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Microsatellite marker analysis of peach–potato aphids (*Myzus persicae*, Homoptera: Aphididae) from Scottish suction traps

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Abstract

The peach–potato aphid *Myzus persicae* (Sulzer) is an important vector of plant viruses. A network of suction traps collects aerial samples of this aphid in order to monitor and help predict its spatial distribution and likely impact on virus transmission in crops. A suction trap catch is thought to be a good representation of the total aphid pool. Sensitive molecular markers have been developed that determine the genetic composition of the *M. persicae* population. In Scotland, UK, these were applied to field collections revealing a limited number of clones. Molecular markers are less successful when applied to specimens that have been preserved in an ethanol-based trap fluid designed to preserve morphology. An assessment of different DNA extraction and PCR techniques is presented and the most efficient are used to analyse *M. persicae* specimens caught in the Dundee suction trap in 2001, a year when exceptionally high numbers were caught. The results reveal that the majority of the *M. persicae* caught belonged to two highly insecticide resistant clones. In addition, it was possible to compare the relative frequencies of genotypes caught in the trap with those collected at insecticide treated and untreated field sites in the vicinity. These results indicate that, in addition to suction trap data, the ability to sample field sites provides valuable early warning data which have implications for pest control and virus management strategies.

Keywords: *Myzus persicae*, suction trap, microsatellites, DNA extraction

Introduction

The peach–potato aphid *Myzus persicae* (Sulzer) has a world-wide distribution and is an important vector of plant viruses, spreading diseases among many crops (Blackman & Eastop, 2000). The importance of *M. persicae* as a vector of potato viruses, particularly potato leafroll virus (PLRV), means that it is the major insect pest of seed potatoes. In Scotland, UK, seed potatoes are an important agricultural export making *M. persicae* ecology and movement the subject of detailed scrutiny. Part of this process includes the regular sampling of flying aphids by a series of suction traps. Apart from their practical value in monitoring current aphid activity, the suction trap catches provide valuable information on the spatial and temporal distribution of aphids that can be used for retrospective analysis.

In addition to its primary host, peach, on which some clones of *M. persicae* produce overwintering eggs, it feeds on a wide range of secondary host plants. Some of these

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secondary hosts are crops subjected to insecticide treatments aimed at control of *M. persicae*, some are crops where insecticides are used to control other insects and some are not treated with insecticides as they are not crops or, if they are, insecticide treatment is not appropriate. It is believed that this complex environment could make it difficult to collect field samples that are a true representation of the *M. persicae* population (Foster *et al*., 2002). Aerial surveys, which collect aphids at a height where they are thought to be randomly distributed, are more likely to be representative of the population from the total plant pool (Foster *et al*., 2002). Winged forms of aphids can travel long distances (Simon *et al*., 1999; Cocu *et al*., 2005c). Studies have also shown that genetically diverse winged *M. persicae* populations have been found in suction traps in north-east France, far from the main peach growing areas in the south, suggesting that the influence of sexual populations can extend over considerable distances (Guillemaud *et al*., 2003). This is not the case in Scotland where the *M. persicae* population is restricted to large numbers of a few genotypes (Fenton *et al*., 2005).

Estimates of the representative range of a suction trap vary from a 30 km radius (Halbert *et al*., 1998), 80 km or more (Taylor, 1974), 100 km (Hullé & Gamon, 1990) and even up to 290 km (Cocu *et al*., 2005a). It is thought that this range does not depend on how far individual aphids move, but on aphids being similarly abundant, and demonstrating similar behaviours over the range (Cocu *et al*., 2005c). Variables such as host plant availability, type of trap, topography of the region and climate are all thought to influence the area represented by the trap (Cocu *et al*., 2005c). However, most studies have only considered total aphid numbers and have not investigated the relative distribution of genotypes caught in the trap.

By examining the genetic composition of *M. persicae* in a trap it will be possible to gather considerably more information about local *M. persicae* ecology. It has been possible to develop enzyme-based insecticide resistance testing for trapped samples (Tatchell *et al*., 1988; Foster *et al*., 2002) and while this is extremely useful, it is becoming clear that different genotypes can exhibit the same insecticide resistance characteristics. For example, differences in the ecology of the two insecticide sensitive types I and J (Fenton *et al*., 2005) could lead to different abundances at different times of year. In addition, many specimens collected by the suction traps have been preserved for decades and are beyond enzymatic analysis. Developing a reliable DNA extraction technique would allow the analysis of the genetic composition of these historical populations.

In recent years, several highly sensitive molecular markers have been developed that reveal the genetic structure of natural aphid populations (Fenton *et al*., 1998; Loxdale & Lushai, 1998; Sloane *et al*., 2001). One of the most successful markers uses a polymerase chain reaction (PCR) method to amplify repetitive DNA sequences throughout the aphid genome (locus specific microsatellites). The number of repeat units at any given locus can vary and it is therefore possible to identify different genotypes within the population. The utility and resolving power of these markers for aphids has been well described (Wilson *et al*., 2003).

In this study we report an improved and simple DNA extraction method that works on fresh aphid material and material that has been preserved in suction trap fluid. The method was then used to analyse *M. persicae* from a suction trap located in Edinburgh in 1995 and a suction trap at the Scottish Crop Research Institute (SCRI), Dundee in 2001, a year when unprecedented numbers of *M. persicae* were caught. By combining the results with field collected specimens in the same year (Fenton *et al*., 2005) we attempt to determine the contributions from different field sources to the overall alate population of *M. persicae* in eastern central Scotland that year.

