Retrospective estimation of the date of infection with beet yellowing viruses in sugar-beet under field conditions

W. VAN DER WERF[1,2], F. BONNIER[1] and D. PETERS[1]

[1] Department of Virology, Wageningen Agricultural University, P.O. Box 8045, 6700 EM Wageningen, the Netherlands
[2] Department of Theoretical Production Ecology, Wageningen Agricultural University, P.O. Box 430, 6700 AK Wageningen, the Netherlands

Abstract

Sugar-beet 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 the date of appearance of the oldest systemically-infected leaf of a plant. This approach was tested in the field and gave good results.

Additional keywords: beet yellows virus, closterovirus, beet mild yellowing virus, luteovirus, systemic virus transport, phloem translocation, phyllotaxis, leaf arrangement, leaf appearance, temperature sum, symptom development

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 sugar-beet (Duffus, 1973; Smith, 1986). The most important vector of these viruses is the green peach 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 (e.g. 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 growing conditions and plant age, and by the lack of accurate estimates (Van der Werf et al., 1989). Therefore, it was hitherto impossible to relate the population dynamics and the behaviour of vector aphids to the subsequent increase in the number of yellowed virus-infected 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 had generally just appeared when the plant became infected. They calculated the ratio of the serial number of this leaf and the total number of leaves on the plant. Com-

parison of this ratio to ratios obtained for reference plants, infected on known dates, gave the desired estimate of the infection-date.

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

Materials and methods

Leaf appearance. In 1984, 1985 and 1986, experiments were carried out in sugar-beet crops grown near Wageningen on heavy river-clay soil. In the first and second year the cultivar Regina was sown on 17 and 24 April, respectively. In the third year, cv. Bingo was sown on 25 April. All leaves longer than 3 cm, emerging from the centre of the plant were counted weekly on five groups of five reference plants in 1984, on six groups of ten plants in 1985 and on five groups of ten plants in 1986. Serial 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 sugar-beet (Milford et al., 1985a,b), were calculated by fitting a sine between the measured minimum and maximum temperatures and summing the hourly increments. The number of emerged leaves was calculated using these temperature sums and accumulated temperature equations of Milford et al. (1985b). In Milford's experiments, the first leaf pair unfolded 355 °C days after sowing while each of the next 21 leaves required 29 °C days to unfold and leaf 24 and all following leaves needed 48 °C days.

Relationship between leaf appearance and symptom development. Inoculations with BYV and BMYV were made throughout the season. On the infection-date, the total number of leaves greater than 3 cm was counted (N_0). The development of symptoms was recorded at one to three-week-intervals until the oldest systemically-infected leaves showed intense yellowing and necrosis. The relation between the number of leaves on the infection-date (N_0) and the serial number of the oldest systemically-infected leaf (C) was examined by linear regression.

In 1984, inoculations were made on 2, 15 and 29 June and on 6 and 20 July when the plants had an average of five, ten, 15, 18 and 21 leaves, respectively. On each date, five groups of ten plants were inoculated with BYV and five groups with BMYV. In eight plots sown on 8 June, ten plants having about nine leaves were inoculated on 20 July.

In 1985, eight inoculations were made between 23 May (two-leaf stage) and 8 August (32-leaf stage). The number of plants inoculated varied from 450 in June, when the inoculation conditions were varied (Van der Werf et al., 1989), to 60 in July, when only standard inoculations with BYV and BMYV were made.

The viruses were maintained in beet as described before (Van der Werf et al., 1989). 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-

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viruliferous aphids from oilseed rape on detached virus-infected beet leaves for two to three days. In 1984, the *M. persicae* clone M2 was used, while in 1985 the clone M3 was preferred because it transmitted BYV slightly better (Van der Werf, unpubl. res.). Aphid-proof clip-cages were used to prevent any undeliberate infection of other leaves than those on which the cages were placed. The inoculated leaves were marked with plastic labels to facilitate inspection.

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.

Results

Leaf appearance. The plants produced more than 50 leaves during the growing season. The leaf appearance rate had a maximum 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 and autumn due to lower temperatures and in the latter case also because the plants became older (Fig. 1). Leaf appearance rate showed a large variation between plants; the coefficients of variation of final leaf number were 17, 13 and 16% in the three years, respectively.

