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# APHID AND VIRUS DYNAMICS TO IMPROVE FORECASTS OF BARLEY YELLOW DWARF VIRUS RISK

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by

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CONTENTS

## 1. SUMMARY

The objective of the project was to quantify aspects of the epidemiology of barley yellow dwarf virus sufficiently to be able to contribute to a reliable scheme for forecasting the risk of infection in the different cereal growing regions of Britain. The project took place between August 1992 and July 1995.

A field experiment was continued in the first year from that started in project number 0003/3/89 (see Project Report No. 87), incorporating four sites and five different sowing dates of winter barley at each site. The experiment was designed to study the effects of sowing date on the number and species of aphid vectors of BYDV and on virus incidence in the crop. In addition, monitoring of the numbers of migrant aphid vectors and the proportion carrying virus continued for the three years of the project. The data provide valuable information on variation in aphid and BYDV incidence between regions and years using a standardised sampling method.

An experiment was done to investigate the effect of crop growth stage and temperature on virus progression and detectability in winter barley using artificial inoculations. The relationship between the time that a virus can be detected in a plant by ELISA, and the time at which a plant becomes a source for virus spread was also investigated. Results showed that temperature in the period after inoculation was critical in determining the final virus levels regardless of growth stage at inoculation, but that the younger the plants when inoculated, the higher the level of virus when mature. However, plants inoculated at a later growth stage were a better source for subsequent virus transmission by aphids than those inoculated at an earlier growth stage.

Laboratory experiments were done to examine transmission of the virus from infected plants, through the aphid vector and into uninfected plants at a range of temperatures encountered in the autumn in the U.K. and in the two main vector aphid species. The results showed the crucial importance of autumn and winter temperature at all stages in the virus transmission cycle.

The results will be used, in conjunction with those of other HGCA-funded projects and information from the literature, in the assembly of a weather-driven mechanistic model of virus spread.

## 2. OBJECTIVES

The objectives for the project were to quantify aspects of the epidemiology of barley yellow dwarf virus sufficiently to be able to contribute to a reliable scheme for forecasting the risk of infection in the different cereal growing regions of Britain. This continues work started in project number 0003/3/89 and reported as Project Report No. 87.

The specific objectives were-

a) to monitor the numbers of migrant vector aphids in the 12.2m. suction traps at four core sites: Leeds (Yorkshire), Rothamsted (Hertfordshire), Wye (Kent) and Starcross (Devon);

b) to monitor virus incidence in migrant aphids;

c) to monitor the proportions of the different migrant forms of the bird cherry aphid in autumn at Rothamsted and to validate the method developed for distinguishing between the cereal-colonising and non-colonising autumn winged forms of the bird cherry aphid;

d) to monitor aphids and virus in winter barley planted on five sowing dates at the four different sites;

e) to investigate the effect of crop growth stage and temperature on virus progression and detectability in winter barley planted on three sowing dates;

f) to examine the relationship between the time that a virus can be detected in a plant by ELISA and the time at which a plant becomes a source for virus spread;

g) to examine the effect of temperature on virus acquisition and inoculation by aphids and on the latent period of the virus in the aphid.

## 3. INTRODUCTION

The incidence of barley yellow dwarf virus (BYDV) varies considerably from year to year and between regions. The virus is spread by two principal vectors in the UK, the grain aphid (*Sitobion avenae*) and the bird cherry aphid (*Rhopalosiphum padi*). There are at least three different isolates of BYDV in the UK: the MAV-like isolate, transmitted mainly by the grain aphid, the RPV-like isolate, transmitted by the bird cherry aphid, and the PAV-like isolate, transmitted by both species. The isolates differ in the severity of the symptoms they cause and the aphids differ in key respects, which makes the epidemiology of the disease very complex.

Initial infection of autumn sown cereals with BYDV is usually caused by infective winged aphids flying into the crop in its early stages. Plants inoculated at a younger growth stage show a greater yield loss than those inoculated at an older growth stage (Doodson & Saunders, 1970). The characteristic patches of BYDV are caused by subsequent virus spread as a result of the original crop colonising aphids and their wingless offspring moving between plants in the late autumn and winter. Data from the Rothamsted Insect Survey network of 12.2m suction traps show that the numbers of the two vectors vary annually and regionally. The proportion of vectors carrying virus also varies between years and regions (Tatchell *et al.*, 1994). These components are very important in determining the initial level of BYDV in any one year.

The bird cherry aphid can have a sexual phase in its life cycle which is initiated in autumn. Throughout summer the winged aphids caught in the suction traps are the crop colonising forms (virginoparae). In autumn the non-colonising forms (gynoparae), which are morphologically identical to the crop colonising forms are also found. The non-colonising forms leave the crop to find the primary host (bird cherry trees) on which overwintering eggs are eventually produced. However, some clones do not have a sexual phase and produce cereal-colonising forms all year round. The proportions of these two winged forms vary annually and regionally and have been assessed for four years using host plant choice tests and a further two years using a new method developed at Rothamsted (Lowles, 1995). It is important to know the proportion of colonising forms as they are far more likely to infect a crop with BYDV than non-colonising forms, though they can both transmit the virus (Tatchell *et al.*, 1994).

The grain aphid usually overwinters as the active, parthenogenetic form with no egg phase, so that all the winged forms in the autumn are potential cereal colonisers. The species can often be found in the crop throughout a mild winter and tends to survive for longer than the cereal-colonising form of the bird cherry aphid.

In order to quantify the process of virus build-up in the crop, factors influencing the efficiency of transmission of the virus from infected plants to uninfected plants need to be studied closely. The process of transmission can be separated into three periods: a) the acquisition access period, during which time the vector has access to the source plant although it is not necessarily feeding; b) the latent period, which is the time between the vector acquiring the virus and becoming infective and c) the inoculation threshold period, which is the minimum time necessary for a vector to feed on a plant before inoculation can occur (Federation of British Plant Pathologists, 1973). The duration of the three periods varies

according to conditions, the most important being temperature, and can be measured to assess the chances of virus transmission occurring in the autumn.

The rate of BYDV spread in previous years has not shown a direct correspondence with changes in aphid abundance in field plots. Most aphids were found in plots in the autumn whereas the number of leaves infected with BYDV increased slowly throughout the winter, not reaching a peak until February or March. It is not known how the rate of BYDV spread is affected either by growth stage of the plant at the time of infection, or by weather conditions (especially temperature) during the winter. Because all testing of field plants for virus is done by ELISA (Lister & Rochow, 1979), which cannot detect virus below a threshold concentration, it is important also to estimate the time difference between detection of virus in a plant by ELISA and the plant acting as a source of virus for acquisition by aphids.

# 4 CORE DATA

## 4.1 Materials and Methods

Complete sets of data were collected from four sites throughout England, Starcross in Devon, Wye in Kent, Rothamsted in Hertfordshire and Leeds in Yorkshire, during the 1992/3 and 1993/4 growing seasons as follows.

**4.1.1** Monitoring aerial aphid populations.

Suction traps of the Rothamsted Insect Survey were used to sample the number of insects in a standard volume of air daily throughout the year at a height of 12.2 m (Macaulay, Tatchell & Taylor,1988). Data from traps at the four sites (Fig. 1) from 1 August to 15 December 1992-1994 are used for this project.

**4.1.2** Monitoring virus in migrant aphids.

Suction traps were used to sample live insects at a height of 1.5 m at the four sites (Fig. 1). Samples were collected daily from 1 September to 1 December 1992-1994 and were transferred to the laboratory where BYDV vector species were separated from others and frozen individually in labelled tubes for subsequent assay for virus by ELISA at the Central Science Laboratory, MAFF, Harpenden.

**4.1.3** Monitoring the form of migrant bird cherry aphids.

Suction traps were used to sample female bird cherry aphids at a height of 12.2 and 1.5 m at Rothamsted. Insects were trapped alive and samples collected twice a day (daily at weekends) and sorted as in the previous section. In 1992 the method used was the same as described by Tatchell, Plumb & Carter (1988) where, in order to separate the forms, female bird cherry aphids were given a choice of leaves (bird cherry or barley) on which to produce nymphs. The non-colonising forms (gynoparae) reproduce only on bird cherry leaves and the crop colonising forms (virginoparae) on barley leaves. In 1993 a new method was tested (Lowles, 1995) which separates the two different autumn migrant forms of the bird cherry aphid quickly and easily. The method uses a simple 'squash blot' technique where living or freshly-killed aphids are placed individually on a piece of filter paper wetted with 95% ethyl alcohol. Each aphid is held by the thorax with forceps while the embryos are dissected out into the alcohol. The embryos of colonising forms immediately change colour from yellow/green to brown/dark pink as the pigment reacts with the alcohol. Embryos of non-colonising forms change slightly, to an opaque yellow.

The method was validated during the period of autumn migration in 1993. Aphids were collected alive using suction traps operating at Rothamsted. Samples were collected twice daily and bird cherry aphids were removed by releasing the insects caught into a large open fronted perspex box positioned in front of a window. The insects flew to the back of the box, attracted to the light from the window, allowing aphids to be picked out and placed into individual glass tubes for identification under the microscope. To remove any bias in catches due to time of sampling, alternate morning catches were subjected to choice testing and to squash blot testing. On days where the morning catch was choice tested, the afternoon


Fig. 1. Experimental sites

catch was squash blot tested and vice versa. As a further validation, 13 members of the Rothamsted Insect Survey, unfamiliar with the technique, were given the opportunity to practice on known non-colonising and colonising forms reared in the laboratory, and were then asked to identify ten randomly mixed forms presented in a blind test. In 1994 the squash blot test was the only method used.

### 4.1.4 Crop Validation

In 1992 plots of winter barley cv. Igri (Magie at Rothamsted), were sown on five different dates in autumn, replicated four times in a randomised block design at each of the four experimental sites. Sowing dates were selected to reflect normal practice in the area and to maximise the range of infestation (Table 1). At Rothamsted, an additional set of plots was sown on each occasion. These were sprayed with cypermethrin to control the aphid vectors. Combine yields were taken at harvest on both the sprayed and unsprayed plots. Numbers of aphids were assessed at regular intervals by visual inspection. All unsprayed plots at each of six locations per plot were examined and the number, species and development stage of the aphids recorded. The number of plants sampled was reduced if numbers of aphids were large.

Visual symptoms of BYDV were recorded in spring. Additionally, 20 leaves were collected at monthly intervals from each plot sown in mid September. The leaves were placed individually in plastic bags and deep-frozen for subsequent identification of BYDV strain using ELISA at the Central Science Laboratory, MAFF, Harpenden. In addition, at Rothamsted, two further leaf samples were taken to coincide with each aphid count. These consisted of 100 leaves from plots sown in early September and late September. The leaves

**Table 1. Sowing dates of experimental plots of winter barley on the four core sites, 1992**

| Leeds | Rothamsted | Wye | Starcross |
|---|---|---|---|
| 16 Sept | 8 Sept | 16 Sept | 14 Sept |
| 7 Oct | 15 Sept | 29 Sept | 28 Sept |
| 13 Oct | 28 Sept | 8 Oct | 12 Oct |
| 30 Oct | 12 Oct | 15 Oct | 26 Oct |
| 13 Nov | 31 Oct | 30 Oct | 4 Dec |

were cut in half longitudinally, one half placed individually in plastic bags, the other half into 'pooled' bags containing five half leaves. The pooled samples were tested by ELISA for BYDV presence. Positive results were further investigated by ELISA testing the corresponding individual halves to determine the strain present and the proportion of infected leaves.

