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Measuring and modelling the effects of inoculation date and aphid flights on the secondary spread of Beet mosaic virus in sugar beet

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Summary

The effect of the inoculation date on the spread of *Beet mosaic virus* (BtMV) in sugar beet field plots was studied. Two plants in the centre of each plot were inoculated with BtMV using *Myzus persicae*. The spread of the infection around these sources was monitored by inspecting the plants on two diagonal transects through the centre of the plot. Early inoculations resulted in a greater spread than late inoculations, but any inoculation before the onset of the aphid migration resulted in a similar-sized spread. The spread was concentrated in patches around the inoculated plants, and its rate was explained by vector pressure, as shown by regression analysis and a mechanistic simulation model. This vector pressure was quantified using data obtained by catching aphids in a green water trap in the crop, catching aphids in a 12 m high suction trap at a distant location, and infection of bait plants from adjacent virus source plants. The daily total aphid catches obtained by a suction trap provided the best statistical explanation for the spread of this virus. The parameter *r*, describing the relationship between vector pressure and the rate of disease progress, was remarkably robust. This parameter varied less than 10% between treatments (infection date) within a single experiment, and less than a factor two between four experiments performed at different sites in two years. The robustness of this parameter suggests that the spread of a potyvirus may be predicted on the basis of the initial infection date and vector abundance.

Key words: Potyvirus, non-persistent transmission, simulation model and winged aphids

Introduction

The members of the genus *Potyvirus* of the Potyviridae are non-persistently transmitted by aphids. Acquisition and transmission take place in a matter of seconds or minutes, as the aphids probe successive plants to assess their suitability for colonisation. Different species transmit potyviruses independent of whether they accept or reject the plant as host (van Hoof, 1977; Halbert, Irwin & Goodman, 1981; Sigvald, 1986; Eckel & Lampert, 1993). The extent of potyvirus spread depends on factors such as efficiency by which different aphid species transmit these viruses, their abundance, phenology and behaviour (Irwin & Ruesink, 1986; Raccah, 1986).

The spread of potyviruses has been described in several models to quantify and analyse the different factors involved or to forecast outbreaks (Ruesink & Irwin, 1986; Sigvald, 1986; Madden, Pirone & Raccah, 1987*b*,*c*). Analysis of this spread is, however, often intermingled by the simultaneous occurrence of spread from one field to another (primary infection) and spread within a field (secondary infection). Spread of secondary infections can be analysed using a virus, which does not occur naturally and spreads after the introduction of an inoculum source in the field. Such a virus is *Beet mosaic virus* (BtMV), which is rarely found in the Netherlands since the demise of fodder beets as cattle food. The leftovers of clamps, used to store these beets during winter, provided an important reservoir of beet viruses in the spring (Heathcote & Cockbain, 1966; Heathcote & Byford, 1975).

The studies described were aimed to answer the questions (1) how does the inoculation date affect the spread of BtMV in sugar beet; (2) in which way is the size and pattern of the spread related to abundance, time profile and species spectrum of vectors; and (3) can the spread be described by a simple mechanistic simulation model, using accepted principles of disease epidemics. The first question was studied in four replicated field experiments in which the virus source was introduced at different dates. The second was addressed by regression and correlation analysis between observed spread and aphid flights. The third question was answered by calibrating a basic epidemiological model to the virus spread data, using aphid catch data as forcing function, and studying goodness of fit and robustness of parameter values.

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Materials and Methods

Experiments

Four field experiments were conducted to evaluate the spread of BtMV from artificially inoculated plants, in relation to the date of inoculation and the size and time of aphid flights. All trials were conducted in 25 m x 25 m field plots, laid out in commercial fields of sugar beet, cvs Univers or Auris, with a plant density of approximately 10 pl m^{-2}.

In 1995, an experiment was conducted at the experimental farm De Bouwing (Expt 1), Zetten, The Netherlands. This experiment had a randomised block design with four inoculation dates (16 May, 6 and 27 June, and 18 July) as treatments in five replications. Two plants in the centre of each plot were inoculated by ten viruliferous apterous *Myzus persicae*. In each block, one non-inoculated plot was incorporated to verify the absence of inter-plot interference and to monitor virus introduction from outside the field.

The plots were inspected weekly for the presence of plants showing BtMV symptoms until the end of September. These plants were marked with a bamboo stick and their position was recorded. The spread was determined by monitoring the plants on two orthogonal transects that extended from the inoculated plants at angles of 45° with the rows. Each transect extended to 17 rows at either side of the centre row. The total number of plants showing symptoms within the 12.4 m radius from the central source plants was calculated from the transect observations by multiplying the proportion of infected plants observed at each distance with the number of plants at that distance, and summing over distances:

$$y = y_0 + \sum_i n_i \cdot f_i \qquad \text{(Eqn 1)}$$

where:

y : number of plants showing symptoms in the patch;

y_0 : number of artificially infected sources;

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

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

The limits of a distance class were taken at the intersections of the diagonal transects and mid-points between adjacent rows. Accordingly, the number of plants in distance class i is with R = row distance (*i.e.* 0.5 m), D = plant density (#/m^2) and i the distance class number. The operational form of Eqn 1 is therefore:

$$y = y_0 + 4\pi R^2 D \sum_i i \cdot f_i \qquad \text{(Eqn 2)}$$

A second experiment (Expt 2) in 1995 was conducted at the experimental farm De Minderhoudhoeve, Swifterbant, The Netherlands, approximately 85 km north from Expt 1. This experiment had a randomised block design with four infection dates (8 and 22 June, 6 and 20 July) in five replications. One plot in each block remained non-inoculated.

In 1996, an experiment was conducted at the De Minderhoudhoeve (Expt 3), and another at the experimental farm of Unifarm, Wageningen (Expt 4). Both experiments had a randomised block design with four replications. Three inoculations were made in Expt 3 (18 June, 2 and 16 July) and four in Expt 4 (31 May, 14 and 28 June, and 12 July). A non-inoculated plot was included in each block. Plot size and monitoring procedures were the same as described for Expt 1.

The average patch size, characterised by the mean distance of the plants showing symptoms from the inoculated source plant, was determined as for a negative exponential gradient truncated at a distance class n, using Eqn 3.

$$\bar{i} = \frac{\sum_{i=1}^{n} i \cdot a \cdot q^i}{\sum_{i=1}^{n} a \cdot q^i} = \frac{1}{1-q} - n \cdot \frac{q^n}{1-q^n}, \qquad \text{(Eqn 3),}$$

where:

$\bar{i}$: mean distance (expressed in distance classes)

A : disease incidence near the center

Q : slope parameter

The seed in these experiments was treated with Imidacloprid, which is currently common practise in Western Europe. This insecticide prevents the establishment of aphid colonies on beet seedlings, but does not affect the transmission of BtMV (Collar, Avilla, Duque & Fereres, 1997).

Aphid population

Aphid population data, collected in Tollebeek (Netherlands) with a 12 m high suction trap, following specifications of the Rothamsted Insect Survey (Taylor, 1986), were provided by the Nederlandse Algemene Keuringsdienst (NAK), Emmeloord, The Netherlands. This suction trap is located about 20 km from the Minderhoudhoeve, 80 km from Wageningen

and 85 km from Zetten.

