HGCA

PROJECT REPORT No. 87

MONITORING APHIDS AND VIRUS TO IMPROVE FORECASTS OF BARLEY YELLOW DWARF VIRUS

FEBRUARY 1994

PRICE £6.00

PROJECT REPORT No. 87

MONITORING APHIDS AND VIRUS TO IMPROVE FORECASTS OF BARLEY YELLOW DWARF VIRUS

by

G.M. TATCHELL[1,4], S.A. HILL[2], S. TONES[3], I. BARKER[2] AND A.J. SMITH[1]

[1] AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ

[2] Central Science Laboratory, MAFF, Harpenden, Hertfordshire, AL5 2BD

[3] ADAS, Staplake Mount, Starcross, Devon, EX6 8PE

[4] Present Address: Horticulture Research International, Wellesbourne, Warwickshire, CV35 9EF

This is the final report of a three year project funded by the Home-Grown Cereals Authority. The work commenced in August 1989 and was funded by grants of £87,211 to the Central Science Laboratory (Project No. 0003/3/39A) and £84,017 to Rothamsted Experimental Station (Project No. 0003/3/89B).

CONTENTS.

1. SUMMARY.

1. Sets of data were collected from four locations in England over three seasons (1989/90 to 1991/92) with the objective of providing the basic data necessary for developing a reliable forecasting scheme for barley yellow dwarf viruses (BYDV) in autumn-sown cereals applicable to all the major cereal growing regions of Britain.

2. The sets of data have two major components. Firstly, the numbers of vector aphids flying in autumn were monitored with suction traps, and their virus content and ability to colonize crops was determined by additional tests. Secondly, crop validation experiments examined vector aphid colonization and virus spread within plots of winter barley sown on different dates.

3. Suction trap data indicate that the bird cherry aphid was the most abundant species each autumn. Numbers were largest in 1991 and smallest in 1990.

4. The infectivity index, defined as the product of the number of migrant vector aphids and the proportion that are viruliferous, was greatest for the earliest sown crops indicating a greater risk of primary infection with BYDV in these plots. Infectivity indices were greatest in 1989 and smallest in 1990.

5. Aphid infestations in crops were always greatest on the earliest-sown barley. The largest infestations occurred in 1989/90. Numbers of the bird cherry aphid were generally greater than those of the grain aphid.

6. Early-sown winter barley had greater BYDV infection than that sown later. MAV-like isolates, transmitted by the grain aphid, were dominant in the north (Leeds) whereas PAV-like isolates, transmitted most efficiently by the bird cherry aphid, were dominant in the east (Rothamsted and Wye). In the south west (Starcross) both serotypes were common. RPV-like isolates were rare. PAV infection tended to be less in later-sown crops, while MAV infection was less influenced by sowing date.

7. The increase in infection detected in mid-September sown barley did not correspond well with the increase in aphid infestation. The PAV-like isolates tended to be spread earlier than MAV.

8. The proportion of crop-colonizing forms of the bird cherry aphid differed from one autumn to another.

9. Colonizing and non-colonizing forms of the bird cherry aphid in autumn could not be identified morphometrically, nor by the use of biochemical methods.

10. The colonizing and non-colonizing forms of the bird cherry aphid acquire BYDV at a similar rate in autumn, but the colonizing form transmits the virus more efficiently to new plants.

11. The non-colonizing forms of the bird cherry aphid tend to fly higher and longer than the colonizing forms. Virus sources may therefore be several kilometres from infected fields.

2. OBJECTIVES

The overall project objectives were to obtain the necessary data for the development of a reliable and practical forecasting system for barley yellow dwarf viruses (BYDV) in autumn-sown cereals applicable to all the major cereal growing regions of Britain. This was approached by the collection of core data combined with further appropriate experimentation.

Core data

a. Monitor the numbers of migrant vector aphids in 12.2 m high suction traps at four core sites at Starcross in Devon, Wye in Kent, Rothamsted in Hertfordshire and Leeds in Yorkshire, and their virus content at Rothamsted.

b. Monitor BYDV in individual aphids collected in 1.5 m high suction traps at Starcross, Wye, Rothamsted and Leeds.

c. Monitor the proportions of the different migrant forms of the bird cherry aphid at Rothamsted.

d. Monitor aphids and virus in winter barley planted on different dates at Starcross, Wye, Rothamsted and Leeds.

Further experimentation

e. Examine both morphological and biochemical methods to identify the colonizing and non-colonizing forms of the bird cherry aphid in autumn.

f. Quantify the relative contribution that colonizing and non-colonizing forms of the bird cherry aphid make to BYDV risk, both through their behaviour and their ability to transmit BYDV.

3. INTRODUCTION

The level of aphid transmitted barley yellow dwarf viruses (BYDV) infecting autumn-sown cereals differs from year to year, so causing a variable loss to the industry. This project aimed to provide some of the basic data necessary for the development of a forecasting system applicable to all the major cereal growing regions of Britain.

On farms where in-field sources of virus are eliminated, initial infection of autumn-sown cereals with BYDV is caused by infective aphids flying into these crops from outside. Subsequent virus spread results from the original crop-colonizing aphids, and particularly their offspring, moving between plants in late-autumn and winter, if conditions permit. Appropriately timed insecticides give effective control of the vector aphids, but, as greatest yield loss occurs when plants are infected at an early growth stage (Doodson & Saunders, 1970), spray timing is critical.

Barley yellow dwarf virus is transmitted only by aphids that colonize cereals; 24 species have been recorded as vectors, but in Britain for practical purposes only the bird cherry aphid (*Rhopalosiphum padi*) and the grain aphid (*Sitobion avenae*) are important in autumn-sown cereals (Plumb, 1981). The different vector species transmit different strains of the virus with different efficiencies (Rochow, 1969), and these strains may have a different effect on grain yield. PAV-like isolates are transmitted non-specifically by the bird cherry and grain aphids, but most efficiently by the bird cherry aphid. MAV-like isolates are transmitted specifically by the grain aphid, while the RPV-like isolates are transmitted specifically by the bird cherry aphid.

Data from the Rothamsted Insect Survey network of suction traps distributed around Britain (Taylor, 1986) shows that the numbers of the principal vector species flying in the autumn differ greatly from year to year and in different localities. Similarly, the proportion of these aphids that transmit BYDV also differs from year to year (Plumb, 1986). These two factors are the most important in determining the risk of initial BYDV infection of crops sown into clean seed beds in any one year.

In 1986, a long-term multi-site experiment was established, with ADAS and IACR Rothamsted collaborating closely, to determine how the varying numbers of migrant aphids and their infectivity affected subsequent infection of winter barley sown on different dates in different regions of Britain. The data generated from this series of experiments, partly funded through this project, will be used to develop forecasting methods.

Vector aphid biology, particularly that of the bird cherry aphid, is complex and modifies the ability of an individual aphid to infect and spread BYDV. In autumn two forms of the female bird cherry aphid occur; the first will colonize cereals and spread BYDV, while the second form is in search of its alternative winter host plant, the bird cherry tree (*Prunus padus*), where the virus cycle is broken. However, this second form may land on cereals, and possibly feed, while searching for bird cherry trees. The relative proportions of these two forms differs from year to year and region to region

(Tatchell, Plumb & Carter, 1988a; Tatchell, 1991). The relative importance of these two forms as virus vectors is unclear and requires clarification for inclusion in forecasts of risk. In addition, male bird cherry aphids in search of *P. padus* occur in autumn in large numbers. Their role in BYDV epidemiology is also unclear.

This programme draws together aspects of aphid and virus epidemiology and relates them to aphid and virus incidence in crops to provide the necessary data for the development of a robust system for forecasting BYDV risk to autumn-sown cereals in different regions of England.

4. CORE DATA FROM FOUR WIDELY DISTRIBUTED SITES

4.1. Materials and Methods.

Complete sets of data were gathered for three seasons (1989/90 to 1991/92) from the four collaborating sites throughout England; Starcross in Devon (ADAS), Wye in Kent (ADAS), Rothamsted in Hertfordshire (IACR) and Leeds in Yorkshire (ADAS) (Fig 1). The main methods used to sample for aphids and virus were the same at each site.

4.1.1. Monitoring aerial aphid populations.

Rothamsted Insect Survey suction traps sampled aphids flying at a height of 12.2 m above the ground (Macaulay, Tatchell & Taylor, 1988) at all four sites (Fig. 1) from 1 August to 15 December 1989-1991. Daily samples were sent to Rothamsted where all BYDV vector species were identified and counted.

4.1.2. Monitoring virus in migrant aphids.

A second suction trap at each site sampled aphids alive from 1 September to 1 December each year at a height of 1.5 m above ground (Fig. 1). Individual BYDV vector species were identified in the daily samples in the laboratory (Plumb, 1976) and then deep frozen in wells of microtitre plates for subsequent assay for virus by amplified enzyme linked immunosorbant assay (ELISA) (Torrance, 1987) at the Central Science Laboratory, MAFF, Harpenden. The vector aphids were assayed, using specific monoclonal antibodies, for the isolates of BYDV they are able to transmit.

Secondly, samples of the bird cherry aphid from the 12.2 m suction traps at Rothamsted were collected in an experimental collecting medium (Tatchell, Thorn, Loxdale & Devonshire, 1988b) which had been found to preserve viruses. Following identification of these samples the bird cherry aphids were placed individually in the wells of microtitre plates for subsequent assay by amplified ELISA at Rothamsted. Samples for assay were limited to 50 aphids per day if numbers were greater. The bird cherry aphids were assayed, using specific monoclonal antibodies, for the isolates of BYDV that this species is able to transmit.

The effectiveness of the experimental collecting medium at preserving viruses was determined in the laboratory at Rothamsted by feeding winged bird cherry aphids on BYDV-infected plants for three days. Following acquisition of virus, aphids were placed in the collecting medium for different periods ranging from 0 to 10 days (the maximum time taken to process suction trap samples) at 10 or 18° C. Control aphids were frozen immediately, while treated aphids were frozen after the test period. All aphids were placed individually in wells of microtitre plates for subsequent assay by amplified ELISA as above.

4.1.3. Crop validation.

Plots of winter barley cv. Puffin (Magie at Rothamsted) were sown on five different dates in autumn, replicated four times in a randomized block design at each of the four core sites (Fig. 1). Sowing dates were selected to reflect normal practice in the area and to maximise the range of responses, but also to have some dates that were common to all sites (Table 1).

At Rothamsted an insecticide treatment was also included. Plots were either sprayed in early November with cypermethrin to control the aphid vectors or left unsprayed. Combine grain yields were taken at harvest on both the sprayed and unsprayed plots.

4.1.3.1. Aphid numbers.

The aphids were counted at regular intervals by close visual inspection of the growing plants. During the period of crop establishment, up to December, crops were sampled at intervals of one to two weeks. This interval was then extended to three to four weeks until shortly after the beginning of stem extension (GS 31) when sampling ceased. Twenty consecutive plants in a row at each of six locations per plot were examined on each sampling occasion and the number, species and development stage of aphids recorded. The number of plants sampled was reduced if aphid numbers were very large.

4.1.3.2. Virus infection.

The incidence of BYDV infection in the barley plot was measured by collecting the youngest fully expanded leaf from each of 20 plants selected at random from within each plot. These leaves were put individually into labelled plastic bags and deep frozen for subsequent testing for BYDV, and strain determined, by ELISA at the Central Science Laboratory, MAFF, Harpenden.

During the 1989-90 season, samples were collected from all plots in November and again in April. The objective was to determine the initial level of infection (November) and how much spread took place through the winter (April). Additional plant samples were collected at Rothamsted from the first and third unsprayed sowing of the sequentially-sown barley experiment on every occasion that the plots were

sampled for aphids. The subsequent assay of these samples by ELISA at Rothamsted gave an indication of the progression of the disease through the autumn, winter and spring. These data indicated that two samples timed in November and April did not provide the data necessary to determine when virus spread occurred. Therefore in 1990-91 and 1991-92 the methodology was altered to provide data at monthly intervals from plots sown in mid-September from all four experimental sites. In addition, a sample was taken from all sowing dates in April.

4.2. Results and Discussion.

4.2.1. Numbers of migrant vector aphids.

The 12.2 m suction trap results show that in the autumns of all three years the numbers of the bird cherry aphid were always much larger than those of the grain aphid (Fig. 2). (Note the data are plotted on a logarithmic scale). Overall, the numbers of the bird cherry aphid were largest in 1991 and smallest in 1990. Relative to the bird cherry aphid, the numbers of the grain aphid were always small, but the largest numbers were recorded in 1991. The largest numbers of the bird cherry aphid were generally recorded from Leeds, while the smallest numbers were from Rothamsted. In contrast the largest numbers of the grain aphid tended to be recorded from Starcross. In all years migrations of the bird cherry aphid continued until the end of November, though numbers were small during this month.

4.2.2. Virus infectivity of migrant aphids.

4.2.2.1. From live trapping.

The numbers of vector species trapped each autumn and tested varied from site to site and from week to week (Tables 2-4). The five cereal aphid species assayed have all been used by Plumb (1986) in the calculation of an infectivity index to give an assessment of BYDV risk, but for practical purposes only the bird cherry aphid (*R. padi*) and the grain aphid (*S. avenae*) are thought to be important. Therefore only the results for these two species are presented here. The infectivity index is defined here as the product of the proportion of vector species found to be viruliferous in 1.5 m high suction traps and the number of that species in the RIS suction trap. This is a slight modification of the infectivity index of Plumb (1983), but is used here for simplicity to aid comparison between sites and years. The infectivity index can be accumulated from the sowing date of a crop to give a measure of the risk of primary infection of that crop. Far more bird cherry aphids than grain aphids were tested each year, reflecting the difference in the abundance of aphids recorded in 12.2 m trap (Fig. 2).

1989.

Averaged across the four experimental sites for both species, 23% of individuals were found to be carrying isolates of BYDV that they had the potential to transmit, but this ranged from 20-35% of bird cherry aphids and 0-50% of grain aphids at the different sites (Table 2). Nearly all viruliferous aphids were recorded in October, and as the numbers of migrant aphids were greatest at this time, the highest weekly infectivity indices were also recorded at this time. The accumulated infectivity index at each site was greatest at Starcross and least at Rothamsted (Fig. 3). As there were few viruliferous aphids in September, all September-sown crops at one site were exposed to a similar risk of primary infection by BYDV. There was little or no risk to crops sown in November.

1990.

The overall percentage of aphids that was viruliferous was 11.9%, lower than in 1989, and ranged from 6-15% for the bird cherry aphid and 0-50% for the grain aphid (Table 3). The infectivity index indicates that the overall risk of infection, as measured by the accumulated infectivity index, was lower in the autumn of 1990 than 1989 at all sites except Leeds (Fig. 4). Again, the largest infectivity indices were recorded in October, though at Starcross values for September and October were similar. Viruliferous bird cherry aphids were recorded up to mid-November, but as the total number of migrants was small, the infectivity index at this time was also small. The large infectivity index recorded at Leeds in September declined rapidly during October.

1991.

The infectivity index indicates that crops sown in November were not at risk (Table 4). The largest infectivity index was recorded from Leeds in early September, but this declined rapidly to nothing by the beginning of November (Fig. 5). The index at Rothamsted and Starcross was low.

