ALTERNATIVE INSECTICIDES TO CONTROL CEREAL APHIDS, *SITOBION AVENAE*, THAT ARE RESISTANT TO PYRETHROIDS

A M Dewar[1], A J G Dewar[1], L A Haylock[1], S P Foster[2] and M S Williamson[2]
[1]*Dewar Crop Protection Ltd., Drumlanrig, Great Saxham, Bury St. Edmunds, Suffolk IP29 5JR*
[2]*Rothamsted Research, Harpenden, Herts., AL5 2JQ*
E-mail: alan@dewarcropprotection.co.uk

Summary: Cereal aphids, *Sitobion avenae*, have developed resistance to pyrethroids in the last few years, culminating in control failure at some locations in England 2011 and 2012, associated in the latter year with local epidemics of barley yellow dwarf virus (BYDV). Studies by Rothamsted Research and Syngenta have shown this to be due to target site resistance, which can be detected in populations by using a vial test, and in individual aphids using a DNA diagnostic test. Field trials in autumn 2012 using inoculations of a clone of *S. avenae* that was resistant to pyrethroids showed that cypermethrin at 25 g a.i./ha and deltamethrin at 7.5 g a.i./ha gave significantly poorer control than chlorpyrifos at 450 g a.i./ha, but not lambda-cyhalothrin at 7.5 g a.i./ha. In two trials to test alternative products, the carbamate pirimicarb at 120 g a.i./ha gave moderate control at one site, but good control at another; with neonicotinoids, thiacloprid gave good control at both sites, while acetamiprid at 50g a.i./ha was relatively poor. Pymetrozine at 100 g a.i./ha plus adjuvant oil gave good control at the one site it was tested. Chlorpyrifos gave best control. None of these alternatives are currently approved for use against aphids in cereals in the autumn, but these results will give regulators some evidence for their activity against resistant grain aphids, should alternatives be required.

INTRODUCTION

Control of the grain aphid, *Sitobion avenae* has relied heavily upon the use of pyrethroids in recent years, largely because they are cheap, and readily mixable with fungicides in disease control programmes. As a consequence, the majority of cereals are treated every year in the autumn to control aphids carrying barley yellow dwarf virus, often as an insurance, and sometimes twice a year when cereals are also treated with pyrethroids in the summer to control grain aphids. The most recent survey done on pesticide use in the UK showed that 71% of winter barley and 76% of wheat was treated by one of five pyrethroids in 2012, although no distinction was made between summer and autumn use (Table 1; Garthwaite *et al.*, 2013), and this was largely unchanged since the previous survey in 2010 (Garthwaite *et al.*, 2011).

Table 1. Proportion of cereals (% of census area) treated with pyrethroids in the UK in 2012 mostly to control aphids (Garthwaite *et al.*, 2013).

Insecticide AI	Winter barley	Spring barley	Wheat
Cypermethrin	31	4	33
Lambda-cyhalothrin	19	6	24
Esvenvalerate	10	3	8
Alpha-cypermethrin	6		5
Zeta-cypermethrin	5	2	6
Total	**71**	**15**	**76**

Not surprisingly, this usage, whether or not it is needed, has resulted in selection of resistant aphids. Their occurrence was first described in 2012 (Foster *et al.*, 2013), but have since been recorded in many sites across the UK following a survey funded by Syngenta, backed up by further tests using a DNA-based diagnostic assay developed at Rothamsted (Williamson & Foster, In Preparation). In 2012, of 17 samples collected from East Anglia, and tested using a vial test, which requires live aphids, 35% of the samples collected were classified as resistant, after less than 60% of the individuals in vial tests died at a discriminatory dose of insecticide (Table 2). In 2013, 38% of sixteen samples tested in vials were classed as resistant; of these and a further 14 that were collected that year, but tested only using the DNA assay, 50% contained some individuals that carried the *kdr* mutation conferring resistance to pyrethroids. These results correlate well with similar testing of dead *S avenae* caught in Rothamsted Insect Survey suction traps operating in the same areas, namely the Broom's Barn, Rothamsted and Kirton traps in east and central England (Williamson & Foster, In Preparation).

Table 2. Resistance status of *S. avenae* collected from cereals in the UK in 2012 and 2013.

