The incubation period of beet yellowing viruses in sugar-beet under field conditions

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Abstract

In three years field trials, the incubation period, i.e. the time between infection and the appearance of symptoms, of beet yellows virus (BYV) and beet mild yellowing virus (BMYV) increased with later infection during the growing season. The incubation period of BYV, a closterovirus, increased from 3 weeks in young plants infected before canopy closure, to 9 weeks in old plants infected in August. The incubation period of BMYV, a luteovirus, increased from 4 to 5 weeks in young plants to 9 weeks in old plants. Symptoms were observed c. one week earlier on the inoculated leaves than on those infected systemically, except on young BYV-infected plants. On these plants, symptoms developed in 3 weeks on both leaf types.

The incubation period decreased at increasing temperature, a fixed temperature sum being required for the development of symptoms on plants of a certain age. This temperature sum increased with plant age. Symptom development was related to leaf growth; the systemic symptoms appeared after the infected leaves attained their final size. Young, expanding leaves did not show symptoms. Thus the development of symptoms seems to be related to physiological conditions occurring only in full-grown leaves. A low rate of leaf expansion may constitute the underlying reason for the long incubation period of virus symptoms in old plants and at low temperatures.

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 and (4) the source plant of BMYV, beet or shepherd's-purse, *Capsella bursa-pastoris*. The incubation period can be used to obtain rough estimates of the infection-date of individual plants, given the date on which symptoms appear.

Additional keywords: beet yellows virus, closterovirus, beet mild yellowing virus, luteovirus, *Myzus persicae*, local symptoms, systemic symptoms, plant age, temperature sum, leaf growth, estimation of date of infection

Introduction

Virus yellows is an economically important disease of sugar-beet, *Beta vulgaris* ssp. *saccharifera*. Studies in Europe (Russell, 1958, 1963, 1965b; Björling and Möllerström, 1974; Thielemann and Nagi, 1977; Häni, 1979; Smith, 1986; Smith and Hinckes, 1987) have shown that the disease is most often caused by beet mild yellowing virus (BMYV, luteovirus group). Beet yellows virus (BYV, closterovirus group) is less important (Duffus, 1973; Bar-Joseph et al., 1979). Upon infection with BYV or BMYV beet leaves become

thick and brittle, while their starch and sugar content increases (Watson and Watson, 1951). The photosynthetic capacity of the plant decreases (Hall and Loomis, 1972a,b). Symptoms develop on the inoculated leaves, on which aphids infected the beet plant, and on systemically-infected leaves, to which virus has been transported via the phloem system. Systemic infection extends to a few young leaves present in the heart of the plant on the infection-date and to all leaves that appear later (Roseboom and Peters, 1984; Van der Werf et al., 1989a). Symptoms of BMYV develop with leaf age from pale-yellow to bright-yellow or orange, culminating in necrosis as a result of infection by *Alternaria* spp. (Russell, 1964a,b). BYV causes vein clearing in the first leaves expanding after infection. Systemically-infected leaves become yellow when they mature and develop typical red or necrotic spots subsequently.

The green peach aphid, *Myzus persicae*, is the major vector of beet yellowing viruses in the field (Watson et al., 1951; Björling, 1952; Heathcote, 1966). The black bean aphid, *Aphis fabae*, is a second, much less important vector of BYV. This aphid species does not transmit BMYV (Russell, 1963; Björling and Nilsson, 1966); however, Thielemann and Nagi (1979) and Karl and Giersemehl (1981) came to a different conclusion. The population dynamics of *M. persicae* and the concomitant spread of beet yellowing viruses have been studied by many authors (a.o. Björling et al., 1951; Björling, 1952; Watson and Healy, 1953; Ribbands, 1963; Kershaw, 1965; Watson and Heathcote, 1966). Though these studies have learnt a lot about the epidemiology of beet yellowing viruses, they do not shed much light on the relationship between aphid vector population dynamics in time and space and the concomitant spread of virus, which becomes manifest after the incubation period (the time period between infection and the appearance of symptoms; Van der Plank, 1963; Bos et al., 1985). Therefore, the incubation period of the disease must be known. However, no systematic observations on the incubation period of BYV and BMYV under field conditions have been made.

Reports in the literature indicate that the incubation period of beet yellowing viruses varies widely according to inoculation-date (Wiesner, 1959; Thielemann and Nagi, 1977), weather (Wiesner, 1959), year (Thielemann and Nagi, 1977), vector species (Thielemann and Nagi, 1977) and number of vectors (Steudel, 1958). The work presented here has two aims: (1) to identify the factors which have a great influence on the incubation period, and (2) to provide estimates of the incubation period for analyses of experiments on virus spread (Van der Werf et al., in prep.).

Materials and methods

Arrangement of experiments. The effect of five factors on the incubation period was studied: (1) infection-date: May to August, (2) number of vector aphids, *M. persicae*: 1 to 30, 10 as a standard, (3) number of inoculated leaves: 1 (standard) to 4, (4) development stage of inoculated leaf: expanding, just full-grown (standard) or ageing, (5) sowing date: mid-April (standard), end of May or early-July.

In a few plots, inoculations with BYV were made using *A. fabae* to study a possible effect on the incubation period. Additionally, in 1986, inoculations were made with *M. persicae* which had acquired BMYV from shepherd's-purse, *Capsella bursa-pastoris*, instead of beet. Further details are given below.

Experiment I. Sugar-beets 'Regina' were sown on 24 April 1985 on a riverclay soil

242

at the Binnenhaven in Wageningen. Each combination of the experimental factors 1 to 4 was applied to two duplicate rows of 15 plants. To inoculate a plant 10, 5, 2 or 1 *M. persicae* were used (factor 2). One or three leaves were inoculated (factor 3). Twelve plots of 2.5 × 5 m² were sown on 29 May (factor 5).