The data were used in multiple regression and correlation analyses and calibrations. The supplied data covered the period from the beginning of May until 14 August for 1995 and 22 August for 1996. However, to make the calibration studies, the aphid population data until the end of September were needed. These supplementary data were constructed on the basis of the results of bait plant trials.

While aphid traps measure vector abundance, bait plant trials measure vector activity in the field. This activity was measured in both years by placing batches with non-infected plants in the field every two weeks from May until September. These batches consisted of 24 × 10 uninfected potted beet plants in the fourth leaf stage. They were exposed to an adjacent row of infected sugar beet plants at 30 cm distance and taken to the greenhouse (20-26°C) after one week to produce symptoms. New source plants were placed in the field when needed to standardise their condition throughout the season. The proportion of infected plants was recorded and vector activity estimated using Gregory's multiple infection transformation (Gregory, 1948):

$$v = \ln\left(\frac{n}{n-k}\right), \qquad \text{(Eqn 4)}$$

where:

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

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

A single green water pan trap (GWT), 40 cm × 50 cm, was placed 50 cm above the soil in the middle of the fields used in Expts 1 and 4. The trapped aphids were weekly collected and counted. The explanatory value of these data was compared with that of the suction trap data.

Multiple regression and correlation analysis of the relationship between aphid abundance and the rate of spread

The multiple regression analysis was based on the logistic disease progress model (van der Plank, 1963), i.e. the rate of increase is proportional to the number of sources, the proportion of uninfected plants, and the abundance of vectors:

$$\frac{\Delta x}{\Delta t} = x \cdot (1-x) \cdot \left(\sum_{1}^{n} b_i \cdot N_i\right) \qquad \text{(Eqn 5)}$$

where:

N_1 to N_n : is the number of specimens caught for each aphid species (i) in the time interval t to $t + \Delta t$;

b_1 to b_n : regression coefficients;

x : proportion of plants showing symptoms at time t;

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

Δt : time between samples

This equation relates the change in the proportion of plants showing symptoms (Δx) during a time interval (Δt) to the proportion of plants showing symptoms (x) at the start of that interval, and the length of this interval (Δt). This interval, being the time between the subsequent samplings, varied from 1 wk (Expt 1) to 2 or sometimes 3 wk (Expts 2, 3 and 4) and is of the same order of magnitude as the latent and incubation periods of the disease (Dusi & Peters, 1999). As these periods differ little, infectious plants and plants showing symptoms were equated with each other in this analysis. The number of plants showing symptoms was used to calculate x, and the increase of the number of plants showing symptoms to determine Δx. Proportions are calculated by dividing the number of infected plants by the total number of plants in a plot. The equation is then rearranged to obtain a form in which the coefficients b_i may be estimated by linear regression (Garrett, 1988):

$$\frac{\Delta x}{x \cdot (1-x) \cdot \Delta t} = \sum_{1}^{n} b_i \cdot N_i \qquad \text{(Eqn 6)}$$

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

All variables, except the regression coefficients b_i, were obtained in experiments, while the b_i's were determined by regression. Also, the correlation coefficient between the corrected rate and the aphid catches were determined. The regression analysis used as explanatory variables the population trends, measured in the suction trap, for the aphid species *Acyrthosiphon pisum*, *Aphis fabae*, *Macrosiphum euphorbiae*, *Metopolophium dirhodum*, *Myzus persicae* and *Rhopalosiphum padi*. Two other regression variables represented the number caught for all other species, as well as the sum of all aphids caught. The species that were not included individually in the analysis were too low in number and had no explanatory value as shown by preliminary regression analyses.

Simulation of the spread of BtMV

A basic temporal epidemiological model (Edelstein-Keshet, 1988) was used to verify whether the spread obeyed expected mechanistic principles, and to determine whether rate parameters characterising the spread were stable or variable among experiments and/or treatments. In this model, the system is described by four state variables (Leffelaar, 1999). These are: the number of healthy plants in the plot (H), the number of latent infected plants (L), the number of infectious plants that have not (yet) developed symptoms (I), and the number of infectious plants with symptoms (S) (Fig. 1). The rate of spread is a function of the aphid population caught by the suction trap (A; Fig. 5), the number of virus sources (I+S), the proportion of available healthy plants (H/P) and a rate parameter (r):

$$\frac{dL}{dt} = r \cdot A \cdot (I + S) \cdot \frac{H}{P} - i \cdot L \quad \text{(Eqn 7)},$$

$$\frac{dI}{dt} = i \cdot L - s \cdot I \quad \text{(Eqn 8)},$$

$$\frac{dS}{dt} = s \cdot I \quad \text{(Eqn 9)},$$

$$H = P - L - I - S \quad \text{(Eqn 10)},$$

where:

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

In this study, the latent period (LP) was defined as the period between the inoculation and the moment at which virus can be acquired from the plant, and the incubation period (IP) as the period between inoculation and appearance of symptoms (van der Plank, 1963). The aphid population data collected by the suction trap and complemented as described before were used as a driving function (A) for the model. Initial values for the state variables were $L = 2$, $I = 0$, H = P-2, and P is the number of plants in the plot. The LP was modelled as a function of daily mean air temperature, based on the LPs determined in the laboratory under different temperatures while the IP was set to the LP + 2 days (Dusi & Peters, 1999).

The model was implemented in the FORTRAN-based simulation environment SENECA (SENECA 2.0, Netherlands Institute of Ecology, Centre for Estuarine and Coastal Ecology, 1992). Values for r were determined using the 'Price' calibration algorithm

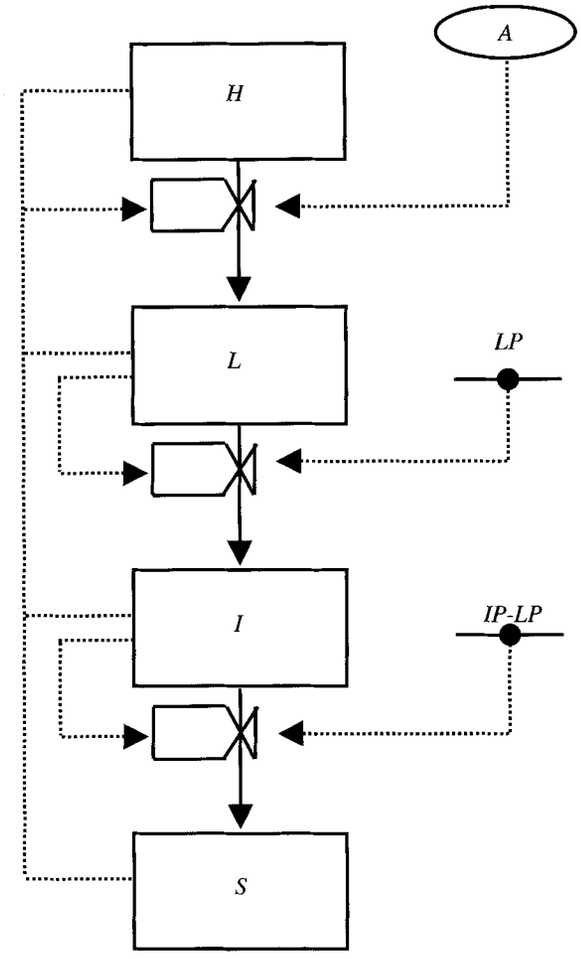

Fig. 1. Relational diagram of a basic epidemiological model for the spread of BtMV. H, healthy plants; L, infected plants before being infectious (latently infected plants); I, infectious plants before symptom expression; S, infectious plants showing symptoms; A, aphid population; LP, latent period; IP, incubation period

of SENECA. This algorithm conducts a controlled random parameter search and avoids local minima. The sum of squared normalised residuals for the variable S (number of plants showing symptoms) was used as the quantity to be minimised during calibration. A normalised residual is the ratio of the residual (simulated value minus observed value) and the observed value for S. The average disease progress for each inoculation date in each experiment was used as 'observed data' in the calibrations. Deviations between simulated and observed disease progress were characterised by the square root of the average squared normalised residual (SRASNR).