## 4.2 Results and Discussion

### 4.2.1 Monitoring aerial aphid populations.

The data from the Rothamsted Insect Survey suction traps for 1992-1994 are displayed in Fig. 2. Migration of the grain aphid and bird cherry aphid in 1994 continued into December in the most southerly sites of Wye and Starcross while at Rothamsted and Leeds, grain aphid migration stopped in November. This was a much longer migration than in other years and was due to higher than average autumn temperatures. The peak numbers for the bird cherry aphid occurred in October in 1994 as is usual (Tatchell *et al.*, 1994) but in 1992 the peak numbers of both species appeared at the end of September at all sites.

### 4.2.2 Monitoring virus in migrant aphids.

The number of the two main vector species trapped each autumn and tested varied from site to site and from week to week (Tables 2 and 3). The infectivity index is defined here as the product of the proportion of vector species found to be viruliferous in 1.5m suction traps (low traps) and the number of that species in the adjacent 12.2m trap. The infectivity index can be accumulated from the time of crop emergence to give a measure of the risk of primary infection of that crop.

In 1992, averaged across the four experimental sites for both species, 13.3% of individuals were found to be carrying isolates of BYDV. This ranged from 10.2% to 22.1% of bird cherry aphids and 0% to 37.5% of grain aphids (Table 2). The accumulated infectivity index was greatest at Leeds and lowest at Rothamsted, the peak in infectivity occurring in the last week of September, significantly earlier than in the previous three years. In general the accumulated serological infectivity indices were much larger in 1992 than in any of the three preceding years, due mainly to much higher numbers of aphids caught in the 12.2m traps. Crops emerging in November appear to have been at little risk from primary infection.

In 1993, the average percentage of individuals found to be carrying BYDV was 13.4%. Overall, accumulated serological infectivity indices were much lower than for 1992 but, as before, Leeds recorded the most virus. The peaks in infectivity occurred earlier in 1993 than in 1992 and, generally, crops emerging after the middle of October were at little risk from migrant viruliferous aphids.

In 1994 data were collected only from Wye and Starcross (Table 3A). The largest numbers of bird cherry aphids collected in the low suction traps at both sites were in October and the largest number of grain aphids were found at Wye in mid to late October and at Starcross in late October and early November. Accumulated serological infectivity indices were similar for both sites, slightly higher than in 1993 but still much lower than in 1992. Peaks of infectivity were however much late than in both 1992 and 1993 with maximum virus pressure occurring in the second week of October.

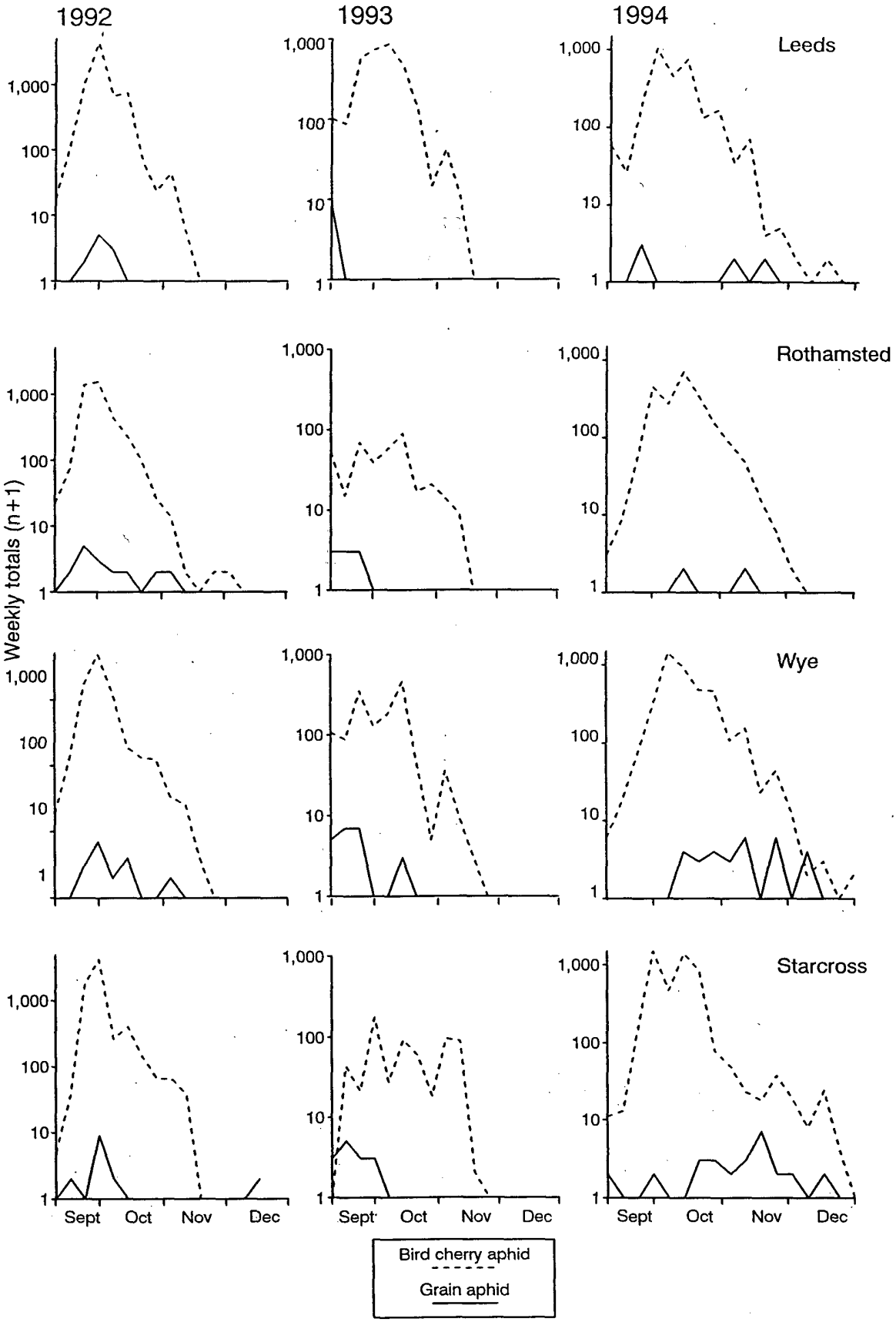


Fig. 2. Aphids in 12.2m suction traps, 1992-1994

TABLE 2. The number of vector aphids trapped weekly and found by ELISA to contain BYDV provide the data for the infectivity index, 1992.

| Trap Site | Week Beginning | Bird Cherry Aphid | | | Grain Aphid | | | Serological Infectivity Index |
|---|---|---|---|---|---|---|---|---|
| | | No. tested | No. positive | No. in RIS trap | No. tested | No. positive | No. in RIS trap | |
| Leeds | 31-Aug | 1 | 0 | 13 | 0 | 0 | 0 | 0 |
| | 07-Sep | 5 | 1 | 58 | 0 | 0 | 0 | 11.6 |
| | 14-Sep | 20 | 5 | 740 | 0 | 0 | 0 | 185 |
| | 21-Sep | 108 | 29 | 1528 | 0 | 0 | 5 | 410.29 |
| | 28-Sep | 88 | 16 | 3258 | 0 | 0 | 0 | 592.39 |
| | 04-Oct | 46 | 6 | 178 | 0 | 0 | 2 | 23.21 |
| | 12-Oct | 84 | 20 | 360 | 0 | 0 | 0 | 85.71 |
| | 19-Oct | 24 | 7 | 27 | 0 | 0 | 0 | 7.87 |
| | 26-Oct | 10 | 3 | 10 | 0 | 0 | 0 | 3 |
| | 02-Nov | 5 | 0 | 7 | 0 | 0 | 0 | 0 |
| | 09-Nov | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| R'sted | 07-Sep | 0 | 0 | 25 | 0 | 0 | 1 | 0 |
| | 14-Sep | 4 | 0 | 740 | 0 | 0 | 1 | 0 |
| | 21-Sep | 4 | 0 | 1508 | 0 | 0 | 4 | 0 |
| | 28-Sep | 7 | 3 | 988 | 1 | 0 | 2 | 423.42 |
| | 05-Oct | 9 | 2 | 156 | 0 | 0 | 2 | 34.66 |
| | 12-Oct | 1 | 0 | 42 | 0 | 0 | 0 | 0 |
| | 19-Oct | 3 | 0 | 5 | 0 | 0 | 1 | 0 |
| Wye | 07-Sep | 5 | 2 | 24 | 1 | 1 | 0 | 9.6 |
| | 14-Sep | 14 | 2 | 456 | 1 | 0 | 1 | 65.14 |
| | 21-Sep | 308 | 38 | 4013 | 1 | 0 | 4 | 495.11 |
| | 28-Sep | 403 | 46 | 2841 | 2 | 1 | 7 | 327.78 |
| | 05-Oct | 113 | 13 | 118 | 0 | 0 | 3 | 13.57 |
| | 12-Oct | 47 | 5 | 26 | 2 | 1 | 0 | 2.76 |
| | 19-Oct | 43 | 8 | 26 | 1 | 0 | 0 | 4.83 |
| | 26-Oct | 19 | 4 | 5 | 0 | 0 | 0 | 1.05 |
| | 02-Nov | 12 | 1 | 11 | 0 | 0 | 0 | 0.91 |
| | 09-Nov | 7 | 1 | 0 | 0 | 0 | 0 | 0 |
| | 16-Nov | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 23-Nov | 1 | 1 | data n/a | 0 | 0 | 0 | 0 |
| | 30-Nov | 0 | 0 | data n/a | 0 | 0 | 0 | 0 |
| Starcross | 24-Aug | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| | 31-Aug | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| | 07-Sep | 14 | 0 | 26 | 0 | 0 | 1 | 0 |
| | 14-Sep | 18 | 3 | 227 | 1 | 1 | 0 | 37.83 |
| | 21-Sep | 203 | 29 | 5139 | 3 | 0 | 8 | 734.14 |
| | 28-Sep | 451 | 31 | 519 | 8 | 0 | 0 | 35.67 |
| | 05-Oct | 77 | 7 | 149 | 0 | 0 | 1 | 13.54 |
| | 12-Oct | 40 | 6 | 121 | 7 | 1 | 0 | 18.15 |
| | 19-Oct | 67 | 11 | 41 | 11 | 1 | 0 | 6.73 |
| | 26-Oct | 7 | 0 | 14 | 7 | 0 | 0 | 0 |
| | 02-Nov | 7 | 3 | 32 | 0 | 0 | 0 | 13.71 |
| | 09-Nov | 4 | 1 | 0 | 0 | 0 | 0 | 0 |
| | 16-Nov | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE 3. The number of vector aphids trapped weekly and found by ELISA to contain BYDV provide the data for the infectivity index, 1993.