Materials and methods

Myzus persicae *field-collected samples*

Myzus persicae from locations within a 30 km radius around the Dundee suction trap (56°27′28″N; 3°04′18″W) were collected during the growing season in 2001 (Fenton *et al*., 2005). Most of these sites were field crops of potato *Solanum tuberosum*, swede *Brassica napus napobrassica* and oilseed rape *Brassica oleifera*, consisting of both insecticide-treated and untreated fields. The site sampled most often was an experimental field approximately 400 m to the west of the Dundee suction trap. Plots of oilseed rape and potato were grown within this site and these were not treated with insecticide but blight control was used on the potatoes. These plots served to attract and maintain local *M. persicae*. The field site and trap are located within a large experimental farm and it was therefore possible to manipulate the agroecosystem for 2 km around the trap. The remaining fields within its vicinity were barley or soft fruit, neither of which are hosts to *M. persicae*.

Myzus persicae *samples used for comparison of DNA extraction and amplification methods*

Twenty-seven *M. persicae* caught in a 1.5 m suction trap (Macaulay *et al*., 1988) in trap fluid (65% ethanol, 30% water, 5% glycerol) at the Scottish Agricultural Science Agency (SASA), Edinburgh in July 1995 were used to test DNA extraction techniques and PCR amplification. After collection, the aphids were identified and stored in fresh trap fluid at room temperature in the dark. Eight years later, for the purposes of this study, they were transferred to 95% ethanol. DNA was extracted using the four methods described below and measured using a nanodrop spectrophotometer (Nanodrop Technologies, USA). Microsatellite loci (M49, M63 and M86, Sloane *et al*., 2001) were amplified using three PCR methods. Once amplified, products were separated using 10% polyacrylamide gel electrophoresis in a discontinous buffer system and then visualized with ethidium bromide (Kumar *et al*., 1999). For more accurate size determination an ABI 377 (96) automated sequencer. Genescan v3.4 and Genotyper v2.5 software (Applied Biosystems, USA) were used to analyse fluorescently labelled PCR products (Fenton *et al*., 2005).

DNA extraction methods

Method 1: urea plus phenol chloroform (Chia *et al*., 1985)

Individual aphids were homogenized in grinding buffer (100 mM Tris-HCl, 350 mM NaCl, 10 mM EDTA, 2% SDS and 7 M urea) and incubated for 10 min at 60°C. Proteins were extracted using phenol: chloroform: isoamyl alcohol in a ratio of 25 : 24 : 1 and chloroform: isoamyl alcohol in a ratio of 24 : 1 and the DNA precipitated in ethanol. DNA was suspended in a final volume of 40 µl TE buffer.

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Method 2: salting out (modification of Sunnucks & Hales, 1996)

Individual aphids were homogenized in TNES buffer (50 mM Tris-HCl pH 7.5, 400 mM NaCl, 20 mM EDTA, 0.5% SDS) and incubated at 37°C overnight in the presence of proteinase K (100 μg ml^{-1}). Proteins were removed using 5 M NaCl, and the DNA was precipitated in ethanol. DNA was suspended in 40 μl TE buffer.

Method 3: Chelex 100 chelating ion exchange resin

Individual aphids were homogenized in 40 μl 5% Chelex 100 (Bio-Rad, Hemel Hempstead, Hertfordshire, UK), mixed well and incubated at 56°C for 35 min. The sample was incubated at 95°C for 15 min. The sample was mixed a second time and centrifuged at 1200 rpm for 2 min. The supernatant was collected and stored at −20°C.

Method 4: sodium hydroxide extraction (modification of Klimyuk *et al.*, 1993) (Stanton *et al.*, 1998)

Individual aphids were incubated at 25°C in 20 μl 0.25 M NaOH for 16 h. After further incubation in a thermocycler at 99°C for 3 min, 10 μl 0.25 M HCl, 5 μl 0.5 M Tris-HCl (pH 8) and 5 μl 2% Triton X-100 were added. The sample was incubated for a further 3 min at 99°C and left to cool to room temperature before storing at −20°C.

PCR amplification

Method 1: PCR Ready-to-go beads

PCR Ready-to-go beads (Amersham, UK) were used according to the manufacturer's instructions. When a bead is reconstituted to a 25 μl volume the concentration of each nucleotide is 200 mM in 10 mM tris-HCl, 50 mM KCl and 1.5 mM $MgCl_2$ and 2.5 units *Taq* DNA polymerase. 100 pmol of each primer was used and 1 μl aphid DNA was used as the target. 25 μl of PCR product was electrophoresed on the acrylamide gels.

Method 2: Taq *polymerase*

The PCR conditions were as follows: 1× strength reaction buffer consisted of (5 mM Tris, 10 mM NaCl, 0.01 mM EDTA, 0.1 mM DTT, 5% glycerol and 0.1% Triton X-100), 2 mM MgCl2, 200 μM dNTPs, 100 pmol each primer and 2.5 units *Taq* polymerase (Promega, UK). PCR was carried out in 20 μl volumes of the reaction buffer. 1 μl aphid DNA was used as the target and 20 μl of PCR product was electrophoresed.

Method 3: high fidelity Taq *polymerase*

The PCR conditions were as follows: high fidelity PCR enzyme mix (ABgene, UK) (2.5 units *Taq* polymerase) 2 mM dNTPs, 2.25 mM $MgCl_2$ (constituents of extensor buffer not disclosed by manufacturer) and 100 pmol each primer. PCR was carried out in 50 μl volumes. 1 μl aphid DNA was used as the target and 40 μl of the PCR product was electrophoresed.

PCR programme

The PCR programme (Touchdown programme PMS2 (Sloane *et al.*, 2001)) consisted of: one cycle 94°C for 2 min, 55°C for 30 s, 72°C for 45 s, one cycle 94°C for 15 s, 53°C for 30 s, 72°C for 45 s, one cycle 94°C for 15 s, 51°C for 30 s, 72°C for 45 s, one cycle 94°C for 15 s, 49°C for 30 s, 72°C for 45 s, 30 cycles of 94°C for 15 s, 47°C for 30 s, 72°C for 45 s and one cycle 94°C for 15 s, 47°C for 30 s and 72°C for 2 min.

