W. van der Werf

Yellowing viruses in sugarbeet; epidemiology and damage

STELLINGEN

1. Kennis van de incubatieperiode is onontbeerlijk voor een goed begrip van de epidemiologie van virusziekten en van de effecten van deze ziekten op de opbrengst van gewassen.
Dit proefschrift.

2. Men zou in de virus-epidemiologie met vrucht gebruik kunnen maken van het feit dat de infectiedatum dikwijls kan worden afgeleid uit de positie van het oudste systemisch besmette blad aan een plant.
Dit proefschrift.

3. Vangplant-experimenten ter vaststelling van vectordruk waarbij herhaaldelijk 100%-infecties worden bepaald zijn even informatief als overgelopen maatglazen bij het bepalen van vloeistofvolumes.
Van Hoof, 1977. Neth. J. Pl. Path. 83, 123-127.

4. Bij het gebruik van gele vangbakken in de studie van vectorgedrag en virusverspreiding dient men er terdege rekening mee te houden dat vangsten in deze bakken een vertekend beeld geven van de aantallen bladluizen die in een terrein landen.
Moericke, V., 1957. Z. PflKrankh. PflSchutz. 64, 507-514.
A'Brook, J.A., 1968. Ann. appl. Biol. 61, 289-294.

5. Het is geenszins aangetoond dat bladluizen worden aangetrokken tot open gewassen noch dat zware virusaantastingen hiervan het gevolg zijn.
Kennedy *et al.*, 1959. Ann. appl. Biol. 47, 410-423.
A'Brook, J.A., 1964. Ann. appl. Biol. 54, 199-208.
Johnstone *et al.*, 1982. Bull. entomol. Res. 72, 289-294.
Jones, A.T., 1987. Ann. appl. Biol. 111, 745-772.

6. Lieveheersbeestjes moeten in staat worden geacht de verspreiding van virussen in gewassen door bladluizen aanzienlijk te verminderen.
Dit proefschrift.

7. Toepassing van het Lotka-Volterra concurrentiemodel op de gelijktijdige verspreiding van meerdere virussen in een gewas biedt geen uitzicht op een beter inzicht in de epidemiologie.
Madden *et al.*, 1987. Phytopath. 77, 974-980.

8. De door Watson en Watson verworpen hypothese dat de opbrengstderving ten gevolge van vergelingsziekte grotendeels te verklaren zou zijn door een sterk gereduceerde fotosynthese in de gele bladeren is toch juist.
Watson, D.J. and M.A. Watson, 1953. Ann. appl. Biol. 40, 1-37.
Dit proefschrift.

9. De geur van Dichlobenil waarmee in het voorjaar sommige stadsplantsoenen onkruidvrij worden gehouden is dermate onaangenaam dat alternatieve methoden van onkruidbeheersing de voorkeur verdienen.

10. Het concept van geïntegreerde bestrijding is evenzeer van toepassing op de menselijke gezondheidszorg als op de gewasbescherming.

11. Het geringe aantal van 5000 studiebelastingsuren als normstelling voor een proefschrift is zelden toereikend en dient er daarom slechts toe de promovendus de geruststelling te geven dat althans aan deze norm is voldaan.

Stellingen bij het proefschrift van W. van der Werf:
Yellowing viruses in sugarbeet; epidemiology and damage.
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Yellowing viruses in sugarbeet; epidemiology and damage

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Co-promotor : **dr.ir. D. Peters,**
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Yellowing viruses in sugarbeet; epidemiology and damage

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
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des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

ABSTRACT

The epidemiology and damage effects of beet yellows virus (BYV) and beet mild yellowing virus (BMYV) were studied.

Chapter 2. The incubation period (time between infection and symptom expression) was determined so that progress curves of the disease (symptoms) could be translated into progress curves of the infection. The incubation period increased during the season from 3 (BYV) or 4 to 5 weeks (BMYV) in June to two months when plants were infected with either virus in August. The incubation period increased with plant development stage and lower temperature.

Chapter 3. Symptoms of systemic virus infection developed on the leaves that appeared after the inoculation. Older leaves (except those inoculated) remained healthy and green. In field experiments the infection date was retrospectively determined by calculating the appearance date of the oldest systemically-infected leaf.

Chapter 4. Theoretical analyses show that high infection percentages must be avoided in bait plant test for the determination of infection pressure. Otherwise the number of viruliferous vectors cannot be estimated accurately. Confidence intervals for the number of vectors are given as well as lower bounds when all plants have become infected.

Chapter 5. The extent of secondary spread of yellowing viruses was strongly affected by the date of primary infection. Inoculations before 15 June resulted in extensive secondary spread while negligible spread occurred in plots inoculated after this date. In early-inoculated plots spread started around 15 June when adjacent plants made leaf contact, so that the vector, *Myzus persicae*, could disperse more readily. Little spread occurred in plots in which the number of *M. persicae* was reduced by coccinellids.

Chapter 6. Inoculations at the end of June in late-sown crops resulted in more extensive spread than inoculations in early-sown crops. The higher rate of spread in young crops was correlated with (1) a higher multiplication rate of *M. persicae* on young plants, (2) a better acceptance of young plants by *M. persicae*, promoting virus transmission and (3) a shorter latency period (time between infection and possibility of virus acquisition).

Chapter 7. Damage by BYV resulted from (1) a smaller size of infected leaves, (2) reduced light absorption by yellow leaves, (3) reduced photosynthesis in yellow leaves and (4) increased respiration in infected leaves. Reduced photosynthesis was the most important damage component. Photosynthesis was almost completely inhibited in bright yellow, infected leaves while healthy leaves on infected plants or infected leaves without symptoms photosynthesized at normal rates. Yield loss decreases with later infection as the proportion of yellow leaves on the plants decreases.

The results demonstrate that plant development stage plays a key role in vector population dynamics, virus spread, symptom development and damage. Thus, the benefit from pesticide applications for the control of virus spread depends on crop development stage. Therefore the development stage of the crop should be considered before control measures are taken.

VOORWOORD

Het hier beschreven onderzoek is geïnitieerd door de co-promotor D. Peters. Aanleiding was de epidemische omvang van de bietevergelingsziekte in Nederland en de rest van Europa in 1974 en 1975. Er kwam toen duidelijk aan het licht dat ons inzicht in de epidemiologie te kort schiet.

Het onderzoek had ten doel meer inzicht in de epidemiologie te verschaffen, allereerst door methoden te ontwikkelen om de infectiedatum van besmette planten te bepalen. Vervolgens bepaalden we de invloed van de zaaidatum van het gewas en de infectiedatum op de mate van virusverspreiding. Tevens werd de gewasfysiologische basis van de opbrengstreductie onderzocht en werden theoretische berekeningen gemaakt over de relatie tussen vectordruk en de mate van infectie.

Dit onderzoek zou niet mogelijk zijn geweest zonder de inbreng van Frans Bonnier, Roland Verweij, Arend Vermazeren, Jiansheng Guan, Corné Kempenaar, Wim Riesebos, Paula Westerman, Joachim Bendiek, Leon Spätjens en Nico Mul. De collegiale samenwerking met deze studenten en met M. Hurej (Universiteit van Wroclaw) en Hans van de Heuvel (Virologie) hebben het werk zeer veraangenaamd evenals de altijd opwekkende commentaren van promotor Rabbinge.

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CONTENTS

1 GENERAL INTRODUCTION

The viruses. Virus yellows is an economically important disease of sugarbeet, *Beta vulgaris* spp. *saccharifera* and other cultural types of *Beta vulgaris*, such as fodderbeet (mangolds), table beet and Swiss chard. The yellows syndrome can be caused by three different viruses, occurring alone or in mixed infections. Two of these viruses are luteoviruses, *viz.* beet mild yellowing virus (BMYV) and beet western yellows virus (BWYV). BMYV is the predominant cause of virus yellows in many European countries such as England (Russell, 1958, 1963, 1965; Smith, 1986; Smith and Hinckes, 1987), Sweden (Björling and Möllerström, 1974), West-Germany (Thielemann and Nagi, 1977) and Switzerland (Häni, 1979). BWYV is the predominant cause of virus yellows in the other continents (Duffus, 1973). BWYV is also widespread in Europe but most European strains do not infect beet (Smith and Hinckes, 1985b). BMYV and BWYV are closely related (Duffus and Russell, 1975; Rochow and Duffus, 1981) and their host ranges show considerable overlap though that of BWYV is wider. The third virus that causes a yellows disease in sugarbeet is beet yellows virus (BYV), a closterovirus (Bar-Joseph *et al.*, 1979). It occurs less often in crops than either of the two luteoviruses but it may be prevalent in the neighbourhood of overwintering places such as fodder beet clamps and beet-seed crops (Russell, 1965; Smith and Hinckes, 1987).

Effects on the plant. The three viruses cause largely similar physiological disturbances in beet plants. Starch and sugars accumulate in the leaves which become yellow, thick and brittle (Watson and Watson, 1951). The discoloration of the leaves results from the breakdown of chlorophyll (BYV) as well as the production of yellow and orange pigments in the case of BMYV (Booth and Russell, 1963). The photosynthetic capacity of the leaves decreases (Hall and Loomis, 1972a, b) and respiration of the plant increases (Löhr and Müller, 1953). The growth of the leaves is impaired (Watson and Watson, 1953). These disturbances have a large impact on production. Maximal yield reductions of 50 to 60 % (Duffus, 1973) and even 70% (Watson *et al.*, 1946) have been reported.

Virus cycle. No seed transmission of the viruses has been demonstrated (Duffus, 1973). They are introduced into the crop by immigrant winged (alate) aphid vectors, originating from infected (winter) host plants. This process is called primary infection. Most primary infections are probably made by the peach-potato aphid, *Myzus persicae*. For instance, Heathcote

and Cockbain (1966) found that *M. persicae* was the most important aphid spreading viruses from clamped fodderbeet though several other aphid species were also found in the clamps. After primary infection, the viruses are disseminated in the crop by dispersing resident aphids, which can be alate or apterous (without wings). This is called secondary spread. Work by Watson *et al.* (1951) and Björling (1952) showed that *M. persicae* is the most important spreader of the viruses. The black bean aphid, *Aphis fabae*, when numerous, may have some importance as a spreader of BYV, but it does not transmit BMYV (Russell, 1963; Björling and Nilsson, 1966). The potato aphid, *Macrosiphum euphorbiae*, which may also occur on beet, plays no role of importance in virus spread. Other aphid species occur seldom in beet crops (Blackman and Eastop, 1984).

Virus transmission. M. persicae transmits BYV in the semipersistent manner (Bennett, 1960; Sylvester, 1956a, b, 1961). Acquisition access periods and inoculation access periods of several hours to a day are needed to obtain maximum transmission success. The aphid retains the virus for a few days. Infectivity is lost with moulting. BMYV is transmitted in the persistent manner (Russell, 1962; Björling and Nilsson, 1966), the virus circulating through the aphid's body. Infectivity is retained for life. Acquisition access periods and inoculation access periods of days are needed for maximum transmission success. The virus cannot be transmitted during a latency period of one to two days after acquisition. BWYV has the same transmission characteristics as BMYV (Duffus, 1973). The different transmission characteristics of BYV and the two luteoviruses, BMYV and BWYV, affect their spread in the field.

Aphid cycle. M. persicae can have three distinct lifecycles around the year (Jepson and Green, 1983; Dixon, 1985; Peters, 1987). (1) Holocyclic; males and females being produced in autumn, the latter laying eggs on woody winter hosts of the genus *Prunus*. In spring, the eggs hatch and a few parthenogenetic generations are produced on the winter host. Then the aphids migrate to herbaceous summer hosts such as beet, potatoes, weeds, etc., on which they reproduce parthenogenetically. (2) Anholocyclic; only parthenogenetic females being produced throughout the year. This cycle favours the carry-over of infectious aphids from one season to the next because many herbaceous plant species are hosts for yellowing viruses, especially for BMYV and BWYV which have wider host ranges than BYV. (3) Androcyclic; parthenogenetic females and sexual males being produced in autumn. The latter mate with the sexual females of

holocyclic biotypes. The parthenogenetic females overwinter on herbaceous hosts like the anholocyclic females. Because the mortality of parthenogenetic female *M. persicae* (Harrington and Cheng Xia-Nian, 1984) and that of virus-infected herbaceous winter hosts depends on winter weather, negative correlations have been found between the number of days with temperatures below -0.3 °C (winter frost days) and the incidence of yellowing viruses in crops (Watson *et al.*, 1975; Heathcote, 1986).

Studies on secondary spread. The degree of infection of a sugarbeet crop with yellowing viruses depends on the number of primary infections and the extent of secondary spread that results from it. A few studies have been made of secondary spread. From the results of mathematical and statistical analyses of the relation between the incidence of yellowing viruses and numbers of alate and apterous aphids in beet fields, Watson and Healy (1953) concluded that that alatae were mainly responsible for spread. However, Ribbands (1963) and Jepson and Green (1983) challenged the assumptions underlying these analyses. Ribbands (1963) concluded from his own work on the spread of BYV and BMYV from experimentally-inoculated plants that apterous *M. persicae*, wandering from plant to plant made the most important contribution. BMYV was spread more than BYV. In similar studies, Björling (1952) showed convincingly that secondary spread by *Aphis fabae* was much less important than that by *M. persicae*. Kershaw (1965), confirming the results of Ribbands (1963), found that BMYV was spread more than BYV. All these authors made only vague assertions about the relation between (1) the number, distribution and behaviour of aphids at a certain moment and (2) the spread of virus. Their estimates of the incubation period of the viruses were too inaccurate to determine the infection date of the plants on which they observed symptoms. Thus, up till now, it is not known at which time *M. persicae* spreads viruses in the sugarbeet crop and which are the factors that affect this spread. Such knowledge is necessary to evaluate the current spraying tactics to control vectors and limit virus spread.

Virus yellows epidemics. Severe epidemics of virus yellows are mostly preceded by mild winters because high winter temperatures promote the survival of the viruliferous anholocyclic aphids that are assumed to introduce the viruses into the crops. The last years with severe epidemics have been 1974-1976 (Heijbroek, 1984; Dunning, 1985; Heathcote, 1986). In 1974, 47% of the plants showed yellows symptoms at the end of August in the Netherlands and 68% of the plants showed symptoms in England, while

the estimated losses were 7 and 18% (Dunning, 1985). Since then yellowing viruses have caused no problems in Western-European beet crops. This is partly explained by the more severe winters prevailing since 1976. It is assumed that yellowing virus epidemics may return if a sequence of mild winters will occur again (Heijbroek, 1984; Dunning, 1985). In non-epidemic years, yellowing virus epidemics still cause problems locally where winter reservoirs are present, e.g. fodder beet clamps, greenhouses or infected breeding material.

Control. Farmers can take several measures to limit the incidence of yellowing viruses and damage to the crop. By sowing early they may create a leaf canopy which closes early in the season. This increases yield because the amount of radiation interception is maximized (Scott and Jaggard, 1985) while the incidence of virus yellows is reduced (Heathcote, 1970, 1972). The reasons why closed leaf canopies have this effect on the incidence of viruses are not known. It is widely assumed, however, that closed canopies are optically less attractive to immigrant alate aphid vectors so that fewer primary infections are made and fewer vector colonies founded (A'Brook, 1964, 1968; Johnstone *et al.*; Jones, 1987). Closure of the leaf canopy early in the season also limits the yield reduction per plant as damage depends on the size of the plant on the infection date. Dense sowing was recommended by Jepson and Green (1983) as a lower proportion of infected plants will be obtained. Application of pesticide granules, mostly aldicarb, in the seed furrow is recommended if a heavy infection pressure is expected after a mild winter.

In most European countries, warning schemes have been set up to advise the growers whether or not and when to apply aphicides to limit virus spread (Dunning, 1985). In most countries the damage threshold, i.e. the number of *M. persicae* above which a spray warning is issued, has a fixed value throughout the season. For example, in England a threshold value of one *M. persicae* per four plants is used. In the Netherlands, however, the crop development stage is taken into account (Barel and Dudok van Heel, 1978; Heijbroek, 1984). In areas with known high virus pressure, due to the presence of virus reservoirs, the threshold value increases from one *M. persicae* per five plants in May when the plants are small, to five *M. persicae* per plant in July when the leaf canopy is closed. In areas with few virus reservoirs the threshold is twice as high. The sliding threshold takes account of the decreasing risk of spread and the decreasing reduction of yield as the plants grow.

Damage. Three types of damage are caused by infection with yellowing viruses: (1) decreased yield of roots (fresh weight), (2) lower concentration of sugar in the roots, and (3) lower processing quality (Jorritsma, 1986). Because of the higher surface/weight ratio of smaller tap roots, the percentage tare increases. Damage expressed as percentage reduction of sugar production per ha depends on the date of infection, early infections causing higher yield reductions. Infections after mid-July (complete canopy closure) cause insignificant reductions of yield. BYV causes slightly more damage than BMYV (Smith, 1986) and mixed infections with the two viruses cause larger damage than either of them alone (Russell, 1963).

Definition of the problem. The current spray warning schemes for virus yellows control are based on experience as well as experiments in which the efficacy of aphicide sprays at different dates was determined (e.g. Hull and Heathcote, 1967). These experiments have not given clear results (see discussion by Jepson and Green (1983)). As a result different damage thresholds are used in different countries. Clearly, more insight is needed into the population dynamics of *M. persicae* and yellowing viruses in sugarbeet crops during a season and into the way in which pesticide sprays interfere with virus spread. Such insight can ultimately result in better spraying tactics for virus yellows control which take account of different sowing dates, development stages, densities and growing circumstances of individual crops. To achieve this goal, more insight is also needed into the way yellowing viruses reduce yield.

Scope of the investigation. In studies of the epidemiology of yellowing viruses, accurate estimates of the incubation period are needed to determine the infection date of infected plants and to correlate spread of virus with the number and activity of aphids. Therefore a study was made of the factors that might influence the incubation period of yellowing viruses under field conditions; *viz.* sowing date, infection date, weather, age of the inoculated leaf, number of inoculated leaves, number of vector aphids, species of vector aphid and source plant of the virus (Chapter 2).

Because the reported estimates of the incubation period differ widely, it was attempted to develop a method to estimate the infection date that takes the morphogenesis of the plant into account. The method is based on preliminary observations by Roseboom and Peters (1983) which suggested that the leaf number of the oldest leaf with systemic virus symptoms was a marker of the infection date because it was mostly one third to one half its final size at the infection date (Chapter 3).

In several studies of virus diseases in crops, bait plants were used to determine the time of primary infection of crops, the aphid species responsible, the infection pressure and the relative importance of apterae and alatae in spread (Peters, 1987). The method has not been applied in studies of sugarbeet viruses because secondary spread is considered to play a greater role than primary infections which would be relatively few in number. In bait plant test, batches of virus-susceptible plants are exposed for short periods in the field, transferred to a glasshouse for symptom expression and scored on the presence of symptoms. If vectors alight *at random*, the number of vectors (v) can be calculated from the proportion of plants infected (k out of n), using the multiple infection transformation (Gregory, 1948): $v = n * \ln(n/(n-k))$. To improve the interpretation and design of these tests it was attempted (1) to derive confidence limits for v and (2) to obtain estimates of v when all plants are infected (Chapter 4).

Studies of secondary spread of yellowing viruses were designed similarly to those of Björling (1952), Ribbands (1963) and Kershaw (1965). A few plants were infected with virus, *M. persicae* were released on them and the subsequent population dynamics of vectors and viruses were monitored. The estimates of the incubation period were used to determine the time plants became infected and to relate virus spread to vector dispersal. With this simple (and laborious) experimental design the impact of two major factors determining secondary spread was studied: (1) date of primary infection (Chapter 5) and (2) crop sowing date (Chapter 6). Detailed measurements of the effect of plant age on the susceptibility of the plants to BYV and the latency period of BYV were made to explain the effects of primary infection date and crop sowing date on the rate of secondary spread.

To gain insight into the nature and level of damage, four damage components of BYV were quantified while the growth of the infected crop was determined simultaneously. The reduced yield of BYV-infected beet was associated with: (1) decreased leaf size, (2) increased canopy reflection, (3) impaired photosynthesis and (4) increased respiration. A simulation model of crop growth (SUCROS87; Spitters *et al.*, 1988) was used (Chapter 7) to calculate the consequences of the different optical and physiological properties of infected leaves for the growth of the crop. The model calculations show that the four damage components can quantitatively explain the observed yield reduction.

2 THE INCUBATION PERIOD OF BEET YELLOWING VIRUSES

Abstract

In field trials with sugarbeet in 1985 and 1986 in the Netherlands, the incubation period (time between infection and appearance of symptoms) of beet yellows virus (BYV), a closterovirus, and beet mild yellowing virus (BMYV), a luteovirus, increased during the season. The incubation period of BYV was 3 weeks in young plants, but increased after crop closure, up to 9 weeks in old plants infected in August. The incubation period of BMYV was 4 to 5 weeks in young plants and increased up to 9 weeks in old plants infected in August. On BMYV-infected and old BYV-infected plants, the symptoms were observed about a week earlier on the inoculated leaves than on the systemically-infected leaves.

The incubation period was shorter throughout the season on late-sown plants but similar thermal incubation periods (°C days) were necessary to develop symptoms on plants sown on different dates and infected in the same development stage. The thermal incubation period increased as the plants grew older. Thus the incubation period increased with plant age and lower temperature. Symptoms of both viruses appeared soon after leaves reached their final size, suggesting that the development of symptoms is associated with physiological conditions characteristic for fullgrown leaves.

The incubation period was not substantially affected by: (1) the number of *Myzus persicae* used to inoculate the plants, (2) the number of leaves inoculated, (3) the development stage of the inoculated leaf or (4) the source plant of BMYV, beet or shepherd's-purse, *Capsella bursa-pastoris*. The relation between the development of virus yellows symptoms and the transport and multiplication of virus is discussed.

2.1 Introduction

Virus yellows is an economically important disease of sugarbeet, *Beta vulgaris* spp. *saccharifera*, causing yield losses of up to 60% (Duffus, 1973; Smith, 1986). Beet mild yellowing virus (BMYV,. luteovirus group) is the predominant cause of virus yellows in Europe (Russell, 1958, 1963, 1965; Björling and Möllerström, 1974; Thielemann and Nagi, 1977; Häni, 1979; Smith, 1986; Smith and Hinckes, 1987). In some years and in some

regions, beet yellows virus (BYV, closterovirus group; Duffus, 1973; Bar-Joseph *et al.*, 1979) may be a second important cause of virus yellows. Upon infection with BYV or BMYV the beet leaves become thick and brittle, while their starch and sugar content increases (Watson and Watson, 1951). The photosynthetic capacity decreases (Hall and Loomis, 1972a). Symptoms develop on the inoculated leaves (on which aphids first infected the beet plant) and on the systemically-infected leaves (to which virus has been transported from the inoculated leaves via the phloem system). Systemic infection occurs in the youngest leaves in the heart of the plant and in all other leaves that develop after the infection (Roseboom and Peters, 1984; Chapter 3). The symptoms caused by BMYV vary from pale to bright-yellow or orange as the leaves grow older and culminate in necrosis which is caused by secondary fungal pathogens. BYV causes vein clearing in the first few expanding leaves which develop after the infection. The systemically-infected leaves become yellow when they are mature and develop subsequently typical red or necrotic spots.

The peach-potato aphid, *Myzus persicae*, is the major vector of yellowing viruses in the field (Watson *et al.*, 1951; Björling, 1952). The black bean aphid, *Aphis fabae*, is a second, much less important vector of BYV and not a vector of BMYV (Russell, 1963; Björling and Nilsson, 1966; but see Thielemann and Nagi (1979) and Karl and Gieselmehl (1981) for a different view). Many studies have been made of the spread of viruses in sugarbeet in relation to the population dynamics of *M. persicae* (e.g. Watson and Healy, 1953; Ribbands, 1963; Kershaw, 1965; Watson and Heathcote, 1966; Watson *et al.*, 1975). Nevertheless, it is still not fully understood when the viruses are spread in the crop and how this spread is related to the behaviour of aphids at that time.

One of the reasons for this lack of understanding is the reported variability of the incubation period of the disease and the lack of accurate estimates. The incubation period is defined as the time needed from inoculation to the appearance of the first disease symptoms (van der Plank, 1963; Bos *et al.*, 1985). Watson *et al.* (1951) observed symptoms of virus yellows 3 to 5 weeks after infection. They did not distinguish between BYV and BMYV at the time because BMYV was not described until 1958 (Russell, 1958). Ribbands (1963), distinguishing BYV and BMYV, also observed symptoms of both viruses after 3 to 5 weeks. Thielemann and Nagi (1977) found similar incubation periods for the 2 viruses. Their

estimates varied from 4 to 6 weeks when *M. persicae* was used as the vector and from 6 to 9 weeks if either virus was transmitted by *A. fabae*. However, Björling (1963) observed no differences in incubation period of BYV between beet plants infected by *M. persicae* or *A. fabae*. Furthermore, Björling found that the median incubation period of BYV in *Chenopodium foliosum* plants in the glasshouse was similar in plants infected by *A. fabae* or *M. persicae*. However, in the group of plants infected by *A. fabae* there were some plants with markedly longer incubation periods. Steudel (1958) reported that the symptoms of BYV appeared earlier and became more intense with higher numbers of *M. persicae* used for inoculation. Hull (1959) criticized Steudel's results because virus could be spread from the experimentally-infected plants to uninfected plants in the plots in which few aphids were used to infect the plants. These naturally-infected plants could be mistaken for plants experimentally-infected, causing an overestimation of the incubation period in the plots in which few *M. persicae* were used for the inoculation. Wiesner (1959) and Björling (1963) found that the incubation period of BYV in glasshouse experiments was much longer in the winter than in the summer with intense radiation and high temperatures. In the glasshouse, BMYV-infected plants may remain symptomless. According to Rochow and Duffus (1981), cool and bright weather is favourable for the development of clear symptoms of beet western yellows virus (BWYV), a luteovirus which is closely related to BMYV and which is widespread in beet crops in the USA and Australia (Duffus, 1973; Johnstone and Duffus, 1984) but not in Europe (Duffus and Russell, 1975).

In the light of the variable estimates of the length of the incubation period of virus yellows in sugarbeet, the work presented here had two aims: (1) to provide estimates of the incubation period for use in the analysis of virus spread and (2) to find out which factors affect the incubation period in the field.

2.2 Materials and methods

2.2.1 Arrangement of the experiments

Most observations were made in two field experiments near Wageningen, the Netherlands, in 1985 and 1986. In these experiments, the effect of several factors on the length of the incubation period of BYV and BMYV

Table 2.1: Field and crop data of experiments on the incubation period of BYV and BMYV in sugarbeet.

Year	Location	Farm	Variety	Sowing date	Soil type	% Silt	N-fertilizer (kg ha^{-1})	Plant density (ha^{-1})	Beet yield (1000 kg ha^{-1})
1984	Wageningen	Binnenhaven	Regina	17 April	river clay	76	120	75.000	54
1985	Wageningen	Binnenhaven	Regina	24 April	river clay	64	135	50.000	47
1985	Wageningen	Haarweg	Monohil	23 April	river clay	53	180	60.000	50
1985	Flevopolder	Minderhoudhoeve	Regina	22 April	sea clay	44	120	80.000	65
1986	Wageningen	Haarweg	Bingo	25 April	river clay	46	170	70.000	70
1986	Betuwe	de Bouwing	Bingo	25 April	river clay	57	135	75.000	70
1986	Flevopolder	Minderhoudhoeve	Regina	18 April	sea clay	39	170	90.000	75

was studied: (1) date of infection: from mid-May to mid-August; (2) number of vector aphids *(M. persicae)* per plant: from 2 to 30, 10 as a standard; (3) number of inoculated leaves: 1 (standard) to 4; (4) development stage of the inoculated leaf: expanding, just fullgrown (standard) or ageing and (5) sowing date: mid-April (standard), end of May or beginning of July. In a few plots, inoculations with BYV were made using *A. fabae* as a vector to study its effect on the incubation period. Additionally, in 1986, inoculations were made with *M. persicae* which had acquired BMYV from shepherd's-purse instead of beet.

In 1985, the variety Regina was sown on 24 April on a 1.8 ha field at the Binnenhaven in Wageningen (Table 2.1). A total of 152 rows of 15 beet plants were allotted to 76 different combinations of the experimental factors 1 to 4. Twelve plots of 2.5 * 5 m^2 were sown on 29 May. The varying numbers of *M. persicae* per plant for inoculation (factor 2) were 10, 5, 2 or 1. The number of leaves inoculated (factor 3) was 1 or 3.

In 1986, the variety Bingo was sown on 25 April on a 2 ha field at the Haarweg in Wageningen. Observations on the incubation period of BYV and BMYV were made in two adjacent parts of the field, each measuring 72 * 36 m^2. Each part was divided into 4 blocks of 9 plots measuring 12 * 6 m^2. Each of the 9 plots in a block was inoculated on a different date. In some plots, beds of 2.5 * 5 m^2 were sown on 28 May and 3 July. All rows in a plot were inoculated on the same date and received a different treatment. The varying numbers of *M. persicae* per plant for inoculation (factor 2) were 30, 10 or 2. The number of leaves inoculated (factor 3) was 1 or 4. In another part of the field, observations on the incubation period of BYV were made in plots, sown on 25 April or 26 May, in which the latency period of BYV was determined (Chapter 6).

More observations on the incubation period of BYV and BMYV were made in five other fields. Inoculations were made on single or duplicate rows of 15 to 30 plants. Field and crop data are summarized in Table 2.1.

2.2.2 *Myzus persicae* culture

Virus-free peach-potato aphids, *M. persicae*, from a clone named M3, were cultured in a glasshouse on the third and fourth leaf of 5-leaved oilseed-rape plants, *Brassica napus* subsp. *oleifera* (leaf 1 is the first leaf following the cotelydons). Every day a new age cohort of 0-24 h old nymphs was started. The temperature in the glasshouse was 20-25 °C and

the photoperiod at least 16 h/day. According to Russell (1965) and Björling and Nilsson (1966), oilseed rape is immune to BMYV and BYV. Thus no BMYV could be introduced into the BYV culture by aphids from rape. However, rape is susceptible to BWYV, but this virus was not detected when *M. persicae* from the culture were periodically tested on *Physalis floridana* plants (Duffes, 1973).

2.2.3 Cultures of BYV and BMYV

BYV and BMYV were maintained on beet in two insect-proof glasshouse compartments located at 200 m distance from each other to avoid contamination. According to criteria given by Björling (1961), the strain of BYV used causes moderately-severe symptoms, *viz.* vein clearing in young leaves and necrotic spots on fully mature leaves. To keep the BYV-culture free from possibly contaminating BMYV, the virus was periodically transmitted to healthy plants by either *A. fabae*, which does not or hardly transmit BMYV, or by *M. persicae* from rape, in a 4-hour acquisition period followed by a 4-hour inoculation access period. In such a sequence BMYV is not transmitted (Russell, 1962; Björling and Nilsson, 1966). BYV-infections in the BMYV culture were not observed during the whole investigation. Occasionally, symptoms resembling those of BMYV were noticed on BYV-inoculated plants in the field, but BMYV was never successfully recovered from these plants, using *M. persicae* as a vector and sugarbeet or *C. bursa-pastoris* as test plants.

2.2.4 Production of viruliferous aphids and methods of inoculation

Infectious *M. persicae* were reared on virus-infected beet plants in the glasshouse. The populations on beet collapsed, however, in June 1985 and 1986, presumably because the plants were no longer acceptable to the aphids. From then on, different methods were employed to produce infectious aphids.

In 1985, *M. persicae* from rape were brushed onto detached BYV- or BMYV-infected beet leaves lying in large petri-dishes or standing upright in small bottles inside a large glass jar, with their petioles submerged in water. The dishes and jars were closed with poly-ethene foil or cheese cloth and their walls were coated with Fluon to prevent aphids from escaping. After 2 or 3 days, the aphids were transferred to the field in

aphid-proof clip-cages. Aphids in these cages had to penetrate a nylon gauze with their stylets to reach the leaf. Using these methods in 1985, 10% of the inoculated plants became infected.

After the decline of the *M. persicae* population on infected beet in 1986, adult *M. persicae* from rape were clip-caged onto yellow leaves of BYV-infected sugarbeet plants in the glasshouse. BMYV-infected aphids were cultured on infected shepherd's-purse. The aphids were caged onto plants in the field in non aphid-proof clip-cages (Adams and van Emden, 1972). In 1986, 90% of the plants became infected.

After an inoculation access period of 1 or 2 days, the aphids were killed manually and the plants sprayed with the carbamate-insecticide pirimicarb. The plots were then treated weekly with insecticide to control immigrant aphids and reduce virus spread from the inoculated plants. In 1985 pirimicarb was used, except in June when aldicarb granules were applied to the soil because rainy weather hindered spraying. In 1986, sprays of pirimicarb and the organo-phosphate oxy-demeton-methyl were alternated.

2.2.5 Evaluation of symptoms

In 1985 the infected plants were inspected for the development of symptoms once a week. In 1986, the inspections were made every 4 or 5 days in May and June, every week in July and August and every 10 days in September and October. In these inspections, the inoculated leaves (which were marked with a plastic label around the petiole) were distinguished from the systemically-infected ones. The latter were considered to be yellowed when the discoloration could be easily observed at a meter distance. The inoculated leaves were judged with the same criterion after later-developed healthy leaves had been moved aside. In some groups of April- or May-sown BYV-infected plants on the Haarweg in 1986, the time needed for the development of vein clearing symptoms was determined.

2.2.6 Selection of plants for analysis

An infected plant was used in the analysis when: (1) symptoms of the inoculated virus were found on the inoculated leaf, (2) no symptoms were found on leaves other than the inoculated leaf and the systemically-infected

leaves, which had just appeared when the plant was infected or appeared afterwards; (3) systemic symptoms developed normally, *viz.* with the first and most severe symptoms appearing on a leaf, implanted above the inoculated leaf (Chapter 3) and (4) no virus infection occurred in neighbouring rows. The presence of symptoms in neighbouring rows or on leaves other than the inoculated leaf and the systemically-infected leaves would indicate natural spread. If one of the 4 requirements was not met, a plant or row was discarded. The *a posteriori* selection of plants was necessary because virus spread by naturally-occurring aphids cannot be controlled completely with pesticides. Because *M. persicae* was scarce in 1985, only a few plants had to be discarded, most of them in the late-sown plots. In 1986, however, 6 of the 8 plots, sown on 3 July and inoculated in the second week of August, were discarded because of natural spread. Few plants were discarded in the early-sown plots.