| Trap Site | Week Beginning | Bird Cherry Aphid | | | Grain Aphid | | | Serological Infectivity Index |
|---|---|---|---|---|---|---|---|---|
| | | No. tested | No. positive | No. in RIS trap | No. tested | No. positive | No. in RIS trap | |
| Leeds | 6-Sep | 3 | 0 | 94 | 0 | 0 | 0 | 0 |
| | 13-Sep | 0 | 0 | 32 | 0 | 0 | 0 | 0 |
| | 20-Sep | 108 | 18 | 1106 | 0 | 0 | 0 | 184.33 |
| | 27-Sep | 34 | 6 | 364 | 0 | 0 | 0 | 64.23 |
| | 4-Oct | 117 | 15 | 592 | 0 | 0 | 1 | 75.89 |
| | 11-Oct | 34 | 5 | 18 | 0 | 0 | 0 | 2.64 |
| | 18-Oct | 30 | 3 | 77 | 0 | 0 | 0 | 7.7 |
| | 25-Oct | 3 | 0 | 4 | 0 | 0 | 0 | 0 |
| | 1-Nov | 1 | 0 | 4 | 0 | 0 | 0 | 0 |
| | 8-Nov | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Wye | 6-Sep | 33 | 4 | 46 | 0 | 0 | 4 | 5.57 |
| | 13-Sep | 13 | 2 | 150 | 3 | 1 | 8 | 25.74 |
| | 20-Sep | 55 | 6 | 254 | 2 | 1 | 0 | 27.7 |
| | 27-Sep | 48 | 7 | 172 | 0 | 0 | 0 | 25.08 |
| | 4-Oct | 61 | 3 | 210 | 0 | 0 | 2 | 10.32 |
| | 11-Oct | 106 | 14 | 82 | 1 | 0 | 0 | 10.83 |
| | 18-Oct | 16 | 5 | 6 | 0 | 0 | 0 | 1.85 |
| | 25-Oct | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1-Nov | 6 | 0 | 10 | 0 | 0 | 0 | 0 |
| | 8-Nov | 1 | 1 | 2 | 0 | 0 | 0 | 2 |
| Starcross | 30-Aug | 118 | 15 | 98 | 0 | 0 | 6 | 12.45 |
| | 6-Sep | 25 | 7 | 12 | 0 | 0 | 6 | 3.36 |
| | 13-Sep | 15 | 2 | 36 | 0 | 0 | 0 | 4.8 |
| | 20-Sep | 7 | 1 | 48 | 0 | 0 | 2 | 6.85 |
| | 27-Sep | 33 | 3 | 128 | 0 | 0 | 2 | 11.63 |
| | 4-Oct | 8 | 1 | 26 | 0 | 0 | 0 | 3.25 |
| | 11-Oct | 22 | 3 | 44 | 0 | 0 | 0 | 6 |
| | 18-Oct | 8 | 0 | 18 | 0 | 0 | 0 | 0 |
| | 25-Oct | 3 | 0 | 2 | 0 | 0 | 0 | 0 |
| | 1-Nov | 2 | 0 | 53 | 0 | 0 | 0 | 0 |
| | 8-Nov | 8 | 1 | 7 | 0 | 0 | 0 | 0.87 |

TABLE 3a. The number of vector aphids trapped weekly and found by ELISA to contain BYDV provide the data for the infectivity index, 1994.

| Trap Site | Week Beginning | Bird Cherry Aphid | | | Grain Aphid | | | Serological Infectivity Index |
|---|---|---|---|---|---|---|---|---|
| | | No. tested | No. positive | No. in RIS trap | No. tested | No. positive | No. in RIS trap | |
| Starcross A | 12-Sept | 5 | 1 | 50 | 1 | 0 | 0 | 10 |
| | 19-Sept | 14 | 0 | 541 | 0 | 0 | 1 | 0 |
| | 26-Sept | 60 | 4 | 919 | 0 | 0 | 0 | 61.27 |
| | 3-Oct | 30 | 3 | 244 | 0 | 0 | 0 | 24.4 |
| | 10-Oct | 49 | 6 | 1059 | 3 | 2 | 2 | 131.01 |
| | 17-Oct | 38 | 4 | 114 | 0 | 0 | 0 | 12 |
| | 24-Oct | 11 | 0 | 40 | 0 | 0 | 3 | 0 |
| | 31-Oct | 5 | 0 | 12 | 3 | 0 | 0 | 0 |
| | 7-Nov | 3 | 0 | 11 | 0 | 0 | 2 | 0 |
| Starcross B | 12-Sept | 1 | 0 | 50 | 0 | 0 | 0 | 0 |
| | 19-Sept | 16 | 0 | 541 | 0 | 0 | 1 | 0 |
| | 26-Sept | 63 | 3 | 919 | 1 | 0 | 0 | 43.76 |
| | 3-Oct | 34 | 2 | 244 | 1 | 0 | 0 | 14.35 |
| | 10-Oct | 51 | 5 | 1059 | 2 | 0 | 2 | 103.82 |
| | 17-Oct | 27 | 2 | 114 | 3 | 0 | 0 | 8.44 |
| | 24-Oct | 3 | 0 | 40 | 1 | 0 | 3 | 0 |
| | 31-Oct | 7 | 1 | 12 | 9 | 0 | 0 | 1.71 |
| | 7-Nov | 2 | 1 | 11 | 0 | 0 | 2 | 5.5 |
| Wye A | 5-Sept | 4 | 1 | 4 | 1 | 0 | 1 | 1 |
| | 12-Sept | 7 | 0 | 63 | 0 | 0 | 0 | 0 |
| | 19-Sept | 21 | 2 | 15 | 0 | 0 | 0 | 1.43 |
| | 26-Sept | 72 | 2 | 259 | 0 | 0 | 0 | 7.19 |
| | 3-Oct | 189 | 17 | 790 | 1 | 1 | 1 | 72.06 |
| | 10-Oct | 102 | 11 | 533 | 0 | 0 | 0 | 57.48 |
| | 17-Oct | 58 | 4 | 266 | 0 | 0 | 0 | 18.34 |
| | 24-Oct | 77 | 3 | 52 | 2 | 1 | 2 | 3.03 |
| | 31-Oct | 16 | 0 | 50 | 0 | 0 | 0 | 0 |
| | 7-Nov | 2 | 0 | 27 | 1 | 0 | 1 | 0 |
| | 14-Nov | 3 | 0 | 12 | 0 | 0 | 0 | 0 |
| | 21-Nov | 18 | 3 | 30 | 12 | 0 | 12 | 5 |
| | 28-Nov | 2 | 0 | 9 | 2 | 1 | 2 | 1 |
| Wye B | 5-Sept | 1 | 0 | 4 | 0 | 0 | 1 | 0 |
| | 12-Sept | 4 | 0 | 63 | 0 | 0 | 0 | 0 |
| | 19-Sept | 28 | 0 | 15 | 0 | 0 | 0 | 0 |
| | 26-Sept | 58 | 1 | 259 | 0 | 0 | 0 | 4.47 |
| | 3-Oct | 145 | 6 | 790 | 0 | 0 | 1 | 32.69 |
| | 10-Oct | 80 | 8 | 533 | 0 | 0 | 0 | 53.3 |
| | 17-Oct | 54 | 6 | 266 | 0 | 0 | 0 | 29.56 |
| | 24-Oct | 59 | 1 | 52 | 0 | 0 | 2 | 0.88 |
| | 31-Oct | 7 | 0 | 50 | 0 | 0 | 0 | 0 |
| | 7-Nov | 10 | 1 | 27 | 3 | 1 | 1 | 3.03 |
| | 14-Nov | 3 | 0 | 12 | 0 | 0 | 0 | 0 |
| | 21-Nov | 8 | 0 | 30 | 8 | 1 | 12 | 1.5 |
| | 28-Nov | 4 | 1 | 9 | 3 | 0 | 2 | 2.25 |

**4.2.3** Monitoring the form of migrant bird cherry aphids.

The percentage of crop colonising forms found at the two heights is shown in Fig 3. The decline in the proportion of crop colonising forms in mid September to early October corresponds with the switch in the clones which produce sexual forms, from crop colonising forms to non-crop colonising forms which return to the winter host, bird cherry. Few crop colonising forms were trapped at 12.2m throughout the period of observation. The apparent increase in the proportions of colonising forms from the end of October and the beginning of November is due to a single individual at each height.

Results from the trial comparing the choice test method and squash blot method showed no significant difference between the two methods. In the blind test, 98.5% of the insects were correctly identified, with only two samples out of the 130 being mis-identified. This method has now been adopted to separate routinely the two forms of the aphid in the autumn.

**4.2.4** Crop validation

Numbers of grain aphid at the Leeds site remained low all season (Fig. 4). When making direct comparisons between sites it is important to remember that the sowing dates differ from site to site (Table 1). Numbers of the bird cherry aphid differed considerably between sites (Fig 5), with many more aphids being found earlier in the season at Starcross than elsewhere. Generally, numbers were greater in earlier sown plots than in later sown plots.

The percentage of plants infested with aphids (Fig. 6) was not monitored at Starcross throughout the autumn and winter as growth of the plants from the earliest sowing was so great that individual plants became impossible to separate. Elsewhere aphids were most numerous on the earlier sowings.

The percentage of BYDV infection was low at all sites except Starcross where PAV increased throughout the season (Fig. 7). Virus incidence reaches a peak after aphid populations have declined, emphasising the need to understand factors affecting transmission and detectability.

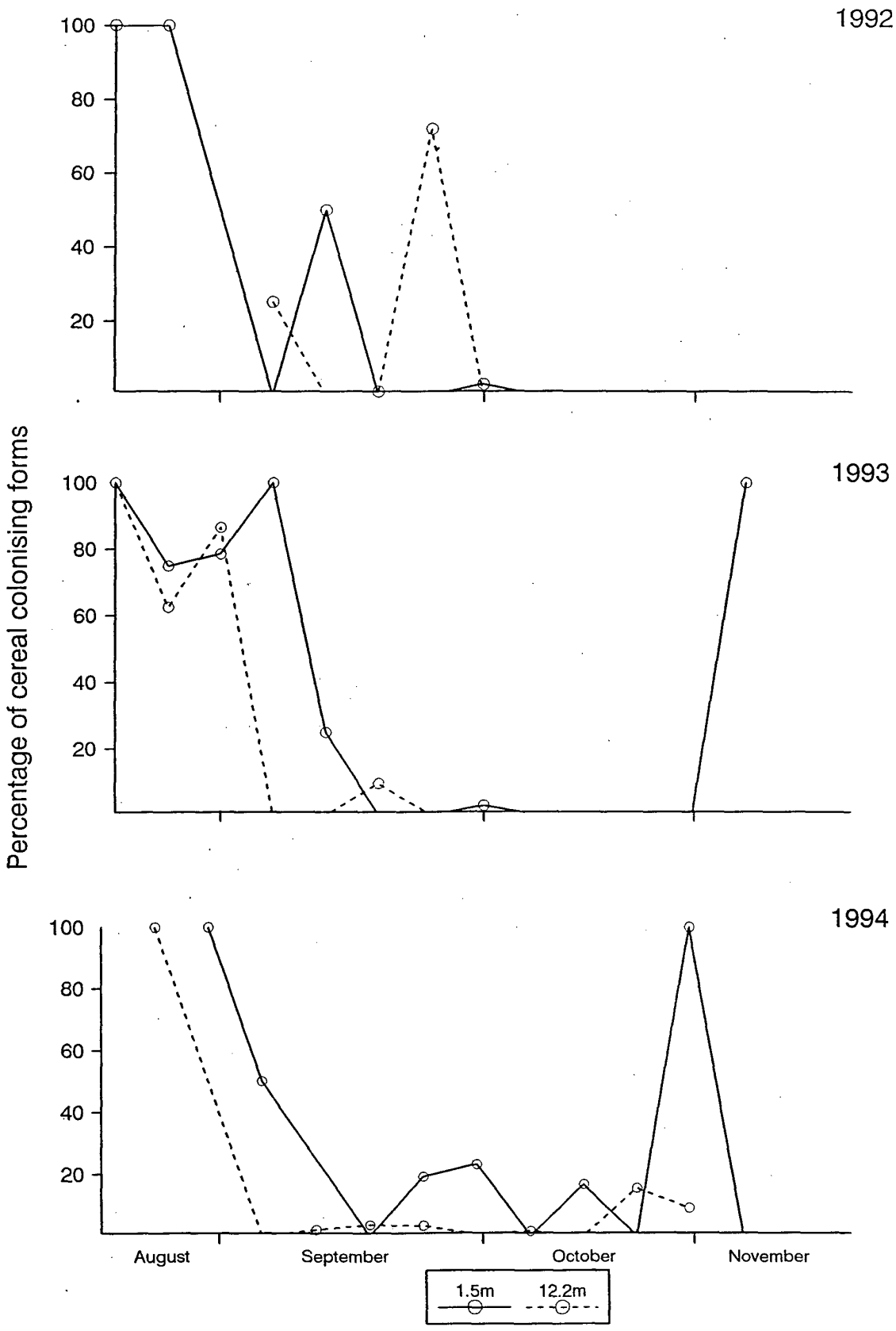


Fig. 3. Percentage of cereal colonising forms of the bird cherry aphid caught at Rothamsted at two heights 1992-1994

