 1983; Mora-Aguilera *et al.*, 1992; Madden *et al.*, 1987a and b; Rivas Platero and Larios, 1994; Watson and Healy, 1953). The methods used varied from a subjective comparison of the temporal pattern of spread with temporal trends of aphid populations to multiple regression analysis, in order to better understand the role of certain aphid species in the spread of potyviruses and to develop more appropriate control strategies. However, the diversity between the several pathosystems makes it difficult to identify consistent and generally applicable relationships between vector population and potyvirus spread. Even for the same pathosystem, the pattern of spread will differ between different years as the aphid species population, the numbers in which they occur and the climatic conditions vary. Subjective comparisons of the overlaying curves describing the fluctuation of aphid populations and the rate of spread (Eckel and Lambert, 1993) suggest that the spread of a potyviruses in different years is strongly correlated with aphid flights.

Spread of potyvirus infections is generally associated with the flight of winged aphids. The alatae of colonizing and non-colonizing species are considered the main morphs responsible for virus spread (Madden *et al.*, 1987a). The infection pressure exerted by the alate aphid population is the result of the number and location of virus sources (within or outside the field), the number of aphids and their efficiency to transmit, which will be affected by the susceptibility, age, mature resistance of the crop and climatic conditions. The spread by apterous aphids, on the other hand, is restricted to movement between leaves of the same plant or a few plants around the virus source.

The proportion of infectious aphids alighting in the field, coming from outside sources, is expected to be very small, as for *Bean yellow mosaic virus* (BYMV), a potyvirus that spreads in legume pastures in a monocyclic pattern (Jones, 1993). The rate at which the newly infected plants appear will be a function of the infection pressure. Due to the short range of movement of the aphids and the short retention period of the virus by the vectors, secondary spread (spread within the field from the primarily and secondarily infected plants to healthy ones) is expected to play a major role in the spread within fields than primary infection (infected propagated material or introduction of the virus by the vector from sources outside the field). The virus will spread in several cycles in the crop during the growing season. This characteristic of spread defines potyvirus infections in herbaceous crops as polycyclic. The development of aphid populations within the crop has little or no impact on the secondary spread of potyviruses (Atiri, 1992; Eckel and Lampert, 1993; Madden *et al*, 1987b; Watson and Healy, 1953). No correlation was found in these studies between colonization of a crop by an aphid species and the spread of a non-persistently transmitted virus.

Studies of the spread of these viruses in annual crops have generally a descriptive nature (Almeida *et al.*, 1994), using statistical or non-linear models (such as logistic or Gompertz) to describe the temporal pattern of the epidemics (Rivas Platero and Larios, 1994; Dahal, 1992; Garrett, 1988). Spatial patterns have been described by distance class analysis of diseased plants (Latorre, 1983; Jayasena and Randles, 1984) and attempts have been made to correlate aphid population with spread by multiple regression analysis (Garrett, 1988).

1.3. Modeling studies involving non-persistently transmitted viruses:

A model is a simple representation of a system that contains elements to characterize the system in a comprehensive manner (de Wit, 1995). Models can be useful for a series of purposes regarding the spread of plant viruses (Mora-Aguilera *et al.*, 1993; Bertschinger *et al.*, 1995) and provide information on the effect of control measures such as roguing, crop density, plant architecture, plant resistance, choice of planting time, use of barriers, intercropping, or changes in environmental or variables such as temperature (Ferriss and Berger, 1993; Jeger and Chan, 1995; Jeger *et al.*, 1998; Nemecek *et al.*, 1994). Models also allow for the evaluation of some parameters such as mode of transmission, vector density and activity, vector behavior, transmission efficiency (Ferriss and Berger, 1993; Jeger *et al.*, 1998; Madden *et al.*, 1990; Marcus, 1991; Marcus and Raccah, 1986). Modeling spread also helps to identify the limitations of available epidemiological data, as well as the lack of information on important parameters to look for (Jeger and Chan, 1995).

Most modeling studies regarding the spread of potyviruses are, in general, theoretical (Ferriss and Berger, 1993; Jeger *et al.*, 1998; Marcus and Raccah, 1986). These studies contribute to understand the effect of the mode of transmission on the spread of the disease. Results of studies using field experimentation usually indicate a weak relationship of spread with a few species and fail to explain differences in virus spread among different fields (Mora-Aguilera *et al.*, 1993). Some models are descriptive statistical models rather than explanatory simulation models and do not attempt to associate spread with the development of an aphid population (Marcus, 1990 and 1991). Few studies have been published in which the parameterization of the models is based on experimentation and/or field observations (Ruesink and Irwin, 1986).

The spread of viruses is a process depending on the interaction between the host crop, the vectors, the virus sources and the environmental conditions. The quantification of these relations is still a difficult task. Simulation models can be used to study these interactions. Field studies on the spread of the disease are necessary in order to collect actual data to test models. Also, a more directed study on the transmission of potyviruses

by aphids and on the infection development, under controlled conditions, can help to elucidate specific aspects of the interaction between vector, virus, and host.

1.4. *Beet mosaic virus* (BtMV) and sugar beet:

Beet mosaic potyvirus (BtMV) is non-persistently transmitted by several aphid species, including *Acyrthosiphon pisum, Aphis appi, A. fabae, Macrosiphum euphorbiae, Metopolophium dirhodum, Myzus persicae,* and *Rhopalosiphum padi* (Sylvester, 1952; Chapter 2). As BtMV is not seed-transmitted in sugar beet, its spread is entirely dependent on aphids. Due to its transmission by transient vectors, control of the disease by insecticides is expected to be rather inefficient, as has been shown for other potyviruses (Eckel and Lampert, 1993; Loebenstein and Raccah, 1980; Raccah, 1986). Control of beet mosaic disease by using resistant cultivars is also not optional as genetic resistance to BtMV is not reported.

Some studies have been made on the virus/host and virus/vector relationships (Severin and Drake, 1948; Smrz, 1972; Summers *et al.*, 1990; Sylvester, 1947; Sylvester, 1949; 1952). Basically, these studies were focused on those aspects, which are related to vector propensity: aspects of transmission efficiency under controlled conditions (Irwin and Ruesink,1986). These studies were made under laboratory conditions to evaluate the vector ability of some aphid species, the optimal acquisition conditions, the retention of the virus by apterae, and also the latency and incubation periods of the virus in the host. Knowledge on the relationships between BtMV and its host and vectors under field conditions is almost lacking and the spread of this virus has not been correlated with any specific aphid species..

In The Netherlands, sugar beet (*Beta vulgaris L.*) is cultivated in fields varying in size from less than 1 to more than 10 ha. The total acreage is 115,000 ha every year with an average yield of 55 ton roots/ha (Centraal Bureau Statistiek, http://www.cbs.nl). Sugar beet is sown in the end of March and the roots harvested from mid September to early December, and are transported to sugar processing plants.

Sugar beet/BtMV is a typical representative of an annual crop/potyvirus pathosystem. Plant density varies from 7 to 10 plants/m^2. The crop cycle lasts 7 to 9 months. The disease is polycyclic and vectored by several aphid species. Studies on this system can, therefore in several respects, be extrapolated to other annual crop/potyvirus systems. BtMV and sugar beet were chosen as the model crop for the proposed studies for the following reasons:

1. The virus is not seed-transmitted and its natural occurrence in the Netherlands is extremely low. Hence, uncontrolled primary infections are very rare and therefore, field experiments using introduced inoculations are reliable;

2. Symptoms evoked by the virus are clearly expressed and characteristic. Disease progress is readily monitored by visual observation;
3. Crop losses resulting from BtMV infections are usually negligible, and infection does not spread to other crops, which allowed us to work on fields without significantly threatening nearby commercial crops.

1.5. Outline and scope of this study:

The spread of non-persistently transmitted viruses in a crop is known to depend on many factors, such as the presence and number of virus sources, the genotype of the crop, the occurrence of vectoring aphid populations, and cultivation practices. Basic parameters describing the virus-vector-host relationship, such as vectoring efficiencies of different aphid species, optimal acquisition conditions, retention of the virus by apterae, and latency and incubation periods of the virus in plants have been comparatively well studied. Also, the temporal development of disease from natural primary infections has been extensively studied. Nevertheless, some aspects of potyvirus disease dynamics and impact on crop growth are not well understood, especially the spatio-temporal spread of disease from known sources under field conditions, and the impact on plant growth and crop productivity.

At the onset of this project, the interactions between sugar beet, BtMV and one of its vectors, *M. persicae*, were studied under controlled (laboratory) conditions, with the aim to collect data which would be instrumental for the correct interpretation of the data obtained in the field experiments later (Chapter 2). The spatio-temporal spread of the virus from deliberately infected source plants in the sugar beet crop was then studied. Since inspection of numerous plants can be time consuming, a time-saving transect inspection method was developed and its reliability compared to full inspection of sugar beet plots (Chapter 3). The spread of potyviruses does not depend only on the availability of virus sources, but also on the number of aphids in search for a new host and the dates at which they make their flights. To evaluate the effect of these aphid flights, spread was analyzed after introducing virus sources at different dates during the cropping season (Chapter 4). The density of the crop plants constitutes another factor, which may affect the rate of spread of infection. The proportion and the number of infected plants around primary infection sources were analyzed in crops with different densities (Chapter 5). The extent by which the soil is covered may be a stimulus for aphids to differentially spread the virus in the plots with different plant densities. The influence of soil coverage will also be elaborated in Chapter 5. It is generally assumed that BtMV does not affect yield significantly. However, a study that simulated crop growth, based on the carbon balance of the crop, taking into account the net photosynthesis in crops with different disease timing and incidences, showed that a considerable reduction of yield can occur when disease

incidence is high early in the season (Chapter 6). Finally, the results obtained in this study are discussed and placed into the context of other studies of the epidemiology and yield of potyviruses (Chapter 7).

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Chapter 2

Beet mosaic virus: its vector and host relationships[1]

[1] A. N. Dusi and D. Peters. Journal of Phytopathology (in press)

2.1. Abstract:

In order to understand the various factors which affect *Beet mosaic virus* (BtMV) epidemics, different aspects of the relationships between this virus, its vectors and sugar beet were studied. The latency and incubation periods, determined under growth chamber and field conditions, responded inversely to the temperature and leaf growth rate. Field infected plants could function as virus sources during the whole growing season. The virus was transmitted by *Acyrthosiphon pisum, Aphis fabae, Macrosiphum euphorbiae, Metopolophium dirhodum, Myzus persicae* and *Rhopalosiphum padi. M. persicae* could retain the virus for at least 16 h. Alatae and apterae of *M. persicae* transmitted the virus with the same efficiency, and in at least two consecutive probes. The proportion of infected plants increased as a logarithmic function of the number of alatae of six aphid species used in the arena tests.

2.2. Introduction:

Beet mosaic virus (BtMV) is a non-persistently aphid-transmitted potyvirus infecting mainly sugar beet and its close relatives. Only a limited number of studies have been made with respect to the relationship between this virus and its host, sugar beet, and vectors (Severin and Drake, 1948; Smrz, 1972; Summers *et al.*, 1990; Sylvester, 1947; Sylvester, 1949 and Sylvester, 1952). Basically, these studies were performed with a few aphid species, focusing on the probability of a single aphid to vector the virus under controlled conditions. This property, defined by Irwin and Ruesink (1986) as vector propensity, does not consider population behavioral aspects.

The aphid transmission of potyviruses is characterized by short acquisition and transmission access periods. The aphids usually lose their inoculativity rapidly, although the virus can, in some cases, be retained for 24 h (Zeyen and Berger, 1990).

The spread of potyviruses has been shown to occur mainly around foci (Shukla *et al.*, 1994), resulting usually in steep gradients. Thus, secondary spread occurs mainly over short distances. Transmission over long distances is often attributed to the introduction of the virus in the crops (primary spread) and has little impact on further development of an epidemic.

Control strategies of non-persistently transmitted viruses must take the various epidemiological aspects into account, as the control of vector populations is solely ineffective (Harris and Maramorosch, 1977), and requires an understanding of the interactions of the factors involved in their spread. Information on the latency and incubation periods, the infectivity of sugar beet plants acting as source plants under

different environmental conditions, the vector aphid species involved and other virus/vector relationships form the basis for the comprehension of the whole epidemiological process.

In this paper, we report the results of experiments designed to determine some BtMV-vector and BtMV-host relationships. Several aspects of its transmission by the different aphid species and morphs of *M. persicae* were evaluated. Also, the response of the host to BtMV infections under controlled environment and field conditions, at different times during the growth period of the crop were studied. The information obtained on both virus-host and virus-vector interactions will grant a better insight into this pathosystem. A better knowledge of the components governing the epidemics is required to develop computer simulation models, that can be used to understand the epidemiological processes and control disease spread (Shukla *et al.*, 1994).

2.3. Material and Methods:

2.3.1. Virus isolate and test plants:

In all experiments an isolate of BtMV, which was collected some years ago from an infected sugar beet plant, and designated 'Wageningen', was used. It was maintained on sugar beet of the cv. Hilton by aphid inoculation. In all transmission studies, seedlings of this sugar beet cultivar were used as indicator plants.

2.3.2. Aphids:

The beet colonizing aphid, *Myzus persicae*, was reared on *Brassica napus* L. subsp. *oleifera* (oilseed rape) under greenhouse conditions at a temperature of 20 ± 3 °C and a photoperiod of 16 h. Cohorts of equally aged nymphs were produced by daily transfer of mature apterae to fresh plants and kept in leaf cages (Van den Heuvel and Peters, 1989). To avoid moulting during the experiments and consequently loss of inoculativity, only apterae adults were used in the experiments. Alatae of this species that naturally developed in the same colonies were used in some studies.

Acyrthosiphon pisum, Aphis fabae, Macrosiphum euphorbiae, Metopolophium dirhodum and *Rhopalosiphum padi* were kindly provided by Dr. Hans van der Heuvel (IPO-DLO, Wageningen). Clones of these species were maintained under climate chamber conditions at 15 °C with a photoperiod of 16 h. *A. pisum* and *A. fabae* were maintained on *Vicia faba*, *M. euphorbiae* on *Lactuca sativa* and *M. dirhodum* and *R. padi* on *Avena sativa*. Only alatae of these species were used. They were collected at the ceiling of the cage, assuming that they had completed their teneral period (Van den Heuvel *et al.*, 1994).

2.3.3. Transmission tests:

Unless otherwise stated, the aphids were starved for 1 to 2 h prior to an acquisition access period (AAP) of 5 to 10 min on an infected sugar beet plant (source). They were then given an inoculation access period (IAP) of 8 to 20 h on healthy test plants by caging them individually. The plants were then sprayed with pirimicarb to kill the aphids, placed in a growth chamber at 24 °C and maintained for 2 weeks for symptom expression.

2.3.4. Transmission efficiency, latency, incubation and acquisition under field conditions:

The field experiments were conducted in a commercial sugar beet crop (cv. Univers) at Binnenhaven, Wageningen, during the growing seasons of 1996 and 1997. The latency and incubation period of BtMV in these seasons were determined in sugar beet plants inoculated at two week intervals.

2.3.4.1.Latency and incubation periods:

Ten beet plants were inoculated with BtMV by placing 10 viruliferous aphids on the youngest expanding leaf, without cages or any other restriction. Starting five days after inoculation, a leaf disc of 0.8 cm in diameter was daily collected from each inoculated leaf. These samples were ground in $^1/_2$ strength phosphate-buffered saline containing 0.5% Tween-20 (0.5x PBS-T) (1:10 w/v), the extracts spotted onto a nitrocellulose membrane and assayed by dotblot-ELISA for the presence of virus. The membranes were incubated for 2 h in 2% non-fat milk powder in 0.5x PBS-T and then 2 h in 1 µg/ml anti-BtMV-IgG (raised at the Department of Virology, Wageningen Agricultural University) in 2% healthy sugar beet leaf sap prepared in 0.5x PBS-T. After washing the membranes 3 times in 5-min steps with 0.5x PBS-T, they were incubated with 0.5 µg/ml goat anti-rabbit IgG (GibcoBRL) in 0.5x PBS-T for another 2 h. The washing steps were repeated and NBT/BCIP (Sigma) substrate (33 µl and 22 µl, respectively) in 7.5 ml of 0.1 M Tris buffer, pH 9.5, containing 0.1 M NaCl and 5 mM $MgCl_2$, was added to develop the reaction. The number of infected plants was determined in each set until three weeks after inoculation.

2.3.4.2. The capacity of infected plants to act as virus sources:

The first set of the inoculated plants from the above experiment was tested for their suitability to act as virus source for virus spread by aphids during the growing season. Aphids were given an AAP of 5 to 10 min on leaf discs of 0.8 cm in diameter, collected at intervals of 2 weeks, and tested on 15 to 20 plants, using 1 aphid/plant.

Also, leaf discs from 4 plants inoculated at 2-week intervals were collected after symptom expression and assayed for their infectivity by aphid transmission. An infected

sugar beet plant kept at 24 °C in a growth chamber was used as reference in the measurements of the transmission efficiency. In all cases, only the youngest expanded leaf showing symptoms were used to sample the leaf discs.

2.3.5. Determining the latency and incubation periods at different temperatures:

Sugar beet 'Hilton' plants in their fourth leaf stage were aphid inoculated as described above using 10 aphids/plant, and kept in growth chambers at 10, 15, 20 and 24 °C (four plants/treatment), respectively. Starting 4 days after inoculation, leaf discs were daily collected from the inoculated leaves and tested by dot-blot ELISA for the presence of BtMV until symptom appearance. This experiment was repeated twice.

2.3.6. Determining the retention of BtMV in *Myzus persicae:*

Apterae adults were fixed to a copper wire using a water based paint to fix the wire on the back of the aphid after the AAP and suspended freely in space at room temperature (18 to 22 °C) for 1, 2, 4, 8, 16, 18 and 24 h. The aphids were prevented in this way to touch any surface with their stylets during the period they were suspended. A drop of water was used to release the aphids from the wire. Single aphids were then caged on seedlings to test their inoculativity. The number of aphids tested increases with the length of the suspension period, from 15 (1 h period) to 90 (12 to 24 h periods) to enhance the chance to get a measurable level of transmission when the rate was low. Each treatment was repeated at least twice. The seedlings were checked for disease symptom expression. Simultaneously, apterae adults were kept in Petri dishes after the AAP for the same intervals as the suspended ones and then transferred to the test plants. The inoculativity of the aphids in the above described treatments was compared with aphids that were directly placed after the AAP on test plants.

2.3.7. Inoculativity of alatae and apterae:

The effect of the AAP on the inoculativity of different morphs of aphids was studied. Groups of 28 apterae were starved for 2 h and then given an AAP of 3 to 5, 5 to 10, 10 to 15 and 15 to 20 min, respectively. The aphids were transferred to test plants to test their inoculativity.

The transmission efficiency of 28 alatae and apterae of *M. persicae* was compared by testing their inoculativity in 2 replications. After a 5-10 min AAP, the aphids were tested for their inoculativity.

The transmission of BtMV was also studied when specimen of both morphs could acquire the virus in a single probe. Twenty-eight alatae and 27 apterae were starved for 2 h. Each aphid was allowed to make a single probe on a virus source, while monitored under a stereomicroscope, and then immediately transferred to test their inoculativity. It was

assumed that the vector makes a probe when it touches the leaf surface with its proboscus and places its antennae backwards (Tarn and Adams, 1982).

The frequency with which a viruliferous aphid can transmit BtMV, was determined by starving a group of 16 apterae for 2 h and allowing them to make a single probe on a virus source monitored under a stereomicroscope. Aphids were individually transferred in a series of 2 to 5 test plants, on which they were allowed to make a single probe in each transfer. They were caged for 12 h on the last plant of the series. The number of test plants in each series varied due to the restless behavior of some individuals. In this case, the number of test plants were smaller. The results of the above mentioned assays were evaluated by the χ^2 test.

2.3.8. Transmission of BtMV by alatae under arena conditions:

Arena experiments were conducted according Fereres *et al.* (1993) and Labone *et al.* (1995). In these experiments, the alatae could freely move between the virus source and test plants and probe on them.

The aim of these experiments was to compare the efficiency by which different species transmitted BtMV (Table 2.2). The winged adults (20, 40 or 80 specimens) were released in 40 x 50 x 60 cm cages containing a tray with 16 test and 4 infected plants in the 2nd leaf stage. The plants were sprayed after 24 h and kept at 24 °C for 2 weeks for symptom expression. The capacity of each species to transmit was determined in 2 to 4 replications.

The efficiency at which *M. persicae* transmitted BtMV was evaluated by releasing a gradually increasing number of alatae (1, 3, 9, 27 and 81 specimens) in cages, as described above, containing a tray with 1 source and 30 test plants, in the 2nd leaf stage. The plants were sprayed after 24 h and kept at 24°C for two weeks until symptom expression. A similar experiment was repeated two times using three groups of 16 test and 4 source plants in the 2nd leaf stage, on which 9, 27 and 81 aphids were released.

2.4. Results and Discussion:

2.4.1. Transmission efficiency, latency, incubation and acquisition under field conditions:

In this study, the term latency is used to define the period between the inoculation and the moment that the virus can be acquired by the vector, and the term incubation is the period between inoculation and appearance of symptoms.

The symptoms in the test plants usually appeared 6 days after inoculation, starting with a vein clearing and followed by a mosaic, leaf distortion and stunting. Several sugar

beet cultivars were tested for their susceptibility, and since no differences were found, the cv. Hilton was chosen as seeds of this cultivar were readily available.

2.4.1.1. Determining the latency and incubation period:

Preliminary experiments showed that as soon as the virus could be detected in the plants, the virus could also be acquired by aphids. Thus, serology was used to determine the latent period of the virus. Figure 1 shows the moment after inoculation at which the first positive dotblot-ELISA was obtained (latent period) and the moment at which the first infected plant exhibited symptoms (incubation period). The end of incubation period, in general, occurs one day later than the latent period. Both showed the same trend during the growing season and appeared to be correlated with the temperature. Shorter periods were found when higher temperatures prevailed (meteorological data not shown). The overall average time for 50% of the infected plants to accomplish the latency and incubation period in the field was 13 and 15 days, respectively. As the age of the plant may affect the susceptibility, the proportion of sugar beet plants that became infected was determined at several moments during the growing season (Figure 2). Until the beginning of August, a high percentage was infected, but decreased significantly in the middle of August and September. The decrease of the susceptibility, late in the season, might be related to a phenomenon known as 'mature plant resistance', which has previously been reported for other potyvirus/host systems (Ferris and Jones, 1996; Sangar *et al.*, 1988).

2.4.1.2. The capacity of infected plants to act as virus sources:

The efficiency at which the virus is transmitted from inoculated plants just after the incubation period was determined. The virus could be acquired from all plants sampled. The proportion of infected plants varied from 0.05 to 0.13 when field infected plants were used as a source, and 0.33 to 0.60 using growth chamber plants. In addition, the efficiency at which the aphid acquires the virus from plants inoculated in the beginning of the season was also determined. The values obtained ranged from 0.02 to 0.05 for field infected plants, and from 0.13 to 0.30 for growth chamber plants. The results show that all infected plants in the field can function as virus source during the whole season. However, the transmission efficiency from these plants was lower than from plants kept in the growth chamber. This lower efficiency may be related to a lower concentration of virus in the field plants as found for potato virus Y (de Bokx *et al.*, 1978) and turnip mosaic virus (Yamamoto and Ishii, 1981). The virus titer in the field plants was not determined. However, the dot-blot ELISA reaction showed that the samples from field plants produced spots with lower intensity than the growth chamber plants, indicating higher BtMV titers in the latter plants

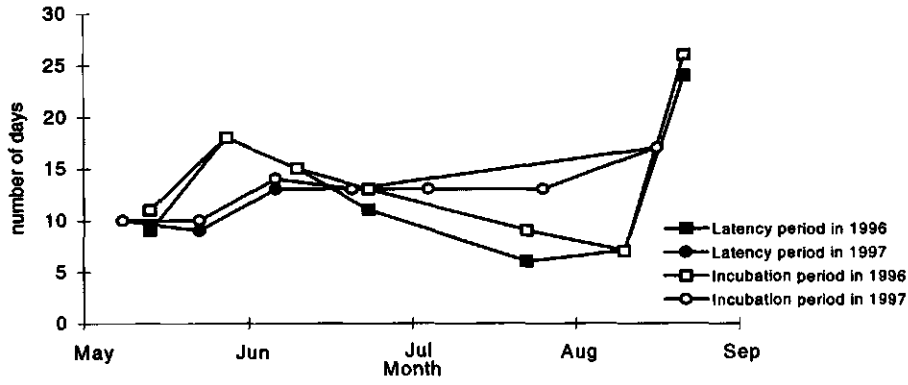

Figure 2.1. Latency and incubation periods of BtMV in sugar beet plants in the field in 1996 and 1997.

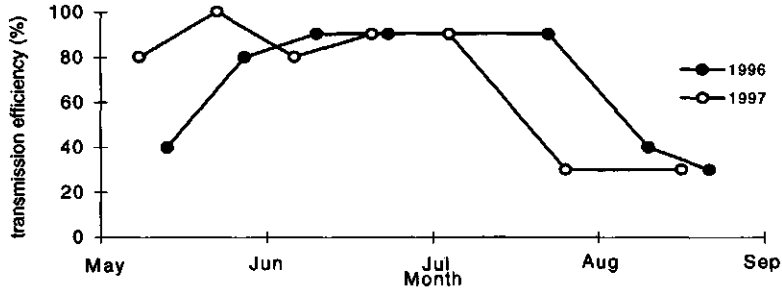

Figure 2.2. Transmission efficiency of BtMV to sugar beet by *M. persicae* under field conditions in 1996 and 1997.

2.4.2. Effect of temperature on the latency and incubation periods:

The disease caused by BtMV is polycyclic in the concept of van der Plank (1963), i.e., virus spread occur within the field in several cycles during the development of the epidemic from the first sources established. The latter infected plants (secondary sources) play a major role in this epidemiological process. The shorter the latency period, the faster the epidemic can develop, as more sources become available in a smaller period for the spread of the virus. As shown in this study, the length of the latency and incubation periods

increased with decreasing temperatures (Table 2.1). Similar observations were also made under field conditions. The latency and incubation periods were shorter during the summer months. Their length varied according to the average temperature of the particular year (data not shown). The variation in the latency and incubation periods is also related to the phenology of the crop. At low temperatures, the rate of leaf growth decreases, whereas no growth occurs below 7°C (Milford and Riley, 1980). Plants kept at 10°C for 4 weeks did not develop any symptom. Upon incubation of these plants at 22°C, symptoms appeared within 2 days on the developing leaves and virus could be detected by dot-blot ELISA. Leaf growth resumes after transferring them from low to high temperatures (Milford and Riley, 1980). These results demonstrate that the development of infection and the replication of virus is strongly correlated with the growth of the plant.