2.2.7 Estimation of the incubation period

Percentages of plants showing symptoms in experimental plots with the same treatment were plotted against time. The incubation period was estimated as the point on the abscis where the curve reached the 50% level on the ordinate. To assess the variation in the length of the incubation period between plants, logistic growth curves were fitted to the data obtained on the Haarweg in 1986:

$$p = 1/(1 + e^{-(t - \mu)/s}) \qquad (2.1)$$

equivalent with

$$\ln(p/(1 - p)) = (t - \mu)/s \qquad (2.2)$$

in which p is the proportion of plants showing symptoms, t is time, expressed as day of the year, μ is the average incubation period and s is the scale parameter. The parameters μ and s were calculated by least squares regression of logit-values, $\ln(p/(1-p))$ on time (Zadoks and Schein, 1979). The standard deviation of the incubation period was calculated by multiplying the scale parameter s with $\pi/\sqrt{3}$ (Finney, 1971).

2.2.8 Temperature measurements

Daily minimum and maximum temperatures were measured in Stevenson screens both in Wageningen and in the Flevopolder. Daily temperature sums above 3 °C, the thermal threshold for leaf expansion in sugarbeet (Milford *et al.*, 1985b), were calculated by fitting a sine between the measured minima and maxima and adding the hourly increments.

2.2.9 Measurements of leaf growth

In 1986, leaf growth in plots sown on 25 April and 26 May on the Haarweg near Wageningen was measured with a ruler on 10 healthy plants. For each leaf, the increase in relative length, expressed as a percentage of final length, was calculated. The relative lengths were averaged for leaves which appeared on the same day. From the averaged relative leaf growth curves, the 95%-point (Milford *et al.*, 1985b) was taken as the moment at which the leaves, appearing at a certain date, reached their final length.

2.2.10 ELISA-measurements

In the same field, the transport and multiplication of BYV in plants, sown on 25 April and 28 May, was studied with enzyme-linked immunosorbent essay (ELISA), using leaf discs (Roseboom and Peters, 1984). Every 2 weeks, from June to August, 20 plants of each sowing date were inoculated. Twice a week, a sample was taken from the inoculated leaf and another from one of the systemically-infected leaves of 3 plants in each group.

In October, the virus content was measured with leaf disc ELISA in leaves of 10 plants showing advanced BYV-symptoms. From the oldest yellowed leaf to the youngest heart leaf every third leaf was sampled.

2.2.11 Translocation of BYV

The translocation of BYV out of the inoculated leaf was studied by removing it at different times after inoculation. On 3 July and 1 August, 4 groups of 20 field-grown plants were inoculated with BYV by 30 *M. per-*

sicae, clip-caged on the plants for 24 hours. In one group the inoculated leaf was not removed and in the other groups it was removed 2, 5 or 8 days after the end of the inoculation access period. In the glasshouse, the first, second or third leaf of 4-weeks-old beet plants in the 4-leaf stage was infected with BYV or BMYV by 20 to 30 *M. persicae* in a 24-h inoculation access period, using aphid-proof clip-cages. From the different groups of 20 plants, the inoculated leaf was removed 0, 24, 48, 72 or 96 hours after the end of the inoculation access period. The experiment contained an untreated control group and a group in which the inoculated leaf was not removed.

2.3 Results

2.3.1 Development of symptoms during the season

Leaves inoculated in May or June generally developed symptoms on their entire leaf blade within a short time, while those infected in July and August developed at first only a small yellow spot which expanded later. On the inoculated leaves, the symptoms of BYV typically spread downwards along the midvein. The yellowing symptoms were confined to leaf sectors, sharply bordered by the midvein and other veins. Later, these yellow areas expanded towards the leaf margin. BYV-symptoms on the systemically-infected leaves differed slightly from those on the inoculated ones. On the latter, the yellowing was mostly more intense and the typical red or necrotic spots became often larger. BMYV-symptoms on the inoculated leaves, spread along the leaf margin to the leaf tip. BYV and BMYV symptoms were brighter late in the season with cool weather than early in the season and the spots caused by BYV were red instead of necrotic.

The symptoms of both viruses appeared at different times on inoculated and systemically-infected leaves. Throughout the growing season, those of BMYV appeared earlier on the inoculated than on the systemically-infected leaves. On young plants, BYV-symptoms appeared at the same time on both types of infected leaves, whereas on older plants they appeared earlier on the inoculated leaves.

The incubation period increased during the growing season (Fig. 2.1). The symptoms of BYV appeared after 3 weeks on plants which were

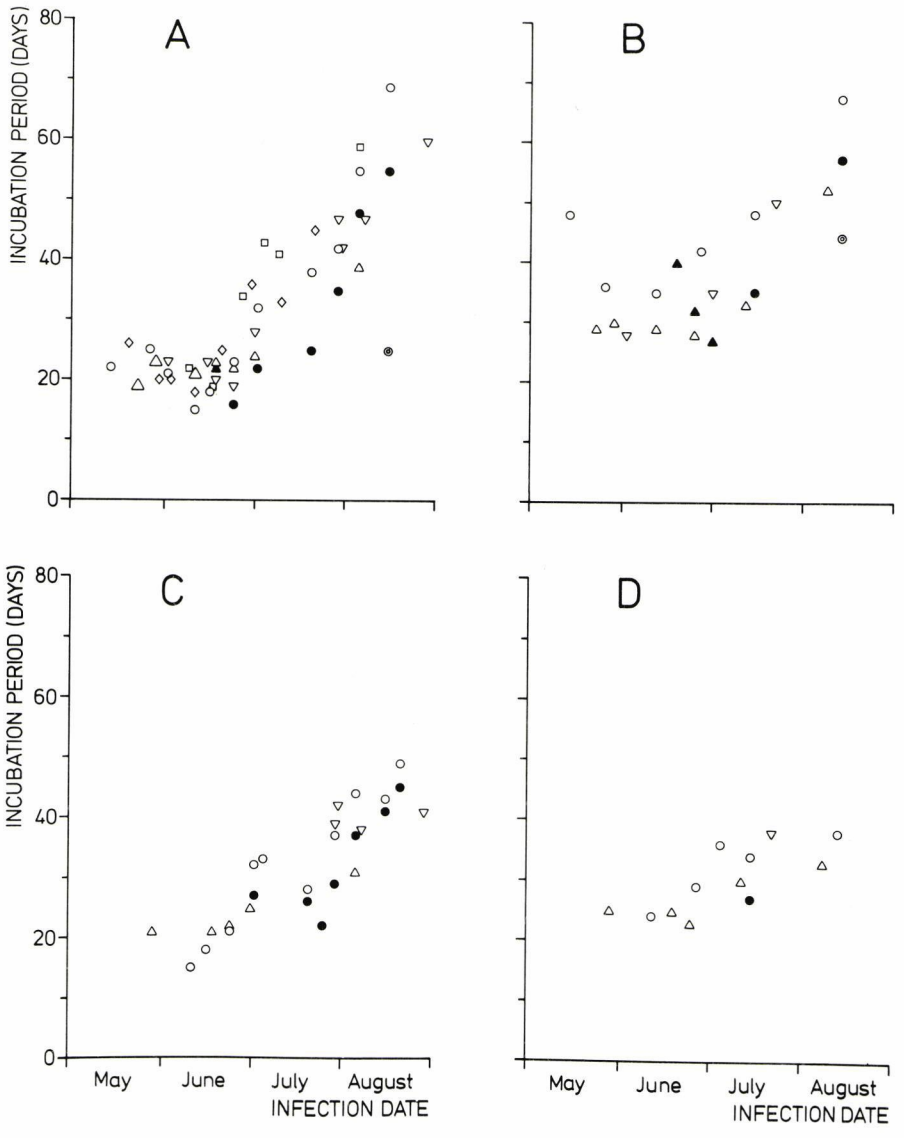


Fig. 2.1: Incubation period of BYV and BMYV on inoculated and systemically-infected leaves of sugarbeet during the growing season. Data from the Haarweg, 1986 (○, ●, ⊙), Binnenhaven, 1985 (△, ▲), Minderhoudhoeve, 1986 (◇), de Bouwing, 1986 (□) or from one of the other fields (▽) listed in Table 2.1. Open symbols denote crops sown in April and solid symbols denote crops sown in May. One observation was made on plants sown in July (⊙). A: BYV, systemically-infected leaves; B: BMYV, systemically-infected leaves; C: BYV, inoculated leaves; D: BMYV, inoculated leaves.

Table 2.2: Median incubation period (days) of BYV and BMYV on systemically-infected leaves, measured at the Binnenhaven in 1985 and at the Haarweg in 1986 for different modes of inoculation.

Mode of Inoculation			Virus and Date of Inoculation								
			BYV				BMYV				
number of *M. persicae* per leaf	growth stage of inoculated leaf	number of inoculated leaves	11/12 June 1986	24 June 1986	29 July 1986	15 Aug. 1986	23 May 1985	29 May 1985	12/13 July 1986	15 July 1986	13 Aug. 1986
10	expanding	1	15	24	42	58[2]	29	29	33	51[1]/49	58[1,2]/58[2]
10	fullgrown	1	16	22	43	-	-	-	32	49	-
10	old	1	15	24	48	-	28	-	36	-	-
10	all stages	4	15	-	-	53[2]	-	-	-	-	67[2]
1	expanding	1		-	-	-	-	30	-	-	-
2	id.	1	15	-	42	42	30	-	35	43	-
5	id.	1	-	-	-	-	29	-	-	-	-
30	id.	1	14	-	40	-	-	-	35	-	-

1) source plant shepherd's-purse, *Capsella bursa-pastoris*, 2) 25% of the plants showing symptoms

infected in May or June, but the incubation period gradually increased after canopy closure, at the end of June. Plants inoculated at the end of August showed BYV-symptoms after 6 to 7 weeks on the inoculated leaves and after 9 weeks on the systemically-infected leaves. When plants were infected with BMYV in May or June, the symptoms appeared on the inoculated leaves after 3 to 4 weeks and on the systemically-infected leaves after 4 to 5 weeks. In the course of the season these incubation periods increased to values of 5 and 9 weeks, respectively.

2.3.2 Effect of inoculation conditions

The incubation period on both categories of leaves appeared not to be affected by: (1) the number of vector aphids, *M. persicae*; (2) the number of leaves inoculated; (3) the developmental stage of the inoculated leaf or (4) the source plant of BMYV, whether beet or shepherd's-purse. The few plants successfully inoculated with BYV by *A. fabae* developed symptoms at the same time and with the same intensity as those inoculated by *M. persicae*. Some results are presented in Table 2.2.

2.3.3 Effect of sowing date

Symptoms of both viruses appeared earlier on late-sown than on early-sown plants. For both sowing dates in 1986, 25 April and 26/28 May, the incubation period of BYV increased during the season, while the difference in the incubation period between the two sowings was maintained (Fig. 2.2). Apparently, the age of the plant affected the length of the incubation period for both categories of infected leaves. This was confirmed in an experiment in the same year in which groups of plants, sown on 3 different dates, were inoculated with BMYV or BYV on 13 and 15 August 1986, respectively. On the infection date the plants sown on 25 April, 28 May and 3 July had 35, 27 and 10 leaves, respectively. The last-sown plants were the first to show symptoms of systemic infection, symptoms of BYV appearing after 35 days and those of BMYV after 43 days. The plants of the second sowing showed symptoms of BYV and BMYV only after 55 and 57 days, respectively. The incubation period of either virus was longer than two months on the plants of the first sowing. When the crop was harvested on 13 October only a quarter of them showed symptoms.

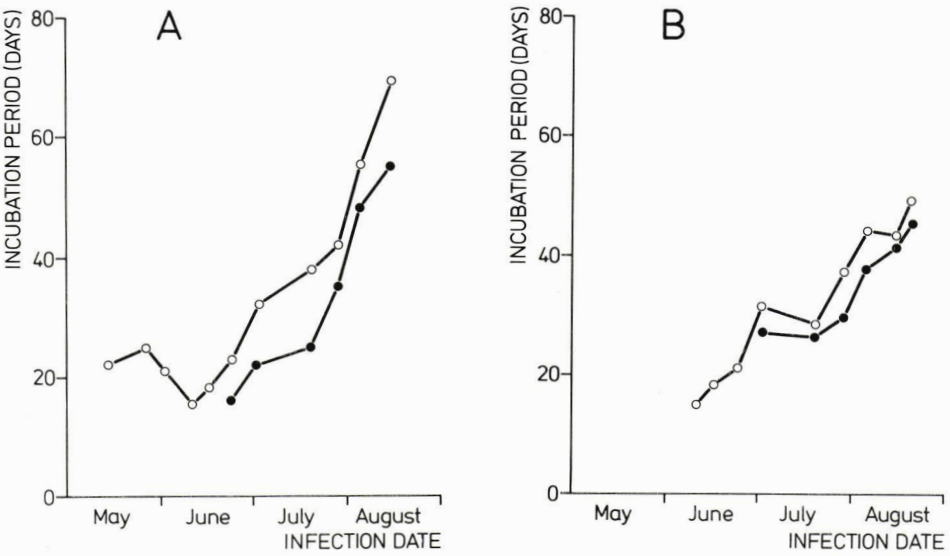


Fig. 2.2: Difference in the incubation period of BYV between early- (25 April; ○) and late-sown plants (26/28 May; ●). Data from the Haarweg, 1986. (A) Systemically-infected leaves; (B) inoculated leaves.

2.3.4 Effect of temperature

Plants sown on 25 April 1986 and infected with BYV on 16 June, when they had 10 leaves, showed symptoms after 18 days. The average temperature during this period was 22 °C. Plants sown on 3 July and infected on 15 August when these had 10 leaves, showed symptoms only after 35 days. In this period the average temperature was 10 °C. These results indicate that the temperature affects the length of the incubation period. Accordingly, the thermal incubation periods, expressed in °C days, differred only slightly (253 °C days for the plants inoculated on 16 June versus 292 °C days for the plants inoculated on 15 August). All measured incubation periods of BYV and BMYV for inoculated and systemically-infected leaves were converted to °C days and plotted against the date of infection (Fig. 2.3). The thermal incubation period of BYV on the systemically-infected leaves increased from roughly 230 °C days on plants inoculated in May and June to about 600 °C days on plants inoculated in August. The thermal incubation period of BYV on the inoculated leaves increased from approximately 230 °C days on plants inoculated in May and June to 400 °C days

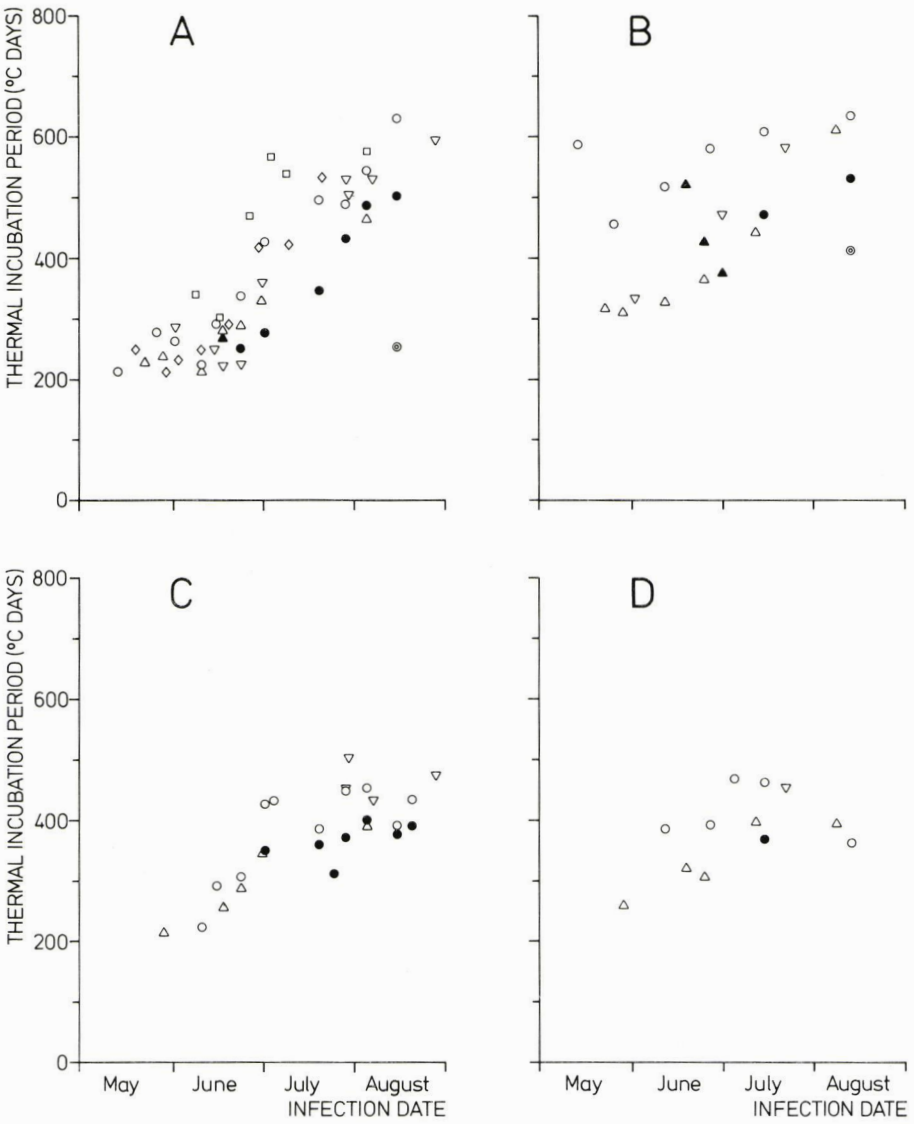


Fig. 2.3: Thermal incubation period (°C days above 3 °C) of BYV and BMYV on systemically-infected and inoculated leaves of sugarbeet during the growing season. Meaning of symbols and lettering as in Fig. 2.1.

on plants inoculated in August. The thermal incubation period of BMYV on the systemically-infected leaves increased from about 300 - 500 °C days in May and June to about 600 °C days in July and August. These data show that the increase of the length of the incubation period is partly caused by the ageing of the plants during the season. Such a trend in the incubation period was not observed with BMYV-inoculated leaves. Thus, the incubation period of BMYV on the inoculated leaves appeared virtually unaffected by the age of the plant, and the lower temperature in autumn might have accounted for the increased incubation period.

2.3.5 Relation between the incubation period and the duration of leaf expansion

The first leaves to show symptoms of systemic infection by yellowing viruses are those that just appear (≧ 3 cm) at the moment of inoculation (Chapter 3). The duration of blade expansion of newly appearing leaves, as measured on healthy plants (Fig. 2.4), increases in much the same way

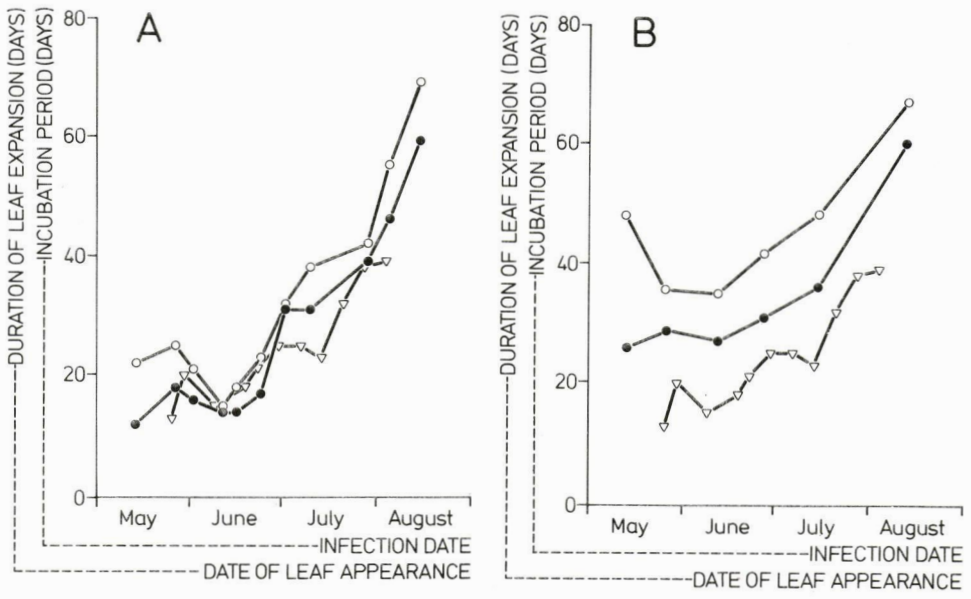


Fig. 2.4: Duration of leaf expansion (▽) and incubation period with regard to clear (○) and incipient (●) symptoms of systemic infection with BYV (A) or BMYV (B) in sugarbeet during the growing season. Data from the Haarweg, 1986.

during the season as the incubation period on leaves infected systemically with BYV or BMYV. The first (incipient) yellowing symptoms of BYV could be observed when leaves of the same age on healthy plants approached their final length. The symptoms were evaluated as 'clear' about a week later. Incipient symptoms of BMYV were noticed one to two weeks after healthy leaves of the same age reached full length, whereas it took another 1 to 2 weeks before the symptoms became clearly visible. These results suggest that the development of yellowing symptoms is associated with physiological conditions characteristic for fully-expanded leaves.

Fig. 2.5 combines the influences of temperature and plant age (as indexed by the number of leaves on the plant) on the incubation period. The temperature sums, which determine the lengths of the incubation periods, appear to depend on the physiological age (leaf number) of the plant. The curves for systemic symptoms of the two viruses are similar to the relation for leaf expansion in sugarbeet as found by Milford *et al.* (1985b). This suggests that the effects of temperature and the age of the plant on the duration of leaf expansion may account for the effect of these factors on the incubation period on systemically-infected leaves.

Further evidence for a relation between the development of symptoms and leaf expansion was obtained by inoculating plants in the cotelydon stage. Though young plants in general showed short incubation periods, such seedlings needed more time to develop symptoms than plants with true leaves infected at the same time. Groups of plants sown on 29 May 1985 were inoculated with BMYV on 19 June, 25 June or 1 July when they were in the early cotelydon stage, late cotelydon stage and the 2-leaf-stage, respectively (Lutman and Tucker, 1987). The plants of these 3 groups showed symptoms simultaneously around 31 July on leaf 3 and 4 when these leaves attained their final size. Vague symptoms were seen earlier on leaf 1 and 2 but these short-lived leaves (Milford *et al.*, 1985a) died before clear symptoms developed. Similar observations were made in 1986.

On BYV-infected plants on the Haarweg in 1986, vein clearing appeared 50 - 60 °C days before leaf yellowing symptoms, throughout the season (Fig. 2.6). April- and May-sown plants showed the same relation between thermal incubation period and leaf number (as an index for plant age).

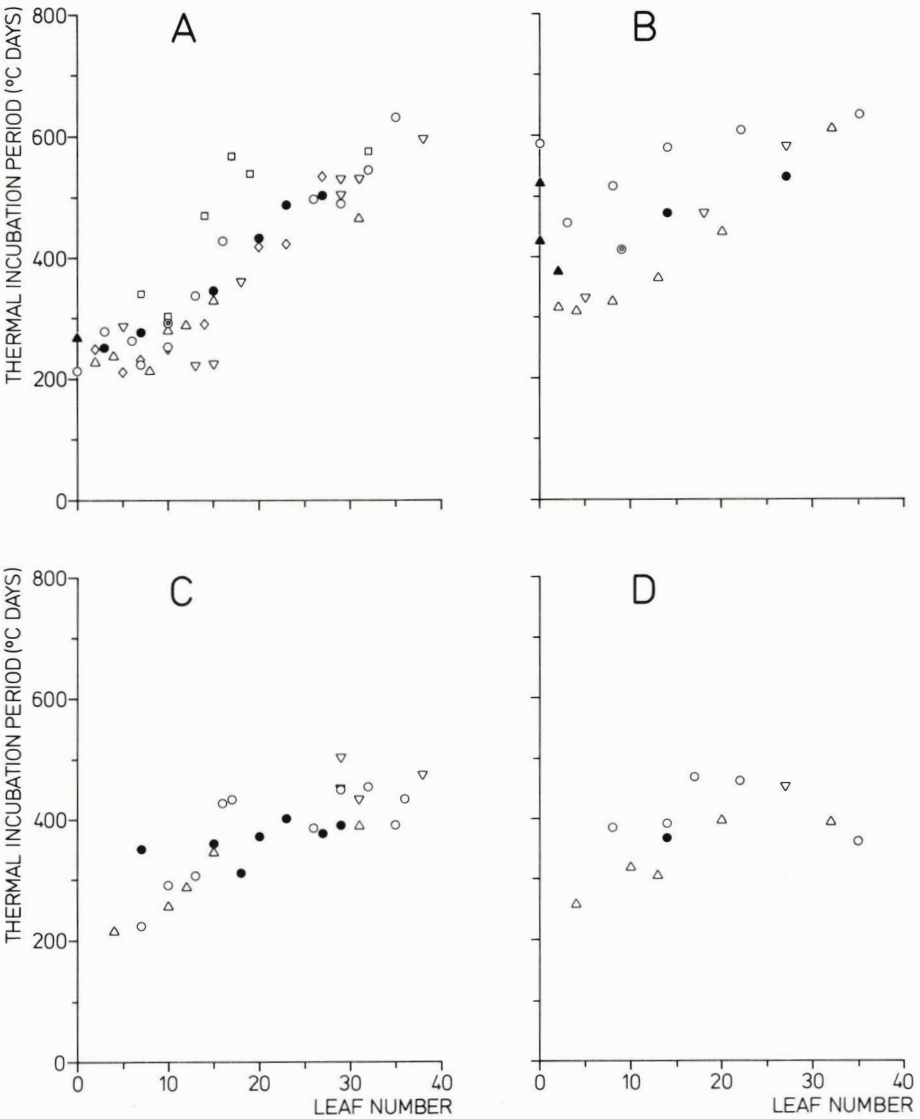


Fig. 2.5: Thermal incubation period (°C days above 3 °C) of BYV and BMYV in sugarbeet as a function of plant leaf number on the infection date. Meaning of symbols and lettering as in Fig. 2.1.

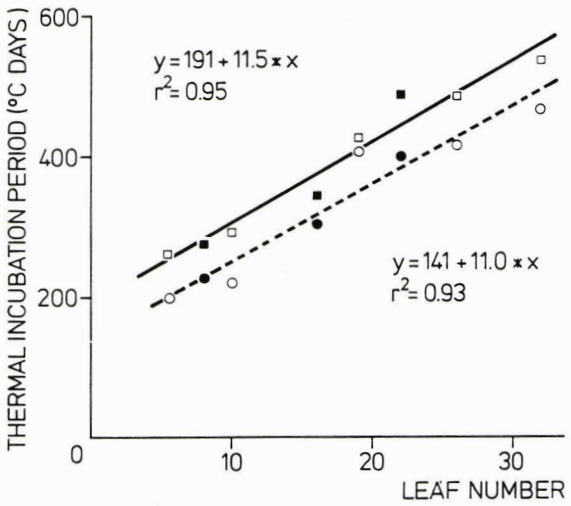


Fig. 2.6: Temperature sum needed for the development of vein clearing (○,●; hatched line) or leaf yellowing symptoms (□,■; drawn line) following inoculation with BYV at different stages of plant development (leaf number). Data obtained on plants sown on 25 April (open symbols) or 26 May (closed symbols) on the Haarweg in 1986.

2.3.6 Variation between plants

Fig. 2.7 shows the development of symptoms in the course of time on leaves infected systemically with BYV or BMYV on plants sown on 25 April or 26/28 May on the Haarweg in 1986. In some inoculated groups the symptoms developed simultaneously within a short period of time on all plants. For instance, 208 plants, sown on 25 April and inoculated with BYV on 11 June developed clear symptoms in a time span of 13 days between 24 June and 7 July. In other groups, however, there was a large variation in incubation period. For instance, 54 plants, sown on 25 April and inoculated with BMYV on 15 July developed symptoms in a time span of 7 weeks between 6 August and 24 September. The smallest standard deviations were obtained when incubation periods were short and the largest at the end of the season when incubation periods were long (Table 2.3).

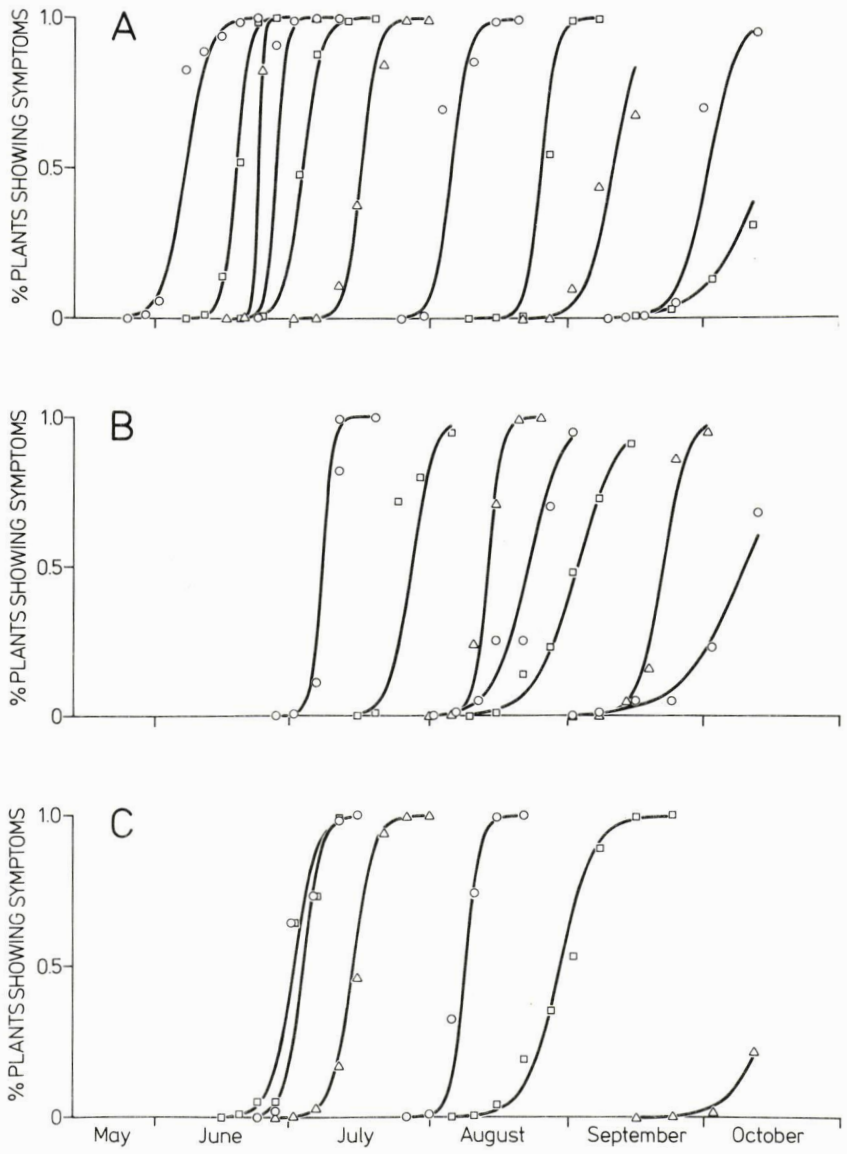


Fig. 2.7: Time-course of the development of yellowing symptoms on systemically-infected leaves of plants sown on 25 April 1986 and infected with BYV (A) or BMYV (C) on different dates and of plants sown on 26 or 28 May and infected with BYV on different dates (B). The symbols ○, □ and △ denote observations belonging to subsequent inoculations. Drawn lines are logistic curves, fitted to the data (Table 2.3).

Table 2.3: Incubation period of BYV and BMYV on systemically-infected leaves of plants sown on 25 April or 26/28 May 1986 on the Haarweg near Wageningen. The mean incubation period (μ) and the variation between plants ($\sigma = s * \pi/\sqrt{3}$) were estimated by fitting logistic growth curves to the data. n = number of infected plants.

Date of infection	BYV; crop sown 25 April			BYV; crop sown 26/28 May			BMYV; crop sown 25 April		
	μ	σ	n	μ	σ	n	μ	σ	n
14 May	25	5	18	-	-	-	51	4	11
26 or 27 May	23	3	21	-	-	-	37	5	22
2 June	22	1	43	-	-	-	-	-	-
11, 12 or 13 June	17	2	208	-	-	-	33	4	143
16 June	22	4	25	-	-	-	-	-	-
24 June	23	3	108	14	2	44	-	-	-
27 June	-	-	-	-	-	-	43	3	31
2 July	35	3	82	26	4	37	-	-	-
15 July	-	-	-	-	-	-	46	7	54
20 July	37	3	87	25	3	46	-	-	-
25 July	-	-	-	29	7	19	-	-	-
29 July	44	5	97	36	9	22	-	-	-
5 August	58	6	85	48	5	79	-	-	-
13 and 15 August	63	12	31	56	13	22	66	9	45

Table 2.4: Percentage of plants showing symptoms of systemic infection by BYV and BMYV in the glasshouse when the inoculated leaf was removed at different times after the end of a 24-h inoculation access period (IAP).

Time of removal of inoculated leaf (hours after IAP)	BYV		BMYV	
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	17[1)]	13	-	0
24	85	42	78	48
48	91	47	85	-
72	91	-	-	87
96	-	29	88	-
control	84	41	97	73

2.3.7 Translocation of BYV and BMYV and detection of BYV with ELISA

In glasshouse experiments, BYV and BMYV were translocated from the inoculated leaf 1 or 2 days after infection (Table 2.4). After this period removal of the inoculated leaf did not prevent systemic infection of the plant. Removal of the inoculated leaf of field-grown plants 2 days or longer after inoculation in July or August likewise did not affect the number of plants infected. Hence it is concluded that BYV is translocated from the inoculated leaf within 2 days after infection, the moment of transport being not clearly affected by the age of the plant.

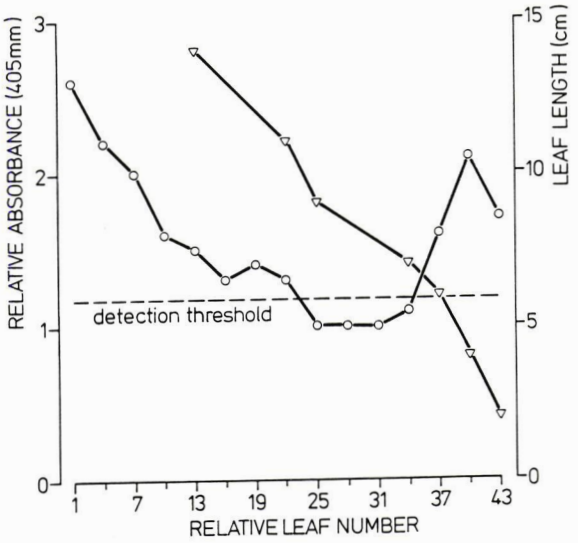


Fig. 2.8: BYV-content as measured by ELISA (○) and leaf length (▽) as measured on 10 October on a range of leaves on a beet plant naturally-infected with BYV around 20 June. The oldest systemically-infected leaf with approximate leaf number 15 was taken as a starting point for leaf numbering. The fully-expanded leaves 1 to 7 showed severe yellowing and necrotic spots. Leaf 10 showed vein clearing. Leaves 13 to 34 were symptomless, expanding leaves. Leaves 37 to 43 were not yet unfolded.