In Fig. 2, leaf appearance is plotted against the temperature sum after sowing. Until 1200 °C days after sowing, the leaf appearance rate was about one leaf per 33 °C days, which is similar to the value obtained by Milford et al. (1985b), one leaf per 29 °C days. When the 28th leaf had appeared at c. 1200 °C days after sowing, the leaf appearance rate decreased to one leaf per 47 °C days while in the experiments of Milford et al. one leaf per 48 °C days appeared after the 23rd leaf. Our observations on leaf appearance
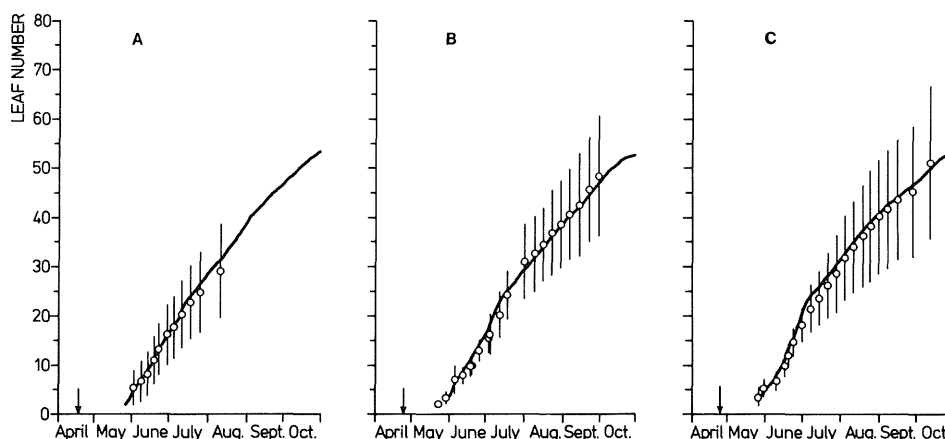

Fig. 1. Leaf appearance (o) in 3 sugar-beet fields in 1984 (A), 1985 (B) and 1986 (C), together with calculated total number of leaves (drawn line) according to accumulated temperature equations of Milford et al. (1985b). Bars denote 95%-prediction intervals with length 2 × 1.96 × $\hat{\sigma}$ where $\hat{\sigma}$ is the standard deviation of observed total number of leaves, increasing through the season. Arrows indicate sowing date.
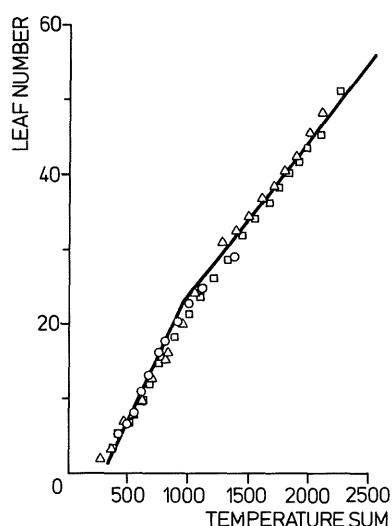
Fig. 2. Leaf appearance in field-grown sugar-beet in 1984 (o), 1985 (Δ) and 1986 (□), respectively, as a function of accumulated temperature above 1 °C. Drawn line according to Milford et al. (1985b).

Table 1. Relation between number of leaves on the date of infection with beet yellows virus (BYV) or beet mild yellowing virus (BMYV) and serial number of oldest systemically-infected leaf, with their respective standard deviations ($\hat{\sigma}$). n is the number of infected plants.

Year	Date	BYV				BMYV				
		$N_0^{1)} \pm \hat{\sigma}^{2)}$		$C^{3)} \pm \hat{\sigma}$	n	$N_0 \pm \hat{\sigma}$		$C \pm \hat{\sigma}$		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[5)]	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	0.0	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	—[6)]	—	—	—	—
	24 June	—[6)]	—	—	—	—	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	—	—	—	—	—

[1)] Average number of leaves on date of infection.
[2)] Standard deviation.
[3)] Average serial number of oldest systemically-infected leaf.
[4)] Number of infected plants.
[5] Plants sown on 8 June.
[6)] Not determined.