2.4.3. Retention of BtMV in *Myzus persicae*:

Retention of the virus after acquisition was determined after suspending individual aphids on a copper wire or keeping them in a Petri dish for different periods. The first group of aphids was prevented, in this way, to touch their stylets to any surface while those in the Petri dish could. According to the ingestion-egestion transmission hypothesis (Harris, 1977), probing on solid surfaces could lead to a loss of inoculativity, due to the egestion of saliva, which is prevented by suspending the aphids. The aphids kept in the Petri dishes, during which they could touch the surface, lost rapidly the capacity to transmit the virus in the first 4 h. The suspended aphids lost their inoculativity at a lower rate in the first four hours. After this period, both groups of aphids show a similar decrease in the residual inoculativity, which persists at least for another 12 h or more (Figure 3). These different rates, by which the inoculativity is lost, can not be explained by the ingestion-egestion of the virus, but by a differential release of the virus from the helper protein-virus complexes. These complexes may dissociate at two, but different, rates or at different places in the food canal. The persistence of some capacity to transmit after a rapid drop of inoculativity has also been explained by a long viability of the helper protein-PVY complex in *M. persicae* (Collar *et al.*, 1997). Working with maize dwarf mosaic virus (MDMV), virus was retained as long as 70 h, under certain conditions (Berger *et al.*, 1987). Previous studies on the persistency of BtMV in the vector showed that the ability to transmit lasted at least 12 h (Karl and Giersemehl, 1977) for apterae kept in a glass vial. This long retention can explain the transport of the non-persistently transmitted viruses over long distances and the introduction of diseases in new areas as has been reported for several potyviruses (Zeyen *et al.*, 1987) and may be significant for epidemiological studies of these viruses (Zeyen and Berger, 1990).

Table 2.1. Latency and incubation periods of BtMV in sugar beet incubated at different temperatures in a growth chamber at 24 °C. Results are the mean of 2 replications, followed by the standard error of the mean.

Temperature (°C)	Latency (days)	Incubation (days)
10	>28	>28
15	7.5 ± 0.5	9.0 ± 1.0
20	6.0 ± 0.0	7.0 ± 0.0
24	5.5 ± 0.5	6.0 ± 0.0

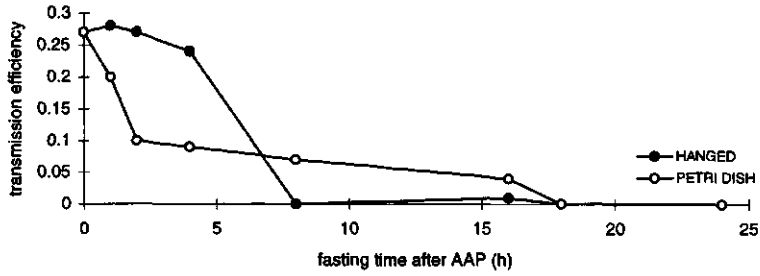

Figure 2.3. Transmission efficiency of BtMV to sugar beet by *M. persicae* when the stylet were either or not prevented, for some time after the AAP, to touch an inert surface.

2.4.4. Infection potential of alatae and apterae:

The transmission efficiency of *M. persicae* after AAPs varying in lengths, did not differ statistically (values ranging from 6/28 to 8/28, infected/test plants, p=0.94). When a 5-10 min AAP was given to the alatae and apterae *M. persicae*, a statistically significant difference in transmission efficiency was observed between both morphs (23 and 20 out of 28 apterae, and 14 and 13 out of 28 alatae, p<0.01). However, when the transmission efficiency was determined in single probe experiments, the vector potential of both morphs did not differ statistically (10 out of 28 alatae and 6 out of 27 apterae transmitted, p=0.42), indicating that their potential to vector BtMV is similar. From these laboratory results, the retention and sequential transmission under field conditions can be inferred. The alatae were more restless in the single probe experiments and rarely probed in the first minutes

after being placed on a leaf. Their main behavioral pattern is to walk and fly from the leaf rather than to probe. The apterae, on the other hand, immediately probed on the leaf in the first moments, leading to the acquisition of the virus during the AAP. These behavioral differences between the different morphs resulted in different transmission efficiencies when alatae and apterae were given the same AAP.

The first successful transmission does not always occur in the first probe of the alatae or apterae. Most of the transmissions occurred in the 1st probe (7/16), followed by the 2nd, 3rd and the 4th probes (5/16, 3/16 and 1/16, respectively). The probability of successful transmission decreases after each subsequent probe. The aphids tested made 1 (14 out of 16 aphids) or 2 (2 out of 16 aphids) infective probes. For other potyviruses (Berger *et al.*, 1989), more infective probes have been reported. Visual assessment of the probing does not necessarily prove that the actual acquisition or transmission occurred. Also, it should be noted that the probability to acquire virus also depends on the presence or absence of virus particles in punctured cells as substantiated by the work of Collar *et al.* (1997). The fact that apterae aphids have the tendency to transmit the virus at higher efficiencies than the alatae when allowed to freely probe on a virus source can be associated with their probing behavior. As alatae tend to be more restless, the lower number of probes they make will consequently result in a lower number of infective punctures. The lack of transmission in the first or second probe might be due to the fact that the aphids do not always transmit the virus during a probe. The average transmission efficiency in this test was 40% for both alatae and apterae. This average ranged from 20 to 50 % for *M. persicae* in the other assays described in this work.

2.4.5. Transmission of BtMV by alatae aphids under arena conditions:

A. pisum, *M. euphorbiae*, *M. dirhodum* and *R. padi*, although they do not colonize sugar beet, can transmit BtMV and be more efficient than the colonizing species *M. persicae* and *A. fabae* (Table 2.2). In the test, in which 40 alatae were release in a cage, the transmission rate for each species significantly differed from each other as shown in χ^2 test. The transmission of potyviruses by non-colonizing aphid species has previously been reported (Katis and Gibson, 1984; Peters *et al.*, 1990; Severin and Drake, 1948; Summers *et al.*, 1990) and corroborates the notion that non-colonizing aphid species play an important role in the epidemiology of potyviruses. The transmission efficiency as determined in arena tests is a reliable parameter to measure the vector propensity (Irwin and Ruesink, 1986). This test allows the vector to behave more naturally (Yuan and Ullman, 1996), and the results can give a better indication of the epidemiological role of the aphids, rather than the purely efficiency measurements obtained through controlled aphid transfers. The transmission efficiency increased with the number of alatae *M.*

Table 2.2. Proportion of BtMV infected sugar beet plants inoculated by an increasing number of aphids of different species in arena tests. Proportions are the average of the replications.

Aphid species	Number of aphids/cage		
	20 (n=3)[1]	40[2] (n=4)	80 (n=2)
Acyrthosiphon pisum	0.44	nt	nt
Aphis fabae	0.25	nt	0.06
Macrosiphum euphorbiae	nt[3]	0.50	nt
Metopolophium dirhodum	nt	0.02	0.02
Myzus persicae	0.23	0.19	0.34
Rhopalosiphum padi	nt	0.00	0.13

[1] Number of replications.

[2] Significant difference among the tested species by the χ^2 test at p=0.05.

[3] nt - not tested

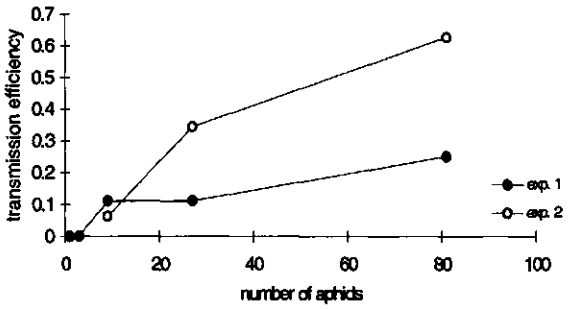

Figure 2.4. Transmission efficiency of BtMV to sugar beet by alatae *M. persicae* under arena test conditions. In experiment 1 (exp.1), 1 source and 30 test plants was used, and in experiment 2 (exp. 2) there were 4 source and 16 test plants. Exp. 2 data are the average of 2 replications.

persicae (Figure 4). The difference in the transmission efficiencies between the two experiments are due to the higher source/test plant ratio in the second one.

Based on the results of these tests, it is concluded that studies on the epidemiology of BtMV must take into account that 1) susceptibility is related to crop phenology; 2) rate of secondary spread is related to the temperature, as the latent and incubation periods increase inversely with the temperature; 3) transmission is expected to occur in the first hour after virus acquisition; 4) long distance spread can take place; and 5) rate of spread depends on aphid species and their number, and not on the capacity of the aphid to colonize the crop.

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Chapter 3

Evaluation of a time saving transect sampling method to assess the spatio-temporal spread of *Beet mosaic virus*

3.1. Abstract:

In three replicated field trials in 1995 and 1996, the spatio-temporal spread of BtMV around artificially inoculated source plants was studied. The progress of the disease was monitored by scoring and mapping all plants within 4.6 m from the source over a period of four months at one to two week intervals. The development, in shape and size, of these patches was characterized by the final number of plants showing symptoms, estimated parameters for a logistic equation describing disease progress, the disease gradient and the average distance of plants showing symptoms from the source. The resulting patches of diseased plants were circular. The incidence of disease decreased with distance from the source according to a exponential decay function. All the above descriptions of patch development were also determined, using only information about plants on two diagonal transects, crossing the center of each patch. The transect method, when applied in the field takes only 10% as much time as a full survey of plot, but is less precise. The results of full and transect counts were compared using ANOVA to investigate whether the lower precision of this transect method could be compensated for by using a higher number of replications. These comparisons show that the transect method gives reliable information for estimating the number of plants showing symptoms and the inflection point of the disease progress curve. To analyze the disease gradient and the size of the patch the transect method requires a 6 fold increase in the number of replications to reduce variance to the level achieved with a full survey, although the overall pattern of the patch could be recognized. It is concluded that, even though a higher number of replications is required, the proposed transect method results in a considerable saving of time in epidemiological field studies of BtMV.

3.2. Introduction:

Beet mosaic virus (BtMV) is a member of the *Potyvirus* genus of the *Potyviridae* family, containing 82 definitive and at least 100 possible members (http://www.scri.sari.ac.uk/vir/ictvhome.html) which are transmitted by aphids in a non-persistent manner (Shukla *et al.*, 1994). BtMV is transmitted by several aphid species (Sylvester, 1952). Since it is not seed-transmitted in sugar beet, the first infections originate from sources outside the crop. The secondary spread by aphids alighting in the crop may result in a clustered pattern of infected plants around the primary sources (Eckel and Lampert, 1993; Scott, 1985). Resident aphids are believed to play a minor role in this process.

A sound understanding of the spatio-temporal spread characteristics of potyviruses may help in the design of appropriate control strategies, such as the choice of the sowing date and plant density. It is commonly accepted that transient winged aphids of colonizing and non-colonizing species are the most important vectors of potyviruses (Madden *et al.*, 1987a). Their restless behavior makes control by insecticides ineffective. Insecticides have a slow mode of action against incoming alatae (Asjes, 1981; Eckel and Lampert, 1993; Loebenstein and Raccah, 1980; Raccah, 1986), and even a high spraying frequency is not effective when aphid numbers are high (Pirone *et al.*, 1988; Roberts *et al*, 1993).

Most studies on the spread of potyviruses focus on the spread in time and neglect its spatial pattern (Dahal, 1992; Irwin and Goodwin, 1981; Madden *et al.*, 1987b; Mora-Aguilera *et al.*, 1996). The shortness of data on spatio-temporal patterns makes it difficult to disentangle the role of primary and secondary infections in the development of epidemics. When studying spatial patterns, an effective sampling method is indispensable and the techniques to analyze this spread are not thoroughly studied for potyviruses. Monitoring all plants showing symptoms around a source may be laborious and time consuming. Under time constraint, it will be more efficient to collect less data points per patch, and increase the number of patches to be monitored. A possible sampling strategy consists of walking along two orthogonal lines passing through the primary virus source and evaluating only the plants on those lines. Transect sampling may be a superior strategy when significant site to site variability occurs with regard to virus spread within a field, or between different fields. This variability may be caused by differences in plant development, soil and environment characteristics, aphid pressure as well as natural enemy populations (Madden *et al.*, 1987b; Mora-Aguilera *et al.*, 1996).

The purpose of this study was to characterize spatial and temporal aspects of the spread of BtMV around known sources of infection and, in addition, to compare the reliability of the proposed transect sampling method, with the 'norm' of a complete spatio-temporal map of infected plants around this source. The evaluation took into account accuracy (absence of bias) as well as precision (repeatability) of parameter estimates based on field collected data (Chatfield, 1983). The knowledge acquired will be used to design further studies on the spread of BtMV under different conditions in the field.

3.3. Material and Methods:

3.3.1. Overall approach:

Three field experiments were established. Two plants in the center of each experimental plot were infected with BtMV at an early growth stage, and the further spread of disease throughout the plot was monitored at intervals of 1 to 2 weeks. Plants showing the characteristic symptoms of the disease were marked with bamboo canes. Samples were taken to the laboratory during the first monitorings and tested by ELISA to confirm infection with BtMV. Further observations were exclusively made visually. One person did all observations to avoid bias. The collected data were analyzed to obtain:

1. the final number of plants showing symptoms per plot;
2. the temporal increase of the number of plants showing symptoms per plot, as described by estimated parameters of the logistic growth equation;
3. the decrease of the proportion of plants showing symptoms with their distance from the source, as described by the estimated parameters of a negative exponential equation.

A more limited data set, representing 'transect sampling', was computer generated from the full count maps and analyzed. The results were compared with those of the full plot data analysis, using Analysis of Variance (ANOVA).

3.3.2. Details of the three field experiments:

Experiment 1 (Exp. 1) was established at the experimental farm 'De Bouwing', in Randwijk, The Netherlands, in 1995. Five square plots, with sides of 5 m and separated by 15 m of sugar beet crop were marked in the center of a 2 ha commercial field of sugar beet cv. Univers. This field was bordered by wheat on two sides, and by roads on the two other sides. Row spacing was 0.5 m and plant density 7 plants/m^2. The *Beet mosaic virus* (BtMV) isolate used was collected in 1983 in Wageningen and maintained in sugar beet under greenhouse conditions. *Myzus persicae*, a known vector of BtMV, was reared on *Brassica napus* L. ssp. *oleifera* under greenhouse conditions through successive transfers (Chapter 2). To infect sugar beet plants, aphids, which had been starved for 2 h., were given an acquisition access period of 5 to 10 min on an infected sugar beet; 10 aphids were then placed on each of the two central plants in the plots on 30 May. These plants, which had about eight leaves, acted as the primary source. Virus spread was weekly monitored until 26 September, counting all plants in the plot exhibiting mosaic symptoms characteristic of the disease. The position of every new plant showing symptoms was marked on a map of the plot. For the analyses, only the plants within 4.6 m from the center of the plot were considered, resulting in a

circular sampled area being used for final analysis (figure 3.1). This radius was chosen to get a sample area directly comparable to transect samples, as discussed in detail below.

Exp. 2 was established at an experimental field of 'Unifarm', Wageningen, in 1996. Four plots of 5 x 5 m were marked in the center of a 3 ha commercial field cv. Univers, as described above. The field was bordered on the four sides by potato, wheat, a fruit orchard and a road. The plots were inoculated on 31 May. The spread was monitored every 2 weeks until 23 September.

Exp. 3 was established at experimental farm 'De Minderhoudhoeve', Swifterbant, in 1996, with 4 plots in the center of a 15 ha field, cv. Auris, bordered by grassland. The virus was inoculated on 4 June, and its spread assessed every 2 weeks until 17 September. In the experiments 2 and 3 (Exp. 2 and 3), row distance was 0.5 m and plant density 10 plants/m^2. The plot size, distance between plots, inoculation procedure, virus and aphid isolates used were the same in Exp. 2 and Exp. 3 as in Exp. 1.

3.3.3. Testing for (an)isotropy:

A program called STCLASS (Nelson, 1995) was used to detect whether the virus was spread in a favored direction (anisotropy). This program performs the analysis in two steps, denominated 2DCLASS and STCLASS. In the 2DCLASS step, the spatial pattern of the disease is analyzed at one single date. In the STCLASS step, two consecutive dates are compared to determined the spatial pattern of the evolution of disease spread to test the hypothesis that the newly diseased plants have a random orientation with respect to the diseased plants of the previous date. In case of significant anisotropy, the infected plants on transects would not be representative for the disease pattern in the whole plot. This would jeopardize the approach using transects.

3.3.4. Transect sampling:

From the original data, a sampling scheme was simulated, considering only plants on, or closest to, the two diagonals traced from corner to corner, passing through the center of the plot (figure 3.1). The plants on the diagonals represent a transect sample. In case of doubles in a row of plants (left or right to the line), only one plant was selected.

3.3.5. Number of plants showing symptoms:

To calculate the number of plants showing symptoms in the plot from the observations made on the transects, an uneven representation of plants for different distance classes in the sample has to be taken into account. In the full counts, the

number of plants at a distance x from the source is proportional to x, as the circumference of a circle is proportional to its radius. In a transect sample, however, all distances are equally represented. A correction must be made when the number of plants showing symptoms for the whole plot has to be estimated from those found on the transects This correction can be made by the use of a weighting factor, representing the number of plants in the different distance classes which are arranged in rings around the source:

$$y = y_0 + \sum_i n_i \cdot f_i \quad \text{, (equation 3.1)}$$

where:

y : estimated number of plants showing symptoms in the patch;

y_0 : number of artificially infected sources;

n_i : number of plants in 'distance class' i;

f_i : observed proportion of plants showing symptoms in distance class i.

The limits of a distance class are defined by the intersections of the diagonal transects and mid-points between adjacent rows (figure 3.1). Accordingly, the number of plants in ring i is $4\pi R^2 Di$, with R = row distance (i.e. 0.5 m), D = plant density (#/m^2) and i the ring number. The operational form of equation 3.1 is therefore:

$$y = y_0 + 4\pi R^2 D \sum_i i \cdot f_i \quad \text{, (equation 3.2)}$$

3.3.6. Fitting a growth curve to describe virus spread in time:

The logistic growth equation was used to characterize the temporal disease progress in each plot:

$$y = \frac{k}{1 + \exp\left(-r(t-b)\right)} \quad \text{, (equation 3.3)}$$

where:

y : number of plants showing symptoms [-];

t : day of the year [d];

k : 'maximum' number of plants showing symptoms [-];

b : day of the year at which y equals $k/2$ [-];

r : relative rate of increase [d^{-1}].

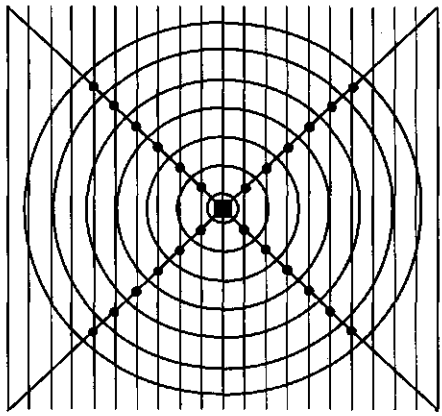

Figure 3.1. Schematic representation of a plot (10 x 10 m). Plants (●) included in the transect sample points occur at the intersection of the plant rows (---) with 2 diagonal transects (—) radiating from the inoculated plants. Concentric circles delimiting sample areas ('distance classes' or 'rings') intersect the diagonals precisely between two adjacent rows, 0.36, 1.07, 1.78, 2.49, 3.19, 3.9 and 4.6 m from the center. The inner ring comprises the source plants (■).

The parameters r and b were obtained by least squared non-linear regression. The parameter representing the maximum number of plants showing symptoms (k) was forced to be equal to the observed final number of plants showing symptoms.

3.3.7. Spatial pattern analysis:

A negative exponential model was used to describe the geometric disease incidence (proportion of plants showing symptoms) with distance from the source:

$y = a \cdot e^{-r \cdot x}$, (equation 3.4)

where:

y : proportion of plants showing symptoms at distance x [-];

x : distance (rows from the source, along a diagonal transect) [-];

A : intercept; theoretical proportion of plants showing symptoms in the center of the patch [-];

R : slope parameter [-];

The model implies that disease incidence at the source equals a, while the proportion of plants showing symptoms on the transect diminishes with a factor $q = e^{-r}$ for each distance class from the initial source. The mean distance class (distance weighted by the proportion of plants showing symptoms) for a negative exponential gradient of disease is:

$$\bar{i} = \frac{\sum_{i=1}^{\infty} i \cdot a \cdot q^i}{\sum_{i=1}^{\infty} a \cdot q^i} = \frac{1}{1-q}, \text{ (equation 3.5)}$$

If the gradient is truncated at a certain class n, the mean distance class within (and including) that radius is:

$$\bar{i} = \frac{\sum_{i=1}^{n} i \cdot a \cdot q^i}{\sum_{i=1}^{n} a \cdot q^i} = \frac{1}{1-q} - n \cdot \frac{q^n}{1-q^n}, \text{ (equation 3.6)}$$

The mean distances, in distance class units, were converted to meters ($\bar{d}$) by multiplication of the mean distance class with the width of each class, $R\sqrt{2} = 0.707$ m.

This mean distance is called here the "weighted mean distance" to the source, having in mind that the distance (class) of an individual plant showing symptoms is inversely weighted with the total number of plants assessed at that distance (class). The expression for $\bar{d}$ based on geometric decay applies to both transect and whole plot counts.

Using the original whole plot data directly, instead of the geometric decay data, the weighted average distance to the source is actually the harmonic mean of the individual distances:

$$\bar{d} = \frac{\sum_i n_i}{\sum_i n_i / d_i} \text{ (equation 3.7)}$$

where:

d_i : distance from source of the plants in distance class i (m)

n_i : number of plants showing symptoms in distance class i

3.3.8. Model fitting and statistical analysis:

Logistic growth curves and geometric disease gradients were fit with the ordinary least squares method, using the non-linear regression procedure of SigmaStat for Windows Version 1.0 (Jandel Corporation, 1992-1994). The purpose of sampling instead of assessing all plants is to estimate the mean value of any characteristic due to a treatment applied to a number of replicated plots. The sampling data is compared with the whole plot data by estimating the mean value of the studied characteristic The full counts are supposed to give true error-free values, so they produce an unbiased estimator of the true population mean value with zero variance. Use of counts obtained in the sampling method will enhance the variability. As the transect method is a systematic sampling method, it may produce a systematic error, or an error that varies with the number of plants showing symptoms. It is assumed that the standard error of the mean of the transect measurements (MSE), used as estimator for the "true" area mean, is build up as:

$$MSE = \sigma^2_{bp} / n + Bias^2 + \sigma^2_{m} / n + 2\, \sigma_{bp} \cdot \sigma_{m} \cdot \rho / n \quad \text{(equation 3.8)}$$

where:

σ^2_{bp} : is the variance between plots of the whole plot measurements. It represents the natural variability between plots ;

σ^2_{m} : is the variance between methods. It represents the random component of the transect sample error;

Bias : is the bias of the transect method. It represents the systematic part of the transect sampling error;

ρ : is the correlation coefficient of the whole plot measurement and the difference between methods (transect minus whole plot).

One-way analyses of variance (with experiments as fixed factor) were conducted for all estimated descriptors of disease spread to estimate bias and (co)variance components under the a priori assumptions that the (co)variance components are constant over experiments and that the errors are normally distributed. The analyses were done using the GLM and ANOVA procedures in SAS for Windows Version 6.11 (SAS Institute Inc., 1989-1995).

3.3.9. Sampling time evaluation:

To determine the usefulness of sampling the plots by the transect method, one experimental plot was inspected laying a rope from the center of the plot (source plants) to each of the four corners. The person who evaluated the field walked along the rope and inspected the closest plant to the rope. The time required to evaluate a plot by both methods was measured a few times during the experimental period.

3.4. Results:

3.4.1. Reliability of visual observations:

In the Netherlands, sugar beet is infected by four virus species - *Beet yellows* closte*rovirus* (BYV), *Beet mild yellowing* luteo*virus* (BMYV), *Beet necrotic yellow vein* furo*virus* (BNYVV) and BtMV. The symptomatology of BtMV distinctly differs form those evoked by the three other viruses. ELISA tests confirmed, in all cases, that the causal agent of the disease was BtMV, warranting the use of visual observations during further evaluations. Natural occurrence of BtMV is very low; hence it may be safely assumed that all observed disease plants had become infected from the artificially inoculated sources. The absence of significant primary infections from other sources was confirmed by observations in non-inoculated plots.

3.4.2. (An)isotropy of disease maps:

During the evaluations, it was noticed that infection in the 13 plots showed an aggregated pattern around the source at all dates that inspections were made. No evidence was obtained for wind effect, which was most often from the south west (Wageningen Agriculture University - Laboratory of Meteorology). The pattern of disease spread was circular in all cases (figures 3.2 and 3.3).

Anisotropy was not detected in any of the 13 plots by the STCLASS program. A characteristic example of an STCLASS output is shown in figure 3.4. The occurrence of a clump of #'s in the STCLASS output indicates a clustered pattern of the spread, as these symbols represent distances between plants showing symptoms that significantly outnumber the expected value if disease spread was at random.

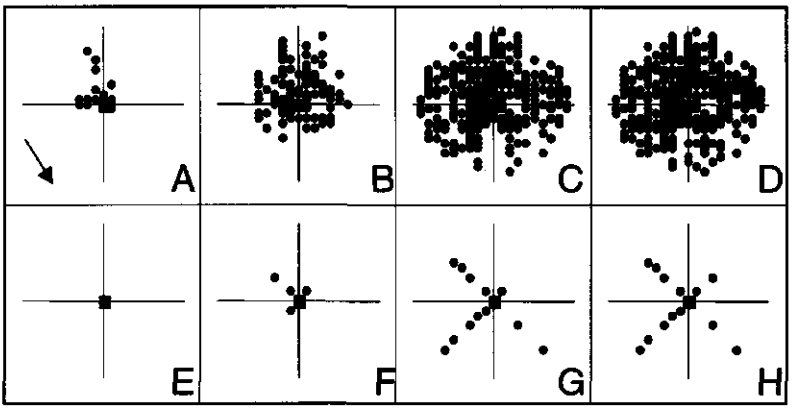

Figure 3.2. Development of the infection in plot 5 of Exp. 1. A-D, full counts at July 17, August 22, September 5 and 26, 1995, respectively; E-H, plants showing symptoms on the transect at the same dates. The arrow indicates the prevailing wind direction (from the South West). The North is to the right.