Values of r were separately calibrated for each treatment in each experiment and used to simulate the spread in these treatments. Spread was also simulated using the average r for the three or four infection dates in each experiment to test whether different treatments within one experiment could be characterised with a model and a single value for the rate parameter. Also, an average r for the four experiments was calibrated to test if a single common rate could characterise the spread observed in these experiments. Again, goodness of fit was determined by SRASNR. A sensitivity analysis for r was made by simulating the spread with the calibrated values and with values, which were 10% higher or lower.

Results

Overview of the field experiments

The first plants showing symptoms in the four experiments were found two weeks after the first aphid catches in the suction trap located in Tollebeek (Figs 2 and 3). The number of infected plants then increased gradually. In 1995, the first aphid migration occurred in substantial numbers in the last week of May (Fig. 2C; day 150 = 30 May), whereas in 1996, substantial numbers of flying aphids were not observed until the second half of July (Fig. 3C; day 200 = 19 July. Consequently, disease spread in 1995 became apparent in the last week of June (Fig. 2A,B; day 180 = 29 June), whereas in 1996 virtually no spread was observed until mid August (Fig. 3A,B; day 230 = 18 August). Hence, broadly speaking, aphid migration and disease spread started 50 days later in the season of 1996 than in 1995.

In all experiments, the spread tended to be less if the initial inoculations were made later in the season (Fig. 4). This trend was most apparent in Expt 1. In this experiment, the first inoculation dates (15 May and 6 June) resulted in substantial disease spread, whereas the later inoculations (in late June or later) did not result in much spread. The inoculated plants in these late inoculated treatments became sources of infection at a time (late July and later) when there were no or few aphid vectors (Fig. 2C; Fig. 4). Likewise, the late inoculated treatments in Expt 2 resulted in little spread (Fig. 2B).

The number of aphids migrating was approximately a factor three higher in 1996 than in 1995 (Fig. 5). This difference in aphid migration is reflected in the size of BtMV spread, which was substantially greater in the 1996 (Fig. 3) than in the 1995 (Fig. 2) experiments. BtMV spread in a spatially aggregated pattern around the source, and the mean distance from the source was smaller with later inoculations in Expt 1 (Table 1). This decrease of the 'distance' of spread with later inoculation is indicative for a decreasing number of infection cycles.

In non-inoculated control plots, only a few infected plants were found in the two last evaluations in 1996. These plants were randomly distributed in the plots and significant interplot interference or virus influx from outside the field may therefore be safely ruled out in the experiments presented here.

During the experiments, randomly selected plants were periodically monitored for the presence of resident aphids. They were not found before mid August, when a few colonies of *A. fabae* were observed on plants in all experiments. Under the experimental conditions in both years, early colonisation of the plants was prevented by the presence of natural enemies (Landis & van der Werf, 1997), and by the use of Imidacloprid treated sugar beet seed. Three (unpublished) trials in which it was attempted to establish vector colonies in pesticide-free sugar beet failed due to this predation pressure. The absence of any colony until late in the season and the occurrence of the early spread show that non-colonising (winged) aphids are responsible for the spread of BtMV. Other authors derived to similar conclusions for other potyviruses (Sigvald, 1984; Scott, 1985; Madden, Louie & Knoke, 1987*a*; Madden *et al.*, 1987*b*; Atiri, 1992).

Correlation between aphid catches and disease spread

The total aphid population, trapped by the suction trap, provided the most stable statistically significant correlation ($0.79 < P < 0.81$) with the rate of spread as estimated with Eqn 6 (Table 2). No consistent statistical relationship was found for any single aphid species with the spread of disease in multiple regression analyses, using stepwise inclusion or exclusion of explanatory variables. Results of stepwise regression for Expt 4 are given to illustrate this point (Table 3). Values for the coefficient of determination of regressions on single species were usually below 0.5. Aphid population trends during the season were quite similar for most species, and hence the matrix of explanatory variables in the multiple linear regression was highly collinear (Tables 4A and B) and explains the failure to find consistent regressions for individual species.

