POPULATION AND INSECTICIDE RESISTANCE DYNAMICS IN APHID VECTORS OF BEET VIRUSES

PROJECT NUMBER 05/14
1st April 2005 to 31st March 2009

FINAL REPORT

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CONTENTS

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2 EXECUTIVE SUMMARY

1) This report concerns monitoring and forecasting of sugar beet yellows and its aphid vectors during the 2005, 2006, 2007 and 2008 growing seasons.

2) Sugar beet yellows is a disease caused by three viruses, *Beet mild yellowing virus* (BMYV), *Beet chlorosis virus* (BChV) and *Beet yellows virus* (BYV). BMYV and BChV are generally far more prevalent than BYV. Both viruses are transmitted by three species of aphid, *Myzus persicae* (peach–potato aphid), *Macrosiphum euphorbiae* (potato aphid) and, to a lesser extent, *Aulacorthum solani* (glasshouse and potato aphid). *Myzus persicae* commonly shows some resistance to a range of insecticides. *Aphis fabae* (black bean aphid) does not transmit the poleroviruses BMYV and BChV but can transmit BYV.

3) There are strong relationships between long-term aphid data from the Rothamsted Insect Survey's national suction trap network and long-term weather data. The timing and size of spring migrations of the two main vector aphids are correlated with temperature in January and February. In early March each year, forecasts of aphid activity and virus incidence were provided to the industry. These helped to determine control measures in crops not sown with insecticide-treated seed.

4) Aphid activity (at 16 trap sites, 5 of particular relevance to sugar beet), BMYV-content (at 3 to 5 trap sites depending on year) and three mechanisms of insecticide resistance status (at 0 to 5 trap sites depending on mechanism and year) were monitored throughout the growing seasons and data passed to the industry via Broom’s Barn to aid decisions on control.

5) On average, 0.26% of the *M. persicae*, 0.41% of the *M. euphorbiae* and 0% of the *A. solani* tested were carrying BMYV. This is similar to the previous four year period.

6) Most *M. persicae* were susceptible or only moderately resistant (S+R1) to insecticides through having elevated levels of carboxylesterases. However, this resistance mechanism is no longer of much consequence as it is mainly to organophosphates. 39.3% of aphids tested (963 out of 2451) showed the more serious `MACE' mechanism, which confers strong resistance to pirimicarb and triazamate (the latter is no longer available as an aphicide for beet). This has risen dramatically from 10.6% in the previous four year period and 0.26% in the four year period before that. 56.3% of *M. persicae* tested (454 out of 807) did not have the kdr resistance mechanism that confers resistance to pyrethroids. The rest were heterozygotes showing partial resistance. None of the 220*M. persicae* tested had the super-kdr resistance mechanism.

3 OBJECTIVES

The general objectives of the project were to optimise and sustain the use of insecticides against aphid pests on beet through monitoring resistance, evaluating potential alternative control strategies, and transferring relevant information to the industry.

Specifically, the objectives were:

1) To monitor nationwide migrant vectors of sugar beet viruses (April to November each year).

2) To provide forecasts of the timing and size of vector aphid migrations, and consequently the potential for virus yellows infection in the absence of control measures (March each year).

3) To assess the status of two insecticide resistance mechanisms (Esterase-based, MACE) in individual *Myzus persicae* from the trap samples and the beet virus content of *M. persicae*, *M. euphorbiae* and *A. solani* (April to November each year).

4) To assess the status of pyrethroid-specific knockdown resistance in samples of *M. persicae* from field sites in beet-growing areas (April to November each year).

5) To assess in field trials the efficacy of existing and novel insecticides against aphids with different resistance characteristics (April – September each year).

6) To disseminate timely and relevant information to growers to aid decisions on vector control (throughout the project, but particularly March to July each year).

During the course of the project, a third resistance mechanism (kdr/super-kdr) was added to the assessments made in (3). It was agreed that field trials (5) would not be done and that, instead, resources would be allocated to collection of *M. persicae* samples for the SA-Link project.

4 INTRODUCTION

Viruses of sugar beet continue to have the potential to cause major economic losses in the UK. The threat is increased by the growing frequency of mild winters which aid aphid survival and tend to lead to early and large migrations (Harrington *et al.*, 1995), and by insecticide resistance which makes vectors difficult to control. The most important vector aphid is the peach–potato aphid (*Myzus persicae*). At least three resistance mechanisms are known in this aphid (Foster *et al*., 2007) and there are few insecticides to which all clones are susceptible.

A UK-wide network of suction traps has been in operation since 1965 to monitor aphids and to help understand their population dynamics (Harrington and Woiwod, 2007). Strong correlations have been found between winter temperature and the timing and size of vector migrations in spring (Harrington *et al*., 1995). These relationships are used to forecast virus risk and to facilitate decision making with respect to vector control (**see Section 5**). The network provides the only unbiased, standardised sample of aphids potentially able to transmit beet viruses. The network is used throughout the beet growing season to monitor relevant aphids and interpret this information for use by beet growers (**see Section 6**). The presence in single aphids of *Beet mild yellowing virus* (**see Section 7**), by far the most important virus in UK beet, can be assessed, as can insecticide resistance levels (**see Section 8**) in *M. persicae* due to elevated levels of two carboxylesterase enzymes (E4 and FE4), to modification of the acetylcholinesterase target enzyme (`MACE') and to changes in the voltage-gated sodium channel protein (`knockdown resistance' or `kdr' and `super knockdown resistance' or `super kdr').

The system provides useful information to help understand the intra- and inter-seasonal dynamics of virus and insecticide resistance in *M. persicae* and is used to inform growers of appropriate control options.

5 FORECASTING APHID ACTIVITY AND VIRUS INCIDENCE

5.1 Methods

Aphid activity

Forecasts of the timing and size of the spring population of *M. persicae* and *M. euphorbiae* were based on simple linear regression relationships between winter weather and aphid data from each suction trap (except for Wellesbourne, Tadcaster and Elgin, where the data runs are insufficient for producing a forecast). The forecasts were updated each year, and the equations for the latest forecasts only (for the year 2009, utilising aphid and weather data from 1965 to 2008) are shown in **Table 1**.

Table 1 - Forecast equations for 2009

	First flight	Numbers trapped
Rothamsted		
Myzus persicae	**y = -15.01x + 192.6**	**y = 0.384x - 0.015**
Macrosiphum euphorbiae	**y = -7.48x + 163.5**	**y = 0.205x + 0.477**
Wye		
Myzus persicae	**y = -14.08x + 198.6**	**y = 0.394x - 0.255**
Macrosiphum euphorbiae	**y = -6.93x + 164.3**	**y = 0.151x + 0.619**
Broom's Barn		
Myzus persicae	**y = -14.73x + 197.3**	**y = 0.430x - 0.136**
Macrosiphum euphorbiae	**y = -12.25x + 190.2**	**y = 0.253x + 0.070**
Newcastle		
Myzus persicae	**y = -20.53x + 261.2**	**y = 0.394x - 1.135**
Macrosiphum euphorbiae	**y = -14.05x + 217.1**	**y = 0.212x - 0.069**
Dundee		
Myzus persicae	**y = -11.46x + 207.2**	**y = 0.270x - 0.448**
Macrosiphum euphorbiae	**y = -11.47x + 192.0**	**y = 0.274x - 0.139**
Silwood		
Myzus persicae	**y = -8.18x + 165.1**	**y = 0.235x + 0.370**
Macrosiphum euphorbiae	**y = -8.45x + 161.2**	**y = 0.125x + 0.952**
Starcross		
Myzus persicae	**y = -14.08x + 198.1**	**y = 0.277x - 0.187**
Macrosiphum euphorbiae	**y = -6.96x + 152.8**	**y = 0.176x + 0.631**
Hereford		
Myzus persicae	**y = -13.28x + 200.3**	**y = 0.392x - 0.469**
Macrosiphum euphorbiae	**y = -9.05x + 171.5**	**y = 0.206x + 0.450**

Table 1 - Forecast equations for 2009 (continued)

	First flight	Numbers trapped
Preston		
Myzus persicae	**y = -4.82x + 157.7**	**y = 0.346x - 0.426**
Macrosiphum euphorbiae	**y = -8.31x + 177.1**	**y = 0.225x + 0.220**
Ayr		
Myzus persicae	**y = -11.76x + 228.6**	**y = 0.165x - 0.453**
Macrosiphum euphorbiae	**y = -9.89x + 194.4**	**y = 0.162x + 0.080**
Writtle		
Myzus persicae	**y = -11.82x + 171.9**	**y = 0.340x + 0.490**
Macrosiphum euphorbiae	**y = -10.70x + 168.7**	**y = 0.188x + 0.810**
Kirton		
Myzus persicae	**y = -11.80x + 186.6**	**y = 0.378x - 0.364**
Macrosiphum euphorbiae	**y = -8.69x + 164.5**	**y = 0.162x + 0.753**
Gogarbank		
Myzus persicae	**y = -12.58x + 211.9**	**y = 0.266x - 0.396**
Macrosiphum euphorbiae	**y = -9.15x + 176.3**	**y = 0.240x + 0.330**

y = julian date of first record or log_{10} (n+1) (where n = number trapped up to 1st July); x = mean temperature in January and February (^oC).

Virus incidence

Forecasts of virus incidence at the end of August were issued by Broom's Barn on the basis of work published by Qi *et al*. (2001). These forecasts took into account crop sowing date, changes in pest management practice, the usage of insecticide-treated seed and forecasts of aphid incidence.

5.2 Results and discussion

Aphid activity

The expected and observed dates of the first trap record and numbers of *M. persicae* and *M. euphorbiae* are shown in **Table 2**. The range of first record dates across all years of trap operation (not shown) spans around three months for most sites and, bearing this in mind, the forecasts were generally accurate, especially in the main sugar beet growing areas of eastern England. On average, the forecasts were out by thirteen days, giving a good indication of whether the migration would be early, average or late.

Because of their potential for exponential rates of change, aphid population levels are best considered in terms of their logarithm. As a result, when showing actual numbers, as in **Table 2**, errors can appear very large. In 2005, many more *M. persicae* were trapped than expected. This was due to a surge of activity right at the end of the forecasting period (late June). In 2008 rather fewer aphids were caught than expected, as a result of poor flying conditions.

Table 2 - Expected and observed aphid activity

Trap and year	*M. persicae*				*M. euphorbiae*			
	First record		No. to 1 July		First record		No. to 1 July	
	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs
2005:								
Rothamsted	121	120	58	506	129	145	29	27
Wye	128	120	62	335	129	134	29	18
Broom's Barn	128	101	76	993	133	121	19	63
Newcastle	164	158	4	20	152	158	7	25
Dundee	152	168	6	8	138	131	13	27
Silwood	122	135	45	39	115	135	53	12
East Craigs	145	151	9	6	129	132	39	46
Starcross	124	130	16	33	116	130	36	16
Hereford	129	134	37	180	123	120	37	60
Preston	134	122	18	39	135	141	21	26
Ayr	168	192	1	0	146	134	7	10
Writtle	111	119	186	1158	110	120	69	92
Kirton	127	108	28	192	121	119	42	82
Average error	12 days		44*		9 days		14*	
2006:								
Rothamsted	137	162	25	23	137	132	17	11
Wye	136	163	35	12	133	129	22	2
Broom's Barn	147	145	21	21	148	132	8	9
Newcastle	175	180	2	1	159	150	6	11
Dundee	162	183	3	0	147	132	8	5
Silwood	133	132	20	4	128	126	29	14
East Craigs	155	194	5	0	136	130	25	12
Starcross	142	149	7	15	125	131	20	44
Hereford	151	134	9	18	138	129	16	19
Preston	135	180	14	1	138	156	18	12
Writtle	124	124	78	29	123	155	40	8
Kirton	136	170	16	17	128	126	30	9
Average error	19 days		6*		10 days		9*	

Table 2 - Expected and observed aphid activity (continued)

Trap and year	*M. persicae*				*M. euphorbiae*			
	First record		No. to 1 July		First record		No. to 1 July	
	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs
2007:								
Rothamsted	94	105	300	443	117	102	74	18
Broom's Barn	106	97	354	537	114	103	49	20
Newcastle	148	131	9	51	141	111	11	42
Dundee	143	129	10	22	127	109	24	45
Silwood	111	95	111	102	102	100	90	18
Starcross	95	97	64	101	102	102	83	107
Hereford	113	111	122	208	111	123	71	20
Preston	131	110	40	123	128	102	40	10
Ayr	156	154	2	20	136	110	12	11
Writtle	93	32	758	735	92	105	150	43
Kirton	112	108	96	191	110	102	77	16
Average error	14 days		45*		15 days		32*	
2008:								
Rothamsted	102	140	218	30	119	129	53	9
Broom's Barn	115	115	198	80	121	129	32	11
Newcastle	152	182	8	1	143	158	11	2
Dundee	153	155	6	9	137	144	14	22
Silwood	115	129	69	9	109	128	55	12
Starcross	114	121	28	21	111	113	48	25
Hereford	124	128	61	37	120	116	43	26
Preston	129	129	45	33	125	148	36	33
Ayr	157	191	3	0	135	153	11	2
Writtle	92	130	534	99	98	106	109	31
Kirton	115	125	83	77	111	123	58	18
Average error	16 days		23*		11 days		19*	

Exp = expected (predicted)
Obs = observed
First record is given as Julian date (days from 1st January). Observed data for 2008 are not yet available.
Average error in prediction of first record is given as the number of days, regardless of whether the prediction was early or late.
**Average error for numbers caught to 1st July is given by:*
(antilog((Σlog(abs(Exp-Obs)+1))/n))-1 (where abs = absolute value and n = number of sites)

Virus incidence

Table 3 shows expected and observed incidence of virus yellows according to the methods of Qi *et al.* (2001). The observed incidences are area averages from the British Sugar specific-field survey and do not distinguish between treated and untreated crops, or sowing dates. The percentage of the crop using insecticide-treated seed is shown.

In 2005 virus incidence was lower than expected in the Eastern region but very close to prediction in the Northern and Western regions. Virus incidence was extremely low in 2006 as predicted. In 2007, virus levels were slightly higher than predicted in the Bury factory area and slightly lower than predicted in the Cantley, Wissington and Newark factory areas. In 2008, virus levels were very low in all areas, and lower than predicted, mirroring the lower than predicted numbers of aphids.

Table 3 - Expected and observed virus incidence (%)

	Expected						Observed
	Without pest Management			With pest Management			(% crop with treated seed)
Sowing date	15/3	30/3	15/4	15/3	30/3	15/4	
2005 East	42.4	53.9	69.1	3.1	3.6	4.3	0.8% (80)
2005 West	12.3	15.4	20.9	2.3	2.6	3.1	3.0% (71)
2005 North	55.3	68.5	82.8	4.5	5.4	6.9	5.1% (63)
2006 East	4.2	5.7	8.6	0.4	0.5	0.6	0.6% (81)
2006 West	6.0	7.3	9.5	1.4	1.6	1.9	0.1% (70)
2006 North	10.0	14.6	23.8	0.8	1.0	1.2	0.2% (64)
2007 Bury	41.2	52.4	67.7	2.3	2.6	3.1	4.6% (90)
2007 Cantley	41.2	52.4	67.7	2.5	2.9	3.4	0.6% (87)
2007 Wissington	41.2	52.4	67.7	3.2	3.6	4.4	1.7% (79)
2007 Newark	61.5	74.0	86.6	3.3	3.8	4.7	2.7% (81)
2008 Bury	37.9	48.8	64.2	2.0	2.3	2.7	0.6%(91)
2008 Cantley	37.9	48.8	64.2	2.1	2.4	2.8	0.6%(90)
2008 Wissington	37.9	48.8	64.2	2.7	3.1	3.6	0.4%(82)
2008 Newark	58.4	71.4	85.1	2.6	3.1	3.7	0.2%(84)

6 AERIAL MONITORING OF KEY APHID VECTORS OF BEET YELLOWS VIRUSES

6.1 Methods

Sixteen suction traps (**Fig. 1**) were operated in all years. Traps were emptied daily and samples from England sent twice a week to Rothamsted where the aphids were separated from the other insects and identified to species by a skilled team of taxonomists. Samples from Scotland were processed by colleagues at the SASA, Gogarbank, and results sent weekly to Rothamsted for inclusion in bulletins. Samples from the traps at Rothamsted, Broom's Barn, Writtle, Kirton and Hereford were collected in a glycerol-based medium (Tatchell *et al.*, 1988) in 2005; from traps at Broom's Barn, Kirton and Writtle in 2006 and from the trap at Rothamsted in 2007 and 2008. This medium allows the detection of insecticide resistance status of individual aphids by immunoassay. From 2006 molecular methods were phased in for diagnosing MACE and kdr resistance and this can be done from alcohol-collected samples. The glycerol-based medium was retained at Rothamsted in order to continue the run of data on carboxylesterase-based resistance. However, in 2006, Rothamsted aphids were used to trial the new molecular technique, so no data are available on esterase-based resistance from Rothamsted in that year. **Fig. 1** also summarises the tests done at each site in each year. Data from all traps were made available routinely to the beet industry through the provision of a weekly confidential bulletin.

6.2 Results and discussion

Weekly totals of the four main species of relevance to beet are shown in **Fig. 2** (bars) together with the average numbers caught in the same weeks since trapping began (lines), for Broom's Barn, Writtle, Kirton, Rothamsted and Hereford. For all species, the average line often shows two distinct flight periods. The first represents those aphids coming from winter hosts into beet in spring and early summer, and merges with summer populations leaving a range of hosts as the aphids become crowded and/or the host plants mature and become nutritionally unsuitable. The autumn peak represents those aphids moving to winter host plants from a range of summer host plants, but rarely from beet.

In 2005 numbers of *M. persicae* were above average at most sites until late June and slightly below average thereafter. Numbers of *A. fabae* were generally below average. Numbers of *M. euphorbiae* were generally close to average until the end of July and then below average. There were very low numbers of *A. solani* as usual, but there were a few more than average at most sites.

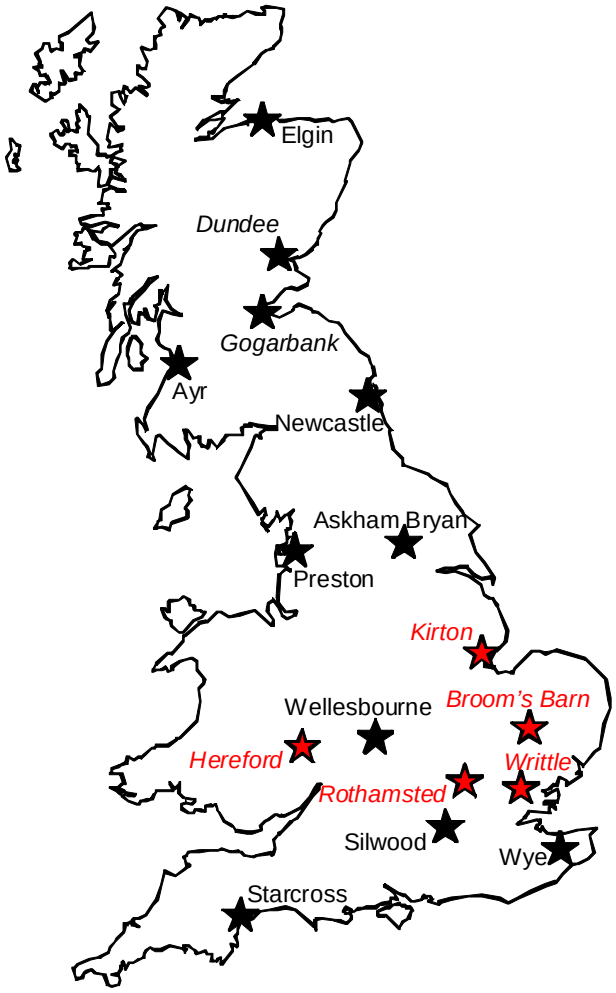
In 2006 the migration of *M. persicae* started later than average but reached average numbers from June onwards. Numbers of *A. fabae* were about average until late July and below average thereafter. Numbers of *M. euphorbiae* were generally below average except for a peak at Broom's Barn and Hereford in mid July. There were very few *A. solani*.