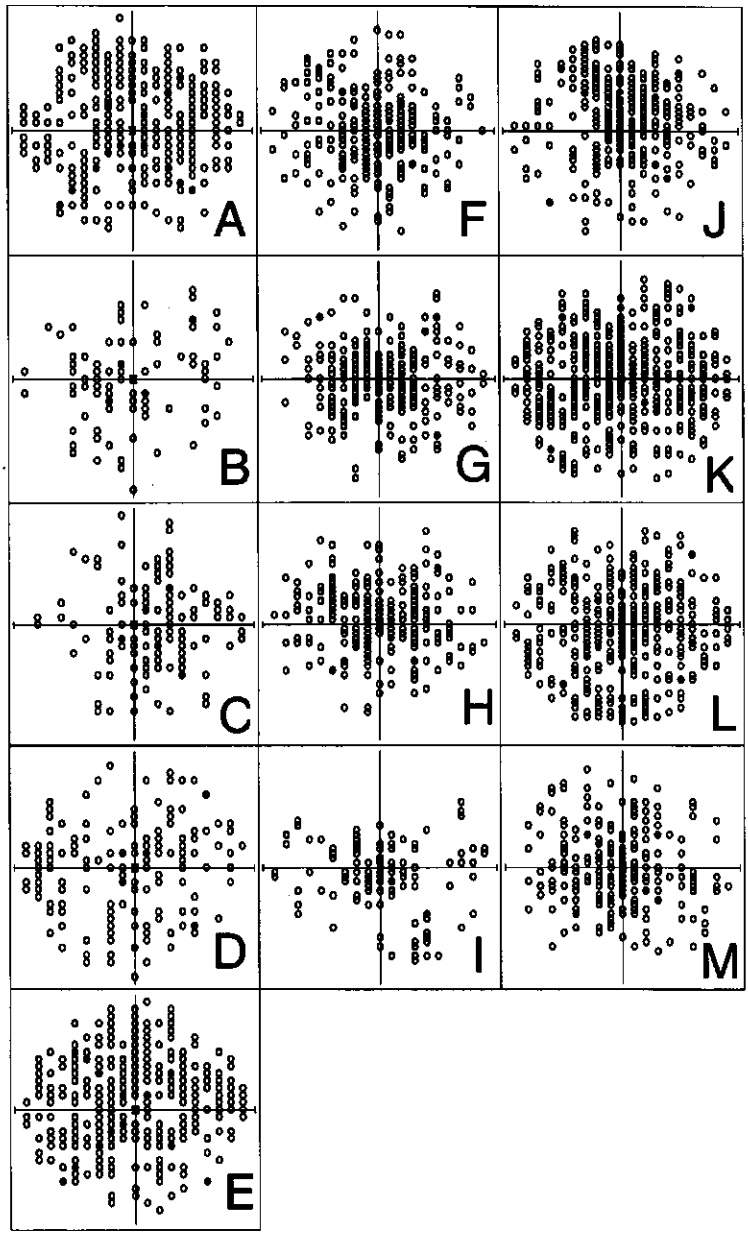

Figure 3.3. Position of the plants showing symptoms in each plot on the last day of evaluation in Exp. 1. (A-E), Exp. 2 (F-I) and Exp. 3 (J-M). (o - plants showing symptoms, • - plants showing symptoms monitored in the transect sample).

Superimposed Distance Analysis Matrices for STCLASS and 2DCLASS

1) STCLASS for dates 236 to 267

2) 2DCLASS for date 267

Dataset = w:\andre\stclass\sheets\plbr42d.prn

```
                                        X-VAL
     01234567890123456789012345678901234567890123456789
Y-VAL
 0   ##################>--$--SSSSSSS<SSSSSSSSS<SSS<<<<---
 1   ##################>-------SSS<SSSSSSSSSSSSSSSSSS<--
 2   ##################>>----S$SSSSSSSSSSSSSSSSSSSSS<S<--
 3   ############>###>>>>->--S$SSSSSSSSSSSSSSSSSSSSS<<--
 4   ################>>->-$--S-SSSSSSSSSSSSSSSSSSS<<---
 5   ###############>#>>----$$-S-S<SSSSSSSSSSSSSS<SS<<--
 6   #############>>>>>----$$$$--SSSSS<SSSSSSSSSSS-<--
 7   ########>##>##>>>>>--$--$S-SSSSSSSSSSSSSSSSSS<<--
 8   ############>>>>>->----$$$$S--<SSSS<SSSSSS<SS<SS<---
 9   -##>>>>>>-#->->->->--$-$-S-SSSSSSS<SSSSSSSSSS<<---
10   ----#-->------>-$$-----SS$SSSSSSSSS<<<<SSSS<S<<<---
11   ---+#----+--#----------SS-S<SSSSSSSS<SSSS<SS<<<---
12   --+<-----<----------<---SSS<SS<<S<<<S<SSS<<S<<----
13   -+<-<---<-<---<---<<--<<-SS<SS<<<S<<S<S<SSS<<-<----
14   -<-S<--<<-<<S--<<SS<<--<-<S<<<-SSS<<-<-<S<S<<<-----
15   --<<<-<<--<<---<-<<S-<<<-<<<-SS<S-SS<<<SS-<<<------
16   <<---<<<--<<--<-<-<<<<<-<<SS<<<<S-<<-<<<--<<<------
17   <S<SS<<<<<<-<<--<-----<<-<---<-<<-----<-<--<--------
18   -S<<<-<-<<-<<<<-<<<<<<-S<-<SSSSS<<<<<---------------
19   -<<<<<----S---<<-<--<----<----<-------------------
20   -------------<-------------------------------------
```

```
# = SCF Significantly Greater than Expected in STCLASS and 2DCLASS (P<=0.05)
S = SCF Significantly Less than Expected in STCLASS and 2DCLASS (P>=0.95)
+ = SCF Significantly Greater than Expected in STCLASS Only (P<=0.05)
$ = SCF Significantly Less than Expected in STCLASS Only (P>=0.95)
> = SCF Significantly Greater than Expected in 2DCLASS Only (P<=0.05)
< = SCF Significantly Less than Expected in 2DCLASS Only (P>=0.95)
- = SCF Not Significant
```

Figure 3.4 (previous page). Characteristic STCLASS output from one of the experiments, comparing two evaluation dates in one plot. The points on the X-VAL axis represent plants in the row and the points in the Y-VAL axis represent the rows. Each relative location among two infected plants represented by the # symbol indicate that the number of observed pairs in this category was greater then expected if disease spread (STCLASS) or incidence (2DCLASS) had a random pattern. The clusters of # identify cluster size and shape, which, in the case of this example, indicates that no anisotropy was detected. S symbolizes pairs of infected plants which were observed in lower numbers than expected in the case of a random pattern for the STCLASS and 2DCLASS tests. The symbols + and $ refer to the pairs that occurred at a higher or lower frequency than expected by the STCLASS test only. The symbols > and < refer to the pairs that occurred at a higher or lower frequency than expected by the 2DCLASS test only. The symbol – refers to non-significant distance class pairs.

Table 3.1. Results of the analysis of variance of the final number of plants showing symptoms of BtMV infections determined by the two sampling procedures.

Method	Experiment			p	$\hat{\sigma}^2$
	1 (n=5)[1]	2 (n=4)	3 (n=4)		
whole plot	159 ± 33[2]	166 ± 23	257 ± 34	0.10[3]	4200
transect	129 ± 41	211 ± 47	278 ± 43	0.09	8183
difference	-30 ± 11	45 ± 24	21 ± 40	0.15[4]	2801
overall-bias[5]		8.9 ± 14.7		0.56	
□[6]		0.17		0.61	

[1] number of replications;

[2] sample mean ± SEM;

[3] p-value for the absence of experiment effect;

[4] p-value for the absence of experiment effect in bias;

[5] estimated mean difference and SE and p-value for absence of overall bias;

[6] correlation between whole plot measurements and difference between sampling methods.

3.4.3. Final patch size:

The final number of plants showing symptoms, determined by the whole plot count, was not statistically different among the three experiments (p=0.10, table 3.1). Estimates of the final number of plants showing symptoms in all experiments, when using the transect data, were of the same order of magnitude as those obtained with the whole plot count, but the standard errors were greater. No significant differences in the final number of plants showing symptoms were detected between the experiments when using the transect method (p=0.09).

The estimates for "between plots" error and the difference error (transect minus whole plot) were: s^2_{bp} = 4200 (with 4+3+3=10 df) and s^2_m = 2801 (with 10 df). These tests showed no significant experiment effect on bias (p=0.15). The overall-bias between the whole plot counts and the estimated number of plants showing symptoms using the transect sampling method in single plots is 8.9 plants/plot, an insignificant number given the associated estimated standard error of 14.7. An upper confidence limit for the bias would be 38.3 (mean + 2*SE). Furthermore, no significant correlation between whole plot count and difference (transect minus whole plot) was found (r=0.17, P=0.61 2-sided). The variability in the number of plants showing symptoms among plots (σ^2_{bp}) and the variability due to the sampling method (σ^2_m) are of the same magnitude. The variance of transect based estimates of the number of plants showing symptoms is a factor 2 greater than for whole plot estimates. This means that the number of plots should be (approximately) doubled to obtain a similar precision when using the time-saving transect method. The time saved per plot will compensate for the required increase in plot numbers. Some precaution is necessary due to possible errors in the estimated variances; for 10 df, a 95% confidence interval for a variance (based on χ^2) runs from about 1/3 to twice the estimated value. Consequently, the variances estimated may be substantially different from their 'true' values, while the factor 2 is only an estimate for the necessary sample size ratio.

3.4.4. Disease progress curves (DPC):

The coefficients of determination (R^2) of the logistic fits ranged from 0.99 to 1.00 for whole plot counts and from 0.75 to 1.00 for the transect estimates. While conducting the field trials, the number of plants showing symptoms did not increase in the last two evaluations. An increase in the final number of plants showing symptoms after that period could not be expected from a biological perspective because the plots were monitored for more than two weeks after the last aphid flight, when all infected plants should have developed symptoms, taking into account the incubation period (Chapter 2). It was decided, therefore, to force k, equating it with the last observed number of plants showing symptoms. Some anomalous fits for transect sampled plots, which resulted in estimated epidemics lasting over one year and having a maximum number of infected plants twice or four times bigger than the plot size, were avoided in this way. Forcing k resulted in adequate fits for those plots while it did not affect R^2 or MSE.

The parameters b (mid point of DPC) and $k*r$ (~ maximum absolute rate of spread) obtained by fitting each individual plot to the logistic equation were also analyzed by ANOVA (table 3.2). The inflection point (b) of the DPC did not differ between the methods used while the estimated variance ratio (transect/whole plot) was

Table 3.2. Results of the analysis of variance of the estimated parameters of the logistic growth equation that describes the disease progress.

Parameter	Method	Experiment			p	$\hat{\sigma}^2$
		1 (n=5)[1]	2 (n=4)	3 (n=4)		
b	whole plot	239 ± 1[2]	240 ± 1	235 ± 4	0.21[3]	20.6
	transect	242 ± 4	237 ± 2	237 ± 1	0.33	30.3
	difference	3 ± 3	-3 ± 2	2 ± 5	0.50[4]	52.9
	overall-bias[5]	0.86 ± 2.02			0.73	
	ρ[6]	-0.65			0.03	
*k*r*	whole plot	5.66 ± 1.46	10.24 ± 2.22	13.08 ± 1.62	0.04	13.33
	transect	7.03 ± 4.11	24.69 ± 11.40	18.91 ± 6.26	0.26	236.31
	difference	1.38 ± 2.92	14.46 ± 10.37	5.83 ±5.58	0.39	183.47
	overall bias	6.77 ± 3.76			0.10	
	ρ	0.40			0.22	

[1] number of replications;

[2] sample mean ± SEM;

[3] p-value for the absence of experiment effect;

[4] p-value for the absence of experiment effect in bias;

[5] estimated mean difference and SE and p-value for absence of overall bias;

[6] correlation between whole plot measurements and difference between sampling methods.

1.5. This result indicates that a two fold increase in replications, recommended for estimating the maximum number of infected plants, would also suffice to estimate *b*. The product *k*r*, was analyzed instead of the rate parameter itself, because it measures the maximum rate of spread. The variability found for the transect method did not allow to detect differences between experiments, which were, however, identified by the whole plot sampling method. The estimated variance ratio found indicated that an 18 fold increase in the number of replicates would be required for the same precision in the estimation of *k*r*. Hence, to estimate the actual 'rate' of spread, transect sampling is found not to be suitable.

3.4.5. Disease gradients and distance of plants showing symptoms from source:

The disease gradients observed in the maps were quantified by fitting an exponential decay model for each individual plot (table 3.3). Good fits, characterized by a high coefficient of determination (R^2), were generally obtained for the exponential decay fits (0.83-0.99 for the whole plot samples and 0.47-0.94 for the transect method)

Table 3.3. Results of the analysis of variance for the estimated parameters of the exponential decay equation that describes the spatial pattern of the disease at the last observation day.

Parameter	Method	Experiment			p	$\bar{\sigma}^2$
		1 (n=4)[1]	2 (n=4)	3 (n=4)		
A	Whole plot	0.90 ± 0.09[2]	1.23 ± 0.05	1.06 ± 0.03	0.01[3]	0.015
	Transect	0.94 ± 0.02	1.49 ± 0.09	1.23 ± 0.11	0.01	0.029
	Difference	0.04 ± 0.09	0.26 ± 0.12	0.16 ± 0.09	0.37[4]	0.044
	Overall-bias[5]		0.16 ± 0.06		0.03	
	ρ[6]		-0.57		0.08	
R	Whole plot	0.288 ± 0.04	0.437 ± 0.06	0.256 ± 0.03	0.03	0.009
	Transect	0.384 ± 0.13	0.456 ± 0.14	0.272 ± 0.05	0.55	0.052
	Difference	0.156 ± 0.11	0.017 ± 0.07	0.016 ± 0.05	0.42	0.027
	Overall-bias		0.063 ± 0.05		0.22	
	ρ		0.47		0.17	

[1] number of replications;

[2] sample mean ± SEM;

[3] p-value for the absence of experiment effect;

[4] p-value for the absence of experiment effect in bias;

[5] estimated mean difference and SE and p-value for absence of overall bias;

[6] correlation between whole plot measurements and difference between sampling methods.

and low residual sum of squares, except for the transect method result for plot 2 of Exp. 1. This plot was therefore eliminated from this analysis. The parameters of the negative exponential equations between sampling methods were compared using ANOVA. Both sampling methods resulted in a steeper profile in Exp. 2. This type of profile indicates a comparatively small average distance of the plants showing symptoms to the source, and, consequently, a smaller patch area. As for the DPC, differences among experiments regarding the decay parameter could not be detected by the transect method.

The average distance of the plants showing symptoms from the center of the plot during the growing season was directly calculated from the counts, using the harmonic mean of all distances to the source (equation 3.7; table 3.4). Also, the average distance of plants showing symptoms from the source was calculated using the rate of the exponential decay gradient estimated at the end of the season (equation 3.6; table 3.4). No difference could be observed between the average distances calculated by the two methods. A difference was detected in the average distances between the counts of the whole plots. Using the transect estimates, the small differences between experiments were not detected due to the higher variance caused by this sampling method.

Table 3.4. Results of the analysis of variance of the estimated distances of plants showing symptoms from the center of the plot at the last observation day.

Variable	Method	Experiment			p	$\hat{\sigma}^2$
		1 (n=5)[1]	2 (n=4)	3 (n=4)		
Distances directly[6]	Whole plot	2.01 ± 0.06[2]	1.68 ± 0.04	1.93 ± 0.08	0.033[3]	0.017
	Transect	1.94 ± 0.15	1.70 ± 0.14	1.91 ± 0.13	0.47	0.093
	Difference	-0.06 ± 0.10	0.02 ± 0.10	-0.02 ± 0.11	0.85[4]	0.046
	Overall-bias[5]		-0.026 ± 0.059		0.67	
	ρ[8]		0.49		0.12	
		(n=4)	(n=4)	(n=4)		
Distances by rate[7]	Whole plot	2.02 ± 0.08	1.68 ± 0.09	1.97 ± 0.06	0.022	0.023
	Transect	1.80 ± 0.20	1.69 ± 0.17	1.94 ± 0.09	0.56	0.103
	Difference	-0.23 ± 0.16	0.02 ± 0.08	-0.03 ± 0.10	0.34	0.056
	Overall-bias		-0.078 ± 0.068		0.28	
	ρ		0.34		0.33	

[1] number of replications;

[2] sample mean ± SEM;

[3] p-value for the absence of experiment effect in the distances;

[4] p-value for the absence of experiment effect in bias;

[5] estimated mean difference and SE and p-value for absence of overall bias;

[6] distance determined by using the collected data directly;

[7] distance determined by the rate parameter of the exponential decay function, corrected for the outlier plot;

[8] correlation between whole plot measurements and difference between sampling methods.

The two methods described to determine the distances between the source and infected plants were compared using the whole plot counts (table 3.5). No differences for distances were found among both methods and the differences between experiments were significant in both methods used.

Variance ratios for the parameter r of the exponential decay equation, for the distance from the center estimated by direct measurements or by the rate were 6, 6 and 5, respectively. These ratios may indicate that a 6-fold increase in the number of replications would result in the same precision of the estimated rates and distances as a full count.

Table 3.5. Results of the analysis of variance of the distance between the source and the plants showing symptoms as calculated by direct measurement and estimated form the rate of the exponential decay equation using whole plot counts.

Method	Experiment			p	$\bar{\sigma}^2$
	1 (n=5)[1]	2 (n=4)	3 (n=4)		
rate	1.98 ± 0.07[2]	1.68 ± 0.09	1.97 ± 0.06	0.03[3]	0.024
direct	2.01 ± 0.06	1.68 ± 0.04	1.93 ± 0.08	0.03	0.017
difference	0.03 ± 0.03	0.00 ± 0.05	-0.04 ± 0.02	0.43[4]	0.005
overall-bias[5]		-0.002 ± 0.02		0.92	
ρ r[6]		-0.54		0.09	

[1] number of replications;

[2] sample mean ± SEM;

[3] p-value for the absence of experiment effect;

[4] p-value for the absence of method effect in bias;

[5] estimated mean difference and SE and p-value for absence of overall bias;

[6] correlation between rate measurements and difference between methods.

3.4.6. Correlation between whole plot counts and difference between sampling methods:

The *b* parameter of the logistic equation was the only parameter that showed a significant correlation between the whole plot counts and the difference between the sampling methods ($\bar{\rho}$=-0.65, p=0.03, Table 3.2). Values for correlation coefficients with the respective statistical methods are presented in Tables 3.1 to 3.5, indicating that the transect sampling method did not bias the estimates for any epidemic descriptor.

3.4.7. Overall temporal and spatial patterns:

Although statistical fits showed a higher variability, and some anomalies in the results of the transect method were found, averaged disease progress curves, exponential gradients and average distances to the source for the transect based results indicate similar spatio-temporal trends as were found using the whole plot information (figure 3.5).

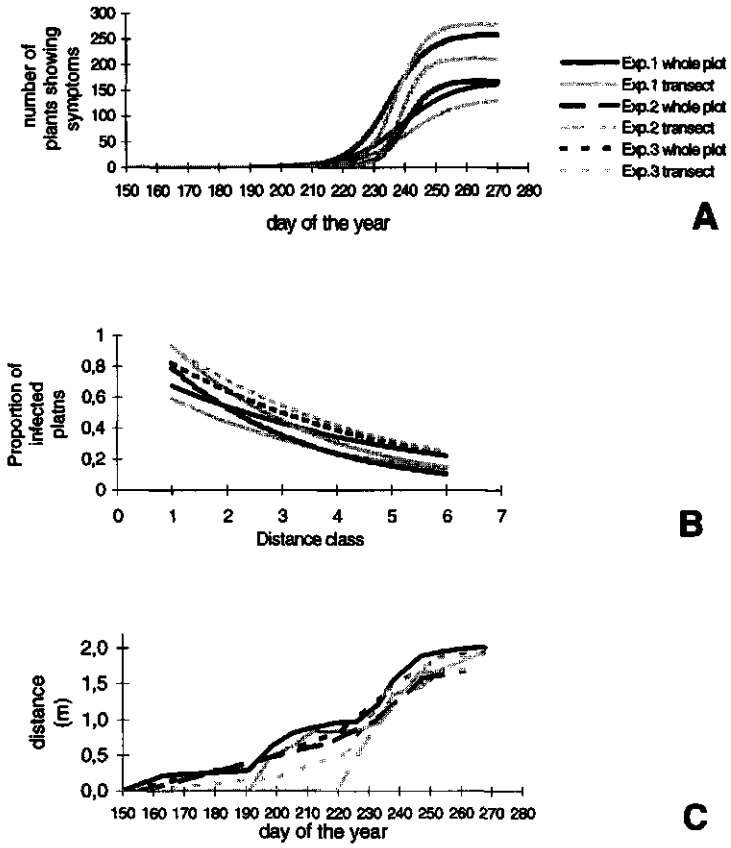

Figure 3.5. Comparison of the spread of BtMV in three field experiments using 2 sampling methods. A, disease progress curves; B, disease gradients and C, distance of plants showing symptoms from the source. Black lines indicate whole plot counts while gray lines indicate transect estimates.

3.4.8. Time required to monitor the plots:

The evaluation of the plot using the proposed transect method, by laying a rope from the center of the plot to each of its corners, took 5 to 10 min. The average time for counting all the plants in a plot, varied from 30 min at the beginning (when few infected plants were present) to 2.5 h from the middle of the season onwards (when most of the infected plants were to be found). This indicates that the transect method indeed results in time savings.

3.5. Discussion:

In the current studies the spread of BtMV in sugar beet was characterized by isotropic patches around the primarily infected source. These patches increased in size during the growing season. The logistic model could fit the temporal pattern. The clustered and isotropic patterns, observed in this study, have also been reported for other potyviruses and other non-persistently transmitted viruses (Dahal, 1992; Eckel and Lampert, 1993; Nelson and Campbell, 1993; Perring *et al.*, 1992). Clustering in the spread of *Soybean mosaic virus* in soybeans was demonstrated by Irwin and Goodman (1981) by analyzing field data using an exponential decay equation. A close analysis of the field maps obtained after each inspection indicated that the spread of BtMV was characterized by an isotropic pattern indicating that wind or an early canopy closure in the row had no effect on the spread. The absence of anisotropy was supported by the analysis of the data using STCLASS (Nelson, 1995), which showed almost circular clusters of the disease (figure 3.3). Nemecek (1993) also observed a clustering of diseased plants in potato infected with *Potato virus Y* (PVY). The main factors that may lead to clustering are the pattern of vector dispersal, inhomogeneities present in the field, unequal susceptibility of the individuals and different attractiveness of plants. The last three factors were likely not to affect the pattern. The fields used were homogeneous, no differences in susceptibility of the crop to the virus occurred and no evidence was observed for differences in attractiveness for aphids of plants in the field. Therefore, the host selection and dispersal behavior of the alatae aphids (Cooke and Scott, 1983; Eckel and Lampert, 1993; Harrewijn *et al.*, 1981; Perring *et al.*, 1992) is the most likely explanation for the aggregation of the spread of BtMV in this study. The contribution of apterae is expected to be rather limited due to short average virus retention and the distances they have to cover to reach another plant.

One of the purposes of this study was to evaluate the suitability of a less labor-intensive method to analyze the spatio-temporal spread of BtMV. In the middle of the season, when the spread reaches its maximum speed, 2 h were, on average, required to monitor a single plot. A new method, designated the transect method, was introduced to reduce the inspection time. This method, laying a rope from corner to corner of the plot, passing through the center, took less than 10 min to assess the same plot. In this way, the maximum number of infected plants, as well as the logistic growth and exponential decay equations could still be estimated, although with generally lower precision. However, the reduction in precision was small enough to be compensated by using more replicates, and still save time. By establishing the rules for the sampling, and making the observations only on the closest plant to the rope, bias in selecting a certain class of plants (healthy or diseased) is effectively avoided. In fact, as for any sampling

procedure, fixed standards must be established prior to applying the method to avoid induced bias by the person who is performing the sampling.

The estimated number of plants showing symptoms at the end of the season (k) obtained by the two sampling methods did not show systematic differences and in both whole plot and transect data series, no differences among the three experiments were detected (ANOVA). However, the values found for the rate parameter, r, which were estimated with the equations 3.3 and 3.4 had a high variance and, as a consequence, no differences could be detected between the experiments using the transect method whereas such differences were detected using the whole plot data. As the sample sizes were equal, we expect less power in tests using transect data.

The logistic equation was selected among different growth equations tested and chosen for its simplicity and capacity to fit the data. There is no sound mechanistic interpretation of the biological process in the used equation, as the rate of spread is not so much limited by a lack of healthy plants (k - y) as by a lack of vectors (related to r). In the preliminary tests, the Gompertz model was also fit to the data, but the logistic model resulted in higher values for R^2 and smaller residual sum of squares (RSS). Therefore, the logistic model was used for the comparisons between the sampling methods. The parameter b, characterizing the time at which half of the spread had occurred, could also be reasonably estimated by the transect method. Fitting single equations to individual plots revealed also that the product $k*r$ showed a systematic difference between the two monitoring methods. In all three experiments, the transect sampling overestimated r (data not shown), thus resulting in a difference observed for $k*r$. This overestimation is not difficult to understand and could be clearly noticed when looking at the fitted disease progress curves (figure 3.5). When only a few plants showing symptoms are found early in the epidemics, the probability of finding infected plants will be low in the first ring due to the low number of sample points. This probability will increase during the season as the number of plants showing symptoms increases. As the spread progresses, infection is detected, resulting in a steeper DPC.

The advantage of using a time saving procedure is that it opens the possibility to study larger plot areas, and to increase the number of treatments, replications and sites. When evaluating all plants in a plot, the number of plants to be examined is so high that one can not manage much repetition. Considering the plot size used in these experiments (650 plants/plot), only 4% of the plants were evaluated by the transect sampling scheme. The size of the plot must be chosen regarding the crop characteristics, such as plant size and the expected size of patches.

The reduction in precision of the data collected in individual plots is acceptable when assessing the temporal aspects of spread. For the spatial pattern evaluation, errors of the estimates were higher. Nevertheless, transect estimates may be applied in

exploratory surveys, provided that the location of primary sources is known. The gain in time gives the opportunity to assess the variation in spread of the disease among different fields, which is valuable as it can provide more information regarding effects of location, cultural practices and other variables that differ between fields on the development of epidemics. An increase in the number of replications by a factor 2 would result in a more precise determination of temporal pattern of the disease, but an increase of a factor 6 is estimated to produce the same precision in the description of the spatial pattern. Even with an increase of the number of replications, it is still a time saving method and enables the sampling of a greater number of objects.

In studies of virus epidemiology, existing field to field variation and variation in virus spread among different spots in the same field must be considered (Madden *et al.*, 1987b; Mora-Aguilera *et al.*, 1996). A time saving sampling scheme that makes a study of several patches distributed over more sites feasible, might give a better insight in the epidemiological process developing in an area than a more detailed but time consuming method that can not be repeated in different fields.

Therefore, usage of transect method to monitor the growth of disease patches from known sources may help to put resources where they may give the most insight in the processes of virus spread and its control.

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Chapter 4

Effect of primary infection date on the secondary spread of *Beet mosaic virus*

4.1 Abstract:

Four field experiments were conducted to study the effect of primary infection date on the spread of *Beet mosaic virus* (BtMV) in sugar beet field plots. In the center of each plot, two plants were inoculated, using *Myzus persicae* as vector. The development of disease patches around these sources was monitored from the moment of primary infection until the end of the season by periodically inspecting all the plants on two diagonal transects through the plot. Early inoculation resulted in greater spread than late inoculation, but any inoculation that was made before the onset of flight of aphid vectors resulted in similar spread. The relation between virus spread and alatae aphid pressure was studied by regression analysis and by calibrating parameters for a mechanistic simulation model. Three measured indicators for aphids pressure were compared with regard to their explanatory value with respect to virus spread: aphid catches in green water traps in the crop, catches in a 12 m high suction trap at a distant location, and infection of bait plants from adjacent virus source plants. Daily total aphid catches by the suction trap provided the best statistical explanation for the development of the disease in our studies. The parameter describing the relationship between vector pressure and the rate of disease progress was remarkably robust, varying in value less than 10% between treatments (infection date) within experiments, and less than a factor 2 between the four experiments. The results show that development of BtMV in sugar beet crops occurs in patches, and that the rate of spread can be well explained by taking into account vector pressure. The results may be used to calculate expected spread of a potyvirus, based on initial primary infection and vector abundance.