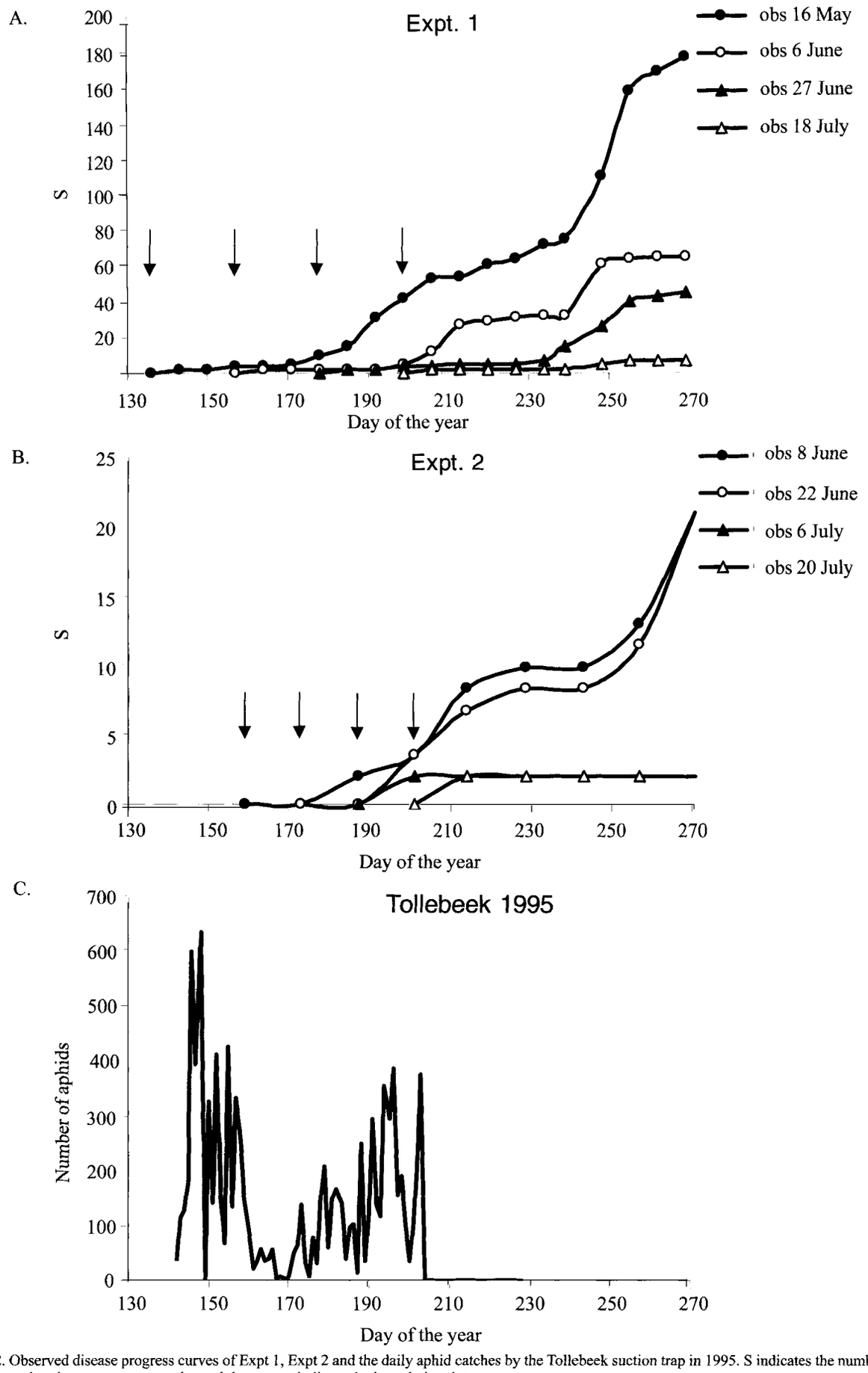

Fig. 2. Observed disease progress curves of Expt 1, Expt 2 and the daily aphid catches by the Tollebeek suction trap in 1995. S indicates the number of plants showing symptoms per plot and the arrows indicate the inoculation dates.

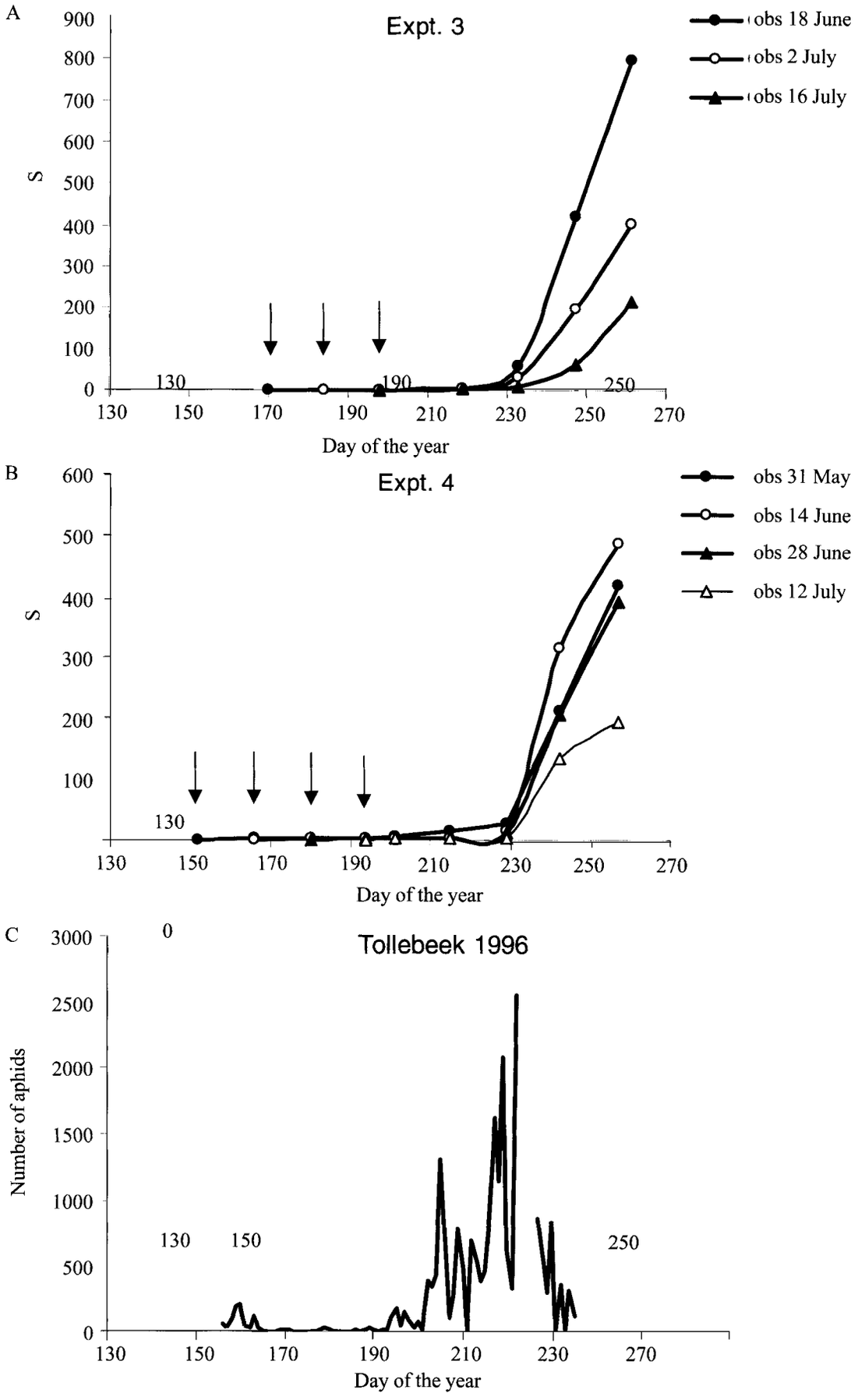

Fig. 3. Observed disease progress curves of Expt 3, Expt 4 and the daily aphid catches by the Tollebeek suction trap (C) in 1996. S indicates the number of plants showing symptoms per plot and the arrows indicate inoculation dates.

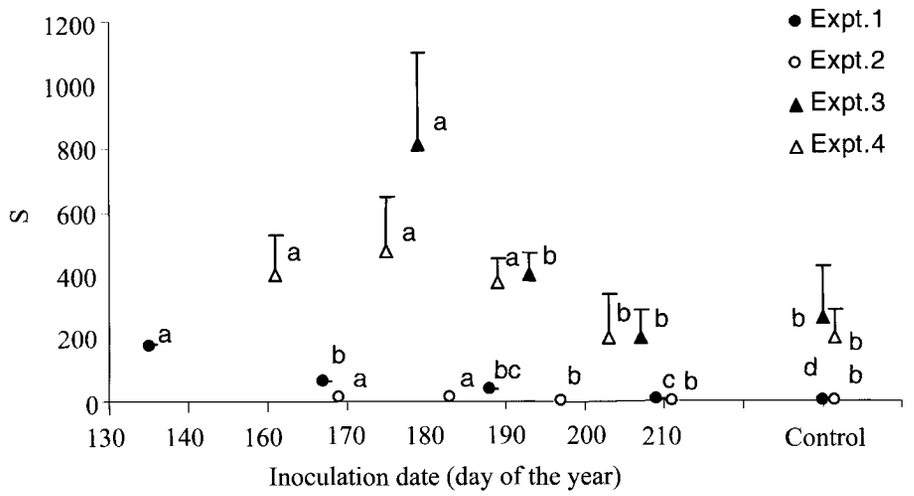

Fig. 4. Final number of BtMV infected plants in the plots inoculated at the indicated dates. Symbols of the same experiment followed by the same letter do not differ statistically at $P = 0.05$. S indicates the number of plants showing symptoms per plot. Infection date is expressed in day of the year (1 January = day 1).