Myzus persicae *suction trap samples*

After determining the optimum method on some Edinburgh samples, DNA was extracted from the remaining samples and 329 *M. persicae* alatae caught in 2001 in a 12.2 m suction trap (Macaulay *et al.*, 1988) located at SCRI, Dundee using method 4 (see above). The 329 aphids were a subsample of the 1235 *M. persicae* caught in 2001. The extractions were carried out in 2003, therefore these specimens had been stored in trap fluid for 2 years. Microsatellite markers were amplified using PCR beads (Amersham, UK) in a multiplex reaction. Each PCR bead was divided into two reactions and used to analyse two specimens. This was carried out by reconstituting the bead in 24 μl of reaction mix (sterile water and multiplex primers). This solution was then divided into two 12 μl aliquots before the target DNA was added. All PCR products were analysed on an ABI genotyper (Applied Biosystems, Foster City, USA). Individuals were analysed from each week beginning 2 July (week 27) when the first *M. persicae* was caught in the trap and ending on 12 November (week 46) when the last one was caught. Sub-samples were taken on days when very large numbers of alatae had been caught.

Results and Discussion

Assessing DNA extraction methods and PCR techniques

PCR Ready-to-go beads

Primers M86F and R were used in conjunction with PCR beads to amplify DNA extracted from alata using the four methods described above. The products were analysed on acrylamide gels. The correct products were clearly visible from specimens extracted using methods 3 and 4, with 4 giving the clearest bands (fig. 1, panel A, lanes 2–9). Specimens extracted using methods 1 and 2 produced only faint bands (fig. 1, lanes 10–13). As stained acrylamide gels are not very sensitive, the amplification was independently repeated using multiplex PCR (primers M49, 63 and 86) with the reverse primer of each pair end-labelled with a different fluorochrome (see Fenton *et al.*, 2005). 0.8 μl of these products were run on an ABI genotyper, which uses a laser to scan for the fluorochrome tagged products as they migrate through an acrylamide matrix. This method is more sensitive than acrylamide gels and it was possible to find clear products in specimens extracted using methods 2–4, with those from method 4 again giving the best results (results not shown).

Taq *polymerase*

DNA from the four extraction methods was subjected to PCR using *Taq* polymerase (Promega, UK) and primers M86F and R. The products were electrophoresed on acrylamide gels and the results are shown in fig. 1, panel B. As with amplification using PCR beads, clear bands were not visible from specimens extracted using methods 1 and 2 and one specimen from method 3 (lanes 10, 11, 12, 13 and 8). The remaining samples worked, but the profiles were not as

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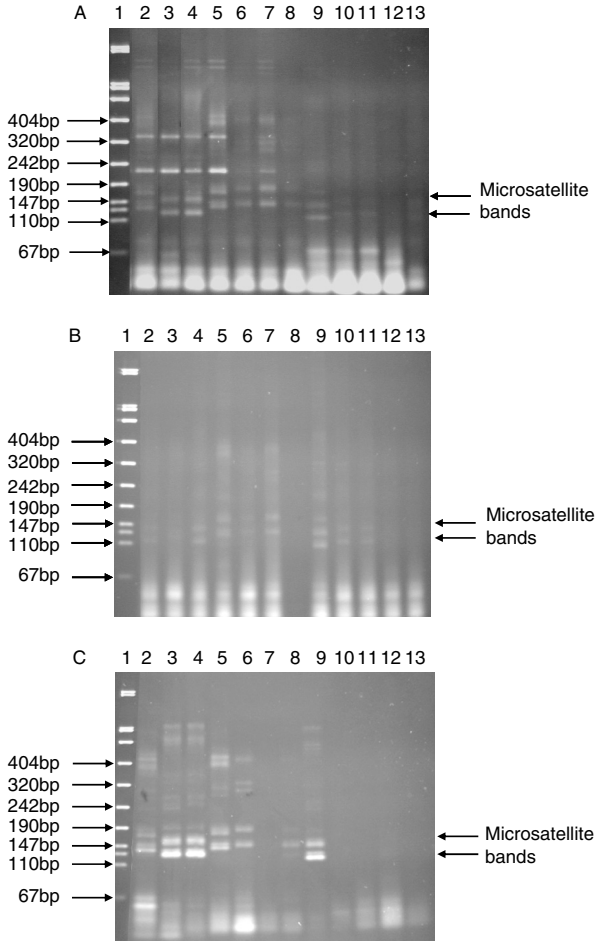

Fig. 1. Assessing the best DNA extraction technique and PCR amplification method using microsatellite analysis *of Myzus persicae* specimens caught in the Edinburgh suction trap in 1995. Panel A, Amersham Ready-to-go beads; panel B, Promega *Taq* polymerase; panel C, ABgene high fidelity *Taq* polymerase. Primers used were (M86F and M86R, Sloane *et al.*, 2001). Lanes are as follows: 1, molecular weight marker VIII (Roche, UK); 2, method 4, sample 1, Clone C; 3, method 4, sample 2, Clone I; 4, method 4, sample 3; Clone I; 5, method 4, sample 4, Clone J; 6, method 3, sample 1, Clone C; 7, method 3, sample 2, Clone C; 8, method 3, sample 3, Clone C; 9, method 3, sample 4, Clone G; 10, method 2, sample 1, Clone J; 11, method 2, sample 2, Clone J; 12, method 1, sample 1, Fail; 13, method 1, sample 2, Fail.

well defined as those from the PCR beads (panel A). PCR products were not analysed with the fluorescent primers and ABI genotyping.