4.2. Introduction:

Like all members of the genus *Potyvirus*, *Beet mosaic virus* (BtMV) is a non-persistently transmitted virus that is vectored by several aphids species at different efficiencies (Chapter 2; Sylvester, 1952; Zitter, 1977). Alatae aphid migrants, which show an active host plant probing behavior while searching for new hosts, are believed to play a major role in the spread of potyviruses as they can acquire and transmit virus during their short probes (Zitter, 1977, Nemecek, 1993). Vector importance under field conditions is determined by a multitude of factors, including vector abundance, vector phenology, transmission efficiency and vector behavior. It is not clear which aphid species are the most important vectors of BtMV (Watson and Healy, 1953). Transient visits of aphids can not be controlled by the use of insecticides, and consequently, chemical aphid control does not

prevent the spread of the non-persistently transmitted viruses (Bayoumi and Kummert, 1986; Pirone *et al.*, 1988; Roberts *et al.*, 1993).

The relation between aphid population trends and spread of potyviruses under open field conditions has been studied by a number of approaches, including the analysis of time profiles, the determination of regressions and correlations and by mathematical modeling. Eckel and Lampert (1993), van Hoof (1977) and Karl *et al.* (1983) compared time profiles of disease progress with those of aphid flight. They did not find a correlation between their catches and the spread of the studied potyviruses. Mora-Aguilera *et al.* (1992) analyzed the correlation between the spread of *Papaya ringspot virus* in papaya with the sum of the number of aphids of potential vector species in each catch. Although the correlations were statistically significant, the correlation coefficients were low. Linear regression between disease progress and aphid counts was used in several studies. Usually, a relationship between spread and number of aphids is found, with significant regression coefficients for the total counts of all species (Madden *et al.*, 1987b) or for some specific species (Garrett, 1988; Rivas Platero and Larios, 1994). In some studies, however, no significant regressions were obtained (e.g. Watson and Healy, 1953). Two general findings in these studies are that the migration of alatae aphid population coincides in time with the disease progress curves, while it is not possible to single out one aphid species as the only or major vector. The spread of potyvirus in a given pathosystem is correlated to the abundance of migrating aphids.

The use of mathematical models of disease dynamics of a disease can help to elucidate the mechanisms involved in the spread of these viruses. The development of such models (Marcus and Raccah, 1986; Ferriss and Berger, 1993) depends on laboratory and field data to provide for parameterization of developed models and validation of their predictions (Jeger *et al.*, 1998). Otherwise it will not be possible to relate the general principles and expectation generated by such models to the actual phenomena in the field. The focus of this study is to provide such validation data, and obtain realistic estimates for parameters.

The majority of field studies on the epidemiology of potyviruses have been done with primary infection (from sources outside the field) and secondary infection (from sources within the field) occurring simultaneously and uncontrolled. In many instances, data on disease development were collected in a spatially non-explicit manner. Due to the methodology used in those studies, it is impossible to disentangle primary infection and secondary spread. In the studies reported here, primary infections were made deliberately, using a virus (BtMV) which is rare in the Netherlands. Therefore, it was possible to study a pure process of secondary spread in a spatially explicit manner.

The raised research questions were how do changes in primary infection date affect the secondary spread of BtMV in sugar beet; in which way is the extent and pattern of

secondary spread related to the abundance, time profile and species spectrum of vectors; and whether it is possible to describe the secondary spread with a simple mechanistic simulation model, using accepted principles of disease epidemics. The first question was addressed in four replicated field experiments in which primary infection was introduced at different dates. The second question was addressed by regression and correlation analysis between observed virus spread in the experiments and aphid catch data from a suction trap. The third question was addressed by calibrating a basic epidemiological model to the virus spread data, using aphid catch data as a forcing function, and studying goodness of fit and robustness of parameter values.

4.3. Material and Methods:

4.3.1. Experiments:

A series of four field experiments was conducted to evaluate the spread of BtMV from two plants inoculated at different dates. Commercial fields of sugar beet, cvs Univers or Auris, with a plant density of 10 pl/m^2 where divided in plots of 25 x 25 m.

In 1995, an experiment was conducted at experimental farm De Bouwing (Exp. 1), Zetten, The Netherlands. This experiment had a random block design with four inoculation dates as treatments (16 May, 6 June, 27 June and 18 July) and five replications. The plots were inoculated by placing 10 viruliferous aphids on two plants in the center of each plot. In each block, one non-inoculated plot was used to verify the absence of inter plot interference and primary infections form outside the field. The plots were weekly monitored for the presence of plants showing BtMV symptoms, using the transect method described in Chapter 3. The transect extended to 17 rows at both sides of the center row, resulting in a circular sampled area of 12.4 m radius and 480 m^2 area. Plants showing mosaic symptoms were marked with a bamboo stick and their position was recorded. The plots were monitored until the end of September.

A second experiment (Exp. 2) in 1995 was conducted at experimental farm De Minderhoudhoeve, Swifterbant, The Netherlands, approximately 100 Km North from Exp. 1. This experiment had a random block design with four infection dates (8 June, 22 June, 6 July and 20 July) and five replications. One plot per block remained non-inoculated.

In 1996, one experiment was conducted at De Minderhoudhoeve (Exp. 3), and another at UNIFARM, Wageningen (Exp. 4). Both experiments had a random block design with four replications. Three inoculations were made in Exp. 3 (18 June, 2 July and 16 July) and four in Exp. 4 (31 May, 14 June, 28 June and 12 July). Each experiment had one non-inoculated control plot in each block. Plot size and monitoring procedure were the

same as described for Exp. 1. Patch size was characterized by the mean distance of the infected plants from the inoculum source (Chapter 3) using equation 3.6.

4.3.2. Aphid population:

Aphid population data were provided by the Nederlandse Algemene Keuringsdienst (NAK), Emmeloord, The Netherlands. These data, obtained with the Tollebeek suction trap, located about 20 km from Minderhoudhoeve and 100 km from Wageningen and Zetten, were used in the multiple regression and correlation analyses and calibrations.

The suction trap data only covered the period from the beginning of May until August 14 for 1995 and August 22 for 1996. However, for the calibration studies of section 4.3.3, the temporal aphid population until the end of September was needed. Supplementary data were constructed on the basis of the results of bait plant trials (below).

While aphid traps measure vector abundance, bait plant trials measure the resulting vector activity. In both years, 12 consecutive batches of 24 x 10 potted uninfected beet plants in the fourth leaf stage were exposed to an adjacent row of infected sugar beet at 30 cm distance, in the field. The exposure period was one week, and new batches were placed in the field every two weeks from March until September. New source plants were placed in the field as needed to keep their condition as similar as possible throughout the season. The proportion of infected plants was recorded and vector activity estimated using Gregory's multiple infection transformation (Gregory, 1948):

$$v = \ln \frac{n}{n-k}, \text{ (Equation 4.1)}$$

where:

v: vector activity;
n: total number of bait plants;
k: number of infected plants.

Vector activity (the estimated average number of inoculations per plant) was plotted against the weekly average of total aphid population data from the suction trap and a linear relationship was fit. Substitute aphid data for the missing period were then estimated by the regression equation using the vector activity data as input.

A single green water pan trap (GWT), 40 x 50 cm, was placed in Exp. 1 and Exp. 4 in the middle of the experimental fields. Aphids were weekly collected and counted. The explanatory value of these data was compared with that of the suction trap data.

4.3.3. Multiple regression and correlation analysis of the relationship between aphid abundance and the rate of epidemic spread:

The multiple regression analysis was based on the logistic disease progress model (van der Plank, 1963), i.e. the rate of increase is proportional to the number of sources, to the number of not yet infected plants, and to the abundance of vectors:

$$v = \ln \frac{n}{n-k} \quad \text{(Equation 4.1)}$$

where:

$N_{1,t}$ to $N_{n,t}$: is the number of specimens caught for each aphid species (i) in the time interval t to $t + \Delta t$;

b_1 to b_n : regression coefficients;

x : proportion of plants showing symptoms at time t;

Δx change of proportion of plants showing symptoms in the time interval t to $t + \Delta t$.

Δx time between samples

This equation relates the change in the proportion of plants showing symptoms (Δx) during a time interval (Δt) to the proportion of plants showing symptoms (x_t) at the start of that interval and the length of the time interval (Δt). The time interval used was the time between subsequent samples, which varied from 1 week (Exp. 1) to 2 or sometimes 3 weeks (Exp. 2, 3 and 4). This time interval is of the same order of magnitude as the latent and incubation periods of the disease (Chapter 2). As latency and incubation period differ very little, infectious plants and plants showing symptoms were equated with each other for the purpose of this analysis. In the analysis, the number of plants showing symptoms was used to calculate x_t, and the increase of the number of plants showing symptoms was used to determine Δx. Proportions are calculated by dividing the number of infected plants by the total number of plants in a plot. The equation is then rearranged to obtain a form in which the coefficients b_I may be estimated by linear regression (Garrett, 1988):

$$\frac{\Delta x}{x \cdot (1-x) \cdot \Delta t} = \sum_{1}^{n} b_i \cdot N_i \quad \text{(Equation 4.2)}$$

This corrected rate represents the number of infections made by the vectors per unit of time, taking into account the proportion of sources and healthy plants available.

All variables, except the regression coefficients b_i, were obtained in experiments, while the b_i were determined by regression. Also, the correlation coefficient between the corrected rate and the aphid catches for each time interval Δt was analyzed. The regression analysis distinguished as variables the number for six aphid species: *Acyrthosiphon pisum*, *Aphis fabae*, *Macrosiphum euphorbiae*, *Metopolophium dirhodum*, *Myzus persicae* and *Rhopalosiphum padi*. Two other regression variables represented the number caught for all other species together, as well as the total aphid catch. Other species were not included because they were low in numbers and/or non-vectors and preliminary regressions had shown that they had no added explanatory value.

4.3.4. Simulation of the spread of BtMV:

A basic temporal epidemiological model (Edelstein-Keshet, 1988) was used to verify whether the growth of epidemics obeyed expected mechanistic principles, and to determine whether rate parameters characterizing disease spread were stable or variable among experiments and/or treatments. The model has four state variables, respectively representing the number of healthy plants in the plot (H), the number of latent infected plants (L), the number of infectious plants that have not (yet) developed symptoms (I), and the number of infectious plants with symptoms (S). The rate of spread is a function of the aphid population caught by the suction trap (A; Figure 4.1), the number of virus sources (I+S), the proportion of available healthy plants (H/P) and a rate parameter (r):

$$\frac{dL}{dt} = r \cdot A \cdot (I + S) \cdot \frac{H}{P} - i \cdot L \quad \text{(Equation 4.3)},$$

$$\frac{dI}{dt} = i \cdot L - s \cdot I \quad \text{(Equation 4.4)},$$

$$\frac{dS}{dt} = s \cdot I \quad \text{(Equation 4.5)},$$

$$H = P - L - I - S \quad \text{(Equation 4.6)},$$

where:

A	: number of aphids captured by a trap per unit of time;
P	: plant population in the plot;
H	; number of healthy plants;
L	: number of infected plants not yet being infectious (i.e. latent);
I	: number of infected plants that have passed the latent period and not yet showing symptoms (incubating);
S	: number of infectious plants showing symptoms;
r	: rate parameter that relates spread with aphid population, sources and available hosts;
i	: relative rate at which latently infected plants become infectious, reciprocal of latent period;
s	: relative rate at which infectious plants without symptoms develop symptoms, reciprocal of [incubation period – latent period];

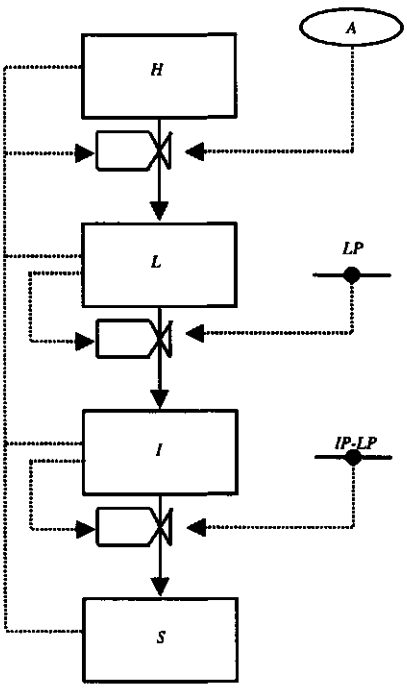

Figure 4.1. Relational diagram. *H*, healthy plants; *L*, infected plants before being infectious (latently infected plants); *I*, infectious plants before symptom expression; *S*, infectious plants showing symptoms; *A*, aphid population; *LP*, latent period; *IP*, incubation period.

In this study, the latent period (*LP*) was defined as the period between the inoculation and the moment at which the virus became available for acquisition, and the incubation period (*IP*) as the period between inoculation and the appearance of the symptoms in 50% of infected plants. The aphid population data collected by the Tollebeek suction trap (section 4.3.4) were used as a forcing function (*A*) for the model.

Initial values for the state variables were L=2, I=0, H=P-2, and P is the number of plants in the plot. The latent period (*LP*) was modeled as a function of daily mean air temperature, based on the laboratory determined latent period under different temperatures (Chapter 2) while the incubation period (*IP*) was set to *LP* + 2.

The model was implemented in the FORTRAN-based simulation environment SENECA (SENECA 2.0, Netherlands Institute of Ecology, Center for Estuarine and Coastal Ecology, 1992). Values for r were determined using the 'Price' calibration algorithm of SENECA. The Price algorithm conducts a controlled random parameter search and is very good at avoiding local minima. The sum of squared normalized residuals for the variable S (number of plants showing symptoms) was used as the quantity to be minimized during calibration. A normalized residual is the ratio of the residuals (simulated value minus observed value) and the observed values for S. The average disease progress for each inoculation date in each experiment was used as 'observed data' in the calibrations. Deviations between simulated and observed disease progress were characterized by the square root of the sum of squared normalized residuals (SRSSNR). Values of r were calibrated separately for each treatment in each experiment, and spread was simulated with the treatment-specific r. Spread was also simulated using the average r for the 3 or 4 infection dates in each experiment in order to test whether different treatments within one experiment could be characterized with one model and a single value for the rate parameter. Again, goodness of fit was determined by the SRSSNR.

4.4 Results:

4.4.1. Overview of the field experiments:

The number of plants showing symptoms in the four experiments started to increase about 2 weeks after the catches of the suction trap located in Tollebeek indicated that alatae aphids had started to fly (Figures 4.2 and 4.3). In 1995, aphids flight occurred in substantial quantity as of the last week of May (Figure 4.2 C; day 150 = 30 May), whereas in 1996, substantial number of flying aphids were not observed until the second half of July (Figure 4.3C; day 200 = 19 July). Consequently, disease spread in 1995 became apparent at the end of June (Figure 4.2 A,B; day 180 = 29 June), whereas in 1996 there was virtually no spread until mid August (Figure 4.3 A,B; day 230 = 18 August). Hence, broadly speaking, aphid

Table 4.1. Average distance of plants showing symptoms (± SE) from the source at the last day of evaluation.

Experiment	Inoculation date	Mean distance (m)[1]
Exp. 1[a]	5/16	1.52 ± 0.267^{a}
	6/6	0.98 ± 0.142^{ab}
	6/27	0.92 ± 0.275^{ab}
	7/18	0.21 ± 0.212^{b}
Exp. 2[a]	6/8	0.75 ± 0.105^{a}
	6/22	0.75 ± 0.105^{a}
Exp. 3[b]	6/18	3.29 ± 1.212^{a}
	7/2	2.74 ± 0.685^{a}
	7/16	2.61 ± 1.396^{a}
Exp. 4[b]	5/31	2.32 ± 0.286^{a}
	6/14	2.86 ± 0.679^{a}
	6/28	3.68 ± 0.565^{a}
	7/12	2.62 ± 1.141^{a}

[1] Values followed by the same letter are not statistically significant at p=0.05.

flight and disease spread started 50 days later in the season of 1996 than in the season of 1995. While the first inoculation dates of Exp. 1 (15 May and 6 June) showed substantial disease spread, all of the inoculation dates in Exp. 2 (from 8 June to 20 July) gave negligible spread. The later inoculations in Exp. 1 and 2 in 1995 (late June or later) did not result in much spread. Apparently, the inoculated plants in these treatments did not become sources on time for the virus to spread when the migrating aphids occurred in mid July (Figures 4.3 C and 4.4).

Vector abundance was approximately a factor three higher in 1996 than in 1995 (Figure 4.5), and this difference translated in a major difference in the extent of spread of BtMV (Figure 4.4). In all of the experiments, the extent of spread tended to decrease with later inoculation (Figure 4.4). This pattern was clearest in Exp. 1, which in retrospect turned out to be the only trial in which the four infection dates were optimally spaced out over the period of aphid flight. Table 4.1 presents the mean distance of all infected plants from the virus source at the last day of evaluation. The spatial pattern of the spread was aggregated around the source. In Exp. 1, the experiment with the clearest trend in number of infected plants in relation to infection date, the mean distance to the source decreased significantly with the infection date, which is indicative of more cycles of infection following the earlier inoculations.

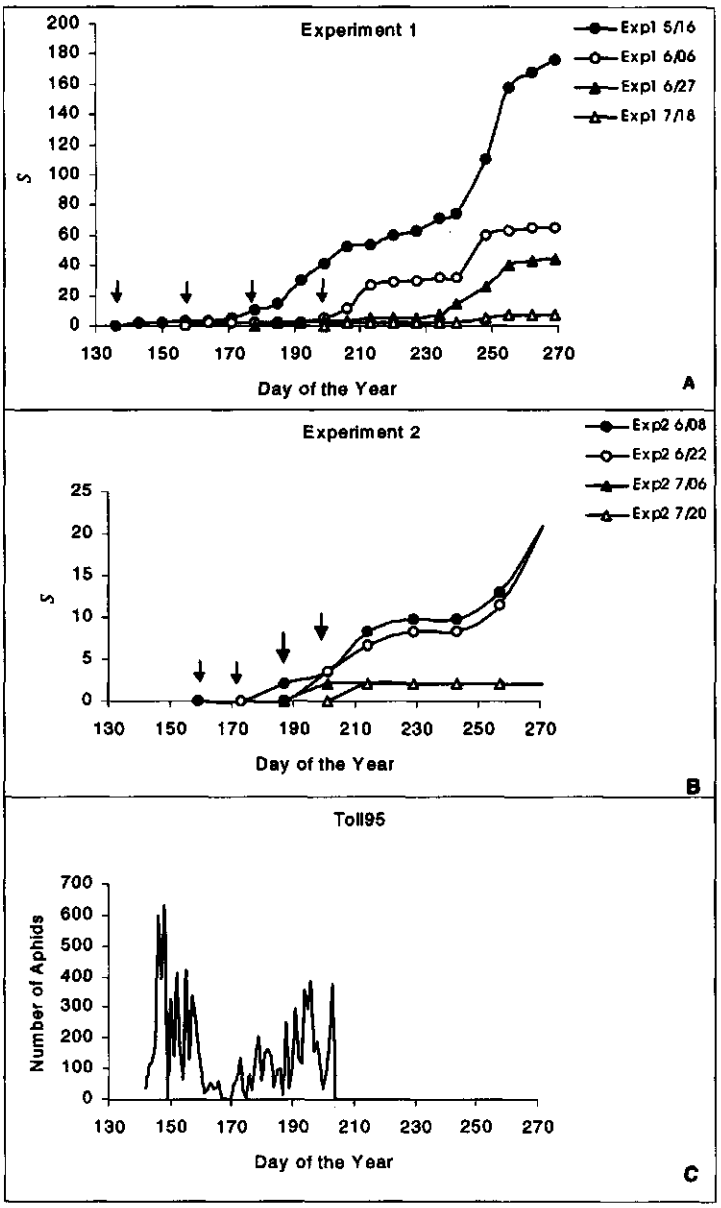

Figure 4.2. Observed disease progress curves of Exp. 1 (A), Exp. 2 (B) and the aphid catches by the suction trap located in Tollebeek (C) in 1995. *S* indicates the number of plants showing symptoms and the arrows indicate the inoculation dates.

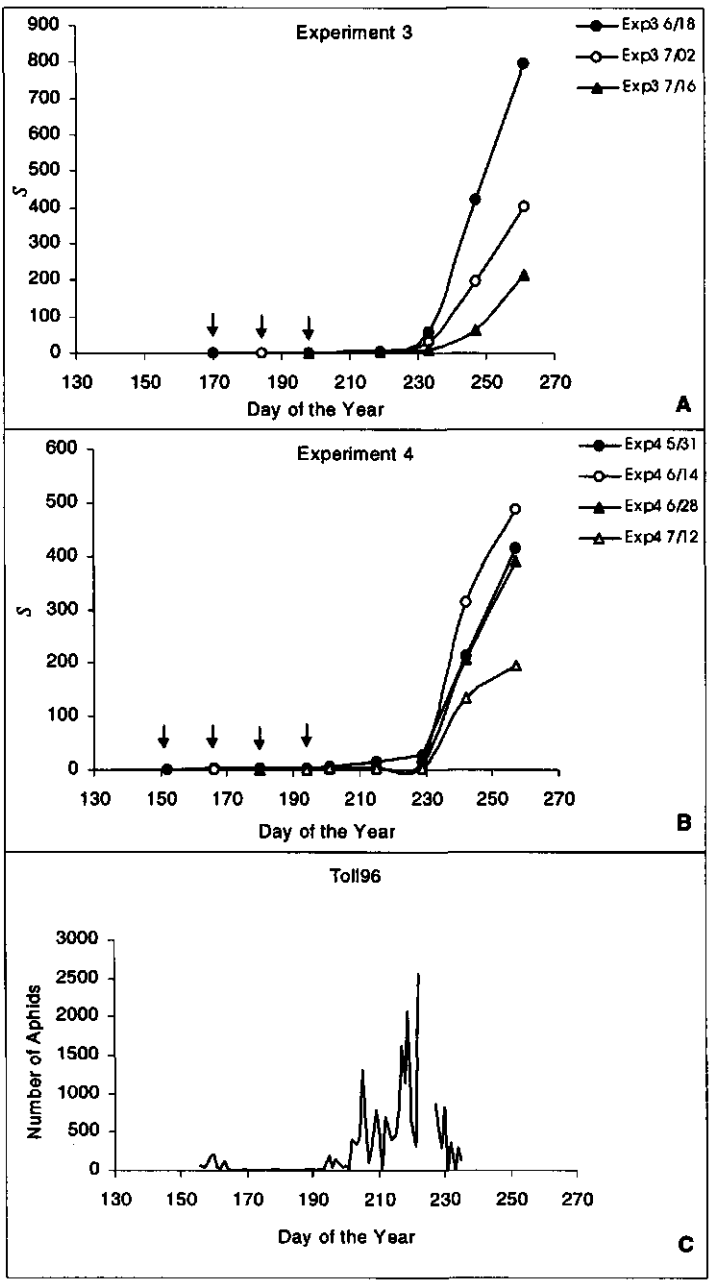

Figure 4.3. Observed disease progress curves of Exp. 3 (A), Exp. 3 (B) and the aphid catches by the suction trap located in Tollebeek (C) in 1996. *S* indicates the number of plants showing symptoms and the arrows indicate inoculation dates.

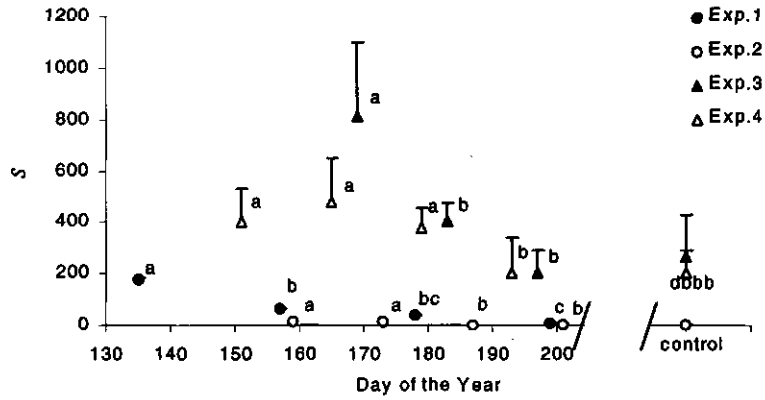

Figure 4.4. Final number of BtMV infected plants in the experimental plots. Symbols of the same experiment followed by the same letter do not differ statistically at p=0.05. *S* indicates the number of plants showing symptoms. Infection date is expressed in day of the year (1 Jan. = day 1)

A few infected plants were recorded in the control plots of the 1996 experiments. These plants were only found in the two last evaluations and their number corresponded with the number of infected plants found in the plots inoculated at the last date. They were randomly distributed in the plot, without any defined spatial pattern suggesting that they resulted from recent infections originating from the earlier inoculated plots in the same experiment.

During the experiments, randomly selected plants were periodically monitored for the presence of aphids. No resident aphids were found before mid August, when a few colonies of *A. fabae* were observed on the plants in all experiments. Under the experimental conditions in both years, early colonization of the plants was prevented by the use of insecticide treated seeds and by the presence of natural enemies (Landis and van der Werf, 1997). In fact, trials in which it was attempted to establish vector colonies in pesticide-free sugar beet failed in 3 years, due to this predation pressure. The absence of colonies until late in the season in combination with the early development of the spread shows, as has previously been reported for other potyviruses (Atiri, 1992; Madden *et al.*, 1987; Madden *et al.*, 1987a; Scott, 1985), that non-colonizing (winged) aphids were responsible for the spread of BtMV.