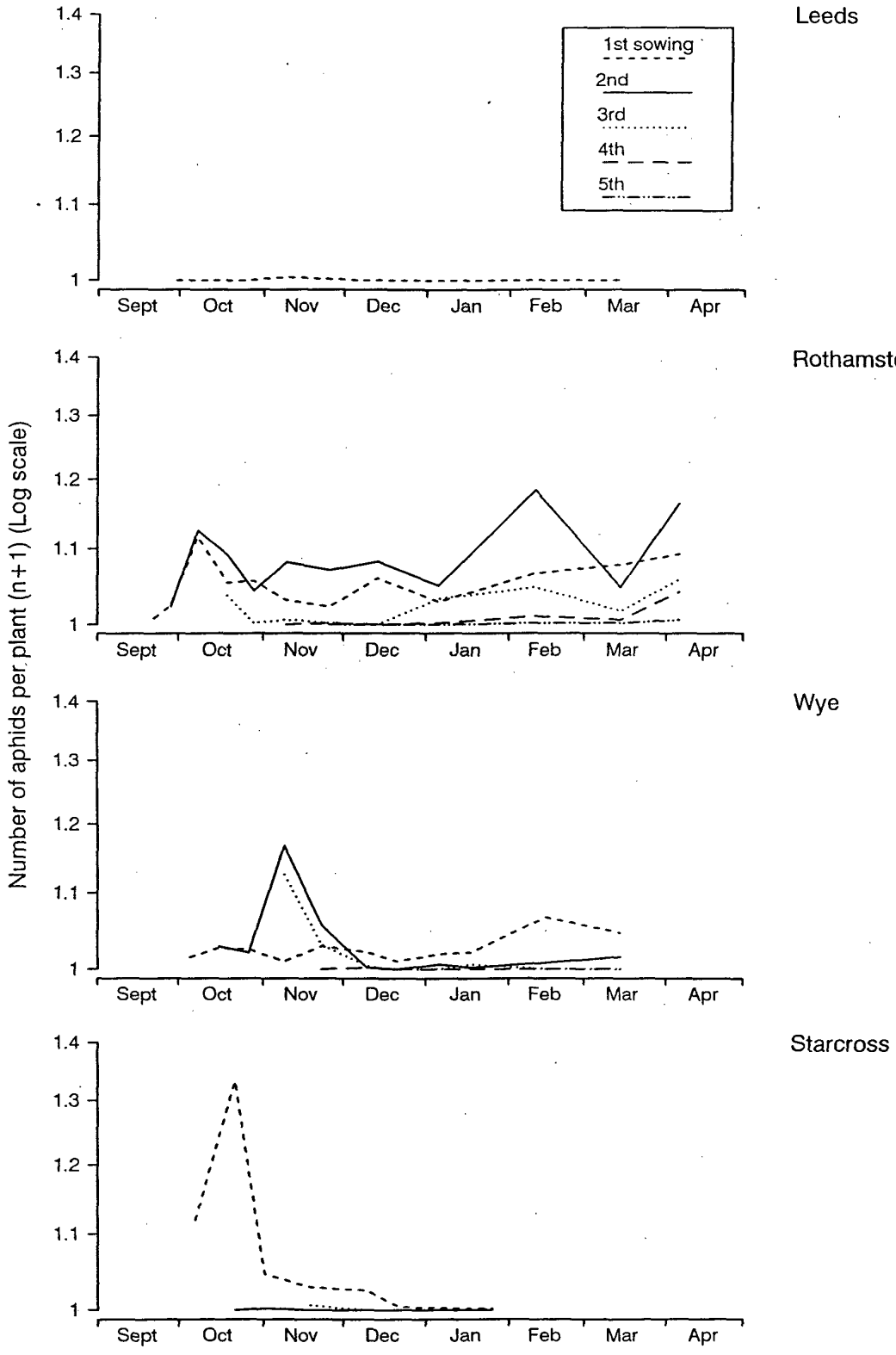


Fig. 4. Numbers of grain aphid per plant on winter barley sown on different dates, 1992-93

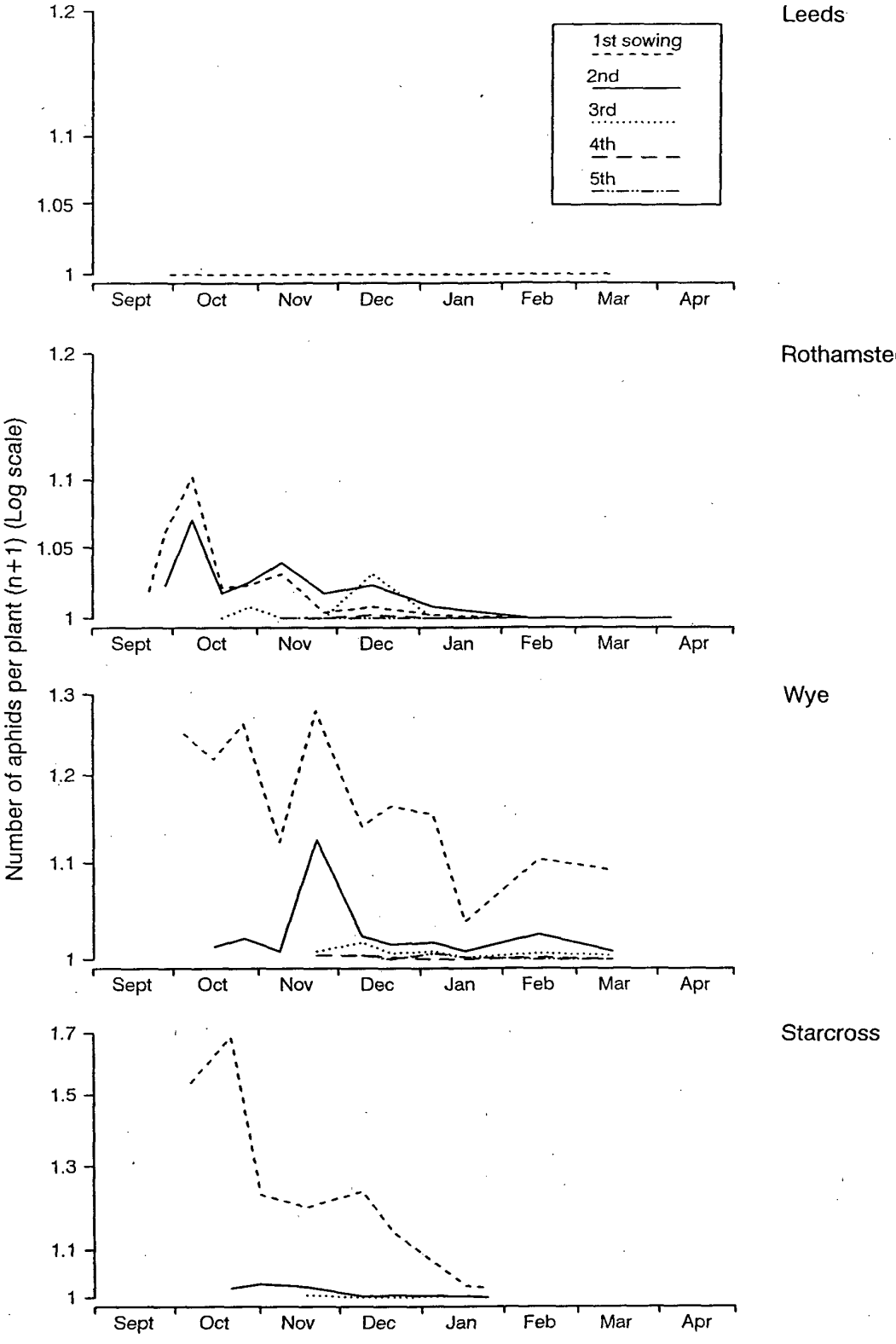


Fig. 5. Numbers of bird cherry aphid per plant on winter barley sown on different dates, 1992-93

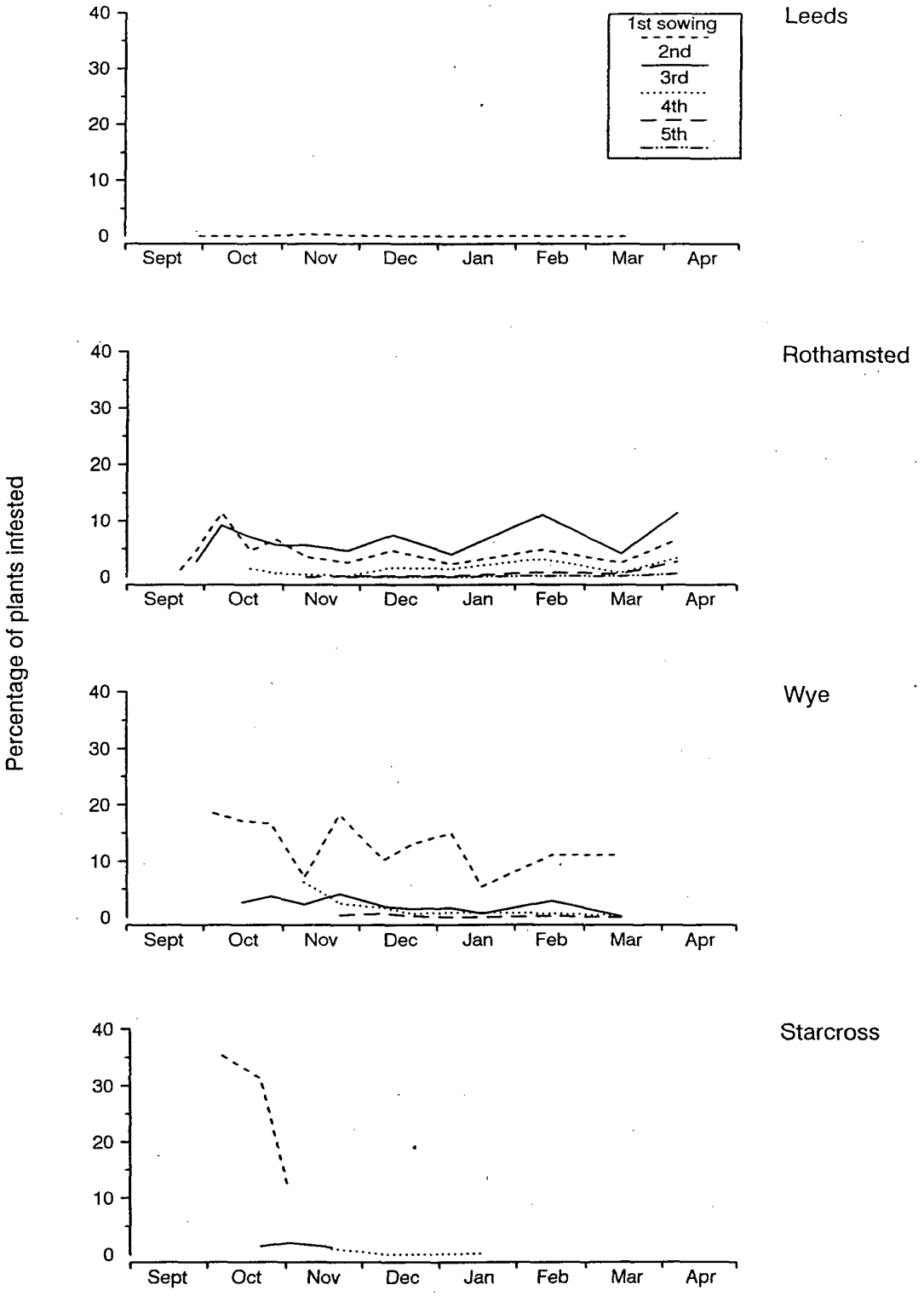


Fig. 6. The percentage of plants infested with aphids on winter barley sown on different dates, 1992-93