High fidelity Taq *polymerase*

DNA from the four extraction methods was subjected to PCR using high fidelity *Taq* polymerase and primers M86F and R. The products were separated on acrylamide gels and the results are shown in fig. 1, panel C. Very good products were obtained from the DNA of all specimens extracted using method 4 and two of those extracted using method 3. However, the other two specimens extracted using method 3, lanes 7 and 8 (fig. 1), gave low yields. Both extraction methods 1 and 2 failed to give visible products. It was noted that the correct microsatellite bands were more pronounced in this analysis (bands 100–200 bp, fig. 1) than the equivalent bands in panel A, where the background non-specific bands were more pronounced (band sizes 250–350 bp, fig. 1).

In conclusion, extraction method 4 produced distinct band profiles with all three of the PCR methods. The high fidelity *Taq* polymerase (ABgene) was the most successful at amplifying the correct target DNA. However, we chose to use PCR beads (Amersham, UK) to analyse the specimens from the Dundee suction trap as they also produced the correct amplicons, but have the advantage of lower cost and ease of use which reduces risk of operator error.

Success rate of the genotyping technique

DNA was extracted from 329 specimens from the Dundee suction trap using method 4 and 239 produced successful PCR products. The PCR failure rate from 2001 was higher (>27%) than the failure rate of the samples from the 1995 Edinburgh suction trap used to assess the technique (15%). The quality and quantity of extracted DNA was measured from a subset of the 2001 samples. There was no clear pattern and successful reactions originated from the complete concentration range of 40 to 150 ng of DNA. It was noted that the high failure rates from the Dundee trap samples appeared to be clustered on certain capture dates and some of the possible causes of this were investigated. The Dundee suction trap collects specimens on a daily basis but the containers, which are on a rotating wheel, are emptied twice a week, on a Monday and Friday. This means collections correspond to four-day (Monday–Thursday) and three-day (Friday–Sunday) intervals. This results in some specimens being stored for several days in the trap fluid, which may be subject to evaporative loss of the alcohol as well as dilution with rainwater, before they are sent for identification and moved to permanent storage in fresh trap fluid. This could take a week for the most delayed samples (Mondays). Suction trap failure and weather data were studied to see if there was any obvious link between high daytime temperatures on the days that the specimens were captured and failed PCR, but none was found. Failures occurred from samples collected throughout the season with the two largest proportions occurring on 25/26 August (week 34, 16/30) and 21/22 September (week 38, 12/50). A chi-squared analysis, assuming that over the entire season each day should have caught approximately equal numbers of alatae, found that there was a significant deviation from this hypothesis ($\chi^2 = 63.58$, 6 df, $P < 0.005$). This revealed that the alatae had been trapped disproportionately, with 628 of the total (1234) trapped on a Friday, Saturday or Sunday when 529 (3/7) would have been expected by chance. Correcting for these catch frequencies revealed that there were more failures associated with Saturday than simply due to larger numbers being caught and analysed from this day. Conversely, there were more successes on a Sunday than would have been encountered by chance. Sunday is the day with the shortest interval between collection and analysis/storage.

In conclusion, it would appear that there are no clear environmental factors in the collection, storage and subsequent extraction of individual alatae that contribute to genotyping failures. However, there were days of the week when more alatae had been collected and these days had

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Table 1. Distribution of *Myzus persicae* clones collected in the suction trap at Dundee in 2001 and at Edinburgh in 1995.

Week	Date collected	Number		Clone							Other	Fail
		collected	extracted	A	B	C	D	E	I	J		
	2001											
27	2 Jul–8 Jul	1	1						1			
29	16 Jul–22 Jul	2	2						1			1
30	24 Jul–28 Jul	5	4						1	2	1	
31	30 Jul–5 Aug	3	3			1				1		1
32	6 Aug–12 Aug	4	4						1	2		1
33	13 Aug–19 Aug	15	13	5	1	1	1		2	2		1
34	20 Aug–26 Aug	131	64	27	2	4		3	1			27
35	27 Aug–2 Sep	164	28	14	1	2	1	2		3	1	4
36	3 Sep–9 Sep	59	8	5		2				1		
37	10 Sep–16 Sep	94	24	13	2	3		1	1			4
38	17 Sep–23 Sep	550	76	41	3	3	2	6	2	3		16
39	24 Sep–30 Sep	156	63	34	5	2	2	5		2		13
40	1 Oct–7 Oct	36	25	14		1			1			9
41	8 Oct–14 Oct	9	9									9
42	15 Oct–21 Oct	4	4									4
43	22 Oct–28 Oct	1	0									
46	12 Nov–18 Nov	1	1					1				
Total		1235	329	153	14	19	6	18	10	17	2	90
	1995		27			7			3	12	1	4

Specimens were trapped from week 27 until week 46. In weeks 34–40 large numbers of flying *M. persicae* were trapped, therefore for these weeks only a sub-sample of the specimens were tested. The number collected in each week is shown (column 2) and the number of specimens on which DNA extraction was carried out is indicated (column 3). The number of each genotype is shown (columns 4–11) and the number that failed to produce PCR products is shown in the final column. A sub-sample of the catch from Edinburgh in 1995 was analysed. These specimens were caught in weeks 28–30.

disproportionately more successes (Sunday) and failures (Saturday). The non-random nature of the collections could be due to a variety of factors and some of these will be discussed after the genotyping results, but human activities on crops (such as spraying) are likely to lead to weekly activity patterns of *M. persicae* living in these crops.

Relative frequencies of each clone in 1995

In addition to providing material to test the extraction methods, the 1995 Edinburgh trap results gave an insight into the clonal composition of the 1995 *M. persicae* population. The analysis found that 52% of the individuals belonged to clone J, 30% to C and 13% to I (see table 1). A single representative of a new genotype (G) was also found. Intergenic spacer (IGS) fingerprinting had been used to characterize Scottish individuals collected from fields in 1995 (Fenton *et al*., 1998). However, the IGS technique failed to distinguish genotypes I and J (Fenton *et al*., 2005). Recent work has shown that the majority of these 1995 samples belonged to the same clones as found in 1995 in the Edinburgh suction trap: J (39%), C (31%) and I (17%) (L. Kasprowicz *et al*., unpublished). The IGS fingerprints of clone C are now believed to display intraclonal diversity (see Fenton *et al*., 2005).

