

4. ASSESSMENT OF APHID PREDATION BY LINYPHIID SPIDERS AND CARABID BEETLES USING PCR TECHNIQUES

4.1. INTRODUCTION

The objective of this part of the project was to develop and use a molecular (PCR) technique to detect the presence of aphid prey within the guts of polyphagous arthropod predators, specifically linyphiids (money) spiders and carabid beetles, collected within crops and field margins. The wider objective was to quantify aphid predation in relation to field margin management treatments that were aimed at encouraging natural predators and parasitoids for the biological control of aphids. Field margins are important habitats for polyphagous predator groups and the diversification and management of margin habitats on farms (e.g. in arable stewardship schemes) will affect these groups (Coombes & Sotherton, 1986; Holopainen, 1995). Previous HGCA research has indicated that these predator groups contribute to cereal pest control, including aphids (Holland, 1997 - HGCA Project Report No. 148). Because polyphagous predators, by definition, eat a range of prey, there is no guarantee that increasing their numbers through diversifying and managing field margin habitats will automatically increase predation of pests such as aphids within the crop. It was therefore important to provide evidence that predatory spiders and beetles foraging in the crop alongside field margins were feeding to a significant degree on aphids, and thereby demonstrate the additional value of margins being managed to enhance the more specific natural enemies of aphids such as parasitoids and hoverflies.

Money spiders (Linyphiidae) are numerically dominant spider species in UK agroecosystems and feed on aphids either directly or via web catches when aphids become dislodged from the plant. The small size of these spiders (less than 5mm) and the fact that they ingest partially digested food means that direct dissection of their gut to examine for aphid contents is not possible. Predation of cereal aphids by linyphiid spiders has been demonstrated in the past using both direct observation in the field and serology techniques to detect aphid remains in field collected spiders (Sunderland et al., 1986, 1987b). Although chemical methods to detect prey in the guts of their predators have been useful in the past (eg chromatography, electrophoresis, radiolabelling of prey, serology), PCR is a particularly attractive means of detection which offers new opportunities to improve the efficiency and accuracy of prey detection in field collected predators. PCR is now routine in many labs, efficient DNA extraction kits are commercially available, candidate target DNA sequences are known for many insects, oligonucleotide primers are cheap to make and reproducible to use, and real-time PCR offers the possibility of quantification.

In this project we developed, tested and applied PCR primers for detecting aphids eaten by spiders and although the test was developed primarily for spiders, it was also successfully applied to carabid beetles collected by project partners at the Game Conservancy Trust as part of their study on the effects of set-aside on aphids and beneficial invertebrates (section 3.5.2).

4.2. MATERIALS AND METHODS

4.2.1. Development of a PCR Test for Detecting Aphids in Predator Guts

4.2.1.1. DNA extraction

DNA was extracted using commercially-available kits: Genome Star (Hybaid) or Purgene (Gentra/Flowgen). DNA was extracted from single whole aphids and spiders, which were stored at -80°C , using the kit protocol for extraction from a single *Drosophila melanogaster*. Carabid beetle guts that had been removed from the beetles and stored at -80°C were extracted using the kit protocol for 100-200mg solid tissue.

4.2.1.2. Primer design

Primers were designed to the aphid mitochondrial COII gene as described in Chen et al. (2000). As a mitochondrial gene, it occurs in multiple copies per cell, increasing the chance of successful amplification in predator guts. Although Chen et al. (2000) described the design of primers to North American aphid species, some of which are common to the UK, in order to ensure success with UK aphids we designed primers to amplify the common UK species by aligning their sequences as found in Genbank with the COII gene sequence. A primer pair was chosen that amplified a number of common UK species but did not amplify DNA from predators, other insects or microbial contaminants found on predator surfaces.

4.2.1.3. PCR cycling conditions and electrophoresis

PCR was carried out in 25ul volumes:

PCR mix

DNA.....	150ng
SDW.....	x for $V_t=25\mu\text{l}$
10x Buffer	2.50 μl
MgCl ₂	1.50 μl
10mM dNTPs	1.00 μl
Primers	1.00 μl
<hr/>	
Total Volume	25.00 μl

Thermocycler program

Cycle 1: 1X	Denaturation.....	94 $^{\circ}\text{C}$ for 15 min
Cycle 2: 35X	Denaturation.....	94 $^{\circ}\text{C}$ for 30 sec
	Annealing.....	51 $^{\circ}\text{C}$ for 30 sec
	Extension.....	72 $^{\circ}\text{C}$ for 30 sec
Cycle 3: 1X	Extension	72 $^{\circ}\text{C}$ for 2 min

The thermocycler was an Applied Biosystems Genamp PCR System 9700

PCR reactions were electrophoresed in 1.5% agarose in 0.5x TBE. Gels were run at 100V for 2h. Marker used was a 1kb ladder.

4.2.1.4. Spider feeding studies

Aphids were fed to spiders (*Lepthyphantes tenuis*), which were then sampled at various times after feeding and subjected to PCR testing to determine a) if aphid DNA could be detected in the gut, and b) the length of time following ingestion after which aphid DNA was still detectable. The aphids used in the tests were the grain aphid *Sitobion avenae*, the rose-grain aphid *Metopolophium dirhodum*, the bird cherry-oat aphid *Rhopalosiphum padi* and the peach-potato aphid *Myzus persicae*. All feeding experiments were carried out in an insectary at a temperature of 20°C +/- 5°C. Spiders were collected from the field, housed individually in small Petri dishes with a plug of moist cotton-wool and left without food for five days. Aphids (approx. 50% body size of the spider) were dropped onto the sheet webs of the spiders after the five day starvation period. Digestion time was recorded from when the spider released the aphid remains after feeding. Spiders were then stored at -80°C until DNA extraction and PCR.

4.2.2. PCR Detection of Aphids Eaten by Linyphiid Spiders and Carabid Beetles Collected within Crops

4.2.2.1. Linyphiid spiders

Linyphiid spiders were collected within spring barley crops at West Fenton Farm, East Lothian, during summer 2001 and 2002, within winter cereals at Unilever's Colworth Farm, Bedfordshire, during summer 2001 and within a crop of vining peas at Muirton Farm, Drem, East Lothian, during summer 2003. Spiders were collected using either a D-Vac or Vortis suction net sampling machine by sweeping along a 100m length within the field margin, and by sweeping along the crop base for 100m at 10, 30 and 100m distances into the crop. These samples were therefore taken along the four sampling transects used in the aphid parasitoid and hoverfly manipulation studies reported in Section 2 above. Spiders were sampled from each of the three field treatments set up in those studies (flower-rich margin, aphid sex pheromone deployment and untreated control) at the selected sites. Spiders were not sampled when the crop was wet because the suction net samplers do not work efficiently in wet conditions. Immediately after collection, linyphiid spiders were picked out of the sampling net using an entomological pooter or forceps and placed in Eppendorf tubes, one spider per Eppendorf, and then frozen in crushed carbon dioxide ice. This procedure was done in the field to halt digestion of prey immediately after collection. The frozen spiders, consisting of several species, were then transported to the laboratory at SAC where they were transferred into a -80C freezer and kept at that temperature until used for PCR.

4.2.2.2. Carabid beetles

The carabid beetles *Pterostichus melanarius* and *Pterostichus madidus* were collected alive, using 6 cm diam. empty pitfall traps, from wheat crops alongside margins with and without set-aside strips at the Cranborne study site (see section 4.5). Traps were opened overnight and contents immediately frozen after collection.

Sampling was conducted once during the aphid population peak. Gut contents were extracted, weighed and refrozen and PCR analysis was done to determine the proportion of beetles that had consumed aphids.

4.3. RESULTS AND DISCUSSION

4.3.1. Development of a PCR Test for Detecting Aphids in Predator Guts

Primer sequences were derived from the aphid COII gene following alignments of the same gene from collembolan, dipteran, coleopteran, hymenopteran and arachnid sequences within Genbank (Fig. 4.1). The derived primer sequences were:

Forward SWA/F: ATAGATGAAATTAAATGTCCAATT

Reverse SWA/R: TAGTTTTATTATCTACTTCAATTAA

These primers were tested initially against DNA extracted from aphids (*S. avenae*, *M. dirhodum* and *M. persicae*) and starved linyphiid spiders (Fig. 4.2) and later against other aphids (*R. padi*, *Acyrtosiphum pisum*), carabid beetle species, a panel of other invertebrates, and bacterial and yeast isolates obtained from the surfaces of *L. tenuis* (results not shown). **No amplification was produced from non-aphid DNA.** Aphid DNA produced a band of 180bp when amplified with the primers (Fig. 4.2). This compares to products of 79 to 386bp from the primers designed by Chen et al (2000) against the COII gene; these authors carried out DNA half-life detection studies in the guts of predators using a 198bp product, which was the reason we designed primers which gave an amplicon of approximately the same size.

Figure 4.1. 5' and 3' sequences of the aphid cytochrome oxidase (COII) gene (bold type) which were used to derive aphid-specific primer sequences, designated SWA/F (forward primer) and SWA/R (reverse primer). These sequences are aligned with selected non-aphid species (non-bold type); bases that differ are underlined.