In 2007 numbers of *M. persicae* were exceptionally high until late May, after which they plummeted as a result of heavy rains. Numbers were high again in autumn. Numbers of *A. fabae* were also high in spring, and especially in autumn,

again with a noticeable lull in summer, a pattern repeated, although less dramatically, for *M. euphorbiae*. Numbers *A. solani* were higher than usual, especially at Writtle.

In 2008, numbers of *M. persicae* were lower than average at most sites until late May and then recovered, except at Rothamsted. Numbers were a little lower than average in autumn. Numbers of *A. fabae* were slightly higher than average until late July and much lower than average thereafter. Numbers of *M. euphorbiae* were about average until late May but below average thereafter except at Hereford, where they continued to be about average until mid July. Numbers of *A. solani* were generally about average.

Figure 1 The distribution of the Rothamsted Insect Survey 12.2 m high suction traps. Those traps used in this programme are labelled in italics. The tables indicate the suction trap sites and years for which different resistance mechanisms and viruses were monitored.



	Esterase				
2005	RT	BB	K	Wr	H
2006		BB	K	Wr	
2007	RT				
2008	RT				

	MACE				
2005	RT	BB	K	Wr	H
2006	RT	BB	K	Wr	(H)
2007	RT	BB	K	Wr	H
2008	RT	BB	K	Wr	H

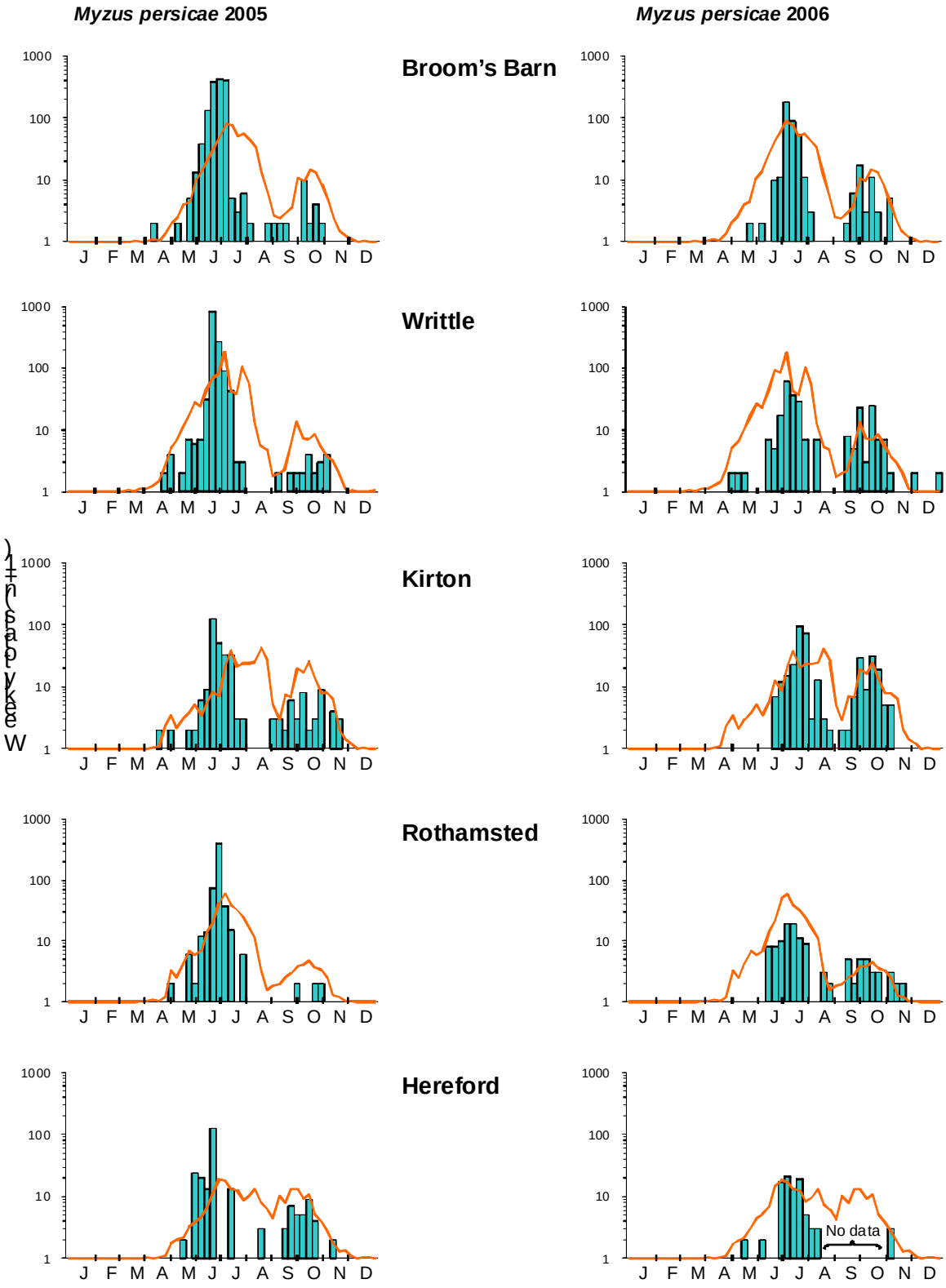
	Knockdown resistance				
2005					
2006	RT				(H)
2007		BB	K	Wr	H
2008		BB	K	Wr	H

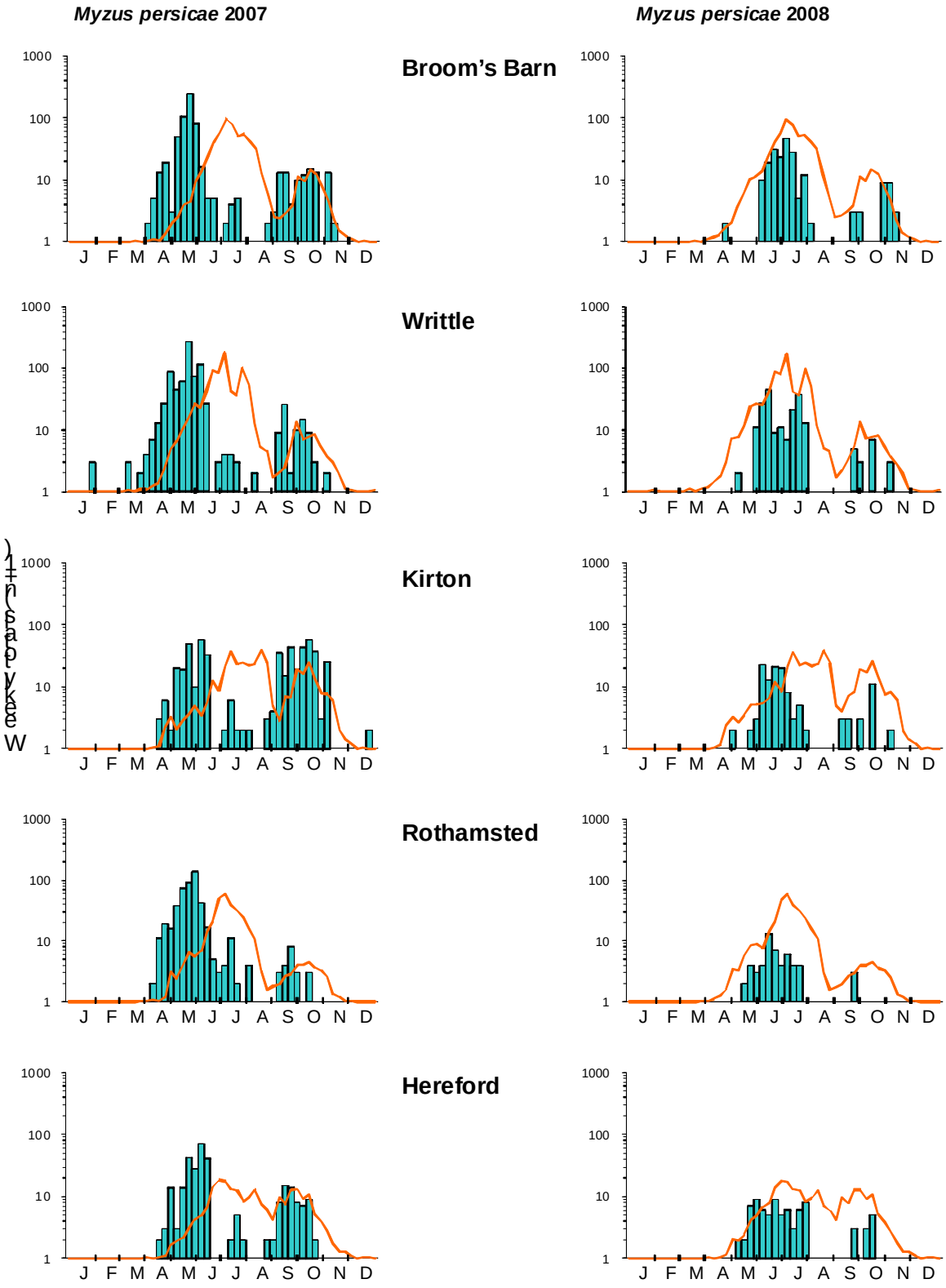
	Superknock down resistance				
2005					
2006	RT				(H)
2007		Very few aphids tested, no skdr			
2008			K		

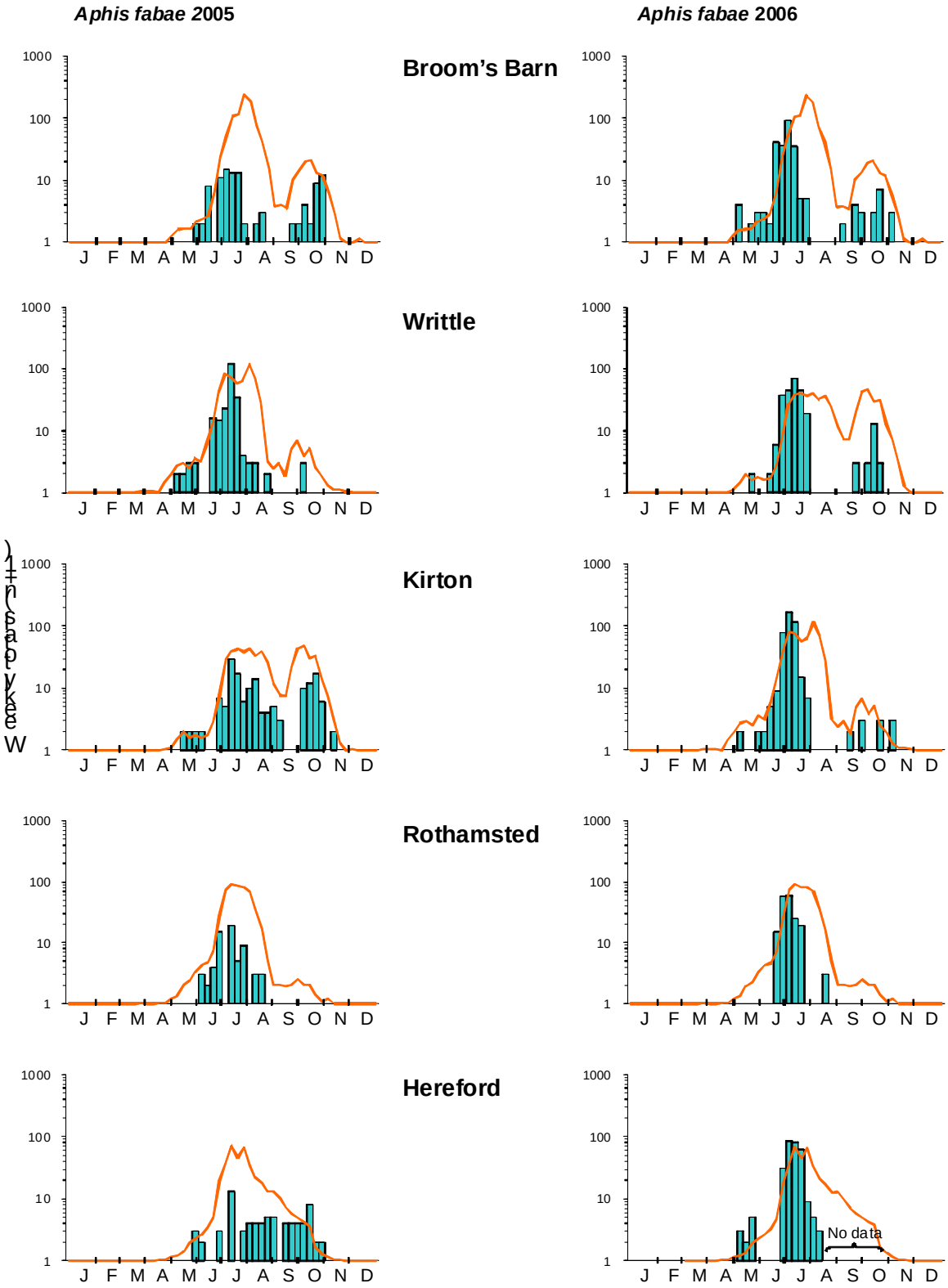
	TuYV and BMYV				
2005	RT	BB	K	Wr	H
2006		BB	K	Wr	
2007	RT	BB	K	Wr	H
2008	RT	BB	K	Wr	H

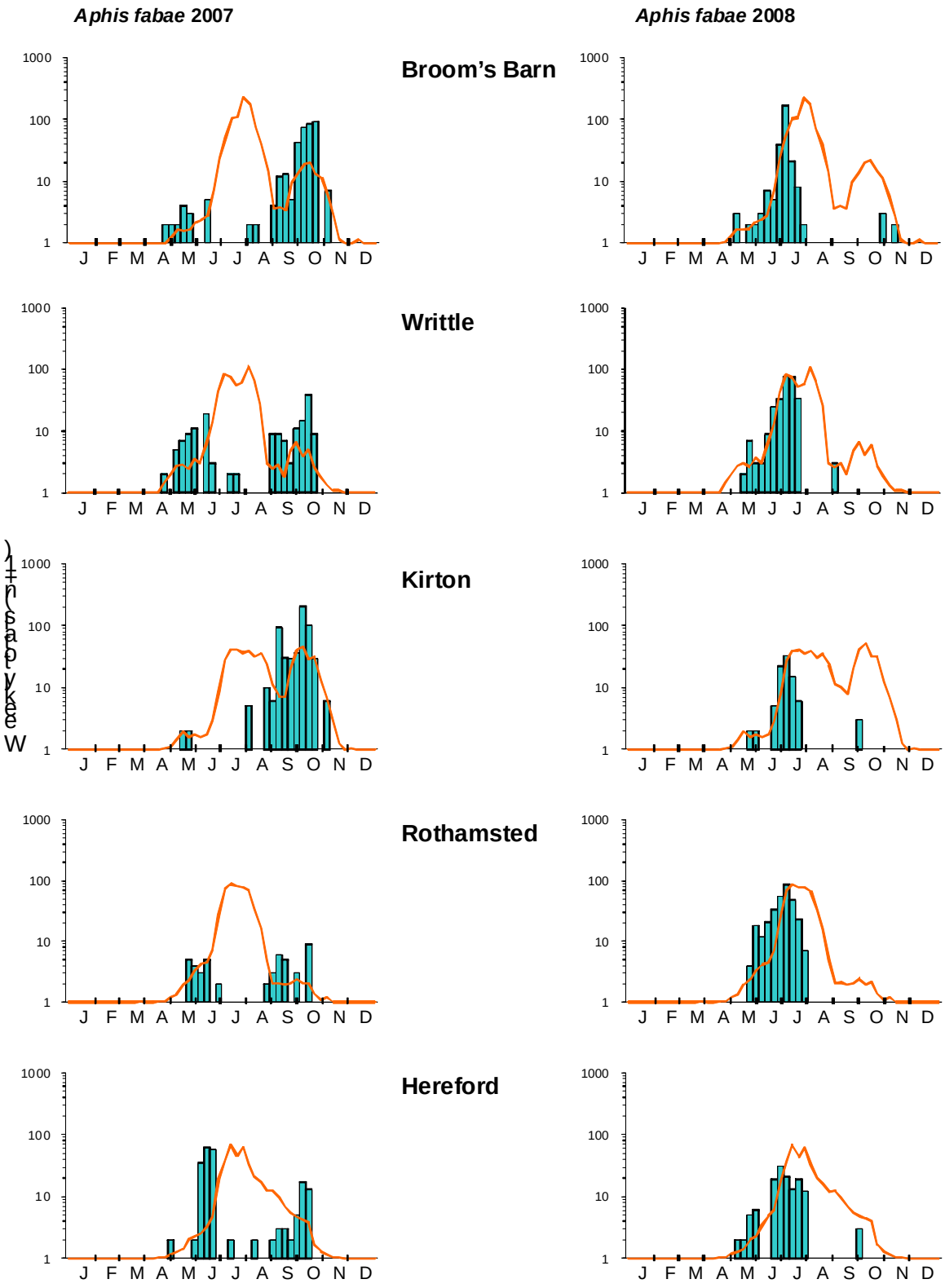
Hereford: no data August – October 2006

Figure 2 **The numbers of aphid pests of sugar beet recorded in suction trap samples at Broom's Barn, Writtle, Kirton, Rothamsted and, Hereford in 2005, 2006, 2007 and 2008 (bars) and the average numbers trapped during the same periods since trapping began (lines).**




Myzus persicae 2007
Myzus persicae 2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
1000
100
10
1
J F M A M J J A S O N D


Aphis fabae 2005
Aphis fabae 2006
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
No data
1000
100
10
1
J F M A M J J A S O N D