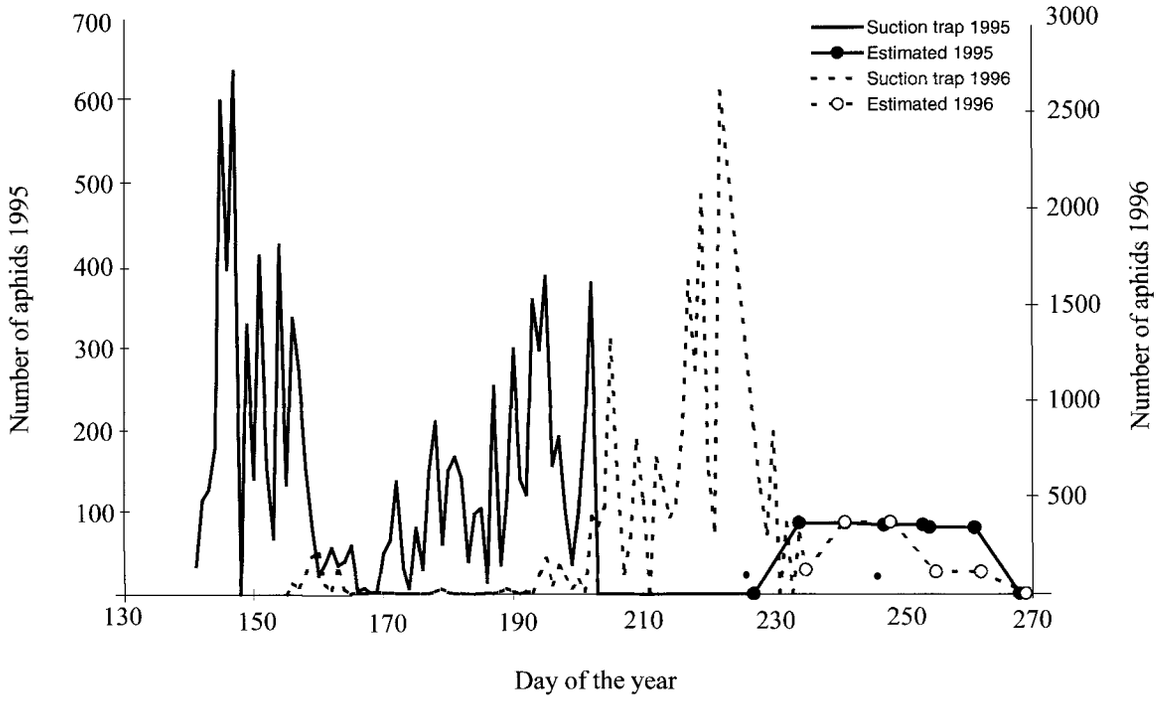

Fig. 5. Daily number of aphids trapped in 1995 and 1996 in the Tollebeek suction trap, complemented with the weekly number of aphids estimated by regression and Gregory's multiple infection transformation for the period between day 225 and 270. Note the difference in vertical axis for 1995 data (left) and 1996 data (right).

The GWT catches showed the same overall fluctuation of the total aphid population (data not shown) as the suction trap catches. Regression equations relating the suction trap catches and the vector activity determined by the bait plants resulted in correlation coefficients of 0.30 in 1995 and 0.93 for 1996.

Modelling the relationship between aphid catches and disease spread

Several calibration runs were made to estimate the parameter r that relates spread with aphid flights, using different measures for aphid abundance or activity as forcing functions. Data from the suction trap, GWT, and estimated vector activity (Eqn 4) were compared. The best fit was obtained with the suction trap data. Adding substitute data for the missing period at the end of the season, using Eqn 4, did not change the values for r and the goodness of fit remained the same. Hence, the suction trap data, complemented with the data based on bait plant trials and Eqn 4, were used as model input in subsequent calibrations.

Model fitting was carried out at three levels of generality (Figs 6-8; Table 5). In the first and least general set of calibrations (Fig. 6), the model was fitted separately for each treatment in each experiment.

Table 1. *Average distance of plants showing symptoms (± SE) to the source at the last day of evaluation*

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

[1] Values followed by the same letter are not statistically significant at

This yielded 13 different estimates for r (Table 5), corresponding to 13 different inoculation dates in four experiments. Fitting the model separately for each inoculation date, gave generally a good fit, a moderate value for the square root of the average square normalised residual (SRASNR; Table 5) and the obtained values for r were in a limited range ($2.86*10^{-4}$-5.78×10^{-4}). In the second, more general, set of calibrations, a single value of r was determined for each experiment, yielding four experiment-specific r-values. The fit was less good (Fig. 7; Table 5), but still acceptable, and the range of r-estimates (2.87×10^{-4}-5.06×10^{-4}) was more restricted than with the inoculation date-specific r-values. At the most general level, the fit of the model was determined when a single overall value of r was used. In this case, all four experiments still showed acceptable predictions of epidemic progress (simulations largely within 95% confidence intervals for the experimental data), although the prediction of epidemics in Expt 1 might

Table 2. *Correlation coefficients between the corrected rate of spread (Eqn 7) and total aphid population catches. Only the species with the highest correlation coefficients are shown. Correlation analysis is presented for the first inoculated treatment of each experiment*

Experiment		*Myzus persicae*	*Metopolophium dirhodum*	Other aphids	Total
Expt 1	r[1]	0.78	0.77	0.77	0.81
	p	< 0.01	< 0.01	< 0.01	< 0.01
Expt 2	Nd[2]				
Expt 3	r	0.97	0.88	0.93	0.79
	p	0.01	0.05	0.02	0.12
Expt 4	r	0.69	0.75	0.75	0.82
	p	0.13	0.09	0.08	0.05

[1] Correlation coefficient.
[2] Insufficient virus spread to conduct a meaningful analysis.

Table 3. *Stepwise multiple regression analysis of the statistical relationship between species-specific aphid catches in the Tollebeek suction trap, and the spread of BtMV in the first inoculated plots in Expt 4*

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

The rate of spread was defined as in Eqn 6.

Table 4. *Correlation matrix of species-specific weekly total aphid catches in the Tollebeek suction trap in 1995 (A) and 1996 (B). Cell contents: Correlation coefficient and significance of the estimate*

A. 1995

	Ap[1]	Md	Me	Mp	Rp	Other	Total
Af	0.218 0.474	0.737 < 0.01	0.846 < 0.01	0.849 < 0.01	0.845 < 0.01	0.578 0.04	0.705 < 0.01
Ap		0.001 0.997	0.336 0.262	0.516 0.07	0.542 0.056	0.171 0.58	0.270 0.37
Md			0.776 < 0.01	0.456 0.12	0.555 0.05	0.466 0.11	0.540 0.06
Me				0.819 < 0.01	0.894 < 0.01	0.460 0.114	0.604 0.03
Mp					0.941 < 0.01	0.277 0.36	0.454 0.12
Rp						0.394 0.18	0.569 0.04
Other							0.980 < 0.01

B. 1996

	Ap	Md	Me	Mp	Rp	Other	Total
Af	0.855 < .01	0.768 < .01	0.284 0.37	0.604 0.038	0.676 0.016	0.611 0.03	0.766 < .01
Ap		0.544 0.08	0.004 0.99	0.469 0.12	0.643 0.02	0.372 0.23	0.584 0.05
Md			0.793 < .01	0.681 0.01	0.777 < 0.01	0.879 < 0.01	0.942 < 0.01
Me				0.620 0.03	0.422 0.17	0.881 < 0.01	0.760 < 0.01
Mp					0.583 0.05	0.855 < 0.01	0.874 < 0.01
Rp						0.550 0.06	0.804 < 0.01
Other							0.932 < 0.01

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

be judged as inadequate, with underpredictions of final disease level in the order of 50% or more. Sensitivity analysis showed that a 10% decrease or increase in r, resulted in a 30% to 40% decrease or increase in the estimated number of plants showing symptoms (Fig. 9).