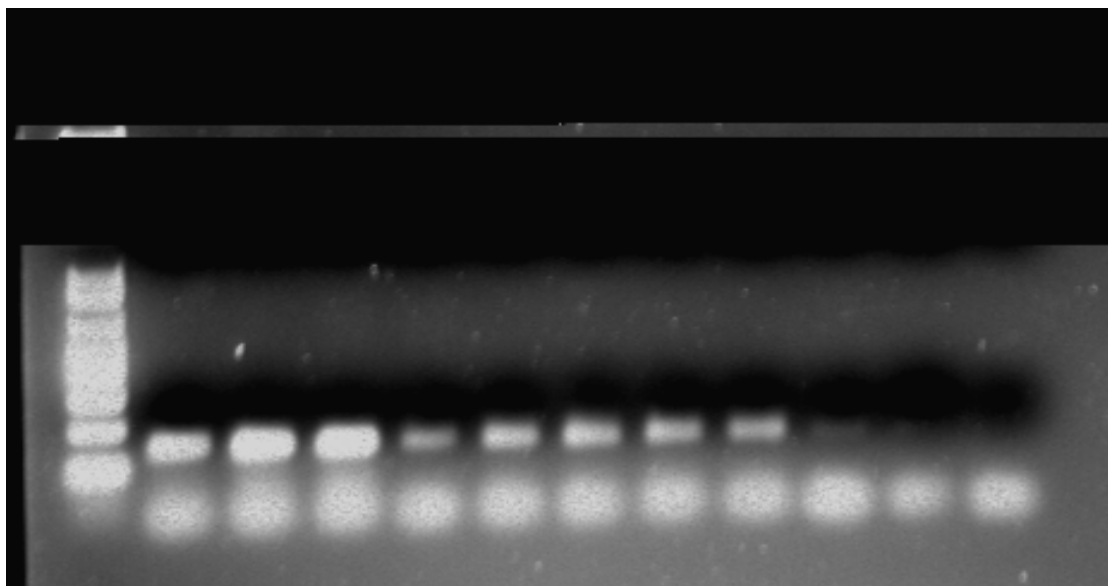
a). 5' end

	5' Forward primer 3'
SWA/F	ATAGATGAAATTAAATGTCCAATT
R. maidis	ATAGATGAAATTAAATGTCCAATT
S. graminum	ATAGATGAAATTAAATGTCCAATT
R. padi	ATAGATGAAATTAAATG<u>C</u>CCAATT
D. noxia	ATAGATGAAATTAAATGTCC<u>T</u>ATT
S. avenae	ATAGATGAAATTAAATGTCC<u>C</u>ATT
M. persicae	ATAGATGAAATTAAATGTCC<u>T</u>ATT
Culex	<u>T</u>TAGATGAAATTAA<u>T</u><u>T</u>C<u>T</u>CC<u>T</u><u>T</u>T<u>A</u>
Chironomus	ATAGATGAAATTAA<u>T</u><u>G</u>A<u>A</u>CC<u>T</u><u>T</u>CT
Drosophila	<u>T</u>TAGATGAAATTAA<u>T</u><u>G</u>A<u>A</u>CC<u>A</u>TC<u>T</u>
Coccinella	AT<u>T</u>GATGAAATT<u>C</u>G<u>T</u>A<u>A</u>TCC<u>A</u>TT<u>G</u>
Drusilla	<u>T</u>TAGATGAAATTAA<u>T</u><u>A</u>ACC<u>T</u><u>T</u>T<u>G</u>
Heliconus	<u>T</u>TAG<u>A</u>C<u>G</u>A<u>A</u>CTTA<u>A</u>T<u>A</u>TC<u>T</u><u>T</u>T<u>A</u>
Neanura	<u>T</u>TAGATGA<u>A</u>G<u>T</u><u>A</u>T<u>A</u>C<u>A</u>CC<u>C</u>CAG<u>C</u>T

Billobella	<u>TTAGATGAAGTTTATAACCCTTCC</u>
Isotomurus	<u>TTAGATGAAGTTTATAACCCTGCA</u>
Harbron	<u>ATAGAAGAGTCTGAGTCTTATGAT</u>
b). 3' end	
	5' Reverse primer 3'
SWA/R	TAGTTTTATTATCTACTTCAATTAA
R.maidis	TAGTTTTATTATCTACTTCAATTAA
S.graminum	TAGTTTTATTATCTACTTCAATTAA
R.padi	TAGTTTTATTATCTACTTCAATTAA
D.noxia	TAGTTTTATTATCTACTTCAATTAA
S.avenae	TAGTTTTATT <u>G</u> TCTACTTCAATTAA
M.persicae	TAGTTTTATTATCTACTTCAATTAA
Culex	TA <u>ATT</u> <u>CG</u> ATTATCA <u>ACA</u> TCTATT <u>G</u> A
Chironomus	<u>CA</u> ATT <u>CG</u> ATTATCA <u>ACA</u> TCTATTAA
Drosophila	<u>CT</u> ACT <u>CGG</u> TTATCA <u>ACA</u> TCTATTAA
Coccinella	<u>CAG</u> TT <u>CG</u> ATTATCA <u>ACT</u> TCTATTAA
Drusilla	<u>CA</u> ATT <u>CG</u> ATTATCA <u>ACA</u> TCTATTAA
Heliconus	<u>CA</u> ATT <u>CG</u> ATTATCTAC <u>AT</u> TCTATTAA
Neanura	<u>AG</u> AT <u>GT</u> <u>GG</u> TT <u>GTC</u> AGT <u>GTC</u> AATA <u>AAG</u>
Billobella	TAGT <u>GCG</u> ATTATCA <u>ACA</u> TCTATT <u>AG</u>
Isotomurus	<u>CTGT</u> <u>ACG</u> ATT <u>GTC</u> <u>GAC</u> TCT <u>AGG</u> AG

Figure 4.2. PCR of the COII gene using primers SWA/F and SWA/R applied to DNA from aphids and spiders. Each lane shows the pcr product from a single individual aphid or spider. A 180bp product is produced from aphid DNA but not from spider DNA. M = marker; SA = *Sitobion avenae*; MD = *Metapolophium dirhodum*; MP = *Myzus persicae*; L = *Lepthyphantes tenuis*; C = control (water, no DNA).

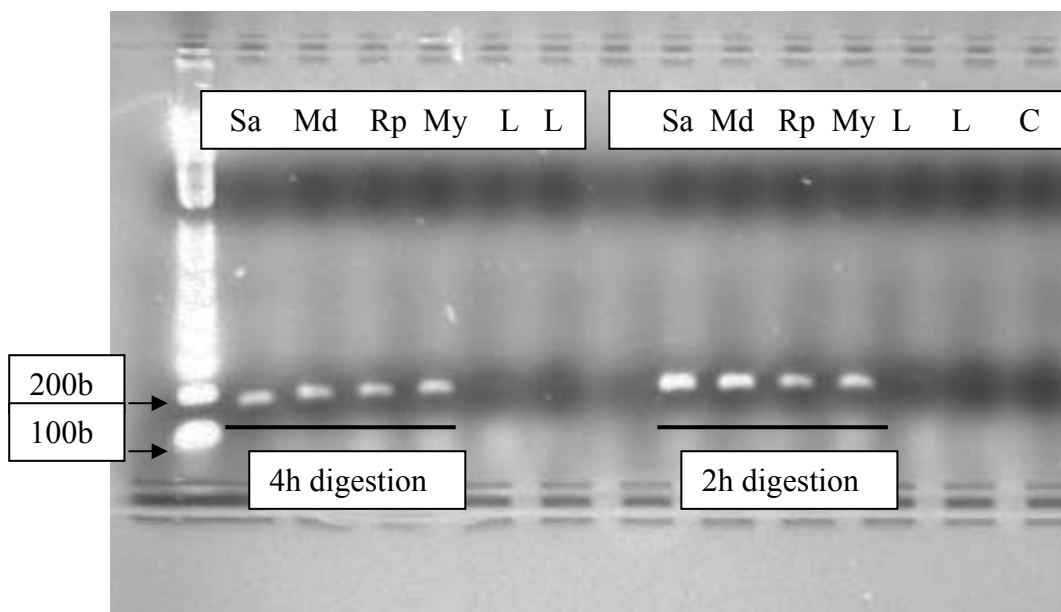
M SA1 MD1 MP1 SA2 SA3 MD2 MD3 MP2 L L C



Single aphids were fed to spiders (*L. tenuis*) and the pcr test applied to the spiders up to 8h following ingestion. **The aphid-specific band was still detectable after 8h**, but we were unable to determine the extinction point owing to the difficulty of coercing spiders to take aphids: many refused to eat in captivity. Fig. 4.3 shows the detection of aphid DNA in the guts of spiders up to 4 hours following ingestion. In the feeding studies of Chen et al (2000), the half-life of aphid DNA in lacewings and lady beetles was determined as 4h and 8.8h respectively, meaning the DNA could no longer be detected in ladybird beetles after 17.6h.

At the present state of this technology, it is only possible to determine whether or not a predator has eaten prey; it is not possible to determine how many have been eaten, or the specific developmental stages of the prey consumed. Although the density of the pcr band could be quantified by real-time pcr, this density is a function of the size of the prey, how many have been eaten, and the time since consumption and it is not possible to separate the effects of these different parameters.

Figure 4.3. Detection of aphid DNA in spiders following 2 and 4 hours digestion. Individual linyphiid spiders (*Lepthyphantes tenuis*) were fed single aphids then total DNA was extracted after 2 and 4 hours and subjected to pcr assay for aphid detection using primers SWA/F and SWA/R. Sa = spider fed *Sitobion avenae*; Md = spider fed *Metapolophium dirhodum*; Rp = spider fed *Rhopalosiphum padi*; My = spider fed *Myzus persicae*; L = unfed spider; C = no DNA



4.3.2. PCR Detection of Aphids Eaten by Linyphiid Spiders and Carabid Beetles Collected within Crops

4.3.2.1. *Linyphiid spiders*

Spiders were caught and subjected to pcr assay for aphid content within spring barley at West Fenton in 2001 and 2002, winter barley at Colworth 2001 and peas at Drem 2003. Results are shown in Tables 4.1-4.3. Results are not tabulated for West Fenton 2002 because numbers of spiders caught, and aphid counts, were very low for that year, with only 27 spiders being caught during the whole of the sampling period (24/6/02 – 30/7/02), of which 4 (15%) contained aphid DNA. A gel showing detection results for field-caught spiders is shown in Fig. 4.4.

Table 4.1. PCR detection of aphid DNA within the guts of spiders caught in spring barley at West Fenton, E Lothian, in 2001. Total numbers of spiders caught at each location, in a 100m sweep with a suction net sampler, over the sampling period (four sampling occasions from 3/7/01 – 31/7/01). Numbers in brackets are spiders giving a positive result for aphid DNA.

Location in field	Field margin treatment			Total
	untreated	flower	pheromone	
Margin	22 (5)	27 (4)	25 (4)	74 (13)
Crop at 10m	14 (4)	14 (4)	10 (3)	38 (11)
Crop at 30m	6 (2)	8 (4)	11 (3)	25 (9)
Crop at 100m	4 (1)	9 (3)	3 (0)	16 (4)
Total	46 (12)	58 (15)	48 (10)	153 (37) = 24%

In general, more spiders were found in the field margins irrespective of crop, and irrespective of treatment (untreated, flower or pheromone). This is presumably due to the denser, undisturbed vegetation and the greater diversity of plant species found within the field margins. **The numbers of spiders caught declined with distance into the crop, but spiders were feeding on aphids with equal efficiency up to 100m into the crop, the maximum distance sampled. At Colworth and West Fenton in 2001, 26% and 24% of spiders, respectively, were positive for aphid DNA.** At West Fenton in 2002, 15% of spiders were positive, but numbers of spiders and aphids were both very low that year, perhaps due to the cool, wet weather conditions prevailing through the summer. Aphid species recorded in the cereal crops from which the spiders were collected at West Fenton and Colworth were *S. avenae*, *M. dirhodum* and *R. padi*. **In the pea crop at Drem in 2003, 88% of spiders caught had eaten aphids (exclusively the pea aphid, *Acyrtosiphum pisum*).** Pea aphid numbers increased rapidly during August, encouraged by favourable temperatures, and local clusters of aphids (20-30 individuals in some groups) on pea shoots provided spiders with an abundant and accessible food supply.