BYV was generally detected by ELISA in young systemically-infected leaves 1 to 2 weeks after the inoculation. The results of this experiment were, however, erratic because the virus was sometimes not detected in the systemically-infected leaves of plants in which it had been positively detected before. Furthermore, the virus could not be detected until 4 weeks after inoculation of plants which were sown on 25 April and inoculated on 18 August, while it was detected within 2 weeks in plants inoculated on 4 August or 1 September. These inconsistencies may have been caused by differences in BYV content in systemically-infected leaves of different age as shown in Fig. 2.8 for a field-grown BYV-infected plant, sampled on 10 October 1986. The pattern is typical for the plants measured in this period. The virus content is high in both very young, pale, not yet unfolded leaves in the centre of the plant and in leaves which show clear yellowing symptoms. On the other hand the virus occurred in concentrations below the detection threshold in the expanding leaves that did not show symptoms. Low virus concentrations were also observed in the expanding symptomless leaves of BYV-infected plants growing in the glasshouse in 1987.

2.4 Discussion

The work described in this paper demonstrates that the incubation period of yellowing viruses in sugarbeet in the field depends largely on the following 4 factors: (1) the virus, BYV or BMYV; (2) the nature of the infection, by inoculation or systemic transport; (3) the developmental stage of the plant and (4) the temperature. Varying inoculation conditions, however, such as the number of vectors, from 1 to 30, the vector species, *M. persicae* or *A. fabae* for BYV, the number of inoculated leaves, 1 to 4, or their development stage, expanding, fully expanded or ageing and the virus source plant, beet or shepherd's-purse for BMYV, did not substantially affect the incubation period. Accordingly, removing the inoculated leaf within a few days after infection in the translocation experiments had no effect on the development of symptoms on the plants which became infected. The results obtained demonstrate a close correlation between the duration of leaf expansion, as determined by plant age and temperature, and the incubation period of yellowing viruses in sugarbeet. Plant age has also a large influence on the incubation period of beet curly top virus (BCTV; gemini virus group) in beet (Duffus and Skoyen, 1977).

BYV and BMYV were shown to be translocated from the inoculated leaf 1 - 2 days after inoculation, consistent with values obtained for BYV in beet (Bennett, 1960) and for barley yellow dwarf virus (BYDV) in cereals (Gill, 1968). In the current study, the age of the plant was found not to affect the period of time needed for the translocation of BYV from the inoculated leaf of field-grown beet plants. This indicates that the extension of the incubation period at the end of the season is underlied by other factors.

The detection of BYV in young systemically-infected leaves by ELISA 1 to 2 weeks after infection is consistent with ELISA measurements by Smith and Hinckes (1985a) and with the results of virus acquisition trials (Chapter 6). The observed inconsistencies in virus detection may have been caused by the strategy to sample the oldest systemically-infected leaves which were expected to have the highest virus content. The sampling of a range of leaves on infected plants in October 1986, however, suggested that BYV is not detectable in leaves which are still expanding but only in the youngest, not yet unfolded leaves on one hand and in yellow, full-grown leaves on the other. Kleczkowski and Watson (1944) also detected less antigen in green leaves at the centre of the plant than in older, yellow leaves. These observations indicate that for monitoring virus infection in symptomless plants the youngest, not yet unfolded leaves should be sampled. However, in plants showing clear symptoms, the yellow leaves should be sampled to detect BYV-infection as opposed to BMYV-infection or other causes of yellowing. The results of the ELISA measurements suggest that the extension of the incubation period in old plants at the end of the season is related to a decreased rate of virus multiplication in the slowly expanding, systemically-infected leaves of these plants.

A considerable variation in incubation period was found between plants (Fig. 2.7 and Table 2.3). This variation may have resulted from differences in growth between individual plants caused by differences in genetic constitution or by different growing conditions throughout the field such as depth of sowing, soil humidity and compaction or proximity of neighbours, etc. Differences in incubation period were also observed among different fields (Figs. 2.1, 2.3 and 2.5). Plants grown on the Bouwing in 1986, for instance, had a relatively high thermal incubation period (Fig. 2.5A). This was probably caused by water stress in July and August which retarded the growth of these plants. Another example is the

great difference in the incubation periods of BMYV on systemically-infected leaves as measured in the experiments at the Binnenhaven in 1985 and the Haarweg in 1986. The reason for this difference has remained obscure. Possible explanations could have been differences in variety or crop husbandry. Though care was taken to judge with the same criterion in all experiments, the difference could also be the result of a different evaluation of the symptoms.

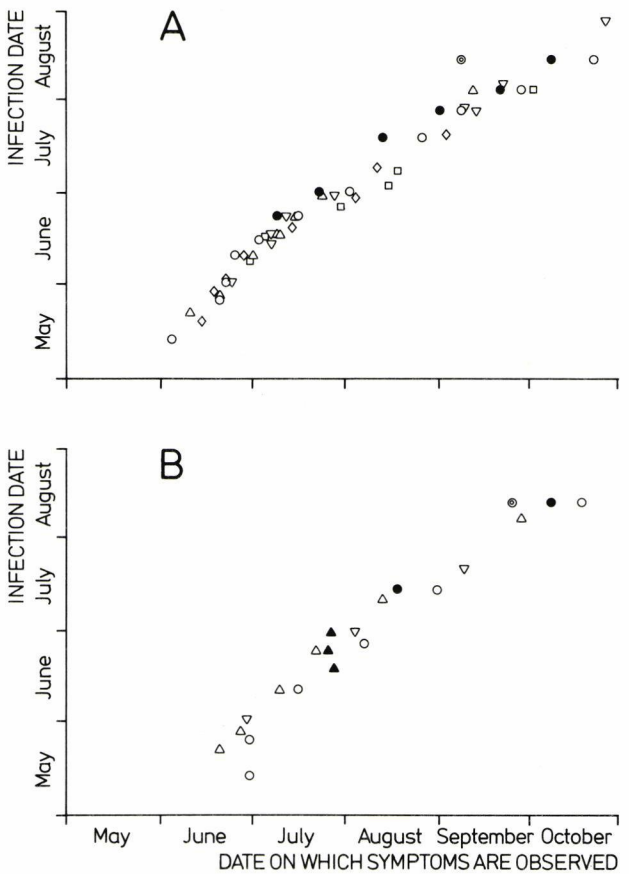


Fig. 2.9: Plot, relating the moment when systemic symptoms of BYV (A) or BMYV (B) are observed to the infection date. Meaning of symbols as in Fig. 2.1.

The incubation period can be used to assess the infection date of beet-plants since it depends only on the virus, the age of the plant and the weather but not on inoculation conditions. In doing so, symptoms on inoculated and systemically-infected leaves should be distinguished as these correspond to different incubation periods. Preferably, symptoms on systemically-infected leaves should be inspected avoiding the problem that symptoms on the inoculated leaves may remain unnoticed when these are covered by other leaves or die before symptoms develop. If no reference observations are made in the crop, Fig. 2.9 (in which the data of Fig. 2.1 are plotted in a different way) can be used to estimate the infection date as long as similar crop husbandry and climate are involved. However, the estimates are not likely to be very accurate because large differences in incubation period can occur between different fields or different seasons. If the sowing date of the crop or the weather circumstances during its growth differ much from what is usual, a reference line, relating the date of infection with the date at which 50% of the plants show symptoms, can be constructed using the relations observed between leaf number and the thermal incubation period (Fig. 2.5). Leaf number can be followed in the field during the season or estimated using accumulated temperatures (Milford *et al.*, 1985a; Chapter 3). This would allow readings by interpolation of the thermal incubation period from Fig. 2.5.

Probably, the rate of leaf growth represents one of the major factors affecting symptom development in virus-infected plants. Hence analysis of this rate may explain and predict symptom development under the influence of weather and other growing conditions and may help to relate virus spread to the behaviour of vectors. For instance, Allen (1978, 1983) was able to predict the effectiveness of roguing for the control of banana bunchy top disease with a model in which the incubation period was calculated as the time needed for the emergence of 2 leaves after the infection.

3 RETROSPECTIVE ESTIMATION OF THE DATE OF INFECTION WITH BEET YELLOWING VIRUSES

Summary

Sugarbeet plants, infected with beet yellows virus (BYV, closterovirus group) or beet mild yellowing virus (BMYV, luteovirus group) develop symptoms on the inoculated leaves on which aphids infected the plant. Symptoms develop also on the systemically-infected leaves to which virus has been transported via the phloem. Systemic infection occurs in the leaves which have just, or not yet appeared at the moment of infection of the plant. All other, older leaves remain uninfected. The infection date can be estimated by assessing when the oldest systemically-infected leaf on a plant appeared. This approach was tested in the field and gave reliable results.

3.1 Introduction

Virus yellows, caused by beet yellows virus (BYV, closterovirus group), beet mild yellowing virus (BMYV, luteovirus group) or beet western yellows virus (BWYV, luteovirus group), may cause important yield reductions in sugarbeet (Duffus, 1973; Smith, 1986). The most important vector of these viruses is the peach-potato aphid, *Myzus persicae* (Sulz.). Since the disease was first described by Quanjer (1934), severe outbreaks have been reported throughout the world (Duffus, 1973; Bar-Joseph *et al.*, 1979) and the epidemiology has been intensively studied (Watson *et al.*, 1951, 1975; Heathcote, 1986). Research into the within-season build-up of the disease has, however, been hampered by the variability of the incubation period under the influence of weather and plant age, and by the lack of accurate estimates (Chapter 2). Therefore, reason, it was impossible to relate the population dynamics and the behaviour of vector aphids to the subsequent increase in the number of yellowed plants in the crop.

Roseboom and Peters (1983) proposed a method for the retrospective determination of the infection date which obviated the use of the incubation period. Their method was based on the observation that the oldest leaf showing symptoms of systemic infection was generally one of the leaves

which had just appeared when the plant became infected. They calculated the ratio of the leaf number of this leaf and the total number of leaves on the plant. The ratio was compared to ratios obtained for reference plants, infected on known dates, to estimate the infection date.

In this paper experiments are described in which the relation between the number of leaves on the plant at the moment of infection and the leaf number of the oldest systemically-infected leaf was studied further. From a study of the appearance of leaves it was possible to modify Roseboom and Peters' method for determining infection date in such a way that the inoculation of reference plants became unnecessary. This updated method was tested in the field.

3.2 Materials and methods

3.2.1 Leaf appearance

In 1984, 1985 and 1986, sugarbeet were grown near Wageningen, the Netherlands, on heavy river-clay soil. In the first and second year the variety Regina was sown on 17 and 24 April, respectively, at the Binnenhaven. In the third year, the variety Bingo was sown on 25 April at the Haarweg. Details of fields and crops are given in Chapter 2. All leaves longer than 3 cm, emerging from the centre of the plant were counted weekly on 5 groups of 5 reference plants in 1984, on 6 groups of 10 plants in 1985 and on 5 groups of 10 plants in 1986. The leaf numbers were written on the leaves with black *Edding 300* felt pens.

Daily minimum and maximum temperatures were measured in Stevenson screens located less than 1 km from the experiments. Daily increments of the temperature sum above 1 °C, the approximate temperature threshold of leaf appearance in sugarbeet (Milford *et al.*, 1985a,b), were calculated by fitting a sine between the measured minimum and maximum temperatures and summing the hourly increments. Leaf number was calculated using these temperature sums and equations of Milford *et al.* (1985b). They found that in 5 beet crops, grown under standard husbandry in England from 1978 to 1982, the first leaf pair unfolded 355 °C days after sowing. Each of the next 21 leaves required 29 °C days to unfold and leaf 24 and all following leaves needed 48 °C days.

3.2.2 The position of leaves with symptoms on a plant as determined by the development stage of the plant (leaf number) on the infection date

The field studies in 1984 and 1985 also included analysis of the relation between leaf appearance and the position of leaves with virus symptoms. From mid-May until mid-July plants were inoculated with BYV or BMYV and the number of leaves was counted. The development of symptoms was recorded at 1 to 3 week intervals until the oldest systemically-infected leaves showed intense yellowing and necrosis. The relation between the number of leaves on a plant on the infection date (N_0) and the number of the oldest systemically-infected leaf (C) was examined by linear regression.

In 1984, the inoculations were made on 2, 15 and 29 June and on 6 and 20 July when the plants had an average of 5, 10, 15, 18 and 21 leaves, respectively. On each date, 5 groups of 10 plants were inoculated with BYV and 5 with BMYV. In 8 plots sown on 8 June, 10 plants having about 9 leaves were inoculated on 20 July. A total of 580 plants were inoculated in 1984.

In 1985, 8 weekly inoculations were made between 23 May (two-leaf stage) and 8 August (32-leaf stage). The number of plants inoculated varied from 60 in July, when only standard inoculations with BYV and BMYV were made, to 450 in June, when inoculation conditions were varied to study the effect on the development of symptoms (Chapter 2). For a 'standard' inoculation, 10 to 15 *M. persicae* were caged for 1 to 2 days on a recently-expanded leaf. Non-standard inoculations were made on an expanding leaf, on an ageing leaf or on 3 leaves per plant or with fewer vectors, *viz.* 1, 2 or 5 on a recently-expanded leaf. Two plants were successfully inoculated with BYV by *Aphis fabae* Scop. A total of 2460 plants was inoculated in 1985.

Viruliferous aphids were collected from infected beet plants in the glasshouse in May and July 1985. When necessary, viruliferous aphids were produced by feeding non-viruliferous aphids from rape on detached virus-infected beet leaves for 2 to 3 days. In 1984 the *M. persicae* clone M2 was used, and in 1985 the clone M3, which transmitted BYV slightly better in glasshouse tests (Van der Werf, unpubl. res.). Aphid-proof clip-cages were used to prevent any accidental infection of leaves not to be inoculated. The inoculated leaves were marked with bright-coloured

plastic labels to facilitate inspection. The viruses were maintained in beet as described before (Chapter 2).

After the inoculations, the plants were sprayed weekly with either pirimicarb or oxy-demeton-methyl to kill naturally-occurring aphids and prevent virus spread to and from the inoculated plants. In June 1985, aldicarb granules were applied to the soil, because the rainy weather did not allow spraying.

3.3 Results

3.3.1 Leaf appearance

The plants produced more than 50 leaves from May to October. Leaf appearance was most rapid in early summer when the plants were young and the temperatures high. The leaf appearance rate was lower both in spring and in late summer/autumn due to lower temperatures and in the latter case also because the plants became older (Fig. 3.1). The variation between plants was high. The coefficients of variation of the final number of leaves in October were 17, 13 and 16% for the 3 seasons, respectively.

In Fig. 3.2, leaf appearance is plotted against the temperature sum since sowing. Until 1200 °C days after sowing, the leaf appearance rate was about one leaf per 33 °C days. This is slightly less rapid than in the experiments of Milford *et al.* (1 leaf per 29 °C days). In our experiments the 28th leaf appeared 1200 °C days after sowing. From that moment onwards leaves appeared less rapidly, 1 leaf per 47 °C days. In their experiments, Milford *et al.* found that the leaf appearance rate was 1 leaf per 48 °C days after the 23rd leaf appeared. Our observations on leaf appearance in beet were adequately predicted with the calculated temperature sums and Milford's equations (Fig. 3.1).

3.3.2 The position of leaves with symptoms on a plant as determined by the development stage of the plant (leaf number) on the infection date

In 1984 about 70-80% of the plants were infected using aphids from rape which had acquired the viruses from detached virus-infected beet leaves. Analyses were made of the symptoms on 155 BYV-infected plants and 145

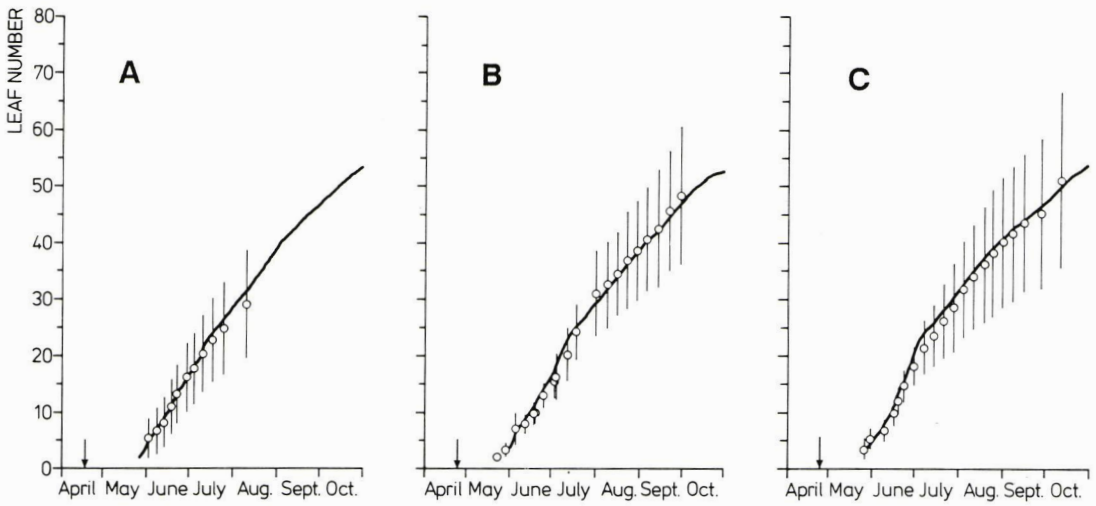


Fig. 3.1: Leaf appearance (○) in 3 sugarbeet fields in 1984 (A), 1985 (B) and 1986 (C), together with calculated leaf number (drawn line) according to accumulated temperature equations of Milford *et al.* (1985a). Bars denote intervals of (μ - 1.96 * σ; μ + 1.96 * σ) in which μ = mean number of leaves and σ = standard deviation. Arrows indicate sowing date.

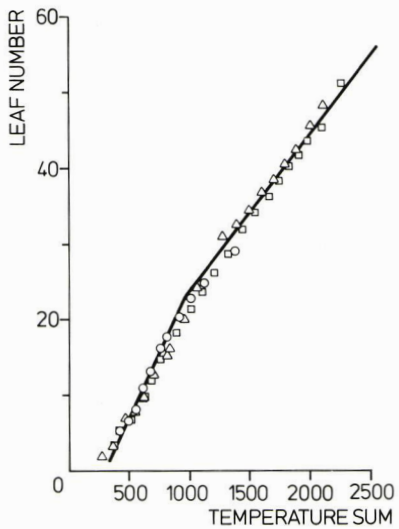


Fig. 3.2: Leaf appearance in 1984 (○), 1985 (△) and 1986 (□), respectively, as a function of accumulated temperature above 1 °C. Drawn line according to Milford *et al.* (1985a).

BMYV-infected plants. In 1985, with aphids from beet, the infection success was also 70-80% but with aphids from rape only 5-10%.

The low virus transmission by aphids from rape in 1985 was presumably due to a combination of factors such as: (1) problems of adaptation to beet of the aphids reared on the more palatable rape; (2) a high air humidity during the acquisition of virus by which the aphids were possibly not bound to feed on the infected leaves; (3) poor contact between the clip-cages and the leaves during the inoculation. In 1985, symptom records were made on 204 plants infected with BYV and on 189 plants infected with BMYV.

The leaf number (C) of the oldest leaf with symptoms of systemic infection moved with the age of the plants up on the phyllogenetic spiral (Bell and Coombe, 1975), following the same course as leaf appearance (Table 3.1). The standard deviation of C increased in a similar way as the standard deviation of the number of leaves on the plants during the season. Because the ranges of C overlap for different dates of infection, determination of C alone is not sufficient to determine the infection date. Account should be taken of the differences in leaf number between plants.

Table 3.1: Number of leaves on the plants (N_0) on the date of infection with either BYV or BMYV and the oldest systemically-infected leaf (C) with their respective standard deviations (σ). n is the number of plants per treatment. [1] Plants sown on 8 June.

		BYV					BMYV				
year	date	N_0	σ	C	σ	n	N_0	σ	C	σ	n
1984	2 June	5.9	1.3	6.3	1.1	15	4.9	2.0	5.6	1.7	29
	15 June	9.8	1.7	9.5	1.5	33	9.7	2.0	10.5	2.0	44
	29 June	15.5	2.3	13.6	2.2	35	14.9	2.6	14.3	2.9	26
	6 July	18.3	2.4	16.8	2.4	18	18.5	2.2	17.6	2.3	18
	20 July	21.5	3.7	19.8	3.4	26	19.3	3.5	18.3	3.6	12
	20 July[1]	8.5	1.8	8.4	1.6	28	8.5	1.8	8.7	1.8	16
1985	20 May	2.0	0.2	3.0	0.2	67	2.0	-	3.0	0.4	98
	27 May	2.9	0.8	3.8	0.8	13	3.5	0.8	4.2	0.8	22
	3 June	8.1	1.0	8.3	0.9	11	8.4	1.0	9.1	1.3	24
	17 June	8.7	0.9	9.5	1.0	20	-	-	-	-	-
	24 June	-	-	-	-	-	13.1	1.0	14.2	1.1	12
	1 July	14.1	2.3	13.5	2.0	51	-	-	-	-	-
	15 July	-	-	-	-	-	20.9	3.1	20.8	3.5	12
	8 Aug.	32.2	3.4	30.8	3.2	22	-	-	-	-	-

A

B

Fig. 3.3: Photograph made in October 1986, showing a plant infected with BYV in an early development stage (A). All living leaves of this plant are infected. The other plant (B) was infected when it had ± 30 leaves. Only the leaves which emerged after the infection date are infected. On both plants, only the fully-expanded leaves show clear symptoms.

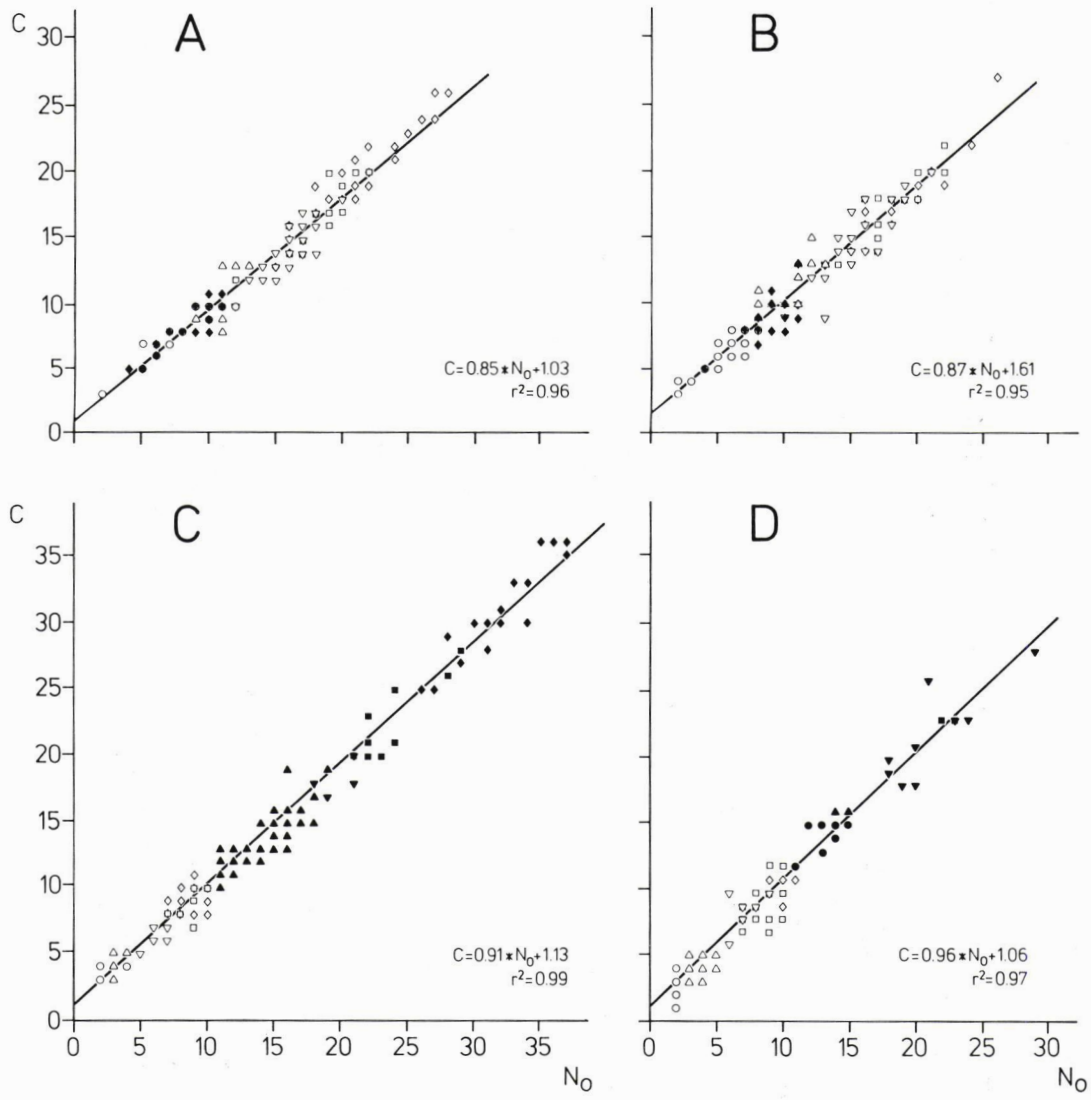


Fig. 3.4: Relation between the number of leaves (N_0) on a plant at the infection date and the oldest leaf (C) showing symptoms of systemic infection. Data for BYV in 1984 (A) and 1985 (C) and for BMYV in 1984 (B) and 1985 (D). In 1984, inoculations with both viruses were made on 2 June (○), 15 June (△), 29 June (▽), 6 July (□) and 20 July (◇) and in 1985 on 20 May (○), 27 May (△), 3 June (▽), 10 June (□), 17 June (◇), 24 June (●), 1 July (▲), 8 July (▼), 15 July (■) and 8 August (◆). In 1984 an inoculation was made on 20 July on plants sown on 3 June, more than a month later than usual (◆).

A close relation exists between the number of leaves on the date of infection (N_0) and the leaf number of the oldest leaf showing symptoms of systemic infection (C) (Fig. 3.4). The coefficients of determination (r^2) range from 0.95 for BMYV in 1984, to 0.99 for BYV in 1985. The residual errors, σ_R, range from 1.04 to 1.19, indicating that a 95%-confidence interval of C around its expected value is only 4 leaves wide. Taking the data of both years together in the regression analysis yields:

$$\text{BYV:} \quad C = 0.89 * N_0 + 1.00 \qquad r^2 = 0.98 \quad \sigma_R = 1.07 \qquad (3.1)$$

$$\text{BMYV:} \quad C = 0.91 * N_0 + 1.24 \qquad r^2 = 0.97 \quad \sigma_R = 1.04 \qquad (3.2)$$

Regression of N_0 on C results in equations which can be used to estimate the number of leaves on a plant when infected, given the oldest systemically-infected leaf:

$$\text{BYV:} \quad N_0 = 1.10 * C - 0.86 \qquad r^2 = 0.98 \quad \sigma_R = 1.19 \qquad (3.3)$$

$$\text{BMYV:} \quad N_0 = 1.06 * C - 1.04 \qquad r^2 = 0.97 \quad \sigma_R = 1.12 \qquad (3.4)$$

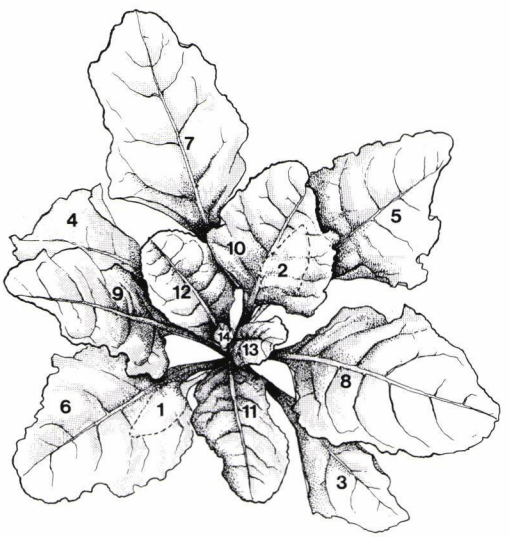


Fig. 3.5: Arrangement of the leaves on a young sugarbeet plant, having 13 leaves longer than 3 cm.

32

33

34

Fig. 3.6: Photographs, taken on 11 October 1985, showing the oldest systemically-infected leaves of a beet plant naturally-infected with BYV on leaf 19 about 1 August. The systemically-infected leaves show typical late season symptoms of BYV: bright yellowing and red spots. Leaf 32 is the oldest systemically-infected leaf (C) and is on a typical position (I + 13). It shows symptoms on two third of its blade area. Leaf 33 is implanted opposite the inoculated leaf. A smaller area is affected and the symptoms are less intense than on leaf 32. Leaf 34 (I + 15) is for the greatest part affected and leaf 35 is entirely yellowed. Leaf 36 and 37 show the decreasing intensity of symptoms typical for later appeared, younger leaves.

3.3.3 The effect of the position of the inoculated leaf (I) on that of the oldest systemically-infected one (C)

Leaves of sugarbeet appear one by one in a 5/13 phyllotaxis (= leaf arrangement; Hayward, 1938), i.e. 13 leaves appear in 5 complete turns of the phyllogenetic spiral, successive leaves spaced at an angle of approximately 138°. The first 2 true leaves are exceptional by appearing at the same time at an angle of 180° (Fig. 3.5). The direction of the phyllogenetic spiral is clockwise in approximately 50% of the plants and anti-clockwise in the other 50%. On a beet plant, one can distinguish several sequences of leaves, called parastichies, which are implanted on almost the same position on the plant and which differ in leaf number by a constant factor (Williams, 1975). The most conspicuous parastichies are those consisting of leaves differing 3 in leaf number (e.g. leaf 1, 4, 7, 10, etc.), 5 or 8.

Different symptoms are developed on leaves which appear on different times and on different positions on the beet plant. It is important to recognize these different symptoms to correctly identify the oldest leaf with symptoms of systemic infection. The first leaf to show symptoms of systemic infection is mostly found on the same side of the beet plant as the inoculated leaf, especially on older plants. Younger leaves than that which first showed symptoms, develop symptoms one after the other as they attain their final size (Chapter 2). The first leaf developing symptoms may remain the oldest yellowed leaf (C). Otherwise, one or a few leaves older than the first leaf showing symptoms may show symptoms after some time. These leaves show generally symptoms only on the base of the leaf. The tip remains green until the leaf dies. On subsequent younger leaves, portions of the blade nearer to the tip will be affected. The first, and consequently most advanced symptoms on a leaf, develop on the oldest infected part of the leaf, i.e. the infected part which is nearest to the tip (Maksymowitsch, 1973). Towards the leaf base the symptoms develop later and they are consequently less pronounced. In the course of time the symptoms become more intense over the whole infected part of the leaf blade.

For BYV the upper margin of the affected area is generally sharply delimited by veins. This phenomenon is known as 'sectoring' (Bennett, 1960). It presumably reflects which parts of the leaf were young enough to

be systemically-infected when virus was transported through the plant and those which were not. Later on, neighbouring sectors may develop symptoms, presumably due to cell-to-cell transport of virus. Sectoring occurs also on BMYV-infected leaves, but is less pronounced than on BYV-infected leaves. For both viruses the spot formed around the site on a leaf where an aphid infected the plant is sharply delimited by veins. Fig. 3.6 gives an example of the types of symptoms on leaves of different age and position on a beet plant infected with BYV.

The effect of the position of the inoculated leaf (I) on that of the oldest systemically-infected one is shown in Fig. 3.7. Young leaves on the same side of the plant as the inoculated leaf differing e.g. 3, 5 or 8

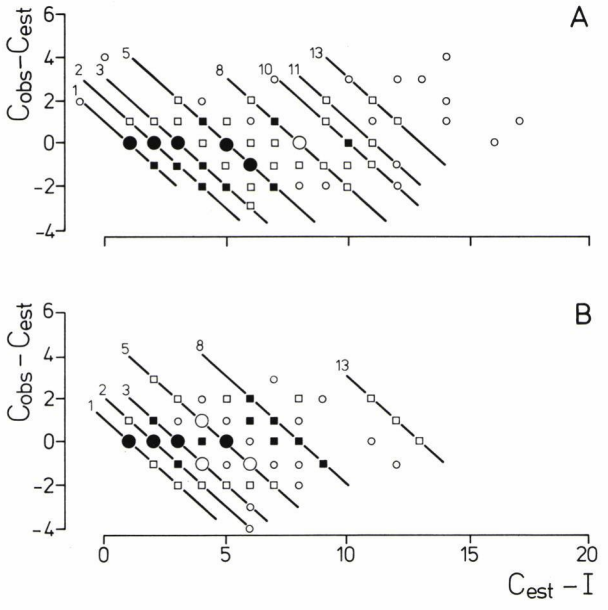


Fig. 3.7: Difference between C_{obs}, the observed oldest leaf with symptoms of systemic infection and the estimated value, C_{est}, for different phyllotactic positions of C_{est} to the inoculated leaf, I. C_{est} is computed with Eqs. 3.3 and 3.4. The symbols denote the number of plots having a given combination of C_{est} - I and C_{obs} - C_{est}: 1 (○), 2 - 5 (□), 6 - 10 (■), 11 - 20 (◯) or more than 20 (●).

leaves with it, have a higher chance of becoming the oldest systemically-infected leaf than other young leaves. The abscis shows the expected position of C, C_{est}, as estimated with Eqs. 1 and 2, relative to I. The difference between the observed value of C, C_{obs} and the computed value, C_{est}, is shown on the ordinate. Leaves on the other side of the plant than the inoculated leaf, are not likely to become the oldest systemically-infected leaf. Thus, when Eqs. 1 and 2 give values of C_{est} which differ 4, 6, 7, 9, 12 or 14 with I, C_{obs} is mostly one or two numbers higher or lower than C_{est} such that C_{obs} is on the same side of the plant as I. C_{obs} - I therefore mostly assumes one of the values 1, 2, 3, 5, 8, 10, 11, 13 or 15 (diagonal lines in Fig. 3.7). When the oldest systemically-infected leaf was at the same side of older plants as the inoculated leaf, it was often observed that the next younger leaf on the other side of the plant escaped infection entirely or developed symptoms on a smaller partion of its base than leaf C (Fig. 3.6).

3.3.4 Retrospective estimation of the infection date

The infection date can be estimated with an observation on the oldest leaf with symptoms of systemic infection (C) and the number of leaves N_{obs} on a plant. A reference leaf appearance curve must be obtained by counts in the field or by calculations based on accumulated temperatures.

As a first step the number of leaves (N_0) on the plant when it was infected with virus is computed with Eqs. 3 and 4. The moment that the plant had N_0 leaves is determined by comparison with the leaf appearance curve of the reference plants. Because beet plants differ considerably in leaf appearance rate, N_0 is corrected for the relative leaf appearance rate of the plant, R. The value of R is estimated with the quotient of the number of leaves on the plant, N_{obs}, and the number of leaves on the reference plants, N'_{obs}, on an arbitrarily chosen moment:

$$R = N_{obs} / N'_{obs} \qquad (3.5)$$

Then, N_0 is corrected to give the approximate number of leaves on the reference plants, N'_0, when the plant under study was infected.

$$N'_0 = N_0 / R \quad (6) \qquad (3.6)$$

The infection date is determined by interpolation with N'_0 in the reference leaf appearance curve.