4.2.2.2. From 12.2 m suction trap.

Both male and female bird cherry aphids were found to contain BYDV in similar proportions (Table 5). The overall percentage of bird cherry aphids that were viruliferous was 9% in 1989 and 1990, but only about 6% in 1991. These levels were lower than those found in aphids trapped alive and frozen immediately without being in a storage solution, particularly in 1989 and 1990 (Tables 2-4). However, the sample size for the aphids trapped alive and then frozen was small in 1989 and 1990.

Tests of the effectiveness of the storage solution to preserve BYDV indicate that the number of positives was reduced by all treatments as compared to the untreated aphids (Fig. 6). After ten days at 10° C the number of positives was reduced to about 50% of the controls, while at 18° C the number of positives was reduced to only 15% of the controls. Most samples of wild-trapped aphids from Rothamsted were frozen

within three days of collection, and kept in a fridge at 4° C as much as possible before then. These results indicate that measurements of the proportion of the aphid population containing BYDV obtained in this way give an adequate indication of the differences between seasons and locations.

Male bird cherry aphids transmit BYDV in infectivity tests, and in some instances do so more frequently than do the females (Halbert *et al.*, 1992). However, their role in transmission in the field where they are in search of *P. padus* rather than cereals is unknown.

4.2.3. Crop validation.

4.2.3.1. Aphid infestations.

The numbers of bird cherry and grain aphids per plant and the percentage of plants infested with aphids for the three seasons are presented in Figs 7-9 respectively. Note that the data for aphid numbers are all plotted on the same logarithmic scale to illustrate clearly the differences both between the sites within a year, and between years at each site. Also note that for the sake of clarity some data have been excluded from Figs. 7-9 where numbers were very low and lines would have been superimposed on each other. However, both aphid species were counted on every sowing at each site.

1989-1990.

The numbers of the bird cherry (Fig. 7) and grain aphids (Fig. 8) differed considerably between experimental sites, and between sowing dates. Earlier-sown barley plots tended to have larger aphid populations than those sown later. These data must, however, be read in conjunction with the dates plots were sown at the different sites (Table 1). Largest numbers in autumn were of the bird cherry aphid on the earliest sowing date at Rothamsted and Wye where peak densities of about 10 aphids per plant were recorded (Fig. 7). In contrast, densities of the bird cherry aphid were very small at Leeds and Starcross, though the first sowing at Starcross coincided with the fifth at Leeds (Table 1). Bird cherry aphid numbers declined through the winter at Rothamsted and Wye, but at Starcross, in the far south west, numbers increased throughout the winter. At Wye and Starcross bird cherry aphid numbers increased dramatically in March and April. At Leeds bird cherry aphid populations did not develop.

Grain aphid numbers were far smaller than those of the bird cherry aphid, especially in autumn (Fig. 8). However, grain aphid numbers increased gradually throughout the winter at the three southern sites, and dramatically in the earliest sown plots at Rothamsted. Largest numbers occurred at Rothamsted and Wye in March and April.

1990-1991.

Aphid infestations on experimental plots were smaller than in the previous season. Aphid numbers were again higher on earlier- than later-sown plots. Aphid numbers were greatest at Rothamsted (two aphids per plant) and Wye, where the bird cherry aphid predominated (Figs. 7 and 8). At Leeds the grain aphid was dominant, though in smaller numbers (Fig. 8). At Starcross both species occurred in similar numbers. The largest numbers of aphids occurred in December, after which populations declined and did not increase again in spring (Figs. 7 and 8), possibly due to the severe cold in February.

1991-1992.

Aphid infestations were the smallest of the three seasons (Figs. 7 and 8). However, it was again the earliest-sown crops that had the largest infestations. Numbers of the bird cherry aphid (Fig. 7) and the grain aphid (Fig. 8) were largest at Starcross where populations of both species persisted through the winter.

Proportion of plants infested

The percentage of plants infested with aphids combines the data for both species (Fig. 9). At Rothamsted and Wye in 1989-90 infestations of greater than 75% were recorded on early-sown barley. Fewer plants were infested on later-sown crops. However, at the beginning of the season infestations were dominated by the bird cherry aphid, but by the grain aphid late in the season (Figs. 7 and 8), indicating that great care is required in interpreting data on the proportion of plants infested. In addition, at Starcross the lush growth of crops made it difficult to separate individual plants so that the proportion of plants infested could only be measured for a short period at the beginning of the season.

4.2.3.2. Virus infection.

1989-1990.

In November virus infection was only detected at low levels in the early-sown plots of winter barley. Infection was greatest in the first two sowings at Leeds (9 and 5%), where only MAV-like isolates were detected. Elsewhere infection was only 1-2%. At Wye and Starcross a greater range of serotypes was detected (MAV, PAV, RPV). At Rothamsted, it was not possible to determine the isolate.

In April the overall levels of BYDV infection were much higher. PAV and MAV were recorded most frequently, while RPV was rare (Table 6). Plots sown earlier had greater levels of infection than those sown later. At Leeds, and to a lesser extent at Starcross, infection with MAV-like isolates were dominant, while at Rothamsted and Wye PAV-like isolates dominated. At the three more northern sites the level of PAV infection tended to be less in the later sowings, while the level of MAV was more

consistent between sowings (Fig. 10). At Starcross both isolates were less abundant in later sowings, but note that all sowings were planted much later than at other sites (Table 1).

In more detailed studies at Rothamsted, plant samples were collected from the first and third sowings of barley throughout the autumn, winter and spring, at the same time as plots were assessed for aphid infestations. Subsequent analysis of these samples by ELISA at Rothamsted showed that the proportion of plants in the earliest sown barley that were infected with PAV increased in December and January while the proportion infected with MAV increased in April and May (Fig. 11). Infection of the third sowing was low in spring (Table 6) so the progression of the disease is not presented here.

As a result of these findings procedures were modified in later years to obtain more information on the time at which detectable virus spread occurred, as detailed in section 4.1.3.2.

1990-1991.

In crops sown in mid-September at Starcross there was a progressive increase in the plants infected with both PAV and MAV up to January, with about 20% of plants infected by each serotype (Fig. 12). There was a large increase in the proportion of plants infected with PAV in February, but MAV infection progressed more gradually, both reaching about 55% infection in March. At Wye PAV infection increased progressively throughout the season to about 65% infection, but MAV and RPV remained at low levels. At Rothamsted only a limited increase in PAV infection occurred, while at Leeds only MAV increased to about 30% in April.

In early-September barley at Rothamsted only limited spread of PAV occurred up to mid-February, but by mid-March about 37% of plants were infected (Fig. 11). MAV remained rare throughout the season.

The results show that in samples collected in March 1991 the distribution of virus serotypes between regions was similar to that in the previous season with MAV dominant in the north and both serotypes present in similar amounts in the south west. Infection, particularly of PAV, was again greatest in early-sown crops (Fig. 13). Only at Starcross was more than one serotype recorded frequently, and it was only there that a significant level of mixed infections of MAV and PAV occurred (Table 7). RPV was rare and occurred more frequently in mixed infections than in sole infections (Table 7).

1991-1992.

In mid-September sown plots, increase in infection was not detected until January at Starcross (Fig. 14). From then on the proportion of plants infected with PAV and MAV increased progressively to 35-45%. At Wye PAV infection increased progressively throughout the winter, but MAV infection increased only in April. At Rothamsted and Leeds little spread occurred, but infection was greatest in spring.

In early-September sown barley at Rothamsted MAV and PAV occurred at similar but low levels up to early January (Fig. 11). By mid-February MAV and particularly PAV infection had increased, but by the end of March infection by MAV and PAV was about 20%. This was the only year in which significant MAV infection occurred at Rothamsted.

In March 1992 infection was less than in either of the two previous seasons, though 58% of the earliest sown plots were infected at Starcross (Table 8). As in other years earlier sowings tended to have higher levels of infection than did later sowings. Both PAV and MAV were common at Starcross, while PAV was dominant at Wye. At Rothamsted and Leeds PAV and MAV both occurred at low levels (Fig. 15). The overall infection in early-sown plots at Starcross approached that of 1990-91, but mixed infection of MAV and PAV occurred less frequently. RPV again occurred most frequently in mixed infections (Table 8).

The disease progress curves (Figs. 11, 12 and 14) must be interpreted with care. The data indicate the times at which BYDV was detectable by ELISA. It is as yet unclear how this measure relates to the time initial infection of the plant occurred and to the ability of infected plants to act as a source for further infections. This requires more detailed investigation under environmental conditions that prevail in autumn and winter.

4.2.3.3. Effect of BYDV infection on yield.

The application of an insecticide to winter barley at Rothamsted in early November resulted in a significant increase in yield in 1989-90 and 1990-91 and a nearly significant increase in 1991-92. Yield increases were largest on earlier-sown plots and ranged from 0-35% depending on the sowing date and year (Table 9).

5. THE DIFFERENT MIGRANT FORMS OF THE BIRD CHERRY APHID IN AUTUMN

5.1. Monitoring the occurrence of the different forms of the bird cherry aphid.

5.1.1. Methods.

Two suction traps at Rothamsted sampled aphids alive at heights of 12.2 and 1.5 m from mid-August to the end of aphid migration in early December 1989-91. Samples were collected twice daily and female bird cherry aphids given a choice of a length of barley leaf or a disc of *P. padus* of equal area on which to produce their offspring (Tatchell *et al.*, 1988a). The host plant selected by the aphid identified which form it was; crop colonizers (virginoparae) preferring barley while the non-colonizers (gynoparae) prefer bird cherry (Tatchell and Parker, 1990).

5.1.2. Results and Discussion.

During each of the three years the proportion of bird cherry aphids that were colonizers declined during September (Fig. 16). This corresponds to the time of the switch from the asexual to the sexual phase of the life cycle. In each year this was more pronounced at 12.2 m than at 1.5 m because the non-colonizers tend to fly higher than the colonizing form (Tatchell *et al.*, 1988a). However, what is of particular interest is the differing proportion of crop colonizers detected after the beginning of October. In the autumns of 1989 and 1990 a significant proportion of the population were colonizers in late autumn, while in 1991 very few were detected at this time, particularly at 12.2 m. It has been proposed that the proportion of colonizers in late autumn is related to the temperature during the previous winter; if it was very cold the colonizers would be killed leading to a very small proportion the following autumn (Tatchell, 1991). The consequence of this would be that during an autumn following a cold winter the risk of colonization of autumn-sown cereals by bird cherry aphids would be much reduced and, conversely, it is increased in autumns following a mild winter. However, more years data are required to determine the precise relationship with temperature during the previous winter before this can be used for predictive purposes.

5.2. Morphometric identification of the different forms of the bird cherry aphid.

5.2.1. Methods.

Female bird cherry aphids that had been identified as either colonizers or non-colonizers by host preference testing were used in a detailed morphometric analysis. Both forms were compared with the winged female form that migrates from *P. padus* in spring (emigrant). Individual aphids were mounted on microscope slides and 23 morphological characters were measured or counted at x75 magnification. Data were analyzed by Canonical Variates Analysis and multiple regression analysis to identify those characters which enabled the identification of the different forms.

5.2.2. Results and Discussion.

The results of these analyses have been published in detail (Taylor, Tatchell and Clark, 1993) and therefore only a brief summary of the findings is presented here. The emigrant aphids from *P. padus* could be separated readily from those aphids leaving grasses and cereals in the summer by measuring the 2nd tarsal segment on the hind leg alone. Accurate identification of the two forms was obtained if this measurement was combined with measurements of the hind femur and base of the 6th antennal segment (Table 10). However, in autumn it was only possible to identify crop colonizers from non-colonizers of the bird cherry aphid with 80-85% accuracy once five morphological characters were considered (width of siphunculae, number of rhinaria on the 5th antennal segment, wing width, length of the cauda and the number of marginal tubercles) (Table 10). This level of accuracy was thought insufficient to identify individuals from unknown populations for the purpose of assessing the risk of aphid colonization and possible BYDV infection of crops. It was therefore decided that alternative biochemical

methods of identification should be investigated.

5.3. Biochemical identification of the different forms of the bird cherry aphid.

Different biochemical techniques were used to try to identify differences between the colonising and non-colonising forms of the bird cherry aphid that could not be identified by more traditional methods of taxonomy. These different forms could be genetically identical as both can arise within a single aphid clone. Biochemical differences between the two forms may therefore be small and difficult to find.

In all instances the aphids used in these studies were from laboratory cultures of *R. padi* reared under the appropriate environmental conditions to produce either crop colonizing or non-colonizing forms. The different methods are outlined briefly below.

5.3.1. Allozyme electrophoresis.

This method aims to identify differences between enzymes within aphids as bands on a gel. Cellulose acetate and polyacrylamide gels were used in combination with 30-40 different enzyme staining systems. No consistent differences were found between the two forms.

5.3.2. Chromatography.

The body fluids (haemolymph) of many aphids contain characteristic fluorescent pigments (Henderson *et al.*, 1976). Homogenates of the two forms were run on a paper chromatographic system, but no differences in pattern were found between forms.

5.3.3. Spectrophotometry.

Experiments were done to measure the quantities of the enzyme ATPase in the flight muscle. ATPase is essential for flight and as the crop colonizing form of the bird cherry aphid flies lower and for shorter distances than the non-colonizers (Tatchell *et al.*, 1988a), it was thought that the amount of ATPase might be greater in the non-colonisers than the colonisers. The quantity of ATPase was determined by a colourmetric reaction and measured with a spectrophotometer. However, any differences between the two forms were too small to differentiate between them.

5.3.4. Poly-acrylamide gel electrophoresis (PAGE).

This technique separates proteins in an aphid homogenate on the basis of size or electrical charge. The methodology can be refined either by varying the concentration of acrylamide in the gel, so changing the pore sizes through which the proteins pass, or by changing the pH of the gel. The aphid homogenate is placed at one end of the gel and an electrical current passed through it which separates the proteins into bands of different molecular weight or electrical charge. These bands are visualised by the use of different stains. Any differences in electrical charge were investigated by using iso-

electric focusing. These methods were tried on micro-gels and slab gels and with a number of different staining techniques. However, although differences in banding patterns were found, none were consistent over time and therefore could not be used for reliable identification of the different forms.

5.3.5. Immunochemistry.

Antibodies were raised to the non-colonizing form of the bird cherry aphid which were used in a number of techniques to try to differentiate between the two aphid forms. One of the most useful was the use of PAGE (see section 5.3.4.) to run homogenates of the two forms on a slab gel. The gel was then Western blotted onto nitrocellulose paper, so transferring the proteins from the gel to the paper. The nitrocellulose paper was then incubated with cross absorbed antibodies. This would identify the bands which were unique to that form. This technique proved successful, showing a different banding pattern for the two forms. However, as with other methods, the differences were not consistent. This may be because aphids had been adult for different lengths of time, or had flown for different periods.

5.3.6. Colour differences.

When the two forms are dissected in alcohol, the unborn nymphs of the colonizing form turn pink, while those of the non-colonizing form remain clear. This technique has yet to be tested fully but appears to work for fresh aphids of different ages from one bird cherry aphid clone maintained in culture. Tests still have to be done on the two different aphid forms from wild populations throughout the autumn season, but the technique looks promising as a quick method to assay fresh aphids.