Aphis fabae 2007
Aphis fabae 2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
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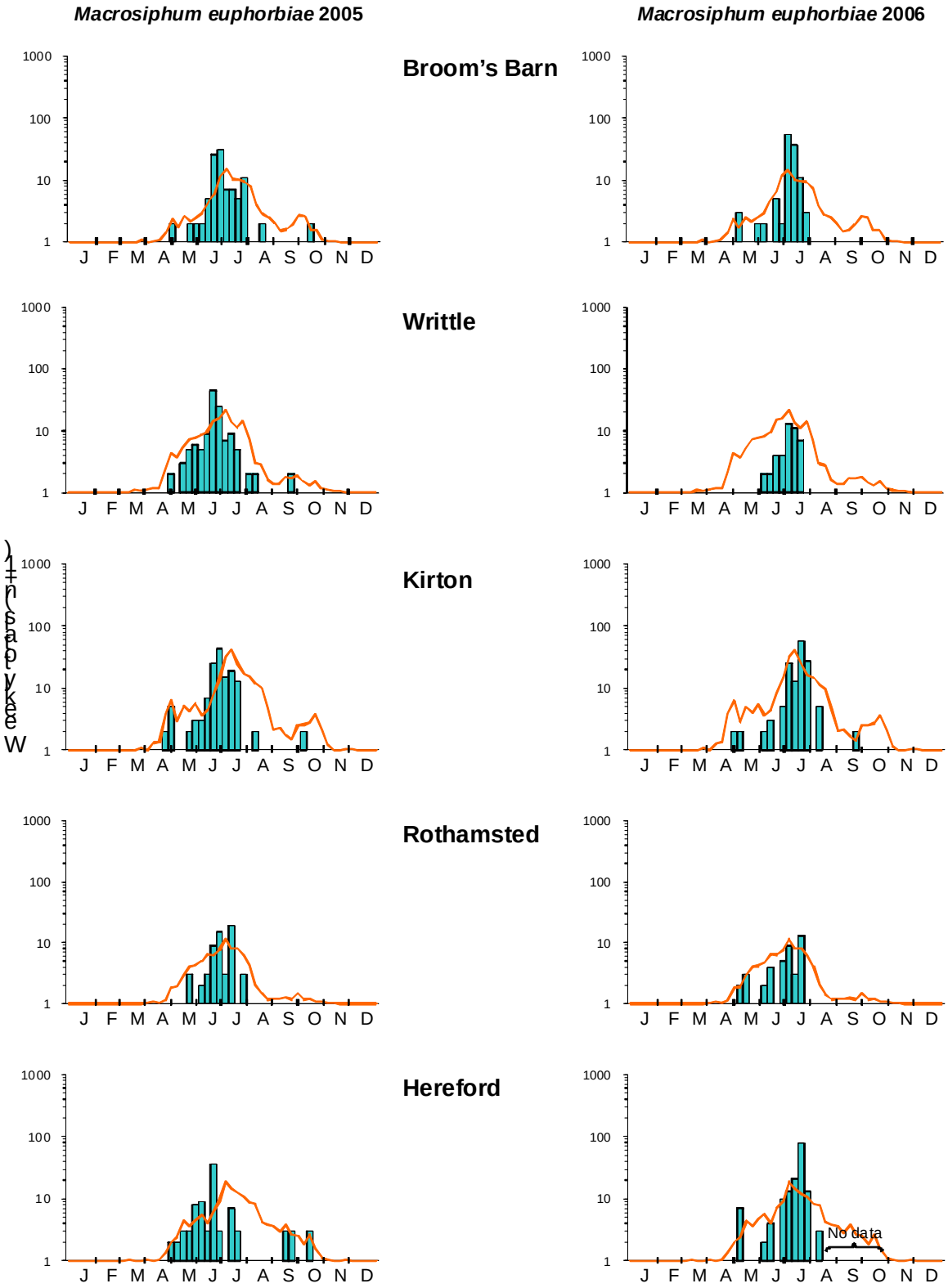
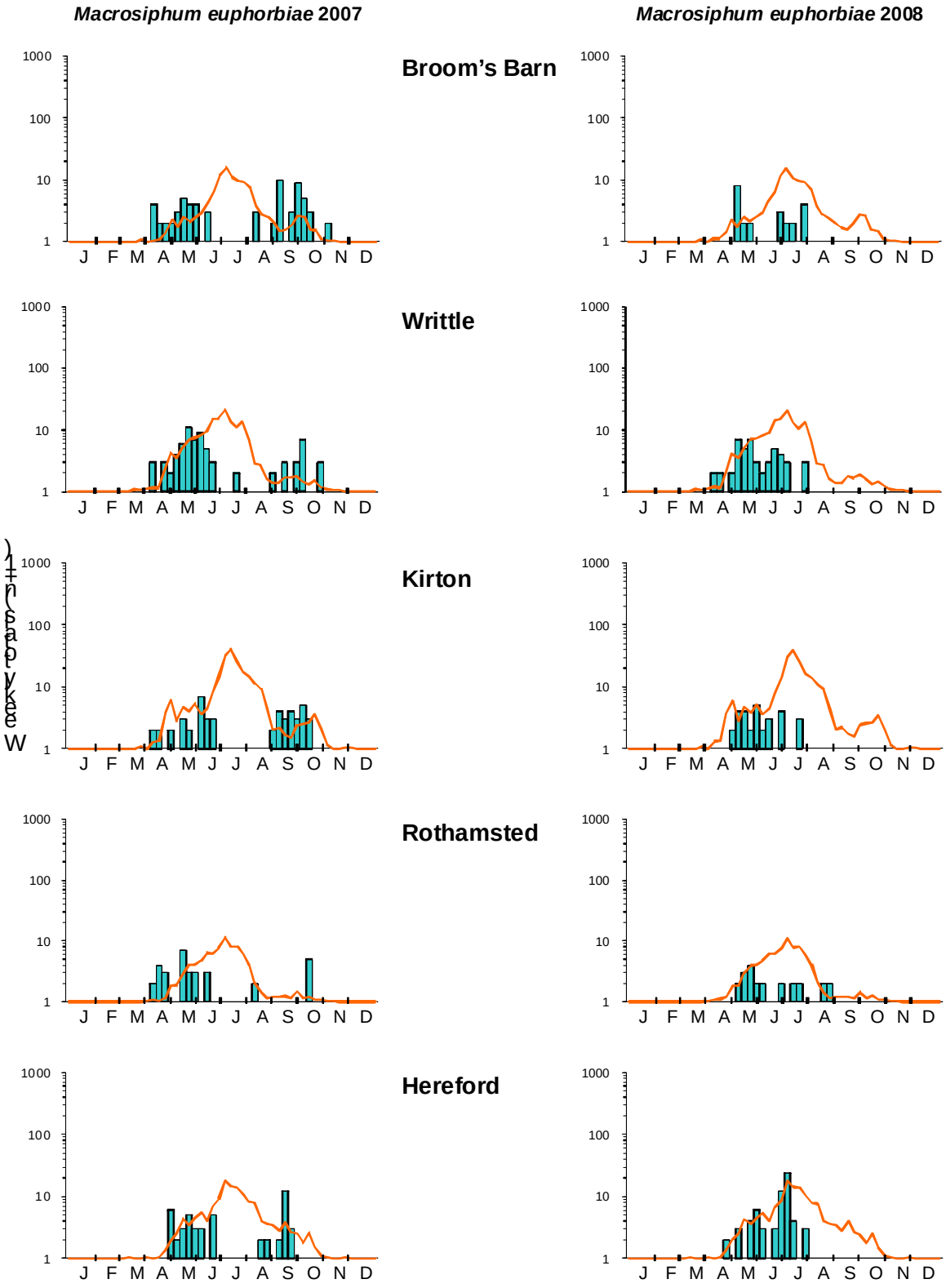
J F M A M J J A S O N D


Macrosiphum euphorbiae 2005
Macrosiphum euphorbiae 2006
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
No data
1000
100
10
1
J F M A M J J A S O N D


Macrosiphum euphorbiae 2007
Macrosiphum euphorbiae 2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
1000
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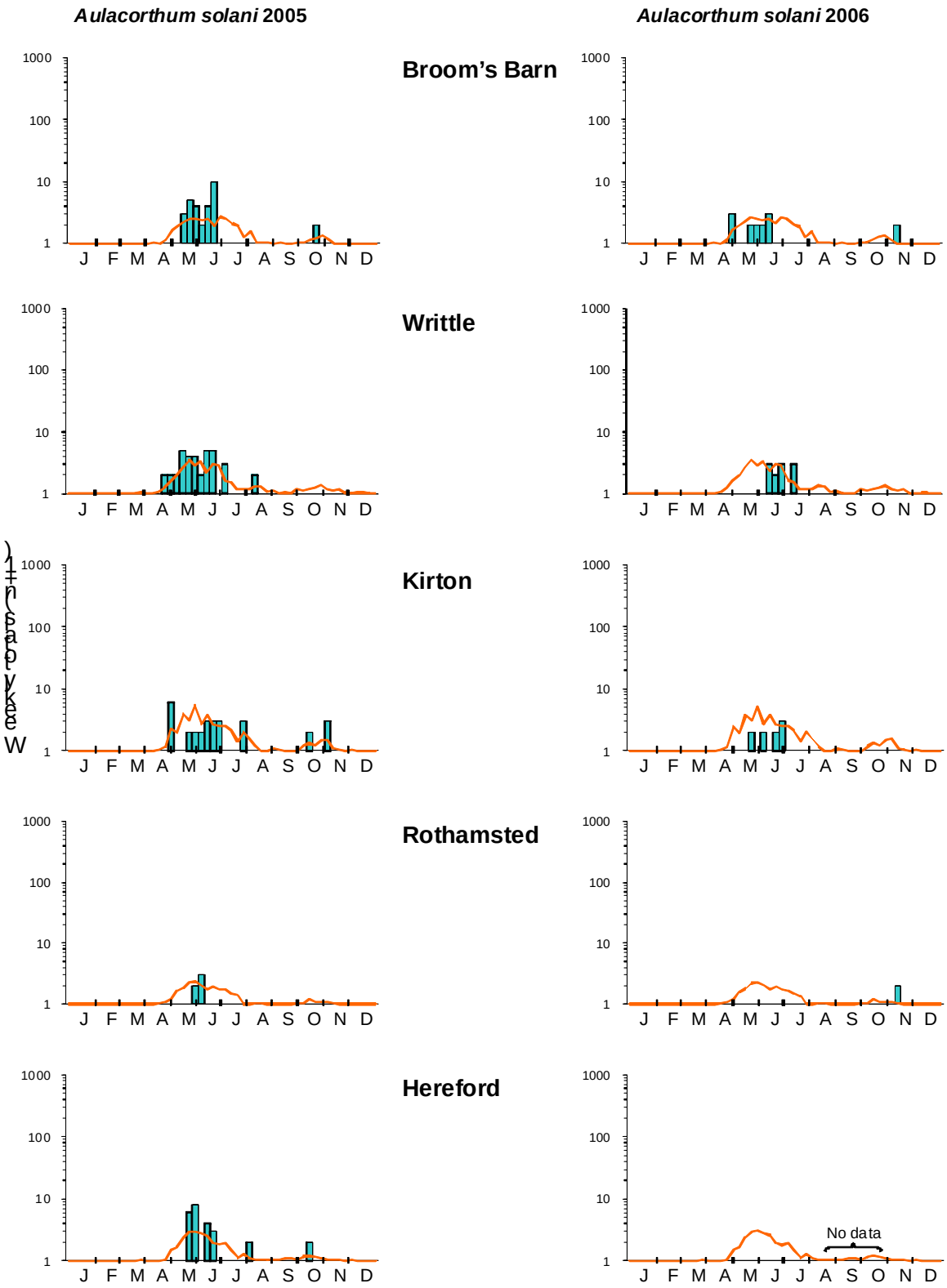
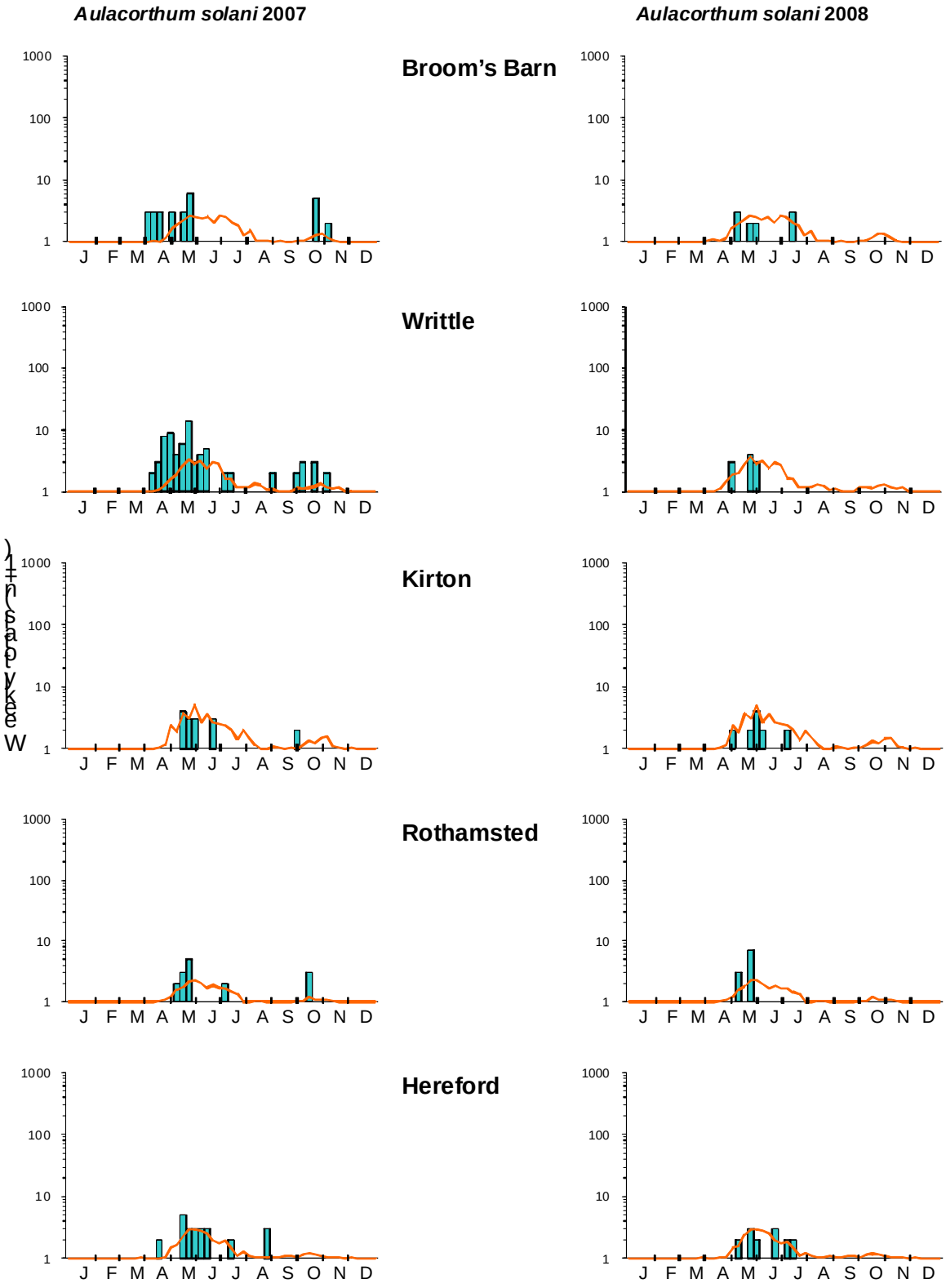
1
J F M A M J J A S O N D


Aulacorthum solani 2005
Aulacorthum solani 2006
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
No data
1000
100
10
1
J F M A M J J A S O N D


Aulacorthum solani 2007
Aulacorthum solani 2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
1000
100
10
1
J F M A M J J A S O N D

7. VIRUS IN AERIAL POPULATIONS OF VECTORS

7.1 Methods

Virus content of vector aphids was tested from the following traps and years:
2005 Rothamsted, Broom's Barn, Kirton, Writtle, Hereford (all from glycerol);
2006 Broom's Barn, Kirton, Writtle (all from glycerol) (Rothamsted and Hereford were not tested in 2006 because it was thought at that time that alcohol denatured the virus. This was subsequently found to be untrue.);
2007, 2008 Rothamsted (glycerol); Broom's Barn, Kirton, Writtle, Hereford (alcohol);

Vector aphid species from samples in the Rothamsted trap were identified daily. Unprocessed samples from Broom's Barn, Writtle, Kirton and Hereford were transferred to fresh storage medium (glycerol or alcohol according to year and trap) and kept at 4^{o}C before being sent twice a week to Rothamsted for separation and identification of aphids. All winged *M. persicae*, *M. euphorbiae* and *A. solani* from the samples were blotted on tissue, placed individually in wells of microtitre plates in 50ul PBS-Tween and then stored at -20^{o}C prior to assay for insecticide resistance status (*M. persicae* only, see next section) and/or virus content. A maximum of 100 *M. persicae* from any one site was tested for virus content in any one week.

Aphids were ground in 50µl PBS Tween and the exudate made up to 210µl in the microtitre plates and 200µl of the aphid extract was used for the assessment of viruses. This fraction was divided into two equal parts to determine the numbers containing *Beet mild yellowing virus* (BMYV) and *Turnip yellows virus* (TuYV) (formerly known as *Beet western yellows virus*). In Britain only a small proportion of TuYV isolates infect sugar beet (Smith *et al*., 1991), but serologically these two viruses are very similar and it is necessary to identify both before the proportion of aphids carrying the more important BMYV can be determined. The method involved the use of monoclonal antibodies in an amplified enzyme linked immunosorbent assay (ELISA) (Smith *et al*., 1991). The semi-persistent virus, *Beet yellows closterovirus* (BYV) cannot be detected in single aphids from trap samples.

7.2 Results and discussion

In total, 3678 aphids (2705 *M. persicae*, 738 *M. euphorbiae* and 235 *A. solani*) were tested for BMYV over the four year period 2005-2008. Only 8 *M. persicae* (0.26% of the total) and 3 *M. euphorbiae* (0.41%) were carrying BMYV. No *A.solani* tested positive for BMYV.