For example; a plant with N_0 = 26 leaves is infected with BYV on 17 July 1985. The reference plants have 22 leaves on that date. An observation is made on 9 September when the number of leaves on the plant, N_{obs}, is 47. The oldest leaf (C) with symptoms is 24. N_0 is then estimated as 1.10 * 24 - 0.86 = 25.5 (Eq. 3.3). The number of leaves, counted on reference plants on 9 September is 42. Thus: R = 47 / 42 = 1.12 and N'_0 = 25.5 / 1.12 = 22.8. The reference plants had this number of leaves on 19 July which is at the same time the estimated infection date. This estimate is close to the actual infection date, 17 July.

3.3.5 Evaluation of the method

In 1985, the method for the retrospective estimation of the date of infection was evaluated on a sugarbeet field, variety Monohil, on the Haarweg near Wageningen (Chapter 2). Inoculations with BYV or BMYV were made on 9 dates from the end of May until the end of July, using 10 to 15 *M. persicae*, clip-caged onto a recently-fullgrown leaf. The number of leaves per plant and the development of yellowing symptoms on individual leaves were recorded on 5 occasions from July till October. In 1986, inoculations and observations were made in the same way on a sugarbeet field, variety Bingo, on the Haarweg.

Two variants of the method were evaluated, one in which the number of leaves on reference plants was counted weekly (1), and another (2) in which the leaf appearance for the reference plants was calculated from accumulated temperatures. Both variants gave good estimates of the infection date (Figs. 3.8A, B).

Variant 1: $y = 0.97 * x + 6.8 \quad r^2 = 0.95 \quad \sigma_R = 4.4$ (3.7)

Variant 2: $y = 0.95 * x + 7.5 \quad r^2 = 0.93 \quad \sigma_R = 4.9$ (3.8)

In these equations, x and y are the real and estimated date of infection, expressed in day of the year (Seem and Eisensmith, 1986). The regressions found do not deviate significantly from the ideal line, y = x

($p > 0.05$). The accuracy of the estimates decreases as the number of leaves on the plants on the moment of infection and its variability increase during the season. Therefore, the best estimates are those for early-infected plants.

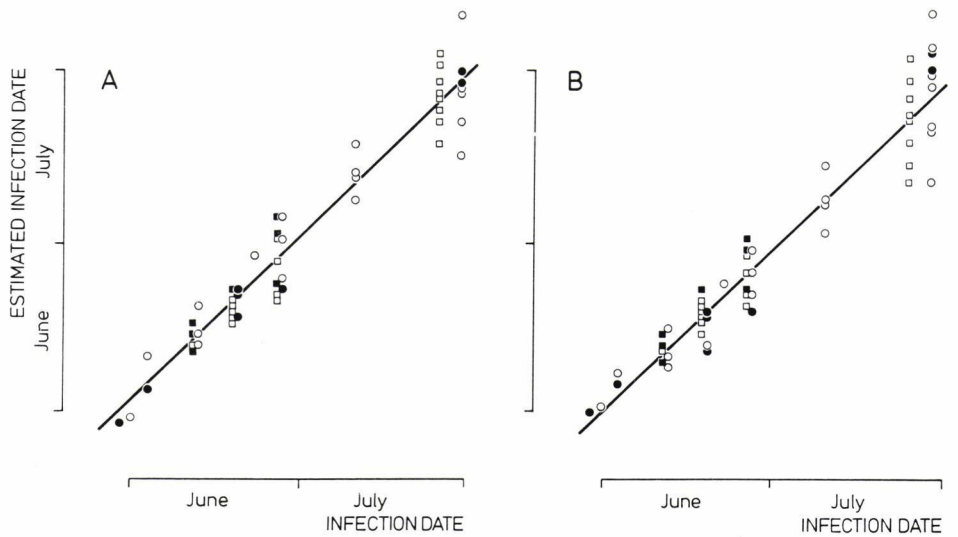


Fig. 3.8: Comparison of estimated with actual dates of infection with BYV (open symbols) and BMYV (solid symbols) in 1985 (○) and 1986 (□), respectively. (A) Reference number of leaves counted in the field. (B) Reference number of leaves calculated with accumulated temperatures.

3.4 Discussion

The observed patterns of yellowing symptoms on (parts of) leaves of different age and position on the plant resemble the pattern of assimilate translocation in plants. Thus, Joy (1964) found that most of the assimilated ^{14}C which was translocated out of a source leaf, was recovered from sink leaves at positions 8, 10, 11 and 13. These leaves are all implanted on the same side of the plant as the source leaf and had, in our experiments, a high probability to become the oldest systemically-infected leaves (Fig. 3.7). In leaves at positions 10 and 11, Joy found most ^{14}C in the leaf halves which were nearest to the source leaf, just as virus symptoms are sometimes found only on the leaf halves which are nearest to the virus source leaf. In our experiments, this was only observed on the oldest

systemically-infected leaves, which soon after the infection switched from assimilate import to export, and most frequently on old plants. Symptoms, restricted to the base of a leaf occurred often on the leaf halve nearest to the inoculated leaf. Similar patterns of translocation of ^{14}C were found in tobacco (Jones *et al.*, 1959; Shiroya *et al.*, 1961; Porter, 1976) and eastern cottonwood, *Populus deltoides* Bartr. (Larson and Dickson, 1973). The latter authors also observed that, in successive younger sink leaves, more ^{14}C was transported to the leaf tip and less to the base, which resembles the pattern of development of virus yellows symptoms on leaves differing in age and position on the beet plant (Fig. 3.6). Fellows and Geiger (1974) observed that assimilate import by the 7th leaf of young sugarbeet plants reached a maximum at 25% of the final leaf length (FLL) and declined to almost zero at 45% FLL. Generally, net assimilate export from a leaf begins when one-third to one-half full leaf expansion is attained. The leaf tip is the first region which switches from import to export and this switch progresses basipetally (Fellows and Geiger, 1974; Larson and Dickson, 1973; Maksymowitsch, 1973). Our observation that on the oldest systemically-infected leaves the symptoms are often restricted to the leaf base is consistent with the timing of the import to export transition in different portions of the leaf and with the evidence that beet yellowing viruses are transported to sink tissues via the phloem (Esau *et al.*, 1967; Esau and Hoefert, 1972).

The estimation of the date of virus infection, using an observation of the position of leaves with symptoms on the plant can be used an alternative to the practice to assess the infection date by substracting the incubation period from the date on which the first symptoms were seen (Chapter 2). Advantages of the method described in this paper are (1) its accuracy for young plants; (2) the necessity of only one observation and (3) the free choice of the moment of the observation. The use of the incubation period might give less accurate results (Chapter 2) and frequent observations must be made at the time the plants are expected to show symptoms. Disadvantages of the described method are, however, (1) its laboriousness and (2) the difficulty to apply it when more than one leaf has been inoculated by aphids and (3) when many leaves have died and cannot be retrieved. The retrieval of the phyllogenetic spiral and the determination of the oldest leaf with symptoms of systemic infection (C) and the number of leaves on the plant (N_t) can be very time-consuming,

especially on old plants with many dead leaves. On such plants, an observation may take 15 minutes or more. Advantages of the use of the incubation period in this respect are the ease of observation and the applicability for multiply-infected and old plants.

The described method is therefore probably most useful to estimate the infection date of isolated primarily-infected plants, early in the season. Knowing the infection date of these plants is important because the earliness of infection is an important factor determining the extent of secondary spread (Chapter 5). Because early in the season the variation in leaf number between plants is small, it may be possible to abandon the adjustment for the leaf appearance rate of the plants.

Pilot studies in the glasshouse showed that the symptoms of systemic infection with beet mosaic virus (BMV) occur also on a few young leaves, present on the plant on the moment of infection and on all leaves appearing afterwards. No symptoms or only a faint mottling was observed on older leaves inoculated by *M. persicae*. These results suggest that the method could also be applied for BMV. Observations in 1986 on the development of BMV symptoms on field-grown plants of different age and naturally-infected with BMV support this suggestion. Application of the method for BMV may, however, be hampered by the vagueness of the mosaic symptoms on fully-expanded, systemically-infected leaves. Only a few symptomless heart leaves were found on BMV-infected plants of all ages, suggesting that the incubation period of BMV is very short throughout the season. Therefore the use of the incubation period could be a more practical method to assess the infection date of BMV-infected plants.

The principle of the described method may be applicable to viruses in a range of crops. It could be particularly useful for viruses with a variable incubation period in crops which are genetically homogeneous and where all plants have equal leaf appearance rates. In such a crop one could simply count the number of leaves appeared since the oldest systemically-infected leaf appeared and calculate how long this has taken, to assess the infection date.

4 CONFIDENCE LIMITS OF THE MULTIPLE INFECTION TRANSFORMATION

Abstract

In virus epidemiology, bait plants are often used to determine the infection pressure with viruses disseminated by alate vectors. Batches of susceptible plants are thereby exposed for short periods in the field, transferred to a glasshouse for symptom expression and scored on the presence of symptoms. If the vectors may be assumed to alight *at random*, the number of viruliferous vectors (v) can be calculated from the proportion of plants infected (k out of n), using Gregories Multiple infection transformation: $v = n * \ln(n/(n-k))$. To improve the interpretation and design of these tests, confidence limits for v are derived and estimates of v are given for situations in which all plants are infected. The results are presented in monograms which are easy to use.

4.1 Introduction

Most plant viruses are spread by vectors and many of these by several vector species, each with its own phenology. To determine when and by which vector viruses are spread, successive batches of bait plants can be placed for short periods in the field. In this way, spread of potato leaf roll virus (PLRV) and potato virus Y (PVY) in potato was monitored with potato bait plants in Britain (Broadbent *et al.*, 1950; Broadbent and Tinsley, 1951) and with tobacco (Van Hoof, 1977a; 1977b; 1979) or potato bait plants (De Bokx, 1979) in the Netherlands. Van Harten (1983) used the results of these tests to determine the optimal date of early haulm destruction in seed potatoes for the control virus spread. Spread of PVY and cucumber mosaic virus (CMV) in peppers was studied with pepper bait plants (Raccah, 1983: Raccah *et al.*, 1985) and spread of CMV in muskmelon with five different bait plant species (Marrou *et al.*, 1979). Bait plants were also used to monitore spread of cirus tristeza virus (CTV; Schwarz, 1955), maize dwarf mosaic virus (MDMV; Knoke *et al.*, 1974; Madden *et al.*, 1983) and strawberry viruses (Posnette and Cropley, 1954).

The expected proportion of bait plants infected is a saturation type function of the number of infections or vectors because the chance of a

vector re-infecting an already infected plant increases as more plants become infected. An estimate of the number of infections can be derived from the proportion of plants infected with the multiple infection transformation (Gregory, 1948). Thus a measure for the infection pressure is obtained which can be directly translated into numbers of viruliferous vectors. Numbers of infections obtained on different locations or in different periods can be more readily compared to each other than the proportions of infected plants. Application of the multiple infection transformation becomes inevitable when the lengths of the exposure times have varied, because the relation between duration of exposure and percentage infection is also not linear. To improve the applicability of the transformation, two extensions are given in this paper: (1) confidence limits for the number of infections, given the number of plants and the proportion of infected plants, and (2) estimates of the number of vectors *at least* needed to infect all the plants.

4.2 Materials and methods

To obtain confidence limits for the number of vectors, the probability distributions of the number of plants infected were calculated for a range of numbers of plants and vectors with a FORTRAN77 program. The same program was used to determine lower confidence limits for and the expected number of vectors at which 100% infection occurs when vectors are added one by one. Approximations were made with the Poisson-distribution.

4.2.1 Calculation of confidence limits of the number of vectors

The multiple infection transformation assesses an unknown parameter v, the number of vectors, given the number of infected plants, k, out of a total of n plants. The value of v can be estimated if the vectors alight *at random*. Thereby, it is assumed that they are distributed over the plants according to the Poisson-distribution (Gregory, 1948; Van der Plank, 1963). This is valid if $n \to \infty$. The probability of i vectors on a given plant is then

$$p_i = e^{-v/n} * \frac{(v/n)^i}{i!} \qquad (4.1)$$

and the probability of zero vectors (i = 0)

$$p_0 = e^{-v/n} * \frac{(v/n)^0}{0!} = e^{-v/n} \qquad (4.2)$$

Under the assumption that each vector causes exactly one infection, the expected fraction of plants remaining healthy equals the probability of zero vectors on a plant, *viz.* $e^{-v/n}$. If k out of n plants are infected, we can solve

$$k/n = 1 - e^{-v/n} \qquad (4.3)$$

for v. In this way v is estimated with (Gregory, 1948)

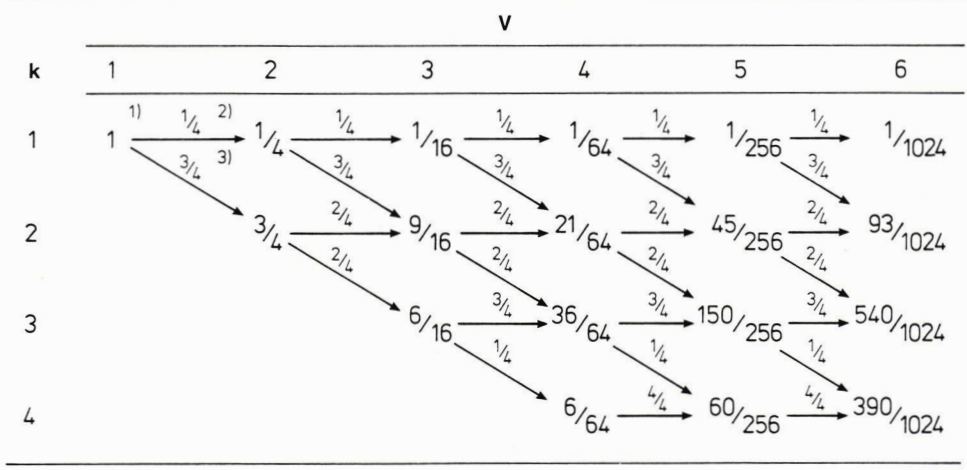
$$v = n * \ln(n/(n-k)) \qquad (4.4)$$

This is a biased estimator, due to the nonlinear transformation involved.

Confidence limits of v can be determined if for a given value of n, the probability distributions of k are known for all v. These probability distributions were calculated with a FORTRAN77-program in which vectors were added one by one to n plants. After the v-th vector is added, the program calculates the probabilities p(k) that k plants are infected, where k assumes all integer values between 1 and the minimum of v and n (k_{max}). Evidently, no more plants can be infected than there are plants (n) or vectors (v). The probability of k infected plants after vector v is added is the sum of the probabilities of two events: (1) The v-th vector super-infects one of the k plants which were already infected by the v-1 previous vectors. Due to the assumed *random* alightment of vectors, this super-infection occurs with a probability of k/n. (2) The v-th vector infects one of n - (k - 1) plants which had up to then remained healthy. Such a new infection occurs with probability (n - (k - 1))/n. Thus the probability of k infected plants after v vectors are added becomes

$$p_v(k) = p_{v-1}(k) * k/n + p_{v-1}(k - 1) * (n - (k - 1))/n \qquad (4.5)$$

Table 4.1: Calculation of the chances on 1, 2, 3 or 4 infected plants when 6 vectors are added *at random* to 4 plants.



1) Probability $p_v(k)$ of k infected plants after v vectors are added.
2) probability (k/n) that the next vector super-infects one of the k infected plants
3) probability (1 - k/n) that the next vector infects one of the n - k plants which are not yet infected.

An example of the computations for n = 4 and v = 6 is worked out in Table 4.1. The first vector infects one plant with probability 1. The probability that the second vector super-infects the same plant is 1/4 and the probability that it infects one of the 3 healthy plants is 3/4. When the third vector is added, 2 initial situations are considered. (1) The first 2 vectors infected only 1 plant, which occurs with probability $p_2(1) = 1/4$. (2) The first 2 vectors infected 2 plants, which occurs with probability $p_2(2) = 3/4$. The probability that only 1 plant is infected after the third vector has been added is then the product of the probability that the first 2 vectors infected only 1 plant and the probability that the third vector infects the same plant, *viz.*

$$p_3(1) = p_2(1) * 1/4 = 1/4 * 1/4 = 1/16$$

The probability that 2 plants are infected after the third vector has been added becomes

$$p_3(2) = p_2(2) * 2/4 + p_2(1) * 3/4$$

$$= 3/4 * 2/4 + 1/4 * 3/4 = 9/16$$

and the probability that 3 plants are infected after the third vector is added is

$$p_3(3) = p_2(3) * 3/4 + p_2(2) * 2/4$$

$$= 0 * 3/4 + 3/4 * 2/4 = 6/16$$

etc. In the program, these calculations were made for n-values ranging from 10 to 1000 and v-values from 1 to 15 times n.

The probabilities f(k) of k or fewer plants infected (cumulative distribution function) were calculated with

$$f(k) = f(k + 1) - p(k + 1) \tag{4.6}$$

where

$$f(k_{max}) = 1 \tag{4.7}$$

The obtained values of f(k) were used to find one-sided critical k-values for a given value of v. These critical values are plotted in Fig. 4.2. The lower critical value, k_{low}, is the largest k for which f(k) does not exceed α; here $1 - \alpha$ is the one-sided confidence level mentioned in the legend of Fig. 4.2:

$$P_v(k \leq k_{low}) \leq \alpha \tag{4.8}$$

The upper critical value, k_{up}, is the smallest k for which f(k-1) equals or exceeds $1 - \alpha$:

$$P_v(k \leq k_{up} - 1) \geq 1 - \alpha \tag{4.9}$$

which is equivalent to

$$P_v(k \geq k_{up}) \leq \alpha \qquad (4.10)$$

Observed k-values between k_{low} and k_{up} will not lead to rejection of the hypothesis that v is the true number of vector for a confidence level $1 - 2\alpha$. Values equal to or lower than k_{low} or equal to or higher than k_{up} will lead to rejection of this hypothesis.

Confidence intervals of v with confidence level $1 - 2\alpha$ can be also read in Fig. 4.2. For a fixed (observed) value of k, the line connecting k_{up}-values gives the value of v for which the probability of at least k infected plants is less than or equal to α. Left of this line, i.e. for smaller v, the probability of at least k infected plants is less than α. Hence the line gives lower confidence limits of v. By analogy, the line connecting k_{low}-values provides upper confidence limits of v. A confidence interval for v with confidence level $1 - 2\alpha$ is bordered by the largest v-value, v_{low}, for which $k_{up}(v_{low}) \leq k$ (lower border) and the smallest v-value, v_{up}, for which $k_{low}(v_{up}) \geq k$ (upper border).

When 100% infection has occurred, the lower confidence limit v_{low} can be obtained from Fig. 4.2, but the upper limit is $+\infty$. Approximate lower confidence limits with confidence level $1 - \alpha$ of the number of vectors at least needed to infect all plants can be derived from the probability distribution of the number of plants remaining *healthy*, as approximated with the Poisson-distribution by Feller (1968; p.101). The probability of m healthy plants is approximately

$$p(m) \cong e^{-\lambda} * \frac{\lambda^m}{m!} \qquad (4.11)$$

with

$$\lambda = n * e^{-v/n} \qquad (4.12)$$

provided that λ does not assume large values.

As k equals n - m, the probability distribution of k is

$$p(k) = e^{-\lambda} * \frac{\lambda^{n-k}}{(n-k)!} \qquad (4.13)$$

100% infection is obtained when k = n, thus

$$p = e^{-\lambda} * \frac{\lambda^{0}}{0!} = e^{-\lambda} = e^{(-n * e^{-v/n})} \qquad (4.14)$$

Rearringing gives

$$\ln(p) = -n * e^{-v/n}$$

$$\ln(-\ln(p)/n) = -v/n$$

$$v/n = \ln(n) - \ln(-\ln(p))$$

Thus, lower confidence limits for the number of vectors when 100% infection is observed are given by

$$v_{low}/n = \ln(n) - \ln(-\ln(\alpha)) \qquad (4.15)$$

This relation is plotted in Fig. 4.3.

4.2.2 Probability distribution of the number of vectors needed to infect all plants

It is impossible to assess the number of vectors when all the plants have become infected. Only the number which is *at least* needed (or *exactly enough*) to do this can be calculated. The situation is therefore considered from a different point of view. Suppose vectors (v) are added one at a time and V is the number at which 100% infection occurs. The expectation of V can be derived from the geometric probability distribution (Feller, 1968) which describes the number of Bernouilli trials needed to obtain the first success. The number of vectors needed to infect the first

plant follows a geometric distribution with probability of success p = 1 and expectation 1/p = 1. The number of vectors needed to infect the second plant follows a geometric distribution with p = (n - 1)/n and expectation n/(n - 1). The expected number of vectors needed to infect the third plant is n/(n - 2), etc. The expected number of vectors needed to infect all plants is the sum of the expected numbers of vectors to infect the first, second through the last plant, *viz.*

$$\mu_V = \frac{n}{n} + \frac{n}{n-1} + \frac{n}{n-2} + \ldots + \frac{n}{1}$$

$$= n * \left(\frac{1}{n} + \frac{1}{n-1} + \frac{1}{n-2} + \ldots + \frac{1}{1} \right)$$

which is a mathematical series approaching

$$\mu_V = n * (\ln(n) + \gamma) \qquad (4.16)$$

for $n \to \infty$ (Spiegel, 1968). γ is the constant of Euler, 0.5772. Eq. 4.16 estimates which vector in a sequence of vectors, added to n plants, infects the last healthy plant. The number can be used (with caution) to obtain a rough (and probably too low) estimate of the number of vectors when 100% infection occurs. In practice, the number of vectors causing a 100%-infection is sometimes estimated by applying the multiple infection transformation to n - 1/2 infected plants (or a similar number), *viz.*

$$\mu_V = n * \ln(1/(1 - (n - 1/2)/n))$$

$$= n * \ln(2n)$$

$$= n * (\ln(n) + \ln(2))$$

$$= n * (\ln(n) + 0.693) \qquad (4.17)$$

This estimate differs only slightly from the outcome of Eq. 4.16.

The expectation of V was also calculated with the program, providing a check on the exactness of the program calculations:

$$\mu_v = \sum_{v=n}^{50*n} v * p_{v-1}(n-1)/n \qquad (4.18)$$

in which $p_{v-1}(n-1)/n$ is the probability that the v-th vector infects the last healthy plant (since the first v - 1 vectors have to infect all but one plant and the v-th vector has to infect the remaining healthy plant. In this case the program was run for 60 n-values between 1 and 500 and for v-values from 1 through 50 times n.

4.3 Results

4.3.1 Comparison of probability distributions of k, as calculated with the program and approximated with the Poisson-distribution.

The program and the Poisson-approximation as defined by Eq. 4.13 give the same mean number of plants infected, but the Poisson-approximation gives a larger variance (Table 4.2). As an example, the probability func-

Table 4.2: Comparison of the mean (μ) and standard deviation (σ) of the number of plants infected (k) for a range of values of the number of plants (n) and vectors (v); (1) as calculated by the program (Eq. 4.5) and (2) as approximated with the Poisson-distribution (Eq. 4.13).

n	v	$\mu_{calculated}$	$\mu_{approximated}$	$\sigma_{calculated}$	$\sigma_{approximated}$
30	15	11.96	11.80	1.28	4.27
	30	19.15	18.96	1.71	3.32
	60	26.08	25.94	1.54	2.01
	150	29.81	29.80	0.42	0.45
	300	29.999	29.999	0.034	0.037
300	150	118.2	118.0	4.0	13.5
	300	189.8	189.6	5.4	10.5
	600	259.5	259.4	4.9	6.4
	1500	298.0	298.0	1.4	1.4
	3000	299.99	299.99	0.12	0.12
3000	1500	1181	1180	12.8	42.7
	3000	1897	1896	17.1	33.2
	6000	2594	2594	15.5	20.1
	15000	2980	2980	4.4	4.5
	30000	2999.8	2999.9	0.38	0.37

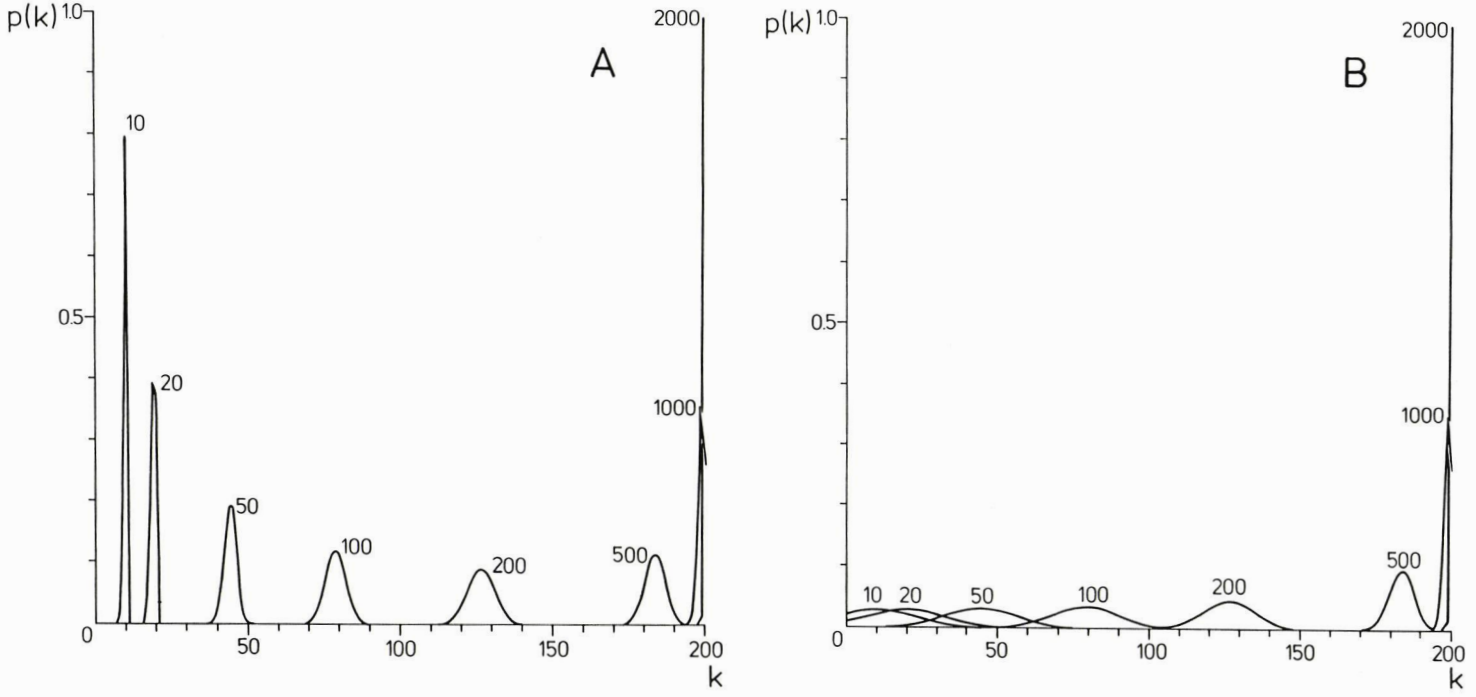


Fig. 4.1: Examples of probability distributions of the number of plants infected (k) when 10, 20, 50, 100, 200, 500, 1000 or 2000 vectors (v) are added to 200 plants (n); (A) as calculated with the program (Eq. 4.5) and (B) as approximated with the Poisson-distribution (Eq. 4.13).

tions (envelopes) of the number of plants infected out of 200 as calculated by the two methods (Eqs. 4.5 and 4.13) are shown in Fig. 4.1. The distributions are very similar for v = 1000 or 2000 when λ is small, 1.35 and 0.009 respectively. When 2000 vectors are added, both methods give the same values for p(200), the probability that all 200 plants are infected, and p(199), the probability that only one plant remains uninfected, *viz.* 0.99 and 0.01, respectively. The chance on 2 or more healthy plants is negligible. For v = 1000, the Poisson-approximation gives the values 0.26, 0.35, 0.24, 0.11, 0.04 and 0.01 for p(200) through p(195) respectively and the program calculated 0.26, 0.36, 0.24, 0.10, 0.03 and 0.01. For lower values of v, however, the approximated distributions become progressively more flattened than the true (calculated) distributions, in accordance with the increasing overestimation of σ as v decreases (Table 4.2). The Poisson-distributions for v = 10, 20 and 50, where λ has high values (190, 181 and 156 respectively), are much too flat and assign positive probabilities to numbers of infected plants exceeding the number of vectors added. The estimates of the variance in Table 4.2 indicate that the fraction of plants infected should exceed 99% (λ smaller than n/100) to obtain reliable results with the Poisson-approximation. Therefore this approximation is only used for to calculate the number of vectors needed to infect all plants (Fig. 4.3) and not for the calculation of confidence limits of v (Fig. 4.2).

4.3.2 Confidence intervals of the number of vectors

Confidence intervals of v, given k and n, are given in Fig. 4.2. For example, let k = 5 and n = 10, then a 95%-confidence interval of v is (4; 13) and a 90%-confidence interval (4; 12). For k = 8 and n = 10, the 95- and 90%-confidence intervals are (8; 34) and (8; 30) respectively. It appears that the width of the confidence intervals increases progressively if the fraction of plants infected approaches 100%. Thus v is inaccurately estimated if k/n is high. Therefore the infection percentage in bait plant tests should not reach high values.

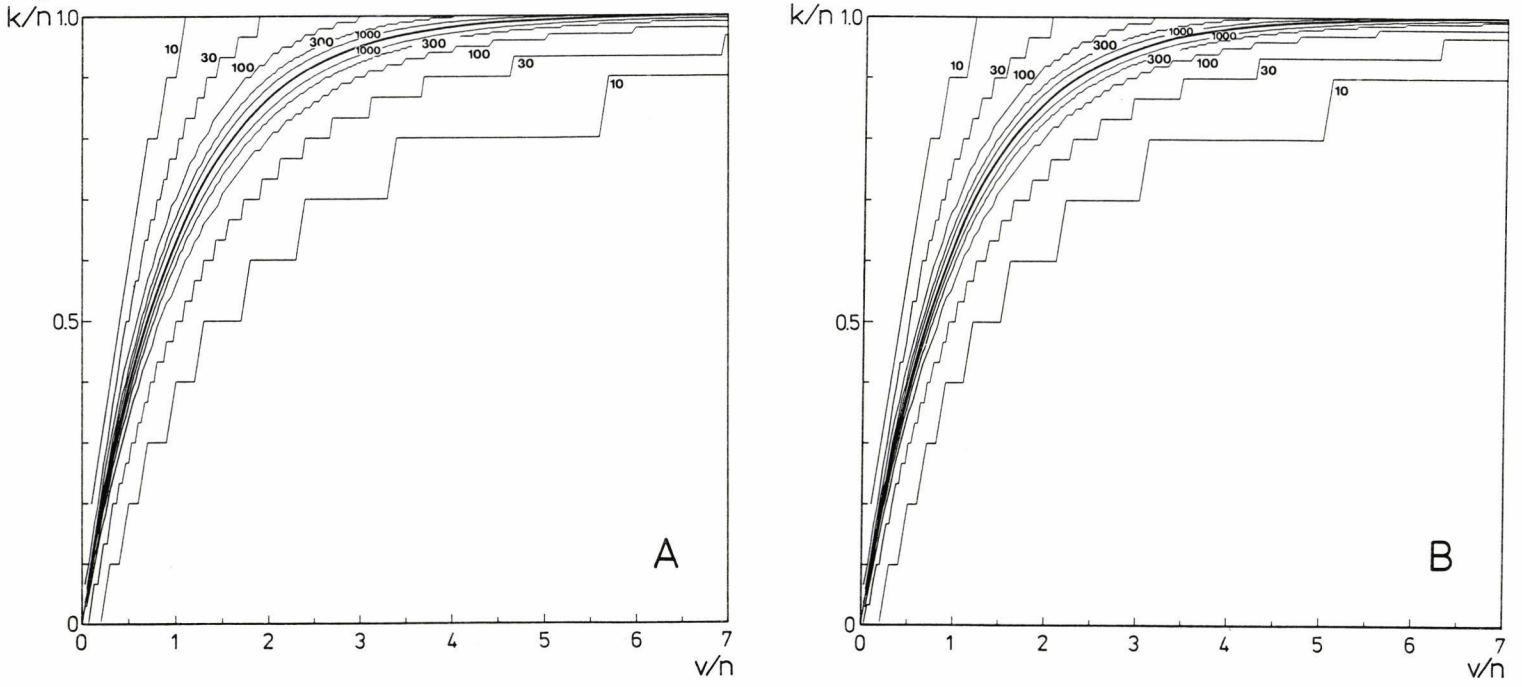


Fig. 4.2: Confidence regions (horizontal) of the number of vectors (v; as a multiple of the number of plants, n) as a function of the number of infected plants, (k; as a fraction of n) for n = 10, 30, 100, 300 and 1000. Critical regions of k are read vertically (see text).
A) α = 0.025 (one-sided) or 0.05 (two-sided)
B) α = 0.05 (one-sided) or 0.10 (two-sided)

4.3.3 Estimation of the number of vectors needed to infect all the plants

The number of vectors needed to infect all plants (as a multiple of the number of plants) increases logarithmically (not exponentially) with the number of plants (Fig. 4.3). The confidence limits calculated with the Poisson-approximation are in good agreement with those calculated with the program. This was expected because the figure deals with situations where λ is small and where the Poisson-approximation proved to be adequate (Fig. 4.1; Table 4.2). The mean number of vectors needed to infect all plants, calculated with the program (Eq. 4.18) is in good agreement with the number derived from the geometric distribution (Eq. 4.16), indicating correctness of the program.

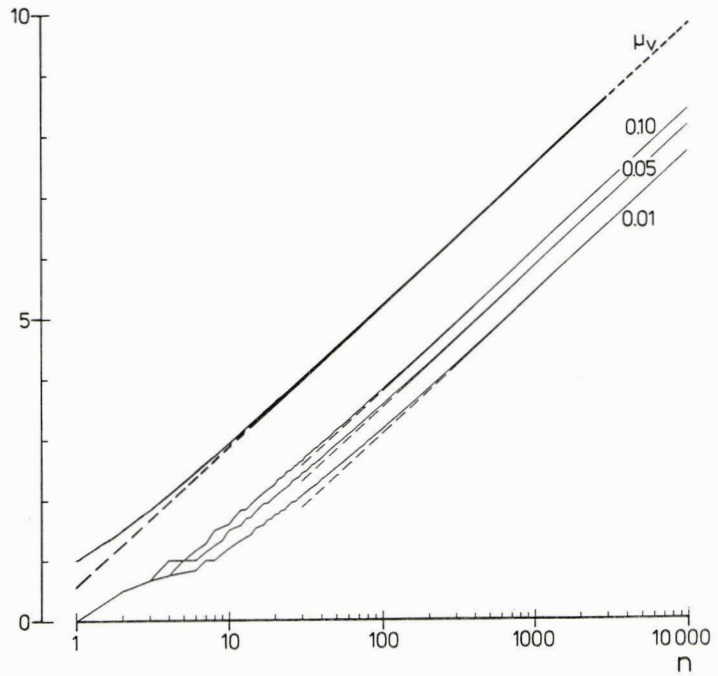


Fig. 4.3: Average number of vectors (bold lines) and lower confidence limits (thin lines) of the number of vectors, *at least* needed to infect all the plants. Drawn lines calculated with the program (Eqs. 4.5 and 4.18) and hatched lines derived from the Poisson distribution (Eq. 4.15) or from the geometric distribution (Eq. 4.16).