Experiment II. Sugar-beets 'Bingo' were sown on 25 April 1986 on a riverclay soil at the Haarweg in Wageningen. Each combination of the experimental factors 1 to 4 was of applied to four duplicate rows of 8 to 10 plants. To inoculate a plant, 30, 10 or 2 *M. persicae* were used (factor 2). One or 4 leaves were inoculated (factor 3). Plots of at least 2.5 × 5 m² were sown on 26 or 28 May and on 3 July (factor 5).

Additional observations on the incubation period of BYV and BMYV were made in five other fields in single or duplicate rows of 15 to 30 plants.

Myzus persicae culture. Virus-free *M. persicae* from a clone named M3, were cultured in the glasshouse on leaf 3 and leaf 4 of 5 to 6-leaved oilseed rape plants, *Brassica napus* ssp. *oleifera* (leaf 1 is the first leaf following the cotyledons). Every day a new cohort of 0-24-hour-old nymphs was started. The temperature in the glasshouse varied between 18 and 30 °C and the photoperiod was at least 16 h day [1]. Because oilseed rape is immune to BMYV and BYV (Russell, 1965; Björling and Nilsson, 1966) no BMYV could be introduced into the BYV culture by aphids from rape. Oilseed rape is susceptible to beet western yellows virus (BWYV), which is closely related to BMYV, but this virus was not detected in periodical tests of *M. persicae* from the culture, using *Physalis floridana* as a test plant (Duffus, 1973).

Virus cultures. BYV and BMYV were maintained in beet in two insect-proof glasshouse compartments located at 200 m distance from each other. The strain of BYV used causes moderately-severe symptoms, viz. vein clearing in young leaves and necrotic spots on fully mature leaves (Björling, 1961). To keep the BYV-culture free from possibly contaminating BMYV, the virus was periodically transmitted to healthy plants either by *A. fabae*, which does not or hardly transmit BMYV, or by *M. persicae* using a 4-hour acquisition access period, immediately 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 recovered, using *M. persicae* as a vector and sugar-beet or *C. bursa-pastoris* as test plants.

Inoculation. When available, viruliferous *M. persicae* were taken from virus-infected beet plants in the glasshouse. During the summer, however, the glasshouse populations on beet collapsed so that other methods had to be used to produce viruliferous aphids.

In 1985, *M. persicae* from rape were brushed onto detached BYV- or BMYV-infected beet leaves lying in large Petri dishes or standing with their petioles upright in small bottles in glass jars. 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 pass a 200 µm mesh nylon gauze with their stylets to reach the leaf. Only about 10% of the inoculated plants became infected.

In 1986, adult *M. persicae* from rape were clip-caged onto yellow leaves of BYV-infected sugar-beet 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). Using this method, about 90% of the plants became infected.

After an inoculation access period of 1 or 2 days, the aphids were killed manually. The plants were sprayed with the carbamate-insecticide pirimicarb. In June 1985 aldicarb granules were added to the soil because prolonged rainy weather hindered the application of foliar insecticides. During summer, the inoculated plots were treated weekly with insecticides, pirimicarb or oxy-demeton-methyl, to control immigrant aphids and limit virus spread from the inoculated plants.

Recording of symptoms. In 1985, the infected plants were inspected for symptoms weekly, if possible with cloudy weather as symptoms are easily overlooked in direct sunlight. In 1986, the inspections were made at intervals increasing from 4 to 5 days in June to 10 days in October. In these inspections, inoculated leaves, which were marked with a plastic label around the petiole, were distinguished from systemically-infected ones. Systemic symptoms which were readily discernible at a meter distance were evaluated positively. Inoculated leaves were judged with the same criterion, lifting healthy leaves covering inoculated ones if necessary. In some plots in Experiment II, the time needed for the development of vein clearing symptoms of BYV was determined.

Selection of plants for analysis. In the type of field study described here, precautions must be taken to exclude naturally-infected plants from the analysis. A plant was included in the analysis if four criteria were met:
(1) symptoms of the inoculated virus present on the inoculated leaf,
(2) no symptoms on leaves already mature on the infection-date, other than the inoculated one,
(3) normal phyllotactic pattern of systemic symptoms, viz. the first and most severe symptoms appearing on a leaf implanted above the inoculated leaf (Van der Werf et al., 1989a), and
(4) no virus infection in neighbouring non-inoculated plants.

Because *M. persicae* was scarce in 1985, only a few plants had to be excluded, most of them in late-sown plots. In 1986, some plants were excluded in early-sown plots and all of six plots sown in July.

Data analysis. Proportions of plants showing symptoms for the first time were plotted against time to determine the median incubation period. Plants from different plots with the same treatment were lumped in this analysis, ignoring minor differences in incubation period between plots. To assess the variation in incubation period between plants, logistic growth curves were fitted to the data obtained in Experiment II:

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

equivalent with

$$\ln(p/(1 - p)) = (t - \mu)/s \tag{2}$$

in which p is the proportion of plants showing symptoms, t is time since inoculation,

244

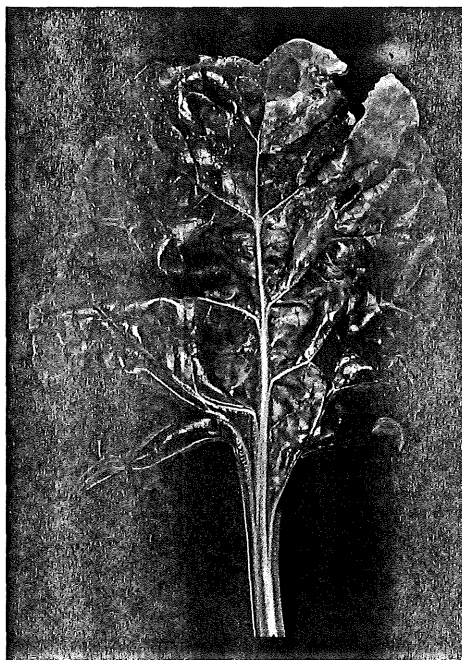Fig. 1. Yellow spot on leaf inoculated with beet yellows virus; photograph taken on 6 September 1985.