5.4. Contribution of different forms of the bird cherry aphid to BYDV risk.

The two forms of female bird cherry aphids found in autumn occur in different proportion in different years. Both forms originate from grasses and cereals, but are in search of rather different host plants. The crop colonizers are in search of grasses and cereals, while the non-colonizers are in search of *P. padus*. It is not known whether the two forms are able to carry BYDV, and if they do, whether they are able to transmit it to cereals. Experiments were therefore done to determine these points.

5.4.1. Methods.

Firstly, it was determined whether the two female forms of the bird cherry aphid, collected in autumn carried BYDV. Live-trapped female bird cherry aphids whose form had been identified by giving them a choice of host plants on which to reproduce, as described above, were placed individually in wells of microtitre plates. These aphids were then deep frozen before assay by amplified ELISA (Torrance, 1987) at Rothamsted. This enabled the relative proportions of the two forms carrying BYDV in the autumns of 1989 and 1990 to be determined.

Experiments were then done to determine whether the two forms are able to transmit BYDV to healthy cereal plants. This was examined firstly in the laboratory and then outside in autumn to relate more closely to field conditions.

The two migrant forms of the bird cherry aphid were reared under the appropriate environmental conditions in the insectary at Rothamsted. The two forms were fed on BYDV-infected oats for three days just prior to becoming winged adults. Once adult, the two forms were divided into two lots. The first group (26 of each form) were placed individually in wells of microtitre plates for direct assay by amplified ELISA to determine the rate of BYDV acquisition by the two forms. The second group were placed individually on oat-test seedlings for inoculation feeds of 0.5, 2, 6, 12, 24, 48 or 72 h; there were 10 individuals of each form for each inoculation period. The experiment was replicated five times. Following the inoculation feed, plants were sprayed with pirimicarb to kill the aphids and the plants were placed in an insect-proof glasshouse for three weeks to allow BYDV symptoms to develop. Plants with unclear symptoms were tested by ELISA.

In an attempt to make the transmission experiments in the laboratory relate more closely to field conditions, a further experiment was done in 60 x 60 x 60 cm cages constructed of terylene gauze placed over sand. In each cage, four 12.5 cm diameter pots each containing 10 oat plants at GS 11 were sunk in the sand so the soil in the pots was level with the sand. Twenty aphids, either crop-colonizers or non-colonizers, were released in the centre of each cage at a height of 40 cm above the sand. Aphids were placed in an inverted filter-paper cone, whose edge was cut to produce serrations to encourage the aphids to take flight, placed in a flower pot attached to the end of a cane pushed into the sand. This arrangement resulted in aphids having to fly before they could colonize plants. Aphids were released in the afternoon and allowed inoculation feeds of 18-24 h. The number of aphids that had not left the filter paper were then counted to determine the number of aphids that could have infected the plants in each cage. Plants were then sprayed with pirimicarb and pots placed in an insect-proof glasshouse for three weeks to allow symptoms to appear. The experiment was arranged in a randomized block design with five replicates and was repeated on three occasions.

5.4.2. Results and Discussion.

Both crop colonizers and non-colonizers of the bird cherry aphid were found to contain BYDV when assayed by amplified ELISA. However, the proportions of the two forms that were viruliferous differed in different years (Table 11). These data equate to those presented earlier for the proportion of males and females of the bird cherry aphid that were viruliferous (Table 5). It is clear that in autumn all the winged forms of the bird cherry aphid may contain BYDV. This is not surprising as all the different forms are born on cereals or grasses, all of which may contain BYDV.

In experiments to assess the relative efficiency with which colonizing and non-colonizing forms of the bird cherry aphid transmit BYDV it was found that there was no difference in the proportion (0.41) of the two forms that acquired BYDV ($F = 1.48$,

P = 0.29). However, significantly more plants were infected by colonizers than non-colonizers (F = 50.0, P <0.001), and there was a significant increase in the proportion of plants that were infected with longer inoculation feeds (F = 26.5, P <0.001) (Fig. 15).

In the experiments done outside both forms of the bird cherry aphid again transmitted BYDV, and the proportion of crop colonizers (0.12) transmitting was also significantly greater than non-colonizers (0.05) (F = 5.24, P <0.05).

These experiments show consistently that both colonizers and non-colonizers of the bird cherry aphid are able to acquire BYDV from infected plants, but that the virus is transmitted to new plants much more efficiently by the colonizers. This is because the non-colonizers are in search of *P. padus* and therefore will tend to leave grasses and cereals as soon as possible while the colonizers will remain on cereals where they will feed and reproduce. The shorter the inoculation feed, the lower the infection level, especially for non-colonizers (Fig. 15). These differences in the ability of the two forms to transmit BYDV highlight the need to be able to identify the two forms in migrant populations if data from trap samples are to be used as a component in the assessment of the risk of infection of crops in autumn.

5.5. Flight behaviour

5.5.1. Methods.

The different seasonal forms of the bird cherry aphid were flown individually in an automated vertical wind tunnel to determine their rate of climb and the time for which they flew before responding to a target. The objectives of these experiments were to provide quantification of behaviour to aid the interpretation of suction trap data and provide information on the distances from which aphids might introduce BYDV to cereal crops in autumn. These experiments have been published in detail (Nottingham, Hardie and Tatchell, 1991) and so are only outlined briefly here.

Emigrant aphids were collected directly from *P. padus* in spring, while the crop colonizing and non-colonizing forms were obtained directly from laboratory cultures reared under appropriate environmental conditions. Newly emerged winged aphids were flown individually in the automated flight chamber. The wind tunnel holds the aphid in the centre of its flight chamber by adjusting the windspeed as aphid flight speed changes. This is achieved through the linking of a video camera monitoring the position of the aphid to a computer which records the position of the aphid and the air speed, and then adjusts the air speed through the wind tunnel to hold the aphid in a central position. A 'target' was presented to the aphid at regular intervals in the form of a green light shone from the side of the flight chamber. The response to this target was measured as the distance the aphid flew towards this target and was recorded by the video camera and computer. The air speed required to hold the aphid in the centre of the chamber gave a direct measure of the aphid's rate of climb, while the time taken before the aphid responded to a green target indicates the probable duration of flight before the aphid will land on a crop.

5.5.2. Results and Discussion.

The non-colonizing form of the bird cherry aphid climbed faster during its first 10 minutes of flight than either the colonizing form or the emigrants from *P. padus* (Table 12). In addition the non-colonizing form flew for 110 minutes on average before responding to a green target, longer than either of the other two forms (Table 13). This suggests that in autumn the non-colonizing forms will tend to fly higher and for longer than the colonizing forms and will therefore form a larger part of the samples at higher altitudes. This agrees with the data for the proportion of the different migrant forms in the population at different heights (Fig. 14). However, the flight duration of the colonizing forms (36 min) indicates that even in relatively light winds the aphids will travel several kilometres. Therefore, the sources of infections of crops could be at some distance from the farm rather than from nearby fields.

6. DISCUSSION

This report provides details of results from three cropping years. Data are currently being gathered for a further season from the four core sites under HGCA sponsorship (0087/1/91). In addition further data have been gathered from a number of other sites through MAFF funding. Therefore a full analysis of the data obtained through HGCA funding alone has not been attempted. This will be undertaken once the final year's date are complete and will combine the data obtained through both HGCA and MAFF funding. The key findings of the work to date are discussed briefly below.

The sets of data from diverse regions of England, that combine information relating to the potential risk of primary BYDV infection of crops (the numbers of migrant vector species and their virus content) with the validation of these data by examining the colonization and infection of crops, provide a unique opportunity to improve forecasts to the industry. However, it is clear from the data obtained so far that the answers are not simple.

An important finding is the prevalence of different BYDV serotype in different areas of England. There is considerable consistency to these regional differences which suggest that different serotype/vector combinations persist in different regions. This contrasts with the overall numbers of migrant vectors which shows that in all areas the bird cherry aphid predominates, while the grain aphid is much less abundant. This finding suggests that as a BYDV vector the grain aphid differs greatly from the bird cherry aphid. This has now formed the basis of project 0041/1/91 currently funded by the HGCA.

The concept of the infectivity index as a measure of the risk of crops to BYDV infection in autumn is well tested. The combining of the data from this study with that from complementary MAFF funded research will provide the most comprehensive testing of the methodology yet undertaken. These data will also be drawn together at the end of the current cropping year. However, a few points warrant comment at this

stage. The numbers of grain aphids recorded in suction trap samples and tested for their BYDV content are small compared to the levels of the MAV isolates that were recorded in crops. This is now the subject of project 0041/1/91. In addition, the bird cherry aphids in suction trap samples comprise forms that transmit BYDV with differing efficiencies, yet the proportions of the different aphid forms differs from year to year. If the infectivity is to be a useful concept it will clearly have to take account both of the different behaviours of the grain and bird cherry aphids, and of the different proportions of colonizing and non-colonizing bird cherry aphids.

The progression of BYDV epidemics within crops is made up of two components. Initially inoculation of the crop takes place by aphids flying into it from remote sources. From these initial foci of infection the disease is then spread within the crop. It is vital to know when these two phases occur. In early-sown crops it is possible for the two phases to occur at the same time, while in those sown later they are probably sequential. The disease progression curves developed in this study have shown when the different serotypes of BYDV are spread. However, these data are based on the detection of infection by ELISA. We lack information on the time it takes for newly infected plants to become detectable by ELISA, and then to become a source for further infection. It is essential to find this out if the spread of BYDV within crops is to be understood fully and controlled. This topic is therefore being addressed within project 0087/1/91.

REFERENCES

Doodson, J.K. and Saunders, P.J.W. (1970) Some effects of barley yellow dwarf virus on spring and winter wheat in field trials. *Annals of Applied Biology*, **66**, 361-374.

Halbert, S.E., Connelly, B.J., Bishop, G.W. and Blackmer, J.L. (1992) Transmission of barley yellow dwarf virus by field collected aphids (Homoptera: Aphididae) and their relative importance in barley yellow dwarf epidemiology in southwestern Idaho. *Annals of Applied Biology*, **121**, 105-121.

Henderson, I.F., Loxdale, H.D. and Greenwood, S.P. (1976) Identification of immature aphids by chromatography. *Ecological Entomology*, **1**, 171-173.

Macaulay, E.D.M., Tatchell, G.M. and Taylor, L.R. (1988) The Rothamsted Insect Survey "12-metre" suction trap. *Bulletin of Entomological Research*, **78**, 121-129.

Nottingham, S.F., Hardie, J. and Tatchell, G.M. (1991) Flight behaviour of the bird cherry aphid, *Rhopalosiphum padi*. *Physiological Entomology*, **16**, 223-229.

Plumb, R.T. (1976) Barley yellow dwarf virus in aphids caught in suction traps, 1969-73. *Annals of Applied Biology*, **83**, 53-59.

Plumb, R.T. (1981) Chemicals in the control of cereal virus diseases. In: Jenkyn, J.F. and Plumb, R.T. eds. Strategies for the control of cereal diseases. Blackwells Scientific Publications, Oxford, 135-145.

Plumb, R.T. (1983) Barley yellow dwarf virus - a global problem. In: Plumb, R.T. and Thresh, J.M. eds. Plant virus epidemiology. Blackwells Scientific Publications, Oxford, 187-198.

Plumb, R.T. (1986) A rational approach to the control of barley yellow dwarf virus. *Journal of the Royal Agricultural Society of England*, **147**, 162-171.

Rochow, W.F. (1969) Biological properties of four isolates of barley yellow dwarf virus. *Phytopathology*, **59**, 1580-1589.

Tatchell, G.M. (1991) Monitoring and forecasting aphid problems. In: *"Aphid plant interactions: populations to molecules"*. Eds. D.C. Peters, J.A. Webster and C.S. Chlouber. Oklahoma Agricultural Experiment Station, Miscellaneous Publication No. **132**, 215-231.

Tatchell, G.M. and Parker, S.J. (1990) Host plant selection by migrant *Rhopalosiphum padi* in autumn and the occurrence of an intermediate morph. *Entomologia Experimentalis et Applicata*, **54**, 237-244.

Tatchell, G.M., Plumb R.T. and Carter, N. (1988a) Migration of alate morphs of the bird cherry aphid (*Rhopalosiphum padi*) and implications for the epidemiology of barley yellow dwarf virus. *Annals of Applied Biology*, **112**, 1-11.

Tatchell, G.M., Thorn, M., Loxdale, H.D. and Devonshire, A.L. (1988b) Monitoring for insecticide resistance in migrant populations of *Myzus persicae*. *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases,* 439-444.

Taylor, L.R. (1986) Synoptic dynamics, migration and the Rothamsted Insect Survey. *Journal of Animal Ecology*, **55**, 1-38.

Taylor, M.S., Tatchell, G.M. and Clark, S.J. (1993) Morph identification in wild populations of alate female bird cherry aphids, *Rhopalosiphum padi* L., by multivariate methods. *Annals of Applied Biology*, in press.

Torrance, L. (1987) Use of enzyme amplification in an ELISA to increase sensitivity of detection of barley yellow dwarf virus in oats and individual vector aphids. *Journal of Virological Methods*, **15**, 131-138.

TABLE 1. Sowing dates of experimental plots of winter barley at the four core sites, 1989-1991.

LEEDS	ROTHAMSTED	WYE	STARCROSS
1989			
15 Sept	5 Sept	18 Sept	10 Oct
20 Sept	18 Sept	27 Sept	16 Oct
27 Sept	29 Sept	6 Oct	24 Oct
4 Oct	9 Oct	16 Oct	1 Nov
11 Oct	18 Oct	23 Oct	6 Nov
1990			
12 Sept	5 Sept	12 Sept	13 Sept
26 Sept	14 Sept	26 Sept	26 Sept
10 Oct	24 Sept	10 Oct	9 Oct
24 Oct	4 Oct	24 Oct	31 Oct
31 Oct	17 Oct	7 Nov	7 Nov
1991			
12 Sept	2 Sept	12 Sept	12 Sept
26 Sept	12 Sept	26 Sept	26 Sept
10 Oct	26 Sept	9 Oct	11 Oct
24 Oct	10 Oct	25 Oct	23 Oct
7 Nov	24 Oct	7 Nov	7 Nov

TABLE 2. The number of vector aphids trapped weekly and found by ELISA to contain BYDV provide the data for the infectivity index, 1989.

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	5 Sept	6	3	18	7	3	8	12.4
	12 Sept	3	1	43	0	0	5	14.3
	19 Sept	0	0	172	0	0	0	0
	26 Sept	0	0	761	0	0	3	0
	3 Oct	16	4	780	0	0	2	195
	10 Oct	20	8	147	0	0	1	58.8
	17 Oct	16	2	154	0	0	0	19.2
	24 Oct	6	1	42	0	0	0	7.0
Rothamsted	19 Sept	1	0	20	0	0	0	0
	26 Sept	0	0	75	3	2	0	0
	3 Oct	2	0	37	0	0	0	0
	10 Oct	5	2	62	0	0	0	24.8
	17 Oct	4	3	163	1	0	1	122.3
	24 Oct	4	1	108	0	0	0	27.0
	31 Oct	0	0	15	0	0	1	0
	7 Nov	0	0	27	0	0	0	0
	14 Nov	1	0	10	0	0	1	0
Wye	5 Sept	1	0	2	0	0	0	0
	12 Sept	4	0	12	5	2	1	0.4
	19 Sept	3	0	77	0	0	0	0
	26 Sept	9	4	100	0	0	0	44.4
	3 Oct	20	5	75	0	0	0	17.8
	10 Oct	43	10	265	1	0	0	61.6
	17 Oct	27	11	118	1	1	0	48.1
	24 Oct	54	13	138	0	0	1	33.2
	31 Oct	3	2	74	1	0	1	29.6
	7 Nov	2	1	23	0	0	0	11.5
Starcross	5 Sept	5	0	6	0	0	0	0
	12 Sept	1	1	3	0	0	0	3.0
	19 Sept	78	17	79	1	0	0	17.2
	26 Sept	22	2	211	2	0	4	19.2
	3 Oct	28	1	118	1	0	1	4.2
	10 Oct	97	22	359	2	0	1	81.4
	17 Oct	69	21	504	3	0	1	153.4
	24 Oct	60	12	302	4	0	2	60.4
	31 Oct	64	13	85	3	0	0	17.3
	7 Nov	0	0	12	0	0	0	0
	14 Nov	0	0	8	0	0	1	0
	21 Nov	1	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, 1990.