Relative frequencies of each clone in 2001

A 12.2 m suction trap will collect a sample of flying aphids representing an area around the trap depending on the aphid species and the surrounding geography and vegetation. In 2001, the results of detailed field studies within 50 km of the Dundee trap were available and this made it possible to identify the likely contribution of different sources to the flying *M. persicae*. Seven main *M. persicae* clones were found in both the suction trap and the field in 2001 (table 2 and Fenton *et al*., 2005). Three of these clones (C, I and J) were detected in 1995, but the others (A, B, D and E) were not (table 1). The proportions of the clones caught in the trap and the field are shown in fig. 2a, b. Both collections consisted of all clones, but the trap collected considerably greater proportions of clone A than had been collected in fields. The relative proportions of genotypes in the trap and the field were compared statistically using a χ^2 analysis. The result was a $\chi^2 = 54.54$ (13 df, $P < 0.005$) indicating that the two distributions are significantly different. After removing clone A the two distributions were more similar (fig. 2c,d) and the test was no longer significant ($\chi^2 = 14.3$, 11 df, $P > 0.05$). This suggests that the samples caught in the trap and in the field consist of a similar proportion of genotypes apart from clone A. There is no evidence of local crop types in Scotland influencing *M. persicae* clone distribution at a field scale, but the types of insecticide used on a crop have a very strong influence, as they will select for different types of resistant clone (Foster *et al*., 2002; Fenton *et al*., 2005). The field samples were therefore divided into those originating from insecticide treated and untreated fields (fig. 2e,f).

The treated fields, which were mostly seed potatoes, were dominated by a highly insecticide resistant clone A (fig. 2e), which carried three resistance mechanisms, modified acetylcholinesterase (MACE), kdr and R_3 esterase (table 2). It was found alongside a second multiply resistant clone B (table 2), which was red in colour whereas all the other clones were green. The mechanisms found in clones A and B rendered them resistant to all the insecticides available for use in 2001 (see Fenton *et al*., 2005). In addition to A and B, clones D and E were present in treated areas. Representatives of

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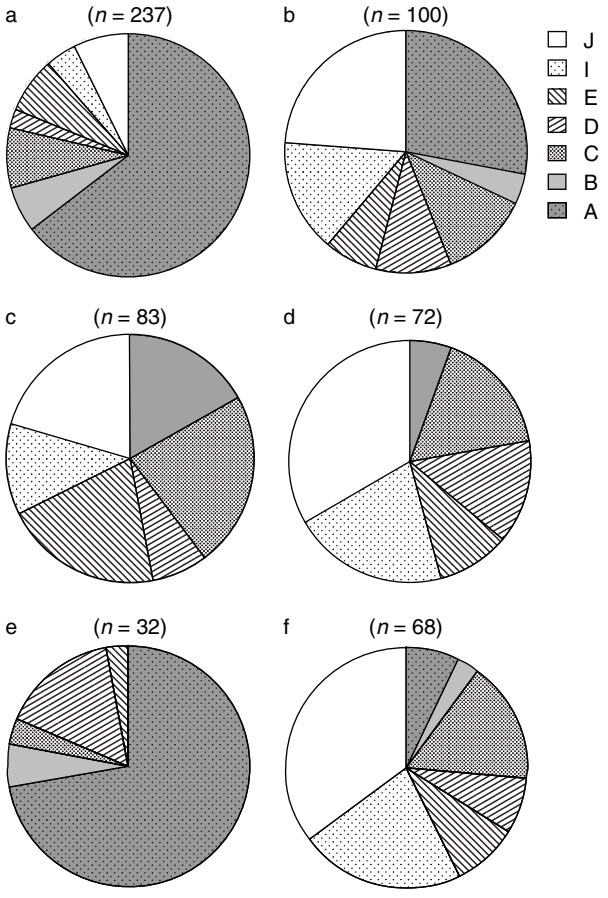

Fig. 2. Relative distribution of *Myzus persicae* clones in the Dundee suction trap (a), at all sampled field sites within a 50 km radius (b). c and d are the same as a and b but without clone A. Clone distribution in insecticide treated (e) and untreated (f) field sites within a 50 km radius.

these clones have high levels of esterase-based insecticide resistance (R_3) and clone D also has kdr. Clone C was present at very low levels in treated fields, presumably gaining an advantage over completely sensitive clones (I and J) from low levels of esterase and kdr, but it was found significantly more often in untreated fields (Fenton *et al*., 2005).

The untreated potato, swede and oilseed rape fields had quantitative and qualitative differences in clonal composition from treated fields (fig. 2f). Not surprisingly, the two sensitive clones I and J, were present and in large numbers whereas they had been totally absent from the treated fields. Clone A was less frequent in untreated than in treated crops while clone C was more frequent in untreated than in treated crops. Clones B, D and E were also present in untreated fields. Thus, despite its advantage in treated crops, clone A did not do well in untreated crops. This was not because clone A appeared late in the season as it was one of the earliest to be detected in the field in 2001 (see fig. 4a). Its lack of success may be due to fitness costs associated with insecticide resistance such as a reduced response to alarm pheromones and an increased susceptibility to parasitoids (Foster *et al*., 2005). Overwintering ability, known to be a disadvantage to resistant clones (Foster *et al*., 1997), is not a factor in the current, single season study.