Table 4.2. PCR detection of aphid DNA within the guts of spiders caught in spring cereals at Colworth, Bedfordshire, in 2001. Total numbers of spiders caught at each location, in a 100m sweep with a suction net sampler, over the sampling period (four sampling occasions from 5/6/01 – 26/6/01). Numbers in brackets are spiders giving a positive result for aphid DNA.

Location in field	Field margin treatment			Total
	untreated	flower	pheromone	
Margin	15 (2)	14 (6)	17 (3)	46 (11)
Crop at 10m	2 (2)	0	5 (2)	7 (4)
Crop at 30m	4 (1)	3 (2)	3 (0)	10 (3)
Crop at 100m	3 (0)	3 (0)	0	6 (0)
Total	24 (5)	20 (8)	25 (5)	69 (18) = 26%

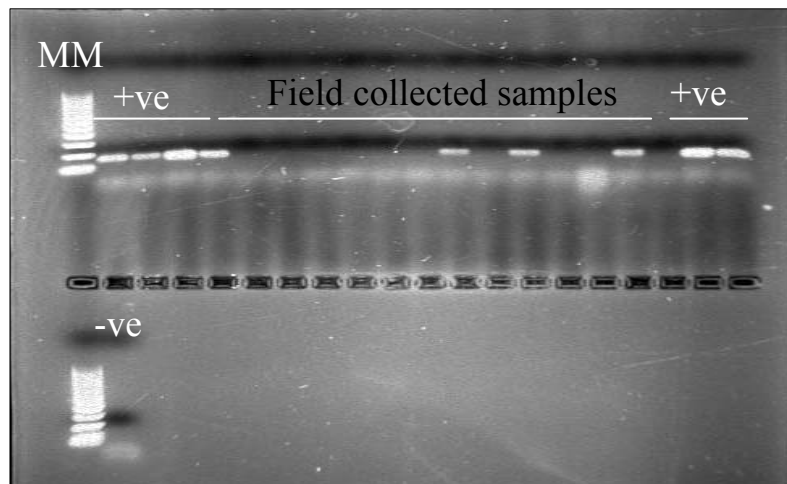
Table 4.3. PCR detection of aphid DNA within the guts of spiders caught in vining peas at Drem, E Lothian, in 2003. Total numbers of spiders caught at each location, in a 100m sweep with a suction net sampler, over the sampling period (four sampling occasions from 2/7/03 – 1/8/03). Numbers in brackets are spiders giving a positive result for aphid DNA.

Location in field	Field margin treatment			Total
	untreated	flower	pheromone	
Margin	30 (23)	27 (24)	8 (8)	65 (55)
Crop at 10m	3 (2)	10 (8)	10 (10)	23 (20)
Crop at 30m	3 (3)	7 (5)	3 (3)	13 (11)
Crop at 100m	5 (5)	11 (11)	4 (4)	20 (20)
Total	41 (33)	55 (48)	25 (25)	121 (106) = 88%

These results provide evidence that Linyphiid spiders are consuming a significant proportion of crop aphid pests, at least up to 100m away from botanically-diverse field margins. It is probable that the proportion of spiders feeding on aphid prey is influenced by aphid abundance, but even at low aphid densities in cereal crops spiders are functioning as important aphid predators. The much higher proportion of spiders detected feeding on aphids in the pea crop, compared with the cereal crops, is almost

certainly due to the much greater aphid density in the former. Establishment of diverse field margins that provide valuable habitats for linyphiid spiders would increase the overall density of spider populations in arable ecosystems that could impact on pest populations as they develop on adjacent crops. The ability of these small spiders to disperse rapidly by ‘ballooning’ on silk threads ensures rapid colonisation of crop areas.

Figure 4.4. An example of pcr results from field-sampled linyphiid spiders. “+ve” and “-ve” are controls consisting of DNA extracted from a single aphid per lane (*Sitobion avenae*, four lanes on left of gel; *Metapolophium dirhodum* two lanes on right of gel) and of water respectively. MM = molecular marker. Band shown is 180bp.



4.3.2.2. Carabid beetles

A total of 233 carabid beetles (*Pterostichus madidus* and *Pterostichus melanarius* combined) from the Cranborne study site were tested for the presence of aphid remains. Of these, 21% were found to have consumed aphids; 23% collected from fields with a set-aside strip and 18% from fields without a set-aside strip (Table 4.4). The proportion of beetles that had consumed aphids was not significantly affected by distance from the margin, at least up to 100m, regardless of the presence of a set-aside strip. These results are discussed further in section 3.5.2.

Table 4.4. Number and percentage of *Pterostichus madidus* and *P. melanarius* than tested positive or negative for aphids in fields with and without the set-aside strips.

		10m		30m		100m		Total	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Control	No.	5	30	8	28	7	33	20	91
	%	14	86	22	78	18	82	18	72
Set-aside strip	No.	9	29	11	33	8	32	28	94
	%	24	76	25	75	20	80	23	77

4.4. ACKNOWLEDGEMENTS

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5. HOVERFLY BEHAVIOUR STUDIES

5.1. HOVERFLY FLORAL PREFERENCES

5.1.1. Introduction

The amount of nectar and pollen available to adult hoverflies can have a significant effect on the egg load of the females (Scholz and Poehling, 2000) and thus also on the potential of hoverfly populations in agricultural fields to reduce cereal aphid numbers. When insufficient food sources are available, fewer eggs are laid near aphid colonies.

Adult hoverflies can be divided into two groups, those that are polyphagous and those that are highly specific to a small range of flowers (section 2.1.2). The former group change their feeding behaviour to ensure that the flowers that currently provide most resources are visited most often (Cowgill *et al.*, 1993). Ensuring that floral margins provide a range of host plants that offer suitable resources for aphidophagous hoverflies throughout their period of activity in agricultural fields is therefore of primary importance if the full potential of these natural enemies is to be realised in the conservation biological control system investigated in this project.

When establishing floral margins, the use of a diverse seed mixture that includes carefully selected species, is therefore a prerequisite of any attempt to manipulate populations of hoverflies for natural control. However, the relative value of candidate native perennial wild flowers to the most common species of hoverflies was poorly understood and required further investigation as part of this project. Existing published data were used to design the initial seed mixture (sown at 15 Kg/acre; 80% grass; 20% flowering plants) used in the trials at Manor Farm, which contained a range of different flowering types including umbellifers, compositae, caryophyllaceae and other taxonomic groups (Table 5.1). This diversity of flower types ensured that hoverfly species with different preferences, either based on nectar or pollen quality or determined by morphological characteristics of the flowers (e.g. exposed nectaries), were considered. In addition, it incorporated a range of species that had hitherto not been tested as a hoverfly resource but were known to be of importance for general farmland biodiversity.

To provide a wider range of plants known to be effective in providing resources for hoverfly adults and which will grow in a range of soil types within the UK, a method of screening their efficacy was developed as part of this study. This was used to test native perennial wildflowers (including some selected from the initial seed mix) that had not been previously investigated and others that were not considered initially. As *Episyrphus balteatus* is the most common aphidophagous hoverfly species in arable habitats and its larvae are known to feed on cereal aphids, this species was selected for use in the floral preference experiments and investigation of the effects on egg load.

Table 5.1. Plant species sown in the flowering margin at Manor Farm, North Yorks.

Common Name	Species
Betony	<i>Betonica officinalis</i>
Birdsfoot	<i>Ornithopus perpusillus</i> = Least; or <i>Lotus angustissimus</i> = Long fruited
Common meadow buttercup	<i>Ranunculus acris</i>
Cornflower	<i>Centaurea cyanus</i>
Cowslip	<i>Primula veris</i>
Crested dog's tail	<i>Cynocurus cristatus</i>
Red fescue / Creeping fescue	<i>Festuca rubra</i> subsp. <i>commutata</i>
	<i>Festuca rubra</i> subsp. <i>pruinosa</i>
	<i>Festuca rubra</i> subsp. <i>rubra</i>
Field scabious	<i>Knautia arvensis</i>
Hoary plantain	<i>Plantago media</i>
Kidney vetch	<i>Anthyllis vulneraria</i>
Knapweed	<i>Centaurea nigra</i>
Lady's bedstraw	<i>Galium verum</i>
Meadow barley	<i>Hordeum secalinum</i>
Meadow fescue	<i>Festuca pratensis</i>
Meadow foxtail	<i>Alopecurus pratensis</i>
Musk mallow	<i>Malva moschata</i>
Oxeye daisy	<i>Chrysanthemum leucanthemum</i>
Ragged robin	<i>Lychnis flos-cuculi</i>
Red campion	<i>Silene dioica</i>
Ribwort plantain	<i>Plantago lanceolata</i>
Rough hawkbit	<i>Leontodon hispidus</i>
Salad burnet	<i>Sanguisorba minor</i>
Self heal	<i>Prunella vulgaris</i>
Smooth meadow grass	<i>Poa pratensis</i>
Sorrel	<i>Rumex acetosa</i>
Timothy	<i>Phleum pratensae</i>
White campion	<i>Silene alba</i>
Wild carrot	<i>Daucus carota</i>
Yarrow	<i>Achillea millefolium</i>
Yellow oat grass	<i>Trisetum flavescens</i>
Yellow rattle	<i>Rhinanthus minor</i>

5.1.2. Hoverfly Flower Preference and Egg Load - Pilot Study

5.1.2.1. Materials and methods

To establish a screening method, initial studies investigated the feeding preferences of female hoverflies and their resultant egg load on three species of flowering plant (*Centaurea cyanus* – cornflower; *Calendula officinalis* - pot marigold; and *Phacelia tanacetifolia*), each selected for ease of production but not intended for eventual use in margins.