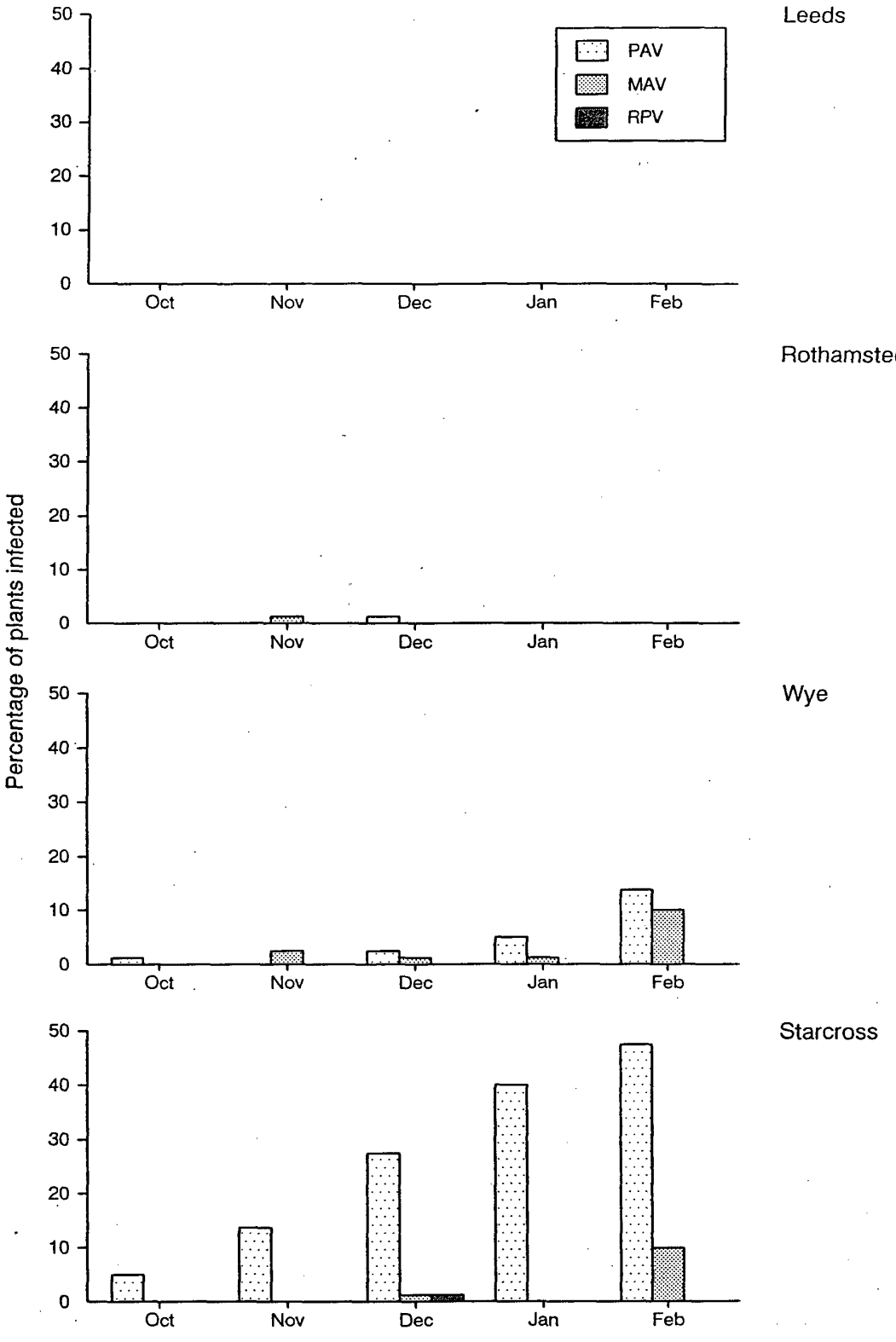


Fig. 7. Percentage of BYDV infection in mid-September sown winter barley, 1992-93

# 5. THE EFFECT OF GROWTH STAGE AND TEMPERATURE ON VIRUS PROGRESSION IN THE FIELD

## 5.1 Introduction

The experiment follows from the sequentially sown plots experiment (Tatchell *et al.*, 1994) and examines the relationship between virus inoculation at different growth stages and times in autumn and the development of the virus through the winter.

## 5.2 Materials and Methods

An experiment using artificial inoculations with the bird cherry aphid and the PAV-like isolate was carried out in the 1993-1994 crop of winter barley and in the following year the experiment was repeated with the grain aphid and the MAV-like isolate. There were three drilling dates in both years. In both years aphids were added on three occasions for each drilling date, based on the growth stage of the plants at the time. Dates and growth stages at inoculation and the drilling dates in both years are shown in Table 4.

The aphids used to infest the plots had been reared on plants infected with the virus. The aphids were placed in tubes and mixed with a little talcum powder. This makes it easy to remove them individually and hence place the required numbers in the plots (Comeau, 1992). Aphids were applied using a fine paint brush as close as possible to the base of the plants at a rate of between three and five per plant and readily crawled from the soil up the nearest plant. Plots were sprayed approximately 1 week after infestation with 260g "Aphox" (Pirimicarb) in 220 l water. The final infestation on the late drilling of both years was not achieved as the stems of the plants had begun to elongate, at which stage the plants are no longer susceptible to virus acquisition.

The first leaf samples were taken 2-3 weeks after infestation and every 2-4 weeks subsequently. Twenty random leaf samples per plot were taken, the youngest fully expanded leaf on each sample plant was removed and tested for virus presence by ELISA.

For the first year's experiment, sub-plots were harvested on 21-22 July. Three blocks were harvested. All heads from within the inoculation area of 1m x 2m were removed and threshed.

In addition to leaf samples for testing by ELISA, leaf samples from the middle sowing were taken for testing as a source for aphid acquisition. Non-viruliferous aphids were placed singly on excised leaf samples from the field and kept in "aphid ampules" (Austin *et al.*, 1991) at room temperature (18-20°C) and left for a 72 hour acquisition access period. Aphids were removed from the ampules and then confined individually on oat test seedlings (cv Dula), at one per pot, with a cellulose acetate cylinder pushed into the soil around the plant and closed at the other with terylene gauze. Plants were kept in the glasshouse and aphids were allowed a 72 hour transmission period at 18°C. Plants were then sprayed with Pirimicarb to kill the aphids. Visual symptoms were assesed at intervals from two to three weeks later in the case of the plants infected with the PAV-like isolate and those infected with the MAV-like isolate were tested by ELISA.

**Table 4: Drilling and inoculation dates for 1993 and 1994 with growth stages (GS).**

| PAV | **Early Drilling (E) 17/9/93** | **Middle Drilling (M) 8/10/93** | **Late Drilling (L) 28/10/93** |
|---|---|---|---|
| **Growth stage and inoculation dates** | GS12 7/10/93<br>GS22-23 11/11/93<br>GS23-24 18/1/94 | GS12 11/11/93<br>GS21-22 18/1/94<br>GS22-25 11/3/94 | GS12-13 18/1/94<br>GS21-22 11/3/94<br>----- |
| **MAV** | **Early Drilling (E) 8/9/94** | **Middle Drilling (M) 26/9/94** | **Late Drilling (L) 17/10/94** |
| **Growth stage and inoculation dates** | GS12-13 4/10/94<br>GS23-25 25/10/94<br>GS24-25 24/11/94 | GS12-13 25/10/94<br>GS22-23 24/11/94<br>GS22-26 2/2/95 | GS12-13 24/11/94<br>GS21-23 2/2/95<br>----- |

## 5.3 Results and Discussion

The results (Fig. 8) show that the growth stage at time of artificial inoculation with PAV by bird cherry aphid had a big influence on determining the final levels of virus, as plants of a younger growth stage inoculated at the same time as older plants had higher levels of virus at the end of the experiment. However, the temperature at and soon after inoculation also appears to have a large influence on the final virus levels as the plots used for the third inoculation had higher final virus levels than those used for the second inoculation, regardless of growth stage. This may be explained by comparing the accumulated day degrees (above 0°C) for the ten days after the inoculations: 28.7 in the case of the second (November) inoculation and 57.6 in the case of the third (January) inoculation.

The winter of 1994-1995 was uncharacteristically warm which led to very high virus levels after inoculation. As in previous years, the age of the plant at the time of inoculation proved important, with those inoculated at earlier growth stages giving higher initial virus levels than those inoculated at later growth stages. However, due to the high temperatures throughout the winter, all inoculations led to virus levels approaching 100 percent at the end of the winter making it very difficult to draw any useful comparisons between inoculation dates.

In 1993-4 the level of transmission of virus by aphids was much lower than the levels found by ELISA directly from field collected leaves (Fig. 9). The leaves from the plots inoculated at growth stage 12 on the middle sowing (M1) showed a maximum of 20-25 percent containing virus when tested by ELISA, while those leaves used for the aphid transmission tests showed a maximum of 5 percent positive. Those plots inoculated at growth stages 21-22 (M2) showed a closer relationship, with the virus levels in leaves inoculated by test aphids previously fed on the field collected samples increasing later and to almost the same level as the leaves tested by ELISA directly from the field.

In the following year, the warmer winter weather increased the virus levels for both methods. Again, the second inoculation at growth stage 22-23 gave results much closer together than the first inoculation at growth stages 12-13: the first inoculation of younger plants giving virus levels of 100 percent in the field while the transmission tests gave virus levels of around 60 percent. The second inoculation produced results of at or near 100 percent for both methods. It would appear that if a plant is inoculated with either the PAV- or MAV-like isolates at an earlier growth stage, it provides a less effective source of virus for subsequent transmission by aphids than plants inoculated at later growth stages.