A

B

Fig. 3. Photograph made in October 1986, showing a sugar-beet plant infected with beet yellows virus in an early development stage (A). All leaves are infected. The other plant (B) was infected when it had c. 30 leaves. Only the leaves which emerged after the infection-date are infected. On both plants, only the infected leaves which are fully-expanded show clear symptoms.

in beet are adequately described by Milford's equations (Fig. 1), using the actual weather data.

Relationship between leaf appearance and symptom development. The leaf number (*C*) of the oldest systemically-infected leaf and the total number of leaves on the infection-date (N_0; Table 1) increased during the season in a parallel fashion. Thus, on early infected plants virtually all leaves are systemically infected (Fig. 3A) while on late-infected
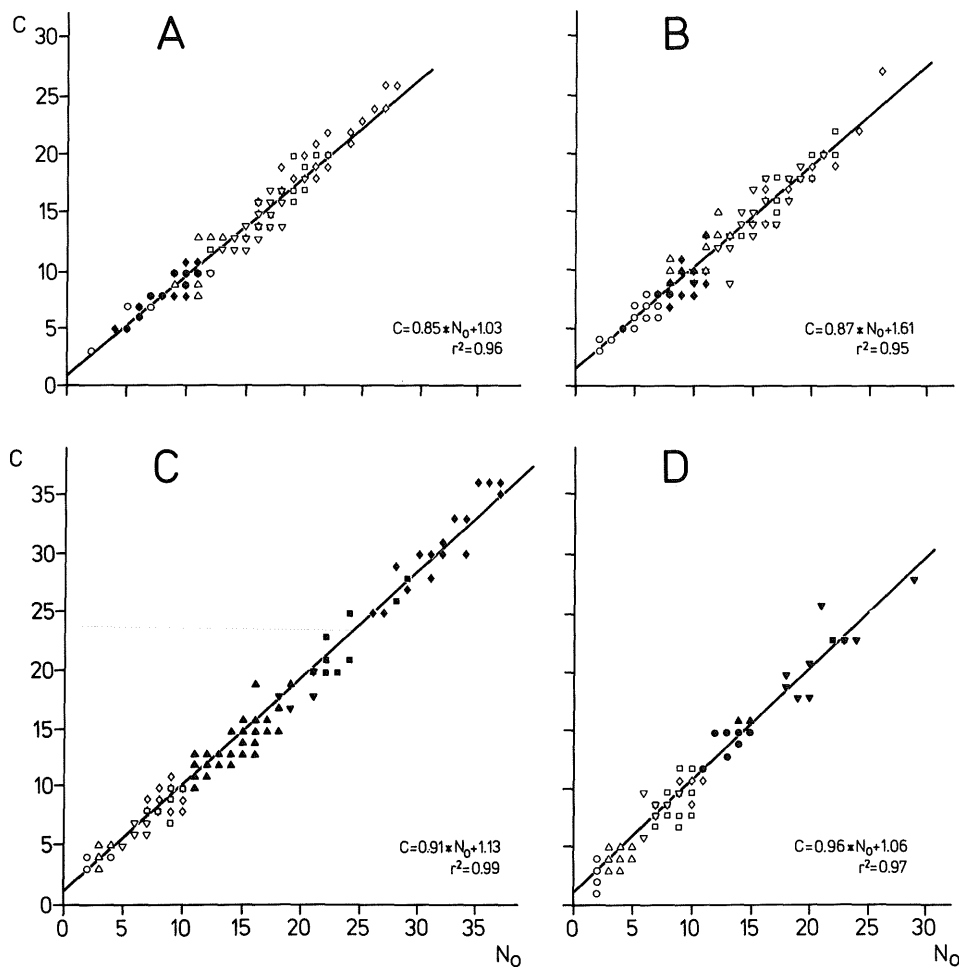

Fig. 4. Relation between the number of leaves on a plant at the infection-date (N_0) and the oldest leaf showing symptoms of systemic infection (C). Data for beet yellows virus in 1984 (A) and 1985 (C) and for beet mild yellowing virus in 1984 (B) and 1985 (D). In 1984 inoculations with both viruses were made on 2 June (o), 15 June (Δ), 29 June (∇), 6 July (□) and 20 July (◇) and in 1985 on 20 May (o), 27 May (Δ), 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 (◆).