A circle of twelve plants (all at the flowering stage) was arranged in flight cages (1m³) such that each was equidistant from the centre of the cage and from its neighbours. The cage consisted of a wooden frame with mesh sides that was lit from above and maintained at 22°C ±1°C throughout the experiments. A single newly emerged adult female hoverfly (*E. balteatus*) was released onto a platform in the centre of the cage. After a 5 minute settling period, the hoverfly was observed for a period of 30 minutes and the number of feeding visits to each plant and the length of each visit recorded. The experiment was replicated 20 times, using different hoverflies (to avoid problems of flower constancy) and different plants. Three experimental arrangements were used

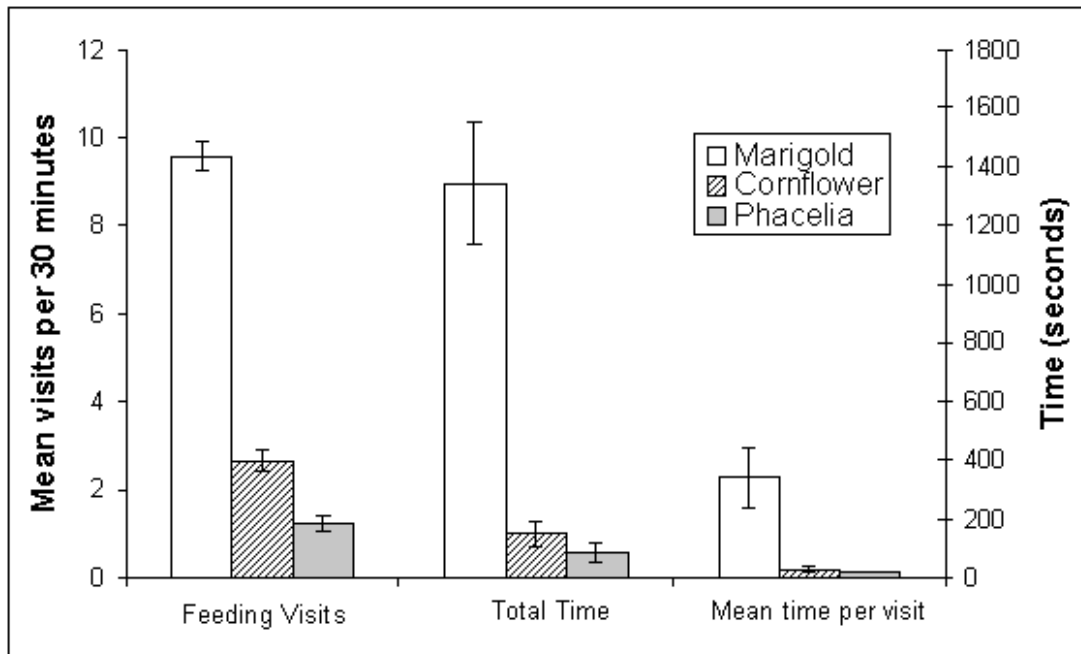
- (i) No-choice: All 12 plants within the set up were from one of the three plant species.
- (ii) Two plant species choice: Two plant species were presented simultaneously (6 plants each).
- (iii) Three plant species choice: All three plant species were presented simultaneously (4 plants each)

To investigate the oviposition rates of female hoverflies feeding on these species, flight cages were set out with a circle of six plants, each equidistant from its nearest neighbour. Cages were lit from above and maintained at 22°C with a 16:8 Light:Dark daylength regime. Four wheat plants that had been infested with a similar number of *Sitobion avenae* 7 days previously, were placed over a tray of water and detergent (to prevent escape of aphids) and positioned in the centre of the circle of plants in the flight cage to act as oviposition sites. Two, newly eclosed, adult male and female hoverflies were released onto a platform at the centre of the cage, and the cage sealed and left undisturbed for 12 days. After this period, two pots of seedlings were removed and the number of hoverfly eggs counted. The other two pots were removed after 14 days and processed in the same way.

5.1.2.2. Results

In no-choice tests, in which only one plant species was offered to adult hoverflies, there was a significant difference ($P < 0.001$) between the three plant species tested in the number of feeding visits recorded in 30 minutes (Fig. 5.1). The highest feeding activity was recorded on pot marigold, followed by cornflower. *Phacelia*, a plant that has been widely cited in the literature as being particularly attractive to hoverflies (Hickman & Wratten, 1996), was the least effective in these experiments with a mean of only 0.5 feeding visits. Similar differences between plants were recorded when the mean length of individual feeding visits and total time spent feeding on each plant were compared. In each case strong preferences ($P < 0.001$) for pot marigold compared to either cornflower or *Phacelia* were recorded.

Figure 5.1. Mean (\pm standard error) number of feeding visits in 30 minutes, the total time spent feeding in 30 minutes and the mean length of each feeding visit, when host plants were offered in no-choice tests to adult hoverflies (*Episyrphus balteatus*).



When the hoverflies were provided with a choice of host plants, a preference pattern consistent with the no-choice results was recorded. Pot marigold was visited more frequently than both cornflower ($P < 0.01$) and *Phacelia* ($P < 0.001$) and cornflower was visited more frequently than *Phacelia* ($P < 0.05$; Fig 5.2). Analysis of data describing mean length of feeding visits and total time spent feeding confirmed the preference for pot marigold in comparison with both cornflower and *Phacelia* (Fig 5.3). However, the data from the two-plant choice experiment should be interpreted with care as they were conducted late in the flowering cycle of the cornflower plants, which consequently displayed reduced pollen and nectar production. As a result, the differences between *Phacelia* and cornflower that were recorded in both of the other experiments were not so clearly apparent in this trial series, whereas those between cornflower and marigold were magnified.

When all three host plants were offered simultaneously, the hoverfly preference pattern was again evident. Both mean number of visits to each plant and the time spent visiting the plant was significantly ($P < 0.001$) greater for pot marigold than for either cornflower or *Phacelia*, and a greater preference was shown for cornflower than *Phacelia* ($P < 0.05$) (Fig 5.4).

Figure 5.2. Mean (\pm standard error) number of feeding visits in 30 minutes, when host plants were offered to adult hoverflies (*Episyrphus balteatus*) in two plant species choice tests. Test 1 = Pot marigold offered together with cornflower; Test 2 = Pot marigold offered with *Phacelia*; test 3 = Cornflower offered with *Phacelia*.

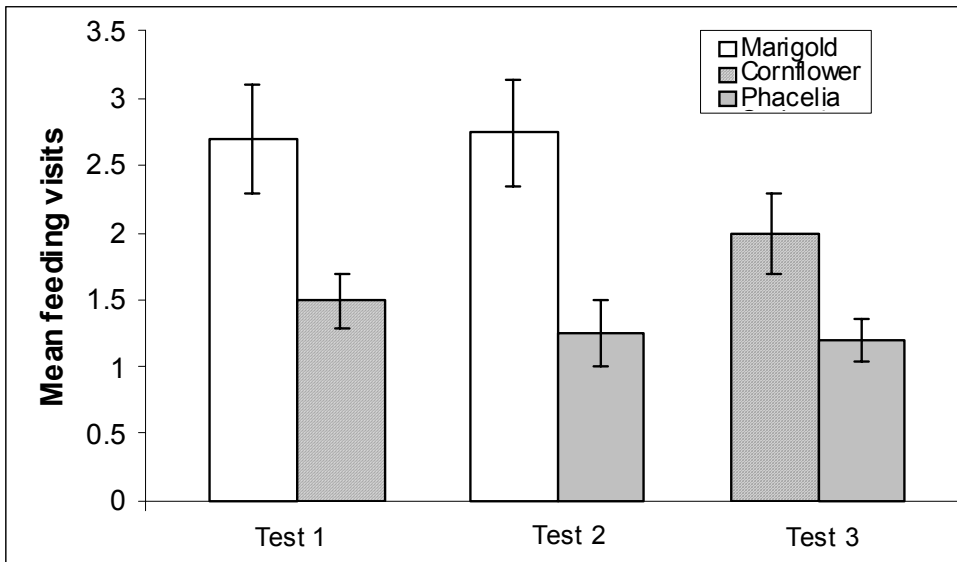


Figure 5.3. The total time spent feeding in 30 minutes and the mean length of each feeding visit when host plants were offered to adult hoverflies (*Episyrphus balteatus*) in two plant species choice tests. Bars = \pm 1 standard error.