Fig. 4.3 gives lower confidence limits of the number of vectors when all plants are observed to be infected (Eq. 4.17). Fig. 4.3 also gives the average number of vectors *at least* needed to infect all the plants (Eq. 4.17). This number should be used with much caution because the true number may have been much higher.

4.4 Discussion

The exact calculation in this paper of probability distributions related to (multiple) infection of plants by vectors allows the construction of nomograms for the determination of confidence intervals for the number of vectors, given k plants are infected out of n. The Poisson-approximation cannot be used for this purpose because it over-estimates the variance of the number of plants infected if the number of vectors does not exceed the number of plants with a factor 5 or more. The program calculations as well as Poisson-approximations could be used to estimate lower confidence limits of the number of vectors needed to infect all plants. However, the true number of vectors which has caused a 100% infection cannot be assessed. For instance, if 100 plants are all observed to be infected, 500 as well as a million vectors may have caused this. Though the former number may seem biologically more relevant, the latter number is more plausible from a statistical point of view.

Both the program calculations and the Poisson-approximations are based on the assumptions that the vectors are distributed *at random* over the plants and that each of them makes exactly one infection. The results are also valid if a fraction of the vectors is viruliferous or if the efficiency of transmission is lower than 100%. In these cases, the calculations apply to infections in stead of vectors. The results are, however, no longer valid if the infections are not distributed *at random* over the plants, e.g. if vectors are attracted to or repelled from infected plants. The assumption of randomness is not likely to be satisfied if the source of inoculum is within the field or if there is secondary spread from infected plants, resulting in a clustered distribution of infected plants. Infections can also be clustered if vectors infect several neighbouring plants or if some parts of a field have a higher infection pressure than others, e.g. because of different growth of the plants or the presence of windbreaks. When the infections are clustered, the multiple infection transformation underestimates the number of infections needed to infect a given proportion of

the plants because the chance on multiple infections is increased. When vectors are repelled from already infected plants, the multiple infection transformation will over-estimate the number of infections. The requirement of randomness may be often fulfilled in bait plant test, designed to measure the infection pressure with viruses, carried into the crop by flying vectors.

The nomograms can be used in the design and analysis of these bait plant test. Fig. 4.2 shows that the accuracy of the estimation of v decreases progressively when the proportion of plants infected increases. Therefore tests should be designed such that the infection percentage remains moderate, e.g. not above 80. This can be achieved by keeping the plants for only short times in the field.

Fig. 4.2 also shows that the accuracy gained by increasing the number of plants from 30 to 100 is not greater than the gain by increasing it from 10 to 30, despite the great input of materials and labour. Increasing the number of plants above 100 probably does not justify the investment of plants and labour. The figure suggests that - if the infection pressure is high - more accurate results may be obtained with 3 batches of 30 plants each week than with only one batch of 100 plants.

When all plants become infected, only lower bounds can be given for the number of vectors. The impossibility to assess the true number of vectors is not a shortcoming of the transformation, as suggested by Madden *et al.* (1983), but stems from the inadequacy of the observation. The lower confidence bounds given by Eq. 4.15 specify the number of vectors at least needed to reach a certain small probability α of 100% infection. The true number may have been much higher however. In fact, an upper bound is $+\infty$. The same is true for μ_V, the estimate of the average number of vectors needed to infect all plants when the vectors are added sequentially. (Eq. 4.16). When μ_V is used as an estimate of v, a reasonable estimate of the number of vectors may be obtained when a complete infection of all plants occurs for the first time or when it occurs only occasionally. Otherwise, Eqs. 4.16 and 4.17, the multiple infection transform of n - 1/2 infected plants, are likely to under-estimate the number of vectors. Reliable estimates of the infection pressure can only be obtained with bait plant tests if the infection percentage remains moderate and if 100% infections are avoided.

5 SECONDARY SPREAD OF BEET YELLOWING VIRUSES. I. EFFECT OF PRIMARY INFECTION DATE

Abstract

In field experiments, the effect of the primary infection date on the secondary spread of beet yellowing viruses (BYV and BMYV) was studied. Extensive spread occurred only when the primary infection was made before the plants made leaf contact (mid-June). Limited spread occurred when the primary infection was made later or when the vector, *Myzus persicae*, could not establish populations due to predation by coccinellids.

Higher numbers of *Myzus persicae* developed in plots inoculated before mid-June. This was probably due to enhanced multiplication of *M. persicae* on the infected plants. Dispersal of apterous aphids and virus spread from infected plants to healthy neighbours started when plants in adjacent rows touched each other. Most dispersal occurred in the first 3 weeks of July when the number of aphids decreased. Dissemination of virus by the aphids dispersing in July was responsible for the observed rapid increase of the number of plants with symptoms in August.

5.1 Introduction

Yellowing viruses of sugarbeet are not transmitted with seed but introduced into the crop by immigrant aphid vectors (primary infection). Subsequently, the viruses are disseminated in the crop by resident aphids (secondary spread). Experience has shown that the number and earlyness of primary infections are important determinants of the amounts of spread and damage that will occur (Barel, 1975; Thresh, 1983). Therefore, in the Netherlands, the warning threshold for *M. persicae* is very low in May, one aphid per five plants, while in July, when the plants are older and the canopy is closed, five aphids per plant can be tolerated (Barel and Dudok van Heel, 1975; Heijbroek, 1984). Several studies have been made to determine the relation between infection date and yield loss (Chapter 7). However, no experiments have been reported on the relation between the date of primary infection and the extent of secondary spread that results. The aim of this study was to determine this relation.

2	5	(1)	6	3	1
6	-	(-)	-	6	-
3	1	(-)	5	2	5
(-)	-	(2)	3	-	4
1	(-)	(-)	1	4	-
5	6	(-)	-	6	3
6	(2)	(-)	2	5	-
3	(-)	(-)	-	2	1

N

Fig. 5.1: Outline of experimental field. Numbers 1 through 6 denote inoculations on 20 and 30 May, 10, 20 and 30 June and 10 July, respectively. Not-inoculated plots or those unsuccessfully-inoculated on 20 June are denoted with dashes. Plots marked with brackets are not included in the analysis of the experiment because they were damaged by the herbicide glyphosate, locally applied on 28 May to eradicate patches of coltsfoot (*Tussilago farfara*).

5.2 Materials and methods

5.2.1 Effect of primary infection date on spread of BYV

In sugarbeet, var. Regina, sown on 18 April 1986 on a sea-clay soil in the Flevopolder, 48 plots of 12 * 12 m^2 were laid down in 6 blocks (Fig. 5.1). The 6 plots in a block were each inoculated on a different date: 20 or 30 May, 10, 20 or 30 June, or 10 July. Three central plants per plot were inoculated with BYV by putting an aphid-proof clip-cage with 10 - 20 viruliferous *M. persicae* on each of 3 different leaves of each plant. Viruliferous *M. persicae* were reared on infected beet or prepared by feeding non-viruliferous aphids for 2 - 3 days on detached infected beet

leaves (Chapter 2). Viruses were maintained in beet and non-viruliferous *M. persicae* were reared on rape (Chapter 2). Two plots per block served as uninoculated control.

To establish vector populations in the field, 3 young apterous non-viruliferous adult *M. persicae* were clip-caged for three days onto each of the 3 central plants in all plots on 20 May when the crop was in the 2 - 3 leaf stage. These adults produced a total of 63 nymphs.

Aphids were counted every 10 days from the end of May through mid-August on the 3 central plants and on 6 other sample plants, less than 2.5 m from the central plants. They were classified as L1 - L3, apterous L4, alate L4 (recognized by their size and shape, red colour and wing pads), apterous adult or alate adult. All leaves on a sample plant were examined. The occurrence of predators, parasites, parasitic fungi or other aphid species was noted.

The plants that showed obvious systemic symptoms (Chapter 2 and 3) were marked with bamboo sticks weekly. The infection date of these plants was determined with the incubation period (Chapter 2). Additionally, true colour transparencies (6 * 6 cm^2; Kodak EPR 6017 on 21/8 and Kodak safety film E64 on the other dates) were made on 21 August, 19 September and 15 October from a microlight aircraft, an American Aerolights 'Eagle' (Clevers, 1986), flying at an altitude of 250 - 350 m. The area of BYV patches was determined by tracing their circumference on projections of the transparencies and processing these images with a numerical computer-program which adjusted for distortions caused by slightly oblique camera exposure angles.

A second experiment on the effect of the primary infection date on secondary spread of BYV was carried out in a sugarbeet field, var. Bingo, sown on 25 April 1986 on a river-clay soil in the Betuwe. The field was divided into 6 blocks each with 4 plots measuring 12 * 12 m^2. Each plot was inoculated with BYV on a different date: 9, 19 or 29 June or 9 July.

On each of the 3 central plants 5 young apterous adult *M. persicae* were clip-caged for 3 days, producing a total of 96 nymphs. Since few *M. persicae* were found in the next count as a result of predation by coccinellids, extra introductions were made on plants adjacent to the central plants on 29 June (5 adults/plot) and 9 July (7 adults/plot).

Weekly aphid counts were made on the central plants and on 6 other sample plants in June and July. Two counts were made in August.

5.2.2 Effect of primary infection date on spread of BMYV

In 1985, the effect of the primary infection date on spread of BMYV was studied in a sugarbeet field, var. Regina, sown on 23 April on a river-clay soil in the Betuwe. The field was divided into 6 blocks, each with 7 plots measuring 12 * 12 m^2. In each of six plots in a block one central plant was inoculated with BMYV, but only the inoculations on 23 and 30 May and on 20 June were successful.

From the date of inoculation through 28 June, every week (except on 31 May in the plots inoculated on 23 May), one young apterous adult *M. persicae* was clip-caged onto the inoculated central plant for 3 days to establish and maintain a vector population. The introductions of *M. persicae* were repeated because one introduction proved to be insufficient to establish a colony, due to predation by coccinellids. At each introduction, approximately 6 nymphs were produced. In June and July aphids were counted weekly on the central plant and 6 other sample plants per plot.

5.3 Results

5.3.1 Effect of primary infection date on spread of BYV

Virus spread. In the Flevopolder, most virus spread (as judged by the number of plants developing symptoms) occurred in the plots inoculated on

Table 5.1: Numbers of yellowed, BYV-infected plants counted on 7 August 1986.

	Inoculation date					
	20 May	30 May	10 June	20 June	30 June	10 July
mean	213	178	90	5	4	3
STD	17	41	20	3	2	1
SEM	8	20	9	2	1	1
n	5	4	5	2	5	6

mean = average number of plants with symptoms per plot
STD = standard deviation
SEM = standard error of mean
n = number of plots

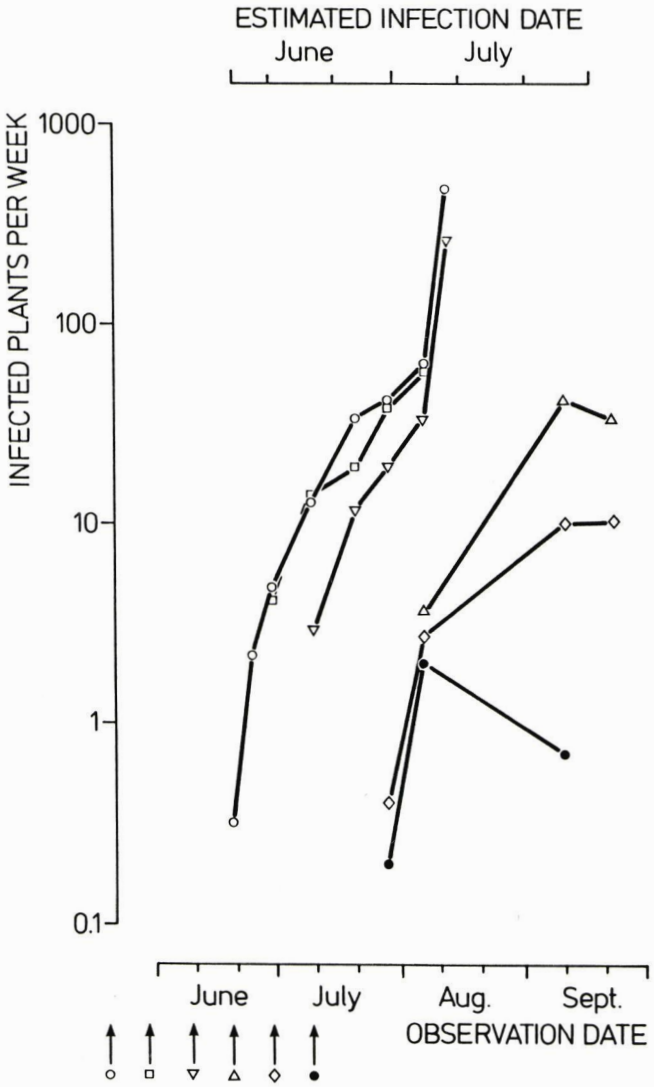


Fig. 5.2: Rate of secondary spread of BYV plotted against the date symptoms were observed (lower abscis) and against the estimated infection date (upper abscis; Chapter 2). Inoculation dates (indicated with arrows): 20 May (○), 30 May (□), 10 June (▽), 20 June (△), 30 June (◇) and 10 July (●). In the plots inoculated on 20 or 30 May or 10 June, counting and marking the numerous plants with symptoms became impracticable in August.

20 May, 30 May or 10 June (early-inoculated plots), i.e. before canopy closure. Considerably less spread occurred in the plots inoculated on later dates (late-inoculated plots; Table 5.1). These two groups of infection dates will be distinguished in the discussion of virus spread and aphid population development.

In the early-inoculated plots, the first yellowed, secondarily-infected plants were observed at the beginning of July (Fig. 5.2), whereas in the late-inoculated plots they were found at the beginning of August. These observations, taking into account the length of the incubation period (Chapter 2), show that secondary spread started between 10 and 20 June in the early-inoculated plots and around 5 July in the late-inoculated plots. From the second week of August onwards, high numbers of secondarily-infected plants were found each week in the early-inoculated plots. These plants were infected from the second week of July onwards. However, in the late-inoculated plots, the number of secondarily-infected plants with symptoms never attained high values. In sum, inoculations before approximately 15 June resulted in an initial dissemination of virus between 10 and 20 June and an extensive virus dissemination in the course of July. The inoculations made after 15 June gave rise to an initial dissemination around 5 July and a limited dissemination during the remainder of this month. The observations indicate that the crop passed a critical development stage around 15 June, after which primary virus infections did not result in early or extensive spread.

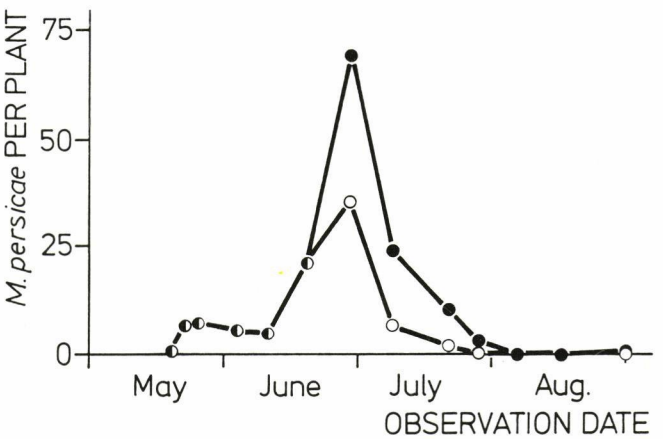


Fig. 5.3: Population development of *M. persicae* in early- (●) and in late- or not-inoculated plots (○).

Aphid population development and dispersal. Significantly more *M. persicae* developed in early-inoculated plots than in late- or not-inoculated ones (Fig. 5.3), despite the fact that the aphids were introduced in all plots on 20 May. The aphid numbers were similar in late- and not-inoculated plots. These observations show that the enhanced reproduction of *M. persicae* on BYV-infected plants as reported by Baker (1960) and Williams (1988) actually results in higher numbers of aphids on infected plant populations in the field.

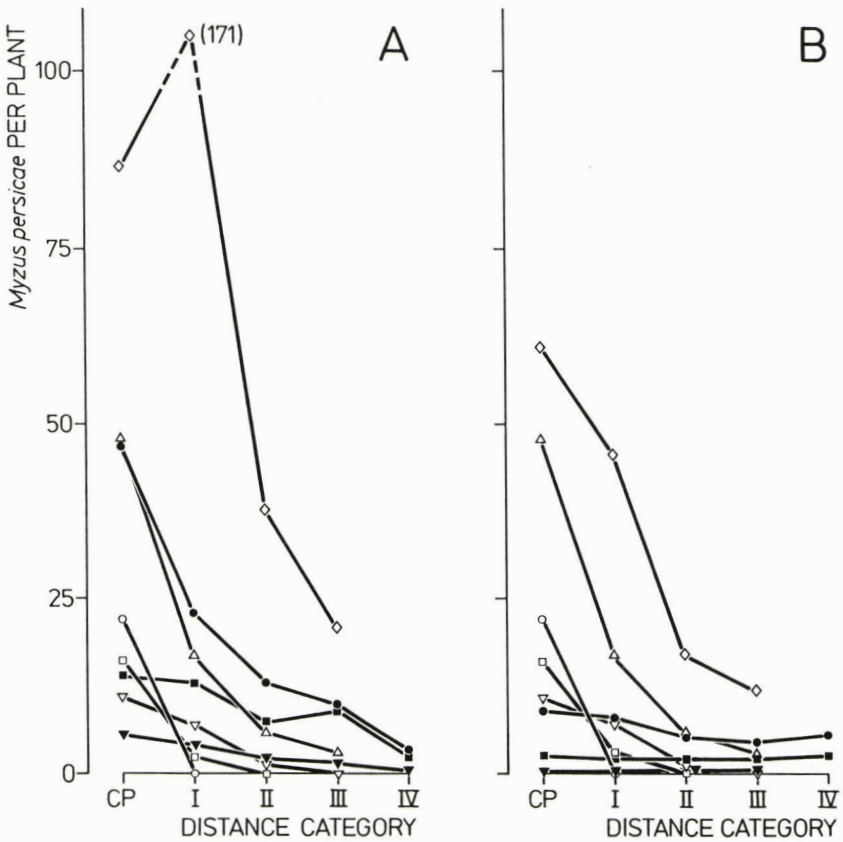


Fig. 5.4: Numbers of *M. persicae* on different sample plants in (A) early- and (B) late- or not-inoculated plots. Observation dates: 26 May (○), 4 June (□), 11 June (▽), 20 June (△), 30 June (◇), 10 July (●), 20 July (■) and 30 July (▼). Counts were made on the central plants (CP) on which aphids were released on 23 May and on sample plants (in the same row or in parallel rows) at distances of ± 50 cm (distance category I), ± 100 cm (II), ± 150 cm (III) or ± 200 cm (IV) from the central plants.

The differences in virus dissemination resulting from inoculations made before and after 15 June are explained by the course of aphid dispersal in relation to the development of the plants (Fig. 5.4): on 26 May, 3 days after release, aphids were found only on the central plants. On 4 June, aphids occurred also on 50% of the adjacent plants within the row, and on 11 June they were found on most immediate neighbours and also on some plants at a distance of 1 m. On 20 June, ± 5 days after leaves of plants in adjacent rows started to touch, aphids were found on virtually all plants examined within a radius of 2 m from the central plants. Apparently, dispersal of the aphids was strongly promoted by the formation of 'leaf bridges' between 10 and 20 June. The onset of dispersal determined by aphid counts coincides with the onset of secondary spread of BYV in the early-inoculated plots as determined in Fig. 5.2. In the plots inoculated on or after 20 June, the aphids dispersed also around 15 June but they did not disseminate virus because the plants were not yet inoculated.

Table 5.2: Approximate numbers of yellowed, BYV-infected plants per patch as assessed on 15 October 1986 by estimating patch diameters.

	Inoculation date					
	20 May	30 May	10 June	20 June	30 June	10 July
diameter (m)	19	17	13	6	3	1
number of plants	2600	2100	1200	250	60	10

Dispersal must have spanned distances larger than 2.5 m (the largest distance on which plants were sampled) as the patches in early-inoculated plots reached diameters of up to 20 m (Table 5.2). The rapid dispersal between 10 and 20 June demonstrates that wandering apterous *M. persicae* have considerable spreading capacities; few alatae were present at that time (Figs. 5.5 and 5.6). These results show that the virus was initially spread by apterae. Alatae may have played a role later on. The low number of alate adults on the sample plants (Fig. 5.6) indicates that alatae spread virus predominantly over larger distances. Spread by apterae, walking from plant to plant on the one hand and by alatae making short flights in the field (Harrewijn *et al.*, 1981) at the other hand can explain the two distinct types of virus spread that were observed: (1) spread over patches in which virtually all plants were infected and (2) 'diffuse'

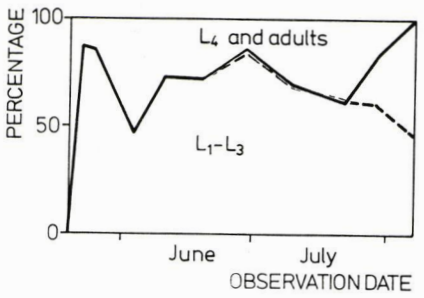


Fig. 5.5: Percentage L1 - L3 of the total *M. persicae* population. Drawn line: late- or not-inoculated plots. Hatched line: early-inoculated plots.

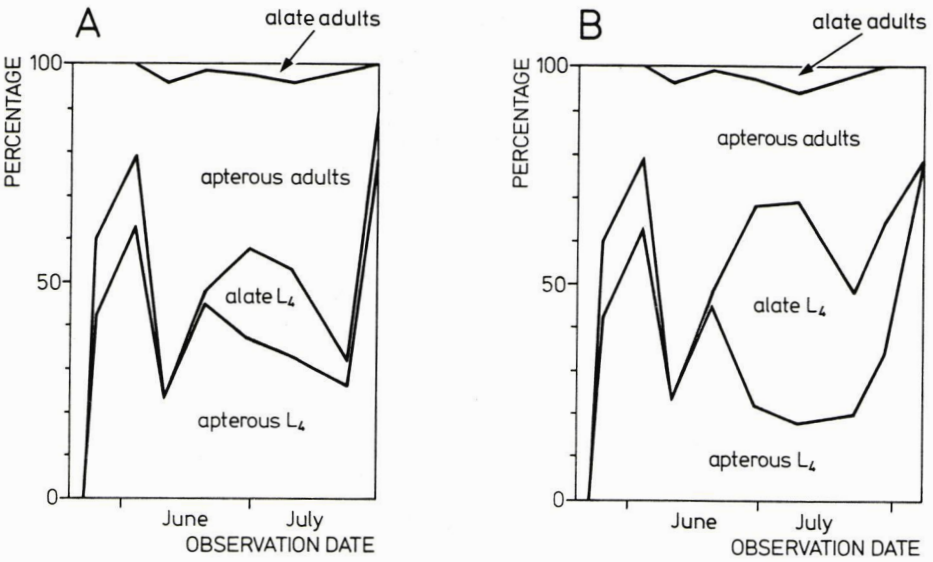


Fig. 5.6: Percentages (of total L4 + adults) of apterous and alate 4th instar and adult *M. persicae* in (A) early- and (B) late- or not-inoculated plots.

Fig. 5.7: Aerial photographs (reprints of true colour transparencies) showing field experiment on the effect of primary infection date on secondary spread of BYV. Patches in the plots inoculated on 20 or 30 May or 10 June are readily observed. See Fig. 5.1 for an outline of the experiment. Photographs were made on (A) 21 August, (B) 19 September and (C) 15 October. The photograph of 15 October shows 'diffuse' spread in the easterly part of the field, presumably as a result of an effect of the prevailing westerly wind direction on the movement of alatae.

spread; individual infected plants being scattered over the field. The last type of spread was observed in the second half of August and September whereas 'patchy' spread was observed already in July. Diffuse spread is observed on the aerial photograph of October but not on the earlier pictures (Fig. 5.7). This timing of diffuse spread is in accordance with the hypothesis that alatae were responsible.

Aerial photography. Infected plants with symptoms were counted and marked each week in all plots until 7 August (Table 5.1). From then on, counting and marking the numerous infected plants individually became impossible and virus spread was monitored by aerial photography. On the photographs the patches caused by the 3 early inoculations were readily observed (Fig. 5.7) and the number of yellowed plants could be inferred by multiplying the estimated area of the patch by the crop density of 9.1 plants/m^2 (Table 5.2). The number of yellowed plants, N_{phot}, determined in this way by aerial photography on 21 August was similar to the field counts, N_{cnt}, made on 7 August ($N_{phot} = 14 + 0.99 * N_{cnt}$; $r^2 = 0.75$). This indicates that yellowed plants infected in the beginning of July were identified approximately 2 weeks later on the photographs than in the field. The photographs made on 19 September and 15 October, however, showed much less expansion of the patches than was assessed in the field (Table 5.3). This discrepancy can be readily explained by the assumption that only early-infected plants which have numerous yellow leaves (e.g. Fig. 3.3A) were detected on the photographs. Plants which were infected later, and which showed symptoms on only a few leaves (e.g. Fig. 3.3B) were apparently missed.

Virus spread and aphid population development in the Betuwe. Little spread occurred in this experiment (Table 5.4) though one inoculation was made before the plants made leaf contact. The first secondarily-infected plants with symptoms were found at the beginning of August, which is late, and the greatest increase in their number occurred at the end of that month. Thus, spread occurred at the same (late) time as in the late-inoculated plots in the Flevopolder in which spread was also limited. Apparently a small amount of BYV spread is correlated with a late timing of spread.

The low level of spread in the Betuwe was also correlated with low numbers of *M. persicae*, probably as a result of predation by coccinellids (see below). The highest number, 3 to 4 *M. persicae* per plant on the sample plants, was found on 13 July. Slightly more aphids developed in

Table 5.3: Numbers of yellowed, BYV-infected plants as inferred from 3 successive aerial photographs.

Date of flight		Inoculation date					
		20 May	30 May	10 June	20 June	30 June	10 July
21 August	mean	241	170	104	5 - 10	-	-
	STD*	47	37	28	-	-	-
	SEM	21	18	12	-	-	-
	n	5	4	5	1	-	-
19 September	mean	349	301	189	± 10	5 - 10	-
	STD	72	78	75	-	-	-
	SEM	32	39	33	-	-	-
	n	5	4	5	1	2	-
15 October	mean	617	547	387	± 40	± 20	-
	STD	122	142	153	-	-	-
	SEM	54	71	68	-	-	-
	n	5	4	5	1	2	-

Table 5.4: Final number of yellowed, BYV-infected plants, counted on 6 October 1986 in the Betuwe.

	Inoculation date			
	9 June	19 June	29 June	9 July
mean	38	34	16	6
STD*	17	19	6	5
SEM	7	8	3	2
n	6	6	6	6

Table 5.5: Final number of BMYV-infected, yellowed plants, counted on 20 September 1985.

	Inoculation date		
	23 May	30 May	20 June
mean	59	52	15
STD*	24	26	2
SEM	10	11	2
n	6	6	2

*see Table 5.1

early-inoculated plots than in late-inoculated ones. The numbers declined in the second half of July when most spread occurred.

5.3.2 Effect of primary infection date on spread of BMYV

Results of the experiment on the effect of the primary infection date on spread of BMYV (Table 5.5) were similar to those obtained in the second experiment with BYV (Table 5.4). The first plants with symptoms were found at the end of July. These plants were infected at the end of June after adjacent plants made leaf contact. Most plants developed symptoms in August and these were infected in early July. Limited spread occurred because aphid numbers were low, up to 4 per plant in mid-July, as a result of predation by coccinellids.

5.3.3 Effect of coccinellids on *M. persicae* and virus spread

Observations in the field strongly suggest that the restricted population increase of *M. persicae* in the experiment with BMYV and the second experiment with BYV was mainly due to predation by Coccinellids. These predators were sometimes even found foraging inside the non-aphid-proof clip-cages with aphids. Initially, the incidence of coccinellids in the 3 experiments was similar: in May and early-June about 1 to 5% of the plants carried coccinellids. The most numerous species was *Coccinella septempunctata*, followed by *Adalia bipunctata* and *Propylea quatordecimpunctata*. The incidence of coccinellids increased in June and July as the summer generation was produced by the adults that had hibernated. The highest number of coccinellids developed in the Flevopolder in 1986, probably as a response to the high number of aphids present. Possibly coccinellids had less impact in the experiment with BYV in the Flevopolder than in the two others because *M. persicae* were introduced earlier and in higher numbers such that aphid colonies were established before coccinellids were active.

5.3.4 Role of *Aphis fabae* in virus spread

A. fabae occurred in all 3 experiments. Its incidence never exceeded 10% infested plants and it did not play any role of importance in virus spread as indicated by the absence of virus-infection in plants carrying large numbers of this aphid.

5.4 Discussion

The experiments described in this chapter indicate that secondary spread of yellowing viruses in sugarbeet is limited to a relatively short period of about 6 weeks between mid-June and the end of July. The development of yellow patches in the field spans a longer period, from early-July untill the end of September because the incubation period increases with the age of the plants (Chapter 2). Little spread occurs before mid-June because dispersal of apterae is hampered by the absence of leaf contact between the plants. Spread stops at the end of July because numbers of *M. persicae* decrease due to the adverse nutritional quality of older plants (Williams, 1988). Additionally, BYV has a longer latency period in older plants and the virus is probably less readily transmitted to older plants (Chapter 6). To provide a conceptual framework for the analysis and interpretation of beet yellows epidemics spread may be divided into three phases: (1) an introduction phase, (2) an establishment phase, and (3) a dispersal phase. These phases may overlap each other.

Ad 1. The introduction phase starts when seedlings emerge and ends when the plants make leaf contact. During this phase, vectors and viruses enter the crop and start their multiplication.

Ad 2. When the plants make leaf contact (LAI 0.5 à 1), dispersal of the aphids and dissemination of viruses becomes easier. Little dispersal occurs, however, because the plants are suitable hosts for the aphid. Because aphids (Williams, 1988) and viruses (short latency period; see Chapter 6) multiply quickly on young plants, a reservoir of infectious plants and aphids is built up during this period. *M. persicae* and beet yellowing viruses have a mutualistic relationship (both partners benefitting from the other) because the aphid spreads the virus and the virus increases the suitability of infected plants to the aphid. Therefore, a slightly earlier introduction of virus and vectors may strongly increase the size of the reservoir.

Ad 3. The dispersal phase begins when the leaf canopy closes completely (LAI > 3). Decreasing host suitability of the plants is probably a major factor causing dispersal during this phase. Dispersal may also be promoted by the increasing incidence of natural enemies (Frazer, 1977; Roitberg *et al.*, 1979) and/or by crowding of the aphids (Dixon, 1985). Extensive dissemination of viruses may occur when an important reservoir of infectious plants and aphids has been built up during the establishment

phase. This happened in the early-inoculated plots in the experiment in the Flevopolder.

Early primary infection and vector infestation were not the only prerequisites for extensive spread in the present experiments. In 2 experiments, early primary infections caused only limited spread because vectors were low in number due to predation by coccinellids. Hence, a low number or late invasion of predators seems to be a second prerequisite for extensive secondary spread. Our observations are in agreement with those of Heathcote (1963, 1978b) who found that foraging coccinellids could prevent immigrant aphids from establishing colonies. Ribbands (1963) also observed that coccinellids reduced the number of *M. persicae* in virus-infected sugarbeet. Wratten and Pearson (1982) and Putman and Wratten (1984; p. 257) reported that predator exclusion from sugarbeet plots increased numbers of aphids as well as the incidence of beet western yellows virus. The possibility of natural control of vectors and virus spread by natural enemies deserves further study.

Ajayi and Dewar (1983) suggested that attraction of immigrant alate cereal aphids to yellowish or reddish cereal plants infected with barley yellow dwarf virus (BYDV; luteovirus-group) was the main cause of the increased aphid numbers in virus-infected plots, while increased population growth on virus-infected plants was considered less important. Though *M. persicae* is attracted by yellow surfaces (Moericke, 1955, 1957), it is unlikely that colour attraction of alatae has played a role of any importance in the present experiments as few plants showed symptoms in June (before the peak in aphid numbers was reached) and only few *M. persicae* were flying at that time (Chapter 6). Therefore, enhanced multiplication of the aphids on BYV and/or BMYV-infected plants as described by Baker (1960) and Williams (1988) is the probable cause of the higher numbers of aphids observed in early-inoculated plots.

The negligible importance of *A. fabae* as a virus vector in our experiments confirms earlier reports. For instance, Watson *et al.* (1951) found for a range of years no correlation between the numbers of *A. fabae* and the severity of virus yellows. Björling (1952) observed only little secondary spread of BYV in plots infested with *A. fabae*, but rapid spread in plots infested with *M. persicae*. Similar results were obtained by Peters c.s. (unpubl. res.). *A. fabae* may play an indirect role in virus spread by attracting predators that also attack *M. persicae*.

The severe beet yellows outbreak in Europe in 1974 was initiated by early and numerous primary infections (Barel, 1975; Heijbroek, 1984) while secondary spread started earlier than normal (Thresh, 1983). Because yellowing viruses have long incubation periods (Chapter 2), spray warnings based on the observation of symptoms caused by widespread early primary infections (before mid-June) can only be issued in early July when aphid dispersal in the crop has already started. Spraying at that time is too late to prevent secondary spread. Forecasts based on regressions of the incidence of yellowing viruses in August on the number of winter frost days and April mean temperatures (Watson *et al.*, 1975) can predict the necessity of control measures more timely. These regressions indicate the survival of virus and vectors through the winter and the earliness of aphid flights and primary infections. It would be desirable, however, to determine the earliness of aphid flights and primary infections and the proportion of aphids that carries virus directly. It is possible to monitore the number of vectors. It is, however, still difficult to screen vectors for virus serologically (Roseboom and Peters, 1984; Govier, 1985). Sensitive tests would be required to detect low proportions of viruliferous aphids. This may be necessary because, as shown in this chapter, one viruliferous vector that infects a beet plant early in the season and founds a vector population on it, may lay the basis for the secondary infection of approximately 1000 plants. Therefore, a few viruliferous aphids may in theory suffice to initiate the infection of a whole crop.