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plants a large whorl of full-grown healthy leaves that appeared before the infection-date, is present (Fig. 3B). During the season, the standard deviations of C and N_0 increased. Because overlapping ranges of C were obtained for different dates of infection, determination of C alone is not sufficient to establish the infection-date. Account should be taken of differences in leaf appearance rate between plants.

Relations between N_0 and C are given in Fig. 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, implicating a close relation between C and N_0. Taking the data of both years together in the regression analysis yields:

$$\text{BYV:} \quad C = 0.89 \times N_0 + 1.00 \quad r^2 = 0.98 \quad \sigma_R = 1.07 \quad (1)$$

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

Regression of N_0 on C results in equations which can be used to derive the total number of leaves on the infection-date from the serial number of the oldest systemically-infected leaf:

$$\text{BYV:} \quad N_0 = 1.10 \times C - 0.86 \quad \cdot r^2 = 0.98 \quad \sigma_R = 1.19 \quad (3)$$

$$\text{BMYV:} \quad N_0 = 1.06 \times C - 1.04 \quad r^2 = 0.97 \quad \sigma_R = 1.12 \quad (4)$$

Relationship between leaf arrangement and symptom development. Leaves of sugar-beet appear one by one in a 5/13 phyllotaxis (Hayward, 1938), i.e. 13 leaves appear in five complete turns of the phyllogenetic spiral, successive leaves being spaced at angles of approximately 138°. The first two true leaves are exceptional by appearing simultaneously at an angle of 180° (Fig. 5). The direction of the phyllogenetic spiral is clockwise in approximately 50% of the plants and anti-clockwise in the other 50%.
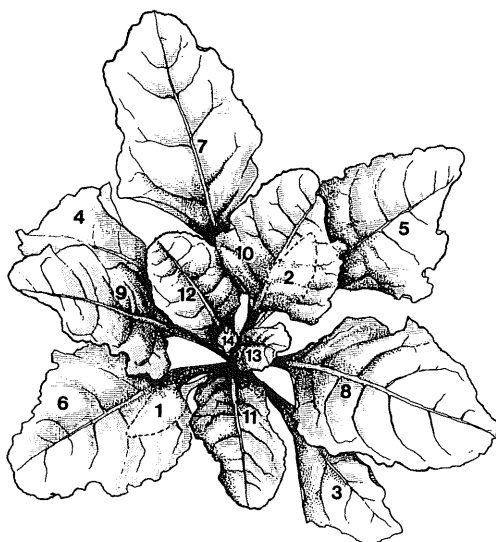

Fig. 5. Arrangement of the leaves on a young sugar-beet plant, having 13 leaves longer than 3 cm.

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33

34

35

36   37

Fig. 6. Photographs, taken on 11 October 1985, showing the oldest systemically-infected leaves of a sugar-beet plant naturally-infected with beet yellows virus on leaf 19 about 1 August. The systemically-infected leaves show typical late season symptoms of beet yellows virus: 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 portion of the blade is affected and the symptoms are less intense than on leaf 32, presumably as a result of a delay in virus transport to leaf 33 due to its distant position relative to the inoculated leaf 19. Leaf 34 (I + 15) is for the greatest part affected and leaf 35 is entirely yellowed. Leaf 36 and 37 show the lesser intensity of symptoms typical for later emerged, younger leaves. On all leaves symptoms become vaguer towards the leaf base.

Leaves differing in serial number by 3, 5, 8, 10 and 13 make small angles with each other and seem to form more or less vertical rows, which are called parastichies (Williams, 1975). Leaves which share a parastichy have short vascular connections.