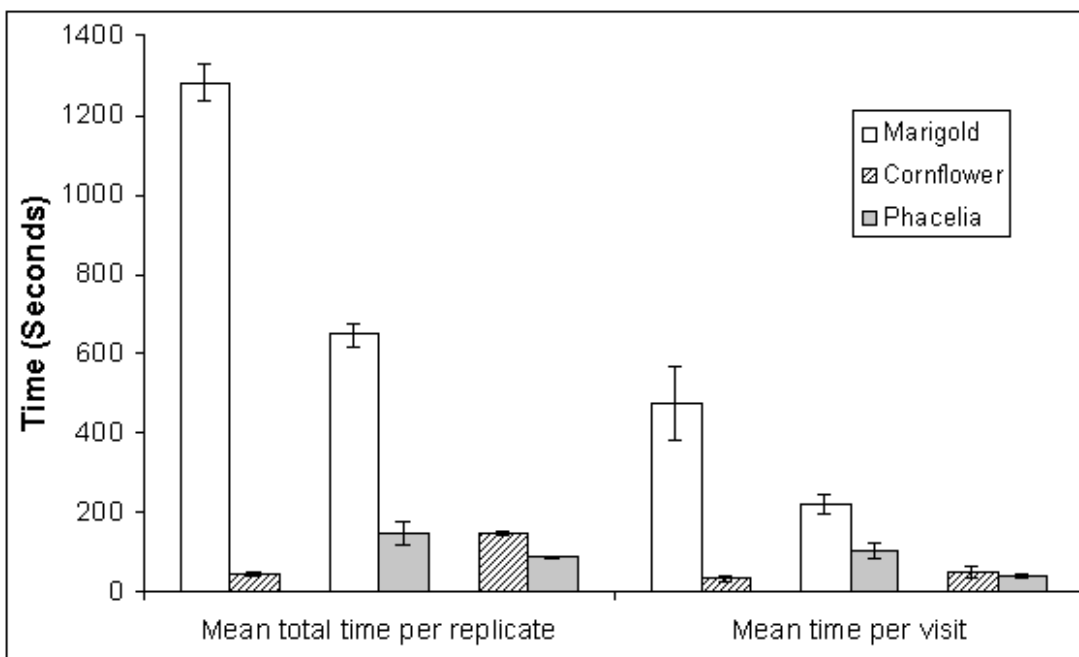
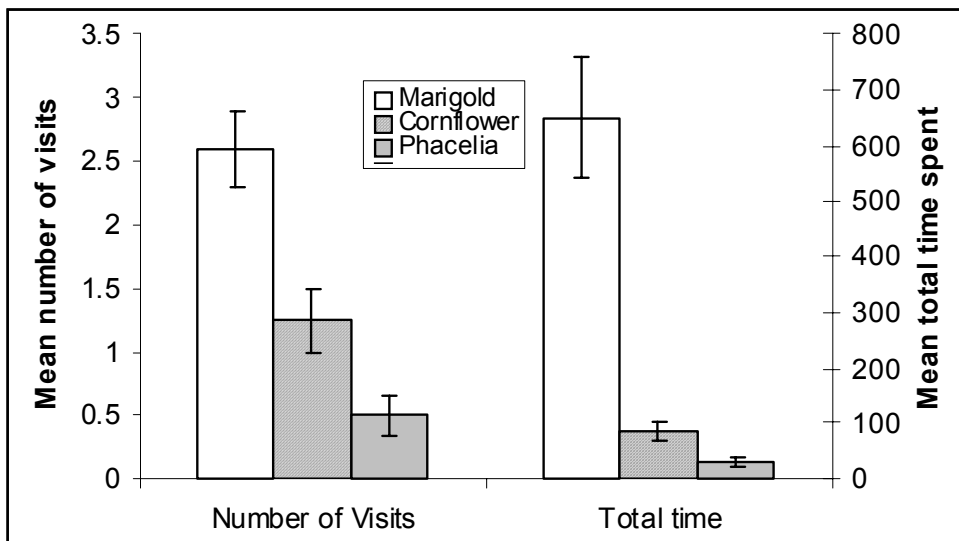
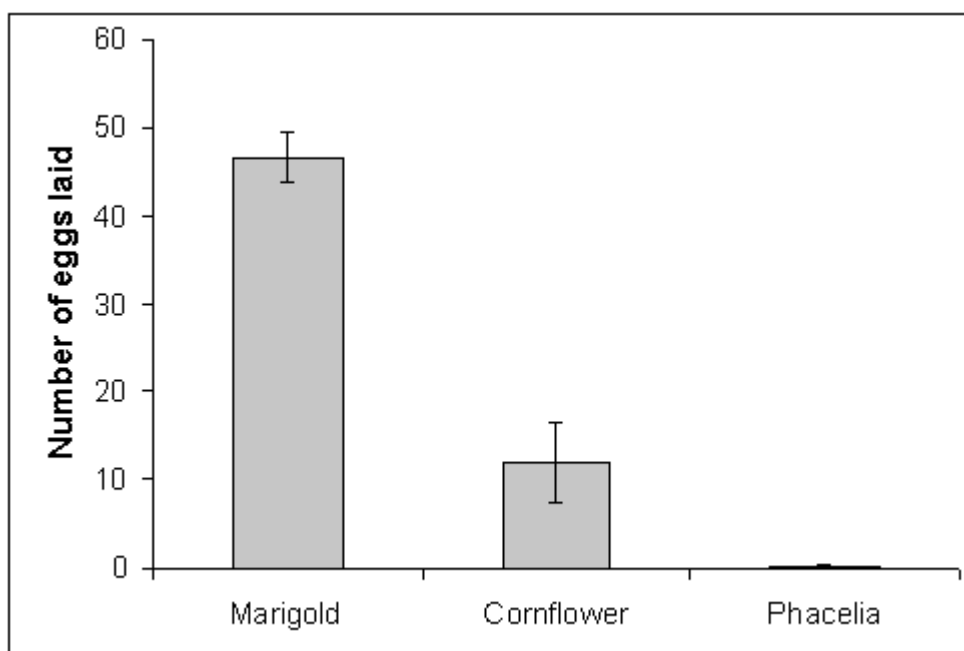


Figure 5.4. Mean (\pm standard error) number of feeding visits in 30 minutes, and the total time spent feeding in 30 minutes, when host plants were offered to adult hoverflies (*Episyrphus balteatus*) in three plant species choice tests.



The number of eggs laid by adult hoverflies on cereal seedlings infested with *S. avenae* after both 12 ($P < 0.001$) and 14 ($P < 0.001$) days varied with the nectar and pollen source available. Hoverflies offered pot marigold laid significantly ($P < 0.001$) more eggs than those offered cornflower, which in turn laid more eggs ($P < 0.01$) than those offered *Phacelia* (Fig. 5.5).

Figure 5.5. Mean (\pm standard error) number of eggs laid after 14 days when adult hoverflies were offered pollen and nectar from pot marigold, cornflower and phacelia flowers.



5.1.3. Hoverfly Flower Preference

5.1.3.1. *Materials and methods*

Modifications of the no-choice and two-plant species choice tests above were used to screen UK native perennial wildflower species.

As before, a circle of twelve plants (all at the flowering stage) was arranged in flight cages (1m³) such that each was equidistant from the centre of the cage and from its neighbours. The cage consisted of a wooden frame with mesh sides that was lit from above and maintained at 22°C ±1°C throughout the experiments. A single newly eclosed adult female hoverfly (*E. balteatus*) was released onto a platform in the centre of the cage. After a 5 minute settling period, the hoverfly was observed for a period of 30 minutes and the number of feeding visits to each plant recorded. The experiment was replicated 20 times, using different hoverflies (to avoid problems of flower constancy) and different plants.

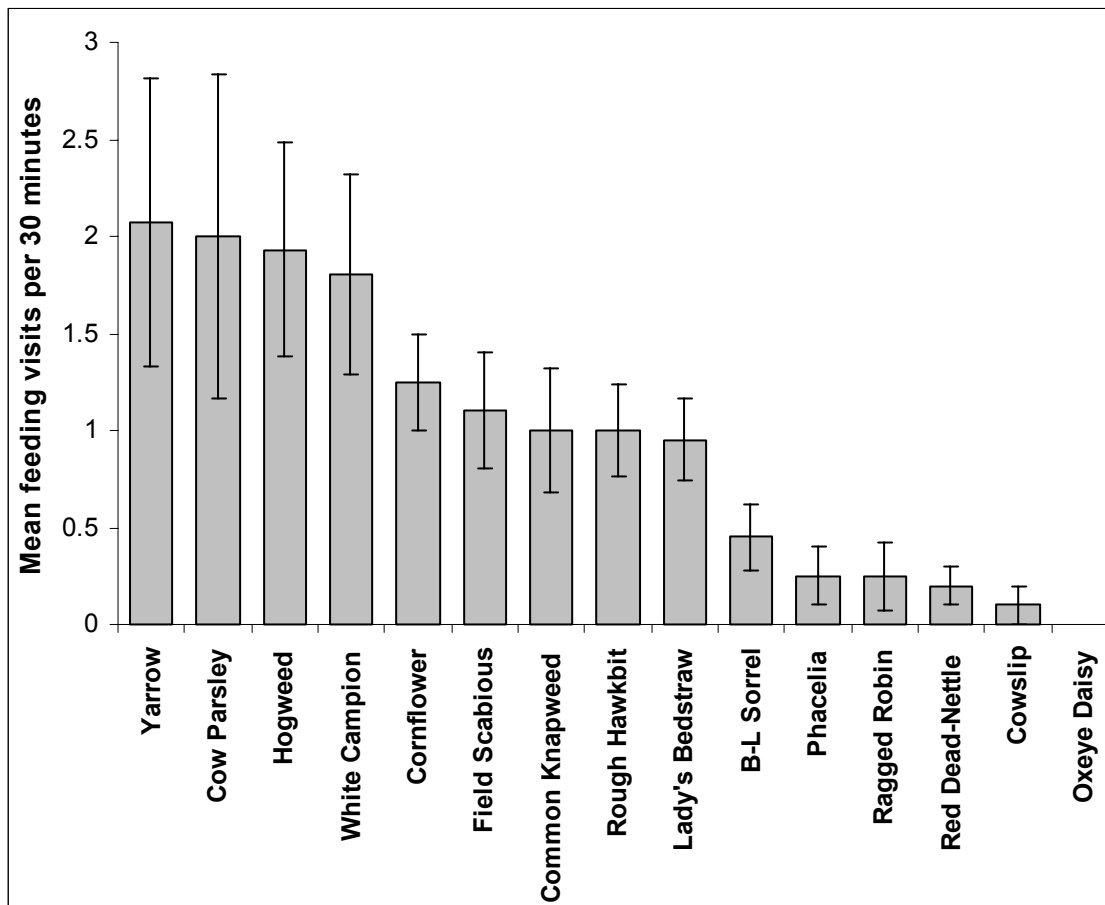
Each plant species screened was subjected to two tests. In the first, plants were offered in a no-choice experiment in which 12 plants of the same species were offered in a screen cage. In the second, 6 plants of the test species were offered in conjunction with 6 plants of a standard. *Phacelia tanacetifolia* was used as the standard in all experiments, as it is widely cited in the scientific literature and trade press as a useful nectary plant for the attraction of hoverflies. No-choice tests of the standard were also conducted.

The revised protocol enabled the rapid screening of a range of candidate wild plant species within the financial and time resources available.

5.1.3.2. *Results*

In no-choice tests, significant differences ($P < 0.001$) were recorded between flower species in the number of feeding visits made during the 30 minute exposure period (Fig. 5.6). Three groups of plants were identified. The most frequent plants on which hoverflies fed were species with umbelliferous or umbel-like flowers (yarrow (*Achillea millefolium*), cow parsley (*Anthriscus sylvestris*) and hogweed (*Heracleum sphondylium*)) and white campion (*Silene alba*). The second grouping consisted of three members of the daisy family with similar flower structures (cornflower, (*Centaurea cyanus*) common knapweed (*Centaurea nigra*) and rough hawkbit (*Leontodon hispidus*)), as well as field scabious (*Knautia arvensis*) and lady's bedstraw (*Galium verum*). Hoverflies fed on the third group, which included *Phacelia tanacetifolia*, ragged robin (*Lychnis flos-cuculi*), red dead-nettle (*Lamium purpureum*), cowslip (*Primula veris*) and ox-eye daisy (*Chrysanthemum leucanthemum*), least often.

Figure 5.6. Mean number of feeding visits by hoverflies (*Episyrphus balteatus*) to flowers of different plant species during a 30 minute exposure period in no-choice experiments.