Fig. 2. Basipetal development of symptoms on sugar-beet leaf inoculated with beet yellows virus; photograph taken on 6 September 1985.

Fig. 3. Acropetal development of symptoms on sugar-beet leaf inoculated with beet mild yellowing virus; photograph taken on 6 September 1985.

μ is the median incubation period and s is the scale parameter of the logistic distribution. 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 obtained by multiplying the scale parameter s with the standard deviation of the logistic distribution, $\pi/\sqrt{3}$:

$$\sigma = s \times \pi/\sqrt{3} \tag{3}$$

Measurements of leaf growth. In Experiment II, leaf growth was measured with a ruler on 10 healthy plants sown on 25 April and on 10 plants sown on 26 May. For each leaf, leaf expansion was calculated relative to final length. Relative leaf growth curves were averaged for leaves appearing on approximately the same day. Leaves were taken to reach final length at the 95%-point of the average relative leaf growth curve (Milford et al., 1985b).

Results

Development of symptoms during the season. Leaves inoculated with BYV or BMYV in May or June generally developed symptoms on a large portion of the blade within one or a few weeks, while those inoculated in July and August developed only a small yellow spot which expanded slowly (Fig. 1). Throughout the season, BYV- and BMYV-symptoms on inoculated leaves differed from each other in that BYV-symptoms mostly expanded basipetally along the midvein (Fig. 2) while those of BMYV spread acropetally along the leaf margin (Fig. 3). Areas with BYV-symptoms were often more sharply bordered by veins ('sectors'; Figs. 1 and 2) than those of BMYV. Local BYV-symptoms became often brighter yellow than systemic symptoms, thus resembling those of BMYV. The spots typical for BYV-infection became larger on the inoculated leaves than on the systemically-infected ones and exhibited a red colour instead of brown.

The brightest yellowing symptoms, especially those of BMYV, were observed when the plants were grown under cool conditions, e.g. in a cooled glasshouse or at the end of the season, while vaguer symptoms developed under warm conditions in the glasshouse or in the field in mid-summer. However, necrosis and vein clearing, caused by BYV, was more pronounced at high temperatures.

The incubation period increased during the growing season (Fig. 4). Symptoms of BYV appeared in 3 weeks on plants infected in May or the first two decades of June, while the incubation period gradually increased with plant age after canopy closure, at the end of June. Plants inoculated at the end of August showed local BYV-symptoms after 6 to 7 weeks and systemic symptoms after 9 weeks. When plants were infected with BMYV in May or June, local symptoms appeared after 3 to 4 weeks while systemic symptoms developed about a week later. In the course of the season the incubation periods of local and systemic BMYV-symptoms increased to 5 and 9 weeks, respectively.

Effect of inoculation conditions. The incubation period of systemic BYV- and BMYV-symptoms was not significantly affected by inoculation conditions, such as (1) the number of vector aphids, *M. persicae*; (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 (Tables 1 and 2). Though inoculation conditions have a small but significant effect on the incubation period under controlled conditions (Björling, 1963),
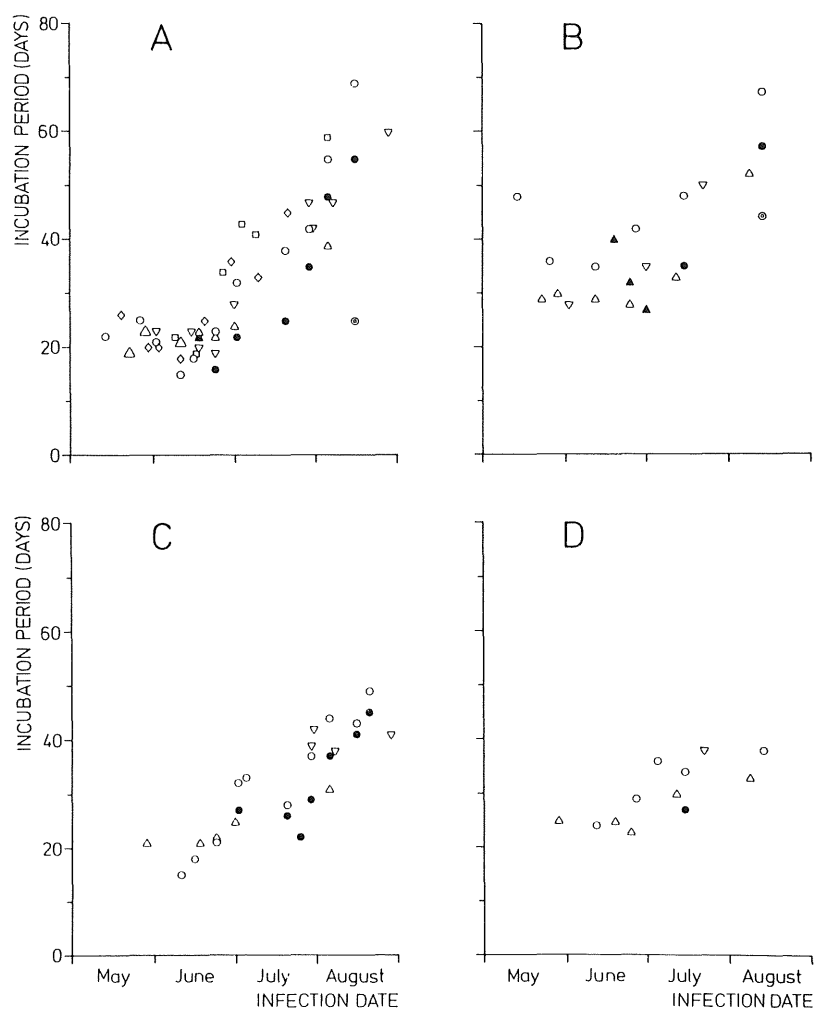
246