These comparisons showed that the closest match to the overall proportion of clone A *M. persicae* in the suction trap (fig. 2a) was material from insecticide treated seed potato fields (fig. 2e), which must have been producing very large numbers of alate *M. persicae*. χ^2 analyses were carried out comparing the proportions of A in the trap relative to treated or untreated fields. There was no significant difference between the observed and expected proportions of A from the treated areas and the trap ($\chi^2 = 0.21$, 1 df, $P > 0.05$). However, there was a significant difference between the proportions of A in untreated areas and the trap ($\chi^2 = 33.6$, 1 df, $P < 0.001$). At this time the treated crops were the only source of alate clone A as, apart from one example at the start of the season, it was not found in untreated areas until after the large flights detected by the trap. The similarity between the proportions of type A found in the treated fields and the proportions found in the trap suggests that it was not producing significantly more alate forms than other genotypes. The insecticide spray regime on the seed potato crop is the most intense of any crop in the local area. For the fields sampled, the regime was known to consist of carbamate and carbamate/pyrethroid mixtures, with up to six applications per crop. These were the ideal conditions for selecting MACE, esterase and kdr-carrying *M. persicae* such as clones A and B. Any farmers noticing these aphids were likely to have applied further doses of carbamate or pyrethroid insecticides, which would only have succeeded in killing insect aphid predators and parasitoids as well as other insecticide sensitive *M. persicae* genotypes.

The temporal distribution of clones in 2001

To help investigate temporal changes in clone frequencies, trap samples were subdivided into time periods and the proportions of each clone plotted on a bar chart (fig. 3). Each date falls into a week, with 1 January counted as beginning week 1. Within the bar chart is a graph of the numbers of alatae caught in the trap during that period. To help relate the temporal information from the trap to field populations, supplementary graphs show the cumulative build up of each clone in treated and untreated crops (fig. 4). However, unlike the constant operation of the suction trap, the amount of material collected from a field was influenced by factors such as our choice, treated vs. untreated, crop availability, access, resources for collection, weather, etc. The SCRI experimental field was visited on a regular basis. This was also the closest field to the suction trap, and the alatae arriving and colonizing it should have been representative of the material arriving at the trap.

Prior to 14 Aug (week 33) the suction trap collected only clones C, I and J. The first of the entire season was a J type collected in the trap in week 27. An I type followed in week 29 and a C type in week 31. While the initial numbers of individuals of these clones in the trap were small, many lines of evidence point to these being the locally abundant clones (Fenton *et al*., 1998, 2005). They formed almost the entire collection in 1995 (see above) and subsequent years (unpublished observation). In 2001, they were detected in oilseed rape crops as early as week 26 (J) or 27 (C and I) and their frequency remained high on these and other untreated crops throughout the season (fig. 4c,f,g). At this early time it is likely that these clones were moving from winter brassica

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Table 2. Microsatellite allele sizes and insecticide resistance properties for the seven main *Myzus persicae* clones found in Scotland in 2001.

Clone	Carboxylesterase	kdr	MACE	35		49		63		86	
				1	2	1	2	1	2	1	2
A	R_3	+	+	196	196	149	156	174	184	113	138
B	R_1/R_2	+	+	196	202	156	159	169	204	99	138
C	R_1/R_2	+	−	186	196	153	165	167	172	136	140
D	R_2/R_3	+	−	186	196	153	153	174	204	125	138
E	R_2/R_3	−	−	198	202	156	163	167	172	101	107
I	S/R_1	−	−	186	196	153	204	169	169	125	140
J	S/R_1	−	−	186	186	153	165	169	172	115	140

The first column identifies each clone or genotype. The clones were designated using microsatellite markers (35, 49, 63 and 86 Sloane *et al*., 2001). The size of each allele pair is shown in columns 5–8. Columns 2–4 show the results of three insecticide resistance tests.

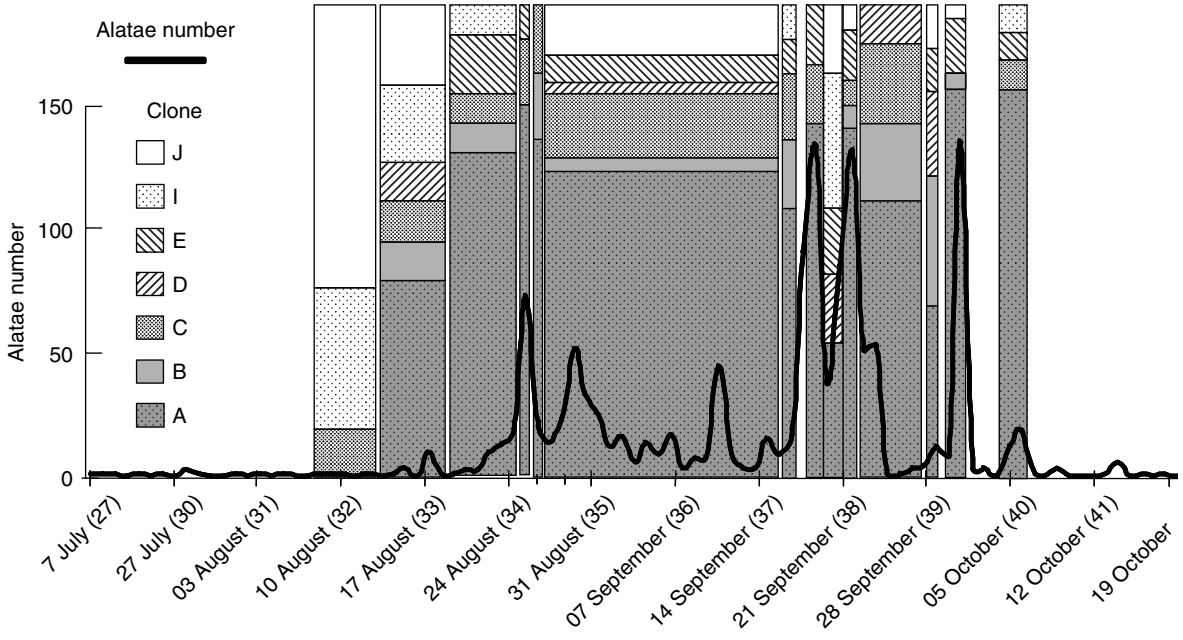

Fig. 3. Suction trap data for 2001. The numbers of alate *Myzus persicae* caught in 2001 are plotted against time (line). Week 1 corresponds to 1–7 January. The X axis is truncated at the start. The relative distribution of genotypes as a proportion of the total catch for the week is shown as a bar-chart (see key for clones on left). Genotype A (a highly insecticide resistant clone) predominates from week 33–40 forming >50% of the total catch.

crops (Jacob, 1940; Cocu *et al*., 2005c) where insecticides are not used for *M. persicae* control.