4.4.2. Correlation between the aphid catches and disease spread:

The total aphid population, trapped by the suction trap, provided the most stable statistically significant correlation ($0.79<R<0.81$) with the rate of spread as estimated with

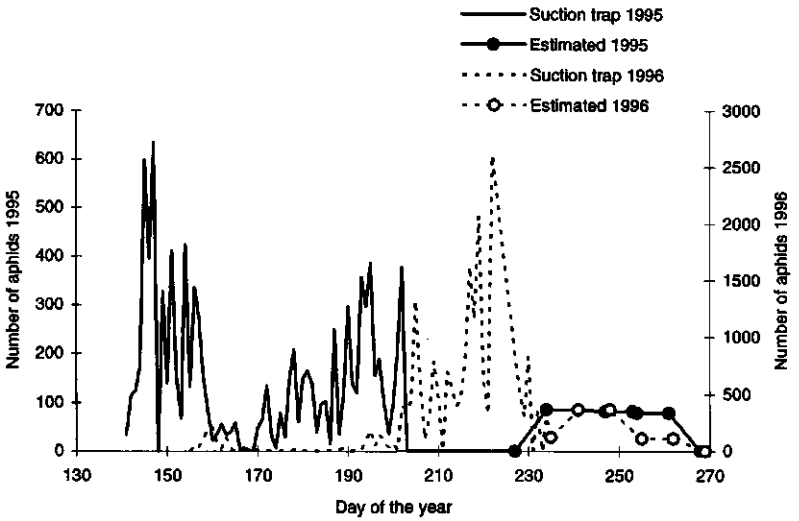

Figure 4.5. Daily number of aphids trapped in 1995 and 1996 in the Tollebeek suction trap, complemented with the weekly number of aphids estimated by regression and Gregory's multiple infection transformation for the period between day 225 and 270. Note the difference in vertical axis for 1995 data (left) and 1996 data (right).

equation 4.2 (Table 4.2). No relationship was found for any single aphid species with the spread of disease in multiple regression analyses, using stepwise inclusion or exclusion of explanatory variables. Results of stepwise regression for Experiment 4 are given to illustrate this point (Table 4.3). Values for the coefficient of determination of regressions on single species were usually below 0.5 (Table 4.3) and the regressions were mostly not statistically significant ($p>0.50$). Aphid population trends through the season were quite similar for most species, and hence the matrix of explanatory variables in the multiple linear regression was highly collinear (Tables 4.4A and 4.4B). This explains why it was not possible to find consistent regressions for individual species.

The data from GWT catches showed the same overall fluctuation of the total aphid population (data not shown) as the suction trap catches. Regression equations relating the suction trap catches and the vector activity determined by the bait plants resulted in coefficients of determination of 0.30 in 1995 and 0.93 for 1996. From the equations, the missing values at the end of the season for the suction trap data, that were needed for the model calibration in section 4.4.3, were calculated (Figure 4.5).

Table 4.2. Correlation coefficients between the corrected rate of spread (Equation 4.2) and total aphid population catches. Only the species with the highest correlation coefficients are shown. Correlation analysis is presented for the first inoculated treatment of each experiment.

Experiment		*Myzus persicae*	*Metopolophium dirhodum*	Other aphids	Total
Exp. 1	□[1]	0.78	0.77	0.77	0.81
	p	< 0.01	< 0.01	< 0.01	< 0.01
Exp. 2	**Nd[2]**				
Exp. 3	□	0.97	0.95	0.92	0.79
	p	0.01	0.05	0.02	0.12
Exp. 4	□	0.69	0.75	0.75	0.82
	p	0.13	0.09	0.08	0.05

[1] Correlation coefficient

[2] Insufficient virus spread to conduct a meaningful analysis

Table 4.3. Multiple linear regression between Garrett's corrected rate of spread and aphid species. Data of the spread in plots inoculated at the first inoculation date plots at Exp. 4.

Number of species	R^2	Constant	Species	Regression coefficient	p
5	0.35	0.521	*Aphis fabae*	-0.02243	0.58
			Acyrthosiphon pisum	-0.03236	0.66
			Macrosiphum euphorbiae	0.72544	0.63
			Myzus persicae	-0.00417	0.73
			Rhopalosiphum padi	0.00152	0.61
4	0.25	0.451	*Aphis fabae*	-0.00374	0.50
			Acyrthosiphon pisum	-0.00342	0.92
			Myzus persicae	0.00165	0.49
			Rhopalosiphum padi	0.00011	0.82
2	0.40	0.447	*Aphis fabae*	-0.00381	0.33
			Myzus persicae	0.00177	0.30
2	0.44	0.506	*Metopolophium dirhodum*	-0.00007	0.92
			Rhopalosiphum padi	0.00008	0.69

Table 4.4A. Correlation matrix for the different studied aphid species. Aphid numbers are the sum of the daily catches for a week. Cell contents: Correlation coefficient and p value of the correlation.

a. 1995

	Ap[1]	Md	Me	Mp	Rp	Other	total
Af	0,218	0,737	0,846	0,849	0,845	0,578	0,705
	0,474	<0.01	<0.01	<0.01	<0.01	0,04	<0.01
Ap		0,001	0,336	0,516	0,542	0,171	0,270
		0,997	0,262	0,07	0,056	0,58	0,37
Md			0,776	0,456	0,555	0,466	0,540
			<0.01	0,12	0,05	0,11	0,06
Me				0,819	0,894	0,460	0,604
				<0.01	<0.01	0,114	0,03
Mp					0,941	0,277	0,454
					<0.01	0,36	0,12
Rp						0,394	0,569
						0,18	0,04
Other							0,980
							<0.01

B. 1996.

	Ap	Md	Me	Mp	Rp	Other	total
Af	0,855	0,768	0,284	0,604	0,676	0,611	0,766
	<0.01	<0.01	0,37	0,038	0,016	0,03	<0.01
Ap		0,544	0,004	0,469	0,643	0,372	0,584
		0,08	0,99	0,12	0,02	0,23	0,05
Md			0,793	0,681	0,777	0,879	0,942
			<0.01	0,01	<0.01	<0.01	<0.01
Me				0,620	0,422	0,881	0,760
				0,03	0,17	<0.01	<0.01
Mp					0,583	0,855	0,874
					0,05	<0.01	<0.01
Rp						0,550	0,804
						0,06	<0.01
Other							0,932
							<0.01

[1] Aphid species: Af, *Aphis fabae*; Ap, *Acyrthosiphon pisum*; Md, *Metopolophium dirhodum*; Me, *Macrosiphum euphorbiae*; Mp, *Myzus persicae*; Rp, *Rhopalosiphum padi*.

4.4.3. Modeling the relationship between aphid catches and disease spread:

Several calibration runs were made to estimate the *r* parameter that relates spread with aphid flights, using different measures for aphid abundance or activity as forcing functions. Data from the Tollebeek suction trap, green water pan trap, vector pressure from proportion of infected bait plants/week and estimated vector activity, using Equation 4.1 were compared. The best fit was obtained when the suction trap catches were used. Substitute data were constructed using the results of the bait plant experiments and equation 4.1. Preliminary simulations with varying estimates for the number of aphids at the end of the simulation period indicated that the model was not too sensitive to this parameter. Then, when the estimated missing data were added to the suction trap data, the *r* values did not change and goodness of fit remained the same. Hence, we may conclude that the estimated population could be used to simulate the spread of BtMV.

Based upon the results of regression analysis, only total aphid counts were used as forcing function in the calibrations. The average number of plants showing symptoms in plots of each inoculation date and experiment was used to estimate the goodness of fit of the model. The *r* values estimated by calibration are presented in Table 4.5. The range of values found for each calibration was very narrow (less then 0.1%). To calculate the average *r* to simulate disease spread, only the values from the plots in which spread actually occurred were used. The last two inoculation dates in Exp. 2, in which no spread occurred at all, were eliminated from this calibration.

Figure 4.6 compares simulated disease progress curves for each inoculation date using the treatment-wise calibrated *r*, with the average observed disease progress curves per treatment. Simulated final number of plants showing symptoms and disease development matched the field experimental data. Generally, small variations were observed between the different dates of the same experiment. The results of these simulation studies, using experiment-wise averaged values of *r*, showed that the spread of the disease could be correlated with the aphid population by a rate parameter which is conserved for all inoculation dates. This single rate variable could be used to simulate the observed virus spread in the field for each experiment (Figure 4.7). Goodness of fit characterized by SRSSNR indicated that, generally, the model could describe the spread of the disease (Table 4.5).

Table 4.5. Overview of calibration results. For each of the four experiments, values of r were determined by calibration. The goodness of fit is characterized by the Square Root of the Sum of Squared Normalized Residuals (SRSSNR). Additionally, for each experiment, goodness of fit is given when simulations are made with an experiment-wise average value of r.

EXP	Inoculation date	Treatment-specific r ($* 10^{-4}$)	SRSSNR with treatment-specific r	Experiment-specific r ($* 10^{-4}$)	SRSSNR with experiment-specific r
Exp. 1	5/16	5.78	0.41	5.06	0.62
	6/6	5.57	0.29		0.41
	6/27	5.47	0.33		0.38
	7/18	3.40	0.48		0.74
Exp. 2	6/8	2.88	0.23	2.87	0.23
	6/22	2.86	0.25		0.25
	7/6[1]	-	-		3.91
	7/20[1]	-	-		1.51
Exp. 3	6/18	4.04	0.14	3.49	0.46
	7/2	3.42	0.14		0.16
	7/16	3.02	0.28		0.69
Exp. 4	5/31	3.29	0.13	3.56	0.32
	6/14	3.98	0.26		0.38
	6/28	3.11	0.29		0.52
	7/12	3.86	0.37		0.37
Average ± SE		3.73 ± 0.47			

[1] No spread occurred in these plots.

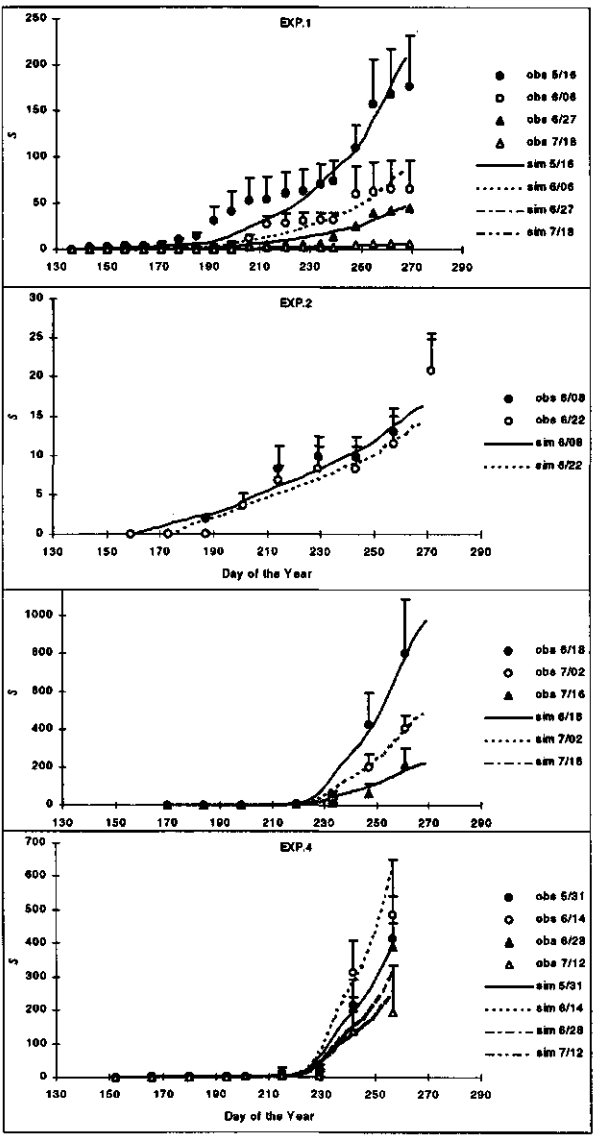

Figure 4.6. Simulated disease progress curves, using the calibrated r for each experiment, compared to the experimental field data found in the plots of each inoculation date. Dots represent the observed spread while lines represent the simulated spread. S is the number of plants showing symptoms. Bars represent the standard error of the mean.

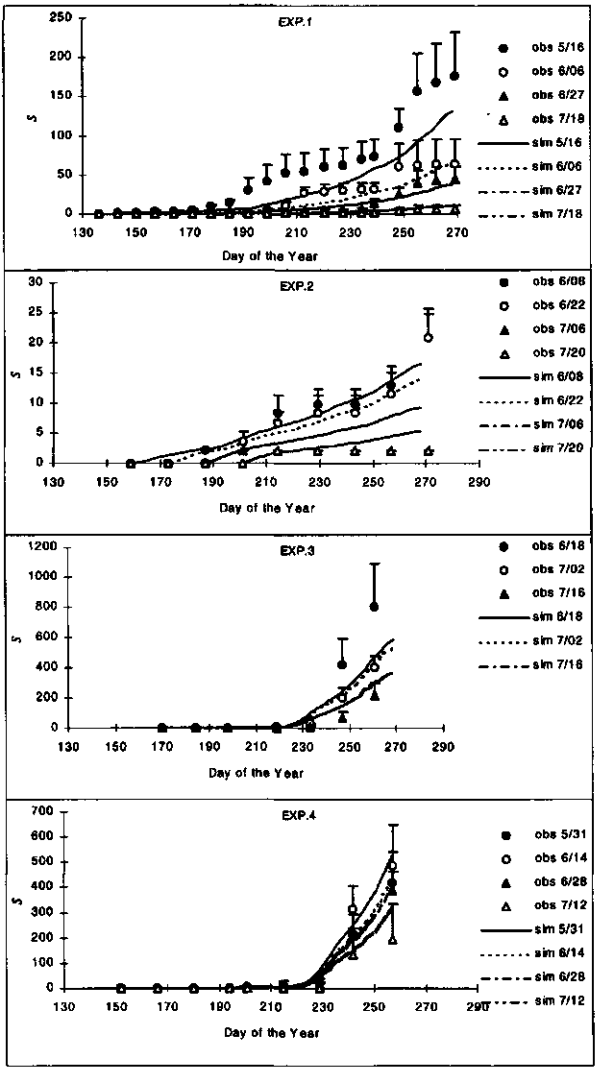

Figure 4.7. Simulated disease progress curves, using the common averaged r over all treatments per experiment, compared to the experimental field data found in the plots of each inoculation date. Dots represent the observed spread and lines represent the simulated spread. S is the number of plants showing symptoms. Bars represent the standard error of the mean of the replications of the treatments.

4.5 Discussion:

The work described in this paper aimed to verify: how did changes in primary infection date affect the secondary spread of BtMV in sugar beet; in which way the extent and pattern of secondary spread was related to the abundance, time profile and species spectrum of vectors; whether it was possible to describe the secondary spread with a simple mechanistic simulation model, using accepted principles of disease epidemics; and how robust were the parameter values in such a model.

The results of the field experiments show that, expectedly, the extent of spread over a season decreases with later inoculation. The effect of infection date is strongly dependent upon the time profile of aphid flight, which was very different among the two years of study; a 50 days earlier start of significant aphid flight in 1995 than in 1996, a bimodal flight profile in 1995, compared to a unimodal flight profile in 1996, and a factor three greater flight intensity in 1996. All these differences affected the spread in manners that made biological sense. For instance, the extent of spread decreased with later inoculation, except when earlier inoculation did not make source plants available to aphids at an earlier moment. This was because aphids were not yet flying by the time the earlier inoculation resulted in infectious plants. Another easily explained result was the increase of spread with higher vector numbers in the second year. Another easily explained result was the increase of spread with higher vector numbers in the second year.

A question that was not resolved in this study is the quest for which aphid species is or are (mainly) responsible for the spread of BtMV. Garrett (1988) used regression analysis to sort out the role of different aphid species in the spread of *Clover yellow vein virus* (CYVV). In that study, the aphid counts must not have suffered to much from collinearity, as in our case, otherwise singling out the importance of single species would not have been possible. The 1995 and 1996 aphid data were distinctly collinear (Table 4.4). As a consequence, analyses of the relationship between virus spread and vector abundance, while leaving out the counts for certain aphid species, resulted in inconsistent regression coefficients for single species. As shown in Table 4.3, negative regression coefficient were found for indisputable vector species, such as *M. persicae,* depending upon the combination with other aphid species, which is a biologically meaningless result. Although several aphid species are reported as vectors for BtMV (Chapter 2; Sylvester, 1952) many more species may contribute to the dissemination of this virus in the field. The collinearity of the aphid trends makes it impossible to identify which species were the most important vectors (assuming that some species were more important vectors than others). Nevertheless, the lack of evidence for the major importance of a few or only one vector species does not completely rule out the possibility that a single or very few aphid species were in fact mainly responsible for the spread. The best way to find this out would be manipulative experiments with exclusion of introduction of vectors, which would be

obviously very difficult to do at the field scale. Another option would be the analysis of very large data sets, which sufficient discrepancies in aphid trends between years or regions. However, such data sets would need to be complemented with reliable data on secondary spread of plant viruses. Most field based studies do not distinguish primary from secondary spread, and this may limit the conclusions that may be drawn from such studies, based on larger data sets.

Potyviruses are transmitted by aphids in a non-persistent manner. This implies that even aphid species that do not colonize the crop have an important role in the transmission of these viruses, including BtMV (Chapter 2; Katis and Gibson, 1984; Summers *et al.*, 1990). The relative transmission efficiency differs between the various aphid species which transmit BtMV (Chapter 2). A low number of aphids of a highly efficient species may lead to a higher rate of spread than a high number of a low efficient species. However, the number of specimens of each species varies from year to year. Although each year one or two species might play a major role in the spread, it can be concluded, based on the simulation studies and the regression analysis, that the relationship between vector dynamics and potyvirus spread can be studied using total aphid population counts rather than the counts of a single species. Madden *et al.* (1987b), Mora-Aguilera *et al.* (1992) and Di Fonzo *et al.* (1997) came to a similar conclusion. Using total aphid counts rather than single species counts saves a substantial amount of time consuming and skill demanding identification labor.

The use of the total number of caught aphids as a forcing function of the simulation model resulted in quite stable estimates for r between inoculation dates within each experiment (Table 4.5). The r values between locations and years varied within a narrow range with the same order of magnitude. These results show that a single value of r could be used to simulate the spread in each experiment and that the studied epidemiological process could be characterized by this parameter.

The meaning of r is quite complex. It is a single rate parameter that represents all aspects of the vector activity in the disease dynamics (Jeger *et al.* 1998). This parameter was estimated using experimental field data and appeared to be quite robust, though differences between the fields used, such as crop stature, densities and species of weeds, presence of trees and other crops on the field borders, wind, latitude, could all affect the spread and the parameter that describes its relationship to aphid abundance in the model. Such variations have been observed for other pathosystems (Madden *et al.*, 1987; Mora-Aguilera *et al.*, 1996). All simulated epidemics fit between the average field data ± SE (the standard error of the mean for the replications) (Figure 4.7). The above mentioned variations might have affected the landing activity of migrating aphids and thus the variation in the disease progress curves of the replications of each treatment. Hence, the

rather small variability of r between the four experiments is an encouraging results, that suggests that models of this sort may have predictive or management value.

The correlation analysis results suggested also that the quality of aphid data to be used as a forcing function in the model may still be improved. The use of daily catches from suction traps as a forcing function in the model, under the evaluated conditions, produced the best simulations, followed by the use of infection pressure from bait plants (Chapter 5). Halbert *et al.* (1990) indicated that suction trap data probably reflects the aphid flight activity over an area with a radius of 80 km. Considering the flat topography of the Netherlands, the area that this suction trap might cover is expected to be larger than for topographically more rugged areas (Dr. R. Harrison, personal communication). Accepting this assumption and considering that the Tollebeek suction trap is located about 100 km from Exp. 1 and 4 and about 20 km from Exp. 2 and 3, data from this trap were assumed to be representative for the experimental locations. Our results could demonstrate that daily catches from the suction trap, of all aphid species, could be correlated to the spread of BtMV. Also, this suction trap data could provide detailed information regarding species composition of the daily catches.

As shown in Chapter 3, BtMV is spread in an aggregated spatial pattern around the primary source. This aggregation certainly influences the availability of healthy plants for the subsequent infection cycles, as the vectors may move over small distances after alighting in the field. Any subsequent study to improve the model represented by Figure 4.1 has to take the spatial distribution of the disease into account. Also, the role of individual aphid species can be included in the model, decomposing r into parameters that represent their behavior in relation to virus transmission.

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Chapter 5

Effect of plant density on the spread of *Beet mosaic virus*

5.1 Abstract:

Field experiments were conducted in 1995 and 1996 to study the effect of crop density on the spread of BtMV in sugar beet. Primary inoculum was introduced in the center of 8 x 8 m field plots in late May or early June, and the spread of the disease was monitored by regular inspection of all plants per plot until the end of September. Virus spread followed the time profile of aphid flight, which was earlier in 1995 than in 1996, but more numerous in the latter year. Consequently, spread started earlier in 1995 than in 1996, but the extent of spread (both spatially and numerically) was greater in the latter year. Initially, more plants became infected in low density plots, but as the number of uninfected plants in low density plots became more limiting to the spread, this trend was weakened or even reversed.

A dynamic epidemiological model was fitted to the data to test whether two hypotheses could explain the observed patterns: (1) as plant density decreases, vectors are concentrated on fewer plants, so the rate of transmission from sources becomes inversely proportional to plant density; and (2) as plant density decreases, the increasing contrast between isolated plants and surrounding bare soil attracts greater aphid numbers per unit crop area, resulting in a further increase in the rate of virus transmission from sources in the crop. The model also took account of host limitation as more plants in the plot became infected. Rate parameters fitted for the epidemiological model during the initial phases of virus spread, when the number of uninfected hosts was not limiting the spread, were consistent with the vector concentration hypothesis (1). Rate parameters for the whole of the epidemic, however, were not consistent with the vector concentration hypothesis, and show that the rate of spread in low density plots is lower than would be predicted on the basis of this hypothesis. Hence these results support hypothesis 1 for the first part of the season, while they give no support to hypothesis 2.

Additionally, the effect of the soil background was tested in experiments in which healthy plants were placed next to infected plants against a background of either bare soil or a grass. The number of infected bait plants was higher in bare soil plots than in plots with grass. This result is consistent with both hypotheses.

5.2 Introduction:

The spread of non-persistently transmitted viruses is affected by several factors including plant resistance to virus or vector, the number and proximity of inoculum sources, the time within the growing season at which the first plants are infected, and the abundance, species composition and activity of the vector population (Jones, 1993; Bwye *et al.*, 1994; Berlandier *et al.*, 1997). The spread of plant viruses may also be affected by plant density. This aspect has been studied for a few polycyclically transmitted viruses outside the potyvirus group, e.g. *Groundnut rosette virus* in groundnut (A'Brook, 1964 and 1968; Booker, 1963; Davies, 1976), and *Beet yellows virus* and *Beet mild yellowing virus* in sugar beet (Heathcote, 1974). It has been found that, in general, increased plant density results in a reduction of the proportion of infected plants, while the absolute number of infected plants may either decrease or increase. Similar effects have also been found for *Tomato spotted wilt virus*, which is introduced into tomato and pine apple crops from outside virus reservoirs, yielding a combination of monocyclic disease introduction from outside sources and polycyclic disease progress within the crop (van der Plank, 1947; Linford, 1943). The only potyvirus for which plant density effects have been studied is *Bean yellow mosaic virus* (BYMV) in *Lupinus angustifolius*. This virus kills the infected lupin plant soon after becoming systemic. As the period in which the virus can be acquired from infected plants is rather brief, spread of BYMV in this crop has largely a monocyclic nature. A low plant density resulted in a considerably higher proportion of infected plants (Jones, 1994), and the number of infected plants was also higher in low density crops. Jones (1994) attributed the effect of low plant density on virus spread to delayed canopy closure affecting crop attractiveness to aphid vectors. This delay in closure will be aggravated by the collapse of infected plants. Effects of plant density on polycyclically spread viruses has not been studied in great detail, and it has not been ascertained which mechanisms are responsible for the reported effects of plant density.

Several mechanisms may underlie the effects of plant density on virus spread. A spurious effect is the relationship between density and disease incidence; naturally the proportion of diseased plants will increase as density goes down even if the number of diseased plants remains constant. Therefore, Burdon and Chilvers (1982) argued in their review of plant density effects on disease ecology that rates of spread rather than final levels of incidence should be studied. Studies should focus on the processes and mechanisms rather than on the final results alone.

Two mechanisms that may increase the rate of virus spread in low density crops are (1) vector concentration on fewer plants, and (2) behavioral responses of vectors to crop-soil contrasts. The first mechanism is almost as spurious as the increased incidence at lower plant

densities. If vector landing rate (number per unit area per unit time) is unaffected by plant density, then the number of vectors that alight on a source plant should become twice as high if the number of plants per unit area is halved. As the rate of spread (number of newly infected plants per unit area per unit time) is proportional to the number of viruliferous vectors per unit area, it is expected that the rate of spread should be proportional to the number of vectors per infected hosts, and hence inversely proportional to plant density. This phenomenon is here labeled 'vector concentration'. Vector concentration may substantially affect rates of virus spread, as it effects the spread per source, i.e. the per capita rate of spread (percentage increase per unit time).

Additionally, several studies have indicated that aphids show behavioral responses to crop-soil contrasts, which might be stronger in unclosed low density crops than in higher density closed canopies (Liewehr and Cranshaw, 1991). It is not clear, however, over which scales such attraction acts, i.e. if aphids are attracted to a certain type of canopy, they should be attracted away from other potential landing sites. The scale of this process, e.g. whether it acts at the between or within field level, is unknown.

A third mechanism which is involved in plant density effects on the spread of systemic viruses is host limitation, which is earlier to occur at low densities because there are fewer hosts available (hypothesis 3). How all the above mentioned mechanisms interact is not clear, and few studies have been conducted in a controlled manner, with known infection sources and absence of disturbing primary infections to elucidate these interactions.

Beet mosaic virus (BtMV) in sugar beet provides a suitable system to study plant density effects on virus spread because BtMV has become extremely rare in The Netherlands since the demise of the fodder beet culture. Previously, fodder beet clamps were an important source for beet viruses in spring, but they have become rare. Since it can be expected that field experiments will not be disturbed by a natural occurrence of BtMV, the spread of this virus may be studied as a role model for the effect of plant density on the formation of clusters around a virus source.

The aim of this study was to determine in which way the spread of BtMV in sugar beet reacts to differences in plant density, and to ascertain which of the above formulated hypotheses may be held responsible for the observed effects. Field studies were conducted in which spread around established sources was monitored through the season. Bait plant trials were done to determine effects of background on vector activity. Epidemiological models, which included the above mentioned hypotheses 1 and 3, were fitted to observed data, to determine whether such models could explain observed trends in virus spread, or whether hypothesis 2 also had to be also accounted for in these models.

5.3 Material and Methods:

5.3.1. Secondary spread of BtMV in plots with different plant densities:

In 1995 and 1996, four field experiments were conducted to determine the effect of plant density on the spread of BtMV. In 1995, at De Bouwing (Exp. 1), Zetten, a field experiment with a random block design with three plant densities in five replications was established in the middle of a commercially grown sugar beet cv. Univers field of approximately 2 ha. Net plots measured 8 x 8 m in size, and they were bordered on all sides by a 1 m wide strip of plants with the same plant density, yielding a 10 x 10 m gross plot area. Standard density plots measured 0.5 m between rows and 7.6 plants/m^2. The other densities were 4.1 plants/m^2 and 2.3 plants/m^2. These densities were obtained by roguing one row out of every two rows, or three rows out of four rows, respectively. The centers of the plots were 25 m apart and the plots were bordered by sugar beet of the standard density. Two plants in the center of each plot were inoculated using 10 aphids after 1 h starvation and a 5 min acquisition access period on an infected host. Inoculations were made on May 30, when the plants were in the 6 leaf stage. Weekly, all the plants in each plot were checked for BtMV symptoms. Infected plants were marked with a bamboo stick and their position was recorded to prevent double counting and monitoring the spatial development of patches.