Discussion

The work reported in this paper was directed to analyse the effect of the inoculation date on the spread of BtMV in the sugar beet crop, the extent and pattern of this spread and its relation to the population dynamics and migration of aphids. The spread observed could be described by a simple mechanistic simulation model using accepted principles of disease epidemics (Edelstein-Keshet, 1988).

The results of the field experiments show that, expectedly, the extent of spread during a season decreases with the later inoculation dates. The effect of inoculation date depends strongly upon the time profiles of the aphid flights, which were different in the two years of this study. The flight started 50 days earlier in 1995 than in 1996 and had a bimodal profile in 1995. The flight of 1996, which had a unimodal profile, was three times greater than in 1995. The large spread in the second year could well be explained by the vector numbers.

Table 5. *Overview of calibration results. For each of the four experiments, values of* r *were determined by calibration. The goodness of fit is characterised by the Square Root of the Average Squared Normalised Residuals (SRASNR). Additionally, for each experiment, goodness of fit is given when simulations are made with an experiment-wise average value of* r

EXPT	Inoculation Date	Treatment-specific *r* (* 10^{-4})	SRASNR with treatment-specific *r*	Experiment-specific *r* (* 10^{-4})	SRASNR with experiment-specific *r*	Overall *r* (* 10^{-4})	SRASNR with overall *r*
Expt 1	16 May	5.78	0.41	5.06	0.62	3.73	1.05
	6 June	5.57	0.29		0.41		0.86
	27 June	5.47	0.33		0.38		0.67
	18 July	3.40	0.48		0.74		0.49
Expt 2	8 June	2.88	0.23	2.87	0.23		1.00
	22 June	2.86	0.25		0.25		0.81
	6[1] July	-	-		-		-
	20[1] July	-	-		-		-
Expt 3	18 June	4.04	0.14	3.49	0.46		0.31
	2 July	3.42	0.14		0.16		0.41
	16 July	3.02	0.28		0.69		1.08
Expt 4	31 May	3.29	0.13	3.56	0.32		0.62
	14 June	3.98	0.26		0.38		0.19
	28 June	3.11	0.29		0.52		1.29
	12 July	3.86	0.37		0.37		0.18

[1] No spread occurred in these plots.

The question, which aphid species was or were (mainly) responsible for the spread of BtMV, could not be resolved in this study. Using multiple regression Garrett (1988) distinguished the role of different aphid species in the spread of *Clover yellow vein virus* (CYVV). This was impossible with our data because of collinearity in temporal trends of aphid flight. (Table 3). As a consequence, analyses of the relationship between virus spread and vector abundance, while leaving out the counts for certain aphid species, resulted in inconsistent regression coefficients for single species. Negative regression coefficients were found for indisputable vector species, such as *M. persicae* (Table 4), depending upon the combination with other aphid species. Such regression coefficients are biologically difficult to interpret. Although we cannot ascertain the role of a single species in the spread using the data at hand, it cannot be ruled out that a single or a few aphid species are the main spreaders.

Potyviruses are transmitted in a non-persistent manner and can therefore be spread by aphid species that do not colonise the crop (Sylvester, 1952; Katis & Gibson, 1984; Sigvald, 1984; Summers, Newton, Kirk & Temple, 1990; Dusi & Peters, 1999). The relative transmission efficiency differs between aphid species (Dusi & Peters, 1999) and a high number of vectors may compensate for a low efficiency, and *vice versa*. The abundance of species varies from year to year. The simulation studies and the regression analysis have shown that the relationship between vector dynamics and potyvirus spread can be studied using total aphid population counts rather than counts of a single species), Madden *et al.* (1987*c*), Mora-Aguilera *et al.* (1992) and Di Fonzo *et al.* (1997) came to a similar conclusion. Using total aphid counts rather than single species counts saves a substantial amount of time and training in aphid identification. However, specific knowledge on the species composition can also be required as shown in the analysis of the spread of PVY[0] under the conditions prevailing in Sweden (Sigvald, 1987).

The use of daily catches from suction traps as a driving function in the model, under the evaluated conditions, produced the best simulations, followed by the use of infection pressure from bait plants (data not shown). Halbert, Connelly & Sandvol (1990) indicated that suction trap data probably reflect aphid flight activity over an area with a radius of 80 km. Considering the flat topography of the Netherlands, the area that the Tollebeek suction trap might cover, will be larger than assumed for topographically more rugged areas (Dr R Harrison, personal communication). Accepting this assumption and considering that this suction trap is located about 85 and 80 km from Expts 1 and 4 and about 15 km from Expts 2 and 3, data from this trap can well be considered to be representative for the experimental locations.