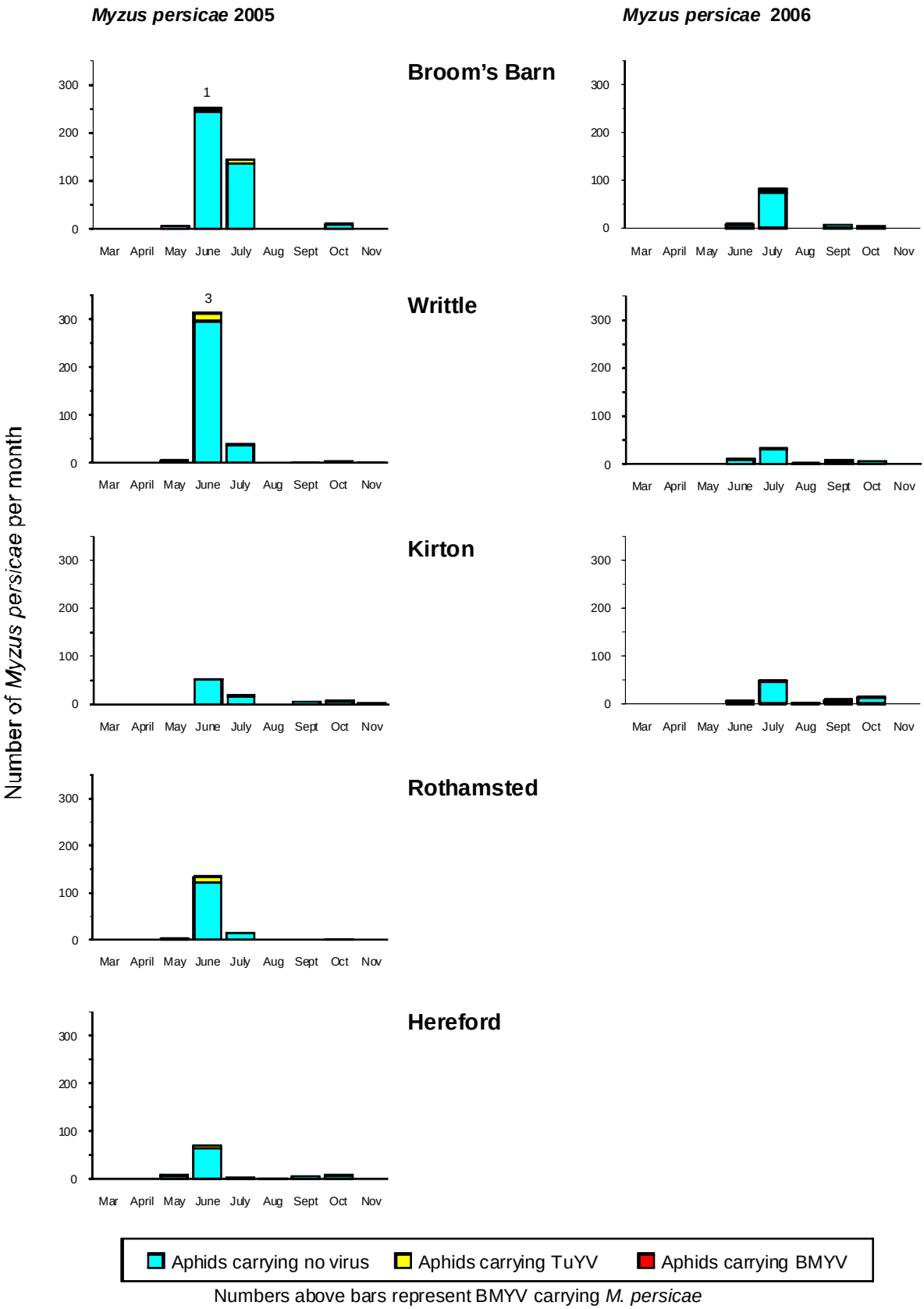
Monthly totals of aphids tested at each of the six trap sites are shown in **Fig. 3.** In 2005, 4 *M. persicae* of 1105 tested and 3 *M. euphorbiae* of 288 tested were carrying BMYV. In 2006 the figures were 1 out of 234 and 0 out of 151, respectively. In 2007 figures were 2 out of 1161 and 0 out of 174, respectively. In 2008figures were 0 out of 205 and 0 out of 125, respectively.

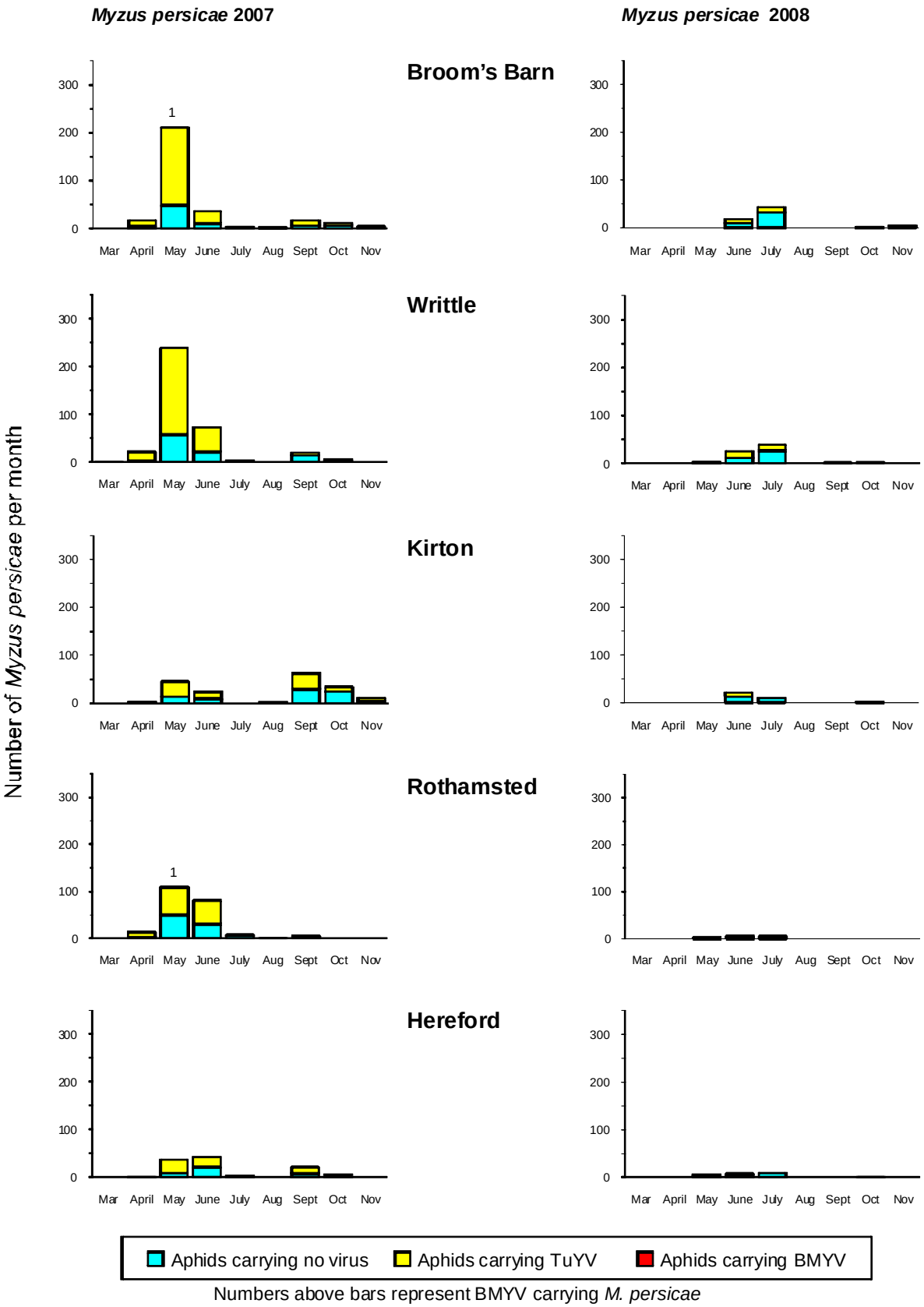
Of the 10 records of aphids carrying BMYV, 1 was at Rothamsted out of 462 aphids tested (0.23%), 3 at Broom's Barn out of 1101 tested (0.27%), and 6 at Writtle out of 1110 tested (0.54%).

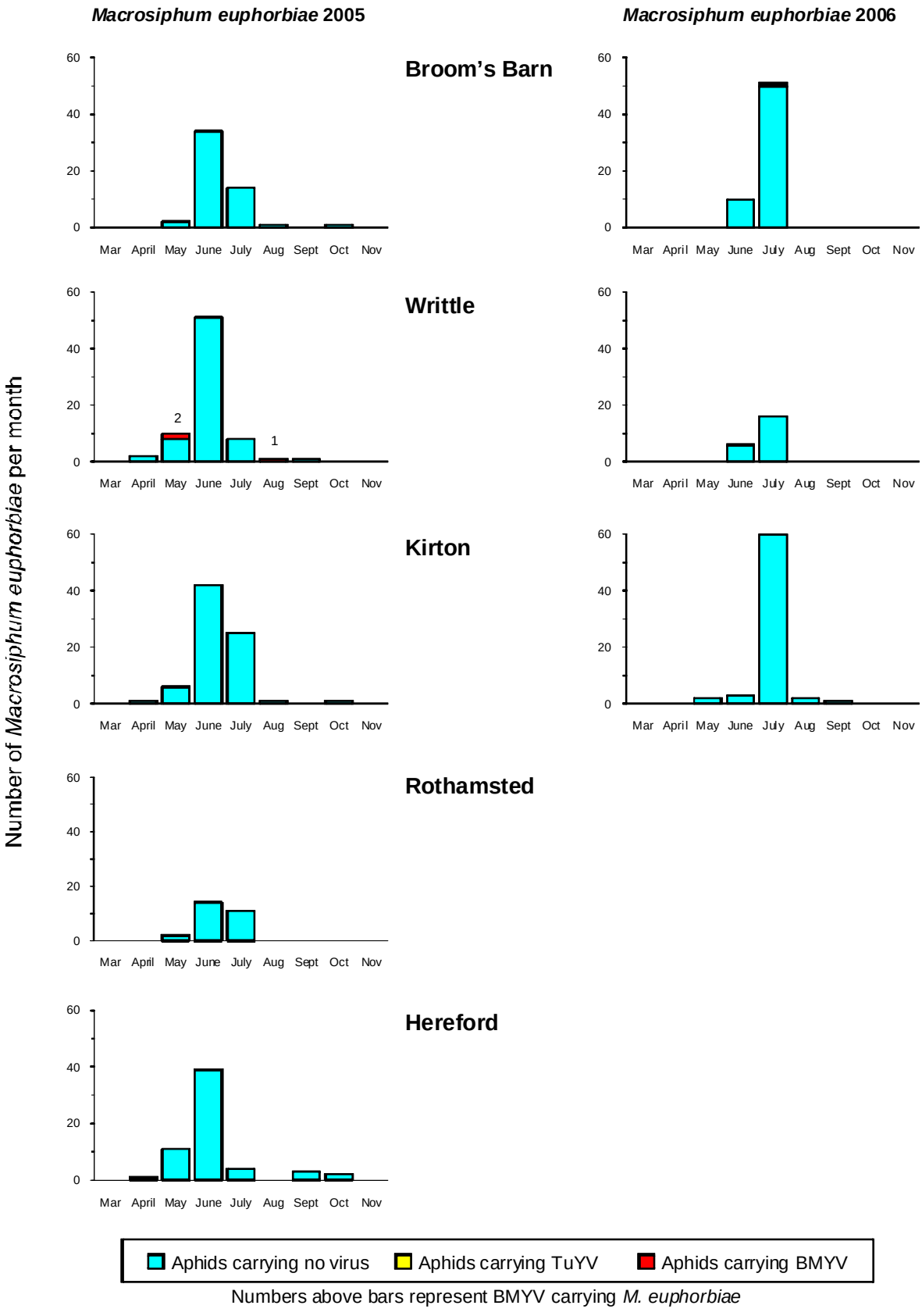
The percentage of aphids carrying BMYV may appear low. However, when seen in terms of the number that will have infested the crop, it ensures a high potential number of primary virus foci. It should be remembered that single aphids from the trap samples cannot be tested using ELISA for BYV, the other contributor to sugar beet yellows disease. However, this virus is not usually significant compared to BMYV. It is also impossible to distinguish BMYV and BChV by ELISA. However, a new RT-PCR method for detecting both BMYV and BChV has recently been developed at Broom's Barn (Vigano & Stevens, 2007).

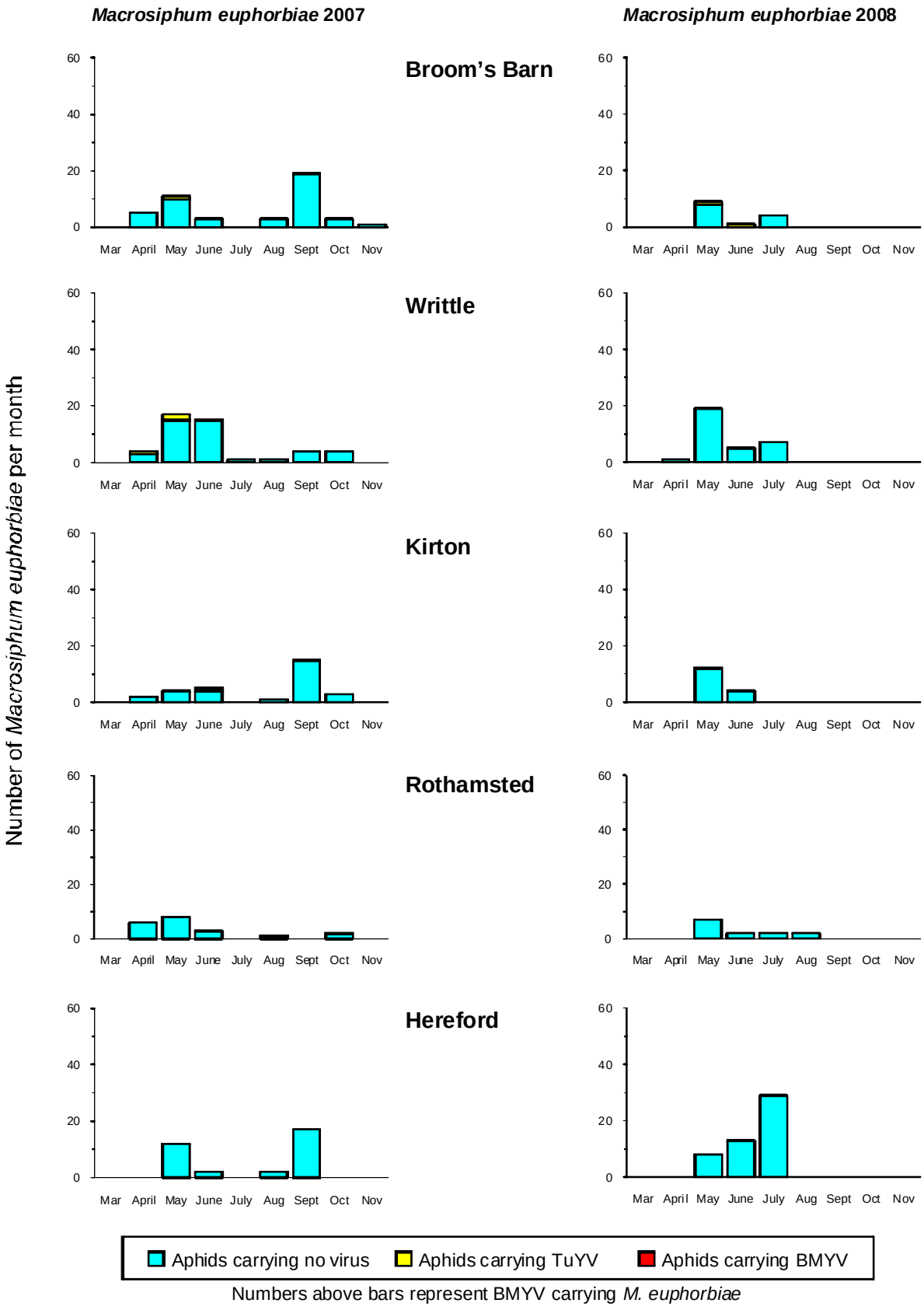
The percentage of aphids carrying BMYV was also low in the previous four year period (2001 to 2004) (**Fig. 4**) but between 1997 and 2000 incidence was much higher at Broom's Barn and Writtle. The reason for the decline is not clear, but it may help to explain why actual virus incidence has tended to be lower than forecast.

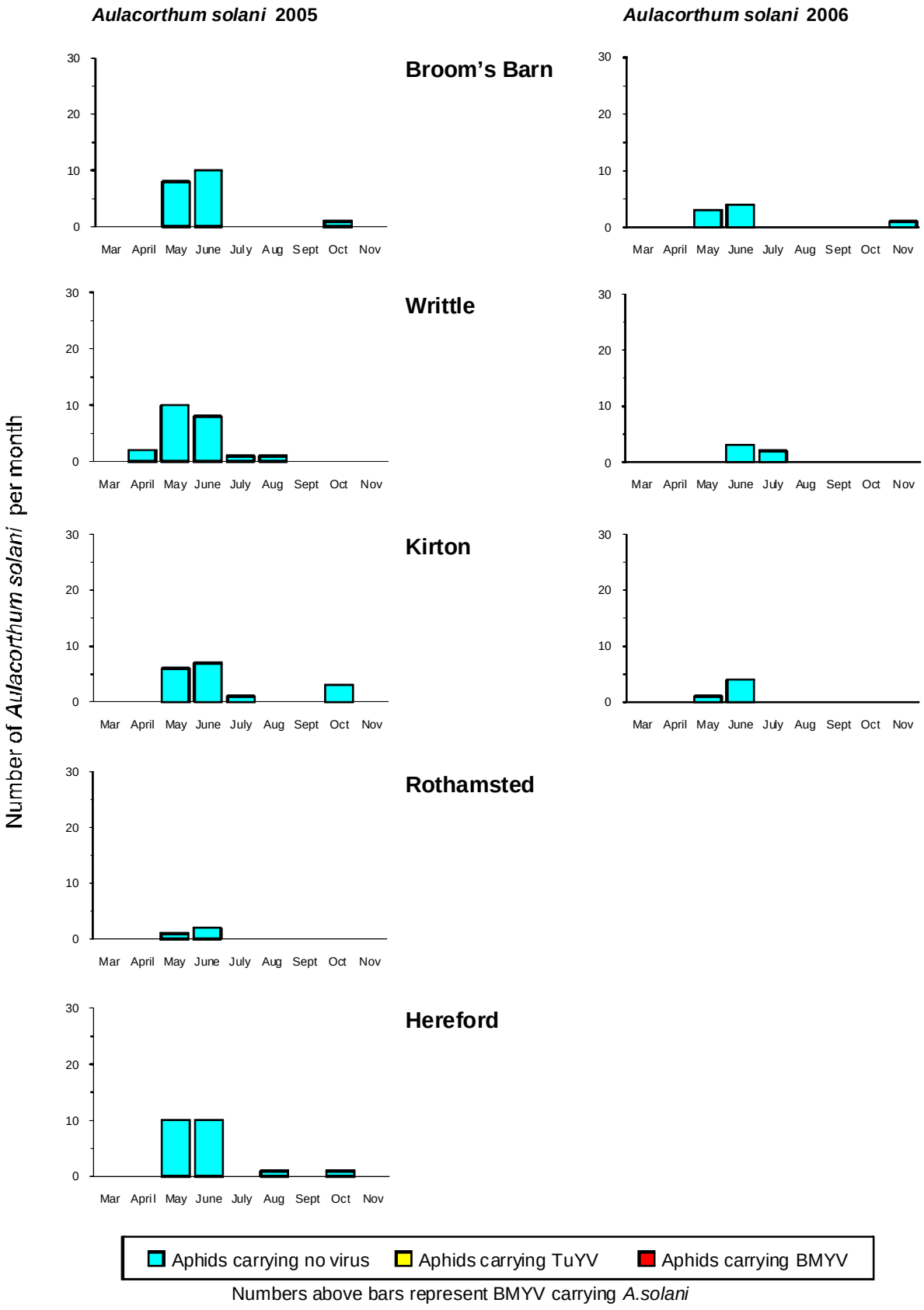
Figure 3 The sugar beet virus content of *Myzus persicae*, *Macrosiphum euphorbiae* and *Aulacorthum solani* in suction trap samples from Broom's Barn, Writtle, Kirton, Rothamsted and Hereford in 2005, 2006, 2007 and 2008.