Year	Test method Insecticide-coated vials		DNA-based assay	
	No of samples	% resistant (mortality <60%)	No of samples	% resistant (>50% heterozygotes)
2012	17	35	20	80*
2013	16	38	30	50

*in 17 of these samples tests were done on survivors of the vial test, hence the higher percentage

Thus, resistance has now become a feature of cereal aphid control, but perhaps has not been seen on a catastrophic scale yet because summer epidemics of aphids are relatively rare these days compared to the 1970's, due to the activities of predators. However, there is some circumstantial evidence that the mini-epidemic of BYDV seen in England in the spring of 2012, following a mild winter that would have allowed overwintering aphids to survive quite well, may have been exacerbated by resistant *S. avenae* that survived the traditional autumn sprays of pyrethroids used to control another aphid species, the bird-cherry aphid, *Rhopalosiphum padi,* which is regarded as the main vector of BYDV.

In response to this situation, experiments were set up to investigate the efficacy of three commonly used pyrethroids against resistant *S avenae* in winter sown cereals, and to test alternative insecticides that might be useful for controlling them if they become a serious problem in the future.

MATERIALS AND METHODS

Comparison of aphid clones

Two clones of *S. avenae,* one susceptible to insecticides (SS, homozygous susceptible), and one containing a single copy of the kdr gene (i.e. it was heterozygous for the *kdr* mutation-SR), were inoculated into plots of winter barley, cv Cassia not treated with an insecticidal seed treatment, sown on 21 September 2012 at Brooms Barn Research Centre in Suffolk. These were compared to a clone of susceptible *R. padi.* Each 2 x 6 m plot was inoculated with each of the three clones in a randomised position down the centres of the plot. Inoculation was achieved by placing a tiller of barley infested with 50-100 aphids along a short length of row in which at least 8 plants were present. As the tiller dried up, aphids moved from these tillers onto the young seedlings. Treatments were applied on 5 November using a Trials Equipment 'Lunchbox' sprayer with a 2 m offset boom sprayer delivering 200 L/ha through Tjet flat fan nozzles.

Alternatives to pyrethroids

In two larger trials only the resistant clone of *S. avenae* was used for inoculation. Trials were carried out at two sites, one in the same field at Broom's Barn as the clone trial, and the other at Stetchworth Estates, Cambridgeshire (sown on 29 September), using the same winter barley seed. In these trials there were 6 inoculation points in each plot, 3m from either end and one in the centre, 1 m in from each side – i.e. two rows of three inoculation points. Insecticides were applied as before, but two passes were made with the sprayer, one up each side of the plot, treating half the plot with each pass. Plots at Broom's Barn were infested on 17 October at GS 13 and sprayed on 20 October; plots at Stetchworth were infested on 19 October at GS12 and sprayed on 23 October.

Treatments

In the clone trial, three pyrethroids, lambda-cyhalothrin (Hallmark Zeon from Syngenta), cypermethrin (Toppel 10 from United Phosphorus Ltd) and deltamethrin (Decis Protech from Bayer) were compared with the organophosphorus product, chlorpyrifos (Dursban WG from Dow Agrosciences). In the efficacy trials comparing alternatives to pyrethroids, seven treatments including an untreated control were tested at Broom's Barn, but an additional treatment was included at Stetchworth that had not been available when the trial at Broom's Barn was sprayed. Treatments included lambda-cyhalothrin and cypermethrin, the carbamate pirimicarb (Aphox from Syngenta), chlorpyrifos, two neonicotinoids, thiacloprid (Biscaya from Bayer) and acetamiprid (InSyst from Certis), and, at Stetchworth only, pymetrozine (Plenum from Syngenta). Rates of application are shown in Table 4.

Assessments

In all three trials, aphids were counted on 8 plants at each inoculation point, on three occasions 3, 6-8 and 13-15 days after sprays were applied. Only selected but representative data are presented here.

Data analyses

Data were analysed by analysis of variance using Genstat XV. Aphid numbers were transformed (log N+1) prior to analysis.

RESULTS

Comparison of aphid clones

All treatments reduced aphid numbers in each clone 3 days after spraying, but this was surprisingly not significant. However, 8 days after application, all treatments gave significant reductions compared to untreated (Table 3). In the susceptible clone of *S. avenae* control was over 85% three days after sprays were applied, rising to over 95% 8 days after application, with no significant differences between treatments. Similar results were observed with the susceptible clone of *R padi*, although deltamethrin gave slightly poorer control at the first assessment. However, with resistant *S. avenae*, , control by cypermethrin was significantly poorer than lambda-cyhalothrin and chlorpyrifos eight days after application; the performance of deltamethrin was intermediate between these treatments.