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	3 Sept	0	0	116	0	0	0	0
	10 Sept	54	1	237	3	1	5	6.1
	17 Sept	12	0	15	0	0	0	0
	24 Sept	21	5	354	0	0	4	84.3
	1 Oct	73	6	123	2	1	0	10.1
	8 Oct	20	6	794	0	0	0	238.2
	15 Oct	33	2	317	1	1	3	22.2
	22 Oct	35	5	386	0	0	0	55.1
	29 Oct	23	6	111	1	0	0	28.9
	5 Nov	1	0	67	0	0	1	0
	12 Nov	4	2	51	5	3	0	25.5
	19 Nov	0	0	1	0	0	0	0
Rothamsted	3 Sept	0	0	6	0	0	0	0
	10 Sept	0	0	8	1	0	0	0
	17 Sept	0	0	0	0	0	0	0
	24 Sept	0	0	11	0	0	0	0
	1 Oct	0	0	3	0	0	0	0
	8 Oct	2	1	48	0	0	0	24.0
	15 Oct	7	1	522	0	0	2	74.6
	22 Oct	4	0	75	0	0	2	0
	29 Oct	0	0	17	0	0	0	0
	5 Nov	0	0	28	0	0	0	0
	12 Nov	0	0	6	0	0	1	0
	19 Nov	0	0	0	0	0	0	0
Wye	3 Sept	0	0	11	1	0	0	0
	10 Sept	0	0	23	1	1	1	0
	17 Sept	0	0	4	0	0	1	0
	24 Sept	8	0	27	0	0	0	0
	1 Oct	1	0	53	4	2	0	0
	8 Oct	24	0	139	0	0	1	0
	15 Oct	47	5	344	2	1	1	37.1
	22 Oct	16	1	41	1	0	1	2.6
	29 Oct	4	0	7	0	0	0	0
	5 Nov	9	1	26	0	0	1	2.9
	12 Nov	4	0	12	0	0	0	0
Starcross	3 Sept	16	0	66	2	0	2	0
	10 Sept	112	19	126	2	2	2	23.4
	17 Sept	30	11	19	3	1	0	6.9
	24 Sept	66	7	93	10	0	3	9.9
	1 Oct	66	3	103	12	1	3	4.7
	8 Oct	72	6	114	10	1	1	12.1
	15 Oct	100	15	73	12	1	1	11.0
	22 Oct	32	6	28	136	7	1	16.3
	29 Oct	1	0	2	2	0	0	0
	5 Nov	0	0	5	0	0	0	0
	12 Nov	15	2	14	0	0	1	1.9

TABLE 4. The number of vector aphids trapped weekly and found by ELISA to contain BYDV provide the data for the infectivity index, 1991.

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	2 Sept	1	0	160	1	0	3	0
	9 Sept	9	1	655	1	0	2	72.8
	16 Sept	24	1	1293	2	0	0	53.9
	23 Sept	113	11	1058	1	0	0	102.9
	30 Sept	78	8	288	2	0	0	29.5
	7 Oct	24	3	511	0	0	4	63.9
	14 Oct	29	1	113	0	0	0	3.9
	21 Oct	10	3	232	0	0	0	69.6
	28 Oct	54	2	235	3	0	0	8.7
	4 Nov	4	1	4	0	0	0	1.0
Rothamsted	9 Sept	1	0	150	0	0	1	0
	16 Sept	4	1	290	0	0	4	72.5
	23 Sept	1	0	103	0	0	3	0
	30 Sept	2	0	98	0	0	1	0
	7 Oct	5	0	575	0	0	5	0
	14 Oct	17	1	481	0	0	1	28.3
	21 Oct	12	1	203	0	0	0	16.9
	28 Oct	1	0	125	0	0	1	0
Wye	9 Sept	12	1	55	0	0	10	4.6
	16 Sept	10	0	73	0	0	7	0
	23 Sept	30	1	491	6	0	7	16.4
	30 Sept	77	0	809	0	0	5	0
	7 Oct	87	4	911	2	0	4	41.9
	14 Oct	65	8	425	0	0	1	52.3
	21 Oct	40	5	820	1	0	0	102.5
	28 Oct	69	6	212	1	0	1	18.4
	4 Nov	10	0	34	0	0	0	0
	11 Nov	10	0	42	0	0	0	0
	18 Nov	1	0	15	0	0	0	0
Starcross	26 Aug	19	0	413	0	0	15	0
	2 Sept	43	0	254	4	0	14	0
	9 Sept	95	0	646	7	1	7	1
	16 Sept	111	3	314	6	0	12	8.5
	23 Sept	64	0	542	13	0	5	0
	30 Sept	51	4	403	2	0	0	31.6
	7 Oct	69	6	351	10	0	6	30.5
	14 Oct	14	3	153	2	1	0	32.8
	21 Oct	23	0	529	5	2	0	0

TABLE 5. The number of bird cherry aphids collected from 12.2 m suction trap samples in a storage solution at Rothamsted that were found by amplified ELISA to contain BYDV, 1989-1991.

Year	Week beginning	Female		Male	
		No. tested	No. positive	No. Tested	No. positive
1989	27 Aug	0	-	0	-
	3 Sept	0	-	0	-
	10 Sept	0	-	0	-
	17 Sept	13	0	3	0
	24 Sept	40	2	5	1
	1 Oct	48	1	0	-
	8 Oct	27	2	32	4
	15 Oct	62	5	57	9
	22 Oct	83	10	89	9
	29 Oct	10	0	10	2
	5 Nov	5	0	2	0
	12 Nov	24	4	4	0
	19 Nov	2	0	1	0
1990	27 Aug	3	1	0	-
	3 Sept	6	2	0	-
	10 Sept	7	2	1	0
	17 Sept	0	-	0	-
	24 Sept	11	0	0	-
	1 Oct	3	0	0	-
	8 Oct	43	5	5	0
	15 Oct	164	21	151	9
	22 Oct	33	1	42	3
	29 Oct	10	0	3	0
	5 Nov	8	0	20	5
	12 Nov	2	0	4	0
	19 Nov	0	-	0	-
1991	27 Aug	2	0	0	-
	3 Sept	6	1	0	-
	10 Sept	105	9	6	0
	17 Sept	130	9	11	0
	24 Sept	84	3	24	0
	1 Oct	62	3	33	4
	8 Oct	211	15	158	7
	15 Oct	91	4	78	3
	22 Oct	84	9	149	11
	29 Oct	28	2	45	3
	5 Nov	1	0	2	0
	12 Nov	0	-	3	0
	19 Nov	0	-	0	-

TABLE 6. Level of infection by different serotypes of BYDV in April in plots of winter barley sown on different dates the previous autumn, 1989-1990.

Sowing date	Total level of infection (%)(*a*)	PAV	RPV	MAV	Unidentified (*b*)
Leeds 1989-1990					
15 Sept	100.0	3.8	0	100.0	-
20 Sept	97.5	0	0	97.5	-
27 Sept	82.5	0	0	82.5	-
4 Oct	76.3	0	3.8	76.3	-
11 Oct	80.0	0	0	80.0	-
Rothamsted 1989-1990					
5 Sept	48.8	37.5	0	12.5	-
18 Sept	15.0	10.0	0	5.0	-
29 Sept	6.3	0	1.3	5.0	-
9 Oct	6.3	1.3	0	5.0	-
18 Oct	5.0	2.5	0	2.5	-
Wye 1989-1990					
18 Sept	97.5	76.3	0	27.5	6.3
27 Sept	82.5	46.3	0	31.3	13.8
6 Oct	83.8	45.0	0	28.8	12.5
16 Oct	38.8	16.3	0	17.5	7.5
23 Nov	65.0	36.3	0	22.5	13.8
Starcross 1989-1990					
10 Oct	93.8	38.8	1.3	75.0	7.5
16 Sept	88.8	35.0	5.0	61.3	13.8
24 Oct	45.0	13.8	0	18.8	13.8
1 Nov	11.3	0	0	6.3	5.0
6 Nov	15.0	0	0	6.3	8.7

a some plants were infected with a mixture of serotypes.

b It was not possible to determine the BYDV serotypes infecting some plants from Wye and Starcross. A refinement of methodologies avoided this for samples from Leeds and Rothamsted.

TABLE 7. Level of infection by different serotypes of BYDV in March in plots of winter barley sown on different dates the previous autumn, 1990-1991.

Sowing date	Total level of infection (%)	PAV	RPV	MAV	PAV + MAV	PAV + RPV	RPV + MAV	PAV + MAV + RPV
Leeds								
12 Sept	18.8	1.3	0	17.5	0	0	0	0
26 Sept	18.8	1.3	0	17.5	0	0	0	0
10 Oct	7.5	0	0	5.0	1.3	0	1.3	0
24 Oct	2.5	0	0	2.5	0	0	0	0
31 Oct	5.0	1.3	0	1.3	0	0	0	0
Rothamsted								
5 Sept	40.0	37.5	0	2.5	0	0	0	0
14 Sept	15.0	15.0	0	0	0	0	0	0
24 Sept	3.7	3.7	0	0	0	0	0	0
4 Oct	1.3	1.3	0	0	0	0	0	0
5 Oct	0	0	0	0	0	0	0	0
Wye								
12 Sept	62.5	57.5	0	2.5	2.5	0	0	0
26 Sept	33.8	31.3	0	2.5	0	0	0	0
10 Oct	8.8	5.0	0	3.8	0	0	0	0
24 Oct	6.3	5.0	0	1.3	0	0	0	0
7 Nov	5.0	1.3	0	2.5	1.3	0	0	0
Starcross								
13 Sept	85.0	28.8	0	27.5	26.3	0	0	2.5
26 Sept	67.5	27.5	1.3	23.8	15.0	0	0	0
9 Oct	17.5	6.3	1.3	2.5	2.5	1.3	1.3	0
31 Oct	0	0	0	0	0	0	0	0
7 Nov	0	0	0	0	0	0	0	0

TABLE 8. Level of infection by different serotypes of BYDV in March in plots of winter barley sown on different dates the previous autumn, 1991-1992.

Sowing date	Total level of infection (%)	PAV	RPV	MAV	PAV + MAV	PAV + RPV	RPV + MAV	PAV + MAV + RPV
Leeds								
12 Sept	7.5	0	0	1.3	0	0	2.5	3.8
26 Sept	7.5	1.3	0	1.3	0	1.3	0	2.5
10 Oct	0	0	0	0	0	0	0	0
24 Oct	0	0	0	0	0	0	0	0
7 Nov	2.5	2.5	0	0	0	0	0	0
Rothamsted								
2 Sept	5.0	5.0	0	0	0	0	0	0
12 Sept	0	0	0	0	0	0	0	0
26 Sept	2.5	0	0	0	0	0	0	2.5
10 Oct	1.3	0	0	1.3	0	0	0	0
24 Oct	5.0	1.3	1.3	1.3	0	0	0	0
Wye								
12 Sept	26.3	17.5	0	6.3	2.5	0	0	0
26 Sept	0	0	0	0	0	0	0	0
9 Oct	0	0	0	0	0	0	0	0
25 Oct	0	0	0	0	0	0	0	0
7 Nov	1.3	0	0	1.3	0	0	0	0
Starcross								
12 Sept	57.5	30.0	0	18.8	8.8	0	0	0
26 Sept	16.3	5.0	0	7.5	0	0	1.3	1.3
11 Oct	3.8	0	0	3.8	0	0	0	0
23 Oct	0	0	0	0	0	0	0	0
7 Nov	0	0	0	0	0	0	0	0

TABLE 9. The yield (t/ha) of winter barley cv. Magie sown on different dates and treated or not with cypermethrin, Rothamsted 1989-1991.

Sowing date 1989	Cypermethrin -	+	% yield increase
5 Sept	4.42	5.95	34.6
18 Sept	6.51	7.77	19.4
29 Sept	7.29	7.75	6.3
9 Oct	7.40	7.69	3.9
18 Oct	7.02	7.42	5.7
s.e.d. = 0.132			
F = 35.6			
P < 0.001			

Sowing date 1990	Cypermethrin -	+	% Yield increase
5 Sept	6.31	7.09	12.4
14 Sept	7.73	8.09	4.7
24 Sept	8.17	8.94	9.4
4 Oct	7.74	7.82	1.0
17 Oct	7.44	7.65	2.8
s.e.d. = 0.128			
F = 11.9			
P = 0.002			

Sowing date 1991	Cypermethrin -	+	% Yield increase
2 Sept	6.59	7.19	9.1
12 Sept	7.10	7.34	3.4
26 Sept	8.36	8.56	2.4
10 Oct	8.35	8.58	2.8
24 Oct	8.02	7.99	-0.37
s.e.d. = 0.138			
F = 3.22			
P = 0.084			

TABLE 10. Percentage of individuals misclassified using a sub-set of the original 22 morphological characters. 95% confidence limits in parentheses.

Variables included	% Misclassified Emigrants		Colonizers	
Separation of emigrants from colonizers				
Hind tarsal segment II	4.3	(0, 9.04)	5.3	(1.48, 9.12)
+ hind femur	1.4	(0, 4.21)	0	
+ base of antennal segment VI	0		0	
All 22 variables	0		0	
Number of individuals	70		132	

	% Misclassified Colonizers		Non-colonizers	
Separation of colonizers and non-colonizers.				
Width of siphunculus	47.6	(26.25, 68.98)	28.9	(19.85, 37.89)
+ no. rhinaria on antenna V	33.3	(13.17, 53.49)	27.8	(18.92, 36.76)
+ wing width	33.3	(13.17, 53.49)	27.8	(18.92, 36.76)
+ cauda length	23.8	(5.59, 42.03)	15.5	(8.27, 22.66)
+ no. marginal tubercles	14.3	(0, 29.26)	18.6	(10.82, 26.30)
All 22 variables	14.3		12.8	
Number of individuals	21		97	

TABLE 11. The proportion of crop colonizing and non-colonizing forms of the bird cherry aphid that were viruliferous, Rothamsted 1989 and 1990.

	1989		1990	
	Colonizers	Non-colonizers	Colonizers	Non-colonizers
Number tested	103	112	63	105
Fitted proportion	0.20	0.21	0.40	0.13
95% confidence interval	0.132, 0.287	0.148, 0.297	0.282, 0.524	0.080, 0.215

TABLE 12. The rate of climb and duration of migratory flight in different forms of the bird cherry aphid.