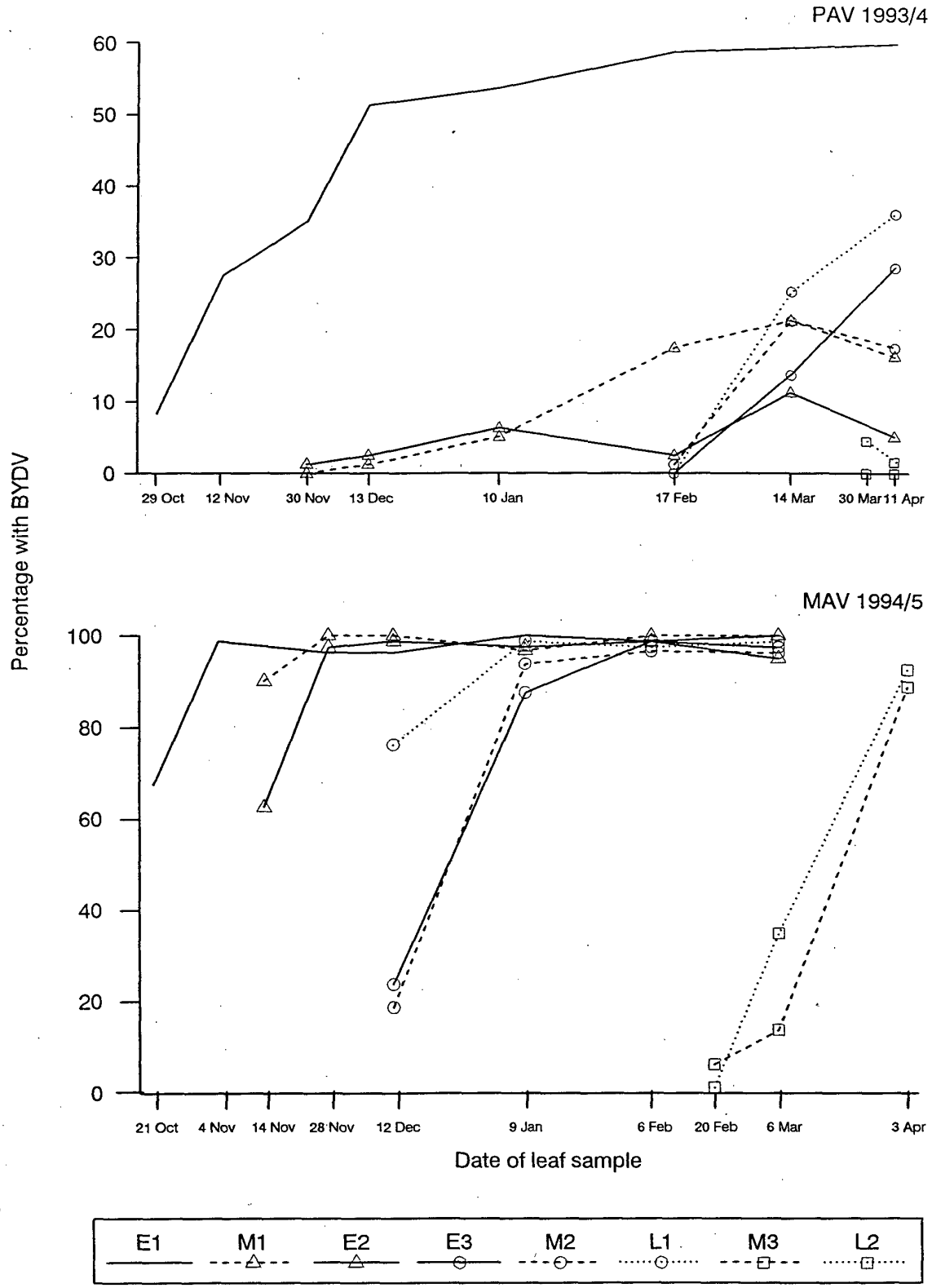


Fig. 8. Percentage of leaf samples with virus after artificial inoculations

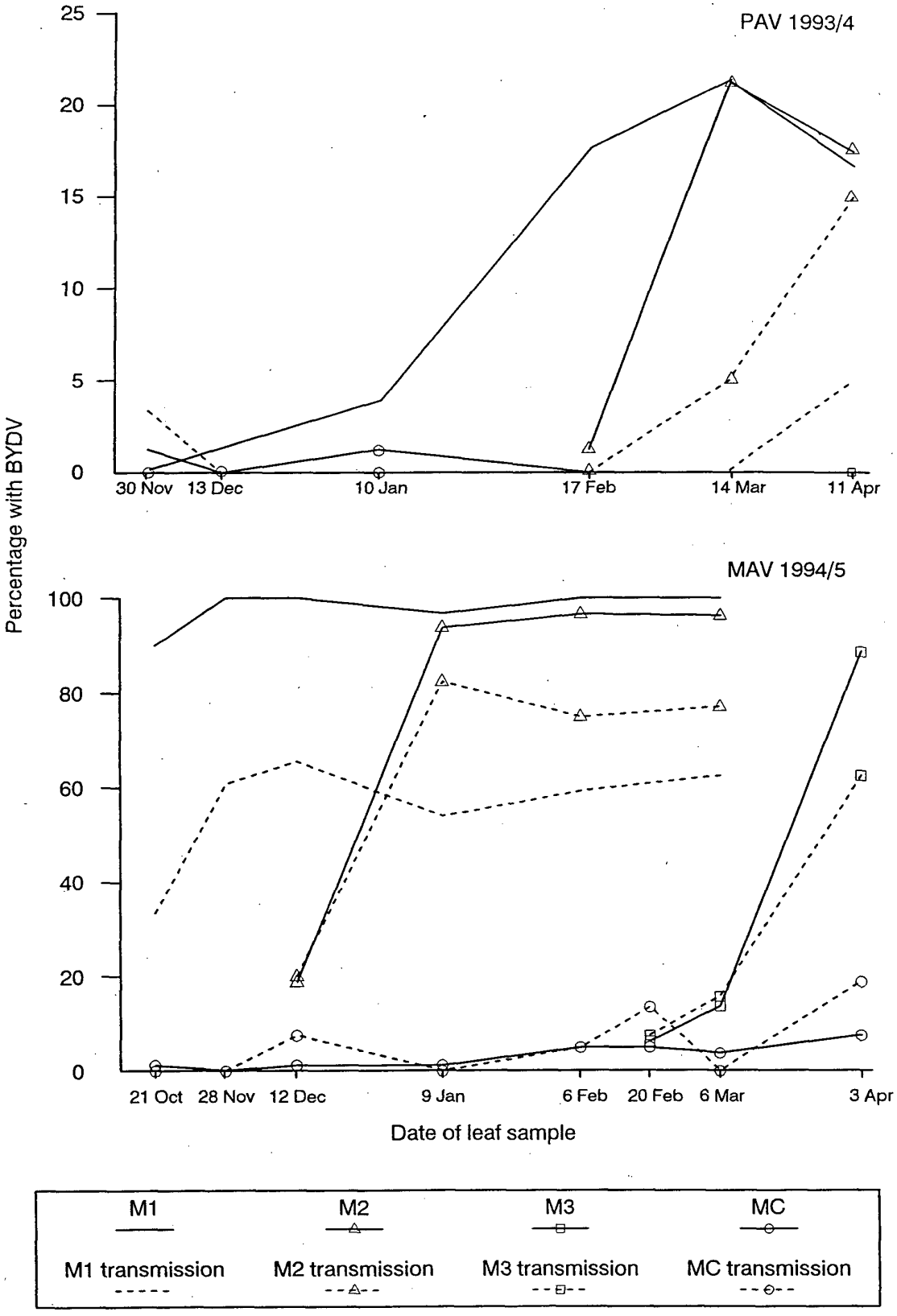


Fig. 9. Leaf samples tested by ELISA (—) and transmission by aphids (--). Percentage of samples with BYDV

# 6 EFFECT OF TEMPERATURE ON VIRUS ACQUISITION AND INOCULATION BY APHIDS AND ON LATENT PERIOD IN THE APHID

## 6.1 Introduction

The following studies examined the three phases of virus transmission: acquisition access period, latent period and inoculation access period.

All transmission studies used the bird cherry aphid to transmit the PAV-like isolate and the grain aphid to transmit the MAV-like isolate. All test aphids used were winged adults, the aphid cultures were reared on winter barley cv. Puffin at a day length of 16 hours light, 8 hours dark. The transmission test plants were oats, cv. Dula for ease of symptom recognition.

## 6.2 Acquisition access period

### 6.2.1 Materials and methods.

Virus acquisition was tested at two temperatures, 6°C and 12°C. Approximately 100 virus-free winged aphids were placed on virus source plants. Twenty aphids were placed on uninfected control plants at the same time. The pots of plants were covered with a cylinder of acetate with a netting top secured with an elastic band.

Aphids were removed from the virus source plants after acquisition access periods of 2, 6, 12, 24, 48 and 72 hours. Control aphids were removed from the uninfected plants after 72 hours. Twenty aphids were removed at each period, ten were placed individually in ELISA plates and subsequently tested by ELISA for virus content, ten were placed individually on oat plants in the glasshouse for two-three days before spraying off with Permethrin. Visual symptoms were assessed at three weeks and leaf samples were tested by ELISA. The experiment was repeated four times for each isolate.

### 6.2.2 Results and Discussion.

The percentages of leaves and grain aphid individuals found positive by ELISA at each acquisition access period are shown in Fig. 10. For any given acquisition access period and at both temperatures, a greater proportion of aphids than leaves tested positive for MAV using ELISA suggesting that not all aphids successfully transmitted the virus to the plants or that virus detection in the plants was imperfect. The relationship between acquisition of virus by the aphid and subsequent transmission to the plant is less consistent for bird cherry aphid and PAV (Fig. 11), with the percentage of leaves with virus being higher than the percentage of aphids with virus at many of the acquisition access periods, suggesting better virus detection in the plants than the aphids.

For both species, the virus can be found at a detectable level in the body of the aphids after only six hours of feeding, the implications for virus levels in the field being that after a short time feeding on an infected plant, a winged aphid is then potentially able to transmit the virus to any plant it feeds on for the rest of its life.