    The position of symptoms on a leaf is related to its development stage on the infection-date and its position relative to the virus-source leaf. The first leaf to show symptoms of systemic infection (F) is often one which has close vascular connections to the source leaf of virus, especially on older plants. Younger leaves than F develop symptoms one after the other as they reach maturity. F is often the oldest one with symptoms (C), but sometimes one or two leaves older than F develop symptoms in due course, such

that $C = F - 1$ or $C = F - 2$. On old plants, the oldest systemically-infected leaves show mostly symptoms on the leaf base only, while the tip remains green until the leaf dies. On subsequent younger leaves, greater portions of the blade are affected until the leaf is infected as a whole. The first and consequently most advanced symptoms develop on the oldest infected portion of a leaf, i.e. the infected part which is nearest to the tip (Maksymowitsch, 1973). The leaf basis is the youngest part of a leaf and hence the last systemic symptoms that appear on a leaf are found on this part. Due to their later development and the smaller amounts of light reaching the leaf basis, base symptoms are often vaguer than symptoms near the tip. In time, the symptoms become gradually more intense over the whole infected area of a leaf. Fig. 6 gives a typical example of systemic symptoms on leaves of different age and position on a beet plant infected with BYV.

For BYV the upper margin of the affected area is generally sharply delimited by veins. This phenomenon, known as sectoring (Bennett, 1960), indicates that a sharp borderline exists between portions of a leaf which are young enough to become systemically-infected when virus is first transported through the phloem and those that are too old. 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 those infected with BYV. Sectoring is also found at the site of inoculation.

The oldest systemically-infected leaf (C) is mostly situated on the same side of the plant as the inoculated leaf, thus having short vascular connections to the virus source-leaf, while leaves on the opposite side of the plant are are unlikely candidates for C (Fig. 7). In Fig. 7, the abscis shows the difference in serial leaf number between I and C_{est}, calculated with Eqs 1 and 2, thus neglecting influences of phyllotaxis on the position of C, while the ordinate shows the difference between C_{obs}, the observed value, and C_{est}. When Eqs 1 and 2 yield values of $C_{est} - I$ of 4, 6, 7, 9, 12 or 14 with I (i.e. C_{est} opposite I), C_{obs} is often still found on the same side of the plant as I, such that $C_{obs} - I$ is 1, 2, 3, 5, 8, 10, 11, 13 or 15 (diagonal lines in Fig. 7). For instance, when Eq. 1 predicts $C_{est} - I = 6$ (phyllotctically distant), then in most cases, $C_{obs} - I = 5$, such that $C_{obs} - C_{est} = -1$ (Fig. 7A). A prediction of $C_{est} - I = 5$ (phyllotactically close), however, is generally confirmed in the experiment (Fig. 7A).

Retrospective estimation of the infection-date. Three data are needed to estimate the infection-date: (1) the serial number of the oldest systemically-infected leaf (C), (2) the total number of leaves on the plant, N_{obs}, on an arbitrary moment, and (3) a reference leaf appearance curve. This curve can be obtained by counts in the field or by calculations based on accumulated temperatures.

As a first step the number of leaves on the infection-date (N_0) is calculated with Eqs 3 and 4. The infection-date is then determined by interpolation with N_0 in the reference leaf appearance curve after a correction has been made 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 arbitrary moment:

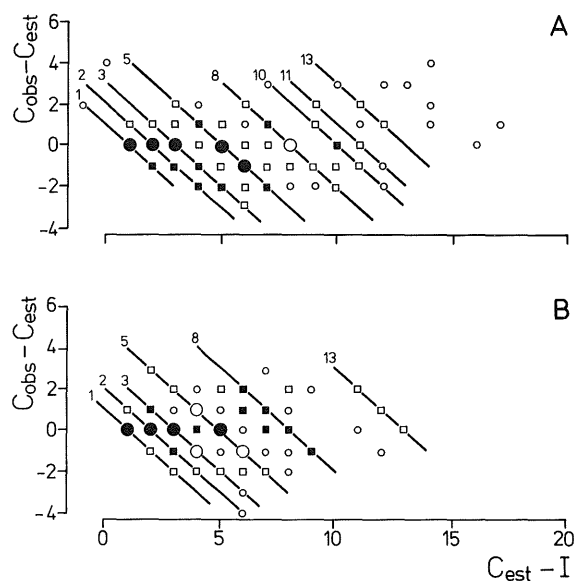$$R = \frac{N_{obs}}{N'_{obs}} \tag{5}$$

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Fig. 7. Difference between C_{obs}, the observed oldest sugar-beet leaf with symptoms of systemic infection and the value, C_{est}, estimated with Eqs 1 and 2 for different phyllotactic positions of C_{est} relative to the inoculated leaf, I. The symbols denote the number of plants having a given combination of $C_{est} - I$ and $C_{obs} - C_{est}$: 1 (o), 2 - 5 (□), 6 - 10 (■), 11 - 20 (o) or more than 20 (●).