Myzus persicae 2007
Myzus persicae 2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
Number of *Myzus persicae* per month
0
100
200
300
Mar
April
May
June
July
Aug
Sept
Oct
Nov
1
Aphids carrying no virus
Aphids carrying TuYV
Aphids carrying BMYV
Numbers above bars represent BMYV carrying *M. persicae*


Macrosiphum euphorbiae 2005
Macrosiphum euphorbiae 2006
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
Number of Macrosiphum euphorbiae per month
0
20
40
60
Mar
April
May
June
July
Aug
Sept
Oct
Nov
2
1
Aphids carrying no virus
Aphids carrying TuYV
Aphids carrying BMYV
Numbers above bars represent BMYV carrying M. euphorbiae


Macrosiphum euphorbiae 2007
Macrosiphum euphorbiae 2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
Number of Macrosiphum euphorbiae per month
0
20
40
60
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Aphids carrying no virus
Aphids carrying TuYV
Aphids carrying BMYV
Numbers above bars represent BMYV carrying M. euphorbiae


Aulacorthum solani 2005
Aulacorthum solani 2006
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
Number of Aulacorthum solani per month
0
10
20
30
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Aphids carrying no virus
Aphids carrying TuYV
Aphids carrying BMYV
Numbers above bars represent BMYV carrying A.solani

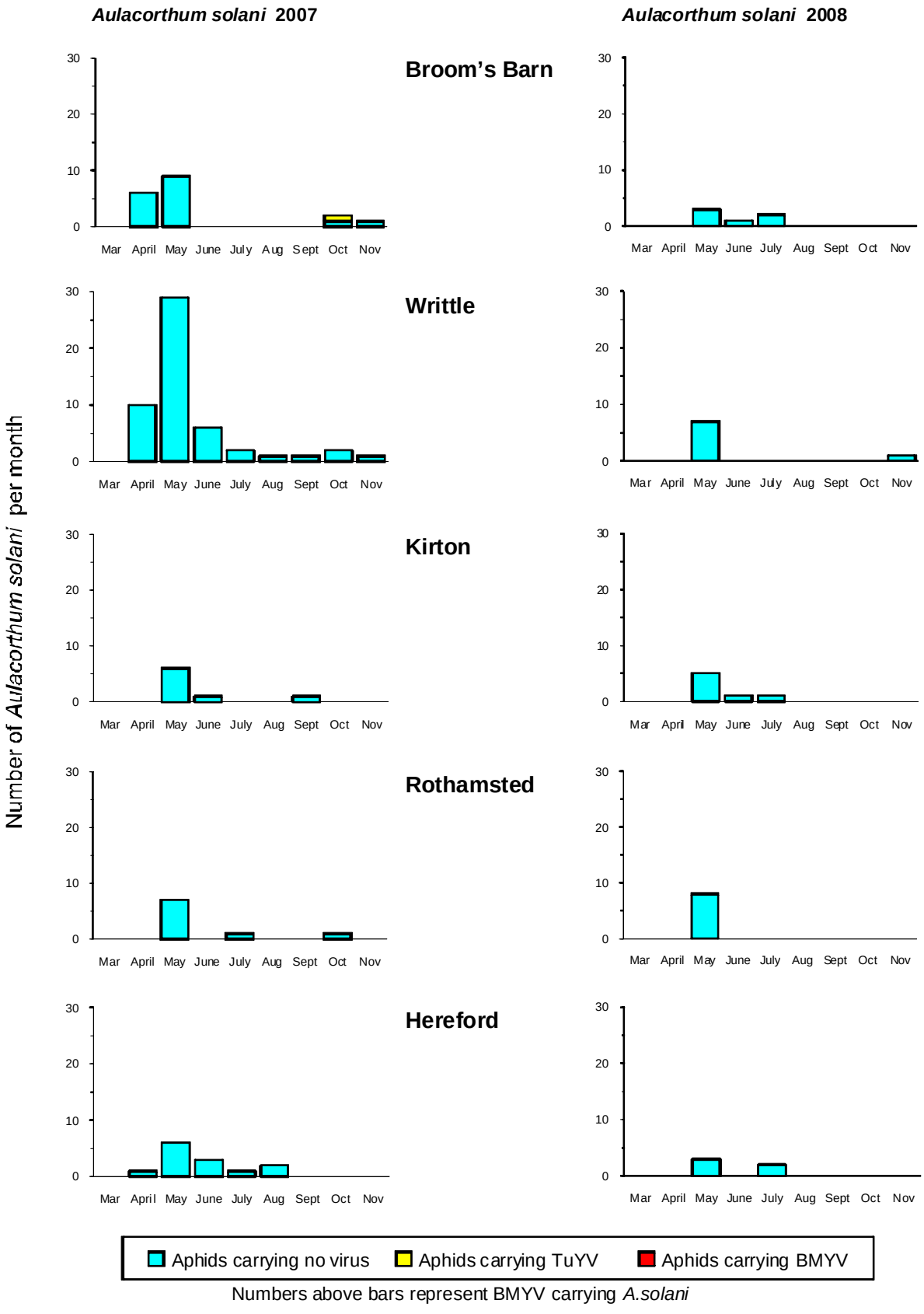
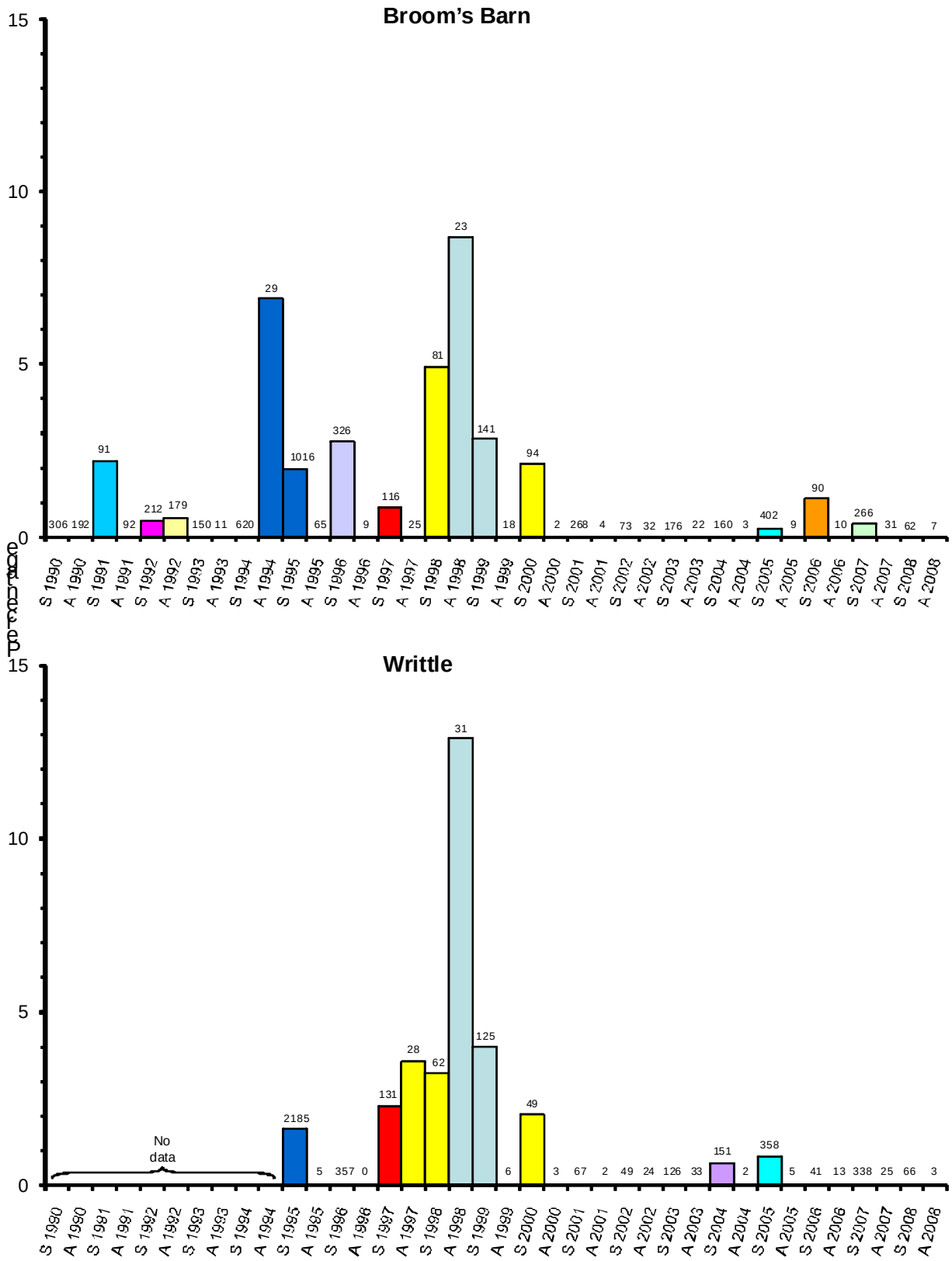
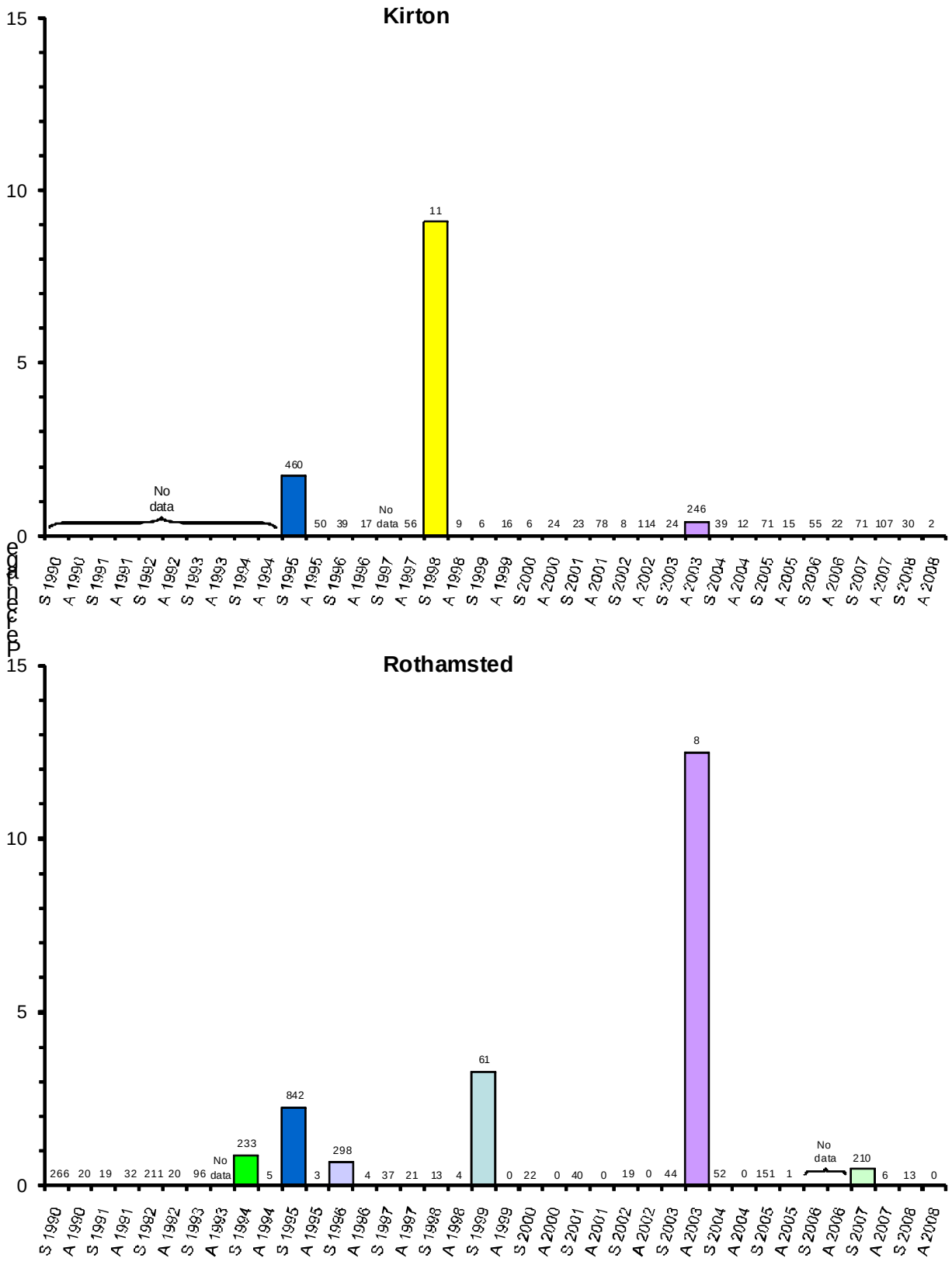
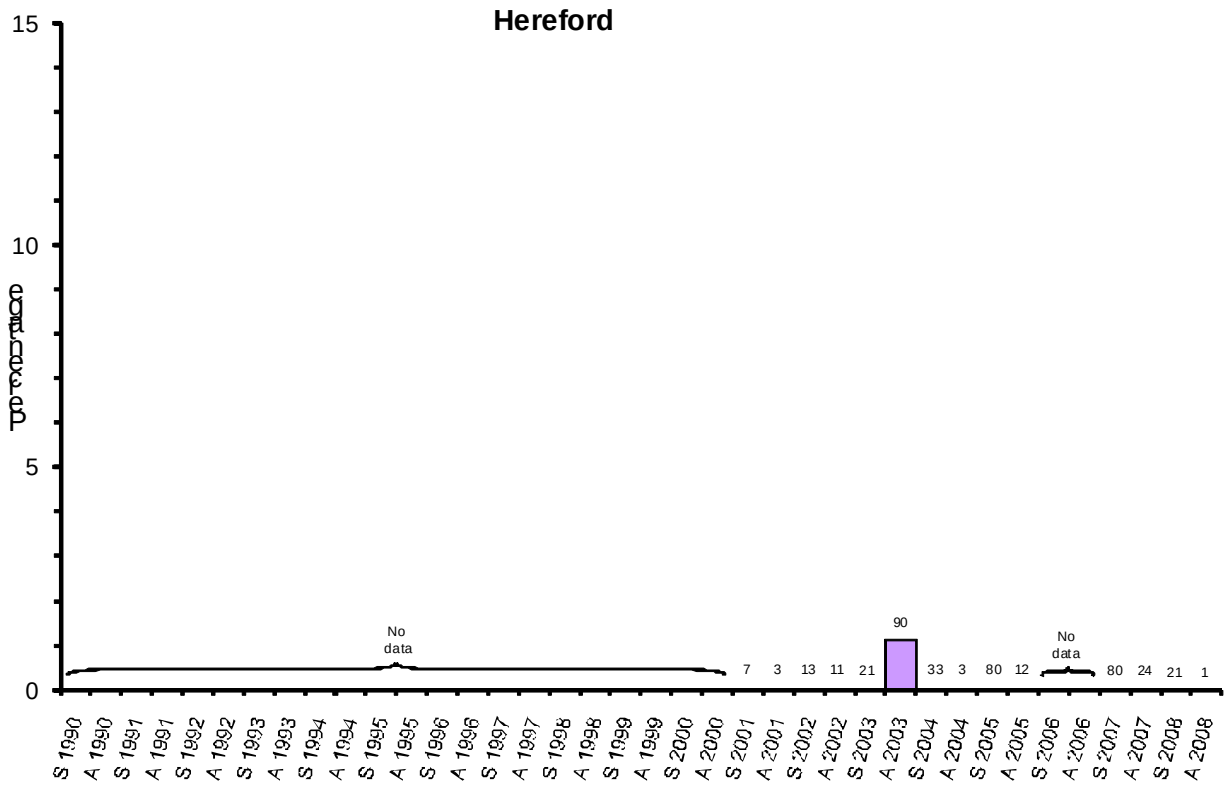

Aulacorthum solani 2007
Aulacorthum solani 2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
Number of Aulacorthum solani per month
0
10
20
30
Mar April May June July Aug Sept Oct Nov
Aphids carrying no virus
Aphids carrying TuYV
Aphids carrying BMYV
Numbers above bars represent BMYV carrying A.solani

Figure 4 The long term trend in percentage of BMYV-carrying *Myzus persicae* in spring and autumn migrations at Broom's Barn, Writtle, Kirton, Rothamsted and Hereford.





Numbers above bars are the number of aphids tested



Numbers above bars are the number of aphids tested

8 INSECTICIDE RESISTANCE STATUS OF AERIAL *Myzus persicae*

8.1 Methods

The insecticide resistance status of *M. persicae* was tested from the following traps and years:
2005 Esterase and MACE: Rothamsted, Broom's Barn, Kirton, Writtle, Hereford (all from glycerol);
2006 Esterase: Broom's Barn, Kirton, Writtle (all from glycerol);
2006 MACE: Broom's Barn, Kirton, Writtle (all from glycerol); Rothamsted, Hereford (from alcohol);
2006 kdr/super-kdr: Rothamsted, Hereford (from alcohol);
2007, 2008 Esterase: Rothamsted (from glycerol);
2007, 2008 MACE: Rothamsted (from glycerol); Broom's Barn, Kirton, Writtle and Hereford (from alcohol);
2007, 2008 kdr/super-kdr: Broom's Barn, Kirton, Writtle and Hereford (from alcohol).

Esterase-based insecticide resistance was tested using the total esterase test of Grant *et al*. (1989). The total esterase test cannot distinguish between susceptible (S) and moderately resistant (R_1) aphids. The results were presented as an optical density reading for each aphid, indicating the level of enzyme activity and hence insecticide resistance.

As a routine control to check the ability of the storage medium to preserve the activity of the carboxylesterases E4 and FE4 (which confer esterase resistance) under normal trap operating conditions, seven wingless aphids from standard laboratory cultures of an extremely resistant (R_3) *M. persicae* clone, reared at Rothamsted, were placed in the suction trap collecting bottles once a week from May to November at Rothamsted, Broom's Barn, Kirton, Writtle and Hereford in 2005, Broom's Barn, Kirton and Writtle in 2006 and Rothamsted in 2007 and 2008. The use of wingless aphids enabled their easy distinction from any wild winged aphids trapped. At the same time as control aphids were placed in the traps, seven more of the aphids from laboratory culture were deep frozen at Rothamsted in PBS-Tween. Both sets were tested for levels of E4 and FE4 in the same way as the wild trapped aphids.

Testing for MACE resistance in 2005 was done using a biochemical kinetic enzyme assay in the absence and presence of a diagnostic concentration of pirimicarb according to the method of Moores *et al.* (1994). New DNA-based assays for two mutations causing knockdown resistance to pyrethroids (kdr and super-kdr) and the MACE mutation (Anstead *et al*. 2004 and unpublished data) were trialled on aphids from the Rothamsted trap. These tests, which have the advantage of distinguishing MACE genotype, were subsequently rolled out to all the traps except Rothamsted (in order to allow carboxylesterase testing at this site) for 2006 2007 and 2008.

8.2 Results and discussion

At all sites in all years for the elevated carboxylesterase resistance mechanism, moderately resistant (R_1) and susceptible (S) aphids (these two forms are not

distinguished in the assay) dominated the samples (**Figs 5, 6**). A few very resistant (R_2) and extremely resistant (R_3) aphids were found.

A comparison of the optical density readings of control R_3 aphids placed in the trap bottles and those frozen into the glycerol-based medium at the same time is shown in **Fig. 7**. In all years there was very little loss of enzyme activity at any site, with very few of the trap control aphids falling below the R_3 level.