Fig. 4. Incubation period of local and systemic symptoms of beet yellows virus and beet mild yellowing virus during the growing season. 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: beet yellows virus, systemic symptoms; B: beet mild yellowing virus, systemic symptoms; C: beet yellows virus, local symptoms; D: beet mild yellowing virus, local symptoms. Observations were made in Wageningen in 1985 (Experiment I; ⊿, ▲), in Wageningen in 1986 (Experiment II; ○, ●, ⊙), in sugar-beet 'Bingo' growing on heavy river-clay near Randwijk in 1986 (□), in sugar-beet 'Regina' growing on sea-clay in the Flevopolder in 1986 (◊) or in other beet crops growing on clay-soils in 1984 and 1985 (⊿).

the size of these effects appears to be small compared to the variation between plants in the field. These influences can therefore be neglected in field studies. 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*.

Table 1. Influence of inoculation conditions on incubation period of systemic beet yellows virus-symptoms (data from Exp. II; see text).

number of M. persicae per plant	development stage of inoculated leaf	number of inoculated leaves	11/12 June			24 June			29 July			15 August		
			$\hat{\mu}$[1]	$\hat{\sigma}$[2]	n[3]	$\hat{\mu}$	$\hat{\sigma}$	n	$\hat{\mu}$	$\hat{\sigma}$	n	$\hat{\mu}_{25}$[4]	$\hat{\sigma}$	n
10	expanding	1	15	2.0	60	24	3.1	40	42	5.4	23	58	11.6	17
10	full-grown	1	16	2.0	32	22	3.1	39	43	5.4	27	–	–	–
10	ageing	1	15	2.0	31	24	3.1	29	48	5.4	19	–	–	–
10	all stages	4	15	2.0	27	–[5]	–	–	–	–	–	53	11.6	42
2	expanding	1	15	2.0	26	–	–	–	42	5.4	21	–	–	–
30	expanding	1	14	2.0	32	–	–	–	40	5.4	21	–	–	–

[1] $\hat{\mu}$ = estimated median incubation period (days).
[2] $\hat{\sigma}$ = estimated standard deviation of incubation period.
[3] n = number of plants observed.
[4] $\hat{\mu}_{25}$ = estimated date on which 25% of the plants showed symptoms (shortly before harvest).
[5] No observations.

Table 2. Influence of inoculation conditions on incubation period of systemic beet mild yellowing virus-symptoms (data from Exp. II; see text).

number of M. persicae per plant	development stage of inoculated leaf	number of inoculated leaves	virus source plant	12/13 July			15 July			13 August		
				$\hat{\mu}$	$\hat{\sigma}$	n	$\hat{\mu}$	$\hat{\sigma}$	n	$\hat{\mu}_{25}$	$\hat{\sigma}$	n
10	expanding	1	beet	33	4.5	25	49	6.7	28	58	8.5	25
10	full-grown	1	beet	32	4.5	22	49	6.7	13	–	–	–
10	ageing	1	beet	36	4.5	22	–	–	–	–	–	–
10	all stages	4	beet	–	–	–	–	–	–	67	8.5	16
2	expanding	1	beet	35	4.5	14	43	6.7	9	–	–	–
30	expanding	1	beet	35	4.5	30	–	–	–	–	–	–
10	expanding	1	Capsella	–	–	–	51	6.7	9	58	8.5	7

For explanation of symbols see Table 1.

Effect of plant age and temperature. May-sown plants exhibited shorter incubation periods than April-sown plants throughout the growing season while plants sown in July developed symptoms most rapidly throughout the season (Figs. 5 and 6). Apparently, the age of the plant affects the incubation period for both categories of infected leaves. High temperature accelerates the development of symptoms. For example, 10-leaved plants sown on 25 April 1986 (Experiment II) and inoculated with BYV on 16 June, showed symptoms after 18 days at an average temperature of 22 °C, while
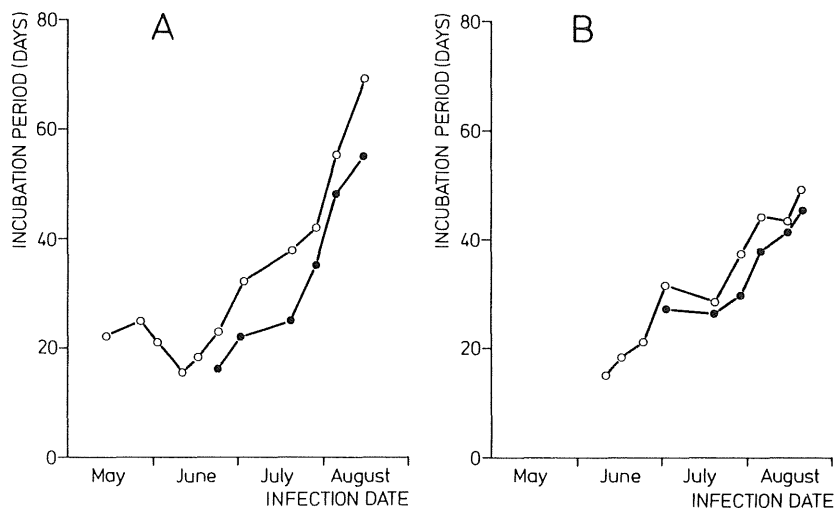
248