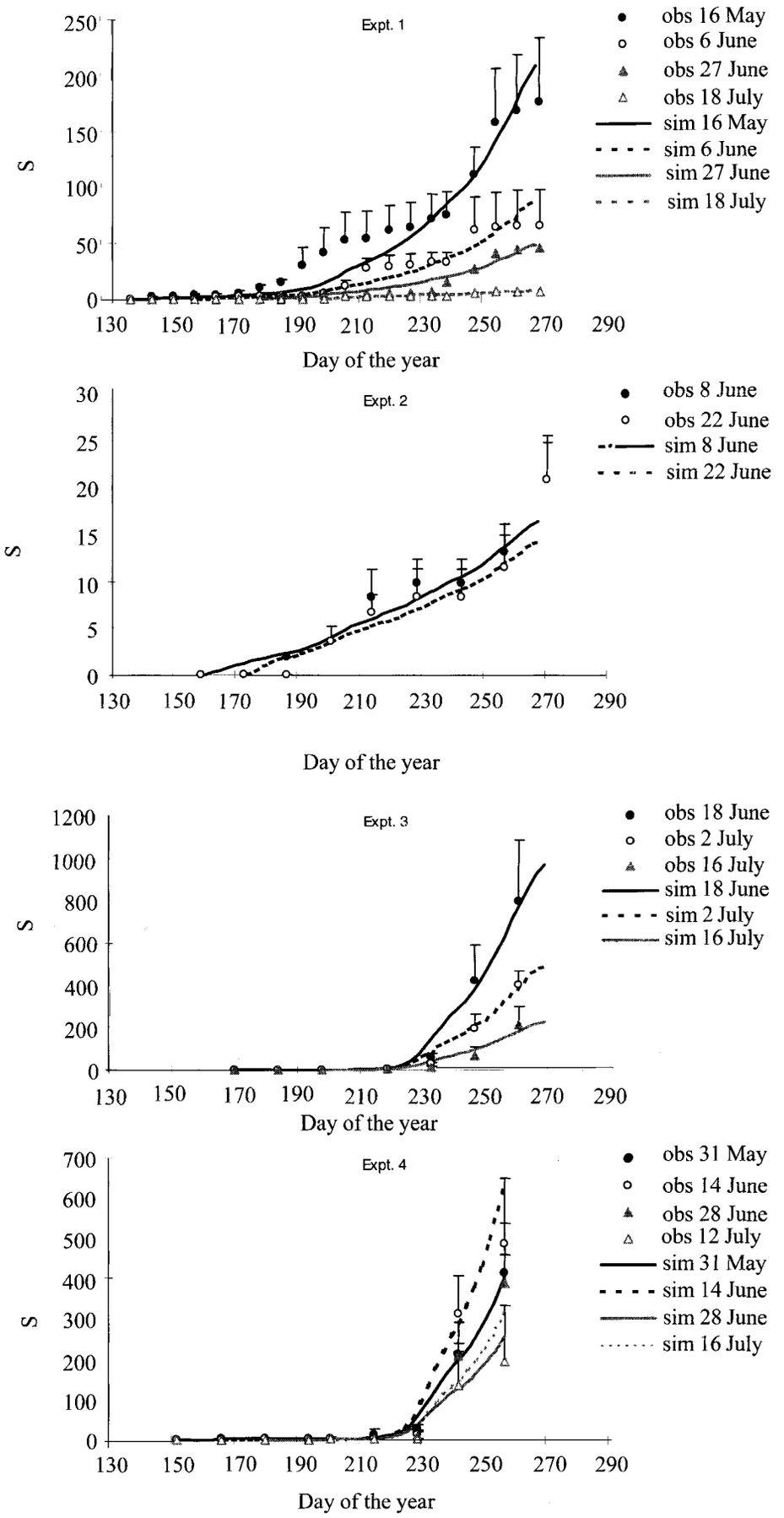

Fig. 6. Simulated disease progress curves, using *r* values calibrated specifically for each experiment, compared to field data. Circles and triangles represent the observed spread for each inoculation date, and lines the simulated spread. S is the number of plants showing symptoms per plot. Bars indicate the standard error of the mean.

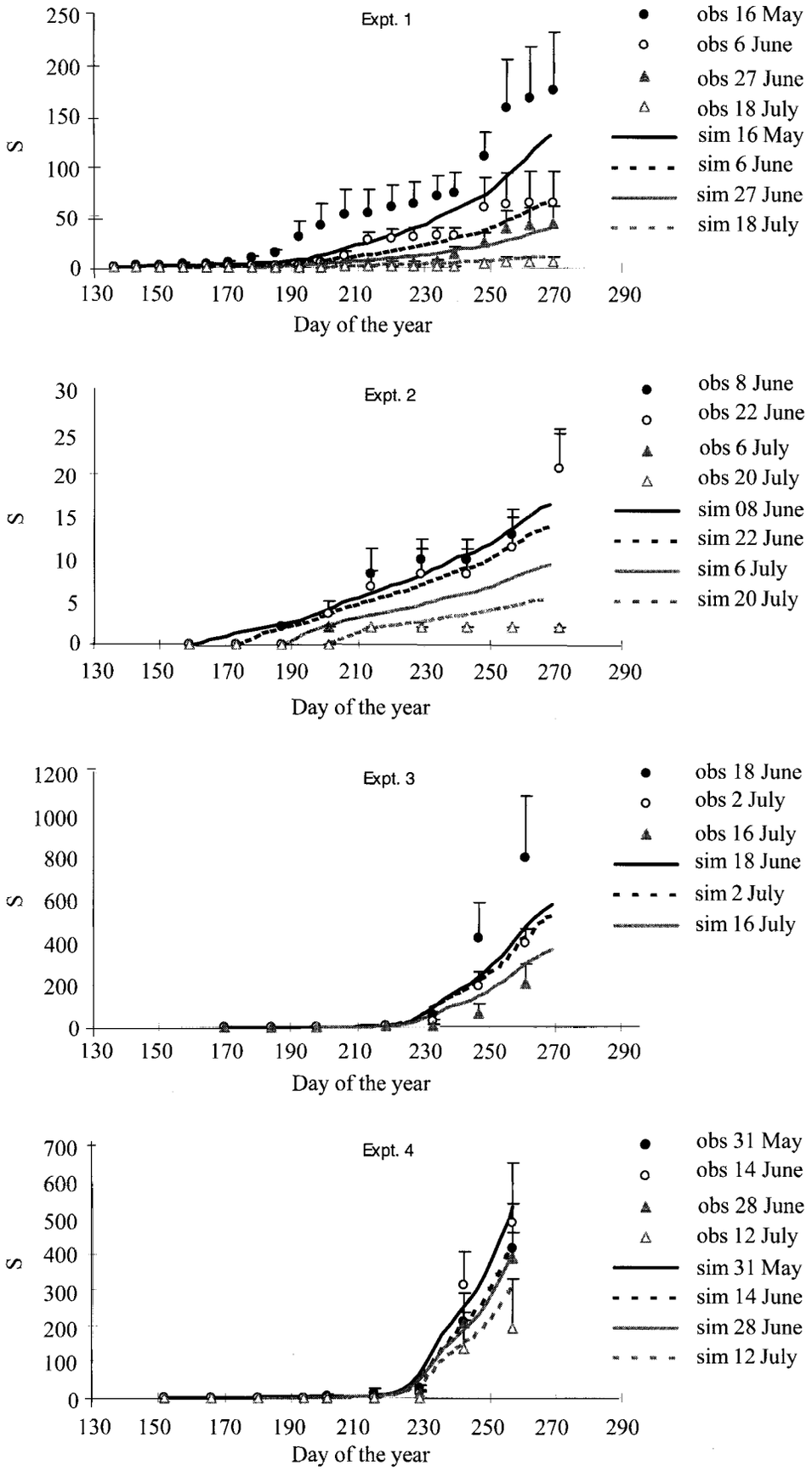

Fig. 7. Simulated disease progress curves, using a common r for all inoculation dates per experiment, compared to field data. Circles and triangles represent the observed spread, and lines the simulated spread. S is the number of plants showing symptoms. Bars represent the standard error of the mean.