The proportion of R_2 and R_3 aphids in the population was generally higher in autumn than spring (**Fig. 8**), continuing a long-term trend. However, with very few R_2 and R_3 aphids present in general, that pattern is becoming less easy to detect. Resistance due to elevated carboxylesterase levels is no longer a significant constraint on sugar beet production because it is effective mainly against organophosphates, which are not used any more. It would therefore be expected that there would be reduced selection for R_2 and R_3 aphids during the summer. The decrease in this type of resistance between autumn and spring appears to be partly a result of fitness costs associated with esterase resistance status during the winter (Foster *et al*., 1996, 1997, 2000).

Numbers and proportions of `MACE' resistant aphids (carrying resistance specifically to pirimicarb) have increased significantly since 2002 at all sites tested (**Figs 9, 10, 11**). 39.3% of aphids tested (963 out of 2451) showed this mechanism. This has risen dramatically from 10.6% in the previous four year period and 0.26% in the four year period before that. Whereas the MACE mechanism used to appear mainly in autumn populations when it was of no relevance to beet, it is now also prevalent in summer. It is the rise and spread in MACE that has rendered pirimicarb largely ineffective against *M. persicae* and led to a worrying reliance on neonicotinoid seed treatments.

56.3% of *M. persicae* tested (454 out of 807) did not have the kdr mechanism that confers resistance to pyrethroids (**Fig. 12**). The rest were heterozygotes showing resistance. Interestingly, none of the 220 *M. persicae* tested was homozygous (RR) for kdr, having the resistance mutation on both chromatids of the relevant chromosomes, and which have been seen in sexually reproducing *M. persicae* populations abroad. None had the super-kdr resistance mechanism. This supports the hypothesis that kdr in the homozygous form, and probably super-kdr, impose fitness handicaps in the absence of insecticides, at least in the UK. For these mechanisms this appears to be through maladaptive aphid behaviour (Foster *et al*., 2007b).

Fig. 13 summarises the combined status of MACE and kdr resistance, the two mechanisms of current practical importance to the beet industry. A few aphids were susceptible homozygotes (`SS' with no resistance mutations for either mechanism). Like kdr, all aphids scoring as MACE were heterozygotes suggesting that homozygotes suffer from some sort of fitness cost (as yet unknown). Collaborative research with SCRI in Scotland suggests that in the UK, MACE is now being found in aphid clones with new genotypes (O and P) that carry neither kdr nor high carboxylesterase resistance. This would mean that MACE aphids are no longer being handicapped by an association with these other insecticide resistance mechanisms and may be better adapted to our climate and ecological conditions. This is supported by the findings that suggest that the Scottish *M. persicae* population consists of waves of

clonal lineages occurring over time that can have a periodicity of up to several years, each potentially carrying fitness advantages and costs conferred by insecticide resistance mechanisms and other genes.

Assessments of the status of pyrethroid-specific knockdown resistance, conferred by the kdr and super-kdr mechanisms, and MACE were also done in samples of *M. persicae* collected directly from field sites in beet-growing areas (April to November each year) as part of an SA-Link project (LK 0953) that BBRO funding towards this project fed into. In agreement with the suction trap data, this showed that kdr and MACE heterozygotes remained relatively common over the last several years while super-kdr was very rare. This reinforces the importance of neonicotinoids for controlling kdr and MACE resistance in *M. persicae* in the UK.

Figure 5 **The level of insecticide resistance of individual *Myzus persicae*, as indicated by their optical density following immunoassay, from 12.2 m high suction traps from Broom's Barn, Writtle, Kirton, Rothamsted and Hereford in 2005, 2006, 2007 and 2008. The dashed lines indicate the separation of standard laboratory clones of S/R_1 (susceptible to moderately resistant); R_2 (very resistant) and R_3 (extremely resistant) *M. persicae*.**

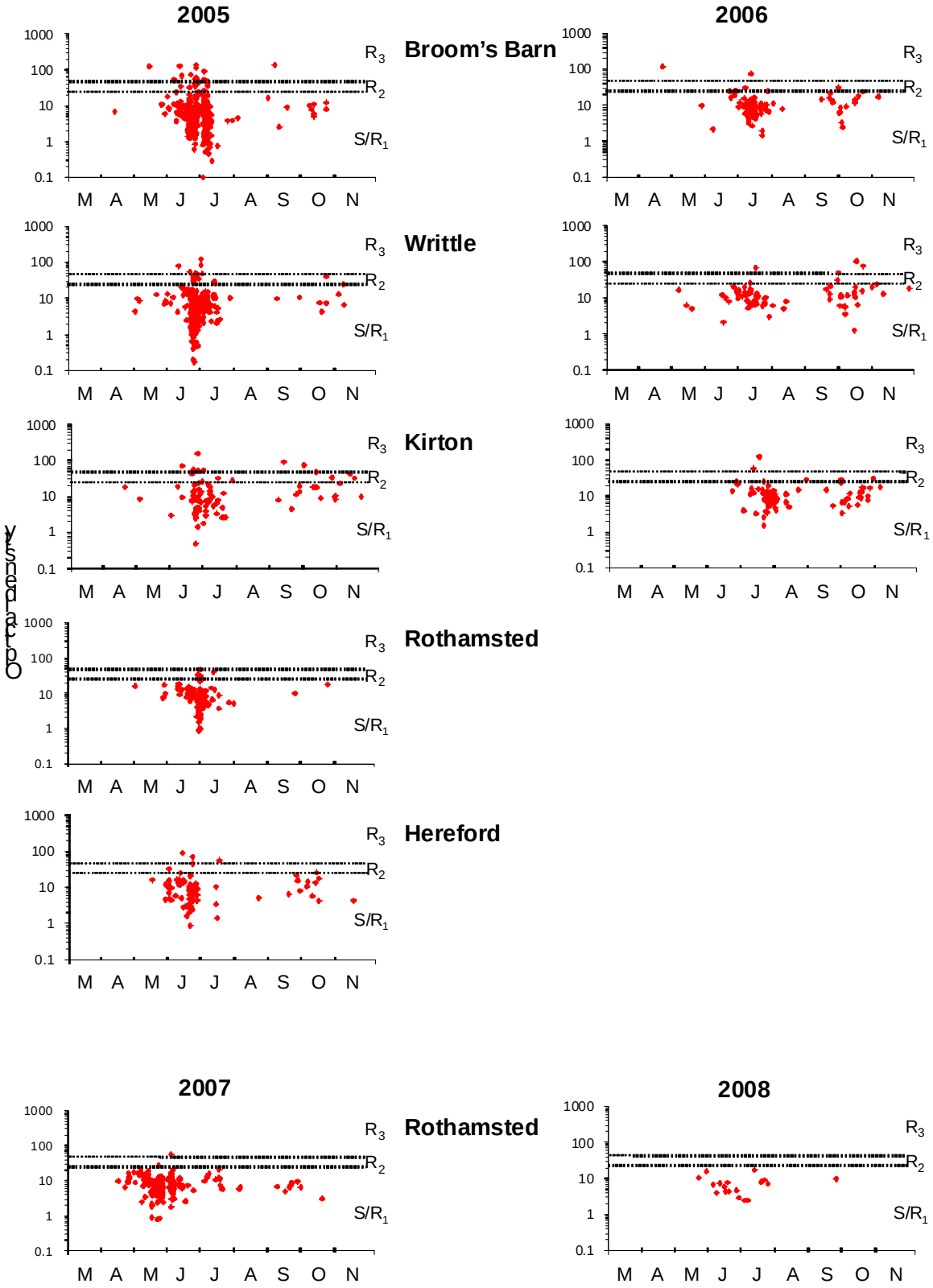


Figure 6 The numbers of *Myzus persicae* of different categories of resistance to insecticides per month during 2005, 2006, 2007and 2008 at Broom's Barn, Writtle, Kirton, Rothamsted and Herford. The resistance categories are defined as in Fig. 5.

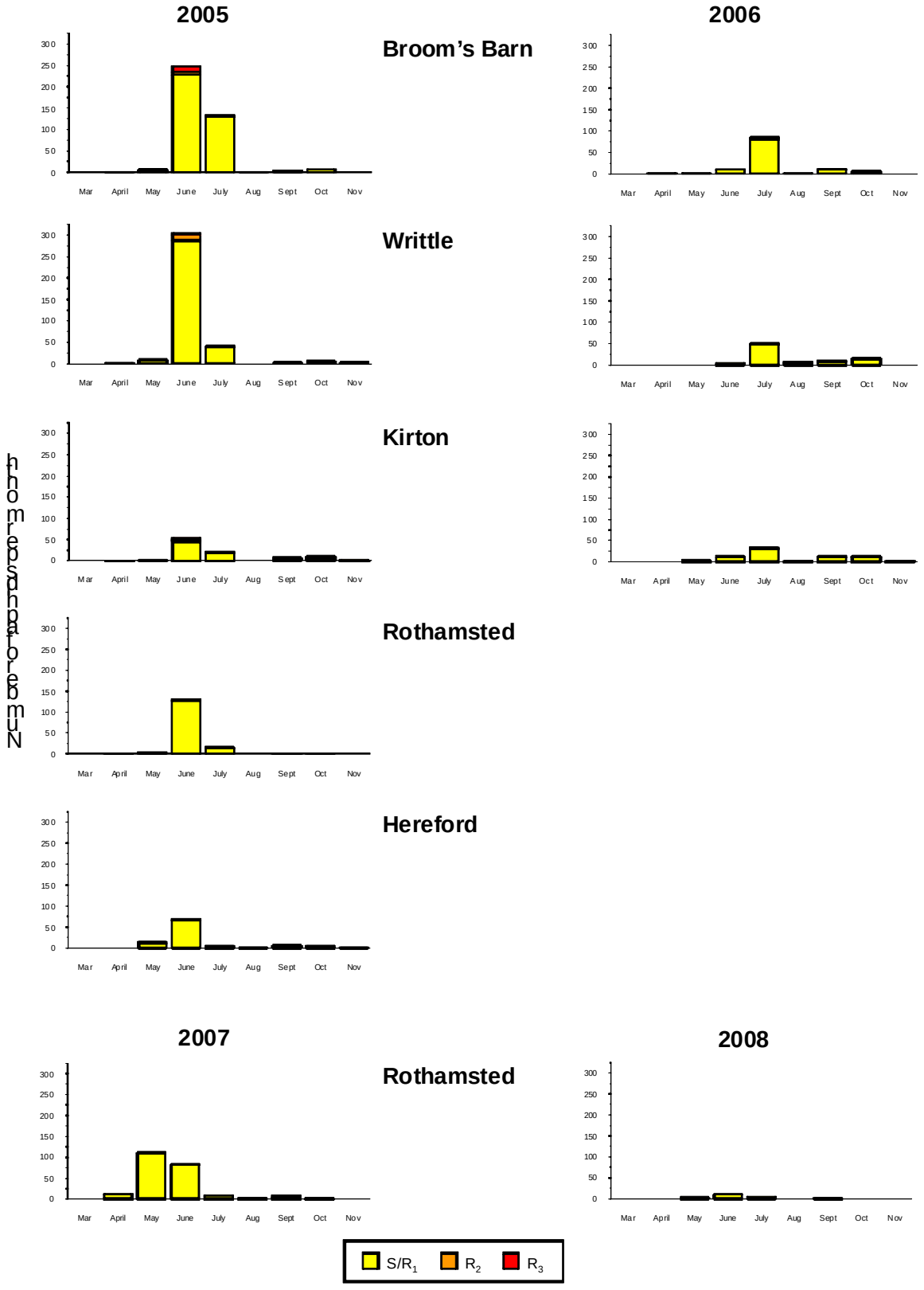


Figure 7 Comparison of mean resistance levels in batches of 7 control *Myzus persicae* placed in traps and 7 control aphids frozen direct into assay plates at the same time, for Broom's Barn, Writtle, Kirton, Rothamsted and Hereford throughout 2005, 2006, 2007 and 2008.

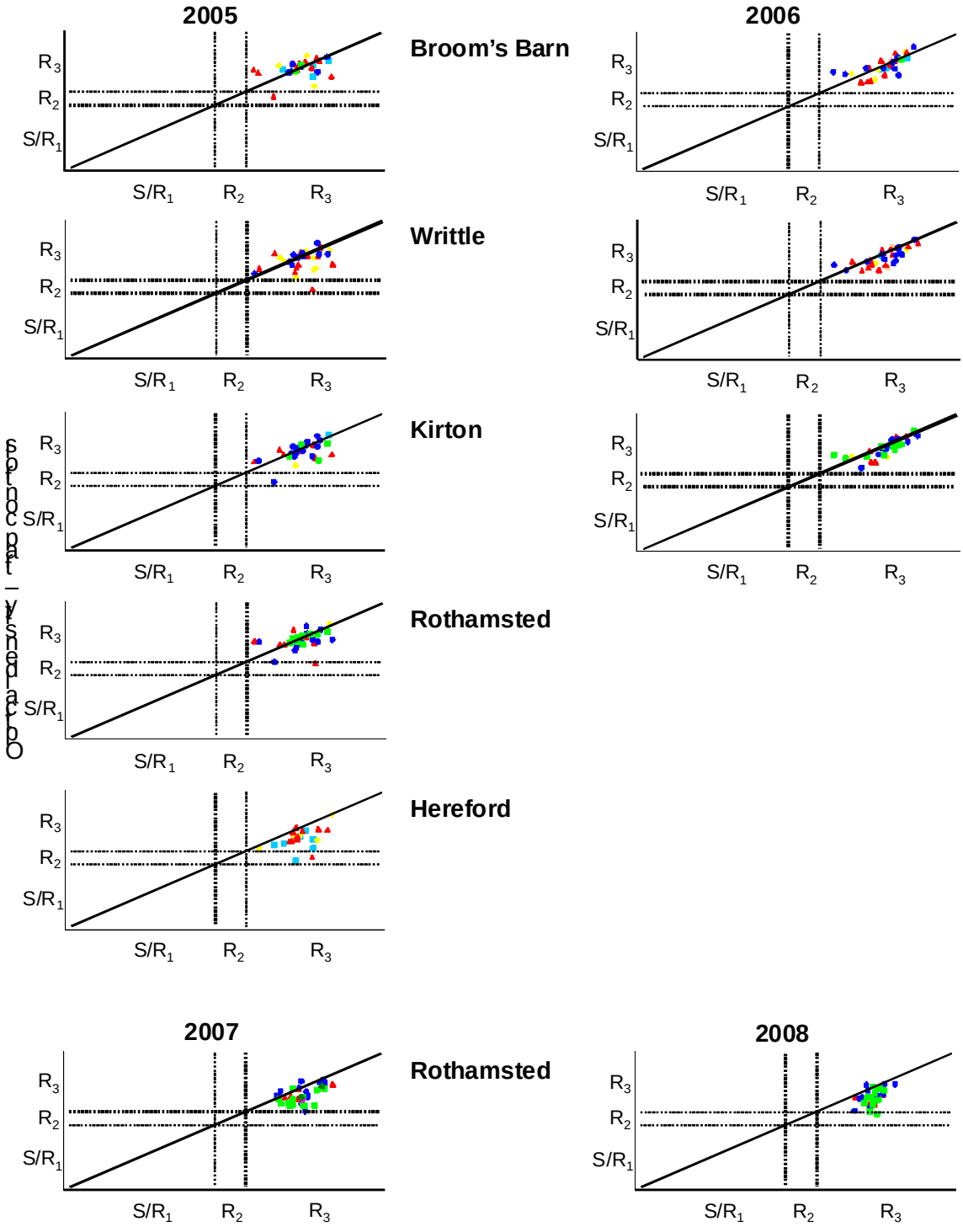
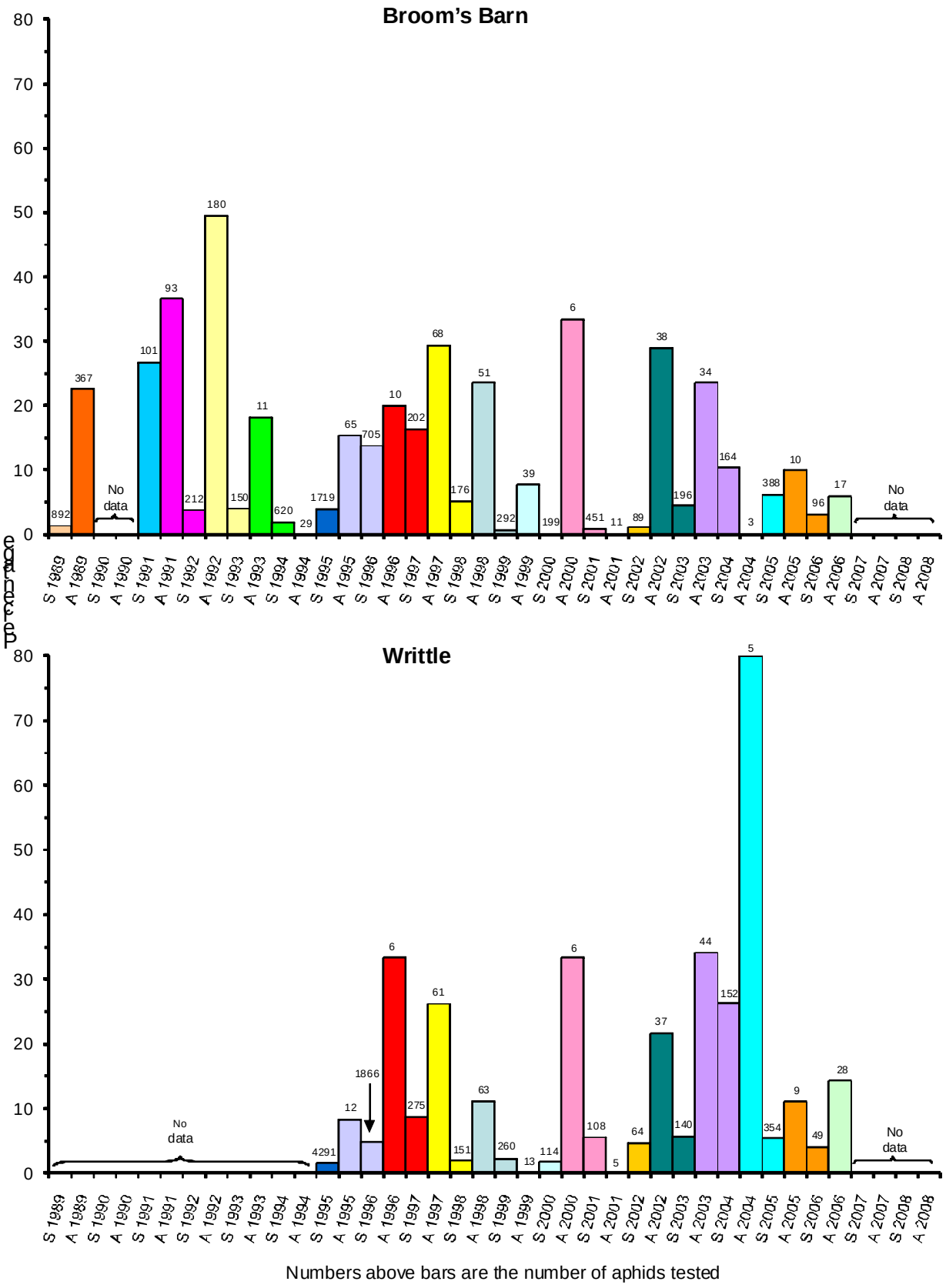
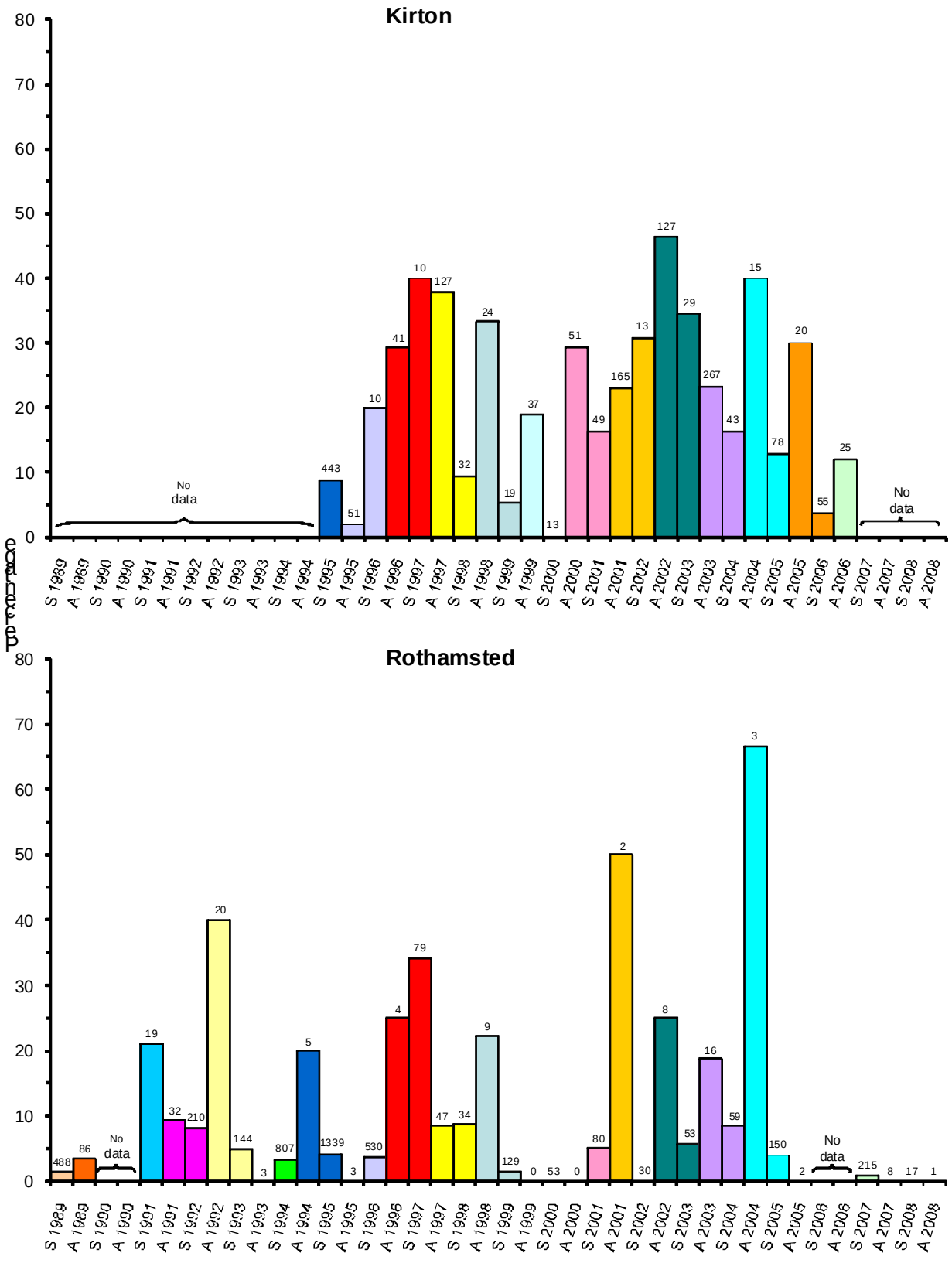
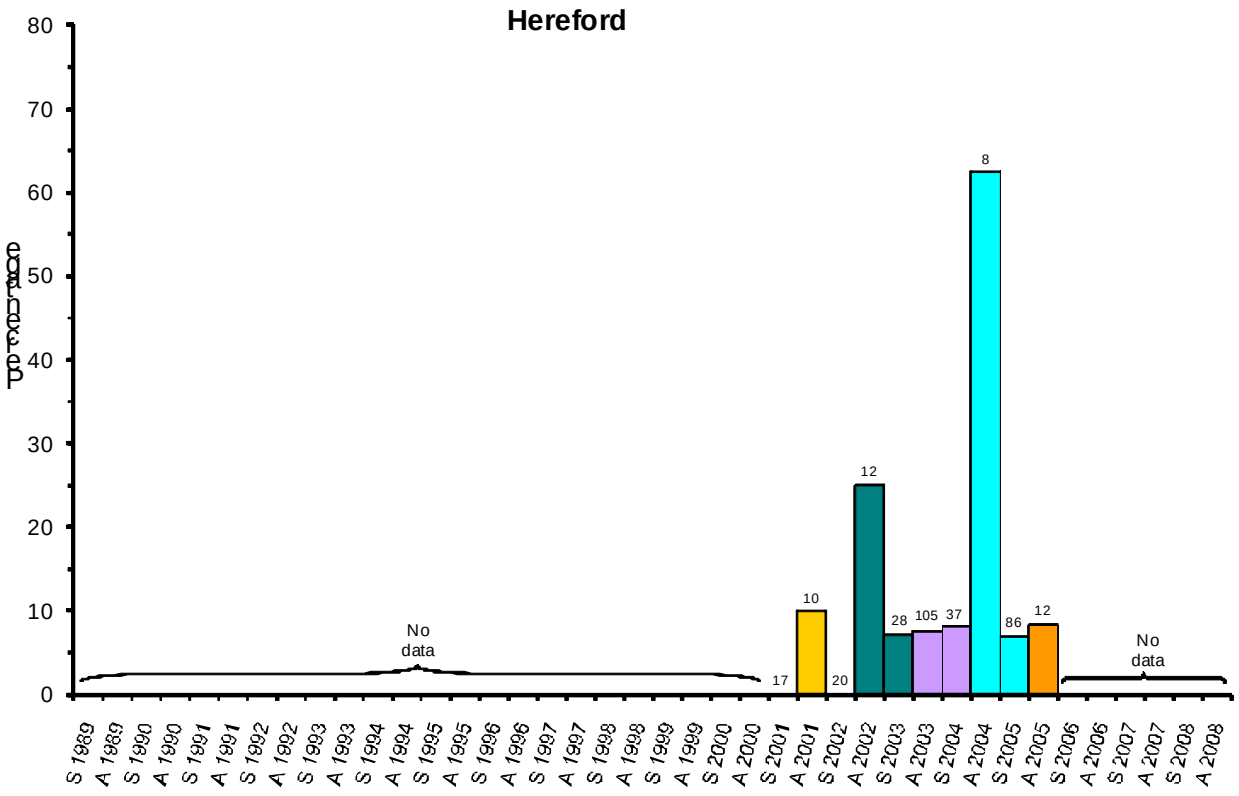