Fig. 5. Effect of sowing date, 25 April (o) or 26/28 May (•), on the incubation period of (A) systemic and (B) local beet yellows virus-symptoms throughout the season. Data from Experiment II (see text).
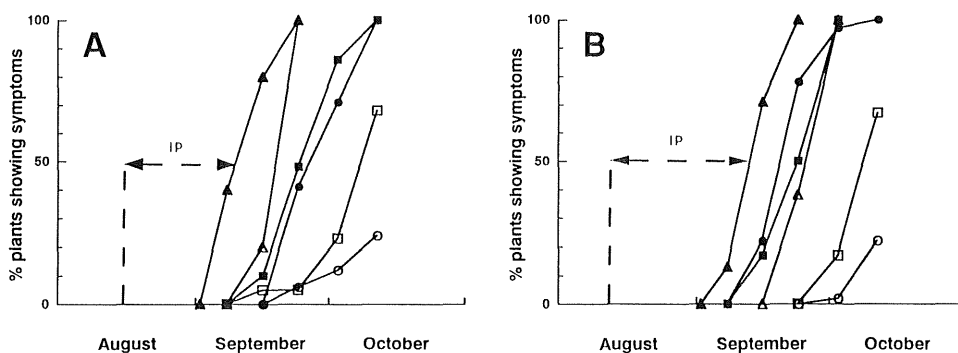

Fig. 6. Effect of plant age on the appearance of symptoms of (A) beet yellows virus, inoculated on 15 August and (B) beet mild yellowing virus, inoculated on 13 August 1986. Sowing dates: 24 April (o, •), 28 May (□, ■) and 3 July (△, ▲). Open symbols refer to systemic symptoms while solid symbols denote local symptoms. The median incubation period (IP) of local symptoms on plants sown in July is indicated by the horizontal hatched arrows. Data from Experiment II (see text).

10-leaved plants sown on 3 July and inoculated on 15 August showed symptoms after 35 days at temperatures averaging 10 °C. Allowing for the temperature effect by expressing the incubation period in °C days (De Wit and Goudriaan, 1978) shows that the thermal incubation periods were approximately similar, 253 °C days for the plants inoculated on 16 June and 292 °C days for those inoculated on 15 August. Thus, it is shown that temperature and plant age are major factors determining the incubation period. Fig. 7 combines the influences of temperature and plant age, as indexed by the
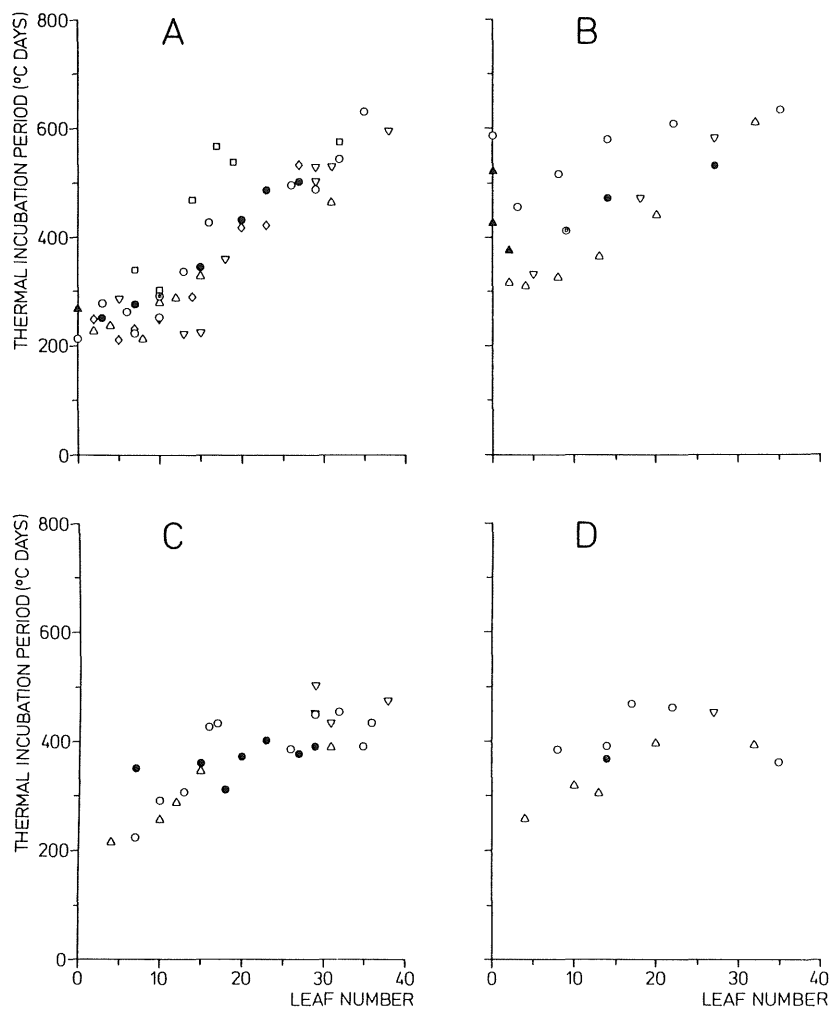
Fig. 7. Thermal incubation period (°C days above 3 °C) of beet yellows virus and beet mild yellowing virus in sugar-beet as a function of plant leaf number on the inoculation-date. Meaning of symbols and lettering as in Fig. 4.

number of leaves on the plant, on the incubation period, showing that a good relation exists between plant leaf number and the thermal incubation period.

Relation between symptom expression and leaf expansion. The first leaves to show symptoms of systemic infection by yellowing viruses are those that just appear ($\geq$ 3 cm) at the moment of inoculation (Van der Werf et al., 1989a). The course of the incubation period during the growing season showed much the same trend as the duration of blade expansion of newly appearing leaves, as measured on healthy plants (Fig. 8). Incipient symptoms of BYV were observed when leaves of the same age on healthy plants approached final length. A week later, the symptoms were evaluated as 'clear'. Incipient
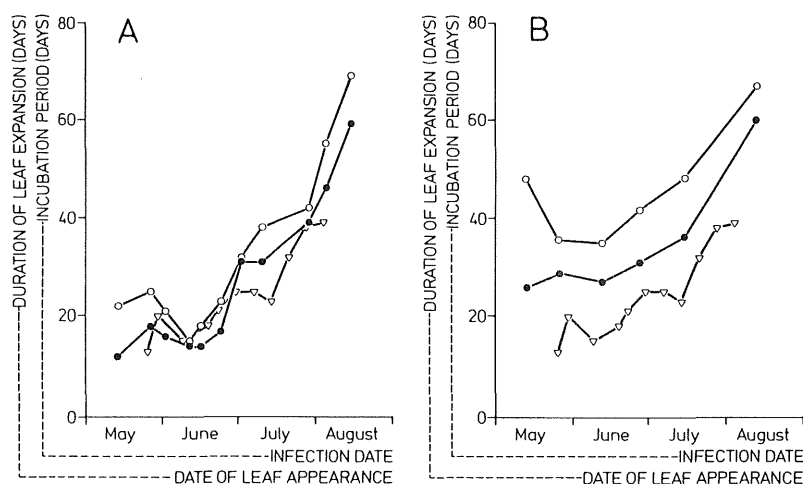
250   *Neth. J. Pl. Path. 95 (1989)*