A second experiment (Exp. 2) was performed in a 15 ha commercial sugar beet field of the cv. Auris at the Minderhoudhoeve, Swifterbant, in the polder Oostelijk Flevoland. There were three treatments in four replications. The experimental design was the same as in Exp. 1, except for the plant densities, which were 10.6, 5.6 and 3.1 plants/m^2, respectively. The plots were inoculated on June 8 and monitored for diseased plants every two weeks.

In 1996, a third experiment (Exp. 3) was done in the center of a 15 ha commercial sugar beet field cv. Auris at the Minderhoudhoeve. The trial had four treatments in four replications . Plants densities in treatments 1-3 were the same as in Exp. 2, while a fourth treatment was added with a density of 3.45 plants/m^2. This density was obtained, not by eliminating three out of four rows as in treatment 3, but by eliminating three plants out of every four within each row. The plots were inoculated on June 4 and evaluated every two weeks as described above.

A fourth experiment (Exp. 4) was conducted in a 3 ha commercial sugar beet field cv Univers at Unifarm, Wageningen, using the same four plant densities as in Exp. 3, in 4 replications. Plants were inoculated on May 31 and evaluated every 2 weeks.

Plots in the four experiments were monitored until the end of September. From September onwards, temperatures become generally too low for development of symptoms.

Hence, the observations at the end of September represent practically the final situation, even for crops which are not harvested until two months later.

5.3.2. Effects of crop background on vector activity:

Vector activity was determined in plots with backgrounds of either bare soil or grass in an experiment in Wageningen in 1997. This experiment was located in the middle of a 4 ha sugar beet crop cv. Elisa at Unifarm. An area of 70 x 70 m was cleared in the middle of this crop and divided into 4 blocks of 35 x 35 m. Two blocks were kept in bare soil while two other blocks were cultivated with the forage grass *Lolium perenne*. In the middle of each block, 6 experimental plots of 5 x 5 m were established, three with bare soil and three with *L. perenne* (Figure 5.1). Ten BtMV infected plants (acting as source plants) and ten healthy sugar beet plants, in 1 liter pots, were placed in two parallel rows at 0.3 m of each other in the center of each of the 24 experimental plots. Bait plants were exposed for periods of one week at intervals of two weeks, then transferred to a greenhouse, sprayed with Pirimicarb and kept for 3 weeks to develop symptoms. The first plants were placed in the field on May 21 and the last ones on September 10. The infected source plants had between 6 and 16 leaves. They were replaced when necessary to maintain a more or less uniform source of virus during the experiment. The number of plants obtained at each date were analyzed by the two-way ANOVA procedure of SigmaStat using the backgrounds of the blocks and experimental plots as independent variables and the proportion of infected plants at each date as the dependent variable.

5.3.3. Characterizing the spread of BtMV at different plant densities with the logistic equation:

Equation 5.1 gives a logistic equation for the rate of spread of BtMV, which is based on the premises that this rate is proportional to the number of sources, the number of vectors per source per day; and to the proportion of available hosts; i.e. those plants which are not yet infected:

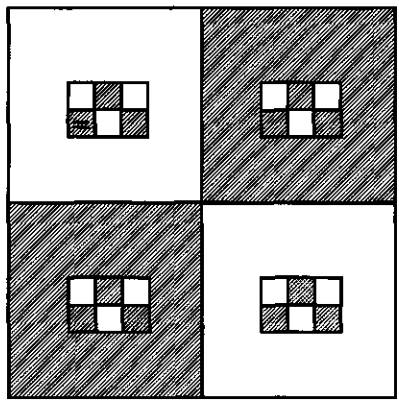

Figure 5.1. Design of the experiment to evaluate the effect of soil or grass background on virus spread. White areas indicate areas with bare soil while gray stripes areas are sown with *L. perenne*. Lines in the top left hand grass plot represent rows of healthy bait and infected source plants.

$$\frac{dV}{dt} = \frac{a}{P} \cdot V \cdot \left(1 - \frac{V}{P}\right) \text{ (equation 5.1),}$$

where:

V : is the number of infected plants (Victims) in the plot;

P : is the total number of plants (Population) in the 8 x 8 m net plot;

a : is the vector pressure (aphids per unit of area per unit of time), quantified as the daily aphid catch in traps and considered to be independent of plant density.

This equation resembles the logistic growth equation. However, the first term is inversely proportional to plant population P, which means that the relative rate of disease increase, brought about by a given vector pressure is inversely proportional to plant population P (plant density). Integration of equation 5.1 yields:

$$V = \frac{P}{1 + \frac{P - V_0}{V_0} \cdot Exp\left(-\frac{a}{P} \cdot t\right)} \text{ (equation 5.2)}$$

Equation 5.2 expresses the hypothesis that plant density affects disease progress by decreasing the intrinsic rate of increase with a factor $1/P$. To describe the observed disease progress curves, the observed final number of infected plants (V_{max}) was used as the end of season asymptote, rather than the total plant population (P):

$$V = \frac{(V_{max})}{1 + \frac{V_{max} - V_0}{V_0} \cdot Exp\left(-\frac{a}{P} \cdot t\right)} \quad \text{(equation 5.3)},$$

where:

V_{max} : is the final (maximum) number of infected plants in the field.

The disease progress curves monitored in the four experiments were fit to equation 5.3, using the non-linear regression procedure of SigmaStat for Windows Version 2.0 (SPSS Inc., 1992-1997). The estimated values for the parameter a for each treatment in an experiment were compared. Assuming that plant density would affect spread according to the hypotheses of vector concentration (1) and host plant limitation (3), values of a are expected to be the same for different density treatments within each experiment. An increase of a at lower density would provide evidence for effects of crop density and crop-soil contrast on aphid behavior, hypothesis 2.

5.3.4. Characterizing the spread of BtMV at different plant densities with a mechanistic epidemiological model:

As in the previous section, constancy of the rate parameter in an epidemiological model was used to test the hypothesis that vector concentration and host plant limitation can explain the effect of plant density on the spread of BtMV in the field experiments. In this section, the more elaborate simulation model of Chapter 4 was used. To account for the hypothesis of vector concentration, vector pressure is expressed in Equation 5.4 as A/P, rather than A, as was done in chapter 4 (where P was not variable). The full model is:

$$\frac{dL}{dt} = r \cdot \frac{A}{P} \cdot (I + S) \cdot \frac{H}{P} - i \cdot L \quad \text{(Equation 5.4)},$$

$$\frac{dI}{dt} = i \cdot L - s \cdot I \quad \text{(Equation 5.5)},$$

$\frac{dS}{dt} = s \cdot I$ (Equation 5.6),

$H = P - L - I - S$ (Equation 5.7),

where:

A	: number of aphids captured by a trap;
P	: plant population in the plot;
H	; number of healthy plants;
L	: number of latent (not infectious) plants;
I	: number of infectious plants without symptoms;
S	: number of infectious plants showing symptoms;
r	: constant that relates spread with the number of aphids per plant, the number of available virus sources and the proportion of healthy plants;
i	: relative rate at which latent plants become infectious; function of latent period;
s	: relative rate at which infectious plants without symptoms develop symptoms, function of latent period and incubation period;

As described in Chapter 4, the model was implemented in SENECA (SENECA 2.0, Netherlands Institute of Ecology, Center for Estuarine and Coastal Ecology, 1992), and values for the only parameter r were determined by calibration, using controlled random search.

5.3.5. Aphid data:

Daily aphid catches were obtained in a suction trap located in Tollebeek (30 km from the location of Exp. 2 and 3 and 100 km from Exp. 1 and 4). The aphid population for the second half of August and September was estimated as described in Chapter 4. In addition, green water pan traps were placed in the middle of the plots of Exp. 1 and 4 and in the soil background experiment. Aphids were weekly collected from the water traps and preserved in 70% ethanol.

5.4. Results:

5.4.1. Secondary spread of BtMV in plots with different plant densities:

In both seasons, 1995 and 1996, spread of the disease was observed earlier in low density than in standard density plots (Figure 5.2). In the standard density plots the first plants showing symptoms were only found when aphid numbers increased substantially as shown by the daily catches of the suction trap (Chapter 4). A higher proportion of infected plants was found in low-density plots than in standard density plots, but the final number of infected plants was in most instances higher in standard density plots than in low density plots (table 5.1).

The first plants showing symptoms in low density plots, were generally located in the same row as the sources. Later in the season, this directional effect disappeared. Such row effects were not observed in standard density plots. The canopy was already closed in these plots when the vector pressure increased.

5.4.2. Effects of crop background on vector activity:

With the increase of the alate aphid population detected by the green water pan trap positioned in the middle of the experiment, the proportion of infected plants increased (Figure 5.3). Analysis of variance showed that the background of the blocks did not affect the proportion of infected plants during the growing season (p values ranging from 0.06 to 1.00). However, significantly greater numbers of test plants became infected in plots with bare soil background than in plots with grass background. The differences were greatest when the vector pressure was high. There was a significant correlation between the proportion of infected plants and the weekly aphids catches in the green water pan: $R^2 = 0.89$ with p=0.003 for bare soil plots, and $R^2 = 0.81$ with p=0.015 for grass plots.

5.4.3. Characterizing the spread of BtMV at different plant densities with the logistic equation:

Logistic growth curves fitted the observed disease progress in the field experiments quite well, with coefficients of determination varying from 0.85 to 0.98 (table 5.2). The logistic growth equation (5.3) included both the vector concentration and host plant limitation mechanisms. Therefore, if these two mechanisms explained differences in spread between plots with different plant densities, we would expect the fitted values of a (the slope parameter) to be similar in the different density treatments within one experiment. In reality, the best fitting values of a were significantly smaller in low density plots than in high density plots, suggesting that the vector concentration hypothesis is not correct. If it were assumed that

Table 5.1: Effect of plant density and plant pattern on spread of BtMV in four sugar beet field experiments

Density (plants/m^2)	Distance between rows x mean distance within rows (cm)	Final density of infected plants ± SEM (plants/m^2)	Final incidence of infected plants (%)
Experiment 1; De Bouwing 1995; inoculated on May 30			
7.6	50 x 26	180 ± 33	37
4.1	100 x 24	149 ± 10	57
2.3	200 x 22	103 ± 4	88
Experiment 2; Minderhoudhoeve 1995; inoculated on June 8			
10.6	50 x 19	9 ± 3	1
5.6	100 x 18	34 ± 8	10
3.1	200 x 16	74 ± 13	37
Experiment 3; Minderhoudhoeve 1996; inoculated on June 4			
10.6	50 x 19	169 ± 24	25
5.6	100 x 18	267 ± 14	74
3.1	200 x 16	150 ± 23	76
3.45	50 x 58	93 ± 10	42
Experiment 4; Unifarm 1996; inoculated on May 31			
10.6	50 x 19	261 ± 33	39
5.6	100 x 18	270 ± 10	75
3.1	200 x 16	169 ± 8	85
3.45	50 x 58	205 ± 14	93

plant density did *not* affect the rate of spread through the vector concentration hypothesis, then the ratio *a/P* would be expected to remain stable among treatments. This is indeed the case for three of the four experiments (table 5.2). This result would suggest that at the lower densities, the incoming aphids would not be concentrated on the fewer sugar beet plants, but that they would alight on either the soil, or on remaining weeds between the plants.

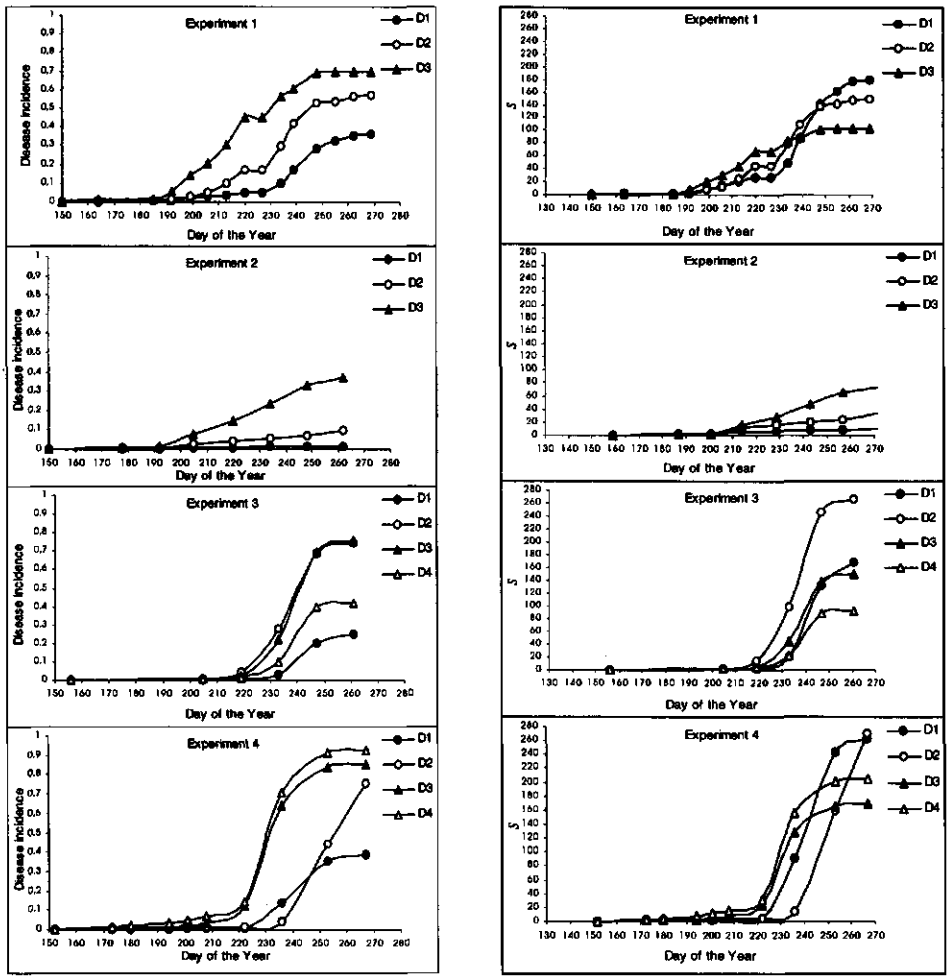

Figure 5.2. Spread of BtMV in plots with different plant densities in field experiments in 1995 (Exp. 1 and 2) and 1996 (Exp. 3 and 4). Spread is characterized by the proportion of infected plants (left side) or by their number S (right side). D_1 indicates standard density plots while D_2, D_3 and D_4 indicate 1/2, 1/3 and 1/3 density plots. D_2 and D_3 were created by eliminating rows, whereas D_4 was created by eliminating plants in rows, but keeping all the rows.

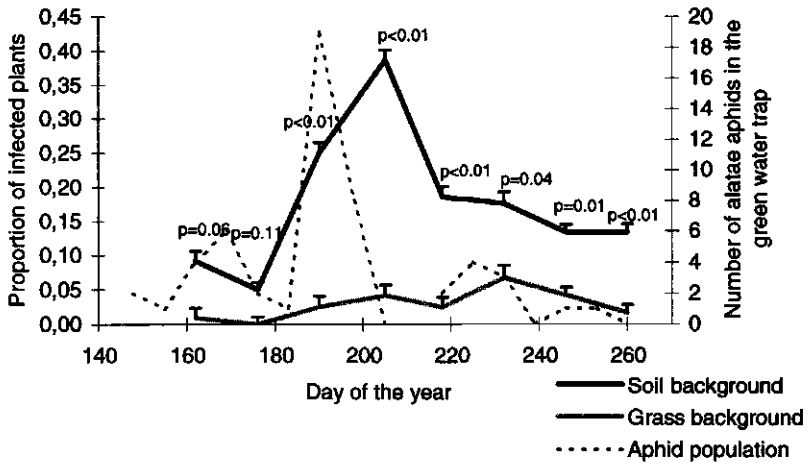

Figure 5.3. Proportion of infected plants in experimental plots with backgrounds of bare soil and grass. Bars show the standard error of the mean. *p*-values indicate the significance of the background effect in t-test.

5.4.4. Characterizing the spread of BtMV at different plant densities with a mechanistic epidemiological model:

The calibration of the epidemiological model tests the same hypotheses as the parameter fitting, using the logistic equation, the difference being that the model represents the system in more detail. The results are presented in table 5.3. If the vector concentration and host limitation hypotheses, expressed in equation 5.4, had been correct, the values for r should have been constant. However, r varied with plant density in the same manner as the rate parameter a of the logistic growth curves (previous section), i.e. the r values were lower for plots with lower densities. The lower values in the low-density plots indicate that, in comparison to the rate of spread in standard density plots, the rate of spread in low-density plots was slower than expected according to the model and its underlying hypotheses. This result is in complete agreement with the results of the previous section.

In order to evaluate whether there was a difference between the early season (with low number of infected plants) and the late season (with disease incidence nearing high levels in low density plots) in this respect, the same calibrations were run again, however, now until the

Table 5.2: Parameter for logistic growth curves (equation 5.3) describing disease progress in four sugar beet field experiments

Plant density (plants/m^2)	V_{max} (plants per plot)	a	R^2	a/P
Experiment 1; De Bouwing 1995; inoculated on May 30				
7.6	180	22.00	0.94	0.044
4.1	149	12.90	0.95	0.049
2.3	149	8.32	0.98	0.057
Experiment 2; Minderhoudhoeve 1995; inoculated on June 8				
10.6	180	12.50	0.94	0.018
5.6	149	10.40	0.95	0.029
3.1	149	8.38	0.98	0.042
Experiment 3; Minderhoudhoeve 1996; inoculated on June 4				
10.6	169	28.70	0.86	0.042
5.6	267	20.00	0.90	0.056
3.1	150	9.10	0.86	0.046
3.45	93	8.36	0.85	0.039
Experiment 4; Unifarm 1996; inoculated on May 31				
10.6	261	33.10	0.92	0.049
5.6	270	15.10	0.92	0.042
3.1	169	10.30	0.90	0.052
3.45	205	12.12	0.93	0.055

time at which the number of infected plants in the plot reached a level of 20% of the final level, *V*max. When calibrations were limited to the initial section of the disease progress curves, the low density plots exhibited a faster relative rate of increase of disease, and the expected constancy of the parameter r in the epidemiological model was indeed found (table

Table 5.3. Calibrated values of the rate parameter r in the epidemiological model of Equations 5.4-5.7. Average disease progress curves for each treatment were used in the calibrations (Figure 5.2). Distinction is made between r values applicable to the whole of the epidemic, or to only the initial period.

Exp.	Plant Density (plants/m^2)	r whole period ($*10^{-3}$)	r initial period ($*10^{-3}$)
1	7.6	5.61	1.53
	4.1	3.19	1.31
	2.3	1.95	1,81
2	10.6	2.19	-
	5.6	2.26	-
	3.1	1.96	-
3	10.6	2.79	0.56
	5.6	2.22	2.38
	3.1	1.02	0.49
	3.45	0.85	0.36
4	10.6	3.40	1.19
	5.6	1.52	6.48
	3.1	1.27	1.27
	3.45	1.62	1.62

5.3). This result suggests that the hypotheses underlying the model (vector concentration and host limitation) are true during the early part of the epidemic, but not later on.

Simulation of disease spread using the calibrated r for each density resulted in good fits with goodness of fit (calculated by the square root of the sum of squared normalizes residuals) varying from 0.04 to 0.28 (Figure 5.4).

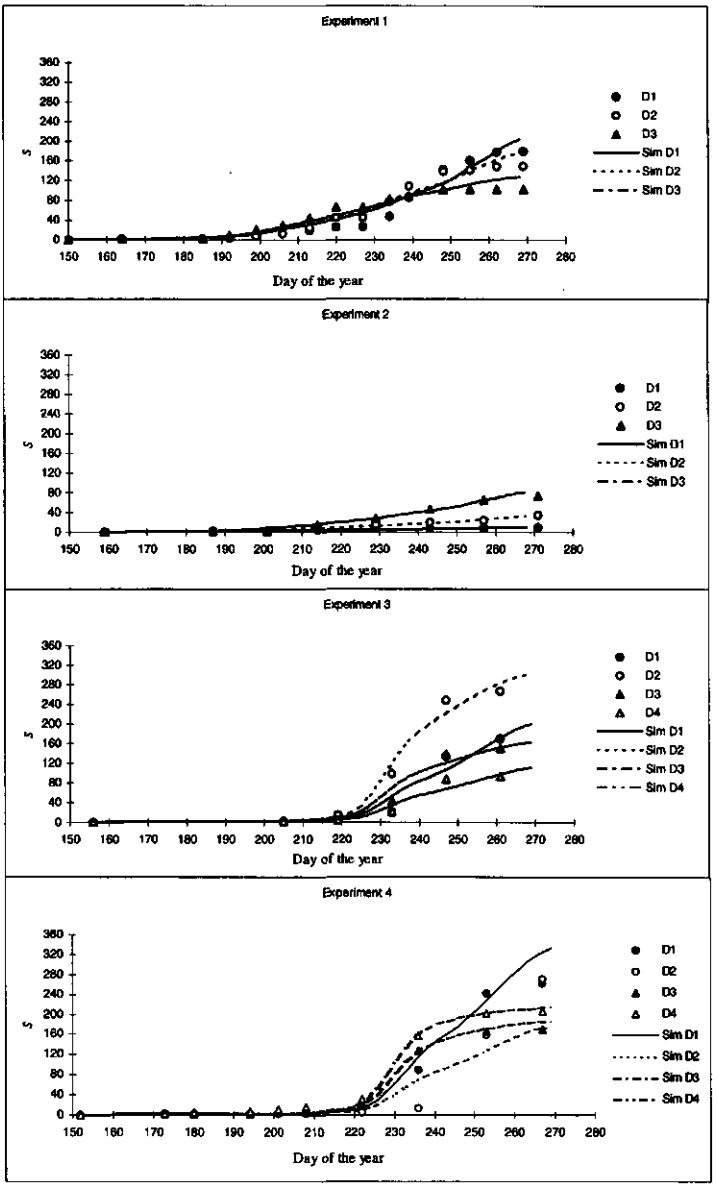

Figure 5.4. Effect of plant density on spread of BtMV in sugar beet; experimental results (symbols) and simulation results (lines), calibrated to the data. S is the average number of plants per plot showing symptoms.

5.4. Discussion:

The studies presented in this chapter aimed to describe in which way plant density affects the spread of the potyvirus BtMV around primary sources of infection in sugar beet. The results show that the spread tracks the time profile of aphid vectors, as determined in a suction trap. Lower density plots got a higher final incidence of infection, but the final number of infected plants was mostly greater in higher density plots. Two different models for disease dynamics were both successful in describing the temporal pattern of spread, and parameters in these models were used to test whether the vector concentration in low density crops resulted in an increased relative rate of spread. This hypothesis was confirmed for the early part of the growing season, when disease patches were small, but it did not hold up during the whole season. This is a puzzling result. One possible explanation why the vector concentration hypothesis did not hold up is that fewer aphids landed in low density plots. This has, however, been never reported in the literature. Alternatively, if the aphids landed in equal numbers in low density plots as in higher density crops, they might have moved less or moved as much but infected less often in the low density plots, due to larger plant distances. Aphids in low density plots might also have dwelled more extensively on soil or weeds. The experimental results do not show whether one or more of these speculations is more plausible than others.

Another possible explanation why vector concentration did not work out as expected is that the formulated models did not truthfully represent the epidemiological processes. One obviously oversimplifying assumption which was made in both models was that host limitation would be proportional to the ratio of non-infected plants over all plants in the plot. In reality, vectors may only move short distances. Thus, the area which the vector would cover in the search for a host may only include a few plants in all directions. If this is true, then the spatial pattern of disease would have a big impact on the proportion of inoculations that is made on not yet infected hosts. As a result, source plants in the center of patches might even be effectively removed from the 'spreading' host population because vectors originating from them would have only a small chance of reaching an uninfected host before they lost the ability to transmit. Unfortunately, there is only scant knowledge about how vectors move and, as a matter of fact, although it has been convincingly demonstrated that transient vectors are responsible for the spread of potyviruses in crops, it is not well known what these winged aphids do while they are moving in the crop. As potyviruses are retained by the aphids for only a short time and easily lost during probes, vector behavior may be a key to understanding how plant density affects spread. Low plant densities create large plant distances, and this in itself may result in fewer moves between virus sources and available hosts, or in longer transit times or distractions on the way, such as probes on weeds that would lower transmission

efficiency among crop plants. Studies of vector behavior may throw some light on these hypotheses.

In Exp. 2, disease incidence was considerably lower than in the other experiments. A similar low spread was observed in another experiment done on that location in 1995. It is not known which factors are responsible for this location vs. year interaction, but it could be weather. The location in the Minderhoudhoeve is comparatively open, cool and windy, and this may have impacted the behavior of vectors. As disease incidence in Exp. 2 was low, multiple infections probably played a smaller role here than in the other experiments, with potential consequences for the spread.

The bait plants experiments resulted in higher transmission in soil background plots then in grass background plots. Whether this is a consequence of vector concentration in soil background plots (grass competing with sugar beet for aphids) or vector attraction (due to beet – soil contrast) is impossible to say. A similar effect would be expected in low density plots in the epidemiological experiments. Indeed, during the early phases of spread in the epidemiological experiments, the vector concentration hypothesis was confirmed, but later on it did not hold up, and the contrast hypothesis was neither supported. According to this reasoning, it would be suggested that vector concentration was responsible for the difference in transmission in response to background. A difference between epidemiological experiments and the bait plant trials, measuring vector activity through time, is that bare soil background is a constant in the bait plant trials, whereas the area of exposed soil diminished during the season in the epidemiological experiments.

The distance covered by an aphid upon leaving the plant will determine how plant pattern and density affects virus spread. An increase in plant density will result in a greater number of host targets within the effective hopping range of the vectors (Burdon and Chilvers, 1982). The models used to simulate the spread of BtMV at different plant densities do not account for the clumped spatial distribution of the disease (Chapter 3) and no information on the hopping range of aphids in sugar beet is available. The observations on the initial spread in the low-density plots showed that the virus is often spread from a plant to its neighbor, indicating that vectors cover mostly short distances. Once plants became infected in an adjacent row, the virus started to spread over the plot. Spread to the neighboring rows increased as distance between the rows decreased due to plant growth. The original row effect then disappeared. A row effect was not observed in the standard density treatment in either year (Chapter 3) because, when spread started, the canopy was already closed. Although plant and row distances are expected to affect vector behavior and spread, comparison of the third and fourth treatments in Exp. 3 and 4 (these treatments featured low plant densities with respectively a 1:16 and 1:1 ratio in plant: row distance) did not result in very different values

for the rate parameters a and r of the two epidemiological models. Hence, the studies reported here shed no light on how modified vector behavior may be responsible for effects of plant density on virus spread. Further experiments may be specifically designed to evaluate vector dispersal distances in relation to the clustering of the disease and the spatial pattern of hosts to further resolve the issues addressed in this paper.