Figure 8 The long-term trend in percentage of *Myzus persicae* showing R_2 or R_3 levels of insecticide resistance in spring and autumn migrations at Broom's Barn, Writtle, Kirton, Rothamsted and Hereford.

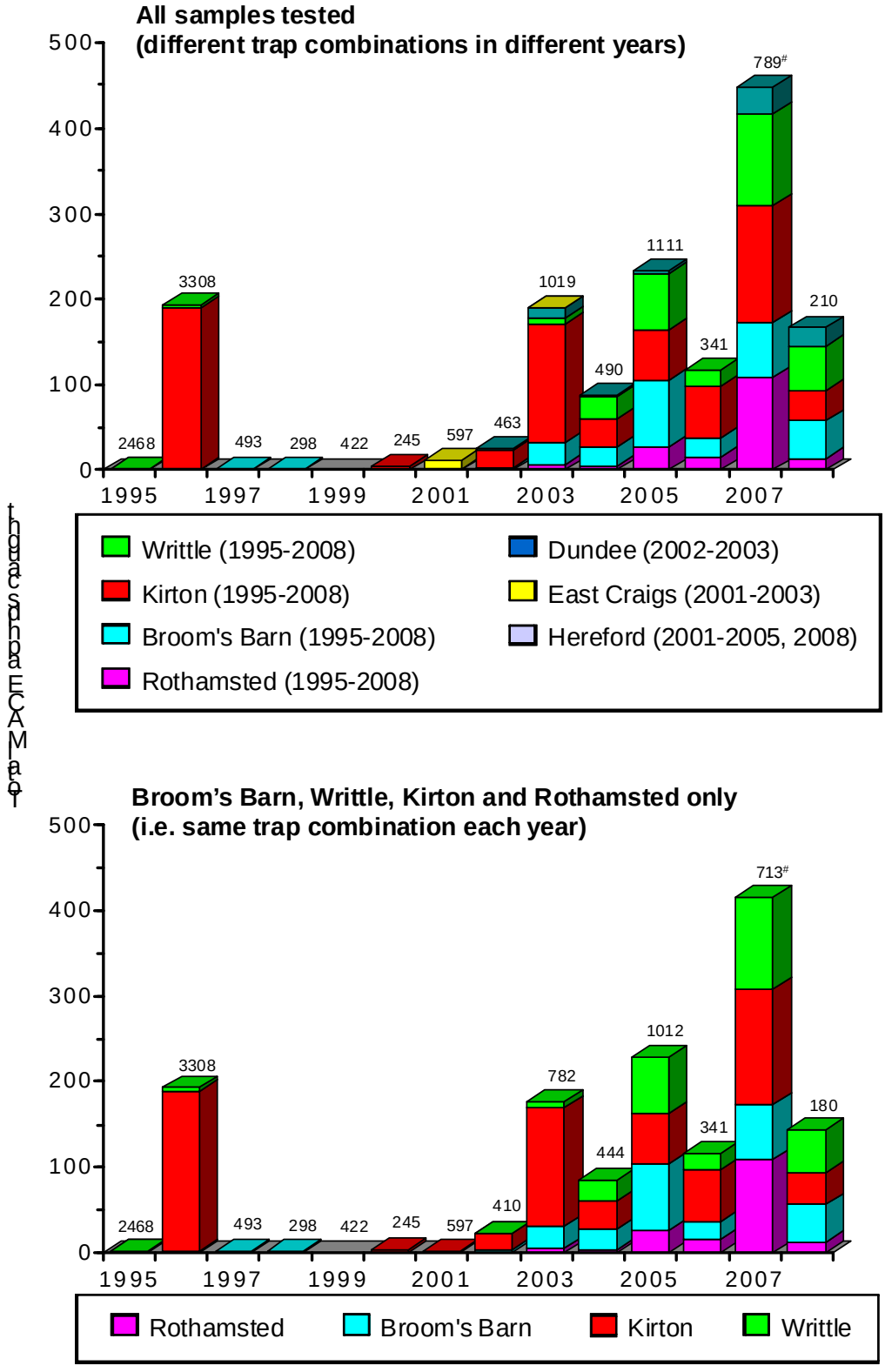



Kirton
Rothamsted
Percentage aphids
No data
Numbers above bars are the number of aphids tested



Numbers above bars are the number of aphids tested

Figure 9 **Total MACE *Myzus persicae* caught in suction traps in 1995 – 2008.**



(numbers above bars are number of aphids tested
for sites except Rothamsted no data available for 7 – 20 May 2007)

Figure 10 Percentage MACE *Myzus persicae* caught in suction traps in 1995 – 2008.

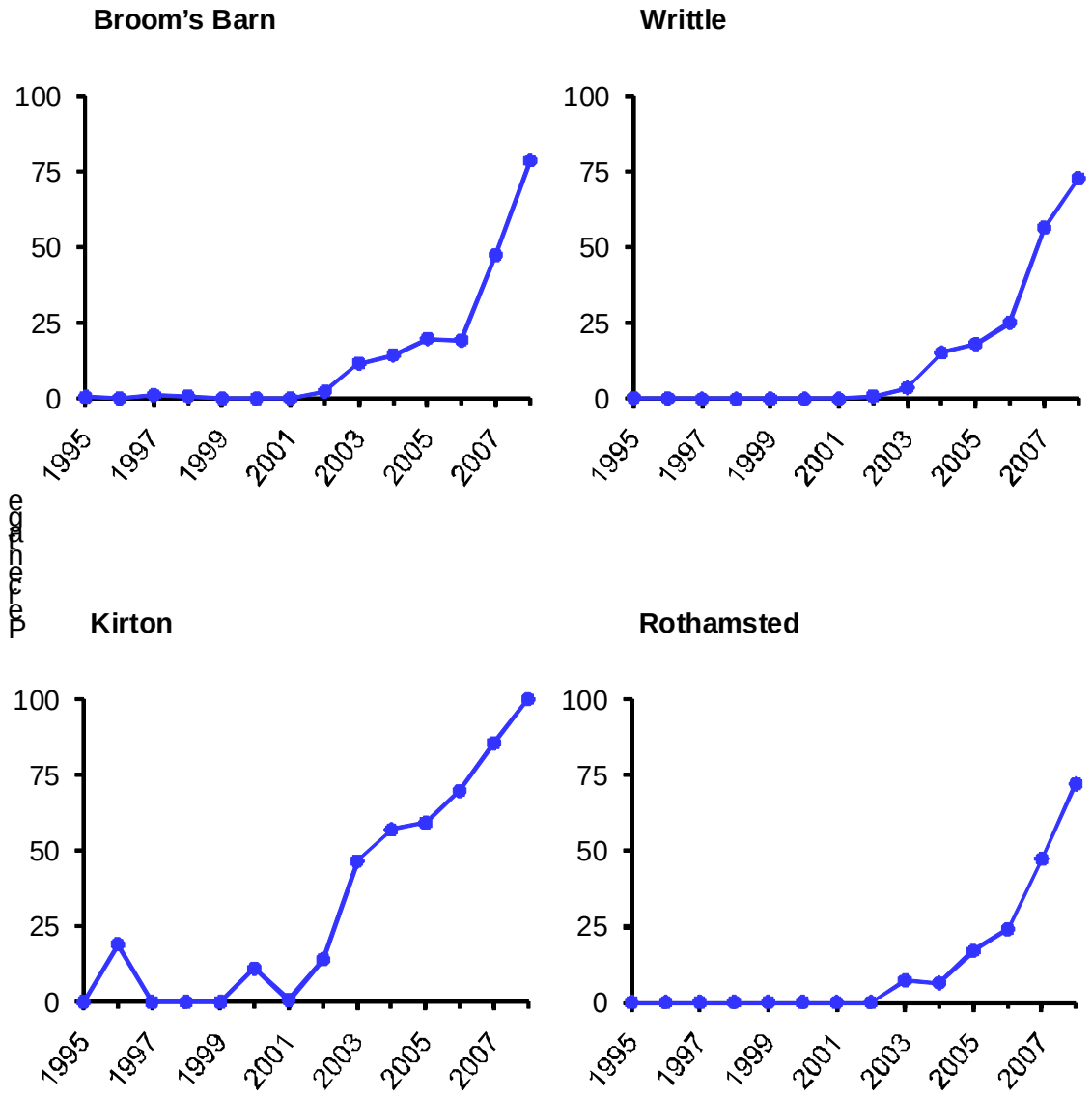
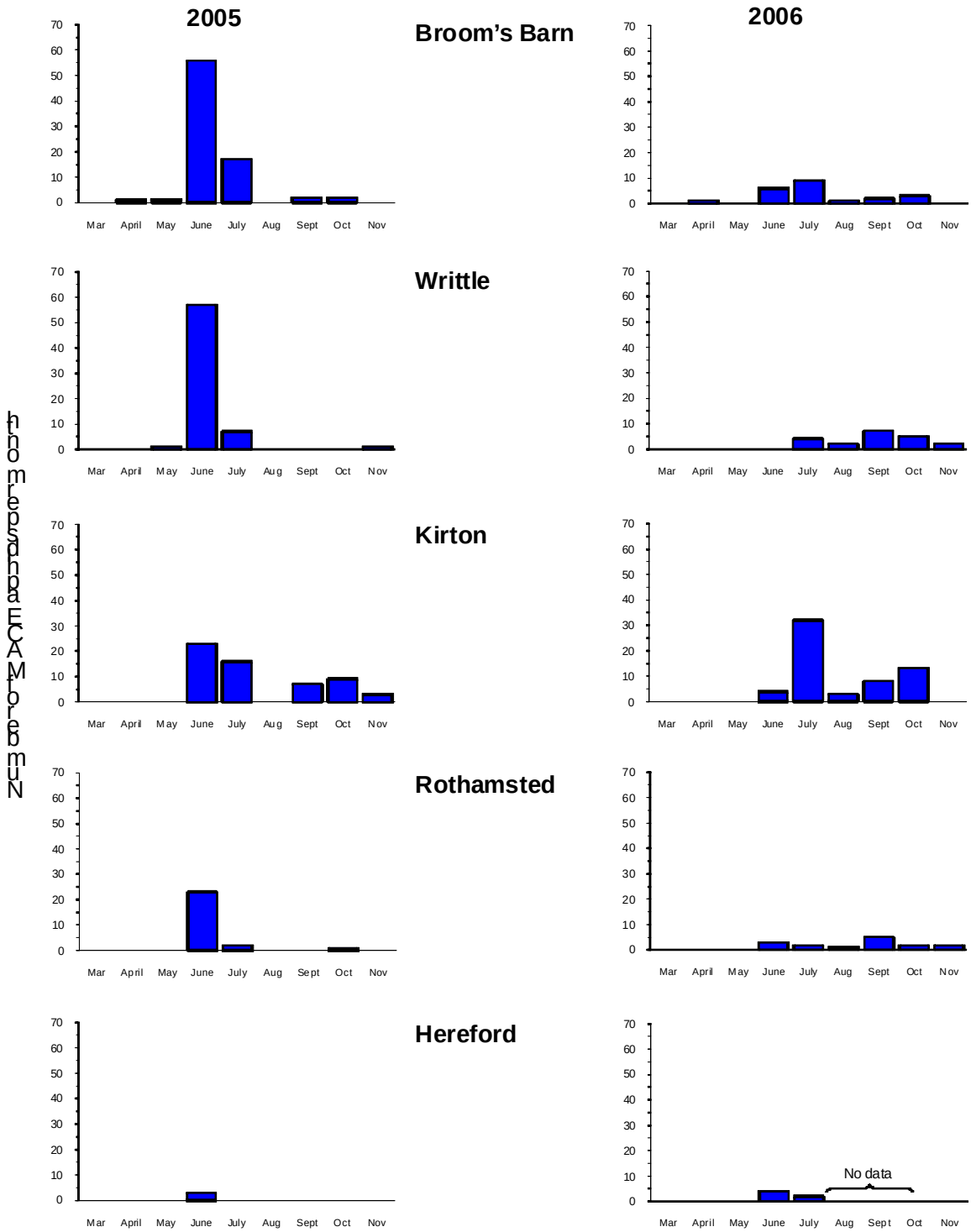


Figure 11 The numbers of MACE *Myzus persicae* per month during 2005, 2006, 2007 and 2008 at Broom's Barn, Writtle, Kirton, Rothamsted and Hereford.