Week 33 represented a transition in the clones being caught in the trap with the capture of the first resistant clones (A, B and D, figs 3 and 4). Clone A immediately arrived in bulk with numbers equivalent to the clones C, I and J, which had been building up gradually. This is represented by a sharp increase in clone A at week 33 (fig. 4a) and a small peak in the graph (fig. 3). In the field the situation was different, as both clones A and B had been collected from untreated fields near the trap early in the season in week 27, the same time as clones C, I and J. However, despite their early arrival (they were collected as alatae) these clones did not increase in numbers on the untreated crops during the first six weeks nor were they detected in the trap during this time. These observations demonstrate the potential early warning value of field sampling from crops as the breakdown in insecticide control later in 2001 could have been predicted.

By week 34, with the addition of E, all clones had been collected in the trap. Clone E was the only clone detected in the trap before the field (fig. 4e). From this week onwards the proportion of clone A in the suction trap far outnumbered all other clones. The peaks of alatae on the 25 and 29 August and the 10, 18, 21 and 30 September were almost entirely composed of clone A (fig. 3). The only exceptions to this were the periods 19–20 September and 28 September when more clones representative of untreated areas (C, I and J) were collected (fig. 3). This would be consistent with constant migration from these areas (fig. 2f; fig. 4c,f,g). In the presence of the large flights of clone A the detection of other clones had been proportionately reduced in our samples. In the field, clone A increased in numbers on untreated crops after week 34, almost certainly having

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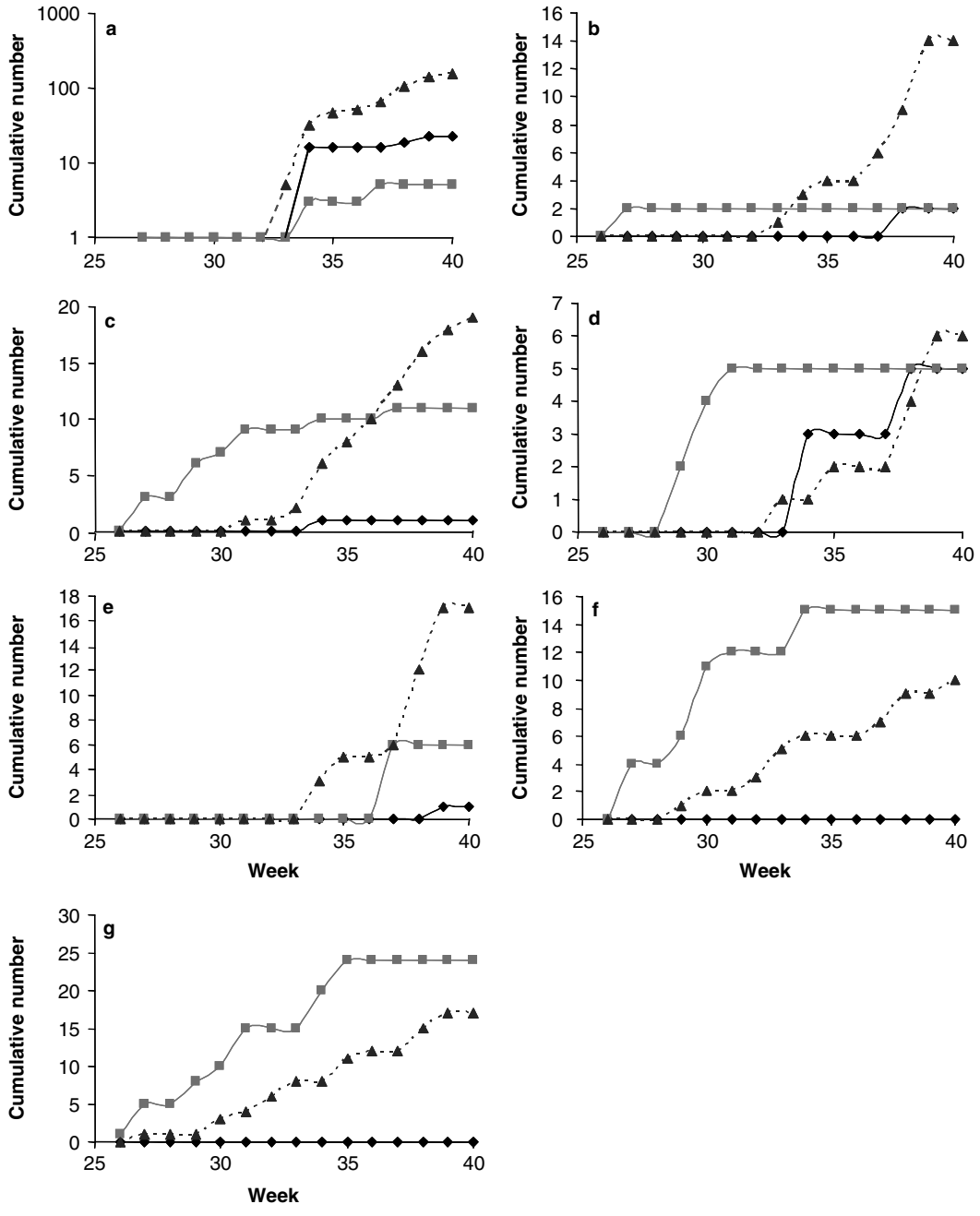