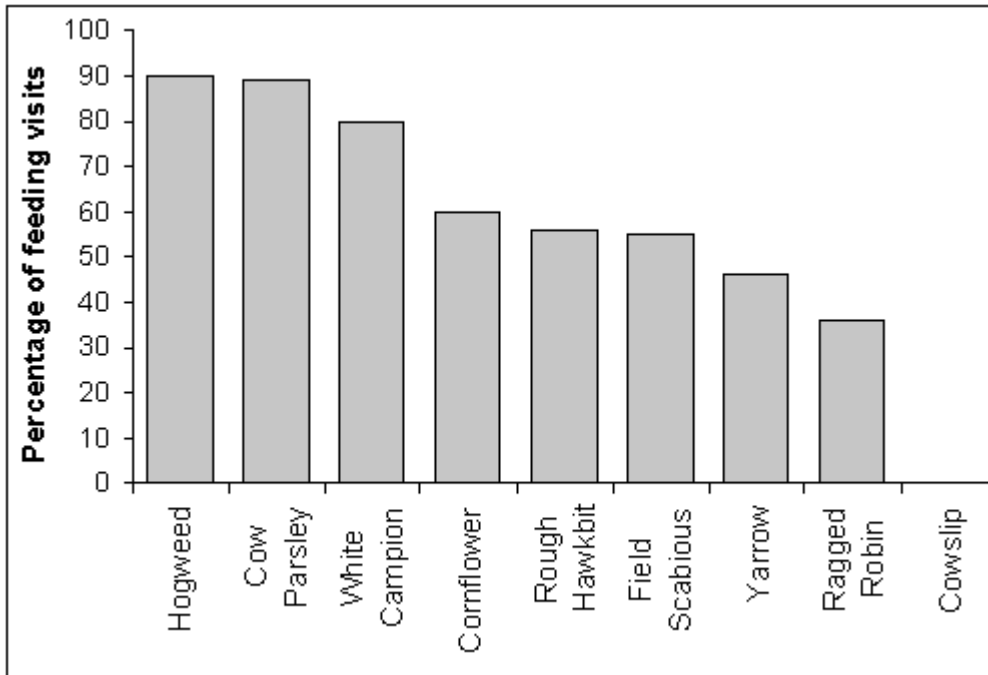


The two plant choice tests largely confirmed the preferences identified by no-choice tests (Fig 5.7). When offered a choice of *Phacelia* (the standard) or one of the plant species from the first group identified by no-choice experiments, more than 80% of feeding visits made were to the test species. For example, when *Phacelia* and hogweed were offered simultaneously, 90% of feeding visits were to hogweed, compared with 89% to cow parsley and 80% to white campion in equivalent tests. Another first group plant from no-choice experiments (yarrow) appeared to be slightly less attractive to hoverflies than predicted by no-choice tests, with only 45% of feeding visits compared to 55% on *Phacelia*.

The second group identified by no-choice tests, were also found to be slightly less attractive than white campion, cow parsley and hogweed, but still preferred by hoverflies. For example, in comparative tests with *Phacelia*, 60% of feeding visits were made to cornflower, and 58% to each of rough hawkbit and field scabious (Fig 5.7).

Those plant species identified by no-choice tests as being relatively unattractive to hoverflies were also confirmed by the two plant choice tests. For example, ragged robin received only 35% of the feeding visits when compared with *Phacelia*, and cowslip received none.

Figure 5.7. Percentage of the total number of hoverfly (*Episyrphus balteatus*) feeding visits observed that were made to selected test plants when offered in two plant choice tests with the standard, *Phacelia*.



5.2. PLANT STRUCTURAL CUES FOR HOVERFLY OVIPOSITION

5.2.1. Introduction

Aphidophagous hoverfly (Syrphidae) species have been classified into two categories, those that oviposit in response to aphid presence/density ('aphidozetic' species) and those that oviposit in response to plant species irrespective of aphid presence; ('phytozetic' species) (Chandler, 1968). Aphidozetic species such as the marmalade hoverfly *Episyrphus balteatus* are able to search out and oviposit close to small isolated aphid infestations and are therefore considered most significant in terms of biological control (Chambers, 1991). Searching involves a sequence of stages, during which aphid mediated cues such as honeydew concentration, volatile chemicals and aphid presence appear to be important in determining whether a plant has sufficient aphids to stimulate oviposition (Bargen *et al.*, 1998; Scholz & Poehling, 2000; Budenberg & Powell, 1992). Initially, focused hovering occurs during which the adult inspects the plant, looking for aphids or signs of aphid infestation (Dixon, 1959). The adult then lands and may either rest, or walk on the plant surface probing with the labella (mouthparts) while searching for aphids. The ovipositor will then be extended and dragged, or used to probe the plant surface. Following selection of a suitable site, the eggs are laid. Soon after eggs hatch, the larvae begin to seek out aphid prey. At emergence they are unable to travel large distances (about 1 metre; Chandler, 1968), and therefore understanding the stimuli that are significant in determining where adults oviposit is important if hoverflies are to be manipulated effectively as components of integrated pest management (IPM) systems.

Previous research investigating oviposition behaviour in the field (Smith 1969, 1976; Pollard 1971) has shown that plant species is a significant factor in the selection of a suitable egg laying site. However, cues leading to the selection of an appropriate plant or small patch of plants for the initial focused hovering inspection have not been investigated in detail. It has been proposed that female hoverflies may utilise olfactory cues, from aphids or plant semiochemicals (Bargen *et al.*, 1998). However, in this case aphid derived volatiles appear to work only over short distances and it has been suggested that cues detectable over longer distances, such as plant structure, may also be important (Scholz & Poehling, 2000). The effect of plant structure (e.g. size and shape) has received little attention and little information on this factor is available.

This component of the study investigated whether plant size/structure was influential in hoverfly searching, to determine if cereal plants at the growth stages present in the field during the period in which hoverflies seek egg laying sites will result in significant focussed hovering and subsequently egg laying.

5.2.2. Materials and Methods

5.2.2.1. *Experimental insects*

A stock culture of *Sitobion avenae* (Fabricus) was maintained on 1-3 week old barley seedlings in cages (60 x 60 x 60cm) maintained in a controlled environment (CE) room at a 16:8 L:D daylength regime and 20±1°C using the method of Huggett *et al.* (1999).

A stock culture of *E. balteatus* was maintained in purpose built flight cages (60 x 60 x 120cm). Barley plants infested with *S. avenae* were provided as oviposition sites for the adult hoverflies. Eggs were allowed to hatch and larvae to feed and develop within the flight cages, but pupae were removed and placed on dampened filter paper in rearing cages before adult emergence. Sources of pollen were provided for adults, to facilitate egg development. Cultures were maintained in a CE room at 22±1°C, and with a 16:8 L:D daylength regime.

For experiments, standard aged cohorts of gravid female adults were reared from pupae taken from the stock culture, in 1m³ cages in a CE room at 22±1°C, and with a 16:8 L:D daylength regime. Emerging adults were provided with tree pollen, sugar cubes and water (offered on cotton wool). Each adult was used only once in experiments (to avoid opportunities for rapid associative learning as described for Lepidoptera by Rausher (1978)) and was transferred directly from the rearing cage to the experimental arenas.

5.2.2.2. Hoverfly searching behaviour

Experiments were conducted in a 1m³ screen cage, in a CE room at 22±1°C and with a 16:8 L:D daylength regime. Experimental plants were arranged in a triangle, equidistant from each other and from a central take off platform.

Individual female *E. balteatus* were placed in a Petri dish, which was positioned on the central take off platform and left undisturbed for 1h prior to the experiment. After this period, each fly was released by removing the lid, and observed for a total of thirty-five minutes. No records of behaviour were made during the first five minutes, but during the remaining thirty minutes the length of time spent in focussed hovering, resting, walking, labella probing, or ovipositor probing, and the number of eggs that were laid, was noted separately for each plant (Table 5.2).

The plants used were grown in 9cm diameter pots in John Innes No 1 potting compost and fell into three categories. ‘Large infested’ plants were at the booting stage and were infested with 0.6g (± 0.04g) of *S. avenae*. ‘Small infested’ plants comprised 10 cereal seedlings with only one leaf between 6 cm and 7 cm long infested with 0.2g (± 0.04g) of *S. avenae*. These infestations provided an aphid density equivalent to 8-10 aphids per leaf. All plants were infested the evening prior to use in experiments. ‘Large un-infested’ plants were also at the booting stage but were not infested with aphids.

Table 5.2. Classes of hoverfly behaviour recorded during hoverfly searching behaviour experiments

Behaviour	Description
Focused hovering	Hovering behaviour associated with particular plant/pot
Resting	Resting on a plant surface
Walking search	Walking across the plant surface - may involve occasional labella probing.
Labella probing	Proboscis protruded repeatedly tasting the plant surface whilst remaining stationary
Ovipositor Probing	The extended ovipositor used to probe the plant surface
Number of eggs laid	Eggs laid on the plants (in each pot) at the end of the 30 minute observation.

All possible combinations of plant categories were presented to the female hoverflies (Table 5.3). (A) In three-choice cage designs one plant of each of the three categories was offered simultaneously to female *E. balteatus*; (B) In two choice cage designs two categories were offered, in a ratio of 2:1; (C) In single treatment cage designs three pots of a single category were offered within the cage. In all experiments the positions of the pots within the cage were randomised. There were 20 replicates of each experiment.

Table 5.3. Cage designs used in hoverfly searching behaviour experiments

Cage design	Treatments			N°. replicates
	Large uninfested	Small infested	Large infested	
(A) 3 - Choice	x 1	x 1	x 1	20
(B) 2 - Choice	x 2	x 1	-	20
	x 2	-	x 1	20
	x 1	x 2	-	20
	x 1	-	x 2	20
	-	x 2	x 1	20
	-	x 1	x 2	20
(C) Single treatment	x 3	-	-	20
	-	x 3	-	20
	-	-	x 3	20

5.2.2.3. Hoverfly oviposition behaviour

Oviposition experiments were conducted in 1 m³ screen cages, in a CE room at 22±1°C, and with a 16:8 L:D daylength regime. Single female *E. balteatus* were released into cages using the method described above and allowed 30 hours for egg laying. Plants were offered using the single treatment and three choice cage designs described above. Pollen, sugar cubes, and water soaked cotton wool were provided throughout the experiment. The total number of eggs laid on each plant was recorded. There were fifteen replicates of each experiment.

5.2.2.4. Statistical analysis

Kruskal-Wallis tests were used to compare pooled data between treatments describing the total time engaged in each behaviour category or the number of eggs laid on each plant category.

5.2.3. Results

5.2.3.1. Hoverfly searching behaviour

Adult female hoverflies spent significantly more time engaged in focused hovering in front of large infested and large uninfested plants than small infested plants (Table 5.4). No significant difference was recorded between time spent engaged in focused hovering in front of large infested and large un-infested plants (Table 5.4).

Table 5.4. Hoverfly searching behaviour directed at three plant size/aphid combinations (Plants at the booting growth stage infested with aphids (LI); Plants at the booting growth stage without aphids (LUI) and seedling plants infested with aphids (SI)). Significance values are based on a Kruskal-Wallis test performed on data across all cage designs separated by plant and behaviour category: df =1 and n=200.