Fig. 8. Duration of leaf expansion (∇) and incubation period of incipient (●) and clear (○) systemic symptoms of (A) beet yellows virus or (B) beet mild yellowing virus in sugar-beet during the growing season. Data from Experiment II (see text).

symptoms of BMYV were noticed one to two weeks after completion of leaf expansion on healthy plants, while it took another one to two weeks before symptoms became clearly visible. These results suggest that physiological conditions characteristic for fully-expanded leaves are prerequisite for the development of symptoms. Furthermore, these results suggest that the low leaf expansion rate in old plants and at low temperatures is the underlying reason for the longer incubation period under these conditions.

The long incubation period in plants infected with BMYV in the cotyledon stage is another example of the relationship between symptom development and leaf expansion. Groups of plants sown on 29 May 1985 (Exp. I) were inoculated with BMYV on 19 June, 25 June or 1 July when they were in the early cotyledon stage, late cotyledon stage and the 2-leaf-stage, respectively (growth stages according to Lutman and Tucker, 1987). The plants of these three groups showed symptoms simultaneously around 31 July when leaf 3 and 4 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.

Vein clearing.   In Exp. II, vein clearing appeared 50 - 60 °C days before leaf yellowing symptoms, throughout the season (Fig. 9). The relation between thermal incubation period and plant age (leaf number) is independent of sowing date (Fig. 9).

Variation between plants.   Fig. 10 shows the time-course of symptom development in Experiment II. The development of symptoms in groups of inoculated plants is adequately described with logistic growth curves, such that the variability of the incubation period among plants can be estimated with Eq. 3 (Table 3). The largest variation between plants is obtained when the incubation period is long, at the end of the season. The coefficient of variation of the incubation period ranges from 5% to 24%
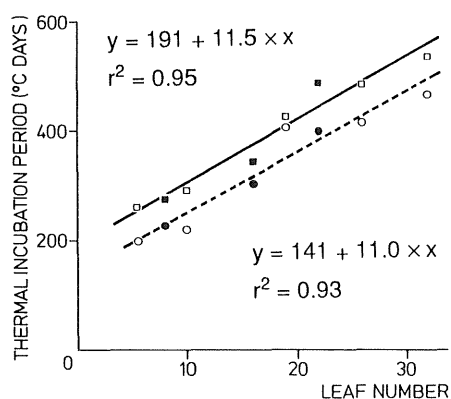
$$y = 191 + 11.5 \times x$$
$$r^2 = 0.95$$

$$y = 141 + 11.0 \times x$$
$$r^2 = 0.93$$

Fig. 9. Comparison of the temperature sum needed for the development of vein clearing (□, ■; hatched line) and yellowing symptoms (o, ●; solid line) after inoculation with beet yellows virus. Data from Experiment II (see text). Plants sown on 25 April denoted by open symbols and those sown on 26 May by solid ones.

Table 3. Incubation period of systemic symptoms of infection by beet yellows virus and beet mild yellowing virus in relation to sowing date (Data from Experiment II; see text).

Inoculation-date	Virus; sowing date											
	BYV; 25 April				BMYV; 25 April				BYV; 26/28 May			
	$\hat{\mu}$[1]	$\hat{\sigma}^{2}$[2]	CV[3]	n[4]	$\hat{\mu}$	$\hat{\sigma}$	CV	n	$\hat{\mu}$	$\hat{\sigma}$	CV	n
14 May	25	4.5	18	18	51	3.6	7	11	−[5]	−	−	−
26 & 27 May	23	2.5	11	21	37	4.5	12	22	−	−	−	−
2 June	22	1.1	5	43	−	−	−	−	−	−	−	−
11 − 13 June	17	2.0	12	208	33	4.5	12	143	−	−	−	−
16 June	22	3.8	17	25	−	−	−	−	−	−	−	−
24 June	23	3.1	13	108	−	−	−	−	14	2.2	15	44
27 June	−	−	−	−	43	2.9	7	31	−	−	−	−
2 July	35	3.4	10	82	−	−	−	−	26	4.4	17	37
15 July	−	−	−	−	46	6.7	15	54	−	−	−	−
20 July	37	2.9	8	87	−	−	−	−	25	2.7	11	46
25 July	−	−	−	−	−	−	−	−	29	6.9	24	19
29 July	44	5.4	12	97	−	−	−	−	36	8.5	24	22
5 August	58	5.8	10	85	−	−	−	−	48	4.9	10	79
13 & 15 August	63	11.6	18	31	66	8.5	13	45	56	12.5	22	22
average CV			12				18				11	

[1] $\hat{\mu}$ = average incubation period (days), determined according to Eq. 2.
[2] $\hat{\sigma}$ = standard deviation of incubation period, determined according to Eqs 2 & 3.
[3] CV = variation coefficient (%).
[4] n = number of plants observed.
[5] No observations.