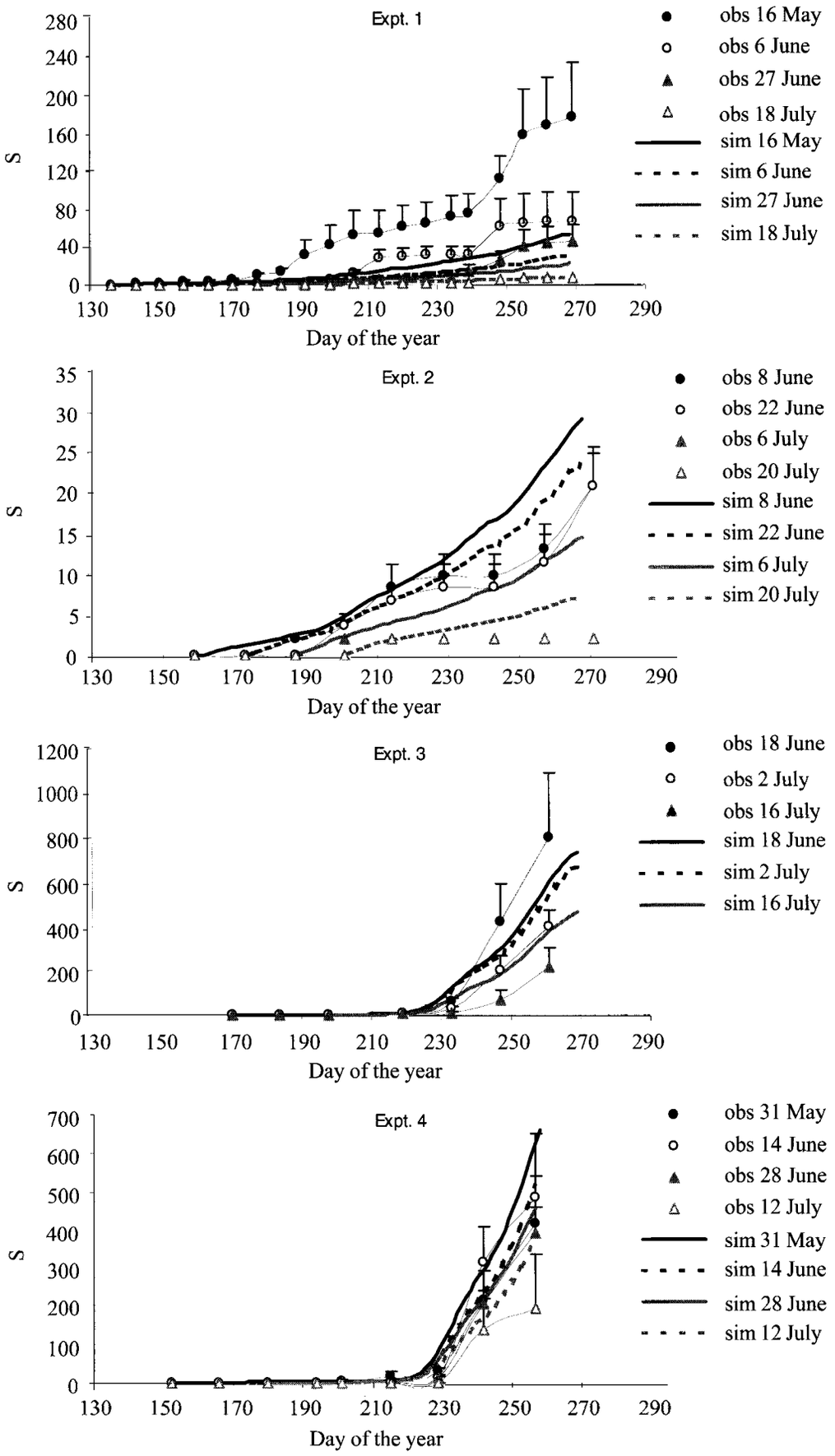

Fig. 8. Simulated disease progress curves, using a single common value of *r*, compared to field data. Circles and triangles represent the observed spread and lines the simulated spread. S is the number of plants showing symptoms per plot. Bars represent the standard error of the mean.

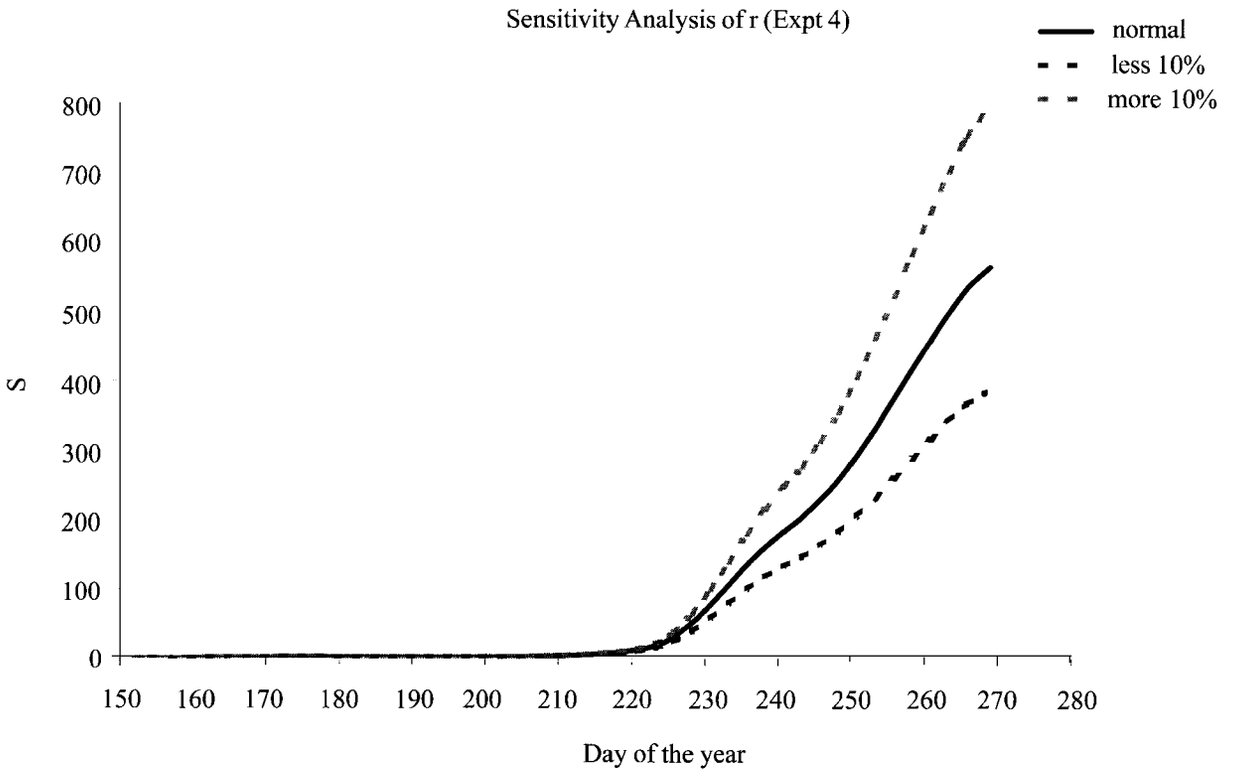

Fig. 9. Sensitivity analysis of *r* with data from Expt 4, inoculation date on 31 May 1996. Solid line is the simulation with the calibrated value of *r*. Top and bottom dashed lines are the simulations with the plus and minus 10% change in the rate parameter. S: plants showing symptoms.

The use of the total number of caught aphids as a driving function of the simulation model resulted in quite stable estimates for *r* for different inoculation dates within each experiment (Table 5). The *r* values between locations and years varied within a narrow range. These results show that a single value of *r* could be used to simulate the spread in each experiment and that the studied epidemiological process could be characterised by this parameter. The meaning of *r* is quite complex. It is a single rate parameter that represents all aspects of the vector activity in the disease dynamics (Jeger, van den Bosch, Madden & Holt, 1998). This parameter was estimated using experimental field data and appeared to be quite robust. This is remarkable because differences between the fields used, such as crop stature, density, weed species, presence of trees and other crops on the field borders, wind, and latitude could all affect the spread and the parameter. Variations in disease incidence within the same field may also occur as have been observed in other pathosystems (Madden *et al.*, 1987*c*; Mora-Aguilera *et al.*, 1996). Most simulated epidemics fit between the average field data ± SE (the standard error of the mean for the replications) (Figs 6, 7 and 8). The rather small variability of *r* between the four experiments is an encouraging result, suggesting that models of this sort may have predictive value in disease management.

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