Form	*n*	Mean rate of climb (cm s^{-1})
Emigrant	15	15.7
Colonizer	15	13.5
Non-colonizer	15	18.4
s.e.d.		0.905

TABLE 13. The duration of migratory flight of different forms of the bird cherry aphid.

Form	*n*	Mean duration of flight (min)	Range (min)
Emigrant	15	72	4-360
Colonizer	15	36	1-93
Non-colonizer	15	110	14-345

Fig. 1. The four core experimental sites

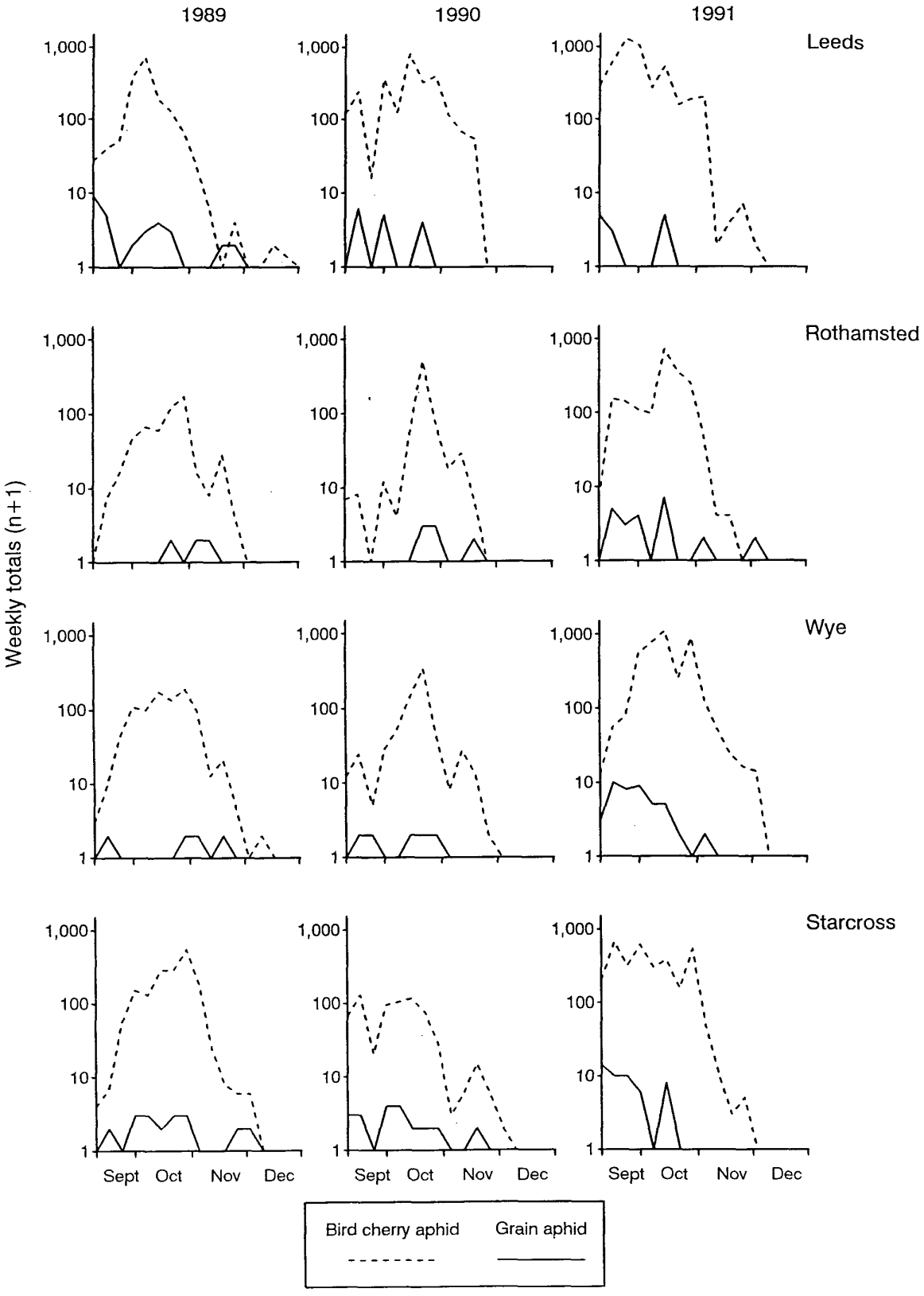

Fig. 2. Aphids in 12.2m suction traps, 1989-91

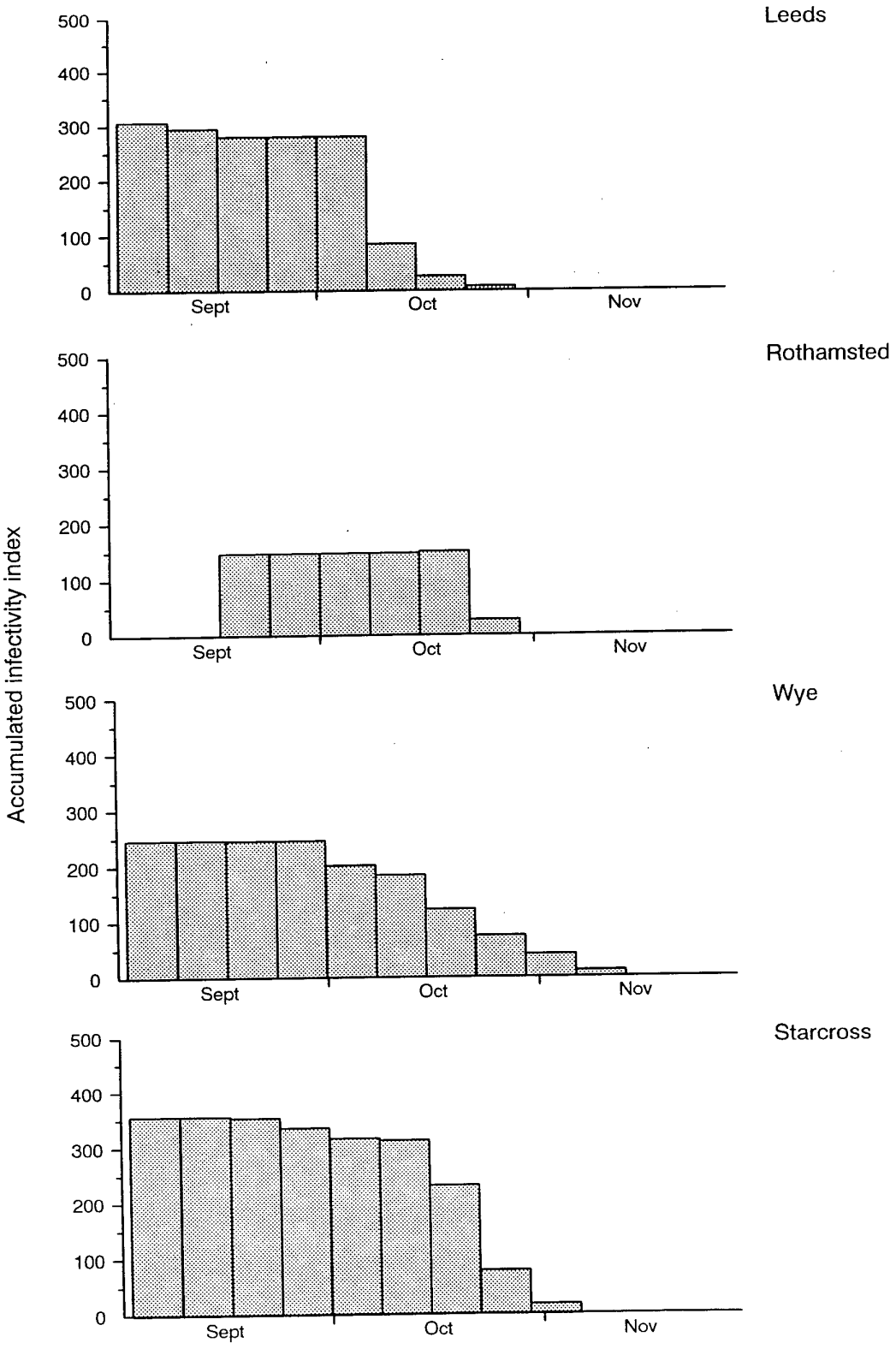

Fig. 3. Accumulated infectivity index 1989