Efficacy of alternative treatments

In the other two trials where only the resistant *S avenae* clone was used for inoculation, best control after 6-8 days at Broom's Barn and Stetchworth was achieved with chlorpyrifos (99%), followed by lambda-cyhalothrin (85 and 94% resp.) and pymetrozine at the latter site (92%). Pirimicarb and thiacloprid gave moderate control at Broom's Barn (73-74%), but good control at Stetchworth (97 and 99% resp.) (Table 4). Poor control was given by acetamiprid (62%) at Broom's Barn, a little better at Stetchworth (82%), but poorest control was given by cypermethrin (47 and 66%) at Broom's Barn and Stetchworth respectively.

DISCUSSION

Indications from the clone trial showed that, for two pyrethroids at least, control of resistant *S. avenae* was significantly poorer than for the susceptible clones of both *S.avenae* and *R. padi*. The fact that lambda-cyhalothrin did not show the same effect as cypermethrin and deltamethrin might be due to the relatively high rate used – 7.5 g a.i./ha is the rate recommended for aphid control in winter oilseed rape, while 5 g a.i./ha is the rate suggested for wheat.

Of the alternatives tested as potential replacements for pyrethroids, chlorpyrifos was consistently the best, considerably better than thiacloprid. Pirimicarb worked well at one site but not so well at the other; acetamiprid was poor at both. Pymetrozine gave relatively poor control 3 days after spraying at Stetchworth, but eventually gave complete control there, suggesting that it is a slow starter, but comes good later.

Although chlorpyrifos and thiacloprid are approved for use in cereals, neither is currently approved for use against aphids in the autumn. This is also the case for acetamiprid and pymetrozine. These results will therefore give regulators some useful information about the comparative efficacy of these alternative compounds for potential future use.

Table 3. Effect of pyrethroids on the number of aphids per plant of susceptible and resistant *S. avenae,* and susceptible *R. padi.*on winter barley in 2012.

Clone	rate a.i./ha	3 DAA Log (n+1)	Back-trans	% control	8 DAA Log (n+1)	Back-trans	% control
Susceptible *S avenae*							
Untreated	-	0.650	3.47		0.487 a	2.07	
L-cyhalothrin	7.5	0.132	0.4	90	0.030 b	0.1	97
Cypermethrin	25	0.182	0.5	85	0.021 b	0.1	98
Deltamethrin	7.5	0.150	0.4	88	0.039 b	0.1	96
Chlorpyrifos	450	0.067	0.2	95	0.042 b	0.1	95
Resistant *S avenae*							
Untreated	-	0.595	2.94		0.484 a	2.05	
L-cyhalothrin	7.5	0.199	0.6	80	0.032 c	0.1	96
Cypermethrin	25	0.303	1.0	66	0.206 b	0.6	70
Deltamethrin	7.5	0.306	1.0	65	0.131 bc	0.4	83
Chlorpyrifos	450	0.141	0.4	87	0.021 c	0.1	98
Susceptible *R. padi*							
Untreated	-	0.852	6.12		0.782 a	5.05	
L-cyhalothrin	7.5	0.070	0.2	97	0.030 b	0.1	99
Cypermethrin	25	0.222	0.7	89	0.087 b	0.2	96
Deltamethrin	7.5	0.406	1.5	75	0.019 b	0.0	99
Chlorpyrifos	450	0.096	0.2	96	0.051 b	0.1	98
F Probability		0.458			0.018		
Significance		NS			*		
SED (68 d.f.)		0.116			0.081		
LSD (5%)		0.233			0.162		

DAA = days after application;
NS = not significant, * = significantly different at $P<0.05$
Data followed by different letters are significantly different at $P<0.05$

Table 4. Efficacy of alternative insecticides against pyrethroid-resistant *S. avenae* 6-8 days after application of sprays in autumn 2012

Treatment	Rate a.i.ha	Broom's Barn Log (N+1)	Broom's Barn Back -trans	Broom's Barn % control	Stetchworth Log (N+1)	Stetchworth Back-trans	Stetchworth % control
Untreated	-	0.412 a	1.58		0.213 a	0.63	
L-cyhalothrin	7.5	0.093 c	0.24	85	0.015 b	0.04	94
Cypermethrin	25	0.262 b	0.15	48	0.085 b	0.22	66
Pirimicarb	120	0.156 bc	0.43	73	0.008 b	0.02	97
Chlorpyrifos	450	0.004 d	0.01	99	0.002 b	0.01	99
Thiacloprid	72	0.149 bc	0.41	74	0.002 b	0.01	99
Acetamiprid	50	0.202 b	0.59	62	0.037 b	0.09	86
Pymetrozine + oil	100				0.023 b	0.05	92
F Pr.		< 0.001			< 0.001		
Significance		***			***		
SED 21 df		0.063			0.039		
LSD (5%)		0.131			0.815		

*** = significantly different at P< 0.001
Data followed by different letters are significantly different at P<0.05

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