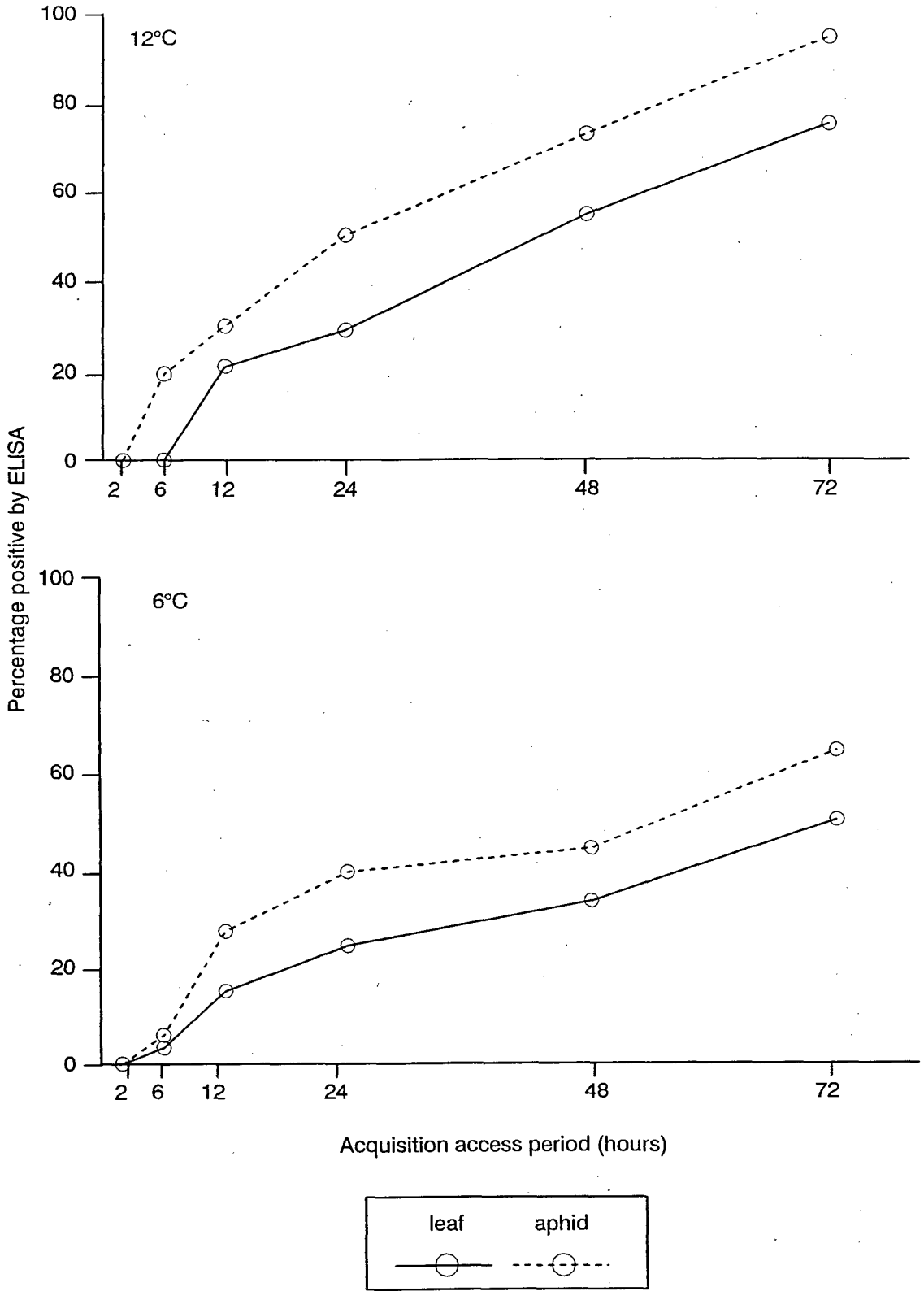


Fig. 10. Acquisition access periods
Grain aphid

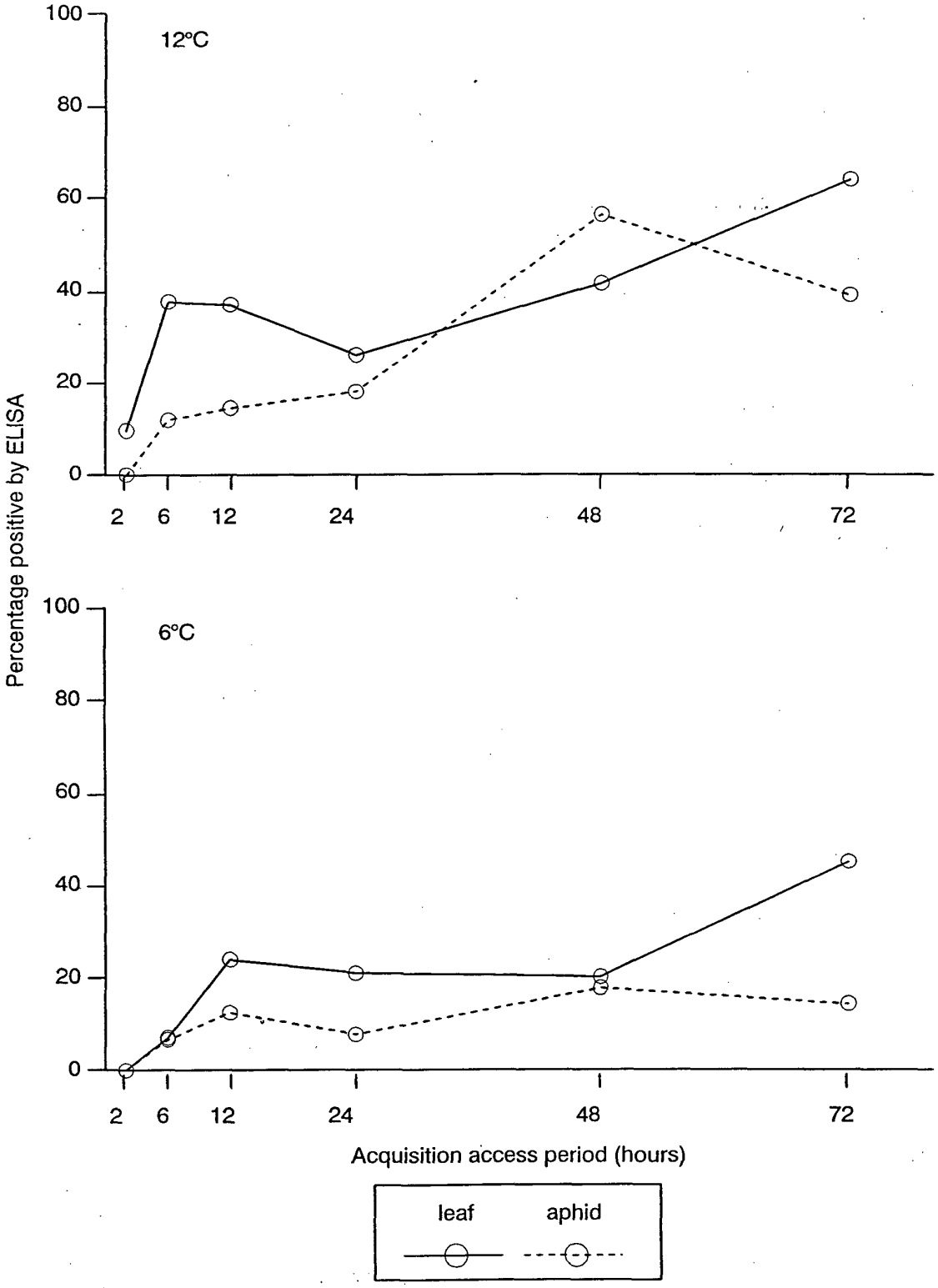


Fig. 11. Acquisition access periods
Bird cherry aphid

## 6.3 Latent period

### 6.3.1 Materials and Methods

Winged aphids of both species were given a 36 hour acquisition access period, removed from the virus source plants and then placed individually on single, week-old oat seedlings for 24 hours. The aphids were then moved onto successive seedlings after each 24 hour period for a total of four days. On the fifth day, half the aphids were moved into the glasshouse and allowed to feed on single seedlings for a further five days. The remaining half were placed into ELISA plates and tested for the virus. The experiment was done for grain aphid and bird cherry aphid at 6 and 12°C. A target of 40 aphids was used for each species/temperature combination, but on some occasions insufficient aphids were available, and some were lost before completion of the serial transfers. The Median Latent Period is defined as the middle of the 24 hour period in which half the test plants have the virus.

### 6.3.2 Results and Discussion

The results are shown in Fig. 12. Temperature has a clear effect on the latent period of grain aphid with the number of test plants acquiring the virus in any 24 hour period reaching 50% on day three at 12°C. The 50% threshold was never reached at 6°C, with acquisition at that temperature remaining low. This emphasises the importance that mild autumns have in BYDV transmission as average temperatures in the southeast of England are 5.9°C in October (Rothamsted weather data, 1965-1991), so that in an "average" year, any aphids which had not acquired the virus by then would remain unlikely to do so for the rest of the winter.

The virus acquisition of bird cherry aphid was very low throughout.

## 6.4 Inoculation access period

### 6.4.1 Materials and Methods

Four temperatures were used to test the inoculation efficiency of the two aphid species: 6°C, 12°C, 18°C and 23°C. Ten aphids were used for each experiment and the experiment was repeated four times for each species.

Single, infective, winged aphids were placed on the oat seedlings for inoculation access periods of 2, 6, 12, 24, 48 and 72 hours and the aphids were contained in a 2.5 cm diameter cellulose acetate tube with a netting top, which was pushed into the soil around the plant. In this way aphids were allowed to find their own feeding site on the source plant.

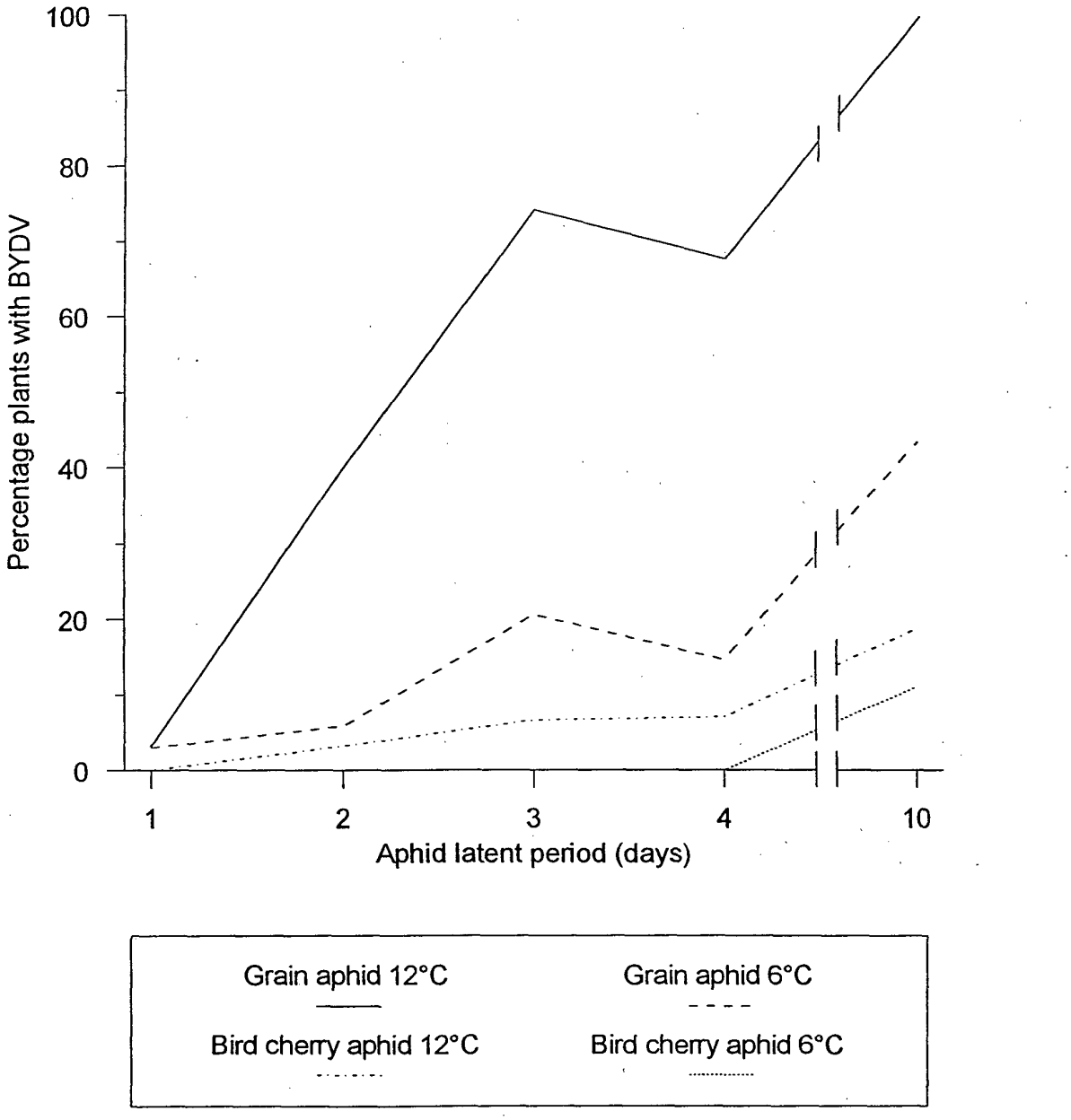


Fig. 12. Percentage of test plants infected with BYDV by aphids against time after virus acquisition

On removal after the appropriate inoculation access period each aphid was classified as dead or alive and the number of missing aphids was recorded. The presence or absence of nymphs and the position of the aphid, ie. whether on or off the plant were recorded. The aphid and any nymphs were squashed and the plants were removed to the greenhouse and watered. Plants were sprayed with Pirimicarb at 0.5g a.i. $l^{-1}$ immediately afterwards to kill any nymphs missed.

The plants were assessed for visual symptoms of virus after 17 and 24 days, and any plants with indistinct symptoms were tested by double antibody sandwich ELISA (Lister and Rochow, 1979).

### **6.4.2** Results and Discussion

The percentages of plants infected with virus over time are shown in Fig. 13 and the percentage of plants infected with virus at different temperatures is shown in Fig. 14. For both isolates the percentages of plants infected with virus at the three highest temperatures were not significantly different, while the percentage of plants infected with virus at 6°C was significantly lower.