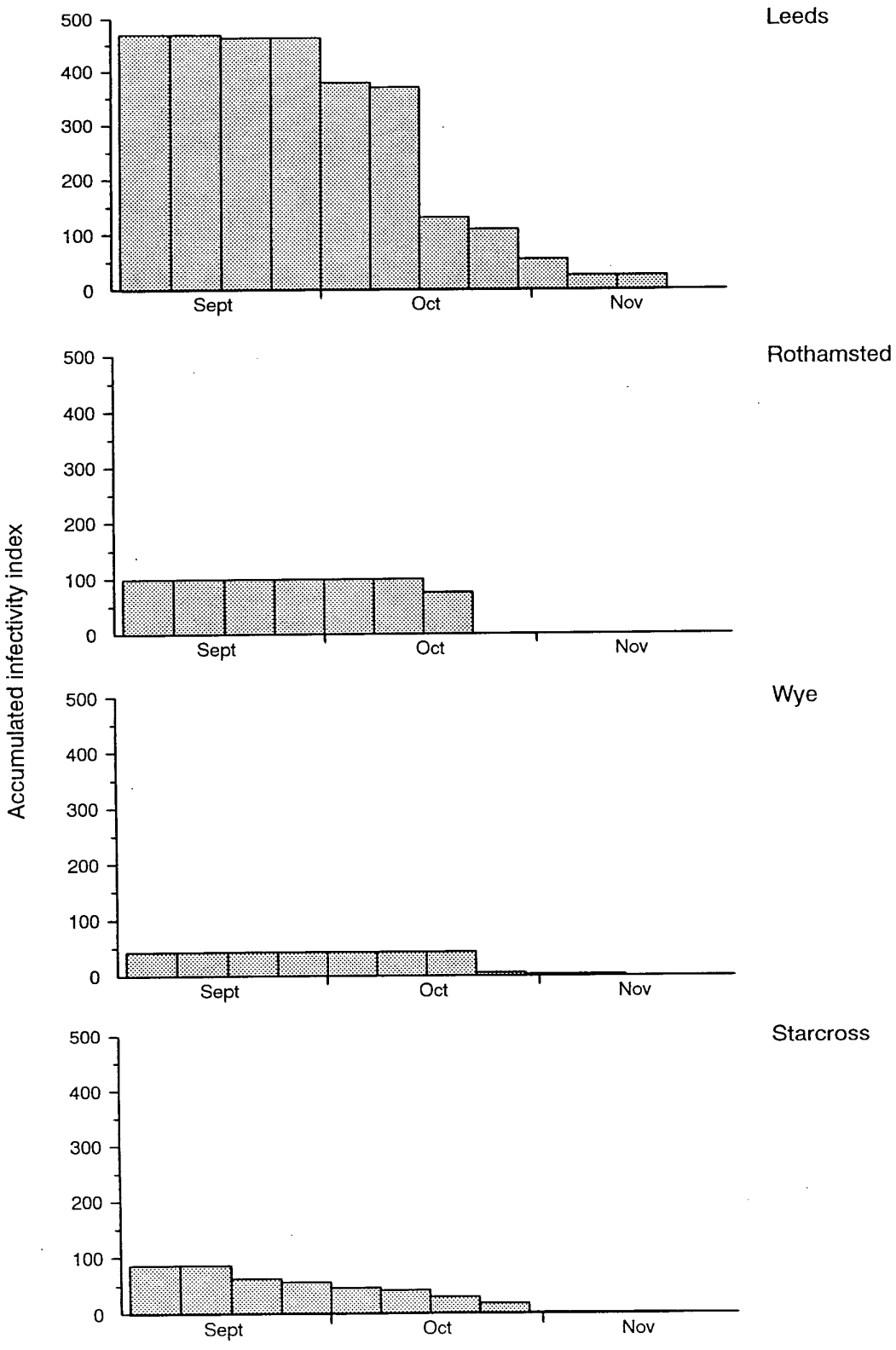

Fig. 4. Accumulated infectivity index 1990

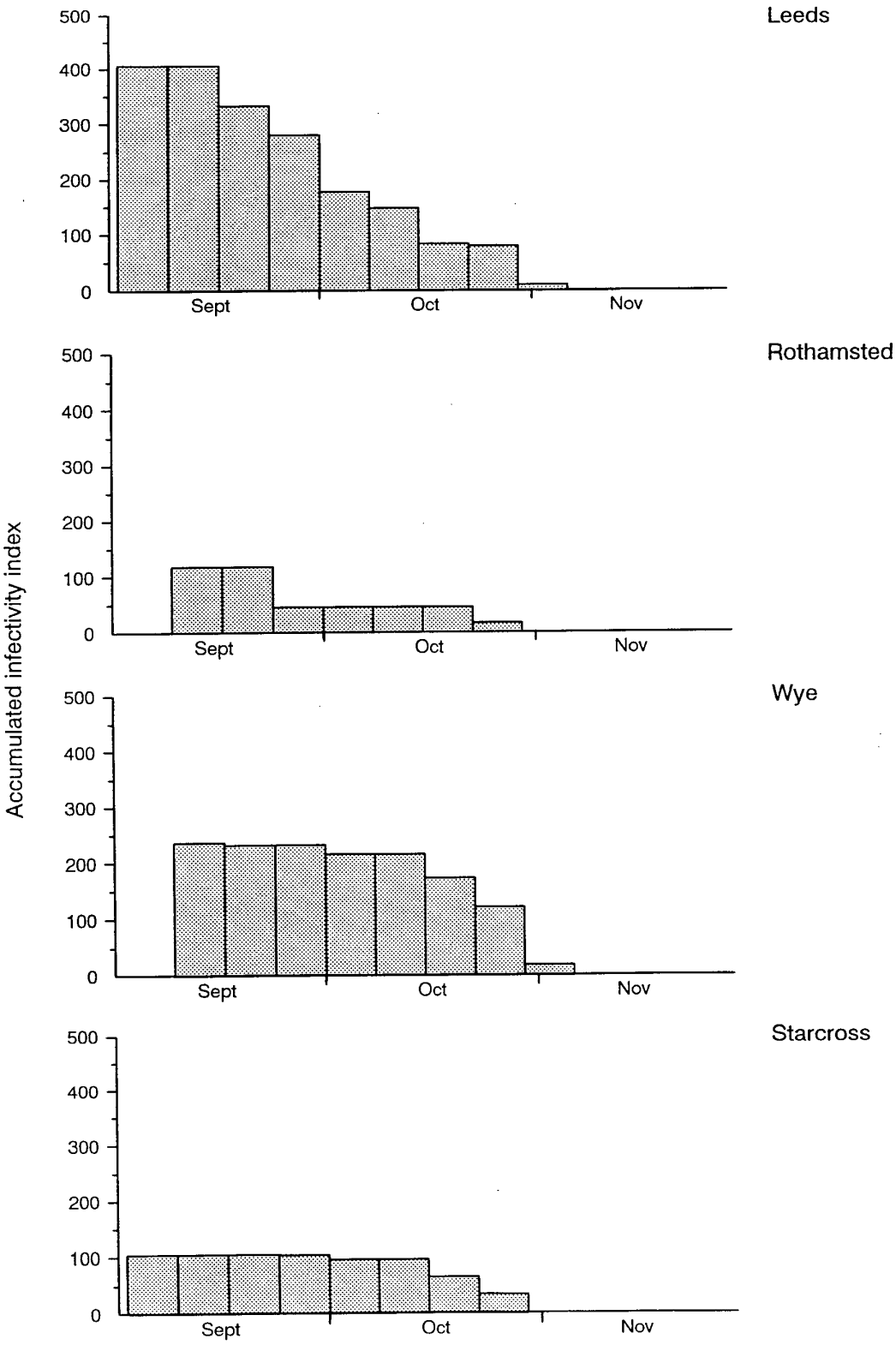

Fig. 5. Accumulated infectivity index 1991

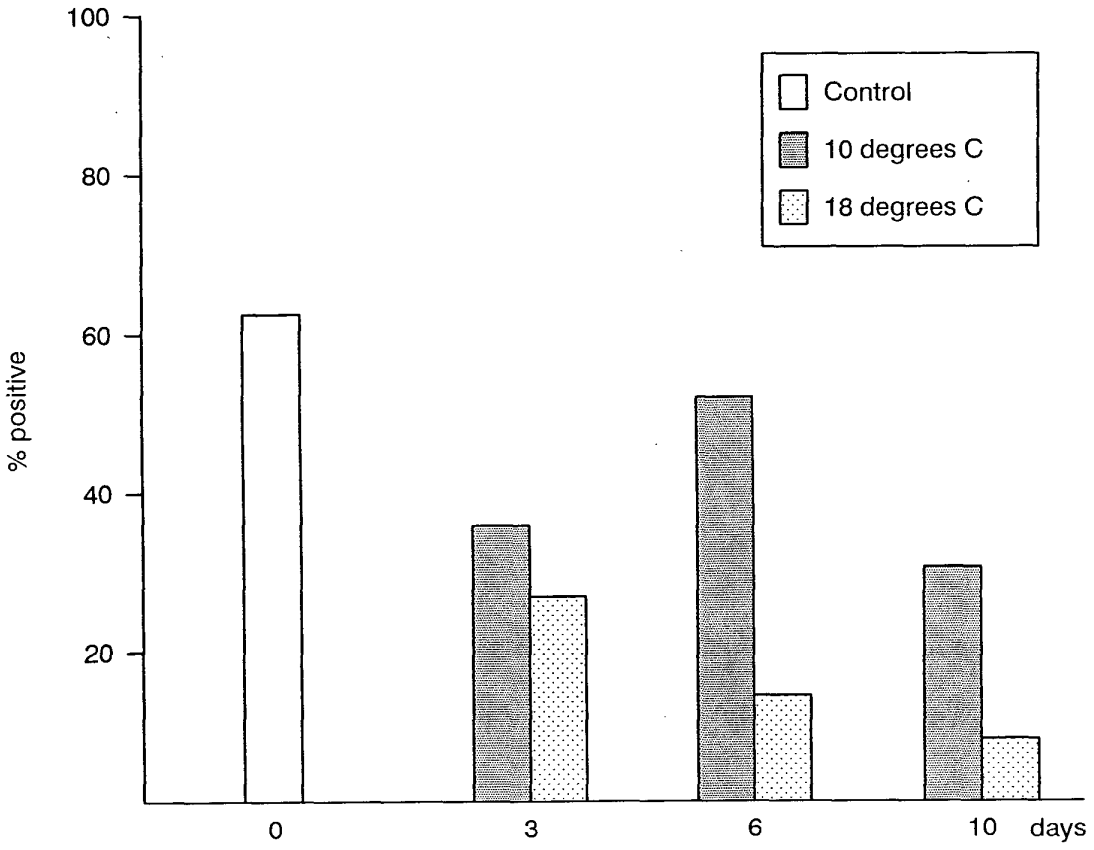

Fig. 6. Effectiveness of storage solution in preserving BYDV in aphids

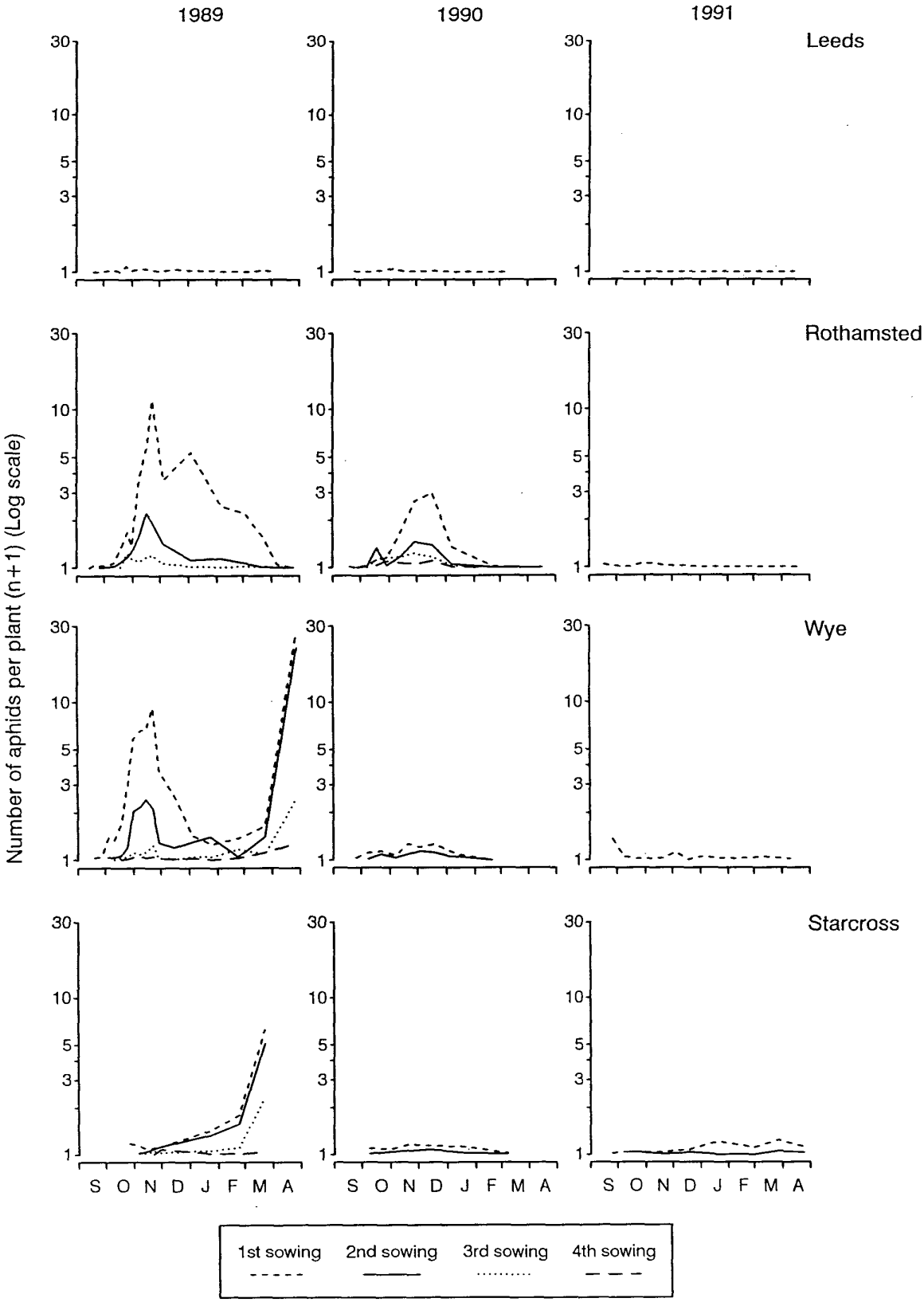

Fig. 7. Numbers of bird cherry aphid per plant, 1989-91

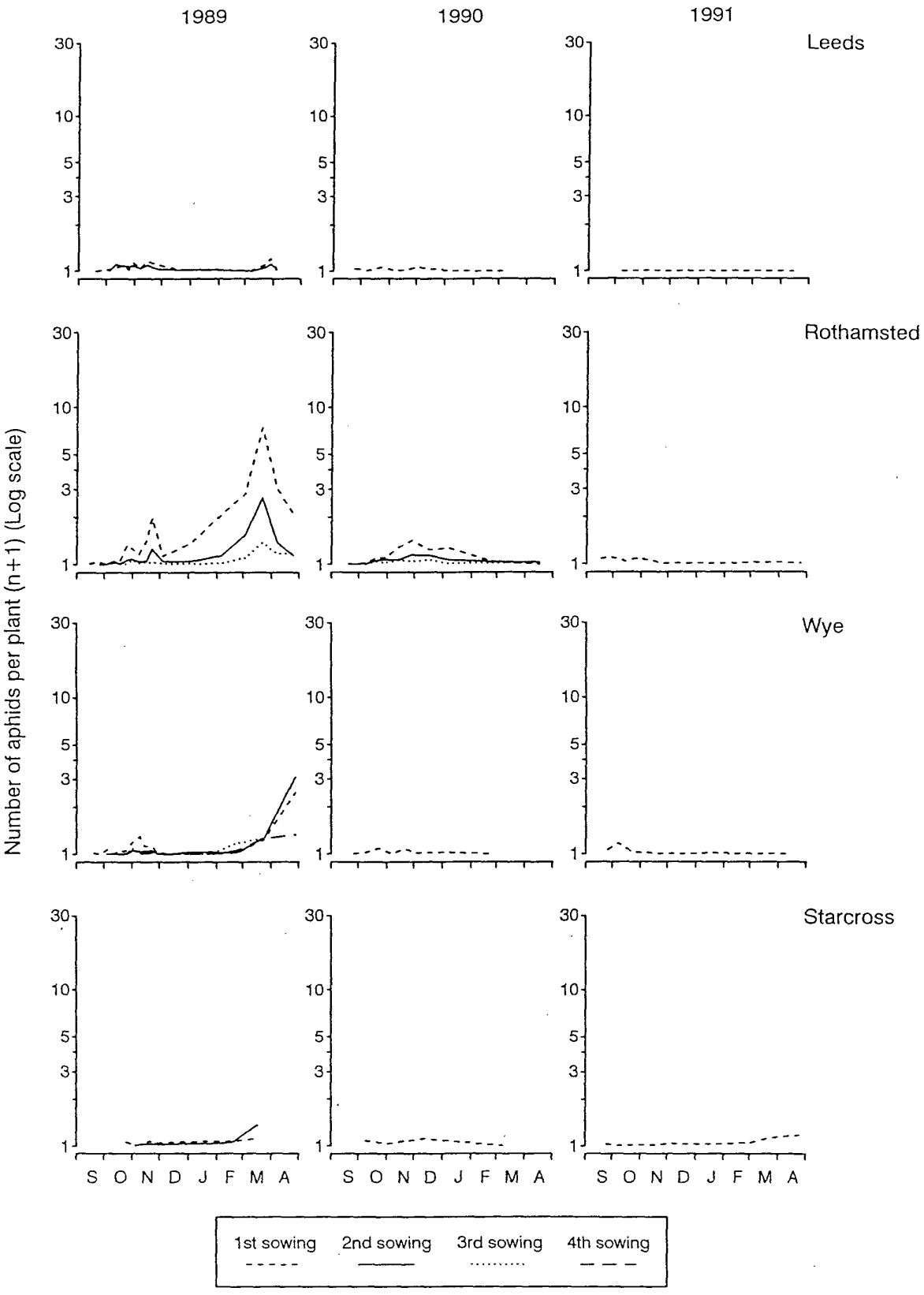

Fig. 8. Numbers of grain aphid per plant, 1989-91

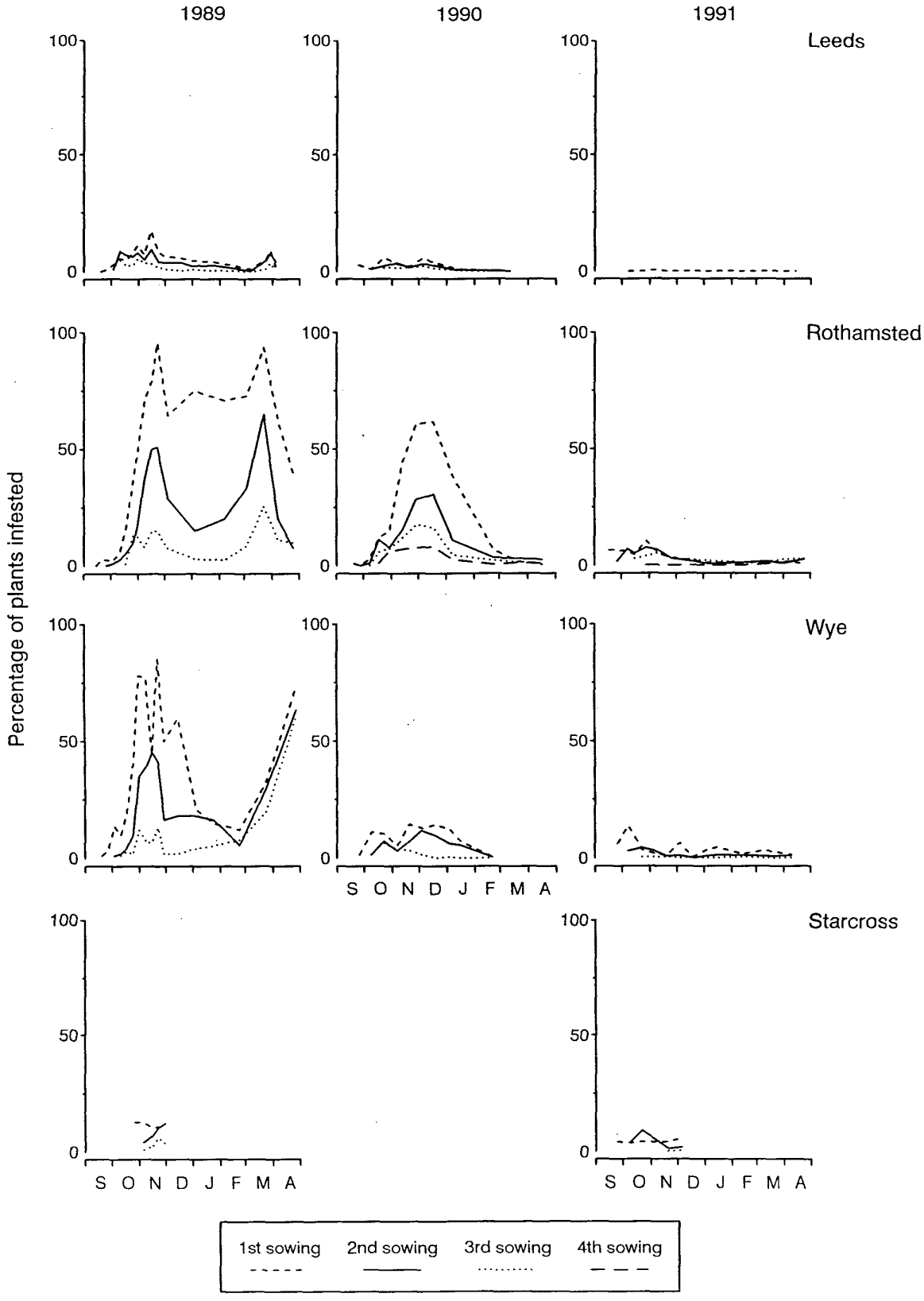