Fig. 4. Temporal distribution of *Myzus persicae* clones in the trap (--▲--) and in treated (–◆–) and untreated (—■—) fields. Samples were not taken from the field in weeks 28, 36 and 37. a, clone A; b, clone B; c, clone C; d, clone D; e, clone E; f, clone I; g, clone J.

moved from the potato crops as this followed the large numbers of clone A flying into the suction trap. It seems likely that any disadvantages clone A may have had in untreated areas were temporarily overcome by very large numbers. Clone A was detected in treated fields at the same time (fig. 4a). At first this seems at odds with their simultaneous arrival in the trap as they will have required a period of reproduction and expansion. However, while untreated sites had been sampled regularly, as these sites avoided problems with selection, the growing commercial seed potato crops, which have a shorter season than winter rape, were only visited from this date onwards. By this time the populations of clone A had already reached high levels in the seed potato crops and it seems highly likely that this was the source of clone A in the trap and untreated fields. Field reports also suggest that these resistant populations had increased rapidly at the end of the seed potato growing season (J. Pickup, unpublished observation).

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The record number of M. persicae *caught in the Dundee trap*

The number of flying *M. persicae* collected in the Dundee suction trap in 2001 (1235) far exceeded every other year and every other trap in Scotland. In comparison, the mean total catch recorded at Dundee between 1984 and 1995 was 106. In recent years larger totals were recorded at Dundee in 1996 (240) and 2000 (227). The trap at Edinburgh, 60 km from the Dundee trap, recorded similar peaks of flying *M. persicae* in weeks 35 and 38 in 2001. However, the total number of *M. persicae* (90) was less than one tenth of the number caught in Dundee.

The exceptionally high *M. persicae* numbers recorded in the Dundee trap in 2001 are consistent with the following: (i) MACE-carrying multiple resistant genotypes A and B arrived for the first time in Scotland in 2000/2001, probably from England as there are close matches to these genotypes in England from 1996 (Fenton *et al*., 2005) and (ii) MACE clone A and to a lesser extent clone B built up rapidly on treated seed potato crops, but not on untreated crops. Large numbers on the crops do not in themselves account for the peaks in flight activity shown on certain days and dates. However, as the composition of the peaks is now known, it is possible to reduce the likelihood of some factors. The distribution and dynamics of *M. persicae* flight is influenced by environmental variables (Harrington *et al*., 1995). Weather, in particular temperature, could have created good flying conditions, but this should have been equally good for all clones, yet the peaks were almost entirely composed of clone A. Maximum daytime temperature, rain and wind were examined for the time periods involved. The six largest *M. persicae* flights occurred when the midday temperatures were 19, 18.1, 15.8, 15.7, 12.8 and 17.2°C, quite a range of temperatures and yet there were warmer days before and after the peaks. The same is true for wind and rain and five of the peaks occurred during a long period of low wind and rain. Aphid count data and its association with climate have been studied using spatial analysis by distance indices (SADIE) (Cocu *et al*., 2005a). High temperature and low rainfall were identified as environmental factors that are positively associated with aphid abundance across north-west Europe. However, the distribution of *M. persicae* in 1989, 2000 and 2001 was different from the other years studied (1990–1999) and climatic conditions did not explain the observed spatial structure of *M. persicae* (Cocu *et al*., 2005a). The authors suggest that other variables such as land-use, food resources, presence of suitable host plants and natural enemies played a more important role in structuring the annual abundance of *M. persicae* in these years. A separate study (Cocu *et al*., 2005b) highlights that specific land use types within agricultural crops play a key role in determining total aphid numbers in north-west Europe. In the current study, we suggest that the failure of insecticide treatments after the arrival of new resistant genotypes played a major role in the abundance of *M. persicae* caught in the Dundee suction trap in 2001. These large *M. persicae* flights also correlated with the time periods for the burning down or natural senescence of either seed or ware potatoes in the local area, where it is now known that *M. persicae* control was likely to have failed. Burn down involves the application of chemicals to destroy the plant haulms which is carried out by local farmers in a very short and synchronized period of time. The haulm of seed crops is destroyed a few weeks earlier than that of ware crops as seed potatoes are usually sold at a smaller size. This process could also explain the noticeable daily effect of high *M. persicae* numbers as local farmers may have carried this out on certain weekdays. Application of herbicides or sulphuric acid for burn down would cause disturbance within the crop and result in large numbers of flying *M. persicae* searching for new hosts.

Ecologists have developed models to examine the spatial patterns of species which help understand the mechanisms that control their distribution. This has important implications for pest management and prevention strategies (Cocu *et al*., 2005b), as well as predicting the effects that global climate change may have on the insect pests and beneficial insects of agriculture in the northern hemisphere (Harrington *et al*., 2001). This can be carried out at a continental scale using suction traps which still provide the most accurate estimates for predicting aphid spray thresholds. However, it is clear from the current study that an understanding of local crops, farming practices and an ability to sample fields will provide valuable early warning information and fill in the gap between the early presence of new types of insecticide resistant *M. persicae* in fields and their detection by suction traps. This will be most effective in regions where there is a delay in the arrival of new mechanisms and where information about clones has already been determined. The ability to analyse the clonal composition as well as abundance of *M. persicae* in suction trap material and relate this to their field distribution will also lead to a new understanding of the population dynamics and agroecology of *M. persicae* and greatly assist in the interpretation of trapping and monitoring data for this species in relation to determining the *M. persicae* populations most likely to contribute to PLRV spread.

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