Behaviour	Total seconds			Significance P		
	LUI	SI	LI	LUI Vs SI	LUI Vs LI	LI Vs SI
Focused hovering	2668	875	3377	<0.001	0.085	<0.001
Resting	9109	6075	52903	0.984	<0.001	<0.001
Walking Search	787	687	12049	0.106	<0.001	<0.001
Labella probing	145	339	3747	0.586	<0.001	<0.001
Ovipositor probing	86	1626	7722	0.002	<0.001	<0.001
No. eggs laid	0	54	261	<0.001	<0.001	<0.001

Adults spent significantly more time engaged in walking search, resting on a plant surface, and labella probing on large infested plants compared with both small infested and large un-infested plants (Table 5.4).

Significantly more time was spent engaged in ovipositor probing on both large and small infested plants compared to large un-infested plants (Table 5.4). Significantly more time was spent engaged in ovipositor probing on large infested plants compared to small infested plants and on small infested plants compared to the large uninfested plants. Significantly more eggs were laid on large than on small infested plants, and on both infested treatments compared with un-infested plants.

5.2.3.2. Hoverfly oviposition behaviour

When all three plant size/aphid combinations were offered to adult *E. balteatus* simultaneously, no eggs were laid on large un-infested plants during any of the replicates. Significantly more eggs were laid on large than on small infested plants, and on large and small infested plants than on large un-infested plants (Table 5.5).

Table 5.5. The number of eggs laid on three plant size/aphid combinations (Plants at the booting growth stage infested with aphids (LI); Plants at the booting growth stage without aphids (LUI) and seedling plants infested with aphids (SI)). Figures = mean number of eggs per plant or pot of seedlings. Significance values are based on a Kruskal-Wallis test performed on pooled data across all cage designs.

Cage design	Mean total eggs/treatment			Significance P		
	LUI	SI	LI	LUI Vs SI	LUI Vs LI	LI Vs SI
Three choice	0	129	617	<0.001	<0.001	0.001
Single treatment	0	296	328	<0.001	<0.001	0.404

When the plant size/aphid combinations were offered to adult *E. balteatus* individually, the mean total egg number per replicate was significantly greater on both large infested and small infested plants when compared with large un-infested plants. No eggs were laid on large un-infested plants during the 15 replicates. No significant difference was observed between mean number of eggs laid per replicate on small infested and large infested plants (Table 5.5).

5.3. DISCUSSION

5.3.1. Hoverfly Flower Preference and Egg Load – Pilot Study

The pilot study showed that the attractiveness of flowering plants to hoverflies is positively associated with the number of eggs that females subsequently develop and lay, supporting the hypothesis that female hoverflies select plant species that currently offer high quality food resources, which will result in increased egg load. *Phacelia tanacetifolia*, a plant that has been widely cited in the literature as being particularly attractive to hoverflies (Hickman & Wratten, 1996), was the least effective in these experiments with a mean of fewer than 5 eggs per female. Cornflower, a once common arable wildflower, provided a better resource, indicating that other **UK indigenous wild plants, which unlike *Phacelia* can be considered for inclusion in field margins, may be equally or more effective at promoting hoverfly predatory impact.**

Hoverfly preferences remained consistent both when plant species were presented individually and when a choice of species was offered, supporting earlier studies (Cowgill *et al.*, 1993), and offering the potential for developing a cost effective laboratory technique for establishing the relative effect of different perennial wildflower species on hoverfly predatory impact. This method was then used to identify important UK perennial wildflowers, as a basis for recommendations for improved species composition of seed mixes used to establish field margins that enable simultaneous promotion of biodiversity and enhancement of naturally occurring pest control agents.

5.3.2. Hoverfly Flower Preference

Adult *Episyrphus balteatus* feed on nectar and pollen from a range of flowering plant species. Previous studies (e.g. Cowgill *et al.*, 1993, Gilbert, 1981) have shown that polyphagous hoverflies are selective in their use of available resources, developing transient flower constancies to ensure that as spring/summer progresses a sequence of plants that offer hoverfly populations the highest quality nectar and pollen are exploited. **The current study indicated that when hoverflies were offered a choice of flowers from different plant species, those selected for feeding most frequently were from plants that were subsequently associated with the development of the highest egg load in females.** These eggs give rise to the aphidophagous stages of the hoverfly, and therefore identification of preferred plant species and their inclusion in seed mixes developed for establishment of flower-rich field margins is important for the optimisation of the approach to conservation bio-control developed in this study.

Phacelia tanacetifolia is often cited in the literature as being highly attractive to hoverflies and a source of high quality pollen and nectar (MacLeod, 1999). This species, however, is not native in the UK and therefore cannot be considered for use in field margins and conservation headlands, but few native species have been tested for suitability as pollen and nectar sources. In the current study, candidate perennial flowering plants native to the UK were compared with *Phacelia* to identify suitable species for inclusion in florally enriched

field margins established in agri-environment schemes and as a component of a conservation biocontrol approach to the control of aphids in arable crops. The objective was to determine if alternative species could be found that offered as high quality resources as *Phacelia*, which has been shown in field trials to encourage hoverfly populations and consequently to contribute to the depression of aphid populations in winter wheat (Hickman and Wratten, 1996).

A range of UK native plant species were shown to be equally or more attractive to hoverflies when compared to *Phacelia*. In particular, a range of umbellifer species, yarrow and white campion were highly attractive to *E. balteatus* in the laboratory experiments, and subsequent observations of the rate at which these species are visited in the field have supported this finding (P. A. S. Mason *pers comm.*). Field observations have also confirmed that hoverfly species other than *E. balteatus* are also attracted by these flower species (e.g. *Metasyrphus corrollae*). A second group of plants were also found to show high potential as components of flower-rich margins for hoverflies, including cornflower, field scabious, common knapweed, rough hawkbit and lady's bedstraw. With one exception (yarrow), a close correlation was obtained between the relative attractiveness of the different plant species, when assessed using no-choice experiments and by comparisons with *Phacelia*. No-choice experiments indicated that yarrow was more attractive to hoverflies than was suggested by experiments comparing yarrow with *Phacelia*. However, no-choice experiments were conducted at the mid-flowering period, whereas resource constraints resulted in choice experiments being conducted towards the end of the plants flowering period, and thus the lower level of attraction recorded may be the result of reduced nectar and pollen availability at this plant growth stage.

These results support and extend the findings of Cowgill *et al.* (1993), in which two of the four species they found to be consistently preferred by *E. balteatus*, during 8 weekly observational sessions in the field, were umbelliferous (fool's parsley and wild carrot), the third was white campion and the fourth was autumn hawkbit (a species from the same genus, *Leontodon*, as rough hawkbit). One apparent difference from the findings of the earlier study and the current work was that yarrow, a preferred species in no-choice tests described above, was only a preferential flower in one week of the four week flowering period. This may indicate that yarrow has a very short period in which it offers high quality nutrition to hoverflies. Colley & Luna (2000) have also reported that umbelliferous flowers (including coriander, fennel), as well as yarrow, were highly attractive to hoverflies, although *E. balteatus* was not one of the species investigated.

The range of species shown to be attractive to hoverflies in the current study have flowering times that collectively span the whole of the period in which aphidophagous hoverflies are both active in and around arable crops, and are developing their eggs (Keble Martin, 1974). Provision of these species as part of the resource offered in managed field margins would therefore offer a plentiful supply of high quality pollen and nectar at the critical point in hoverfly life cycles. If such high quality resources are

associated with increased egg load, then populations of the predatory larvae will be increased. This fact, coupled with behavioural responses to plant structure and signs of aphid presence that enable adult females to lay their eggs near to aphid colonies (see section 5.2), may substantially increase the depression of aphid populations by hoverflies. **Thus the species of perennial wildflowers identified by this study should be considered as either valuable additions to seed mixes designed for establishment of flower-rich field margins or as species to be encouraged in other non-crop habitats**, as they offer advantages for increased farmland biodiversity, and also benefit a group of natural enemies that represent an important component of the beneficial fauna that contributes to conservation biocontrol.

5.3.3. Plant Structural Cues for Hoverfly Oviposition

The characteristic sequence of behaviour displayed by adult female hoverflies searching for aphids or oviposition sites progresses from focused hovering, to walking and labella probing searches on the plant, and culminates in ovipositor probing and egg laying. This study has shown that plant size/structure is an important stimulus, in addition to aphid infestation, for initiation of focused hovering. However, an increasing importance of aphid mediated cues can be traced through the behaviour sequence. Small infested plants stimulate less focused hovering attention than large plants irrespective of aphid infestation, suggesting that size/structure acts as a primary cue in the early stages of searching.

In herbivorous insects, behaviour sequences leading to host selection are not fully prescriptive. Insects will omit steps in the sequence if the relevant environmental cues are not present and proceed with behaviours characteristic of subsequent steps (Kennedy & Fosbrooke, 1973; Kennedy, 1974). In the current study, when only small infested plants were offered to adult females, and hence the large plant structure which usually stimulates focused hovering was missing, random encounter of aphid mediated cues on small plants resulted in a greater incidence of labella and ovipositor probing and egg laying than was recorded on large uninfested plants. Thus females did not reject small infested plants when they were encountered. Where a choice was available the hoverflies responded to the large infested plants and laid more eggs than on the small infested plants suggesting a clear preference for the larger plants. However, when no choice was available, as many eggs were laid on the small infested plants as on the large infested plants. This suggests that **although the preference is for large infested plants when available, the presence of aphids is enough to ensure oviposition on the smaller plants**.

The principal larval mortality factor for aphidophagous hoverflies is starvation, and insufficient food in the larval stage results in decreased fecundity (Cornelius & Barlow, 1980). The adaptive advantage to responding to large plants may be associated with plants large enough to sustain high aphid population growth. Studies of aphid population growth and plant growth stage interaction, indicates that larger cereal plants can sustain maximum aphid population growth rates (Watt, 1979) and are selected preferentially by aphids, which settle less readily on cereals at early growth stages (Walters & Dixon, 1982). Also, large plants

have more complex structures and thus present more refuges from predators and parasitoids for hoverfly larvae. It has been suggested that predation of hoverfly larvae is low (Chambers, 1988), but recent research looking at intraguild predation between aphidophagous predators, found that *E. balteatus* eggs and first and second instar larvae are highly vulnerable to larger aphidophagous predators such as ladybirds and lacewing larvae (Hindayana *et al.* 2001).

Recent work into the effect of egg load (Sadeghi & Gilbert, 2000a), presence of conspecific eggs (Scholtz & Poehling, 2000), female hoverfly age (e.g. Chandler, 1968) and aphid species preference (Sadeghi & Gilbert 1999, 2000b), suggest that all of these factors are also important in oviposition site selection by *E. balteatus*.