Fig. 9. Percentage of plants infested with aphids, 1989-91

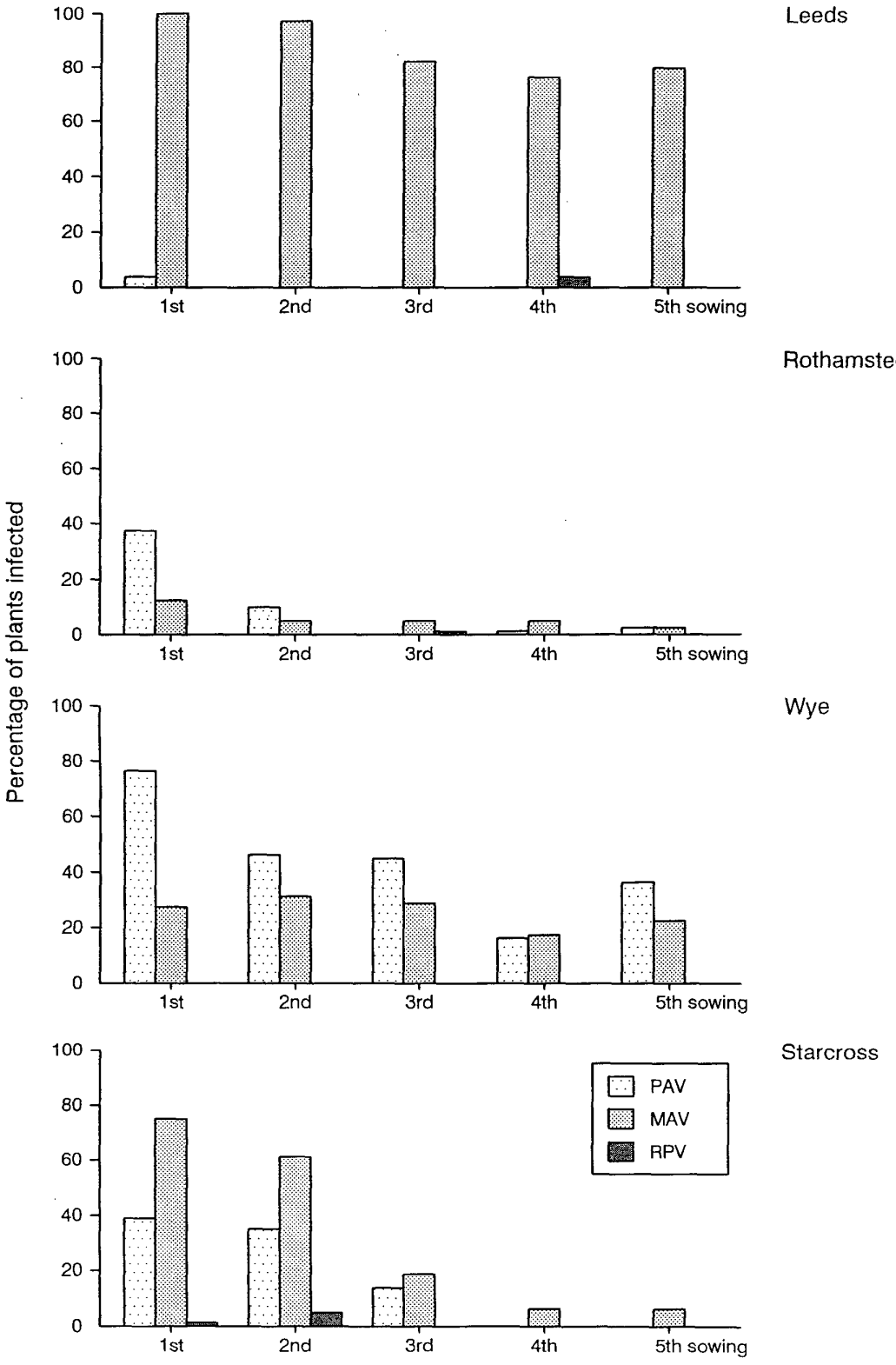

Fig. 10. BYDV infection in April of winter barley sown on different dates, 1989-90

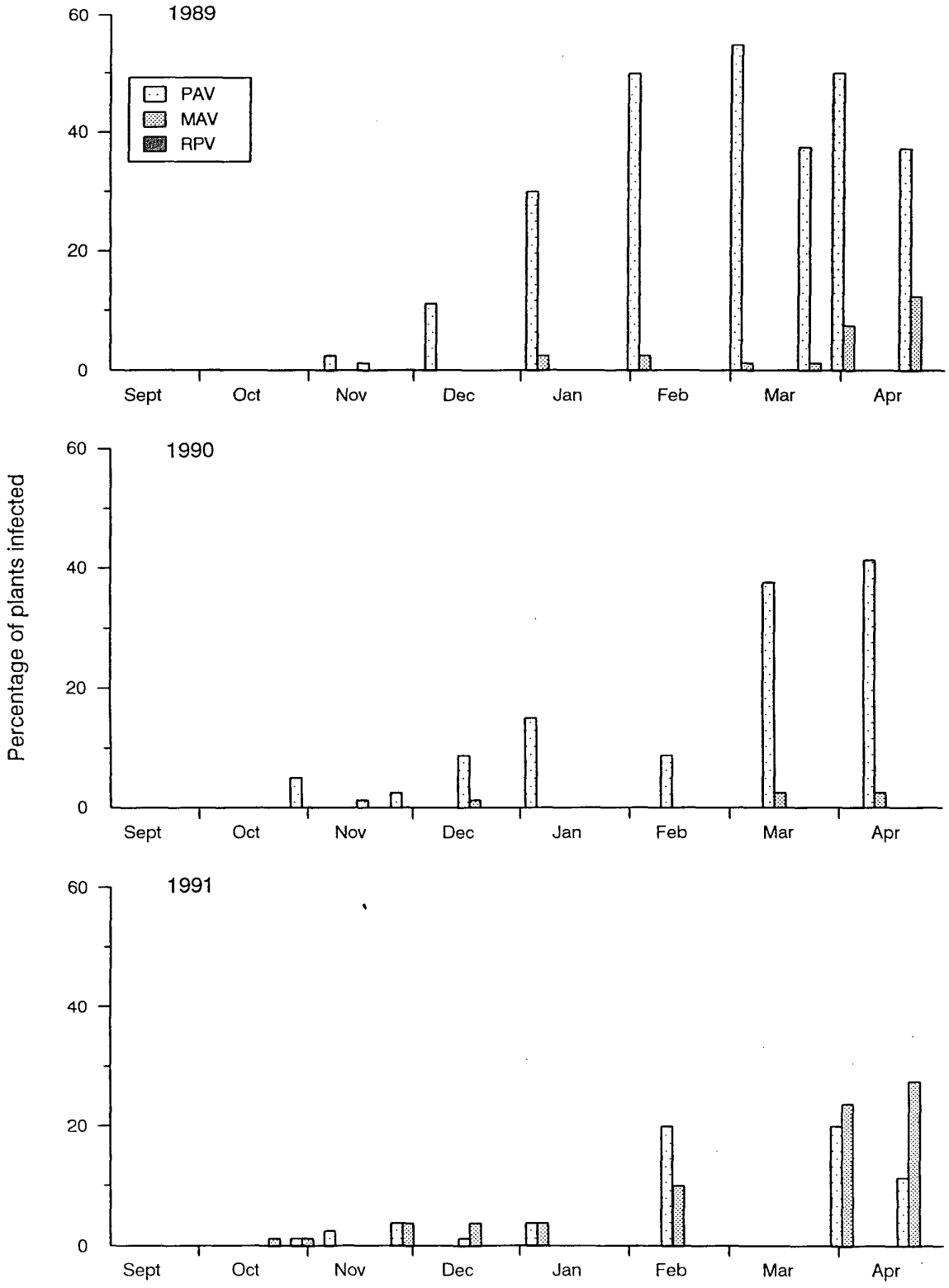

Fig. 11. BYDV progression in early-September sown barley, Rothamsted 1989-91

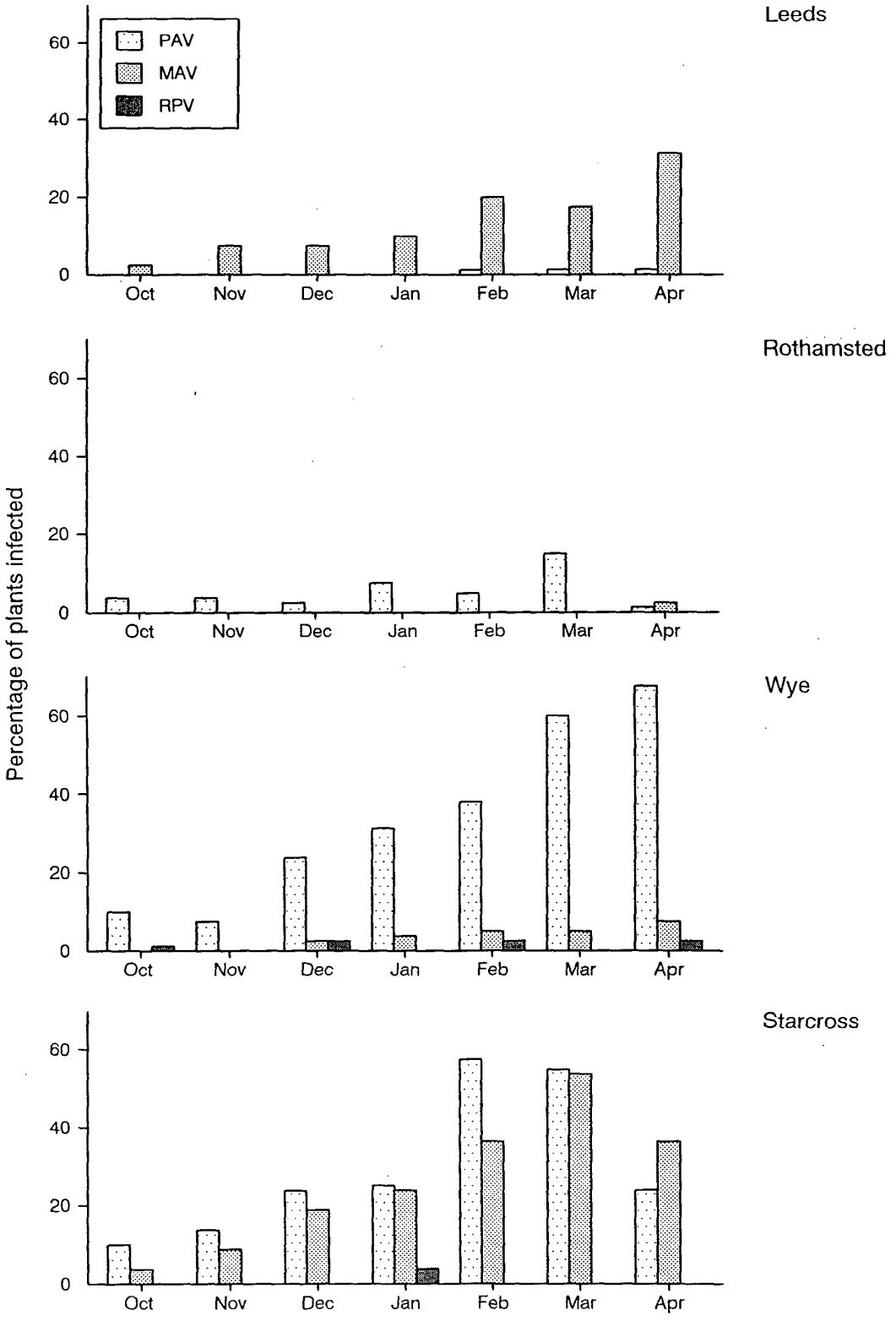

Fig. 12. Percentage BYDV infection in mid-September sown winter barley, 1990-91

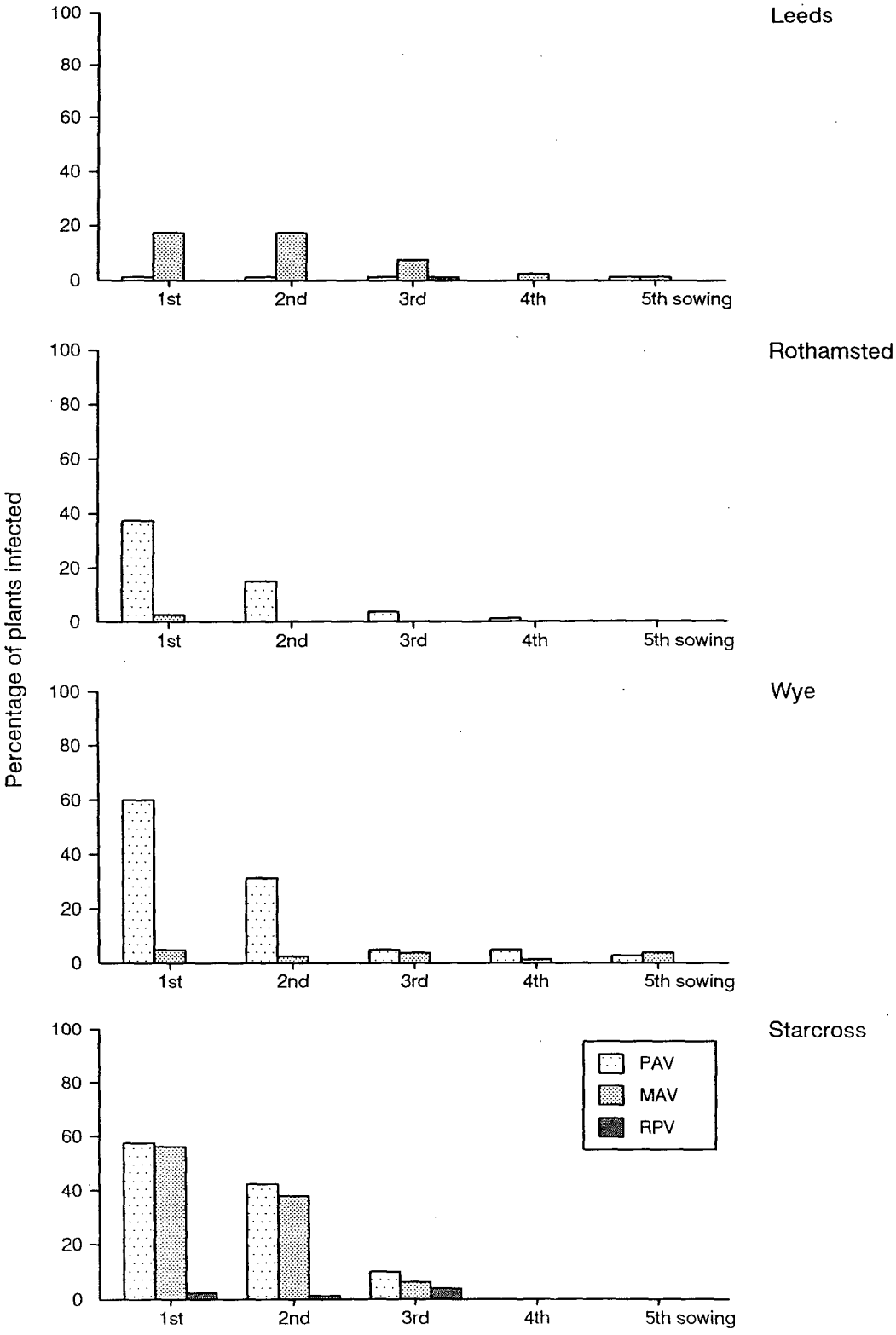

Fig. 13. BYDV infection in March of winter barley sown on different dates, 1990-91