6 SECONDARY SPREAD OF BEET YELLOWING VIRUSES. II. EFFECT OF SOWING DATE

Abstract

The influence of sowing date, plant arrangement and introduced numbers of *Myzus persicae* on secondary spread of BYV and BMYV in sugarbeet was studied in field experiments. Considerably more plants became infected in May-sown plots than in those sown in April. Three factors could explain the increased secondary spread in beet infected in an early development stage:

(1) Higher numbers of *M. persicae* developed on young plants. Higher numbers developed also on plants infected with one of the two viruses.

(2) Virus transmission to older beet plants was impaired, probably because they were not readily accepted as a host plant. The susceptibility of the plants to BYV was equal throughout the season if viruliferous aphids were clip-caged upon them.

(3) The latency period of BYV increased from approximately 5 days in young plants to more than 10 days in old plants. At any time in the season, the latency period was shorter in later-sown, younger plants.

Plant arrangement had no effect on secondary spread of BMYV. Spread of BYV increased only slightly when more *M. persicae* were released. Few immigrant alate *M. persicae*/m^2 were recorded and virus spread was not related to their number.

6.1 Introduction

It is well-known that late-sown sugarbeet crops (e.g. in May) become generally more severely infested with yellowing viruses than early-sown crops (e.g. Watson *et al.*, 1946; Hansen, 1950; Heathcote, 1970, 1972). Widely-spaced or gappy crops are also reported to become more heavily infested (Heathcote, 1969, 1970; Johnstone *et al.*, 1982). In these studies natural primary infections played an important role. Therefore it was impossible to distinguish between effects of sowing date on the number of primary infections and effects on secondary spread. It is widely accepted, however, that attraction of alate aphids to crops showing a 'chequer board' of plants and soil, plays an important role as it would increase the number

of primary infections and promote the colonization of the crop by vectors (A'Brook, 1964, 1968; Heathcote, 1969, 1970, 1972; Johnstone *et al.*, 1982; Thresh, 1983; Jones, 1987).

The idea of aphid attraction to plants surrounded by bare soil is based on work in a flight-chamber (Kennedy *et al.*, 1961) which demonstrated that *Aphis fabae* alights more readily on white papers exposed against a black background and on black papers against a white background than on the same papers exposed against a background with the same colour. These authors concluded that the aphids show, apart from their sensitivity to yellow, a response to objects contrasting to their environment. In the field, more aphids are indeed attracted to yellow water traps standing over bare soil (high contrast) than to traps standing in a crop (Moericke, 1957; A'Brook, 1968). Similar observations were made with whole plants; cabbage aphids, *Brevicoryne brassicae*, released in a large cage in the field, landed more often on Brussels sprouts plants surrounded by bare soil than on plants surrounded by grass turfs (Smith, 1976). None of these analyses shows, however, that more aphids land per unit area in crops which show bare soil between the plants than in crops which cover the soil completely. Halbert and Irwin (1981) even caught, exceptionally, more aphids on green sticky traps above closed soybean canopies than above open ones. We conclude therefore, that other factors may be assumed to be (also) responsible for the higher virus incidence in late-sown and widely-spaced crops, for instance a higher rate of secondary spread.

A few studies of secondary spread of yellowing viruses in sugarbeet have been made in the past (Björling, 1952; Watson and Healy, 1953; Ribbands, 1963; Kershaw, 1965). None of these studies analysed the effects of sowing date or plant arrangement. In this study, secondary spread of BYV was monitored in sugarbeet sown at different dates and infested with different numbers of *M. persicae*. Secondary spread of BMYV was monitored in sugarbeet sown at different dates and in different arrangements and densities. The incubation period was determined in the individual fields to calculate the infection date of infected plants on basis of the time symptoms appeared. Observations were made of the population development of *M. persicae* and the number of immigrant alatae to explain differences in virus spread. The susceptibility of beet plants for BYV and the latency period (time between infection and availability of virus for

acquisition; Van der Plank, 1963) of this virus were determined to explain the different rates of secondary spread in April- and May-sown sugarbeet.

6.2 Materials and methods

6.2.1 Effect of sowing date and numbers of *M. persicae* on spread of BYV

In a sugarbeet field, var. Regina, sown on 18 April 1986 on a sea-clay soil in the Flevopolder, 24 plots of 12 * 24 m^2 were laid down in 4 blocks. In each block, 3 plots were sown on 18 April (regular) and 3 on 20 May (late). Each plot was divided into an 'experimental' area of 12 * 12 m^2, in the centre of which plants were inoculated and aphids released, and a 'control' area in which secondary spread from the experimental area and natural primary infections were assessed. In the centre of each experimental area, 3 plants were inoculated with BYV on 23 June when the April-sown plants had 12 leaves (± canopy closure) and the May-sown ones 4. To establish vector populations of different sizes, varying numbers of young non-viruliferous apterous adults of *M. persicae* were clip-caged on the inoculated plants from 25 to 28 June: 69 in each of 4 April- and 4 May-sown plots, 9 in 8 other plots and 2 in the remaining 8 plots. These adults produced about 400, 70 and 15 nymphs, respectively. Ten nymphs were kept alive in plots which were infested with 2 adults.

To follow aphid population development and dispersal, weekly counts were made on the 3 inoculated plants and 6 other sample plants at a distance of maximally $1\frac{1}{2}$ m from the inoculated plants. Aphids were classified as L1 - L3, apterous L4, alate L4, apterous adult or alate adult.

The plants that showed obvious systemic symptoms were marked with bamboo sticks weekly from the beginning of July until the end of September. Additionally, aerial photographs (true colour transparencies) were made on 21 August, 19 September and 15 October as described before (Chapter 5). To assess the size of the patches, projections of these transparencies were traced on large sheets of paper and redrawn on millimeter-paper, adjusting for slightly oblique camera exposure angles.

Immigration of alate aphids was monitored with green water trays (Fig. 6.1), measuring 30 * 45 cm^2, filled with water containing detergent.

In a separate field segment, plants were inoculated to determine the incubation period.

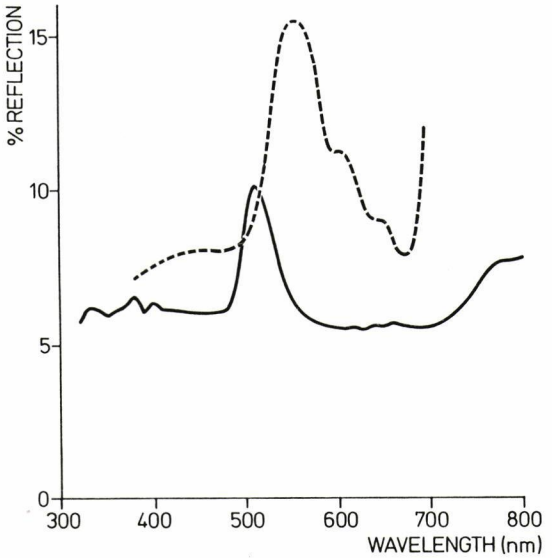


Fig. 6.1: Reflection spectrum of green water trap (drawn line) compared to spectrum of a beet leaf (hatched line).

6.2.2 Effect of sowing date and plant arrangement on spread of BMYV

To test a possible effect of plant arrangement on secondary spread of BMYV, 6 different planting patterns of sugarbeet var. Regina were established on 23 April 1985 on a river-clay soil, using a hand driven sowing machine: (1) 50 * 50 cm^2; (2) 25 * 100 cm^2; (3) 38 * 33 cm^2; (4) 38 * 67 cm^2; (5) 25 * 25 cm^2 and (6) 25 * 50 cm^2, corresponding to densities of 4, 4, 8, 4, 16 and 8 plants/m^2, respectively. The experiment consisted of 6 blocks, each with 12 plots of 9 * 10 m^2, 6 of which were inoculated. The others served as control. A damaging herbicide application necessitated resowing two third of the crop on 24 May. Available seed of the variety Monohil was used.

BMYV was introduced into 36 'treated' plots on 4 July, by transplanting one infected, 4-leaved beet plant from the glasshouse to the centre of each plot. At that date, the plants sown on 23 April had 9 leaves and those sown on 24 May had 5. To establish a vector population, 3 non-viruliferous *M. persicae*, were introduced on the inoculated plant in the treated plots or on a healthy plant in the control plots. Aphids were counted weekly as

described before. Plants showing BMYV symptoms were counted and marked with sticks weekly from the end of July to the beginning of October.

6.2.3 Effect of plant age on susceptibility to BYV and latency period of BYV

Beds of sugarbeet, var. Bingo, were sown on 25 April and 26 May 1986 on a river-clay soil in Wageningen. Inoculations with BYV were made on 2 and 16 June, 2 and 21 July and 5 August. Two stocks of *M. persicae* were used, one reared on rape, acquiring BYV in a 3 days feeding period on detached, BYV-infected leaves from beet plants grown in a climate chamber, and the other grown on infected beet in the glasshouse. Inoculations were made with both batches on fully-expanded or expanding leaves of April- and May-sown beet plants, by clip-caging the aphids for 2 days on the plants. The proportion of plants infected out of 30 inoculated ones was judged by the development of symptoms. A few plants which were apparently naturally-infected (criteria in Chapter 3), were discarded.

In the same field, the plants sown on 25 April or 26 May were inoculated on 16 June, 2 and 18 July, 4 and 19 August and 3 September to investigate the effects of plant age and date of infection on the latency period. To infect the plants, 10 viruliferous *M. persicae*, reared on infected beet in the greenhouse, were caged for 3 days on an expanding leaf, using non-aphid-proof clip-cages. At intervals of 3 to 4 days after inoculation, young heart leaves (± 3 cm) were taken from 20 inoculated plants and placed 2 days into vials with 5 adult, non-viruliferous *M. persicae*. The leaflet with aphids was transferred to a *Chenopodium foliosum* test plant (Björling, 1958). The time between clip-caging infectious aphids on the plant and the moment at which 50% of the young leaves contained virus, acquirable by aphids, was taken as the latency period.

6.3 Results

6.3.1 Effect of sowing date and numbers of *M. persicae* on spread of BYV

Virus spread. About 500 plants became infected with BYV in May-sown plots and 150 in those sown in April, demonstrating a large impact of sowing date on the extent of secondary virus spread (Fig. 6.2). The number of *M. persicae* introduced had some effect on spread (Fig. 6.2)

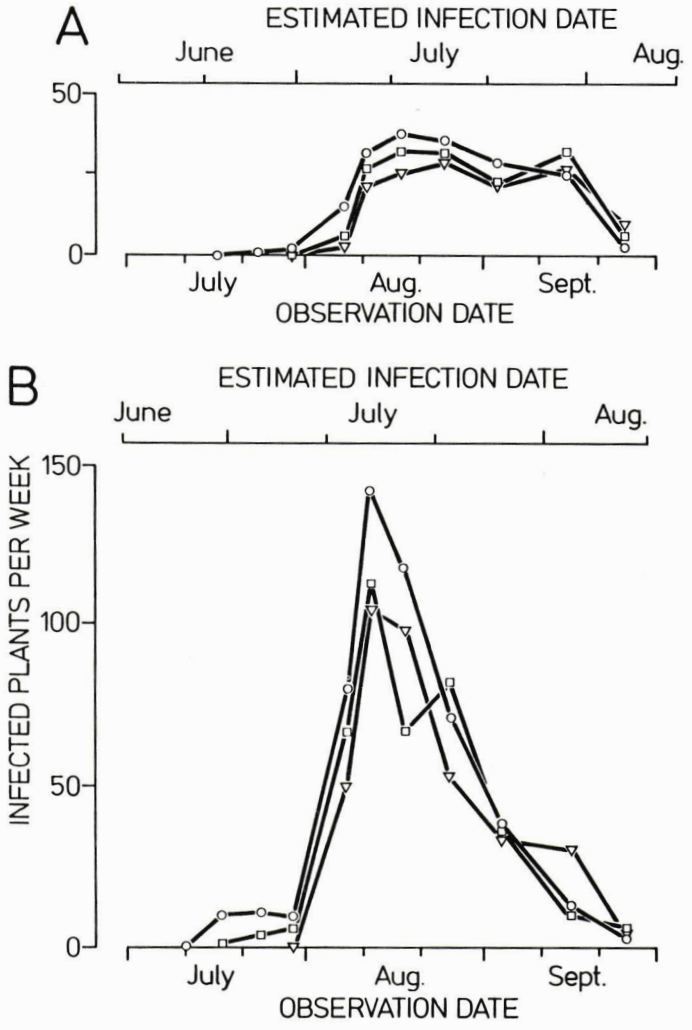


Fig. 6.2: Rate of secondary spread of BYV plotted against the date symptoms were observed (lower abscissa) and against the estimated infection date (upper abscissa). Plants were sown on 18 April (A) or 20 May (B). On 25 June, 69 (○), 9 (□) or 2 (▽) adult apterous *Myzus persicae* were released on the inoculated plants.

but spread increased less than proportionally with the number of aphids introduced or the number of aphids establishing in the field (Fig. 6.5; see below). More virus spread occurred in the eastern part of the field than in the western part (Fig. 6.3). In the two eastern blocks virus invaded the control area of most of the May-sown plots, but it did not invade adjacent April-sown ones (Fig. 6.3). This indicates that the plants in the

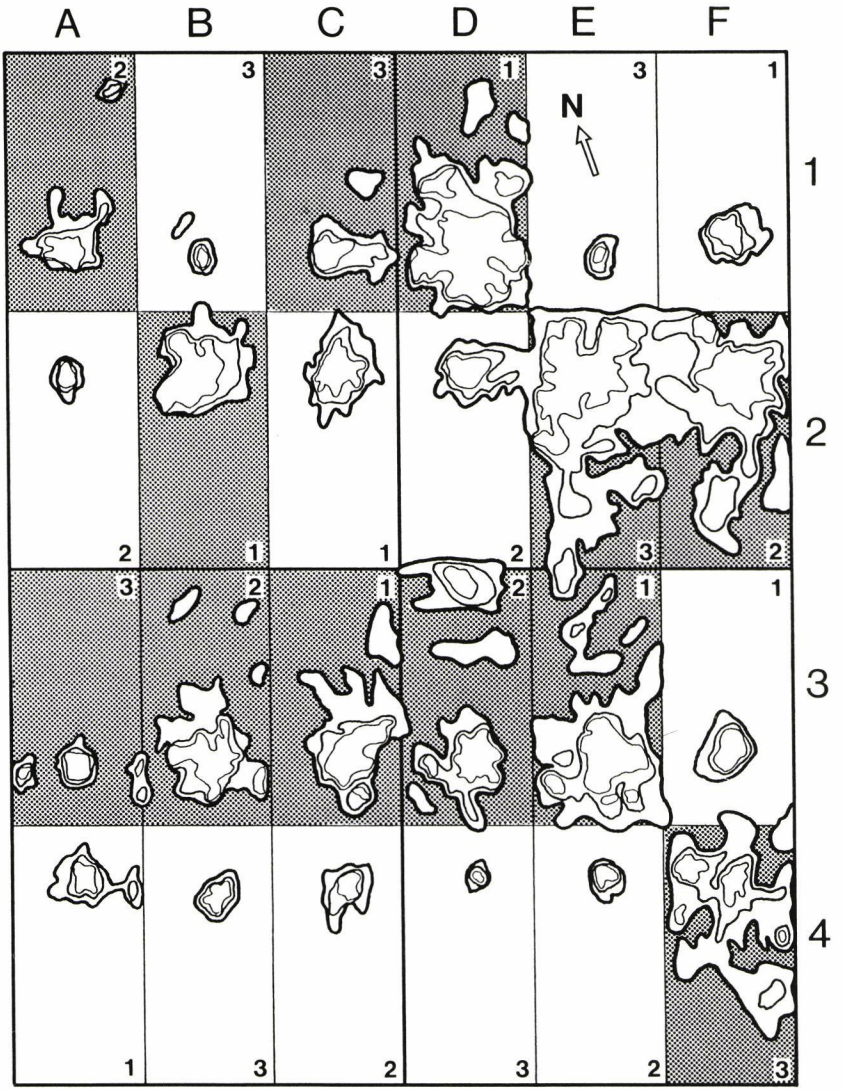


Fig. 6.3: Outline of the experimental field consisting of 4 blocks, each with 3 plots sown in April and 3 plots sown in May (shaded). On 25 June, 69 (plot marked with 1), 9 (2) or 2 (3) *M. persicae* were introduced. Contours of BYV-patches were drawn from projections of aerial photographs made on 21 August (———), 19 September (———) and 15 October (———). More spread of BYV occurred in late-sown plots. In several of these late-sown plots (e.g. D1, E2 and F2) the entire experimental area and a large part of the control area became infected. However, hardly any virus was spread from these plots to adjacent early-sown plots (e.g. E1 and F1). Transmission of virus to older, healthy (April-sown) plants was probably hampered by a preference of the viruliferous *M. persicae* for the younger, infected plants from which they originated.

April-sown plots were either less susceptible to virus infection or were less frequently fed upon by the vector. The latter explanation is favoured because the first one was refuted in the susceptibility test (see below).

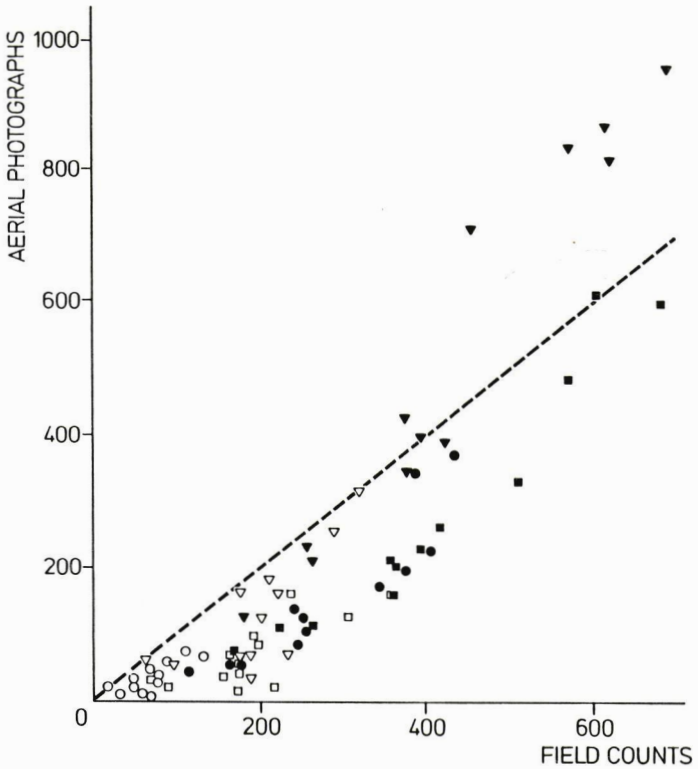


Fig. 6.4: Relation between numbers of infected plants inferred from aerial photographs (ordinate) and numbers counted in the field (abscissa) on 21 August (○), 19 September (□) and 15 October (▽). Open symbols denote observation in April-sown plots, closed symbols in May-sown ones. Hatched line: y = x.

Aerial photography. Numbers of infected plants inferred from aerial photographs were generally lower than field counts (Fig. 6.4). It was concluded in Chapter 5 that plants infected for only a short time were not detected on the photographs because of the small number of yellowed leaves. However, 'aerial' estimates of the number of yellowed plants on 15 October exceeded the field counts. Possibly, this over-estimation was caused by regarding all plants within yellow areas of May-sown plots as infected, whereas, in fact, some plants in these patches showed still no symptoms in October. The resolution of the photographs was insufficient to distinguish between individual healthy and yellowed plants.

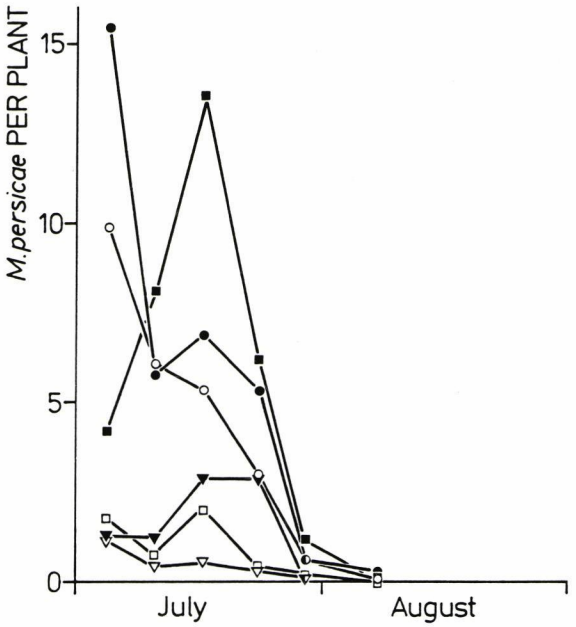


Fig. 6.5: Average numbers of *Myzus persicae* on 9 sample plants in April-sown plots (open symbols) and May-sown plots (closed symbols) in which 65 (○), 9 (□) or 2 (▽) *M. persicae* were introduced on 25 June.

Aphid population dynamics. The higher rate of spread of BYV in May-sown plots was correlated with higher numbers of *M. persicae* developing on the plants (Fig. 6.5). These higher aphid numbers probably resulted from: (1) more rapid reproduction of *M. persicae* on young plants, and (2) enhanced reproduction of aphids on BYV-infected plants (Baker, 1960; Williams, 1988).

Though the numbers of *M. persicae* introduced in different treatments on 25 June differred with a factor 30, the numbers of aphids establishing on the plants differred with a factor 13 in the April-sown plots and a factor 4 in the May-sown plots (Table 6.1). This attenuation of differences may have been caused by density dependent mortality factors, e.g. the observed predation by coccinellids. The different 'vector pressures', calculated as aphid-days (Table 6.1), that resulted from the different numbers of *M. persicae* introduced, had little effect on virus spread (Table 6.1). Sowing date was more important.

Table 6.1: Vector pressure (aphid days) and spread of BYV as affected by sowing date and introduced number of *M. persicae*.

Sowing date	*M. persicae* introduced	vector pressure (aphid days)*		infected plants end September**	
18 April	65	3948	a***	214	a
	9	806	b	188	a
	2	336	c	174	a
20 May	65	6231	a	472	b
	9	4438	a	384	b
	2	1593	b	373	b

* aphid-days calculated for 39 plants within 1½ m from the inoculated plants; data log-transformed for analysis

** data square root-transformed for analysis

*** numbers followed by the same letter are not significantly different (p = 5 %).

Hardly any alate adults were found: a total of only 10 in all counts, though the presence of numerous alatiform L4's indicates that higher numbers of alatae developed in the experiment (Fig. 6.6). Apparently, (1) alatae dispersed outside the sample area soon after their adult moult and (2) immigration of alatae was negligable. In the second half of July, more alate L4's developed in the May-sown plots than in those sown in April. This may be explained by (1) the higher number of aphids in May-sown plots (crowding) and/or (2) an effect of infection of the plant with BYV on wing formation. Gildow (1980) reported an effect of BYDV infection in oats on wing development in cereal aphids.

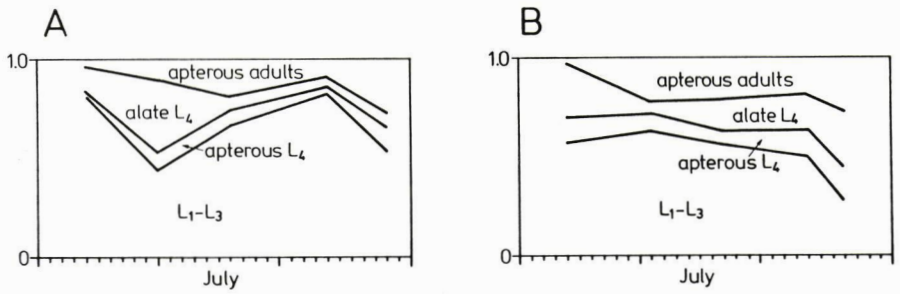


Fig. 6.6: Composition of the *Myzus persicae* population in early- (A) and May-sown plots (B).

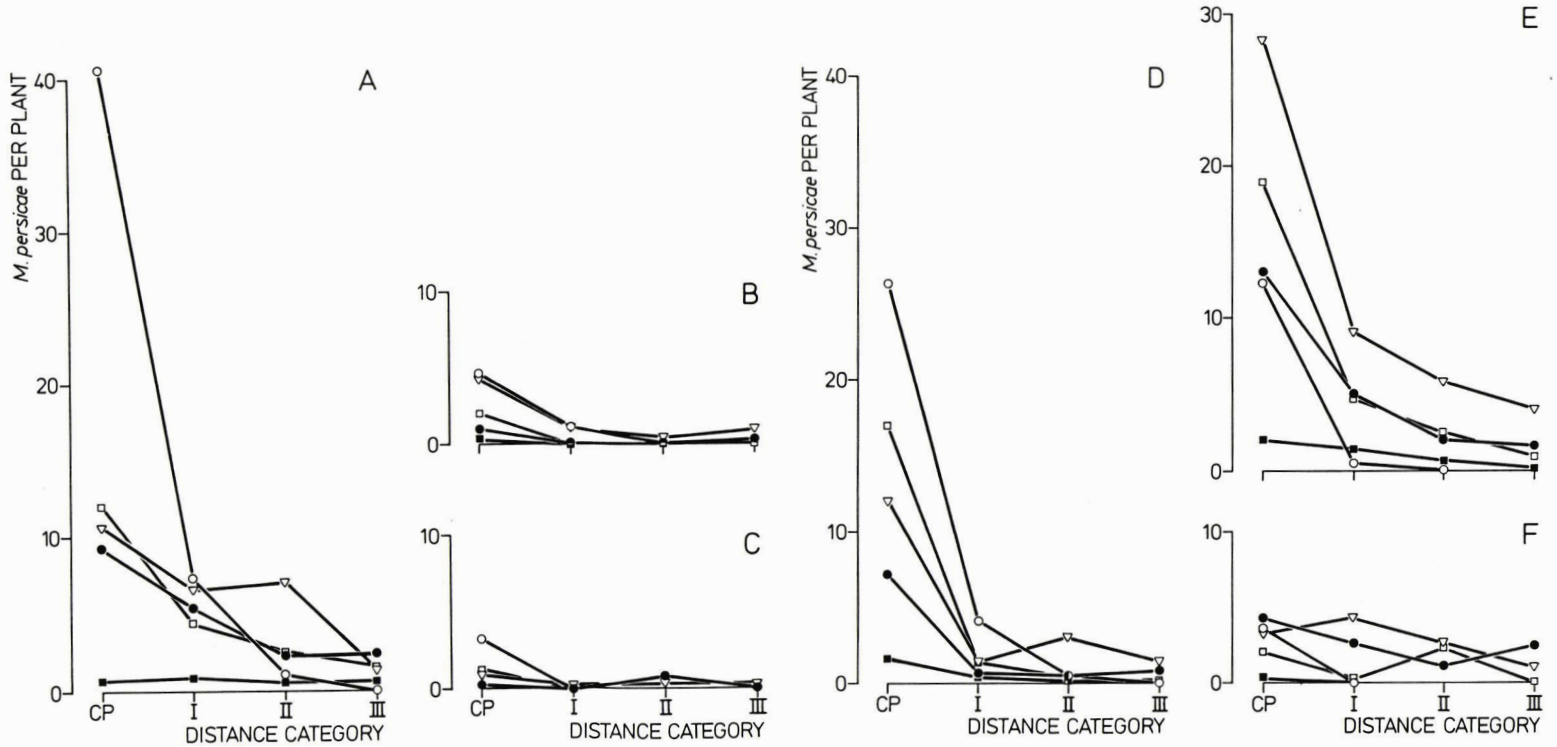


Fig. 6.7: Numbers of *Myzus persicae* on central inoculated plants (CP) and on sample plants at different distances from the inoculated plants. I = adjacent plant in same row and adjacent plant in parallel row. II = second adjacent plant in same row and plant in second adjacent parallel row. III = third adjacent plant in same row and plant in third adjacent parallel row. Plants were sown on 18 April and infested with 65 (A), 9 (B) or 2 (C) *Myzus persicae* on 25 June or sown on 20 May and infested with 65 (D), 9 (E) or 2 (F) *M. persicae*. Observation dates: 5 (○), 11 (□), 17 (▽), 24 (●) and 30 July (■).

Aphid dispersal. In the April-sown plots, few aphids were involved in dispersal and virus spread, as shown by the low number of aphids on some distance from the inoculated plants throughout the counting period (Fig. 6.7 A, B and C). In the May-sown plots, aphids were absent from sample plants at 1 - 1½ m distance from the inoculated plants on 5 July but some were counted on 11 July (Fig. 6.7D, E and F), indicating that dispersal started after the plants made leaf contact around 10 July. Around this time, the rate of virus spread increased greatly (Fig. 6.2). The numbers of *M. persicae* in the 4 blocks did not differ greatly and do not provide an explanation for the observed differences in virus spread between the blocks (Fig. 6.3).

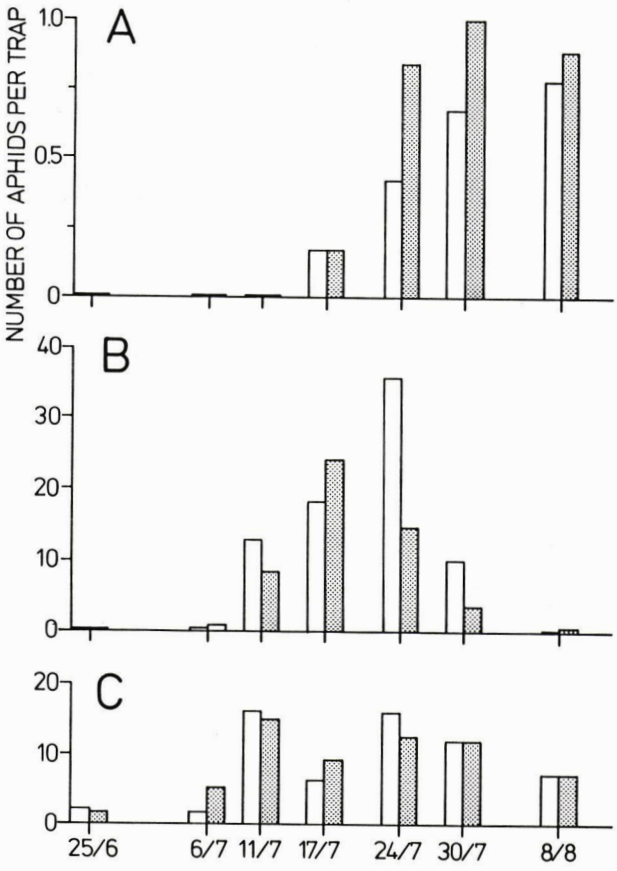


Fig. 6.8: Catches of *Myzus persicae* (A), *Aphis fabae* (B) and other aphid species (C) in green water traps placed in April-sown (white bars) or May-sown plots (Shaded bars).

Aphid trap catches. Slightly more winged *M. persicae* were caught in the water traps placed in the May-sown plots (Fig. 6.8) than in those placed in the April-sown plots, but this difference was not significant. The numbers caught were very low and the first were caught mid-July when alate adults were produced in the experimental plots (Fig. 6.6). Probably the alatae caught originated from within the field.

More alate *Aphis fabae* were captured in April-sown plots than in those sown in May, most probably because they were more numerous in these plots. Catches of other aphid species were not affected by sowing date.

6.3.2 Effect of sowing date and plant arrangement on spread of BMYV

In another experiment, the effect of sowing date and plant arrangement on spread of BMYV, was studied. Most virus spread occurred in May-sown plots (Fig. 6.9), confirming the results obtained with BYV. Plant arrangement had no effect on either virus spread or the numbers of aphids. Little virus spread occurred in the inoculated plots as a result of the late moment of inoculation (Chapter 5) and the low numbers of *M. persicae*.

Numbers of *M. persicae* were low, though aphid reproduction was apparently enhanced on later-sown or BMYV-infected plants (Fig. 6.10), a result which is consistent with those obtained in other experiments with BYV and BMYV (this Chapter; Chapter 5). The transplanted inoculated plants grew poorly and carried few aphids.

Few alatae were counted on the sample plants; in total only 2 to 3 were observed per plant in 8 counts from 8 July to 21 August. Alatiform L4's were virtually absent; less than 0.5 per plant were found over these 8 weeks. Therefore, it is concluded that the alatae counted originated from outside the field. Most alatae were found in May-sown plots and in plots with a high ratio of row distance to plant distance in rows. No relation was found, however, between numbers of alatae and virus spread and only a weak correlation between total aphid numbers and virus spread.

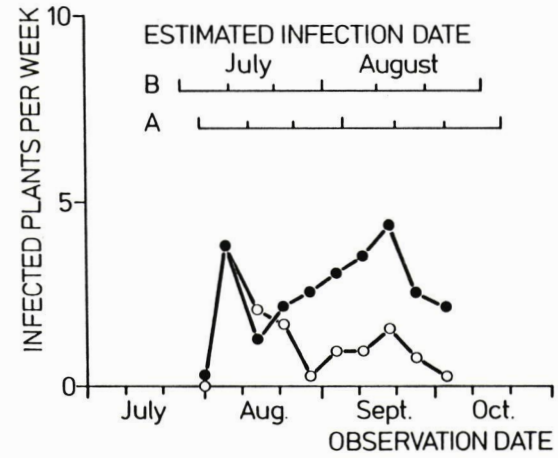


Fig. 6.9: Rate of secondary spread of BMYV in plots sown on 23 April (○) or 24 May (●), averaged for 6 different planting patterns (see text). The rate of spread is plotted against the date symptoms were observed (lower abscissa) and against the estimated infection date (upper abscissae) using the incubation period of BMYV in (A) early- and (B) May-sown plants, respectively.

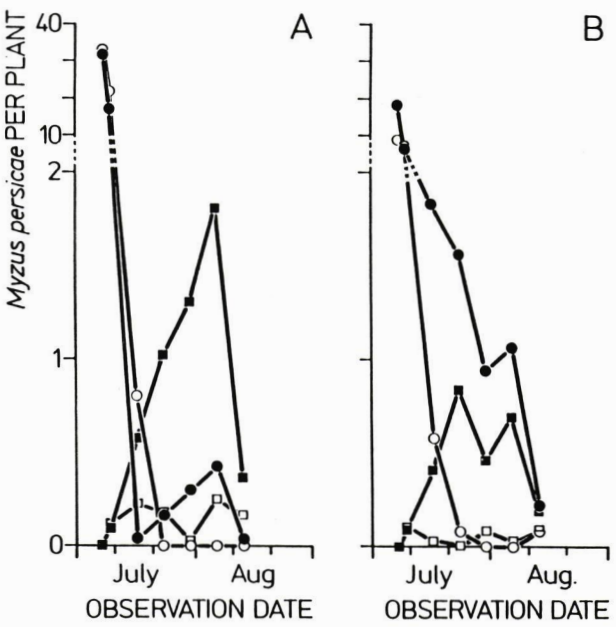


Fig. 6.10: Numbers of *Myzus persicae* on the central plant (○) and on 6 other sample plants (□) in April-sown (open symbols) and May-sown (closed symbols) beet. A: BMYV-inoculated plots; B: control plots.