To estimate the chances of an infective winged aphid transmitting the virus after landing in a crop, factors affecting both inoculation and movement have to be studied as no transmission of virus will occur if the aphid leaves the plant before the minimum inoculation access period. Figure 15 shows the percentages of aphids remaining on the plant at each inoculation access period and for both species there is little movement off the plant before 72 hours. Both species reach their maximum transmission efficiency at 24 hours so maximum inoculation should occur before the aphid moves off the plant. The average September temperature in Southeast England is 13.6°C (as measured at Rothamsted between 1965-1991) and this experiment shows that there is no increase in transmission efficacy above 12°C. Therefore, at average September temperatures, 70-80% of infective bird cherry aphid and 50-60% of infective grain aphid can be expected to transmit BYDV to an uninfected plant. By October the average temperature decreases to 5.9°C when the probability of either species transmitting the virus is 25-35%. Autumn temperature is therefore a critical factor in BYDV epidemiology in the UK. A small increase in average autumn temperatures will lead to greatly increased infection rates. These estimates do not take into consideration the effects of fluctuating temperature, which are difficult to quantify. Other factors, such as the effects of plant growth stage or different clones of the aphid species on inoculation efficacy, will also change the inoculation rate. These data refer to primary inoculation and examination of subsequent movement of the winged colonisers is needed before their contribution to secondary virus spread within the crop can be quantified adequately. This is the subject of further research.

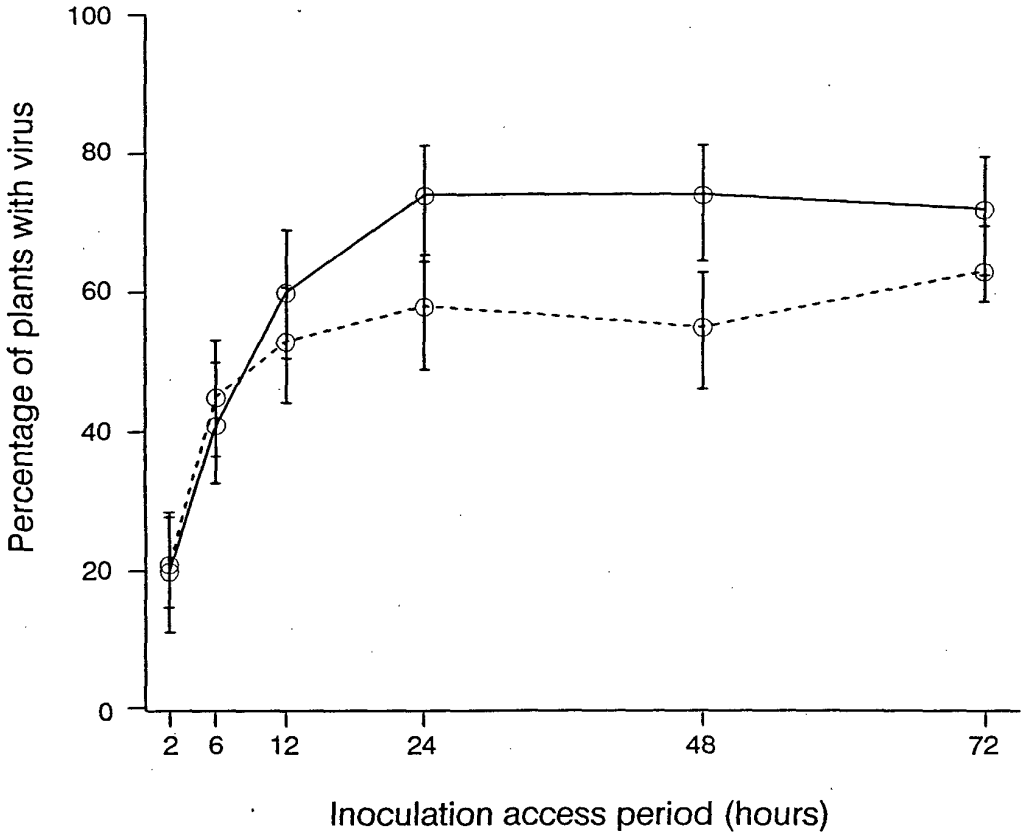


Fig. 13. Percentage of plants with virus for each access period with 95% confidence limits, for MAV-like (---) and PAV-like (—) isolates

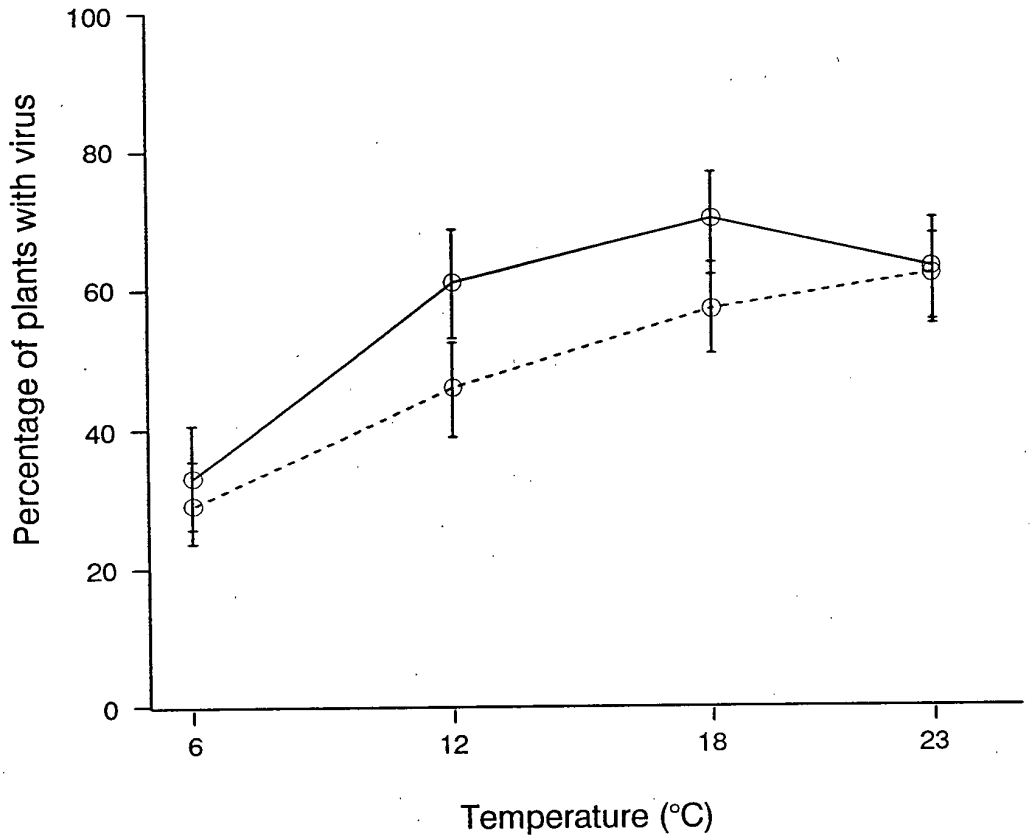


Fig. 14. Percentage of plants with virus for each temperature with 95% confidence limits, for MAV-like (---) and PAV-like (—) isolates

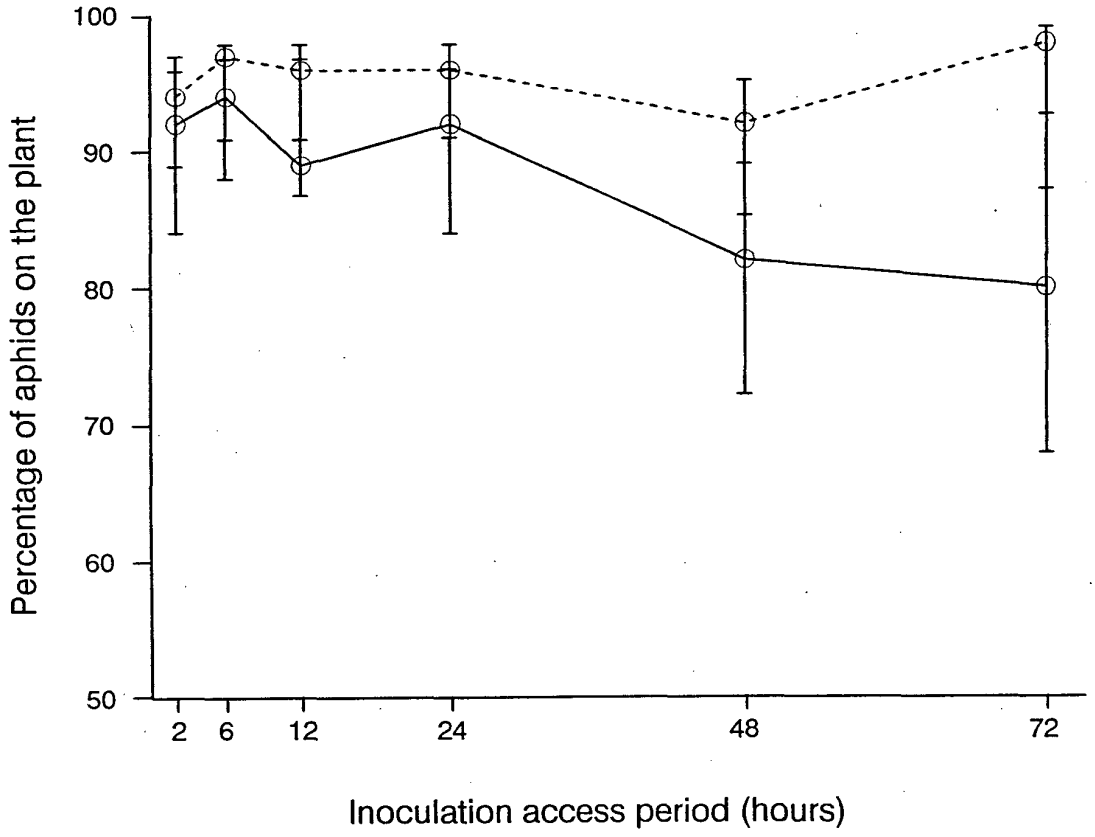


Fig. 15. Percentage of aphids on the plants for each access period with approximate 95% confidence limits for the grain aphid (---) and the bird cherry aphid (—)

# 7 GENERAL DISCUSSION

The results from a diverse range of field and laboratory experiments together emphasise the importance of temperature in determining the initial levels of virus in a crop. Temperature appeared to be the main factor in extending the time of aphid migrations and is certainly an important element in all aspects of virus transmission. This reinforces the importance of later drilling dates in prevention of primary infection of winter cereal crops by BYDV as the later the sowing date, the more likely it is that cooler weather will prevent transmission of the virus by any infective aphids.

Although temperature is very important in determining primary virus levels in a crop, there are other factors involved such as the growth stage of the plant at time of inoculation. However, the results from the field transmission experiments show this relationship to be less clear cut than first thought as the plants inoculated at a later growth stage seem to be a better source for virus than those inoculated at a younger age. There is also a big difference between grain aphid and bird cherry aphid in their ability to acquire and transmit the virus, with grain aphid appearing efficient at acquiring the virus and bird cherry aphid being more efficient at inoculating the virus. The processes of inoculation and secondary spread are complex and factors such as aphid behaviour (especially movement) and aphid survival through winter, which are the subject of separate studies, have to be considered.

The data gathered are being used, in conjunction with results from other HGCA-funded projects and information from the literature, to assemble a weather-driven, quantitative, mechanistic model of BYDV spread which will form the basis of a computerised system to support decisions on the need to control aphid vectors.

## 8. ACKNOWLEDGEMENTS

The authors are grateful to the members of the Rothamsted Insect Survey who assisted with the laboratory and field experiments.

## 9. PUBLICATIONS RESULTING FROM THE PROJECT

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