5.6. Acknowledgments

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Chapter 6

Simulation of damage caused by *Beet mosaic virus*

6.1. Abstract:

The effects of *Beet mosaic virus* (BtMV) infections on CO_2 exchange rates, light absorption and specific leaf area of sugar beet leaves were experimentally determined. The maximum rate of photosynthesis was reduced by about 15% in BtMV infected mature leaves showing symptoms, while it was not significantly affected in young leaves showing symptoms. Light use efficiency at low light intensities was not significantly affected by infection. Dark respiration was approximately doubled in both mature and young infected leaves. Light reflection was the same in healthy and mosaic affected leaves (10%), while light transmission was significantly greater in leaves with mosaic symptoms (6.6%) than in healthy leaves (4.6%). Specific leaf area was not affected by infection with BtMV. The results obtained were incorporated in a mechanistic crop growth model (SUCROS) to assess the likely consequences of these infections for growth and yield of the sugar beet crop. The crop growth model simulates the formation of healthy, latently infected and mosaic expressing leaf canopy and integrates daily net carbon assimilation and allocation over the growth season. A simulated 100% early infection of the crop resulted in 20% reduction in sugar beet root yield. The damage declined with later infections. The reduction in the maximum rate of photosynthesis and the increase in dark respiration in BtMV infected leaves formed the major components (96%) of the damage simulated. Simulations with realistic disease progress curves showed that under Dutch field condition, BtMV is spread at a time when the crop has accumulated enough leaf area to become tolerant to infections.

6.2. Introduction:

Beet mosaic virus (BtMV), a potyvirus transmitted by aphids in a non-persistent manner, is known by farmers as a virus that does not cause serious economical problems (van Steyvoort, 1982). Nevertheless, damage may occur. For instance, Watson and Watson (1953) suggested that little damage is expected and Wiesner (1959) reported a yield reduction in the order of 3.5% for crops harvested early, but not for crops harvested in October. Mixed infections with other viruses are common in sugar beet. A decrease in the sugar content of 10 to 20% and net assimilation rate (dry matter increase per unit of leaf area per unit of time) of 10% was found by Watson and Watson (1953), for early infected plants. However, little effect is expected on yield of naturally infected crops due to the low natural infection levels and late dates at which the crop becomes infected.

BtMV induces mosaic symptoms on the inoculated leaves and on those that developed after inoculation. Leaves already present at the time of the inoculation do not

develop symptoms, and the virus can be neither detected by ELISA nor acquired by its vectors from them (Chapter 2).

Observations during the field experiments in 1995 and 1996 (Chapters 3, 4 and 5) suggest that the overall growth of infected sugar beet plants is little affected when compared to healthy plants, except for those infected early in the season. The crop physiological effects underlying damage caused by BtMV infections in sugar beet, such as photosynthesis and respiration, have not yet been characterized. Effects on leaf formation have been studied to a limited extend (Watson and Watson, 1953). Reduction in chlorophyll content in virus infected leaves has been associated with a reduction in photosynthesis, as shown for some potyviruses infecting *Gramineae* plant species (Tu *et al.*, 1968). Such changes in photosynthesis may be expected in BtMV infected leaves, given the chlorotic spots on leaves expressing the typical mosaic symptoms. This chapter reports the effect of BtMV infections on physiological characteristics of sugar beet leaves that affect crop growth and dry matter production. Damage was simulated and the effect of different injury components (physiological effects at leaf level) was assessed by incorporation of measured effects in the mechanistic crop growth model SUCROS (Goudriaan *et al.*, 1992), which was adapted for this purpose.

6.3. Material and Methods:

In a commercial sugar beet field of the cultivar 'Elisa', at Unifarm, Wageningen, The Netherlands, one set of 200 plants was inoculated with BtMV in the last week of May, and another set in the third week of June, 1998. The plants were then approximately in their six to ten leaf stage, respectively.

Five categories of leaves were distinguished to characterize the effect of BtMV infection on the light response curve, dark respiration, and chlorophyll content. The types were mature (I), and young (II) leaves from healthy plants, and mature leaves showing mosaic symptoms (III), mature leaves from infected plants but without mosaic symptoms (IV), and young leaves showing mosaic symptoms (V).

These leaves were randomly sampled in July and August from the 400 inoculated plants. The mature leaves used were collected between position five and 15 (counting the first leaf pair as numbers one and two), and young leaves between 20 and 30. The young leaves were usually larger than 3 cm.

6.3.1. Photosynthesis light response:

During the experimental period, rainy and cloudy weather prevailed, making the measurements of light saturated maximum photosynthesis in the field, using natural light,

impossible. Measurements were therefore made with artificial light in the laboratory. The plants had between 25 to 35 leaves when leaves were sampled. The leaves were detached early in the morning and brought to the laboratory. They were kept with their petioles in water to prevent wilting. Two leaves of each category were collected each day. Measurements were made no longer than six hours after sampling the leaves. The CO_2 assimilation was measured with a LI-6200 closed system (LI-COR, Lincoln, Nebraska) coupled to a LI-6250 gas analyzer, using a 1 l leaf chamber. Detached leaves were cut to a width of 6 cm and placed perpendicularly across the length of the leaf chamber, so that a 21 cm^2 rectangular leaf segment (3.5 cm length x 6 cm width) was exposed in the chamber. Light was provided by an assembly of three lamps close to the leaf chamber. The main source consisted of two halogen lamps that, with a filter, provided 300 W/m^2 at 5 cm from the lamp with a spectrum from 400 to 700 nm. Two complementary lamps were used (a Philips Ecotone 18 w lamp and a Philips PL-S 11W/84 lamp). These three light sources provided 100% of light intensity. The equipment calibration and measurement conditions were standardized at:

Air flow	: 550 to 700 µmol/s
Relative Humidity (RH)	: 60 to 80%
Temperature of the chamber	: 28 to 30 °C
Stomatal resistance (RS)	: 0.6 cm/s
Initial CO_2 concentration	: 350 to 360 ppm

Two green nylon nets, providing 30 and 50% of shade, were used alone or in combination to give 70, 50 and 35% of the total light intensity. Darkness was obtained by covering the chamber with black fabric. Gas exchange was measured by monitoring the change in CO_2 and water vapor concentration over three subsequent 15 sec depletion periods for each leaf segment and light intensity. A radiometer was fixed in the chamber next to the leave. The light response curved (LRC) was determined for 14 to 16 leaves of each leaf category.

The parameters of LRC were estimated by non-linear regression using the equation:

$$P = -R_d + P_m \cdot \left(1 - e^{-\left(\varepsilon \cdot PAR / P_m \right)} \right),$$

where:

P : net photosynthesis (μmol $CO_2/m^2/s$)

R_d : dark respiration (μmol $CO_2/m^2/s$)

P_m : gross photosynthesis (μmol $CO_2/m^2/s$)

ε : initial light use efficiency (μmol $CO_2/m^2/s$)/($J/m^2/s$)

PAR : radiation

As injuries, due to detaching and cutting the leaves, may increase dark respiration (Jevtic, 1972; Armsbrust, 1982), additional measurements of dark respiration were made on uncut leaves of the leaf categories I, II, III and V under field conditions (14 replications), enclosing 21 cm^2 of leaf area in the chamber. The enclosed area was estimated by assuming a trapezoidal shape of the leaf tip surface. The leaves were detached from the plant and immediately enclosed into the chamber, which was then covered with a black fabric to prevent any penetration of light.

6.3.2. Chlorophyll content determination:

Six to nine leaf discs, 5 mm in diameter, were randomly collected from the leaf categories I, II, III and V, in the third week of August. Chlorophyll was extracted using these disks by incubating 100 mg of leaf material in 10 ml *N,N*-dimethylformamide for 48 h at 4°C (Moran and Porath, 1980). The optical densities at 647 and 664.5 nm were measured for determining the total chlorophyll content (Inskeep and Bloom, 1985).

6.3.3. Light scattering:

Light reflection and transmission values were determined for healthy and infected mature sugar beet leaves (categories I and III), leaves 15 to 25, in the first week of October, using the LI-COR 1800, scanning the spectrum between 400 and 700 nm of five leaves of each category.

6.3.4. Specific leaf area:

The specific leaf area for healthy and infected sugar beet leaves was estimated by determining the dry weight of 11 replicates of 30 cm^2 segments of leaves from the categories I and III in the last week of September.

6.3.5. Modeling damage:

The results of the measurements of the light response curve, light scattering and specific leaf area were used to calculate expected effects of infection with BtMV on crop growth and production with the crop growth model SUCROS (Goudriaan *et al.*, 1992).

This model was parameterized for sugar beet by Kropff and van Laar (1993) and further adapted to simulate the formation of the following categories of leaf area: healthy, infected but with the virus still latent (hereby called latent), and infected and showing mosaic symptoms (hereby called mosaic expressing). External inputs for this model were incident radiation and daily minimum and maximum temperature. The model dynamically simulated the carbon budget and growth of the crop by integrating leaf photosynthesis over time and leaf area, taking into account incident light, leaf area index, proportion of mosaic expressing leaf area, and optical characteristics of leaves and canopy. Respiration losses were calculated on the basis of the weight of plant organs, the effects of BtMV, and effects of temperature on respiration rate. The growth of leaf area was determined by the dry matter accumulation, the temperature sum driven phenology, dry matter allocation, and the specific leaf area. The whole leaf area produced after the day of infection was considered to be BtMV infected. After elapse of the incubation period (Chapter 2), the amount of latently infected leaf area was, all at once, recruited to the mosaic expressing leaf class. Therefore, once the incubation period has passed, the whole newly growing leaf area, in addition to all area formed during the incubation period, was considered to be mosaic expressing. The rate of photosynthesis and respiration, reflection and transmission properties, and leaf thickness were adapted for the leaves in the mosaic expressing leaf category according the results obtained in the measurements described in 6.3.1, 6.3.3 and 6.3.4. All these changes in parameters may be considered as 'injury components' whose isolated and simultaneous effects could be assessed using the model, to provide insight in the relative importance of different components as determinants of crop damage (Rabbinge *et al.*, 1990; van der Werf *et al.*, 1991; Rossing *et al.*, 1992).

Four types of simulations were done with the model:

1. assessment of the effect of BtMV on growth and yield for crops inoculated at different dates (assuming 100% infection at once);
2. assessment of the effect of BtMV on growth and yield for realistic disease progress scenarios;
3. analysis of the relative effects of injury components; and
4. analysis of the importance of direct and indirect effects of BtMV infection on the crop development, through a feedback between leaf area development and CO_2 assimilation.

The realistic disease progress scenario's were observed in two fields in the 1995 and 1996 studies (figure 6.1). The spread in the experiment of 1995 had a bimodal character, with waves of spread in early summer as well as in early autumn, while the spread in 1996 showed only one wave in the late summer (Chapters 4 and 5). In 1995 and 1996, 1.4 and 5.4% of the plants were infected at the end of the growing season. To

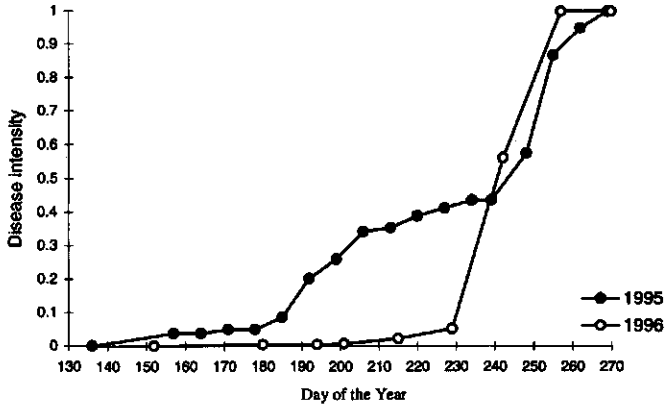

Figure 6.1. Spread of BtMV at De Bouwing, Zetten (1995) and UNIFARM, Wageningen (1996). Final disease intensities were scaled to 1 to allow direct comparison of the temporal pattern in both years.

facilitate interpretation of results, the disease intensities were re-scaled so that the final intensity was set at 1 (figure 6.1).

A component analysis was made to isolate the direct effect of the changed model parameters affected due to BtMV infection (P_m-AMAX, R_d-MAINLV, and light scattering (SCP)) on yield. Several simulations were performed, assuming, at each simulation, one or two of these parameters to be affected by the infection. Also, the model was modified to consider both leaf area index (LAI) and weight of leaves (WLV) to be unaffected by the infection, and the component analysis was repeated. Comparison of the simulation results with and without feedback from crop photosynthesis via leaf growth and light interception back to crop photosynthesis, allowed to separate direct and indirect effects of BtMV on crop production, where the extra reduction in yield with the feedback was defined as an indirect effect.

6.4. Results:

6.4.1. Effects of BtMV infection on photosynthesis, light scattering and specific leaf area:

Estimated values for the parameters of the light response curve, dark respiration and chlorophyll content of healthy and infected beet leaf material are presented in Table 6.1. Infection with BtMV reduced the maximum rate of leaf gross photosynthesis (P_m) in mature leaves by 16% and increased dark respiration (R_d) by 85 to 90% for young as well as mature leaves, when comparing leaf types of the same developmental stage. The initial light use efficiency (ε) was not affected.

Table 6.1. Estimated values for the parameters of the light response curves, dark respiration and chlorophyll content of five leaf types. Values are the average of 14-16 replications (± standard error of the mean).

Leaf type	P_m [1] ($\mu molCO_2/m^2/s$)	ε [2] ($\mu mol\ CO_2$ /μmol photons)	R_d [3] ($\mu molCO_2/m^2/s$)	*Chlor.* [4] (mg/g)
Healthy plants:				
Mature leaves (I)	25.4 ± 0.85^a	0.0741 ± 0.0033^a	1.1 ± 0.19^b	1.9 ± 0.14^b
Young leaves (II)	21.7 ± 1.02^b	0.0773 ± 0.0034^a	1.4 ± 0.23^b	2.4 ± 0.07^a
Infected plants:				
Mature leaves with symptoms (III)	21.4 ± 0.94^b	0.0805 ± 0.0035^a	2.1 ± 0.40^a	1.2 ± 0.12^c
Mature leaves without symptoms (IV)	25.7 ± 0.70^a	0.0756 ± 0.0033^a	nt [5]	nt
Young leaves with symptoms (V)	21.6 ± 1.42^b	0.0714 ± 0.0031^a	2.6 ± 0.20^a	2.2 ± 0.11^a

[1] gross photosynthesis;

[2] initial light use efficiency;

[3] dark respiration in the field;

[4] chlorophyll content;

[5] Not tested.

Outdoor pilot measurements of P_m, ε and R_d under natural light were replicated three times with leaf types I and III to obtain some information about possible side effects of the artificial conditions in the laboratory measurements. Values obtained for P_m and R_d were similar to laboratory readings. However, ε was smaller than for the laboratory measurements (0.056 ± 0.012 for leaf type I and 0.053 ± 0.009 for leaf type III), but not significantly.

Light transmission was significantly increased in infected leaves (t-test; p=0.04) and had values of 0.046 ± 0.007 (SE) for healthy and 0.066 ± 0.005 for infected leaves. BtMV infection did neither affect light reflection (N.S.;p=0.34) nor specific leaf area (N.S.; p=0.88). Light reflection was 0.100 ± 0.003 for healthy leaves and 0.104 ± 0.002 for infected leaves. Specific leaf area was 5.8 ± 0.39 mg/cm^2 in healthy and 5.7 ± 0.27 mg/cm^2 in infected leaves.

6.4.2. Simulation of damage caused by BtMV infections:

The mosaic symptoms induced in sugar beet by BtMV infections affected maximum photosynthesis rate, dark respiration and light scattering (reflection + transmission). These effects were introduced in the simulation model by replacing the healthy leaf values by the infected leaf values for the class of mosaic expressing leaves (table 6.2).

Table 6.2. Parameterization of the model for the effect of BtMV infections in sugar beet. The values were converted to the units used in the simulation model in which AMAX and MAINLV are expressed.

Leaf category	**AMAX**[1] (kg CO_2/ha/h)	**MAINLV**[2] (g CH_2O/gDM leaves/d)	**SCP**[3]
Healthy	40.3	0.030	0.143
Infected	33.9	0.056	0.169

[1] Gross photosynthesis;

[2] Maintenance respiration. Values based on dark respiration measurements;

[3] Light scattering.

Table 6.3. Simulated damage for crops which became completely infected with BtMV at different dates during the growing season.

Day of infection (day of the year)	**LAI at infection** (m^2 leaf / m^2 soil)	**Yield** (kg/ha)	**Yield reduction** (%)
Healthy	-	15811	0
140	0.01	12752	19
160	0.19	13356	16
180	2.72	14762	7
200	5.00	15476	2

The yield reduction caused by BtMV infections was simulated assuming that the crop became completely infected after being inoculated at different dates. The results show that the earlier the crop becomes infected the higher the damage in yield (table 6.3).

The maximum damage was a 20% decrease in storage organ weight (Figure 6.2). LAI in early infected crops is, from day 160 onwards, reduced by 20 to 45 % (Figure 6.3). When virus in inoculated later in the season, the effect on LAI decreases.

Simulated damage caused by the infections in the experiments in 1995 and 1996 resulted in an estimated damage of 2.92% and 0.55% respectively. Simulated damage for the number of plants infected at each date, according the disease progress curve, was added up to estimate final yield damage of the crop as a whole.

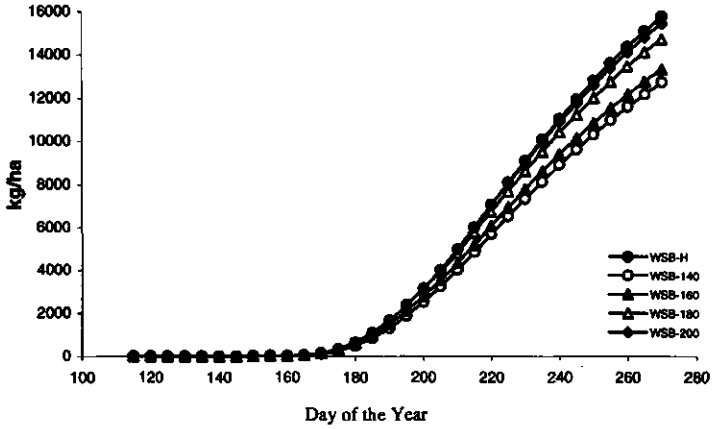

Figure 6.2. Simulated weight of storage organs produced by sugar beet crops which became completely infected using different inoculation dates and a healthy crop.

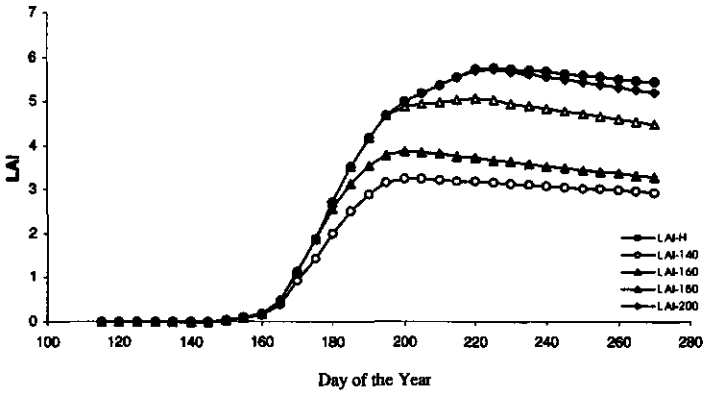

Figure 6.3. Simulated leaf area index (LAI) of healthy and BtMV infected crops, inoculated at different dates during the growing season.

Table 6.4 presents the reduction in yield caused by individual or combined components of the model. Reduction in maximum photosynthesis rate (AMAX) and dark respiration (MAINLV) equally affected yield and could be identified as the major damaging components. When assuming a constant leaf area for healthy and infected crops, MAINLV of infected leaves was of a higher relative importance in decreasing yield than AMAX. Light scattering (SCP) had little effect in reducing yield under both simulation assumptions.

Table 6.4. Isolated effects of individual or combined injury components on the simulated yield of sugar beet infected by BtMV with simulated (s) and forced (f) leaf area. Simulations with simulated leaf area take into account the effect of BtMV on light absorption and crop carbon balance. Simulations with forced leaf area assume that leaf weight and area are unaffected by BtMV infection, light absorption or carbon balance, and they are taken equal to the corresponding values for the control simulation. The yield reductions in the weight of storage organs of sugar beet (WSB) as a proportion of the yield of healthy crops.

Parameter	WSB-140-v	WSB-180-v	WSB-140-f	WSB-180-f
AMAX	0.116	0.022	0.050	0.024
MAINLV	0.127	0.044	0.106	0.056
SCP	0.062	0.003	0.004	0.002
AMAX and MAINLV	0.186	0.065	0.156	0.080
AMAX and SCP	0.124	0.024	0.054	0.026
MAINLV and SCP	0.135	0.046	0.110	0.057
ALL 3	0.193	0.066	0.160	0.082

6.5. Discussion:

Sugar beet plants infected with BtMV show symptoms in all leaves that develop after the infection. All leaves already present on the plant prior to infection will not develop any symptom. This characteristic implies that, to evaluate the effect of infection on photosynthesis, the leaves of the plants should be divided into distinct categories as proposed in section 6.3. The values found for the estimated parameters of the LRC for the different leaf types supported this division (table 6.1).

Previous reports on the 40% damage caused by BtMV (Molz, 1927) were not supported by simulation analyses as done in this study, since under the most severe infection conditions, simulated damage was in the order of 20%. Watson and Watson (1953), however, suggested that a small damaging effect of BtMV is expected, due to the low natural levels of infection. Other plant pathologists, as reviewed by Severin and Drake (1948), came to similar conclusions. The simulation results obtained in our study corroborate the suggestions of the previous studies in which little damage is expected in BtMV infection.

6.5.1. Injury components:

BtMV caused a reduction in P_m only in mature leaves. P_m, like the chlorophyll content, is not affected in young developing leaves. The chlorophyll content, however, was

reduced with 37% in mature leaves of infected plants. Our results show that the reduction of P_m was considerably smaller than the proportional reduction in the chlorophyll content. In corn and sorghum plants infected with *Maize dwarf mosaic virus*, a potyvirus, the reduction in net photosynthesis was greater than the reduction in chlorophyll content, and when subtracting the effect of dark respiration, apparent photosynthesis (comparable to P_m) was still greater than reduction in chlorophyll content (Tu *et al.*, 1968). These authors suggest that efficiency of chlorophyll in mosaic expressing leaves is smaller than in healthy leaves. In the BtMV infected leaves, however, an opposite relation was found. More studies are required to elucidate the relation between virus infection, chlorophyll content and photosynthesis.

Jones *et al.* (1977), studying the effect of *Ryegrass mosaic virus* (RMV) in ryegrass, concluded that changes in both P_m and R_d are responsible for the reduction in net photosynthesis and that initial light use efficiency (ε) was not affected by the infection. Precisely the same results were obtained in this study.

LRC determined under natural light conditions was used only as a control to check whether the laboratory measurements were affected by the quality of light. Indeed, differences were detected in the value of ε when varying the light source, but the comparative differences between leaf types were the same. This higher ε under laboratory conditions may be an artifact caused by the use of an artificial light source at close distance. As the area illuminated by the source was rather small (approximately 100 cm^2), any light reflected by the chamber and falling on the back of the leaf would not be measured by the radiometer placed inside the chamber, but nevertheless increase photosynthesis. When comparing the measurements in natural sunlight conditions, the values obtained were equivalent to previously determined values for sugar beet plants (Kropff and Spitters, 1992). These were, therefore, used in the present model.

The analyses of components performed indicated that the decrease of P_m and the increase of R_d were the major causes of simulated damages and equally important when infections take place early in the season. With later infection, or when the development of LAI is assumed not to be affected by BtMV infections, R_d has a proportionally higher effect than P_m. Light scattering was a minor damaging component as only the reduction in net photosynthesis accounts for 94% of the damage.

The indirect effect of the virus, represented by the effect on the plant development (reduction of the total leaf area) is small. Assuming that the virus infections did not affect plant development, simulated damage was smaller in crops in which LAI was reduced by the infection. This reduction in damage (17%) was smaller than the gain in leaf area (20 to 45%). The damage found in late infected crops was mainly due to an enhanced R_d in infected leaf area. Simulation of infection showed a reduction in LAI in the early infected crops. No or only a small effect was found when the infections which occur late in the

season, and those found in the 1995 and 1996 experiments, were analyzed by simulation. The results are in agreement with those previously reported by Watson and Watson (1953). The limited reduction found in the total leaf area might be an intrinsic characteristic of the crop. For ryegrass infected with RMV, a significant reduction in leaf area was found (Jones *et al.*, 1977).

6.5.2. The late infections under field conditions limits damage:

The simulated damage related to the spread of BtMV is summarized for the years 1995 and 1996 (Fig. 6.4). The continuous line represents the simulated damage as a function of the progress of the disease incidence. The damage decreases steeply when disease establishes later in the crop during the growing season. The rate of spread of the disease in 1995 and 1996 was plotted in the same figure. The low damage found in both years is related to the late spread of the disease. In 1995, two aphid flights, reflected in a bimodal curve for the rate of spread (begin of July and begin of September, respectively), occurred. Only one flight was observed in 1996, which resulted in a sharp rise of infection in the second half of August. The early spread in 1995 is mainly responsible for the damage simulated (Figure 6.4). This indicates that the LAI of the 1996 crop, at the moment that the disease occurred, was large enough to sustain the yield nearly at the level of healthy crops.

In The Netherlands, BtMV epidemics develop late in the season, starting at the end of July and mid August (Chapters 3, 4 and 5). Simulated damage of these epidemics were in the order of 2%, which is small considering the cumulatively potential damage caused by yellowing viruses in sugar beet (van der Werf, 1988), other pathogens and environmental factors. This analysis pinpoints a major reason for the rather harmless nature of BtMV in sugar beet. The disease is spread at a time when the crop has accumulated leaf area to become tolerant for infection. In other potyvirus-crop systems, however, this is not the case as with *Papaya ringspot virus* in cucurbits (Dusi, 1992) or *Potato virus Y* in seed-potatoes (Beukema and van der Zaag, 1979). However, in sugar beet, it does not matter if the roots to be processed come from virus infected plants or not, as long as sugar content and other quality aspects are not affected.

6.6. Acknowledgement :

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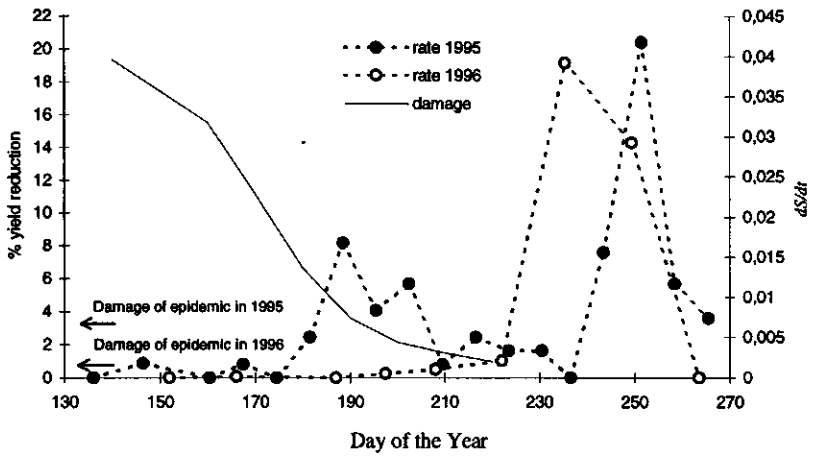

Figure 6.4. Damage as a function of inoculation date (continuous line) and rate of spread of BtMV in 1995 and 1996. Arrows represent the damage level of the epidemics in 1995 and 1996.