Division of N_0 by R gives N'_0, the reference total number of leaves on the infection-date.

$$N'_0 = \frac{N_0}{R} \tag{6}$$

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 $1.10 \times 24 - 0.86 = 25.5$ (Eq. 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.

Evaluation of the method. In 1985, the method for the retrospective estimation of the infection-date was evaluated on a sugar-beet field, cv. Monohil, on heavy clay-soil near Wageningen. Inoculations with BYV or BMYV were made on nine dates from the end of May until the end of July, using 10 to 15 *M. persicae*, clip-caged onto a recently-full-grown leaf. The number of leaves per plant and the development of yellowing symp-

toms on individual leaves were recorded on five occasions from July till October. In 1986, inoculations and observations were made in a similar way in sugar-beet, cv. Bingo, grown on heavy river-clay near Wageningen.

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 8A, B).
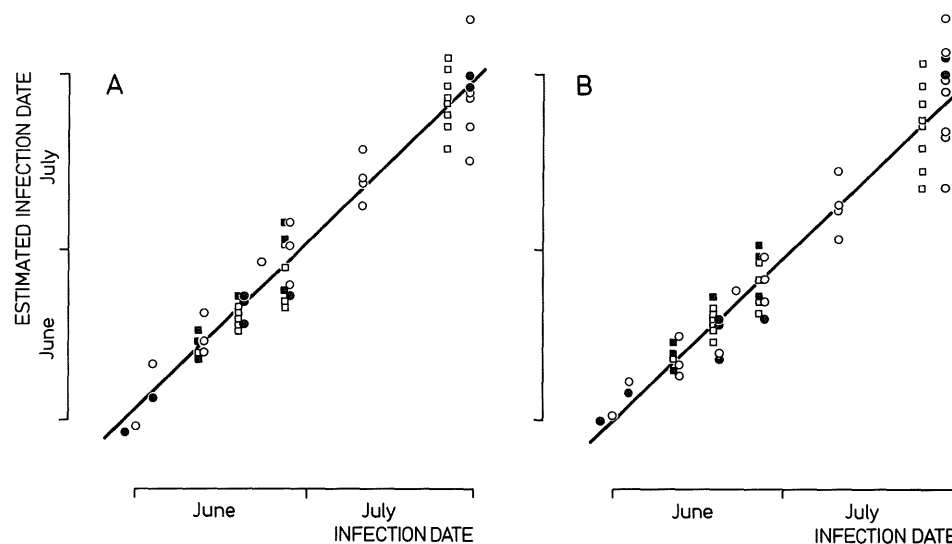

Fig. 8. Comparison of estimated with actual dates of infection of sugar-beet plants with beet yellows virus (open symbols) or beet mild yellowing virus (solid symbols) in 1985 (o) and 1986 (□), respectively. (A) Reference number of leaves counted in the field. (B) Reference number of leaves calculated with accumulated temperatures.

| Variant 1: | $Y = 0.97 \times X + 6.8$ | $r^2 = 0.95$ | $\sigma_R = 4.4$ | (7) |
| Variant 2: | $Y = 0.95 \times X + 7.5$ | $r^2 = 0.93$ | $\sigma_R = 4.9$ | (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 infection-date increases. Therefore the best estimates are obtained in young crops.