6.3.3 Effect of plant age on susceptibility to BYV and latency period of BYV

The susceptibility of the plants to BYV and the latency period of this virus were determined to explain the observed differences in secondary spread between sugarbeet inoculated in an early or a later development stage. Virus transmission by *M. persicae* was not affected by sowing date, infection date or leaf age (Table 6.2). Thus the susceptibility of the plants to BYV remained constant throughout the season. Therefore the observed lack of spread of BYV from May-sown to April-sown beet (Fig. 6.3) was probably caused by a reluctance of the aphids to feed on the older, less suitable plants in the plots sown in April.

Table 6.2: Transmission of BYV by 2 different batches of *Myzus persicae* clip-caged onto young and old leaves of beet plants sown on 25 April or 26 May, throughout the season.

	M. persicae from rape				*M. persicae* from infected beet			
	25 April		26 May		25 April		26 May	
	E	F	E	F	E	F	E	F
2 June	0.80	-	-	-	0.63	-	-	-
16 June	-	-	-	-	0.83	-	-	-
2 July	0.92	0.92	0.77	-	0.53	0.62	0.57	-
21 July	0.95	0.86	0.90	-	0.83	0.77	0.93	-
5 Aug.	0.90	0.93	1.00	0.96	0.50	0.70	0.45	0.79

E = expanding leaves; F = fully-expanded leaves; - = not tested.

Table 6.3: Latency period of BYV (LP) during the season as affected by sowing date.

	Sowing date					
	25 April			26 May		
Inoculation date	N	LP (days)	LP (°C days)	N	LP (days)	LP (°C days)
16 June	10	4½	61	-	-	-
2 July	19	9½	121	8	5½	79
18 July	24	10	132	15	7	84
4 Aug.	32	8	103	22	6	76
19 Aug.	36	10½	112	28	9	97
3 Sept.	40	12	94	32	10½	82

N = number of leaves; LP = latency period expressed in days and in °C days above 3 °C (thermal threshold of leaf expansion in sugarbeet; Milford *et al.*, 1985b)

May-sown plants had consistently shorter latency periods than April-sown ones (Table 6.3). The latency period increased from about 5 days in April-sown plants infected in June to 12 days in plants infected in September. In May-sown plants, the latency period increased from about 5 days in July to 10 days in September. The difference in latency period between the early- and late-sown plants decreased during the season. No relation was found between leaf number (development stage) and latency period, expressed in days or in °C days (Table 6.3).

6.4 Discussion

The experiments described in this chapter demonstrate that later sowing, resulting in younger host plants for virus and vector, increases vector numbers in the crop and rates of secondary spread of BYV and BMYV. Increased vector numbers and rates of spread were also observed when vectors and virus were introduced early in the season in an early-sown crop (Chapter 5). These results demonstrate that crop development stage (plant age) determines the amount of spread to a large extent. At least 3 factors are responsible for this key role of plant age:

(1) Plant age determines the relative growth rate (r_m) of *M. persicae* populations. Williams (1988) found that r_m decreased approximately linearly with the number of leaves on the plant, reaching a value of zero when the plants had about 25 leaves in July. Thus the earlier *M. persicae* arrives during the development of a crop, the greater are its opportunities to multiply and spread virus. Furthermore, virus spread and virus infection form a positive feedback loop because aphid reproduction is stimulated on virus-infected plants and virus spread is promoted by higher numbers of vectors.

(2) As beet plants become increasingly unfavourable to *M. persicae* during their growth, it is plausible that they will be less readily accepted as a host. Decreased acceptability of the plants can account for the observed lack of secondary spread of BYV from late-sown beet plots to early-sown ones (Fig. 6.3), even though young and old beet plants appeared to be equally susceptible to BYV (Table 6.2). In the susceptibility test, the reluctancy of *M. persicae* to feed on older plants may have been overruled by the long period of caging. Similar observations were made by Russell (1966). It is concluded that a decreased acceptability of older beet

plants may interfere with the transmission of viruses by *M. persicae*. This may limit secondary virus spread in early-sown crops. Additionally, this type of aphid-resistance in older plants may affect the number of aphids settling and the number of primary infections resulting from a given influx of viruliferous aphids by an effect on staying times and feeding behaviour (Müller, 1958; Kennedy *et al.*, 1959; Way and Heathcote, 1966).

(3) The latency period of (at least) BYV is related to plant age, increasing from ± 5 days in young plants to ± 2 weeks in old plants (Table 6.2). Thus the number of possible infection cycles increases more than proportionally with the earliness of infection.

Plant age has not only an effect on the rate of virus spread, but affects also the development of disease symptoms (Chapter 2) and the loss of yield (Chapter 7). Therefore control measures are most needed in early-infected and late-sown crops.

Aerial photography may provide a useful tool for monitoring diseases of field crops. Aerial photography was used to monitore epidemics of barley yellow dwarf virus in winter wheat (Hooper, 1978). Our results show that infected plants may not be readily discerned on photographs before a significant number of leaves has been affected. Therefore, estimates of the incidence of diseases, based on aerial photography may differ considerably from field estimates.

Schultz *et al.* (1985) produced evidence that the non-persistently transmitted soybean mosaic virus (SMV; potyvirus group) was spread when transient alate vectors settled for 24 hours on a soybean crop. It cannot be ruled out that the large differences in virus spread in different parts of the field (Fig. 6.3) were caused by a short period of unnoticed activity of transient alate *M. persicae* between 2 observation dates. However, the low number of *M. persicae* caught in the water traps does not support such an explanation. Moreover, it can be questioned if a semi-persistently transmitted closterovirus like BYV can be spread as rapidly as SMV, as two long feeding times (at least several hours) are required to acquire and transmit BYV.

Numbers of alatae entering the crop were low in the present experiments as shown by field counts and trap catches. Alatae had no apparent effect on the population development of *M. persicae* in the plots, or on the introduction and secondary spread of virus. The small impact of the number of *M. persicae* introduced on spread of BYV (Table 6.1), which was

confirmed in another experiment (Kempenaar, 1987), indicates that the number of introduced (immigrant) vectors is not the most important factor determining the incidence of yellowing viruses in crops. Crop development stage and the impact of predators (Chapter 5) were found to be more important factors. This suggests that warning schemes for virus yellows control can be improved by taking account of field to field differences in plant development stage and numbers of predators to estimate the risk of virus spread and damage to the crop and the need of spraying more precisely.

7 COMPONENTS OF DAMAGE BY BEET YELLOWS VIRUS IN SUGARBEET

Abstract

A comparative growth analysis of healthy sugarbeet and sugarbeet infected with beet yellows virus (BYV; closterovirus group) was combined with measurements of radiation absorption and CO_2-assimilation to explain quantitatively the reduction in yield caused by infection with BYV. Plants infected with BYV on 5 June (8 leaf stage; leaf area index (LAI) ± 0.1) produced only 44,800 (± 900 (SEM)) kg of beet roots (fresh weight/ha), whereas healthy plants produced 92,900 (± 900) kg/ha. Plants infected in the 28 leaf stage (LAI > 5), on 14 July, incurred less yield reduction, producing 86,800 (± 1,100) kg/ha.

Four damage components were discerned and quantified:

(1) Reduction of leaf area index as a result of the smaller size of infected leaves.

(2) Decrease of radiation absorption. Virus-infected, yellow leaves reflected or transmitted 40% of the incident photosynthetically active radiation whereas healthy leaves reflected or transmitted only 15%.

(3) Impairment of photosynthesis in infected leaves with disease symptoms. Light saturated net photosynthesis (A_m) decreased from 1.09 ± 0.04 mg CO_2 m^{-2} leaf s^{-1} in healthy leaves or infected leaves without symptoms to 0.16 ± 0.04 mg CO_2 m^{-2} s^{-1} in bright yellow, infected leaves. Light use efficiency, ε, decreased with increasing intensity of symptoms from 11.0 ± 1.0 µg CO_2 J^{-1} absorbed visible radiation in healthy or infected, green leaves to 7.8 ± 1.3 µg CO_2 J^{-1} in yellow leaves.

(4) Increase of respiration, from 0.06 ± 0.01 mg CO_2 m^{-2} s^{-1} in healthy leaves to 0.11 ± 0.02 mg CO_2 m^{-2} s^{-1} in green or yellow, infected leaves.

Simulation of sugarbeet growth showed that the total yield reduction, caused by BYV infection is explained quantitatively by these four damage components. The model predicts a maximum yield reduction of about 50% when the plants become infected before mid-June when a LAI of 1.0 is

reached, and negligable damage for infections after mid-July, when ample leaf area has developed (LAI > 5). According to the model, yield reduction decreases rapidly with later infections between mid-June and mid-July when the LAI increases quickly.

7.1 Introduction

Reported reductions of yield in sugarbeet due to yellowing viruses vary from insignificant to about 60% (Duffus, 1973). The proportion of plants infected and the period the plants show symptoms are the main factors determining yield reduction (Watson *et al.*, 1946; Jepson and Green, 1983). Therefore yield reduction is correlated with the area under the curve of disease progress (percentage of plants showing symptoms) in time (Hull, 1953). This integral, expressed in percentage * weeks, is called 'infected plant weeks' (abbreviated to IPW's). Reported yield reductions per 100 IPW vary from 1 to 5% (Watson *et al.*, 1946; Watson and Watson, 1953; Heathcote, 1978a; Häni, 1979; Heijbroek, 1984). Because of this variability, yield reduction is difficult to estimate on the basis of IPW's.

Apart from differences between observers in what they consider as a plant with symptoms, the variation in yield reduction per 100 IPW's is probably due to differences in sowing date (Watson *et al.*1946; Jepson and Green, 1983), growing conditions, variety tolerance (Russell, 1964a, b; Hall *et al.*, 1972) and agressiveness of virus isolates (Björling, 1961; Russell, 1963). Additionally, the growth reduction incurred for each week with symptoms by late-infected plants is likely to be lower than that incurred by early-infected plants as the former develop only a few yellow leaves. Fig. 7.1 illustrates this larger yield reduction/100 IPW, for early-infected plants.

An analysis of damage by yellowing viruses in beet which can take varying growing conditions, infection dates, sowing dates, etc. into account should be based on knowledge of the physiological effects of virus infection in relation to the growth of the plant. In such an analysis distinction should be made between leaves that become systemically-infected with virus and those that emerged before the plant was infected and remain healthy (Chapter 3). The time needed for symptoms to develop must also be taken into account (Chapter 2). Several effects of infection with

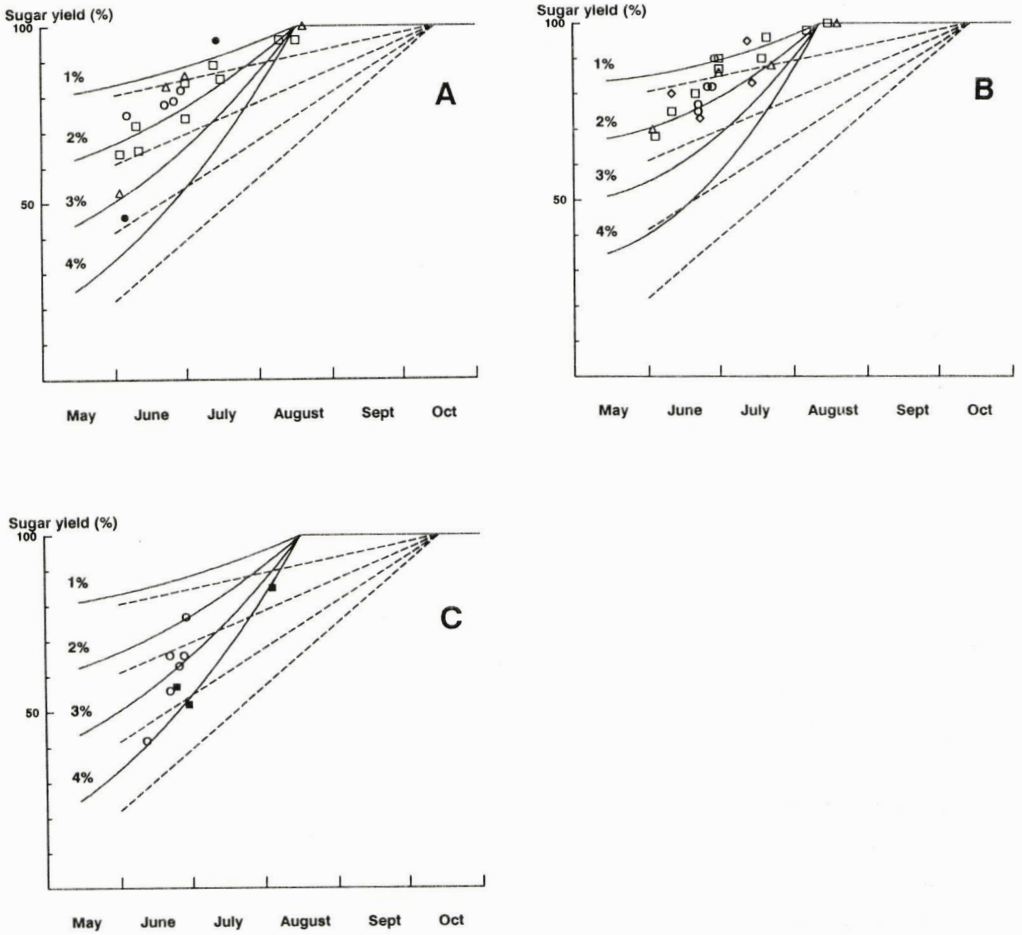


Fig. 7.1: Literature data on the yield reduction caused by infection with BYV (A), BMYV (B) or a mixture of these viruses (C), compared to the yields calculated for yield reductions per 100 IPW of 1, 2, 3 or 4%. Hatched lines give the relation between the date on which symptoms appear and yield calculated with IPW's. Harvest is assumed on 15 October. Drawn lines give the relation between yield and infection date, assuming incubation periods as given in Chapter 2. Data were taken from Häni, 1979 (◇), Heijbroek, 1988 (□), Russell, 1963 (○), Smith, 1986 (△) and from Watson and Watson, 1953 (■). The figure shows a concave relationship between infection date and yield estimated with IPW's, whereas the relationship is in fact convex, converging to a limit value of 100% with late infections. Furthermore, the yield reduction per 100 IPW appears to decrease with later infection. Yield reduction by a mixture of the two viruses appears to be greater than that caused by either of the viruses alone. BYV causes a slightly greater yield reduction than BMYV.

BYV on the growth and physiology of the beet plant have been described in the literature. Hall and Loomis (1972a, b) showed that BYV reduces the rate of photosynthesis and Watson and Watson (1953) showed that the leaf area of the crop is reduced. Furthermore, increased respiration in the leaves (Van Riemsdijk, 1935; Schultz, 1958a, b) and storage roots (Löhr and Müller, 1953) has been reported. All these effects should also be taken into account. In this Chapter, results are presented of a study in which the effects of BYV infection on the physiological characteristics of the leaves were measured and related to the growth and dry matter production of the crop as a whole. A simulation model was used to calculate the theoretical effects on crop growth of all damage components seperately and in combination.

7.2 Materials and methods

Arrangement of the field experiment. On 18 April 1986, sugarbeet 'Regina' were sown to a stand of 75,000 plants/ha on a sea-clay soil (38% silt) near Nagele in the Noordoostpolder. The experiment consisted of 5 blocks, each divided into 3 plots which were each subdivided into subplots of 2 * 3 m^2. One plot in each block was inoculated on 5 June, another plot was inoculated on 14 July and the third plot served as healthy control. Both inoculations extended in fact over 2 days.

Viruliferous *M. persicae* were reared on BYV-infected sugarbeet in the glasshouse. On 5 June, plants were inoculated by clip-caging ± 10 aphids on an expanding leaf of each plant in the plot. After 2 days the aphids were killed by spraying oxy-demeton-methyl. The field was sprayed again on 9 June with pirimicarb and on 28 June with parathion to limit virus spread from the inoculated plants. On 14 July, the plants were inoculated with BYV-infected leaf fragments with ± 10 *M. persicae*. Pirimicarb was applied on 17 and 20 July.

Growth analysis. Periodic harvests and growth analyses were made in the control plots on 11 June, 17 July, 20 August, 29 September and 20 October. To investigate the effect of virus infection on production, harvests were made from the time symptoms had appeared in the inoculated plots. In the plots inoculated on 5 June, the first harvest was made on 17

July and in the plots inoculated on 14 July the first harvest was made on 29 September. All plants in a 2 * 3 m^2 subplot were harvested, except the few naturally-infected plants in control plots or some later-infected plants in inoculated plots. The latter were identified in two ways: (1) by the absence of virus symptoms when the other inoculated plants showed clear symptoms and/or (2) by the absence of symptoms on leaves which had not yet appeared on the inoculation date (Chapter 3). Row length occupied by the harvested plants was measured to calculate the harvested area precisely.

After harvest the plants were divided in leaf blades, petioles, crowns and tap roots. Tap root and crown were separated just below the point of insertion of the first leaf pair, thus revealing the concentric vessel rings of the tap root. The petioles were severed from the crown less than 1 cm from the point of insertion and leaf blades were cut from the petiole at about the point of insertion of the lowermost vein on the midrib. Samples were taken to determine the percentage dry matter in the different plant parts and the percentage sucrose in the tap root.

Estimation of leaf area. On 11 and 27 June, 23 July, 18 September and 24 October, a few representative plants were taken from the field to determine the effect of virus infection on the area of the individual leaves. Leaves were numbered in ontogenetic sequence (disregarding the cotelydons) and compared visually to a series of 33 photographs of standard leaves with known sizes. Dead or senescent leaf area was disregarded.

Measurement of photosynthesis and respiration. On 11 sunny days, between 10 June and 14 October, measurements of CO_2-assimilation and respiration by healthy and infected leaves were made using a portable equipment (The Analytical Development Co. Ltd., 1985), consisting of 4 units: (1) a leaf cuvette (16 ml) which, when clipped onto a leaf, included 2.5 * 2.5 cm^2 leaf area, (2) an air supply unit, equipped with a pump and a mass flow meter to determine the air flow (at least 5 ml/s), (3) an infrared gas analyser (IRGA) to measure the difference in CO_2 concentration between the air flows entering and leaving the leaf cuvette, and (4) a data logger. The leaf cuvette was equipped with a sensor for photosynthetically active radiation (PAR; 400 - 700 nm).

About 3 or 4 leaves per plant were examined. On healthy plants one expanding leaf was selected for measurement, one recently-fullgrown leaf and one older leaf. On infected plants one healthy leaf (which emerged

before infection) and a few leaves with yellowing symptoms of different intensity were examined. The leaves were categorized as (0) not infected, (1) infected but still symptomless, (2) with cleared veins, (3) greenish yellow, (4) bright yellow and (5) yellow with necrotic spots. For each leaf five measurements of CO_2-exchange were made, the first at full illumination by the sun. Three subsequent measurements were made at light intensities of about 60, 30 and 10% and one measurement was made in the dark. The intermediate intensities were created by shading with one or two sheets of nylon gause or white paper. The photosynthesis light response curves of individual leaves (Eq. 7.1) were calculated by fitting negative exponential saturation curves (Goudriaan, 1982) to the measurements made for each leaf, using the non-linear least squares regression procedure NLIN of the SAS statistical software package (SAS Institute Inc., 1985a,b). The equation is:

$$A_n = (A_m + R_d) * (1 - e^{-\varepsilon * H/(A_m + R_d)}) - R_d \qquad (7.1)$$

in which :

A_n is the net CO_2-assimilation rate (mg CO_2 m^{-2} s^{-1}),

A_m is the maximum rate of net CO_2-assimilation, reached at light saturation (mg CO_2 m^{-2} s^{-1}),

R_d is the respiration, measured in the absence of photosynthesis in the dark (mg CO_2 m^{-2} s^{-1}),

ε is the initial light use efficiency for fixing CO_2 (µg CO_2 J^{-1}), and

H is the incident flux of photosynthetically active radiation (400 - 700 nm; W m^{-2})

Measurement of reflection and transmission of visible radiation. On 8 October, the diffuse reflection and transmission of photosynthetically active radiation (PAR) by healthy and infected leaves was measured, using an integrating sphere and compressed $BaSO_4$ as a standard.

7.3 Results

7.3.1 Growth analysis

Healthy beet plants yielded 32,200 (± 500; standard error of mean) kg total dry matter/ha at the final harvest on 20 October. Plants infected on 5 June yielded 15,700 (± 500) kg/ha and plants infected on 14 July 29,900 (± 600) kg/ha ($LSD_{5\%}$ = 1,900 kg/ha). The yield of beet roots (fresh weight) amounted to 92,900, 44,800 and 86,800 kg/ha in the 3 treatments, respectively ($LSD_{5\%}$ = 4,000 kg/ha) and the percentages of sucrose in the roots were 18.6, 17.7 and 19.1, respectively ($LSD_{5\%}$ = 1.2). Clearly, June-infected plants incurred a large yield reduction while the yield reduction in July-infected beet was small. The plants infected on 5 June showed symptoms after 3 weeks. Thus their yield reduction was ± 3%/100 IPW. July-infected plants showed symptoms after 6 weeks, their yield reduction being less than 1%/100 IPW, confirming the mentioned smaller yield reduction per 100 IPW with later infection (Fig. 7.1).

June-infected beet accumulated less dry matter in petioles, crowns and tap roots (Fig. 7.2B, C, E) than healthy beet, while the amount of dry matter in the leaf blades was similar (Fig. 7.2A). Leaf area was, however, reduced by virus infection, infected leaves containing about 40% more dry matter per unit area. The results of this growth analysis are in good accordance with those of Watson and Watson (1953).

7.3.2 Leaf area

June-infected beet plants developed considerably less leaf area (Fig. 7.3), due to decreased expansion of the leaves appearing after the infection (Fig. 7.4). The number of leaves was not clearly reduced. Leaf area production by July-infected plants was less affected (Figs. 7.3 and 7.4C, D) because (1) these plants already possessed ± 20 fullgrown leaves when they were infected, and (2) leaves appearing after mid-July remain naturally small and contribute little to the leaf area of the crop (Milford *et al.*, 1985b; Clark and Loomis, 1978). Leaves emerging from July onwards on June-infected plants expanded more rapidly or to a greater size than leaves appearing at the same time on healthy or July-infected plants. Unfortunately, the method of leaf area estimation used, involving the selection of 'representative plants', does not allow a test on the significance of this observation.

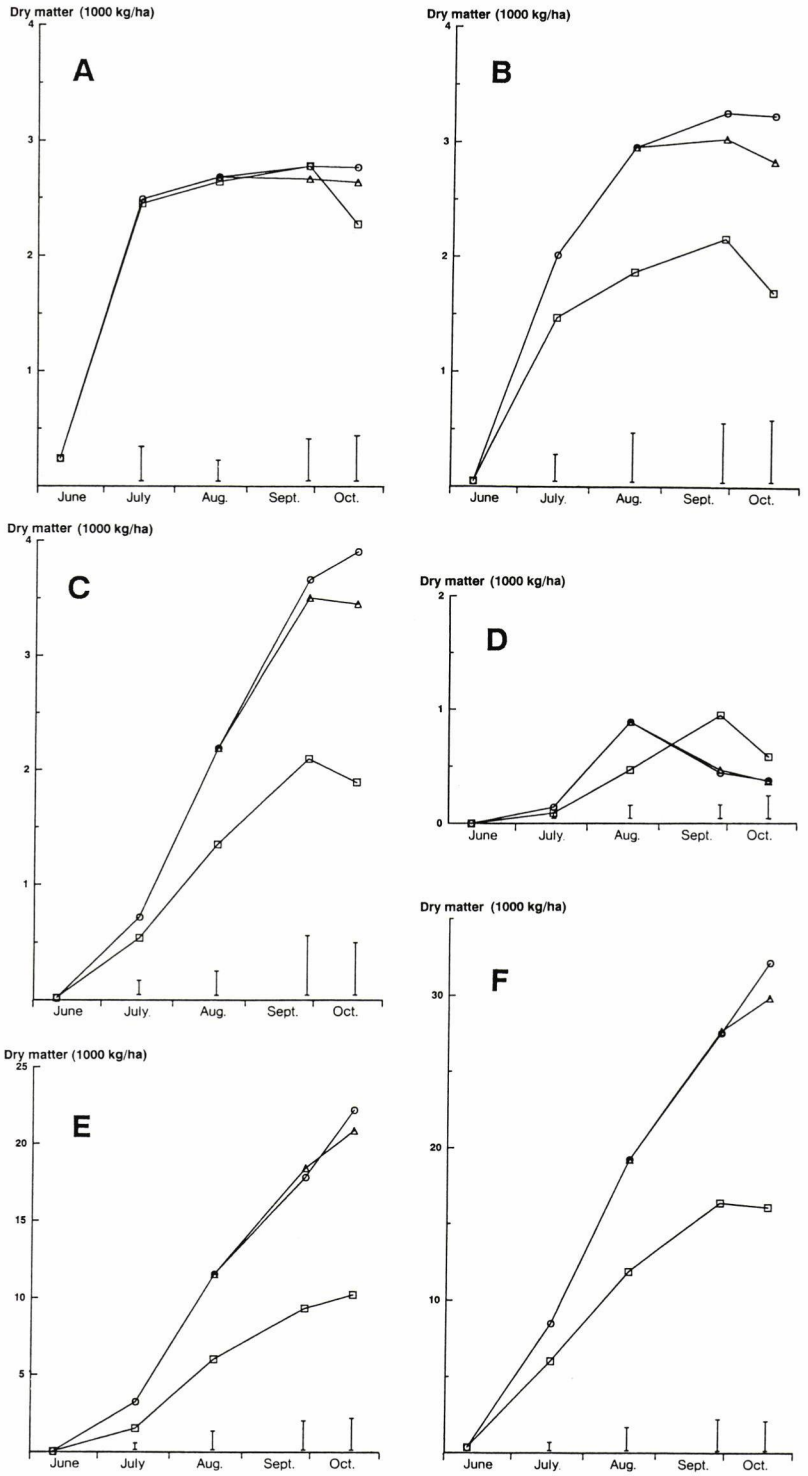


Fig. 7.2: Dry matter production by healthy sugarbeet (○) and by beet infected with BYV on 5 June (□) or 14 July (△). A: leaf blades; B: petioles; C: crowns; D: dead or senescent leaf blades and petioles; E: tap roots and F: whole plants. Bars denote least significant differences (LSD) at the 5% level.

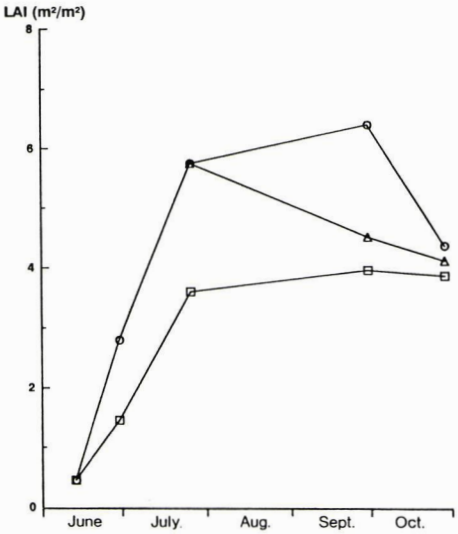


Fig. 7.3: Leaf area index of healthy sugarbeet (○) and beet infected with BYV on 5 June (□) or 14 July (△). Ten representative plants were examined on 11 and 27 June and 23 July, 5 on 18 September and 7 on 24 October.

7.3.3 Photosynthesis and respiration

To provide a standard for the evaluation of the effects of BYV on photosynthesis, the photosynthesis light response curves of healthy leaves were determined on 11 dates. The average parameter values obtained were: A_m = 1.09 mg CO_2 m^{-2} s^{-1}, ε = 10.9 µg CO_2 J^{-1} absorbed PAR and R_d = 0.06 mg CO_2 m^{-2} s^{-1} (Table 7.1). These values are typical of a C_3 species (Goudriaan, 1982; Jones, 1983), though the value of 1.09 for A_m is an underestimate because the method of measurement caused a decrease of the CO_2 concentration in the leaf chamber. Additionally, on some hot and sunny days, the CO_2 concentration at 2 m height, where air was let in, decreased as a result of crop photosynthesis during the day. Higher rates of photosynthesis would have been obtained if the CO_2 concentration in the leaf chamber had been 340 ppm under all circumstances.

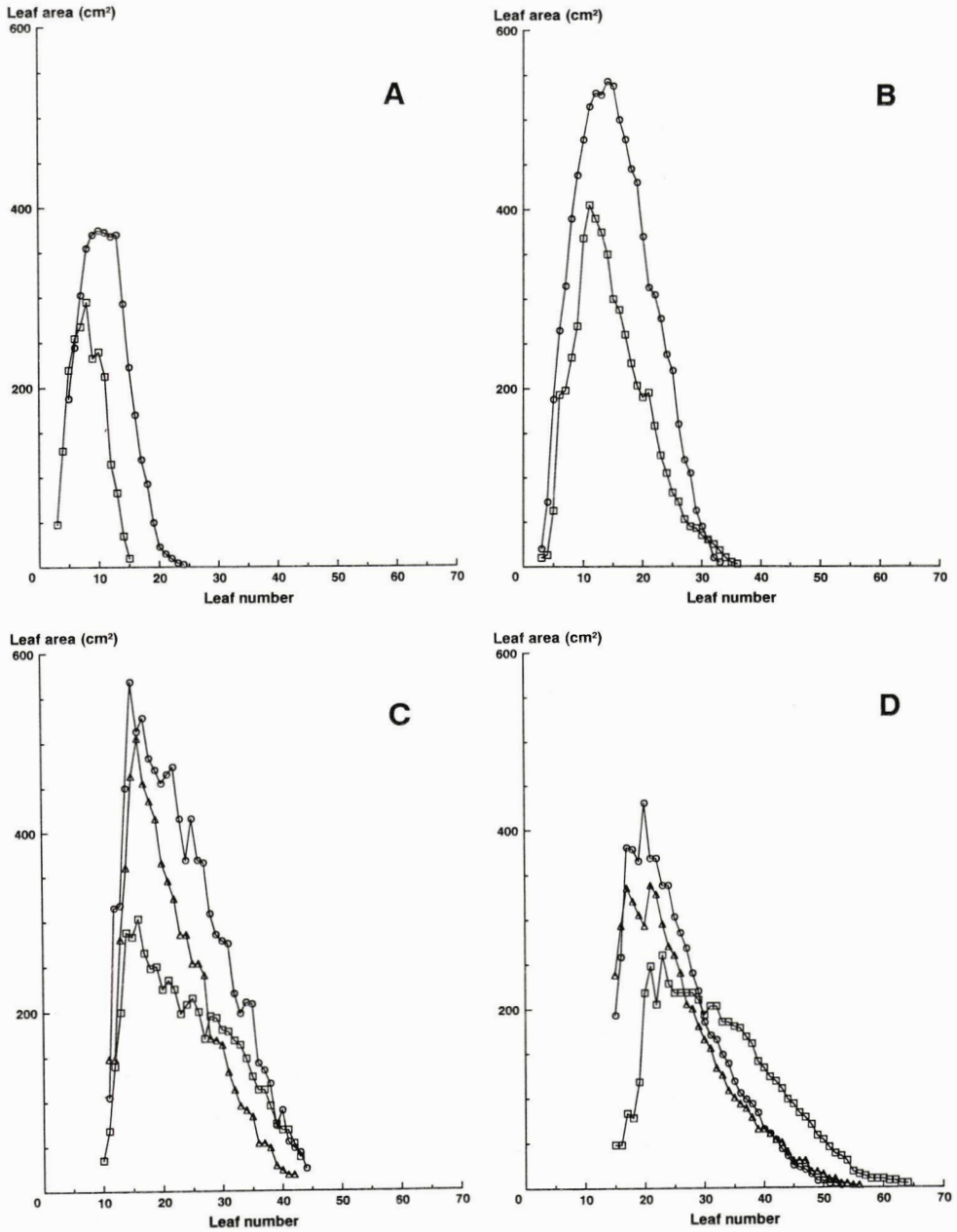


Fig. 7.4: Growth of the area of individual leaves in healthy sugarbeet (○) and in beet infected with BYV on 5 June (□) or 14 July (△). A: 27 June, B: 23 July, C: 18 September, and D: 24 October.