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Chapter 7

General Discussion

7.1. Overview:

The aim of the studies described in this thesis was to obtain a thorough understanding of the main factors determining the spread of a potyvirus in a high plant density crop. The factors studied included the relationships between virus, host and vector, the spread of the virus around an initial virus source consisting of one or more infected plants, the spread of the virus by the prevailing aphid population, and the effect of plant density on the spread of the virus. A time–save sampling technique was developed and the damage caused was estimated. This study was made with the system beet - beet mosaic virus (BtMV), a potyvirus infecting sugar beet, as a model pathosystem. Sugar beet is a herbaceous plant widely cultivated in The Netherlands. The crop, which has a cycle of approximately 8 months, is cultivated in fields at a density of 7 to 10 plants/m^2. The disease in this crop is polycyclic, as several infection cycles occur during the growing season.

The spread of a potyvirus in a crop starts with a primary infection, either introduced by migrating aphids from sources outside the field or by the use of infected seed or propagative plant material. These plants form the sources from which the virus is spread secondarily in the field. The primary infections are in most cases scattered over the field, whereas secondary infections are aggregated around early-infected plants. Studies on the spread of a virus from a known source are few, as primary introductions are difficult to prevent in many crop virus system. Primary infections are frequently introduced at erratic moments and increase in virus incidence, due to plants infected from outside sources is superimposed on the secondary spread ongoing within the field. As BtMV is only rarely encountered in The Netherlands and not seed transmitted, this pathosystem is a good model to analyze secondary spread using a known virus source in the experimental plots. Spread was expected only to occur from these sources and not from outside sources.

7.2. Development of a time saving transect sampling method:

Spread of BtMV occurred around the virus source in a clustered isotropic pattern with a negative exponential gradient. Such a spread is common for polycyclic epidemics of potyviruses in annual crops (Dahal, 1992; Eckel and Lampert, 1993; Nelson and Campbell, 1993; Perring et al., 1992). The isotropic spatial pattern of spread found in all plots showed that a simple sampling method, called transect sampling method, could be developed and used to monitor the development of the infection. This method consisted of monitoring the plants on two orthogonal transects extending diagonally across the rows from the source plants in the plot. In the analysis of transect data, the uneven representation of the sampled plants at each distance class must be taken into consideration. The temporal and spatial spread of the BtMV disease could be described as reliably using the transect method, as by monitoring the whole plot, provided that a lower precision per repetition is compensated by

raising the number of repetitions. This result suggests that by using this less labor intensive and less time consuming sampling method, more sites or more treatments can be studied.

This sampling method can also be applied to study the spread in other pathosystems such as *Papaya ringspot virus* (PRSV) in cucurbits, *Potato* virus *Y* (PVY) in crops of various solanaceous species, and *Soybean mosaic virus* (SMV) in soybeans, when the virus source is known or can be found. This sampling method can potentially also be applied to semi-persistently and persistently transmitted viruses such as *Beet yellows virus* (BYV) and *Beet mild yellowing virus* (BMYV) (van der Werf, personal communication), which form usually clusters with an isotropic spatial pattern around the primarily infected virus source, in a similar fashion as BtMV.

7.3. Modeling spread as a function of migrating aphid flights:

Under natural conditions, aphids transmit potyviruses in a non-persistent manner. Although the interaction between the virus and the vector is specific (Shukla et al., 1994), apparent specificities in the epidemiological relationships between potyviruses and aphid species have not been elucidated. The role of the individual aphid species in the spread of potyviruses has been analyzed by different analytical methods. The simplest approach is to plot virus incidence and number of aphids counted on plants or collected with traps on a common time axis and to subjectively compare the curves obtained for the spread and the number of aphids obtained for each individual species or the total aphid population. Eckel and Lampert (1993), van Hoof (1977) and Karl et al. (1983) used this approach but could not find any relation between the species and the spread of the potyviruses studied. A pitfall of this approach is that population trends of different aphid species over time may be collinear (Chapter 4). Thus, the role of one species might not be isolated from the other. Correlation and regression analysis also usually fail to relate spread to aphid species or total counts (Madden et al., 1987; Mora-Aguilera et al., 1992; Watson and Healy, 1953).

Garrett (1988) demonstrated that, in lupine, *Clover yellow vein virus* was mostly spread by two aphid species (*Aphis craccivora* and *Myzus persicae*) using multiple regression analysis to relate the rate of spread of this potyvirus to the species that compose the aphid population. In the studies described here, no single species could be associated with the spread applying correlation or regression analyses. A good correlation could be detected between the total daily number of alatae caught and the spread of BtMV.

Based on the collected data, a deterministic simulation model was developed to study the spread as a function of the migrating aphid population (Chapter 4). This model was based on a logistic population growth applied to plant diseases (van der Plank, 1963). The rate of the disease was, in this model, proportional to the virus sources, healthy plants, latent and incubation periods of the virus in the plant, the total number of aphids caught in a suction trap (not discriminating species) and a parameter (r) that represented all aspects

of vector activity relevant to virus spread (Jeger et al. 1998). This parameter *r*, describing the relationship between the daily catches of aphids and the number of newly infected plants, was quite robust among experiments. Remarkably, *r* appeared to be independent of the moments at which the primary inoculum sources were introduced, confirming that the chosen model and common parameter value give a seasonable mechanistic description of epidemics started at different dates. These results confirm and extend the conclusions of Di Fonzo et al. (1997), Madden et al. (1987), Mora-Aguilera et al. (1992) and Nemecek (1993), that migrating aphids, regardless of the species, play a major role in the spread of non-persistently transmitted viruses.

The simulation model used in this study only accounted for secondary spread as introductions from external virus sources rarely occur in the Netherlands. The absence of any spread from outside sources allowed inoculating the field at different dates. This simulation model could simulate the final number of plants showing symptoms. A rough approximation, using the averaged obtained value for *r* and aphid catches showed that the number of infected plants to occur could be predicted two weeks in advance. The use of this model as a predictive tool for the whole crop cycle is premature because it does not model the development of the aphid population. Although *r* was conserved between the inoculation dates in each experiment, it varied between experiments. The species composition of the aphid population varies each year. Although the total number of aphids caught could be related to virus spread, the rate by which the virus will be spread will differ among years and among locations. In more elaborate models, *r* must be decomposed into different components representing the behavior of the aphid population such as the acquisition and inoculation rates, the infectious period of the virus in the vector, the vector turn over, the feeding time per vector per day, and the distance hopped by aphids (Jeger et al., 1998).

The simulation models used by Nemecek (1993) and Sigvald (1992), to predict *Potato virus Y* spread in potatoes, included some behavioral characteristics and a more detailed description of the aphid species composition. These models could be used to simulate the final disease incidence in crops, which were initially infected with different numbers of virus sources. The studies in *Soybean mosaic virus* presented by Ruesink and Irwin (1986) also included some behavioral aspects of the vector and could be used to predict yield and level of seed transmission. The complementary information added by the present study was an experimentally demonstration that spread of BtMV is related to the major migrating aphid flight. The calibration studies using a simulation model confirmed that this spread could be described by one absolute rate parameter. It can be concluded that management strategies to control virus spread have to be focused on a delay of virus introductions in the field, or alternatively, to restrain aphid dispersal early in the season.

The deterministic model developed in Chapter 4 was adapted to include a factor that describes the effect of plant density in the rate equation. This factor could be included on the assumption that plant density would affect the spread by affecting the number of aphids per plant, and the number of available plants, while the other parameters related to spread would remain constant. The spread was indeed inversely proportional to the plant density in the first weeks after the virus started to spread. However, analyzing the incidence for the whole growing season, the model failed to explain the observed spread. Values of r estimated by calibration, to experimental field data, showed that the rate of spread in low-density plots was lower than the rate expected by the hypothesis that spread is proportional to the number of aphids per plant. The factors that lead to the strong aggregation of the infected plants around the primarily infected plant might have affected the rate of spread in these low-density plots.

The contrast between bare soil and plants will be larger in low-density plots than in plots with standard density. By the middle of July, when most of the aphid migration occurred in both years, the canopy was closed in the standard density plots while bare soil was still visible in the low-density plots. An attraction exerted on the aphids by the contrast between plants and bare soil might have affected the mobility of the aphids within the plot, reducing the distance hopped between plants and, consequently, the spread of the disease as the infected plants might be re-inoculated frequently. A factor considering this distance and/or the spatial pattern of the spread must be included to improve the model. Improvements of this model must, therefore, concentrate on the inclusion of a set of equations representing the vector behavioral components and the spatial pattern of spread.

7.4. BtMV and damage in sugar beet:

Experiments to determine damage due to virus infections are laborious. It is assumed that yield will depend on date of inoculation, initial inoculum levels, rate at which the virus spreads, disease incidence at the moment of harvest, and others. Since many factors will affect the yield, it will be difficult to estimate the crop losses caused by a pathogen. The use of a crop growth model can overcome these difficulties, assuming that the parameters related to damage can be determined and incorporated in the model. Information on damage caused by BtMV is rare in the literature, but it is generally accepted that this disease has little impact on yield of sugar beet (Watson and Watson, 1953).

The effect of BtMV infections on the yield of the sugar beet crop was evaluated by simulation using a crop growth model (SUCROS). This analysis was experimentally grounded by determination of the light response curve, light absorption and transmission, and other parameters on healthy and BtMV infected leaves showing mosaic symptoms. The model dynamically simulates the carbon budget and growth of the crop by integrating leaf photosynthesis over time and leaf area, taking into account incident light, leaf area index,

proportion of mosaic-affected leaf area, and optical characteristics of the leaves and the canopy. The damage simulated for early-infected crops was estimated to be, under the most extreme situation, approximately 20%. However, as the infection usually starts to spread in the second half of the growing season, the estimated damage in a fully infected crop after July was less than 3%. This value can be neglected considering the damage due to other diseases, harvesting and processing of the roots. Injury component analyses indicated that the direct effect due to both reduction in maximum rate of photosynthesis (P_m) and increase in dark respiration (R_d) were the major causes of the simulated damage (Chapter 6).

The simulation studies demonstrated that the usually observed negligible damaging effect of BtMV is due to the late occurrence of the spread of the disease under field conditions (Chapters 3 and 4). When infection takes place, the crop has already a large enough area of healthy leaves to sustain the yield, even if all plants in the field were infected after the middle of the growing season (Chapter 6). This study is probably the first which simulates crop damage caused by a potyvirus, and it is certainly the first simulation study of the damage caused by BtMV in sugar beet.

7.5. Concluding remarks:

As a model is a simplification of reality, perfection is not expected (Ruesink and Irvin, 1986). Several improvements can be made to the simulation model presented in this study to describe the disease incidence. The present version allowed to test the raised hypothesis that spread was a function of the migrating aphid population, for every date at which the inoculum source was introduced. The results of the analysis of the rate of spread of the field experiments, together with the modeling studies, could indicate the model has to be improved by including an aphid population sub-model that describes vector behavior. It suggested also that the spatial characteristics of spread must be taken in account.

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Chapter 8

Conclusions

8.1. General conclusions:

1) The development of a less labor-intensive transect sampling method provided a tool to study the secondary spatial-temporal spread of viral diseases that develop in an isotropic spatial pattern around a known point source. Crops such as cucurbits, potato, soybean, sugar beet, sweet pepper and tomato are cultivated in large areas throughout the world. This sampling method reduces the time to monitor the growth of disease patches substantially, allowing to include more treatments or field sites in experimental studies;

2) The migration of aphids could be related to the spread of non-persistently transmitted viruses and the daily total aphid population catches could be used as a forcing function in a dynamic simulation model, rather than a single aphid species or the combination of a few key species. The model in this study was a first step to relate spread to aphid population;

3) Control measures that delay the introduction of BtMV in the field reduce loss in two ways: by reducing damage at the plant level and by decreasing disease incidence at the crop level. The simulation studies demonstrated that the general believe that BtMV causes little damage is due to its late natural occurrence. When spread occurs, the crop has already a leaf area enough to sustain yield at commercial levels that do not require control measures against the disease;

4) Definition of planting dates that avoid or delay the exposure of the crop to the migrating aphid population would result in less spread and less damage. For that, an aphid monitoring system must be operating regularly for determination of a time series of data. From the three vector pressure systems studied (suction trap, green water pan and bait plants), the suction trap gave the best statistical relationship with virus spread;

5) Crop management that reduces plant population as a tool to improve soil erosion control, to improve the cosmetic quality of the final product of the crop, or to control soilborne diseases, may enhance the spread and yield impact of potyviruses. In sugar beet, BtMV incidence will increase. However, this increase is proportionally smaller than would be expected if only the ratio aphid numbers/density was affecting the rate of spread;

6) The use of the BtMV spread simulation model (Chapter 4 and 5) as a predictive tool is still premature. To generalize the use of the model for disease forecasting, an aphid behavioral component (Jeger *et al.*, 1998) must be developed. A spatial component must also be included, especially for the modeling of the effect of plant density on the spread.

8.2. References:

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Samenvatting

In dit proefschrift worden de resultaten beschreven van een studie naar de verspreiding van het bietenmozaïekvirus (in het Engels beet mosaic virus, afgekort BtMV) in suikerbieten en de schade die dit virus veroorzaakt. Dit virus, een lid van de familie der Potyviridae, wordt in Nederland tegenwoordig zelden meer in het veld waargenomen en veroorzaakt geen economische schade van belang. Dit geringe voorkomen van dit virus is in wetenschappelijk opzicht een voordeel omdat veldproeven over de verspreiding van het virus daardoor niet of niet noemenswaard worden verstoord door infecties van buitenaf. Het virus kan derhalve model staan om de verspreiding te bestuderen van vele door bladluizen overgedragen potyvirussen, waartoe een aantal economisch zeer belangrijke virussen behoren, zoals het aardappel Y virus. Ten opzichte van een aantal andere potyvirussen heeft het bietenmozaïekvirus verder nog het voordeel dat men geïnfecteerde planten gemakkelijk aan de symptomen kan herkennen.

Allereerst (Hoofdstuk 2) werd de relatie tussen de vector, het virus en de waardplant bestudeerd. Aspecten die werden gekwantificeerd in proeven waren de lengte van de latentieperiode, dat is de tijd die verstrijkt tussen infectie van een plant en het beschikbaar komen van virus voor verwerving door bladluizen uit die plant, en de incubatieperiode, dat is de tijd die verstrijkt tussen infectie van een plant en het verschijnen van de symptomen. Latentie- en incubatieperiode waren nagenoeg gelijk aan elkaar en varieerden met de hoogte van de temperatuur van een ruime week bij een temperatuur van 25 °C tot drie weken of langer bij temperaturen van 15 °C, zoals dat vaak in het naseizoen in Nederland van toepassing is. Ook werd de periode gemeten gedurende welke een bladluis in staat is het virus na verwerving over te dragen. Deze periode, retentieperiode geheten, is voor BtMV in verschillende bladluissoorten vergeleken. Voor de meeste bladluissoorten bedroeg de retentieperiode enkele uren, maar voor *Myzus persicae* liep deze periode op tot wel 16 uur. Deze waarneming doet vermoeden dat er in bladluizen mogelijk twee verschillende mechanismen verantwoordelijk zijn voor de retentie en overdracht. Het ene mechanisme wordt gekarakteriseerd door een korte retentieperiode, terwijl het andere mechanisme een langere retentieperiode tot gevolg heeft.

In de praktijk zijn van buiten het perceel afkomstige vliegende bladluizen verantwoordelijk voor de eerste infecties in een bietenperceel. Rond zulke primair geïnfecteerde planten kan vervolgens secundaire verspreiding optreden. Voor deze secundaire verspreiding zijn vliegende bladluizen, die op zoek zijn naar een nieuwe voedselbron verantwoordelijk, en in het algemeen niet die bladluizen die het gewas koloniseren en er zich op voeden. Zoeken naar een nieuwe voedselplant is bij bladluizen een normaal verschijnsel. Het vormt een integraal deel van hun levensstrategie bestaande uit opeenvolgende fasen van kolonisatie van waardplanten, populatiegroei op deze planten,

vleugelvorming bij de bladluizen wanneer hun dichtheden groot worden, en tenslotte migratie naar nieuwe waardplanten. In Hoofdstuk 3 van het proefschrift wordt beschreven hoe de secundaire verspreiding rond een primair besmette plant zich in de loop van de tijd ontwikkelde. Na enige tijd onstaat er een 'haard', bestaande uit geïnfecteerde planten rond een primair besmette plant. De vorm van deze haarden is min of meer rond. Een neiging om zich sterk in een bepaalde richting (anisotropie) uit te breiden, bijv door wind of sluiten van het bladerdak in een rij planten, werd in deze studie niet gevonden.

Het bemonsteren van bietenpercelen – of delen daarvan – om de verspreiding van het virus in tijd en ruimte te volgen is arbeidsintensief en tijdrovend. Om efficiënter te kunnen werken werd een waarnemingsmethode getoetst waarbij alleen planten worden geïnspecteerd op twee transecten die een hoek van 45 graden maken met de bietenrijen (Hoofdstuk 3). De transecten lopen door de primaire virusbron, die in veldstudies door kunstmatige infectie werd gecreëerd. De nauwkeurigheid van deze bemonsteringsmethode werd gekwantificeerd. Daarbij werd gelet op: (1) het geschatte uiteindelijke aantal besmette planten in een haard; (2) het tijdsverloop van het aantal besmette planten in een haard; (3) de helling van de 'gradiënt', dat is de mate waarin het percentage besmette planten afnam met de afstand tot de primaire bron; en (4) de gemiddelde afstand van besmette planten tot de bron. Op de belangrijkste punten gaf de transect-methode een voldoende nauwkeurige schatting van de gewenste populatie-karakteristiek. Uiteraard was de nauwkeurigheid geringer dan die van een volledig monster, dat verkregen wordt door inspectie van alle planten in een bepaald gebied rond de primaire bron, maar de geringere nauwkeurigheid per waargenomen haard kon voor de meeste karakteristieken uitstekend gecompenseerd wordend door meer haarden te inspecteren, en dan werd er nog steeds tijd bespaard. De nieuw ontwikkelde methode geeft een tijdwinst van zeker 80 %. Door toepassing van deze methode konden de hierna beschreven proeven in een groter aantal percelen worden herhaald.

In een serie van vier veldproeven (Hoofstuk 4) werd nagegaan wat het effect is van de primaire infectiedatum op de mate van verspreiding die vanuit een primaire bron plaatsvindt. De resultaten tonen aan dat het tijdstip waarop de eerste plant besmet wordt bepalend is voor de mate waarin het virus zich verspreidt in het groeiseizoen. De verspreiding bleek sterk gecorreleerd te zijn met de grootte en timing van de bladluisvluchten, en zolang er geen vluchten optreden ligt de verspreiding als het ware stil. Geen correlatie werd gevonden tussen de mate van verspreiding en de vangsten van individuele bladluissoorten. De totale bladluisvangst, de som van alle individuele soorten, bleek de beste voorspeller op te leveren van de mate van virusverspreiding. Op grond van algemeen geaccepteerde epidemiologische principes werd een dynamisch simulatiemodel ontwikkeld dat de mate van virusverspreiding als functie van de intensiteit van bladluisvluchten beschrijft. Het bleek dat de vier genoemde proeven en alle behandelingen

binnen elke proef met dit model goed te beschrijven waren, en dat de enige parameter in dit model in getalswaarde weinig tussen behandelingen en proeven varieert. Deze parameter karakteriseert de relatie tussen de intensiteit van bladluisvluchten en de dientengevolge gelijktijdig optredende verspreiding. De conclusie is dat op basis van bladluisvluchten de waarschijnlijke mate van verspreiding vrij goed te berekenen is.

Verspreiding van virussen kan ook beïnvloed worden door de plantdichtheid van het gewas. Over de relatie tussen plantdichtheid, vectorgedrag en virusverspreiding is nog veel onzekerheid. In vier veldproeven (Hoofdstuk 5) werd de relatie bestudeerd tussen plantdichtheid en verspreiding van het bietenmozaïekvirus. In veldjes van 8 x 8 m werden plantdichtheid en plantverband gemodificeerd door uit een praktijkgewas rijen of planten te verwijderen. Dit resulteerde in duidelijke effecten op de verspreiding. In veldjes met een lage plantdichtheid kwam de verspreiding vroeger op gang, maar raakten uiteindelijk over het algemeen lagere aantallen planten besmet dan in veldjes met een hogere plantdichtheid. Het percentage besmette planten was altijd het hoogst in veldjes met een lage plantdichtheid.

Met behulp van modellen werd nagegaan of op basis van een drietal hypothesen het effect van plantdichtheid op de verspreiding kon worden verklaard. Deze hypothesen waren achtereenvolgens: (1) vector-concentratie; (2) vector-attractie; en (3) waardplant-limitering. De eerste hypothese stelt dat naarmate de plantdichtheid afneemt, er bij gelijkblijvende landingsfrequentie van bladluizen per vierkante meter meer bladluizen per geïnfecteerde plant te vinden zouden moeten zijn in veldjes met een lage plantdichtheid dan in veldjes met een hoge plantdichtheid. Omdat de verspreidingssnelheid evenredig is met het aantal vectoren per besmette plant, valt volgens deze hypothese te verwachten dat de verspreidingssnelheid, gemeten in planten per vierkante meter per dag, omgekeerd evenredig zal zijn met de plantdichtheid; met andere woorden: als de dichtheid halveert, verdubbelt de verspreidingssnelheid. De tweede hypothese doet hier nog een schepje bovenop door te stellen dat een plantverband met veel kale grond tussen de planten een attractief effect heeft op aanvliegende bladluizen. Deze hypothese werkt dezelfde richting op als de eerste, en versterkt deze. Hypothese 3 stelt dat naarmate er meer planten in een veldje besmet zijn er ook meer infecties zullen worden aangebracht op planten die reeds besmet zijn. Deze hypothese impliceert dus dat naarmate de plantdichtheid lager wordt, de verspreiding stagneert ten gevolge van een uitputting van het aantal nog beschikbare gezonde planten. In het begin van het seizoen bleek een model waarin hypothesen 1 en 3 waren verwerkt de werkelijke verspreiding goed te beschrijven, maar over het geheel van het groeiseizoen genomen wezen de resultaten erop dat het aantal vectoren per plant onafhankelijk zou zijn van de plantdichtheid. Mogelijke verklaringen voor dit onverwachte resultaat worden in het hoofdstuk besproken. Onder andere wordt erop gewezen dat de gehanteerde modellen geen rekening houden met het ruimtelijke patroon van besmette en

gezonde planten in een haard en ook geen rekening houden met het ruimtelijk gedrag van de bladluizen. De ruimtelijke aspecten van vectorgedrag en virusverspreiding worden genoemd als een interessant onderwerp voor verdere studie. Hierover is nog weinig bekend, en zonder deze kennis kunnen de resultaten van de in hoofdstuk 5 beschreven proeven niet goed worden verklaard.

In een experiment waarin rijen besmette en gezonde planten op kale grond en op gras naast elkaar werden geplaatst om vector activiteit te kwantificeren, werd gevonden dat op kale grond twee tot drie keer zoveel van de gezonde planten besmet raakten als op gras (Hoofdstuk 5). De resultaten van deze proef tonen aan dat de hypothesen van vector concentratie en/of vector attractie niet uit de lucht gegrepen zijn, maar in proeven bevestigd kunnen worden.

Over de effecten van het bietenmozaïekvirus op de opbrengst van suikerbieten is weinig bekend. Een praktijkervaring is dat aantasting door dit virus weinig schade veroorzaakt, maar waar slechts weinig onderzoek naar is gedaan. In Hoofdstuk 6 wordt beschreven hoe de effecten van virusinfectie op de fotosynthese en ademhaling van bladeren wordt bepaald. Het blijkt dat in bladeren met mozaïeksymptomen de maximale fotosynthesesnelheid met ongeveer 15% is afgenomen t.o.v. gezonde bladeren, terwijl de ademhalingssnelheid is verdubbeld. Verder laten bladeren met mozaïeksymptomen 2% meer licht door dan gezonde bladeren. Alle drie de gemeten effecten beïnvloeden de groei en opbrengst van het gewas, en de mate waarin dit gebeurt wordt berekend met behulp van een simulatiemodel voor de lichtonderschepping, koolstofbalans en groei van een suikerbietengewas. In dit model zijn de gemeten effecten van het bietenmozaïekvirus verwerkt, en wordt ook de ontwikkeling van de symptomen in afhankelijkheid van de infectiedatum op een natuurgetrouwe manier gesimuleerd. Simulaties met het model tonen aan dat de gemeten effecten bij volledige en zeer vroege besmetting van een gewas een opbrengstreductie van om en nabij de 20% veroorzaken. De reductie van de fotosynthese in bladeren met mozaïeksymptomen, en de verhoogde ademhaling zijn in ongeveer gelijke mate verantwoordelijk voor deze schade. In de praktijk echter vinden infecties geleidelijk over het groeiseizoen plaats, en veel planten worden pas in zo'n laat stadium besmet dat slechts een gering deel van de bladeren te lijden heeft van de beschreven effecten op fotosynthese en ademhaling. Daardoor is bij een realistisch, in hoofdstuk 4 beschreven, ziektenverloop in het gewas de mate van opbrengstderving slechts enkele procenten, zelfs als ten gevolge van talrijke primaire infecties het eindniveau van besmetting zou oplopen tot 100%. Deze studie toont daarmee aan dat de geringe opbrengsteffecten van het bietenmozaïekvirus vooral toe te schrijven zijn aan het late moment van infectie, en niet zozeer aan een intrinsieke non-agressiviteit van het virus zelf; immers bij vroege infectie is het effect volgens de simulaties wel degelijk substantieel.

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Curriculum Vitae

July 20. A day in which two big events in history took place. In 1969, Neil Armstrong walked for the first time on the moon. In 1961, André Nepomuceno Dusi was born in Brasília, Brazil. And yes, I had the Apollo 11 on the top of my eighth birthday cake. At the age of 16, I started my studies on Agronomy, at the University of Brasilia. I graduated in March 1983. From April 1983 to January 1984, I worked at Embrapa/Cenargen at the Quarantine Service. This was my first footstep in plant virology. From February 1984 to February 1987 I lived in Viçosa (Minas Gerais state, Brazil) where I got my M. Sc. in Plant Pathology (virology), and met my wife. In July 1987 I joined the staff of Embrapa/Hortaliças, where I still work. From 1987 until 1994 I concentrated my work on the viruses infecting vegetables. Meanwhile, my two daughters were born. I had the opportunity to develop joint programs with several Research Institutes and Universities in Brazil and in other countries such as Argentina, Canada, Japan, Peru, The Netherlands, Uruguay and USA. In December 1994 I arrived in Wageningen for my Ph.D. studies, at the Laboratory of Virology of the Wageningen Agricultural and Research Centre, to work on the epidemiology of an aphid transmitted potyvirus. The results of this work are presented in this thesis.