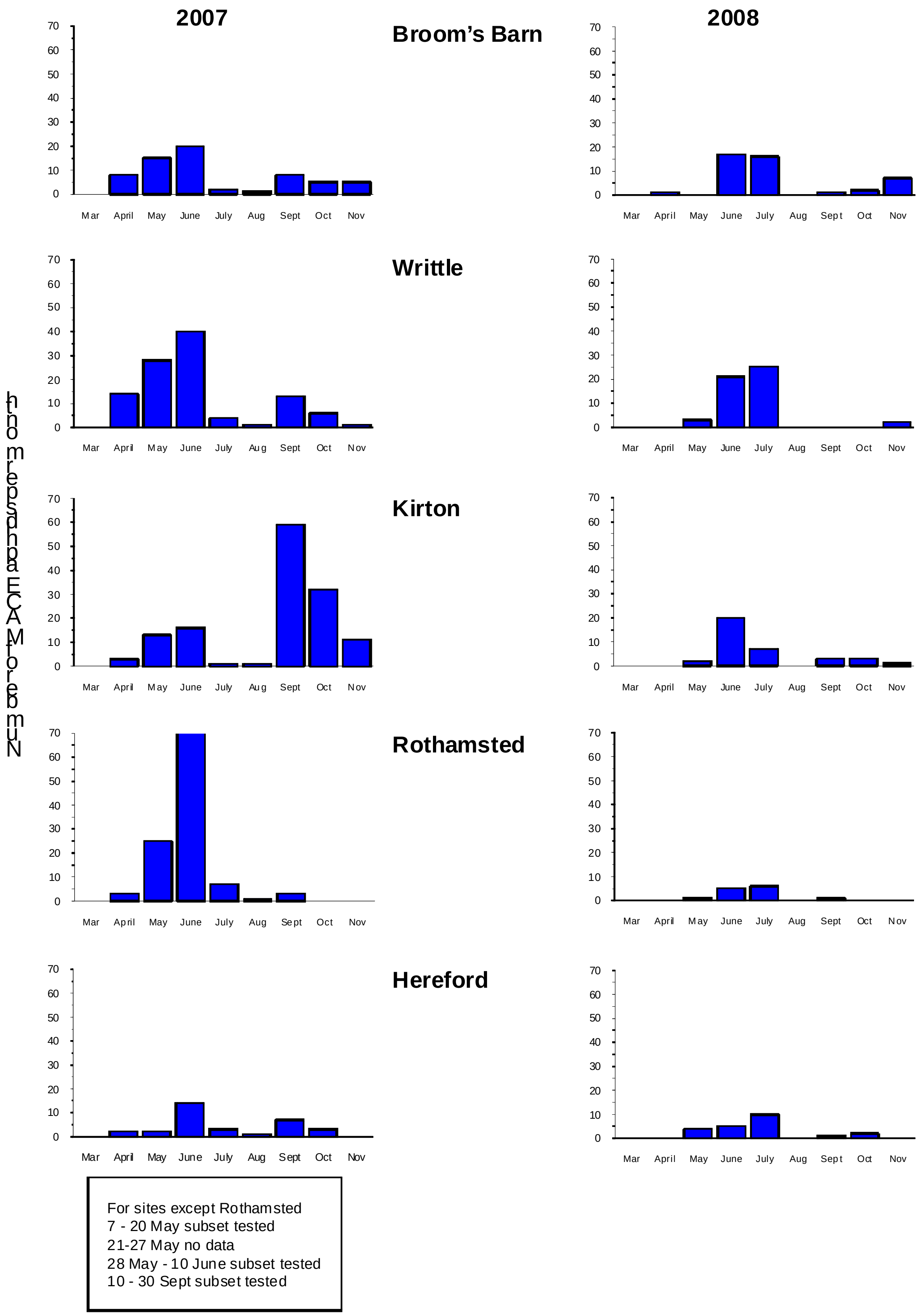
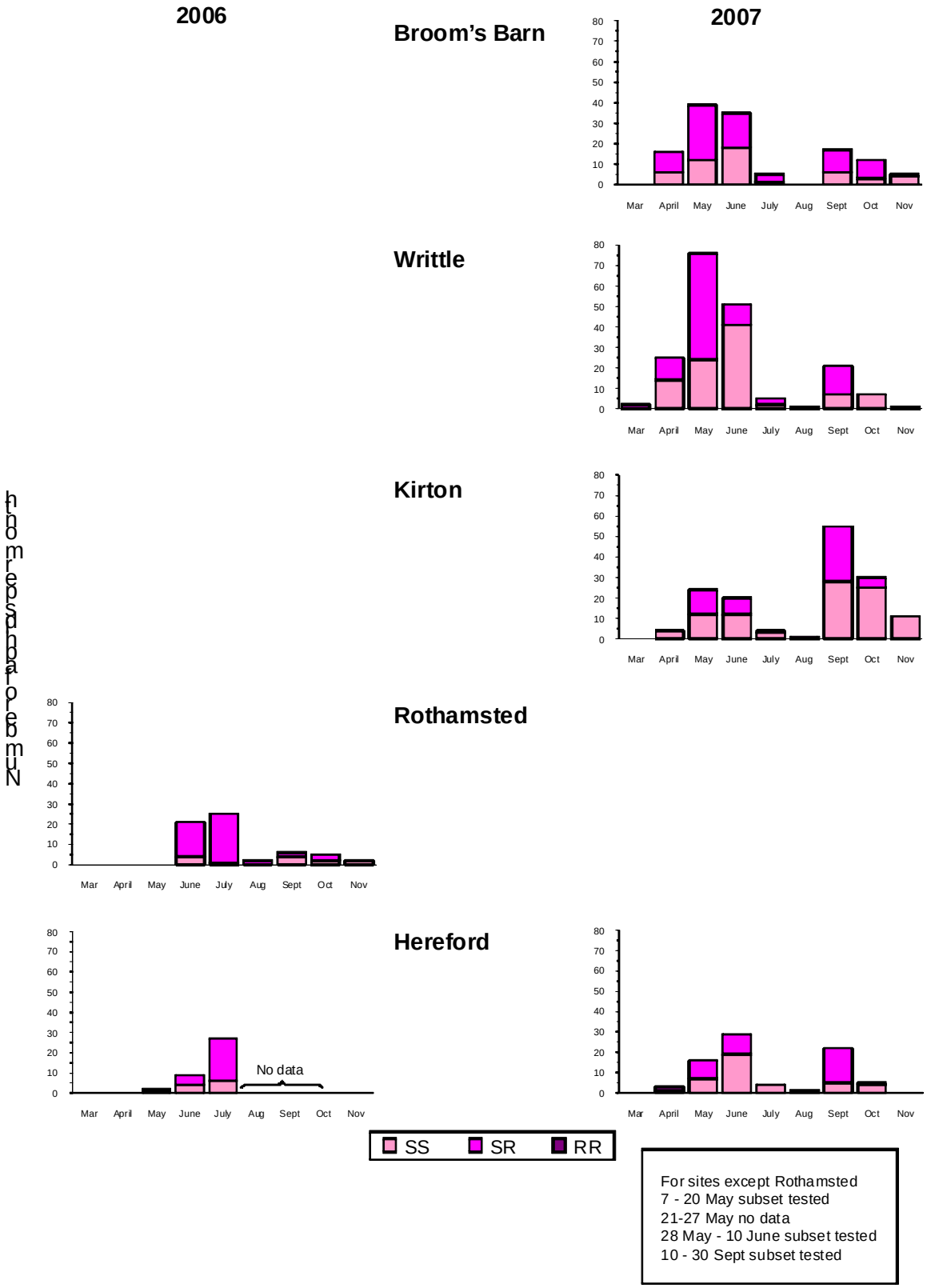

2007
2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
Mar April May June July Aug Sept Oct Nov
0 10 20 30 40 50 60 70
For sites except Rothamsted
7 - 20 May subset tested
21-27 May no data
28 May - 10 June subset tested
10 - 30 Sept subset tested

Figure 12 Knockdown resistance genotypes of *Myzus persicae* at Broom's Barn, Writtle, Kirton, Rothamsted and Hereford during 2006, 2007 and 2008. SS: homozygous susceptible, SR: heterozygous, RR: homozygous resistant.



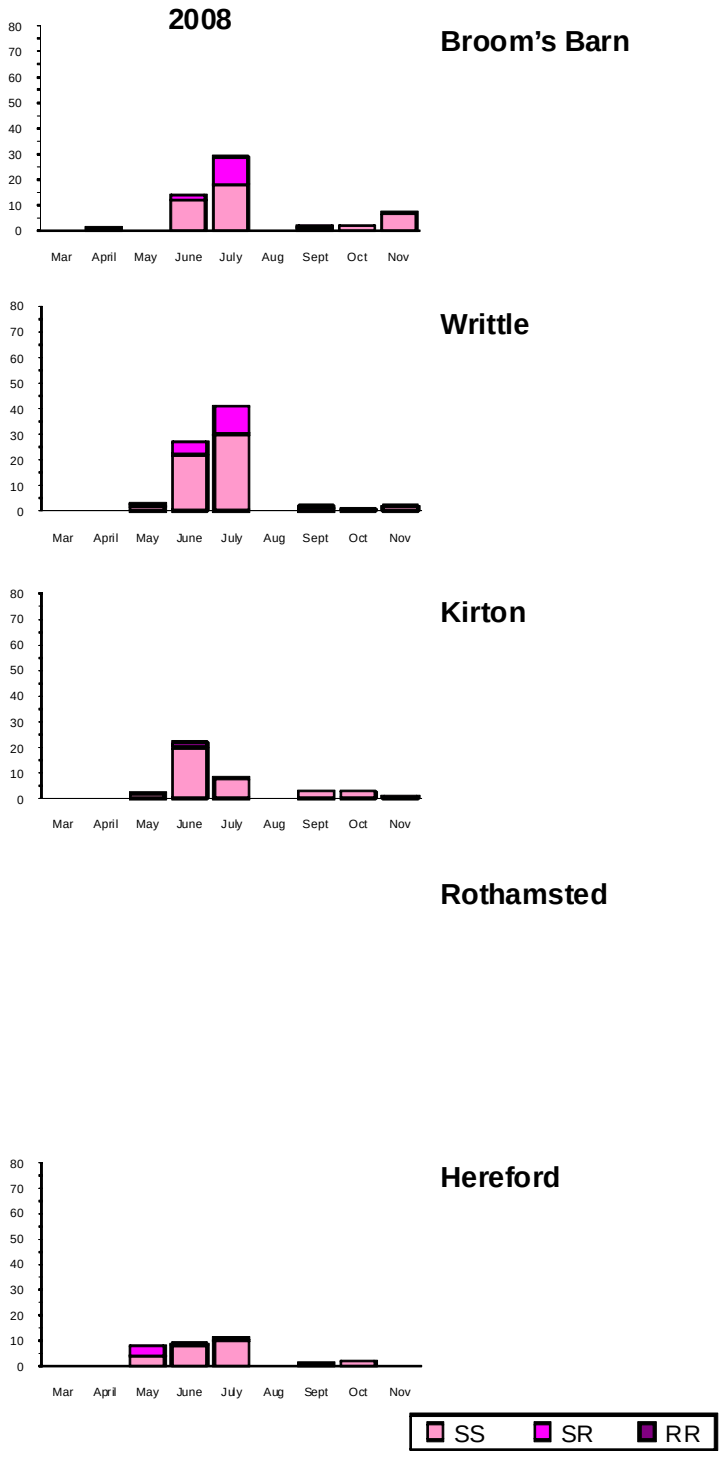

2008
Broom's Barn
Writtle
Kirton
Rothamsted
Hereford
80
70
60
50
40
30
20
10
0
Mar
April
May
June
July
Aug
Sept
Oct
Nov
SS
SR
RR

Figure 13 Combined knockdown resistance and MACE status of *Myzus persicae* at Broom's Barn, Writtle, Kirton, Rothamsted and Hereford during 2006, 2007 and 2008. SS: homozygous susceptible, SR: heterozygous, RR: homozygous resistant.

2006

Broom's Barn

		MACE		
		SS	SR	RR
kdr	SS	7	43	0
	SR	64	14	0
	RR	0	0	0

Writtle

		MACE		
		SS	SR	RR
kdr	SS	7	85	0
	SR	73	20	0
	RR	0	0	0

Kirton

		MACE		
		SS	SR	RR
kdr	SS	7	87	0
	SR	20	31	0
	RR	0	0	0

Rothamsted

		MACE		
		SS	SR	RR
kdr	SS	0	12	0
	SR	45	3	0
	RR	0	0	0

Hereford

		MACE		
		SS	SR	RR
kdr	SS	11	7	0
	SR	30	0	0
	RR	0	0	0

Hereford: no data August – October 2006

2007

		MACE		
		SS	SR	RR
kdr	SS	15	23	0
	SR	28	8	0
	RR	0	0	0

For sites except Rothamsted:
7 - 20 May subset tested
21-27 May no data
28 May - 10 June subset tested
10 - 30 Sept subset tested

2008

Broom's Barn

		MACE		
		SS	SR	RR
kdr	SS	0	38	0
	SR	12	0	0
	RR	0	0	0

Writtle

		MACE		
		SS	SR	RR
kdr	SS	3	49	0
	SR	18	0	0
	RR	0	0	0

Kirton

		MACE		
		SS	SR	RR
kdr	SS	0	34	0
	SR	0	2	0
	RR	0	0	0

Rothamsted

Hereford

		MACE		
		SS	SR	RR
kdr	SS	1	22	0
	SR	6	0	0
	RR	0	0	0

9. PRESENTATION OF RESULTS AND EXPLOITATION

Results were published weekly when relevant through the BBRO/Broom's Barn Advisory Bulletins.

MS gave annual presentations and discussions at BBRO open days, Cereals, Beet 08, Broom's Barn Open Days, as well as presentations to Syngenta, Bayer, Dupont, AICC, Frontier, Dalgety and Masstock.

At least 12 articles were published in popular farming press including Farmers Weekly and Farmers Guardian.

The following publications resulted from the work.

Conference abstract

Qi, A., Dewar, A.M. and Harrington, R. (2005) Forecasting virus yellows incidence in sugar beet – the post-Gaucho era. *Aspects of Applied Biology* **76**, 87-94

Harrington, R. (2007) *Viruses, vectors, host plants and environment: from complexity to control.* Nordic Association of Agricultural Scientists (NJF) Seminar 402: Virus vector management in a changing climate. Kristianstad, Sweden, 9-11 October 2007.

Stevens, M., Adam, N.M. and Harrington, R. (2007) *Turnip yellows virus* (TuYV) in winter oilseed rape: the importance of autumn migrations of the peach-potato aphid, *Myzus persicae*, and the role of insecticide seed treatments in reducing impact of the disease. *Proceedings Crop Protection in Northern Britain* 175-180.

Harrington, R. (2008) *Impacts of climate change on aphids.* International conference on information systems of diagnostics, monitoring and forecasting the major weed plants, pests and diseases of agricultural crops. All Russian Institute of Plant Protection, VIZR, St-Petersburg-Pushkin, Russia, 12-16 May 2008.

Popular articles

Dewar, A., Asher, M., Stevens, M., Harrington, R., Parker, S., Foster, S. and Denholm, I. (2006) Pests and diseases in sugar beet in 2005. *British Sugar Beet Review* **74**, 22-27.

Harrington, R., Anstead, J., Denholm, I., Foster, S., Parker, S. and Cox, D. (2007) Resistance watch. *British Sugar Beet Review* **75**, 37-38.

May, M. J. and Stevens, M. (2008). Crop protection review of 2007. *British Sugar Beet Review* **76**, 37-39.
Stevens, M., Asher, M.J.C. and Dewar, A.M. (2007). Pests and diseases update for 2006. *British Sugar Beet Review* **75**, 16-20.

Stevens, M., Harrington, R., Parker, S., Cox, D., Foster, S. and May, M. (2008) Aphids galore! So how did the industry avert a virus yellows epidemic in 2007? *British Sugar Beet Review* **76**, 20-31.

Papers

Vigano, F. and Stevens, M. (2007). Development of a multiplex immunocapture-RT-PCR for simultaneous detection of BMYV and BChV in plants and single aphids. *Journal of Virological Methods* **146**, 196-201.

10. STAFF INPUT AND COSTS

One Assistant Research Scientist (Band 8) has been funded by the project at 60% time, one Senior Research Scientist (Band 7) at 20% time and one Senior Researcher (Band 5) at 10% time.

08/09 Budgeted expenditure £55,168
08/09 Actual expenditure £56,555

Total project budget £209,766
Total project expenditure to date £215,487

Man months 08/09 Budget 1.18
Man months 08/09 Actual 1.18

Man months project to date Budget 4.72
Man months project to date Actual 4.72

11. REFERENCES

Anstead, J.A., Williamson, M.S., Eleftherianos, I.G. and Denholm, I. (2004) High-throughput detection of knockdown resistance in *Myzus persicae* using allelic discriminating quantitative PCR. *Insect Biochemistry and Molecular Biology* **34**, 871-877.

Foster, S.P., Harrington, R., Devonshire, A.L., Denholm, I., Devine, G.J., Kenward, M.G. and Bale, J.S. (1996) Overwintering success of insecticide-susceptible and resistant peach-potato aphids, *Myzus persicae* (Sulzer) (Hemiptera:Aphididae) *Bulletin of Entomological Research* **86**, 17-27.

Foster, S.P., Harrington, R., Devonshire, A.L., Denholm, I., Clark, S.J., Mugglestone, M.A. (1997) Evidence for a possible fitness trade-off between insecticide resistance and low temperature movement that is essential for survival of UK populations of *Myzus persicae* (Hemiptera:Aphididae). *Bulletin of Entomological Research* **87**, 573-579.

Foster, S.P., Denholm, I. and Devonshire, A.L. (2000) The ups and downs of insecticide resistance in peach-potato aphids (*Myzus persicae*) in the UK. *Crop Protection* **19,** 873-879.

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Foster, S.P., Tomiczek, M., Thompson, R., Denholm, I., Poppy, G., Kraaijeveld, A.R. and Powell, W. (2007b) Behavioural side-effects of insecticide resistance in aphids increase their vulnerability to parasitoid attack. *Animal Behaviour* **74**, 621-632.

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Harrington, R. and Woiwod, I. (2007) Foresight from hindsight: the Rothamsted Insect Survey. *Outlooks on Pest Management* **February 2007**, 9-14.

Harrington, R., Bale, J.S. and Tatchell, G.M. (1995) Aphids in a changing climate. pp 125-155 in: R. Harrington and N.E. Stork (Eds) *Insects in a Changing Environment.* Acadaemic Press, London.

Moores, G.D., Devine, G.J. and Devonshire, A.L. (1994) Insecticide resistance due to insensitive acetylcholinesterase in esterase in *Myzus persicae* and *Myzus nicotianiae*. *Brighton Crop Protection conference - Pests and Diseases*, 413-418.

Qi, A., Dewar, A., Werker, W. and Harrington, R. (2001) Virus yellows forecasting and the impact of `gaucho'. *British Sugar Beet Review* **69**, 36-39.

Smith, H.G., Stevens, M. and Hallsworth, P.B. (1991) The use of monoclonal antibodies to detect beet mild yellowing virus and beet western yellows virus in aphids. *Annals of Applied Biology* **119**, 295-302.

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