in this experiment with an average value of 13%. This considerable variation among plants is a source of error when estimates of the incubation period are used to determine infection-dates of single plants.
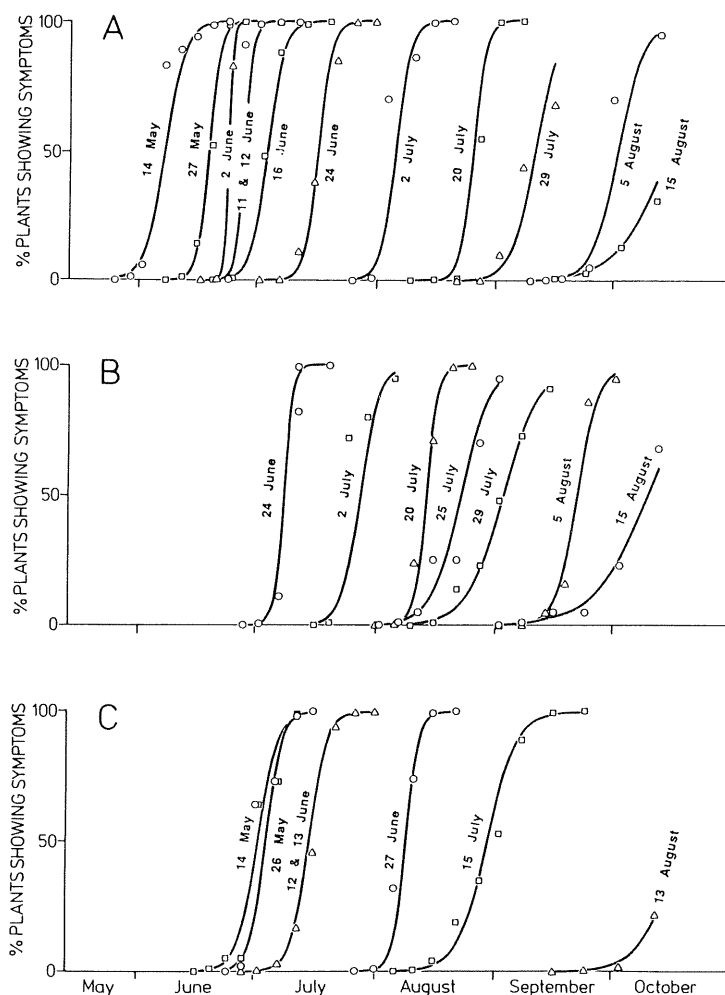
Fig. 10. Time-course of the development of systemic beet yellows virus-symptoms on plants sown on 25 April (A) or 26/28 May 1986 (B) and of systemic beet mild yellowing virus-symptoms on early-sown plants (C), inoculated at different dates. The symbols o, □ and ᴀ denote observations made on batches of plants inoculated at subsequent dates. Data from Experiment II (see text).

Discussion

Incubation periods observed in this study range from less than 3 weeks to more than 9 weeks, confirming the large variation reported in the literature. The present study shows that plant age is the major factor responsible for this variability (Figs. 5 and 6). Glasshouse experiments in sugar-beet by Duffus and Skoyen (1977) show a similar effect of plant age on symptom development of beet curly top virus. In our field experiments, temperature is the second important factor determining the length of the

incubation period, confirming glasshouse studies of Wiesner (1959) and Björling (1963). Ageing of the plants and low temperature explain the long incubation period of yellowing viruses at the end of the season. Two other factors affecting the incubation period are the causal virus, BYV or BMYV, and the nature of the infection, local or systemic (Fig. 4).

Inoculation conditions, such as vector number and species did not substantially affect the incubation period, in contradiction to reports by Steudel (1958) and Thielemann and Nagi (1977). These authors made their observations in field plots, failing to take precautions to exclude naturally-infected plants from their analyses. The inability to distinguish between experimentally- and naturally-infected plants results in an overestimation of the incubation period if a partly successful inoculation is followed by natural spread. Such an erroneous result is likely to be obtained when an inefficient vector is used, e.g. *Aphis fabae* (Thielemann and Nagi, 1977), or when low numbers (1 or 2) of efficient vectors, *M. persicae*, are used (Steudel, 1958). Therefore, it is better, though laborious, to make observations of the incubation period on individual plants in stead of whole plots. In studies with individual plants (Björling, 1963; this study) effects of vector species or number on the incubation period were negligible. The other inoculation conditions studied, age and number of inoculated leaves, did not influence the incubation period.

A considerable variation in incubation period was found between individual plants (Fig. 10; Table 2), probably as a result of differences in heritable properties and growing conditions. Differences in incubation period were also observed among different fields (Figs. 4 and 7). For instance, plants grown near Randwijk in 1986 exhibited a long thermal incubation period (Fig. 7A), probably because drought retarded leaf expansion. Though care was taken to judge with the same criterion in all experiments, inconsistencies in the evaluation of the symptoms could also have made a minor contribution to field to field differences in incubation period.

Because inoculation conditions do not affect the incubation period, estimates of the latter can be used to assess the infection-date, based on observations of the appearance of the first symptoms. In doing so, systemic symptoms should be looked after as local symptoms are easily overlooked. Results from this study may be used as a reference. For this purpose, the infection-date is plotted against the date on which symptoms were first observed in Fig. 11. The formulas given in the legend of the figure yield a point estimate of the infection-date while the hatched lines indicate a 95% confidence interval. The width of the interval is 2 à 3 weeks, indicating considerable uncertainty with respect to the infection-date estimated by means of the incubation period. For early BMYV-infections the uncertainty is even greater than 2 à 3 weeks. In this situation, another method for estimation of the date of infection, based on the position of yellow leaves on the plant (Van der Werf et al., 1989a), may provide a useful alternative. Estimates of the infection-date can be improved by making reference observations in the same crop because $\hat{\sigma}_R$ can then be neglected (see legend Fig. 11). In that case 95% confidence intervals for the infection-date are only 1 à 2 weeks wide, giving sufficient accuracy for application in field studies of virus spread.