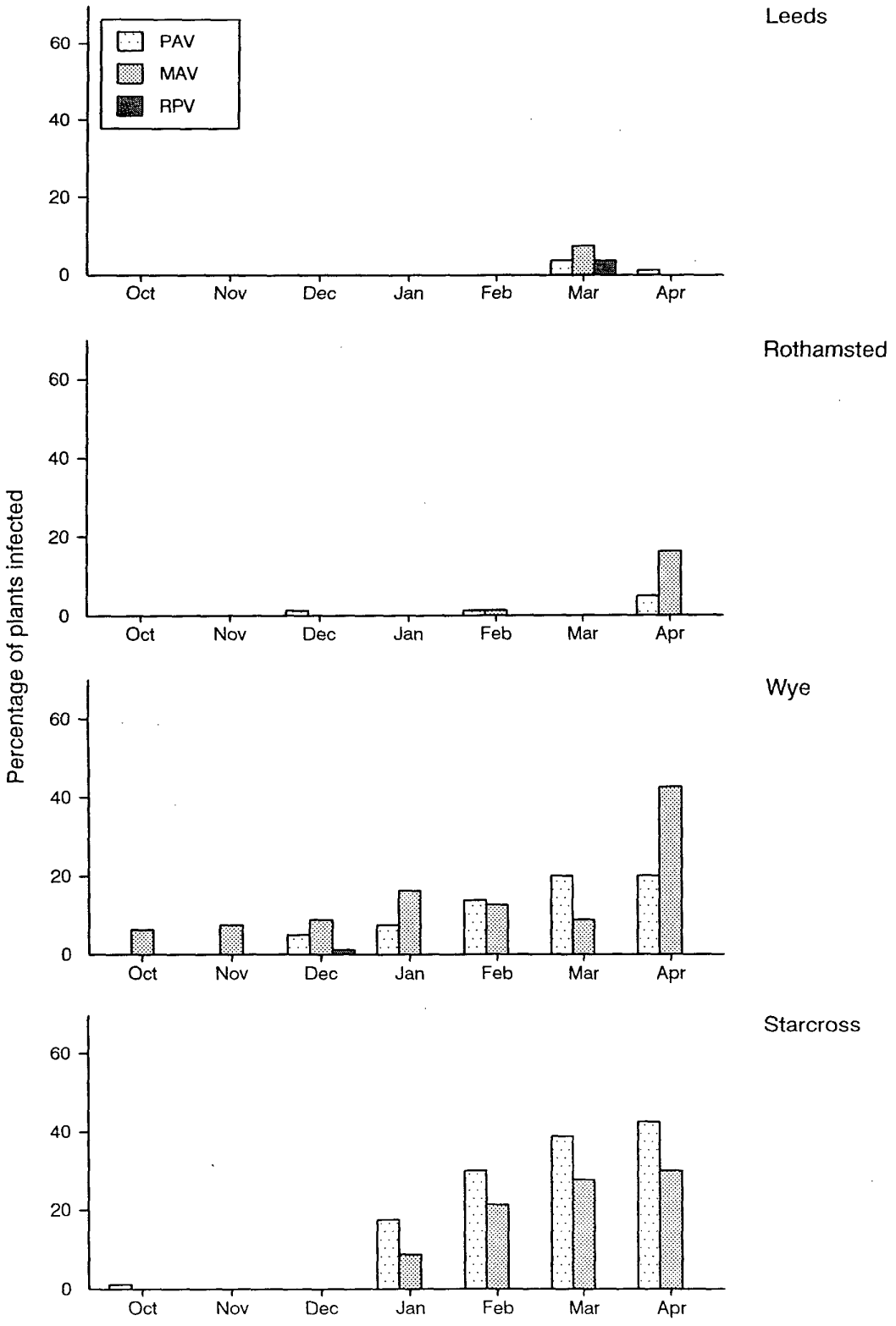

Fig. 14. Percentage BYDV infection in mid-September sown winter barley, 1991-92

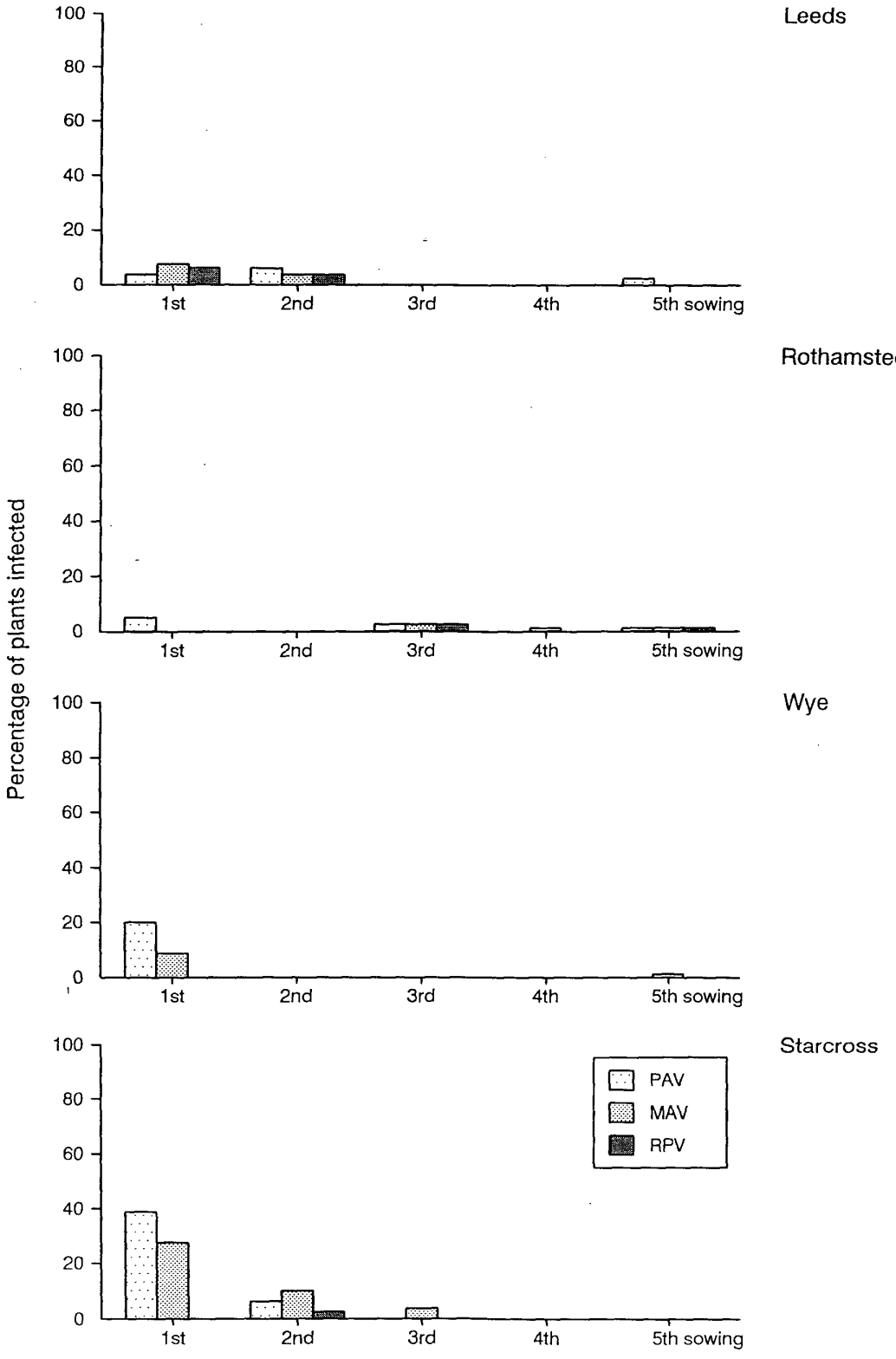

Fig. 15. BYDV infection in March of winter barley sown on different dates, 1991-92

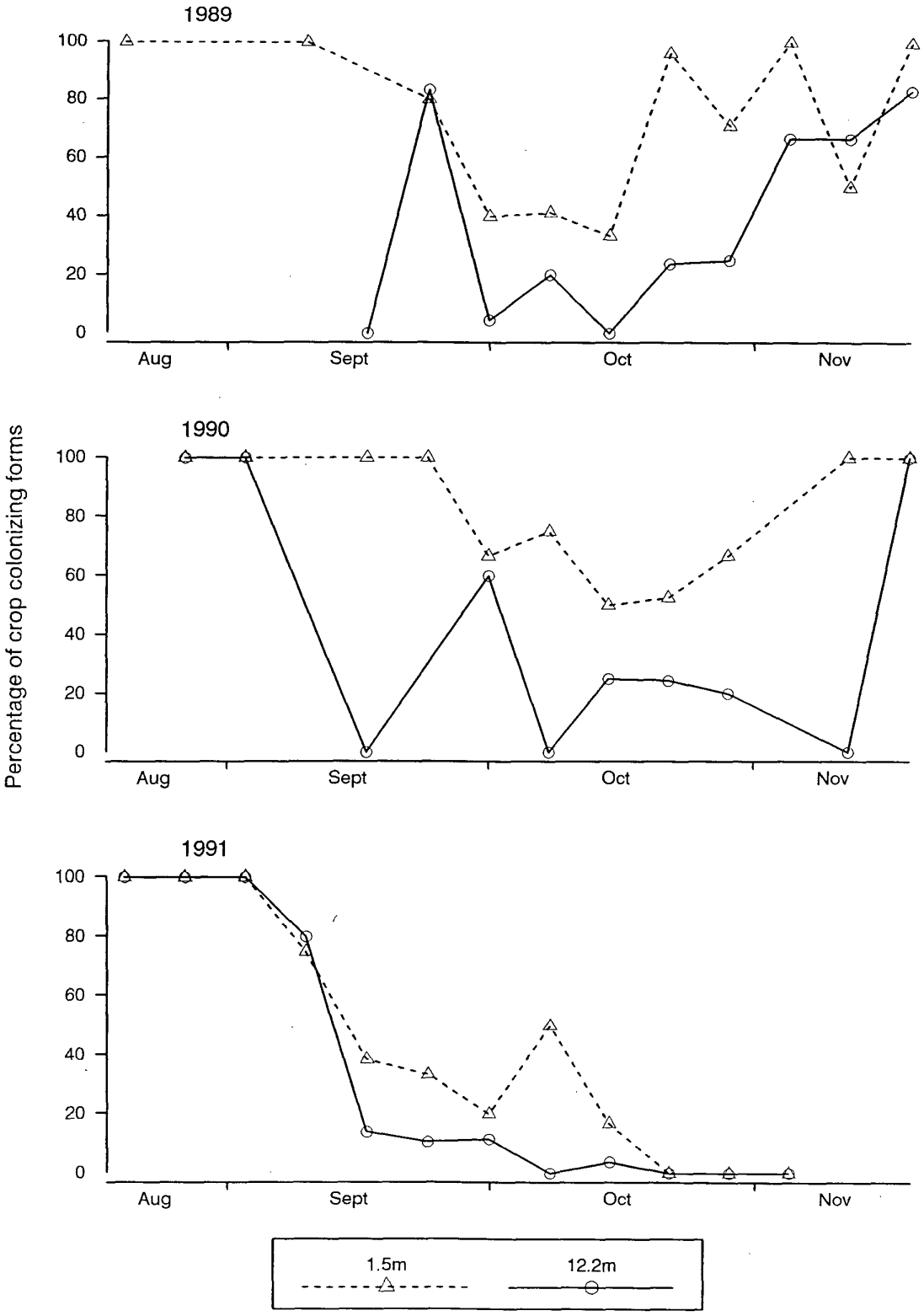

Fig. 16. Percentage of crop colonizing forms of the bird cherry aphid in migrant populations at two heights, Rothamsted 1989-91

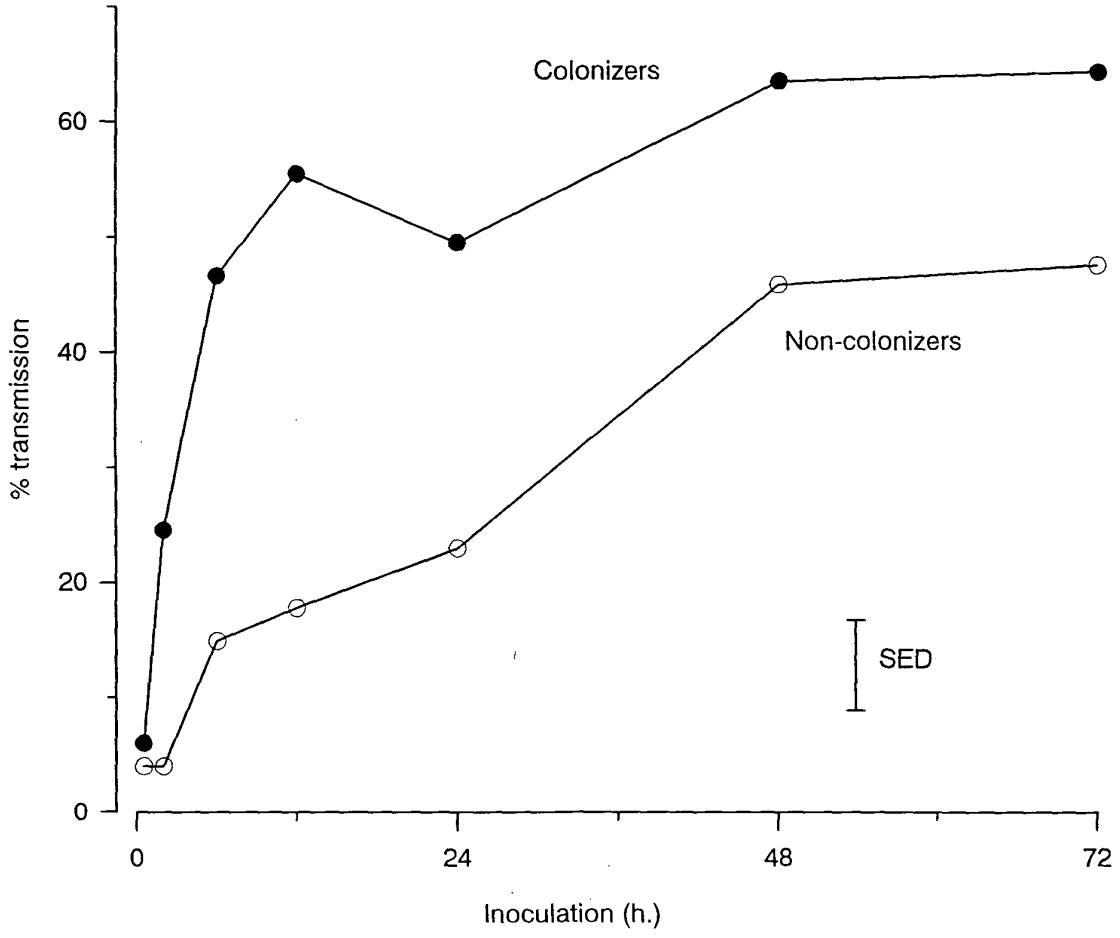

Fig. 17. BYDV transmission by colonizers and non-colonizers