Table 7.1: Photosynthesis parameters of healthy leaves (n = 43)

parameter		mean	STD	SEM	CV
A_m	(mg CO_2 m^{-2} s^{-1})	1.09	0.26	0.04	24
ε	(µg CO_2 J^{-1} abs. PAR)	10.9	2.22	0.34	20
R_d	(mg CO_2 m^{-2} s^{-1})	0.062	0.05	0.008	83

Table 7.2: Photosynthesis parameters (± SEM) of leaves on BYV-infected plants

	A_m (mg CO_2 m^{-2} s^{-1})	ε (µg CO_2 J^{-1} abs. PAR)	R_d (mg CO_2 m^{-2} s^{-1})	n
healthy	1.03 ± 0.08	11.0 ± 1.0	0.03 ± 0.01	8
vein clearing	0.62 ± 0.05	9.9 ± 1.2	0.11 ± 0.03	5
greenish yellow	0.32 ± 0.04	8.8 ± 1.0	0.15 ± 0.02	8
bright yellow	0.16 ± 0.04	7.8 ± 1.3	0.11 ± 0.02	13

Infected leaves showed significant reductions in photosynthetic capability as symptoms developed. The strongest effect was on A_m which decreased to zero in the most severely yellowed leaves, while ε decreased with 30% and R_d approximately doubled (Table 7.2). Leaves of the plants infected on 5 June showed no symptoms and exhibited normal rates of photosynthesis up till 16 June, 11 days after infection. On 19 June, 14 days after infection, the first symptoms were observed. In one leaf (leaf number 10) having cleared veins A_m decreased by 30%, while, on another plant, A_m decreased by 70% in a leaf nr. 10 showing incipient yellowing symptoms. Three weeks after infection, A_m had decreased by 90% in leaves showing clear symptoms. Generally there was a good correlation between the intensity of the symptoms on a leaf and the rate of photosynthesis. On leaves that showed symptoms on only one half, the photosynthesis was only reduced on the yellow part. When several systemically-infected leaves on the same plant were compared, the oldest one, showing the most severe symptoms (Chapter 3), had generally the lowest rate of photosynthesis

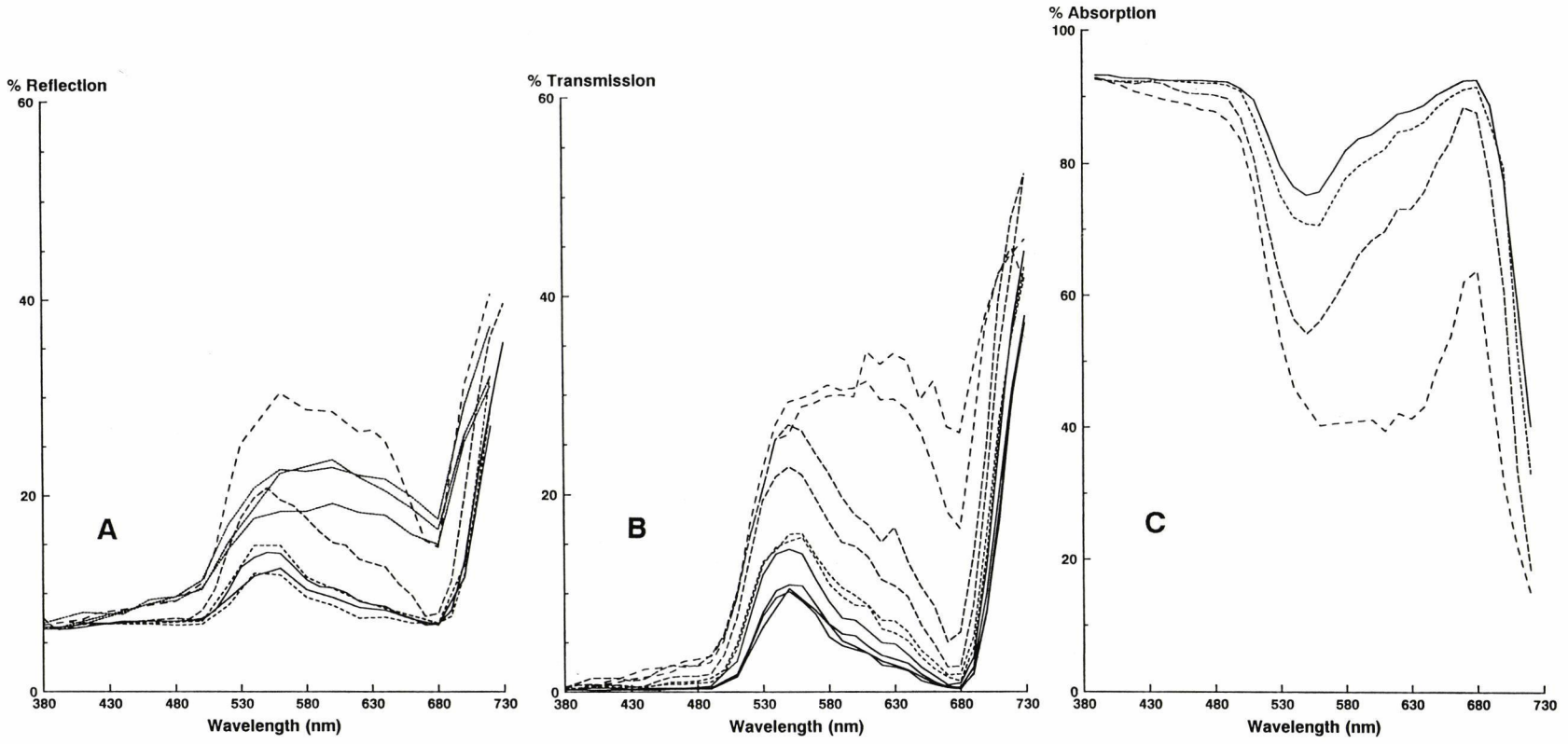


Fig. 7.5: Reflection (A) and transmission (B) spectra of healthy (———) and BYV-infected, green (---------), greenish yellow (-------) or bright yellow (------) beet leaves. C: calculated average absorption spectra.

while the youngest one with mild or no symptoms had the highest rate. Photosynthesis was not impaired in the healthy leaves which emerged before infection.

7.3.4 Absorption of photosynthetically active radiation

Infected leaves reflected and transmitted more of the incident diffuse photosynthetically active radiation (PAR; Fig. 7.5 A, B) than healthy leaves. Leaves with similar symptoms had similar reflection and transmission spectra. From the spectra of the individual leaves, an approximate absorption spectrum was calculated for (1) healthy leaves and for (2) green, (3) greenish yellow and (4) bright yellow, infected leaves (Fig. 7.5C). Integration of these curves over 400 - 700 nm showed that these 4 categories of leaves absorbed approximately 88, 85, 78 and 63% of the incident PAR. This reduction in radiation absorption enhances the effect of the decreased light use efficiency yellow leaves.

7.3.5 Simulation of crop growth

A simulation model was constructed to calculate the consequences of the quantified effects of virus infection on leaf growth, light absorption, photosynthesis and respiration for the growth and production of the crop. The SUCROS87 model (Spitters *et al.*, 1988) was adapted to simulate growth of sugarbeet. Model calculations were made for 7 imaginary crops: one healthy crop with growth parameters derived from the control treatment in the field experiment and 6 crops infected on one of 6 different dates (Table 7.3). To calculate the effects of virus infection, yellow and green leaves are discerned in the model, each with their own photosynthesis parameters:

green leaves: $A_m = 60$ kg CO_2 ha^{-1} leaf h^{-1}
$\varepsilon = 0.50$ (kg CO_2 ha^{-1} leaf h^{-1})/(J abs. PAR m^{-2} s^{-1})

yellow leaves: $A_m = 10$ kg CO_2 ha^{-1} leaf h^{-1}
$\varepsilon = 0.35$ (kg CO_2 ha^{-1} leaf h^{-1})/(J abs. PAR m^{-2} s^{-1})

The leaf area of each of these 7 crops was read or interpolated from Fig. 7.3. The growth of leaf area was estimated by adding to the LAI curves

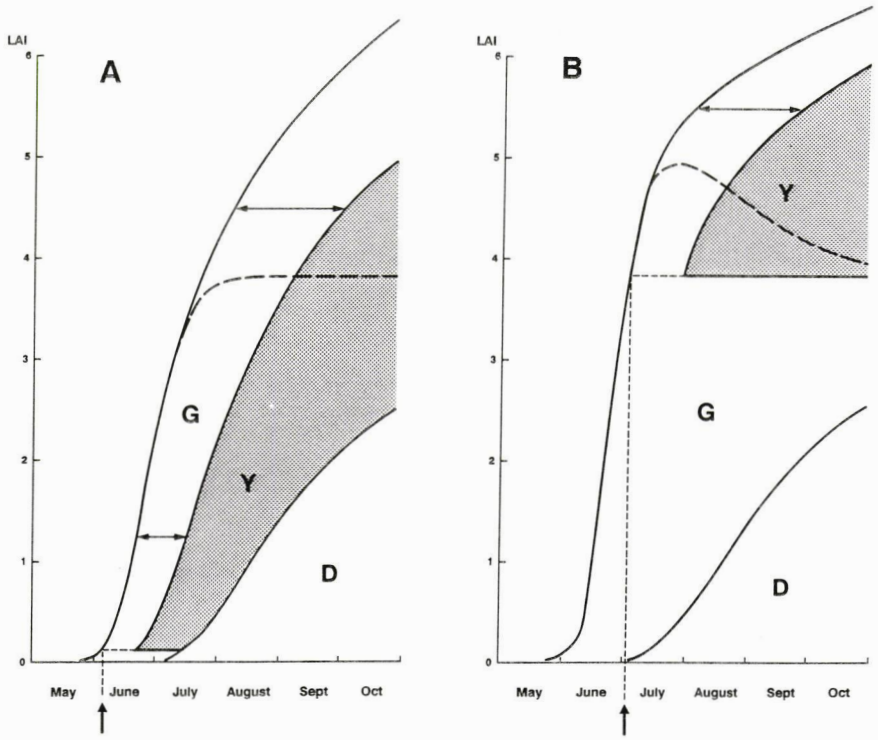


Fig. 7.6: Development of green and yellow leaf area, illustrated for sugarbeet plants infected on 5 June (A) or 3 July (B). Three types of leaves are discerned: (G) green leaves, consisting of (a) healthy ones that appeared before the infection date, and (b) infected leaves that appeared after the infection date but have not yet become yellow, (Y) yellow leaves and (D) dead leaves. The hatched line denotes the course of the leaf area index (LAI) as interpolated from Fig. 7.3. The upper drawn line, which represents the living plus dead LAI is constructed by adding an estimated senescence of about 0.6 LAI per month (lower drawn line) to the LAI curve. All leaf area appearing after the infection date (arrow) becomes yellow after the incubation period has elapsed (horizontal bars). The incubation period increases during the season. The 4 weeks difference in infection date has a large impact on the amount of yellow leaf area and on yield reduction.

Table 7.3. Simulated effect on yield of infection with BYV on a range of dates. Table gives relative yields, expressed as a percentage of 'control' yield (88,000 kg beet roots (fresh weight) ha^{-1}). Four effects of the virus (or combinations of them) were evaluated with the model: (1) effect on LAI; (2) effects on LAI and light absorption; effects on LAI and light absorption combined with effect on either Rd (3), A_m (4) or ε (5); (6) the effect of all factors together; and (7) the effect on photosynthesis alone (A_m and ε). N = number of leaves on infection date. LAI = leaf area index on infection date.

Infection date	N	LAI	Effects included in the model						
			1	2	3	4	5	6	7
22 May	2	0.01	91	87	82	68	76	55	69
*			91	86	80	63	73	49	63
5 June	7	0.1	93	89	83	69	77	56	69
*			93	88	80	64	74	49	63
19 June	14	0.8	96	91	83	70	79	56	69
3 July	21	1.8	97	96	92	87	91	81	88
14 July	26	2.9	99	98	98	97	98	96	98
28 July	30	5.8	100	100	100	99	100	99	100

* Leaf area development of early-infected plants estimated under the assumption of a loss through senescence, 50% of that of healthy plants.

an estimated leaf senescence of 0.6 LAI units per month from July to October (Fig. 7.6). Leaf area emerging before the infection date is considered to remain green (Chapter 3) and photosynthetically active. Leaf area emerging after the infection date becomes yellow when the incubation period (Chapter 2) has elapsed. With these generalizations, the fraction yellow leaf area of each of the 6 infected crops was determined throughout the season. Two examples are given in Fig. 7.6. Photosynthesis of the crop is calculated by taking a weighted average of the photosynthesis by green and yellow leaves.

The increased reflection and transmission by yellow leaves was taken into account by calculating the scattering coefficient (SCP) of the leaves with:

$$SCP = 0.12 * F_g + 0.40 * F_y \qquad (7.2)$$

in which F_g and F_y are the proportions of green and yellow leaf area as determined in Fig. 7.6 and 0.12 and 0.40 are the scattering coefficients of green and yellow leaves. Consequently, reflection (REFL) by the canopy as a whole is calculated with:

$$REFL = (1 - \sqrt{(1-SCP)})/(1 + \sqrt{(1-SCP)})$$

Thus 13% of the incident radiation would be reflected to the sky if all leaves were yellow while 3% is reflected by healthy plants.

Increased respiration in virus-infected leaves was taken into account by computing the maintenance respiration R (kg CH_2O ha^{-1} day^{-1}) for leaves with

$$R = 0.03 * (F_g + 2.5 * F_y) * WLV \qquad (7.3)$$

in which WLV is the dry weight of leaves (kg ha^{-1}).

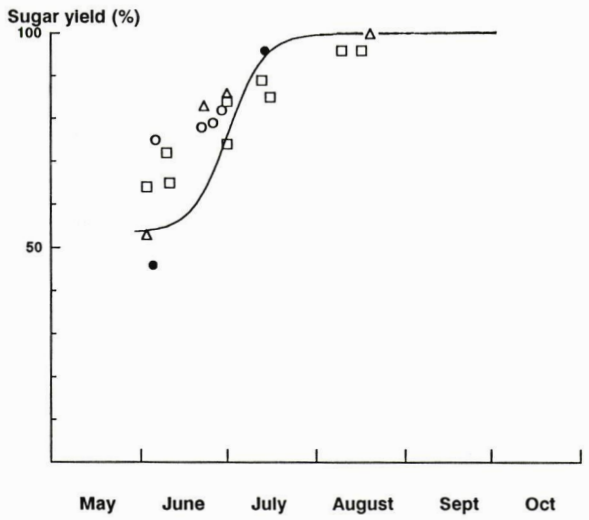


Fig. 7.7: Relative yield (as % of control) as a function of infection date, calculated with the simulation model (——) in comparison to field experiment (●) and data of Heijbroek, 1988 (□, ■), Russell, 1963 (○) and Smith, 1986 (△). Agreement between simulation and experiment is fair but the simulated line differs from the literature data because growing conditions, sowing date etc. in the different experiments are not accounted for.

Model calculations

The model calculations show that the effects of BYV infection on leaf area development, light absorption and respiration cause relatively small reductions in crop production. The impairment of photosynthesis in yellow leaves is the most important damage component (Table 7.3). Both the effects on light saturated photosynthesis, A_m, and light use efficiency, ε, are important. When all effects are included in the model, good agreement is obtained with the results of the growth analysis (Fig. 7.7). The calculations suggest that a maximum yield reduction is incurred when the crop is infected in early-June or before. Yield reduction decreases rapidly with later infections after canopy closure (± 20 June). Infections after mid-July do not cause a substantial yield reduction because a leaf canopy well capable of light interception and photosynthesis has already developed.

7.4 Discussion

Four damage components of BYV were discerned and quantified in this study:

(1) reduction of LAI,
(2) reduction of light absorption,
(3) reduction of photosynthesis and
(4) increase of respiration

These four damage components constitute 4 successive, non-overlapping restraints in the chain of events leading to the fixation of radiation energy in plant biomass. (1) Less leaf area is available to absorb radiation, (2) the leaf area present absorbs radiation less well, (3) the absorbed radiation is less efficiently employed in photosynthesis, and (4) more of the sugars that are produced are consumed. With early infection, these four effects of virus infection are responsible for a reduction in yield of approximately 50%.

The reduction of leaf area in BYV-infected beet plants may be directly caused by the presence of virus or it may result from a reduced supply of developing leaves with photosynthates by older, virus-infected leaves. In hot glasshouse environments, the first leaves emerging after a plant had been infected with BYV were often markedly reduced in size. They remained smaller than older, healthy leaves as well as later developing, infected leaves. This supports a supposedly direct effect of virus infection

on leaf expansion. However, when plants have been infected for a long period, it is plausible that reduced supply of photosynthates plays also a role. Nevertheless, sugarbeet plants infected on 5 June in the present experiment seemed to produce more and larger leaves than healthy plants at the end of the season. This may be explained by the plasticity of leaf growth in sugarbeet (Clark and Loomis, 1978; Milford *et al.*, 1855a, b), leaves growing more rapidly and to a larger size when the leaf area of the crop is lower.

A simulation model was used to calculate the yield reduction caused by the four damage components distinguished. The model is preliminary. Only yellow and green leaves are discerned and intermediate symptoms with associated intermediate effects on photosynthesis are neglected. The growth of the leaves and the development of symptoms can be simulated on the basis of quantitative description of leaf appearance and expansion (Milford, 1985a, b) and symptom development (Chapter 2) to make the model more generally applicable. Increased respiration in the root may be included to account for the lower sugar content of BYV-infected beets. The model calculations give insight into the relative importance of the four damage components. Reduced leaf area, increased reflection and increased respiration are not so important, while the effect on photosynthesis alone would account for about 70% of the damage (Table 7.3). The model takes account of the restriction of the virus to the plant parts that develop after the infection. This important fact was neglected by Hall and Loomis (1972b). The model also takes account of the fact that the incubation period elapses before an infected leaf becomes yellow and shows reduction of photosynthesis. If this was not accounted for, beet plants infected in early development stages would incur much larger reductions of yield than the maximum value of approximately 50% that has been obtained in practice and in these simulations.

If yellow leaves on BMYV-infected plants have also strongly reduced rates of photosynthesis, the difference in yield reduction caused by BMYV and BYV might well be a consequence of the different incubation periods of the two viruses (Chapter 2). Because of this difference, the curve relating relative yield to infection date for BMYV would lie approximately 10 days to the left of the curve drawn for BYV in Fig. 7.7. This implies, for instance, that plants infected with BMYV on 30 June would incur 10% yield reduction whereas those infected with BYV on the same date would incur 28% reduction.

The model shows that yield reduction is closely related to the development stage of the crop on the infection date. Below LAI 1 (± 20 June), the yield reduction is maximal. Between LAI 1 and LAI 5 (mid-July), yield reduction decreases with the growth of the plants from 50% to negligible. Crops which completely cover the soil at the infection date incur hardly any reduction of yield. Farmers are inclined to apply insecticides to control virus yellows when they see a rapid increase of the number of plants with symptoms at the end of July. This is useless as the plants are not or hardly damaged by infections made at this time.

8 SUMMARY AND EPILOGUE

In this epilogue the achievements of the present investigation are summarized and prospects to improve control of yellowing viruses in sugarbeet are considered. Some questions that remain to be answered are discussed.

8.1 Summary of achievements

In a first study (Chapter 2) the incubation period (time between infection and symptom expression) was determined. Estimates of the incubation period are needed to translate observations of disease progress (symptoms) into an approximate time-course of the infection, enabling comparisons with the number and behaviour of vectors at the time of virus dissemination. Literature estimates of the incubation period vary from two weeks to two months. This was confirmed in the present study and explained by the effect of plant development stage and temperature on the expansion rate of the leaves and the development of symptoms. Incubation periods of beet mild yellowing virus (BMYV) were slightly longer than those of beet yellows virus (BYV) and differences in incubation period were found between systemically-infected leaves and leaves inoculated by aphids. Under field conditions, the incubation period was not significantly affected by the age of the inoculated leaf, the number of leaves inoculated, the number of aphids used for inoculation, the aphid species used for inoculation or the source plant of the virus. In a given field (growing conditions) and year (weather) the incubation period was therefore determined only by the infection date, though individual plants differed in the time needed for symptom expression.

Studies described in Chapter 3 demonstrate that all leaves emerging after the infection of a plant become systematically-infected. The other leaves remain green and healthy except the few that have been inoculated by aphids. Starting from the growing point, four categories of leaves can be discerned on infected plants: (1) expanding, green, infected leaves, (2) yellow, infected leaves, the intensity of the symptoms increasing with leaf age, (3) healthy, green leaves and (4) leaves inoculated by aphids. As the plants grow, the oldest leaves in category (1) develop symptoms

and end up in category (2), while the leaves in category (2) develop more severe symptoms. Leaves of category (3) age and drop off the plant.

The number of leaves on a plant on the infection date is approximately equal to the leaf number of the oldest leaf with systemic symptoms (leaves numbered in ontogenetic sequence). Therefore this leaf number marks the infection date. Especially in the early growing season, the infection date can be readily assessed by determing the date at which the plant had a number of leaves equal to the leaf number of the oldest yellow leaf (provided that it is not inoculated by aphids). This method may provide a useful new tool in monitoring epidemics of yellowing viruses. The method may be very time consuming and inconvenient in older beet crops, however, because old leaves drop off the plant and allowance has to be made for the different leaf appearance rates of individual plants.

The theoretical calculations in Chapter 4 demonstrate that high infection percentages should be avoided in bait plant tests for the determination of infection pressure because the number of vectors can then not be estimated accurately. When the infection pressure is high it may be necessary to expose the plants in the field for only a few days instead of a week, which is usual. Smaller numbers of bait plants may then be used. Such an approach will give more accurate estimates of infection pressure which are also better differentiated in time. Attention should be given to the biological principles and uncertainties underlying the results of these tests. For instance, do vectors discriminate between bait plants and crop plants? Is alightment *at random*? Is the susceptibility of bait plants and crop plants equal?

The Chapters 5 and 6 describe studies of the dissemination of yellowing viruses from experimentally-infected plants by the major vector, *Myzus persicae*. The incubation period was used to translate the observed course of disease progress (symptoms) into an approximate time-course of the infection. The progress of the infection was compared to the population dynamics and dispersal of *M. persicae* as assessed by weekly counts on a number of sample plants at different distances from the inoculated plants. These studies, as well as those described in Chapter 2 and 3, were possible due to a low natural infection pressure with yellowing viruses and *M. persicae* in the years 1984 to 1986. The effects of two major factors determining secondary spread were examined: (1) date of inoculation (primary infection) and (2) sowing date of the crop. These studies showed

that plant development stage is a key factor determining the amount of secondary spread resulting from a primary infection. Aphid dispersal and the concurrent dissemination of virus started after adjacent plants made leaf contact. In April-sown sugarbeet this leaf contact is usually made around 15 June. Primary infections made before this critical development stage resulted in rapid and extensive secondary spread throughout the month of July. Thus, about 2000 plants developed symptoms in August and September in the plots in which a few plants were experimentally-infected in May. Primary infections made after 15 June caused negligible spread. However, primary infections made at the end of June in a late-sown crop, in which the plants were in an early development stage, resulted in extensive spread. This higher rate of spread in young crops could be explained by (1) a higher rate of reproduction of *M. persicae* on young plants; (2) a better acceptance of young plants by *M. persicae*, allowing more efficient virus transmission; and (3) a shorter latency period (time between infection and possibility of virus acquisition), at least for BYV.

In some fields the buildup of populations of viruliferous aphids on infected plants was prevented by coccinellid predators. In this way these predators hampered virus spread. The possibility of control of virus yellows epidemics by naturally occurring predators deserves further study.

In Chapter 7 the reduction of yield caused by BYV is explained in terms of four damage components: (1) smaller size of individual infected leaves; (2) reduced light absorption by yellow leaves; (3) reduced rates of photosynthesis in yellow leaves; and (4) increased respiration in infected leaves. The impairment of photosynthesis is, according to model calculations, the most important damage component. Photosynthesis was almost completely inhibited in infected leaves with clear symptoms, while infected leaves which were still green photosynthesized at approximately normal rates. Therefore, the development stage of the plant and the leaf area index on the infection date determine the reduction of yield. Plants infected before the 10-leaf stage (mid-June) incur a maximum yield loss of approximately 50% as almost their entire leaf area becomes infected. In these plants most dry matter is produced by the infected, green leaves in the centre of the plants. Plants infected mid-July or later incur a negligible yield loss because ample healthy leaf area has developed and because the infected leaves remain small naturally. Additionally, they take a long time to become yellow.

8.2 Prospects for improved control of virus spread

The present study was aimed to obtain more insight into the secondary spread of yellowing viruses in sugarbeet, not to improve control measures. Nevertheless some remarks may be made. Studies described in Chapter 5 and 6 showed that the risk of spread depends strongly on plant development stage at the primary infection date; in none of the early-sown crops did primary infection after 15 June result in secondary spread of any importance. Plant development stage strongly affected yield loss as well, infections after 15 July causing negligible damage, while inoculations in early-June cause approximately 50% yield reduction. The Dutch warning scheme for virus yellows takes account of the crop development stage. The warning threshold for *M. persicae* control increases with the development stage of the crop. However, differences in development stage between individual fields are not considered. Furthermore, spray warnings are based on the regional population development of *M. persicae*, not on the situation in the specific field. Because the studies described in this thesis demonstrate large effects of plant development stage on secondary spread and yield reduction, the development stages of individual crops and the vector population in them should be considered in decisions on control measures. In that case, farmers will have to make their own observations on vectors and distinguish between the virus transmitting *Myzus persicae* and the relatively harmless potato aphid, *Macrosiphum euphorbiae* and black bean aphid, *Aphis fabae*. Recommendations taylored to the situation in the individual fields can probably further decrease the number of aphicide applications in sugarbeet without increasing the damage by yellowing viruses. This will optimize the farmers' financial result and protect the environment and public health. Additionally, it will avoid or postpone the selection of insecticide-resistant *M. persicae* (Rice *et al.*, 1985; Ffrench-Constant *et al.*, 1987).

In Chapter 5 three phases of spread were distinguished. (1) An introduction phase in which viruliferous vectors colonize the crop. This phase spans the period from seedling emergence untill mid-June. (2) An establishment phase in which some aphids disperse, thereby spreading virus and increasing the number of infection sources in the crop. This phase approximately spans the second half of June. (3) A dispersal phase during

the month of July, in which viruliferous aphids disperse, possibly in response to decreasing host plant quality, increasing disturbance by predators and/or crowding. Sprays during each of these phases will interfere in a different way with yellowing virus epidemics. Granular insecticides applied in the seed furrow or sprays applied in early-June reduce the number of primary infections. Sprays in the second half of June may hamper the establishment of vector colonies and infection sources in the crop, thereby preventing later epidemic spread. Sprays in July interfere directly with secondary spread. The effectiveness of sprays applied at different dates may be evaluated in the light of these different phases of spread that are affected. The different degrees of damage inflicted upon plants infected in different development stages can thereby be taken into account.

8.3 Directions for further research

The amount of secondary spread is not a simple function of plant development stage on the infection date. In May-sown plots, inoculated on 23 June (4-leaf stage; Chapter 6) 400 to 500 plants became infected, while in April-sown plots inoculated in a similar development stage (30 May; Chapter 5) approximately 2000 plants became infected. This difference in secondary spread may be explained by (1) a more rapid development of the May-sown plants, due to the higher temperatures following inoculation at the end of June, and/or (2) a greater impact of predators later in the season. Probably both explanations are valid. To obtain more and quantitative insight into their relative importance, it would be helpful to evaluate these hypotheses with a simulation model of secondary spread. Such a model has been constructed (Riesebos, 1988) but due to lack of reliable input relations it is as yet difficult to draw conclusions from the simulation results. Important imput relations for a model that need to be better quantified are: (1) walking behaviour of the aphids as a function of plant development stage and acceptability; (2) virus transmission in relation to feeding behaviour and host plant acceptability; and (3) the impact of predators on the population dynamics and behaviour of the aphids.

The work on components of damage by BYV (Chapter 7) suggests that the mechanisms that curtail the production of BYV-infected beet plants

have been adequately described and quantified. To make the model generally applicable, quantitative descriptions of the growth of the leaves, the development of symptoms and the impairment of photosynthesis have to be introduced. The model must be further validated and more sensitivity analyses must be made. It would be promising to make a study, similar to the one with BYV in the present investigation, on the components of damage by BMYV and by infection with a mixture of both viruses. Such an analysis could resolve the confusion that exists with regard to the size of the yield reduction caused by BYV, BMYV or mixed infections. Different isolates of the viruses or different sugarbeet varieties may be compared with regard to the most important damage component, impaired photosynthesis, in order to predict the overall effect of infection on yield.

The damage components of BYV were adequately described on the integration level of the leaves as indicated by the good accordance between model predictions and experimental results (Chapter 7). However, at a lower integration level the sequence of physiological disturbances that results in the accumulation of sugars, yellowing of leaves, impairment of photosynthesis, reduction of leaf size and increase of respiration is poorly understood (Peters, 1988). Studies in this area are needed to obtain a better understanding of the biochemical and cell physiological principles underlying the damage caused by yellowing viruses.

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SAMENVATTING

In dit proefschrift wordt onderzoek aan de epidemiologie en opbrengsteffecten van bietevergelingsvirussen beschreven. Allereerst werd de incubatieperiode bepaald, dat is de tijd die verstrijkt tussen infectie van de plant en het verschijnen van ziektesymptomen (hoofdstuk 2). Kennis van de incubatieperiode is essentieel voor het leggen van een relatie tussen het aantal en het gedrag van virusvectoren in een bietenperceel, voornamelijk de groene perzikbladluis, *Myzus persicae*, en de introductie en verspreiding van vergelingsvirussen, later tot uiting komend in een toename van het aantal planten met symptomen. Gepubliceerde schattingen van de incubatieperiode lopen uiteen van twee tot negen weken. Deze variabiliteit werd in onze proeven ook gevonden en kon worden verklaard door de invloed van de temperatuur en het ontwikkelingsstadium van de plant op de groeisnelheid van de bladeren en de expressie van virussymptomen. Naarmate de plant ouder is of de temperatuur lager, groeien de bladeren langzamer en duurt het langer voordat de symptomen zich manifesteren. Daardoor neemt de incubatieperiode gedurende het groeiseizoen sterk toe. De incubatieperiode van het zwakke vergelingsvirus (BMYV) bleek iets langer te zijn dan die van het sterke vergelingsvirus (BYV). Verder bleek de lengte van de incubatieperiode niet of nauwelijks te worden beïnvloed door de ouderdom van het geïnoculeerde blad, het aantal geïnoculeerde bladeren, het aantal bladluizen gebruikt voor inoculatie, de bladluissoort of de bronplant van het virus.

Proeven beschreven in hoofdstuk 3 tonen aan dat alle bladeren die na succesvolle inoculatie van een plant verschijnen systemisch besmet raken. De andere, oudere bladeren blijven gezond en groen afgezien van de bladeren die zijn geïnoculeerd. De systemisch besmette bladeren vertonen de eerste tijd na verschijnen nog geen symptomen; ze vergelen pas als ze volgroeid zijn. Dientengevolge zijn aan een besmette plant vier categorieën bladeren te onderscheiden (Fig. 3.3B), van jong naar oud: (1) groeiende, systemisch besmette bladeren zonder symptomen, (2) volgroeide, systemisch besmette bladeren met symptomen, (3) volgroeide, niet besmette bladeren en (4) volgroeide, geïnoculeerde bladeren met symptomen. De oudste bladeren van categorie 2 zijn de langdurigst besmette bladeren aan een plant en hebben dienovereenkomstig de zwaarste symptomen. Op bladeren dichter bij het groeipunt worden de symptomen gaandeweg zwakker en is alleen de

bladtop vergeeld. Tijdens de groei van de planten komen er nieuwe bladeren van groep 1 bij en gaan de oudste bladeren van groep 1 over in groep 2. De symptomen op de gele bladeren worden mettertijd steeds intenser. Uiteindelijk ontstaan er bruine vlekken en sterft het blad af. De bladeren van categorie 3 verouderen en vallen van de plant.

Het aantal bladeren aan de plant op de infectiedatum bepaalt welk blad het oudste systemisch besmette blad zal zijn. Daarom kan de infectiedatum afgeleid worden uit de positie van dit blad. Vooral in het vroege groeiseizoen kan de infectiedatum gemakkelijk geschat worden door uit te rekenen op welke datum het oudste systemisch besmette blad verscheen. Toepassing van deze methode in het veld gaf goede resultaten. Een dergelijke methode voor bepaling van de infectiedatum zou ook een nuttig instrument kunnen zijn in de bestudering van de epidemiologie van verscheidene andere virusziekten. In suikerbieten wordt toepassing van de methode bemoeilijkt door de grote verschillen in bladafsplitsingssnelheid tussen individuele planten. Ook kan het vaststellen van het aantal afgestorven bladeren problemen opleveren. Deze bezwaren spelen nauwelijks een rol in jonge gewassen. Hier kan de methode goed worden gebruikt en levert hij nauwkeurige resultaten.

Theoretische berekeningen in hoofdstuk 4 tonen aan dat bij vangplantexperimenten ter bepaling van vectordruk hoge infectiepercentages vermeden dienen te worden omdat anders het aantal vectoren niet nauwkeurig kan worden bepaald. Het kan bij hoge vectordruk nodig zijn meerdere malen per week planten in het veld uit te zetten om zodoende het infectiepercentage te beperken. Eventueel kan dan met kleine aantallen vangplanten worden volstaan. Zo'n benadering leidt tot nauwkeuriger schattingen van het aantal vectoren en bovendien tot een in de tijd beter gedifferentieerde schatting van de vectordruk.

In de hoofdstukken 5 en 6 worden proeven beschreven over de verspreiding van bietevergelingsvirussen door *M. persicae* vanuit kunstmatig besmette planten. Het effect van twee sleutelfactoren werd onderzocht: (1) de datum van inoculatie (primaire infectie) en (2) de zaaidatum van het gewas. De resultaten tonen aan dat het gewasontwikkelingsstadium op het tijdstip van primaire infectie in grote mate de secundaire virusverspreiding bepaalt. Er werd waargenomen dat de verspreiding van de bladluizen en de hiermee samengaande verspreiding van virussen begon nadat naburige planten bladcontact maakten. Dit kritieke ontwikkelingsstadium wordt normaliter bereikt

rond 15 juni. Inoculaties vóór 15 juni resulteerden in grootschalige secundaire verspreiding in de loop van juli. Inoculaties ná 15 juni leidden uitsluitend tot noemenswaardige virusverspreiding in laat gezaaide suikerbietenveldjes waar de planten nog in een vroeg ontwikkelingsstadium verkeerden. De grote mate van verspreiding in jonge gewassen bleek samen te hangen met: (1) een snellere populatiegroei van *M. persicae* op jonge planten, (2) een betere acceptatie van jonge planten door *M. persicae* waardoor de overdracht van virussen wordt vergemakkelijkt, en (3) een kortere latentieperiode, als gevolg waarvan jonge planten spoedig na infectie een verspreidingbron van het virus worden.

In een aantal velden werd de opbouw van bladluispopulaties en, daarmee samenhangend, de verspreiding van virussen belemmerd door de aanwezigheid van predatoren, vooral lieveheersbeestjes. Het nut van predatoren bij de inperking van virusverspreiding dient nader te worden onderzocht.

In hoofdstuk 7 wordt de opbrengstderving veroorzaakt door infectie met BYV, verklaard op basis van vier verstoringen die werden vastgesteld bij viruszieke suikerbieteplanten: (1) kleinere afmetingen van de besmette bladeren, (2) gereduceerde lichtabsorptie door gele bladeren, (3) gereduceerde fotosynthese in gele bladeren, en (4) verhoogde ademhaling in besmette bladeren. Modelberekeningen tonen aan dat component 3, de verminderde fotosynthese, de grootste bijdrage levert aan de schade. De reductie van de fotosynthese was evenredig met de intensiteit van de geelverkleuring van de bladeren; besmette bladeren die nog groen waren vertoonden geen reductie in fotosynthese terwijl de fotosynthese in intens gele bladeren vrijwel tot nul was gereduceerd. Aangezien de schade voornamelijk het gevolg is van gereduceerde fotosynthese in gele bladeren en het aandeel gele bladeren sterk afneemt met latere infectie, neemt de opbrengstderving met latere infecties snel af. Infecties na medio juli zijn goeddeels onschadelijk.

De verkregen resultaten tonen aan dat het gewasontwikkelingsstadium een grote invloed heeft op de populatiedynamica van *M. persicae*, de mate van virusverspreiding, de ontwikkeling van vergelingssymptomen en de grootte van de opbrengstderving. Daarom moet bij het nemen van bestrijdingsmaatregelen het gewasontwikkelingsstadium in overweging worden genomen.

CURRICULUM VITAE

Wopke van der Werf werd 2 juli 1959 te Hengelo (Overijsel) geboren. In 1977 behaalde hij het diploma Gymnasium-B aan het lyceum 'Bataafse Kamp' te Hengelo. Vervolgens begon hij aan de Landbouwuniversiteit te Wageningen de studie in de planteziektenkunde. Hiervan werd in 1983 het diploma met lof verkregen. Het vakkenpakket omvatte de entomologie, de fytopathologie, de theoretische teeltkunde en de wiskundige statistiek. Van januari 1984 tot januari 1987 verrichtte de promovendus bij de vakgroepen Virologie en Theoretische Teeltkunde (thans Theoretische Produktie-ecologie) het onderzoek beschreven in dit proefschrift. Sinds september 1987 is hij verbonden aan de vakgroep Theoretische Produktie-ecologie als universitair docent in de populatiedynamica.