Possible mechanisms underlying the longer incubation period in older plants, e.g. delayed virus translocation or retarded multiplication, are discussed in a following paper (Van der Werf et al., 1990).
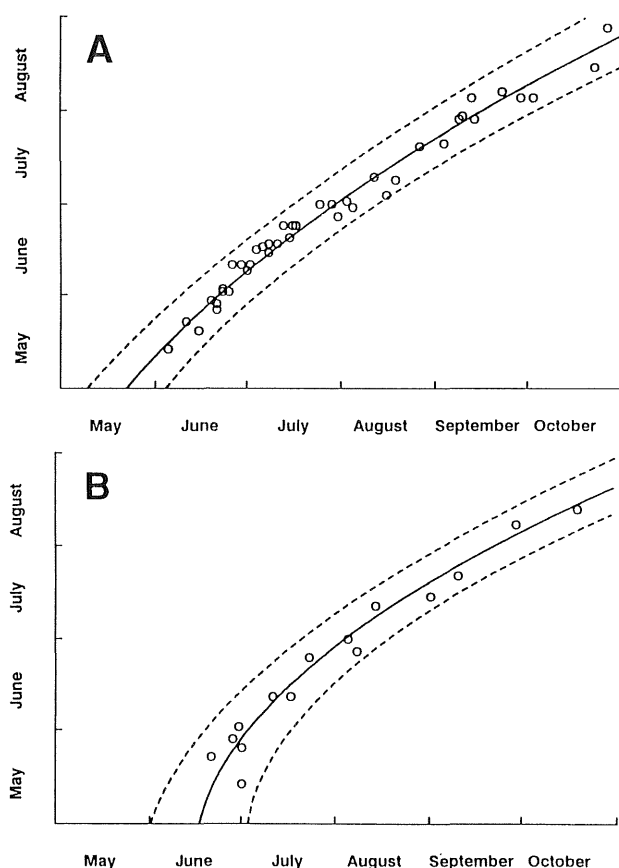
Fig. 11. Estimated date of infection (Y) with beet yellows virus (Fig. 11A) or beet mild yellowing virus (Fig. 11B) as a function of the date on which systemic symptoms are first observed (X), with 95% confidence intervals. Open circles denote observed median dates of symptom expression in April-sown crops (cf. Fig. 4). Drawn lines are defined by:

beet yellows virus: $Y = 35 + 14.4 \times \sqrt{X - 107}$

beet mild yellowing virus: $Y = 107 + 10.7 \times \sqrt{X - 167}$

These equations were obtained by inverting curvilinear least squares regression lines, relating X to Y:

beet yellows virus:

$X = 113 - 0.340 \times Y + 0.00484 \times Y^2$ $r^2 = 0.98$ $\hat{\sigma}_R = 5.6$

beet mild yellowing virus:

$X = 267 - 1.878 \times Y + 0.00878 \times Y^2$ $r^2 = 0.98$ $\hat{\sigma}_R = 6.1$

Reading horizontally in the figures, hatched lines demarcate 95% prediction regions for X, given Y. The width of these regions is:

$$4 \times \sqrt{\hat{\sigma}_R^2 + (CV \times \mu)^2}$$

where $\hat{\sigma}_R^2$ is the average squared deviation between the observed average date of symptom expression and the regression line while $(CV \times \mu)^2$ denotes the between-plants variance of the incubation period (Table 3; Fig. 10). Based on data obtained in 1985 (unpublished results) and 1986 (Table 3), the estimate of CV is 0.14 for beet yellows virus and 0.11 for beet mild yellowing virus. Vertical intervals between the hatched lines denote confidence regions for the infection-date of single plants, given the date of symptom expression.

Acknowledgements

Samenvatting

De incubatieperiode van bietevergelingsvirussen in suikerbieten onder veldcondities

De incubatieperiode van het bietevergelingsvirus, BYV, en het zwakke-bietevergelingsvirus, BMYV, nam toe naarmate suikerbieteplanten later in het seizoen geïnfecteerd werden. Jonge planten ontwikkelden BYV-symptomen na ongeveer 3 weken terwijl na gewassluiting de incubatieperiode geleidelijk toenam tot 9 weken. De incubatieperiode van BMYV nam toe van 4 à 5 weken na inoculatie in juni tot 9 weken na inoculatie in augustus. Geïnoculeerde bladeren ontwikkelden ongeveer een week eerder symptomen dan de systemisch geïnfecteerde bladeren, behalve bij jonge planten, geïnfecteerd met BYV, waar de symptomen zich op beide typen bladeren tegelijkertijd ontwikkelden.

De incubatieperiode nam bij hogere temperatuur af en, afhankelijk van de leeftijd van de plant (aantal bladeren), was een bepaalde temperatuursom nodig voor de ontwikkeling van symptomen. Deze temperatuursom nam toe met de ouderdom van de plant. Van alle systemisch besmette bladeren, vertoonden de oudste, welke juist verschenen op het moment dat de plant werd geïnfecteerd, als eerste symptomen. Dit gebeurde zodra of kort nadat ze hun uiteindelijke grootte hadden bereikt. Groeiende bladeren vertoonden nooit vergelingssymptomen. De trage bladexpansie in oude planten en bij lage temperaturen is een mogelijke oorzaak van de lange incubatieperiode aan het einde van het seizoen.

De incubatieperiode werd niet duidelijk beïnvloed door inoculatieomstandigheden, zoals (1) het aantal groene perzikluizen, *Myzus persicae*, dat werd gebruikt voor inoculatie, (2) het aantal geïnoculeerde bladeren, (3) de ouderdom van het geïnoculeerde blad, (4) de bronplant van BMYV, biet of herderstasje, of (5) de vector species. Omdat de incubatieperiode niet in belangrijke mate afhankelijk is van deze factoren kan bij kennis van de datum waarop symptomen verschenen de infectiedatum worden bepaald op basis van de incubatieperiode.

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