Discussion

The observed patterns of yellowing symptoms on (parts of) leaves of different age and position on the plant are strikingly similar to patterns of assimilate translocation in plants. In experiments with sugar-beet, Joy (1964) recovered most radio-actively label-

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ed carbon translocated from a source leaf from sink leaves at positions 8, 10, 11 and 13 relative to the source leaf. These leaves are all implanted on the same side of the plant as the source leaf and were in our experiments frequently the oldest one that became systemically-infected (Fig. 7). In leaf 10 and 11, Joy found most ^{14}C in the halves nearest to the source leaf. In agreement with this, virus symptoms occur sometimes only on the leaf half nearest to the source leaf. This occurs only in the oldest systemically-infected leaves (symptoms on leaf basis), which soon after the infection switched from assimilate import to export, and most frequently on old plants. These typical patterns of ^{14}C-translocation were also found in tobacco (Jones et al., 1959; Shiroya et al., 1961; Porter, 1976) and eastern cottonwood, *Populus deltoides* (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. 6). Fellows and Geiger (1974) observed that assimilate import by the 7th leaf of young sugar-beet plants reached a maximum at 25 % final leaf length and declined to almost zero at 45 % final length. In many plants, 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). The marked similarity between established patterns of assimilate transport in plants and the patterns of virus symptom expression observed in this study confirms the idea that beet yellowing viruses are transported to sink tissues via the phloem (Esau et al., 1967; Esau and Hoefert, 1972) and indicates that virus translocation and symptom development is intimately related to assimilate transport in the plant.

The estimation of the date of virus infection, by determining the position of leaves with symptoms on the plant can be used as an alternative to the practice of assessing the infection-date by substracting the incubation period from the date on which the first sympoms were seen (Van der Werf et al., 1989). Advantages of the method described in this paper are (1) its accuracy for young plants, (2) the necessity of only one observation of symptoms and (3) the free choice of the moment of the observation. Disadvantages of the described method are (1) its laboriousness, (2) the difficulty of correct application when more than one leaf has been inoculated by aphids or (3) when many leaves have died and cannot be retrieved. These disadvantages are more serious in old plants than in young ones. Moreover, reference observations on number of leaves or temperature are needed.

The described method can provide a useful means to estimate the infection-date of isolated primarily-infected plants, early in the season. Knowing the moment the primary infections are made is of great epidemiological importance as the earliness of infection is a major factor determining the amount of secondary spread and damage (Van der Werf et al., in prep.). Because early in the season the variation in total number of leaves between plants is small, it may be possible to simplify the method by abandoning the adjustment for the leaf appearance rate. Making use of the incubation period in this situation is likely to yield unreliable information because the first plants with first symptoms are hard to detect. Even if the first symptoms have been spotted at the correct time, use of the incubation period for estimation of the date of infection still gives inaccurate results for early BMYV infections (Van der Werf et al, 1989).

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. Field observations on the development of symptoms on naturally-infected plants of different ages support this conclusion. Application of the method for BMV may, however, be hampered by the vagueness of the mosaic symptoms on fully-expanded, systemically-infected leaves. Use of the incubation period provides probably a good alternative as only few symptomless heart leaves were found on BMV-infected plants of all ages. This indicates that the incubation period of BMV is short throughout the season, which facilitates its use for infection-date estimation.

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, especially in genetically homogeneous crops such that all plants have similar leaf appearance rates. In such crops knowledge of the number of leaves appeared since infection suffices to calculate the infection-date. In general, the approach outlined in this paper provides insight that can be used to verify and ameliorate infection-date estimates based on the incubation period.

Acknowledgements

Samenvatting

Retrospectieve bepaling van de datum waarop suikerbieteplanten werden besmet met bietevergelingsvirussen

Suikerbieteplanten die besmet zijn met het bietevergelingsvirus, BYV, of met het zwakke vergelingsvirus, BMYV, ontwikkelen symptomen op de geïnoculeerde bladeren, waarop infectieuze bladluizen virus hebben overgedragen, én op de systemisch besmette bladeren waarheen het virus vanuit de geïnoculeerde bladeren is getransporteerd via het vaatsysteem. Bladeren die op het moment van infectie nog niet verschenen zijn of vlak ervóór zijn verschenen, worden systemisch besmet, terwijl oudere bladeren gezond blijven. De infectiedatum kan worden bepaald door aan de hand van temperatuursommen de verschijningsdatum van het oudste systemisch besmette blad te berekenen. Deze methode bleek bij toetsing in het veld goed te voldoen.

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