In the UK, the large growth stages of the autumn/winter sown cereals coincide with the arrival of *E. balteatus* in the crop. This study has shown that *E. balteatus* females will react to plant structural cues and concentrate their initial searching behaviour (focussed hovering) on the larger plants in preference to the smaller plants, but will only progress through the rest of their oviposition behaviour if signs of aphid colonies are present. This reinforces the hypothesis that these **hoverflies have the potential to provide some control of aphid populations as part of a natural predator complex. However, it is possible that late spring sown cereals may be at a disadvantage, particularly if there are larger, aphid infested plants in the area.** Cereal crops are therefore a suitable subject for the management strategy investigated in this project. The searching efficiency for egg laying sites on other crops may also depend in part on the presence of appropriate visual cues, and therefore further work may be required before the management system developed in this project for cereals can be reliably transferred to new commodities.

5.4. ACKNOWLEDGEMENTS

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6. OVERALL CONCLUSIONS AND KEY MESSAGES

Natural populations of beneficial invertebrates are capable of controlling aphid pests on arable field crops, especially cereals, but this natural biological control can break down, particularly as a result of annual variability in climatic conditions. This project has demonstrated that this natural control can be boosted and therefore made more resilient by management of the agricultural environment and manipulation of key components of the natural enemy fauna. Two essential factors are 1) the maintenance of natural enemy diversity, including parasitoids, specialist predators such as hoverflies and generalist, ground-dwelling predators such as carabid beetles and spiders and 2) the enhancement of early season activity, particularly of parasitoids and carabid beetles. Data has clearly shown that field margins, including those established within agri-environment schemes, can play a valuable role in promoting these two factors. Also, commercially-produced aphid sex pheromones deployed in cereal crops to coincide with summer aphid invasions can significantly enhance the impact of parasitoids at this critical time for control. Native wild flower species that provide essential food resources for hoverflies have also been identified for incorporation into field margin seed mixes and/or conservation in other non-crop habitats around the farm. Important within-crop factors that can help to conserve and boost ground-dwelling insect predators, such as optimum levels of weed cover, have also been identified.

Not surprisingly, there is no single field margin vegetation type that will benefit all components of the natural enemy community, including aphid-pathogenic fungi, which have been shown in a companion Sustainable Arable Link project to benefit from appropriate field margin design and management. It is therefore beneficial to establish and maintain a variety of field margins, including set-aside strips within the farming landscape. It is also proposed that composite margins, comprising a strip of uncut vegetation containing tussocky grasses next to the field boundary bordered by a more botanically diverse strip incorporating key wild flower species and cut annually in late summer, would provide the greatest benefits.

Natural biological control of aphids on other field crops, particularly peas, brassicas and salad crops is more challenging but data collected during this project has highlighted potential approaches that could prove profitable but require further research and development. The success of our approaches in the cereal cropping system offers encouragement to pursue the development of conservation biological control and natural enemy manipulation in other field crops.

The following key messages have arisen from the extensive work done in the 3D Farming project:

- Field margins containing wild flower/grass mixtures can help to reduce aphid densities in adjacent cereal crops.
- Early activity by parasitic wasps (parasitoids), coinciding with aphid colonisation in Spring, is a key component of natural biological control in cereals.

- Field margins and other non-crop habitats provide valuable reservoirs of aphid parasitoids.
- Aphid pheromones stimulate early spread of parasitoids into the crop and increase their impact on cereal aphid populations.
- Flower-rich field margins may increase the impact of aphid parasitoids on aphid populations in field brassicas.
- Umbellifer flowers, such as cow parsley and hogweed, as well as yarrow and white campion provide the best food resources for adult hoverflies, whose larvae feed on aphids. These should be incorporated into field margin seed mixes or conserved in other non-crop habitats such as hedge bottoms and track verges, as appropriate.
- Hoverfly activity in fields with appropriate wild flower margins can result in substantial reductions in aphid numbers in cereal crops.
- Predatory hoverflies can significantly reduce aphid population development during early to mid summer, when the effect of parasitoids is declining.
- Both adult hoverflies and adult aphid parasitoids are highly mobile and can rapidly spread across large fields.
- The distribution of carabid beetles, which are valuable pest predators, varies through both space and time and is influenced by crop type and by crop and margin management.
- Field margins support ground-dwelling predatory invertebrates that subsequently distribute themselves through the crop. Large fields will be more slowly colonised than small fields, and the diversity of these predators will be lower in the centre of large fields.
- Large numbers of predatory invertebrates overwinter within the soil and autumn cultivations can reduce their numbers.
- Some species of generalist invertebrate predators, such as carabid beetles, have localised distribution patterns across and amongst fields and broad-scale insecticide applications should be avoided wherever possible if the chances of reinvasion are to be maximised.
- Predatory invertebrates are encouraged by weeds but 10-14% weed cover is optimal.
- Set-aside strips sown with game cover can encourage predatory invertebrates within the crop but the most appropriate sown mixtures need to be developed for this purpose.
- Ground-active invertebrate predators can contribute to pea aphid control.
- Money spiders are important predators of aphids, feeding on cereal and pea aphids for at least 100m into the crop even when aphid densities are low.
- Field margins provide valuable habitats for money spiders, which can rapidly spread into crops by ballooning on silk threads.
- Maintaining biodiversity on the farm aids natural aphid control, especially if a range of invertebrate predators and parasitoids are encouraged.

- Encouraging a diverse natural enemy community in agricultural ecosystems provides stability for natural biocontrol systems.
- A diverse range of field margins should be maintained on the farm as this adds to the diversity of invertebrate predators. There is not a single margin design that will suit all purposes.
- A dual margin consisting of a narrow strip of grassy uncut vegetation against the field boundary (around 1m), with a broader (at least 2m) flower-rich strip, cut in late summer, would probably benefit the greatest range of beneficial invertebrates.

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APPENDIX 1: COMMUNICATION AND TECHNOLOGY TRANSFER

Scientific Publications

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- Powell, W. (2000) The use of field margins in the manipulation of parasitoids for aphid control in arable crops. *Proceedings The BCPC Conference -Pests and Diseases 2000*, 2, 579-584.
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Other Publications

- Holland, J.M. 2000. 3D Farming- making biodiversity work for the farmer. Farmland Research section. The Game Conservancy Trust Review of 2000. pp.75-76. The Game Conservancy Trust, Fordingbridge.
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- Powell, W. (2001) Increasing beneficial insects in field margins for aphid control. *ARIA Newsletter*, August 2001.
- Powell, W., Pell, J. & Williams, I. (2004) Pest control for free. Rothamsted Research Association Newsletter, Issue 14, June 2004.

Articles about the Project

- ‘Make use of margins for natural aphid control.’ *Farmers Weekly*, May 2001.
- ‘Marginal action.’ *HGCA Research in Action, Crops*, April 2002.
- ‘Marginal success against aphids.’ *HDC News*, October 2002.
- ‘Biodiversity to boost pest control.’ *Agriculture and the Environment, Defra R&D Newsletter*, June 2003.

Presentations at Scientific Meetings

2000

- Platform presentation at the BCPC Pests & Diseases Conference in Brighton, UK.

2001

- Platform presentation at the Annual International Association of Landscape Ecologists (UK) Conference in Birmingham, UK.
- Platform presentation at the 10th European Carabidologist meeting in Poland.
- Poster presentation at the 10th European Carabidologist meeting in Poland.
- Platform presentation at an International Biological Control Symposium in Brazil.
- Platform presentation at an International Pest Control Conference in Cuba.

2002

- Platform presentation at the European Congress of Entomology in Greece

2003

- Platform presentation at EWRS Working Group - Weeds and Biodiversity, Discussion meeting on “Weeds in the food chain” in Bristol, UK
- Two platform presentations at the 1st meeting of the IOBC/WPRS Study group “Landscape Management for Functional Biodiversity” in Italy.

2004

- British Ecological Society, Agroecology group meeting on “The Spatial Distribution of Invertebrates in Agroecosystems” in Fordingbridge, UK.
- Platform presentation at the XXII International Congress of Entomology in Australia.

Presentations at Farming Industry/Environmental Events

2000

- Poster presentation at the Cereals Event.

2001

- Presentation to Chadacre Trust trustees and Defra staff during open day at Allerton Educational Trust, Loddington.

2002

- Poster presentation at Unilever Sustainability Workshop.
- Field demonstration to farmers and advisors at HGCA/LEAF farm day in Cambridgeshire.
- Poster presentation at the Cereals Event.
- Poster presentation and demonstration at Great Yorkshire Show.
- Poster presentation at the HGCA R&D Workshop.
- Talk given to the Vegetable Agonomists Association at PGRO.

2003

- Talk at Unilever Workshop for European field site managers.
- Talk at ADAS Workshop for Lincolnshire Vegetable Growers.
- Field demonstrations to farmers and advisors at HGCA/LEAF farm days in Suffolk and Lincolnshire.
- Site demonstration of project to farmers and advisors at Unilever Colworth.
- Poster presentation at the Cereals Event.
- Poster presentation at the Environment Research Funders Forum at CSL
- Talk to Lincolnshire Brassica Growers.
- Talk and poster presentation at HGCA Roadshow, Oadby Lodge Farm, Leicestershire.
- Poster presentation on the ‘Grain Trail’ at the Royal Show.
- Project presented at Sustainable Arable LINK workshop at Rothamsted Research
- Talk at joint Rothamsted Research Association/HGCA Workshop in Leicestershire.

2004

- Talk given at HDC Workshop for brassica and salad growers at HRI Wellesbourne.
- Poster presentation at PGRO member’s day.
- Poster presentation at the Cereals Event.
- Poster presentation and demonstration at CSL Science Day

In January 2003, prior to the start of the final year of the project, a valuable project workshop was held at Unilever Research Colworth to discuss communication strategies for the project outputs. This was facilitated by Pete Stephenson of 'The Falling Apples Centres Ltd.' This helped to identify key messages, target audiences and routes for dissemination of information. A meeting was also held with Adrian Bell of 'The Mistral Group Ltd.' and Chrissie Davies of Unilever to discuss technology transfer, following which Adrian Bell gave a presentation to a project management group meeting and helped with the design and production of display material for incorporation in the Grain Trail display at the Royal Show.

APPENDIX 2. MINUTES OF A MEETING TO ARRIVE AT A CONSENSUS ON SEED MIXES FOR AGRICULTURAL MARGINS

In recent years, a number of other research projects have looked at the potential value of field margins on arable farmland for a variety of different benefits. Consequently, this is in danger of generating a range of contrasting recommendations for seed mixes to be used when establishing margins, creating confusion for the farmer. Obviously, no single margin design can fulfil all environmental, biodiversity and pest management objectives but it was necessary to collate available information and consider the most appropriate options. Therefore, on May 6th 2004 a meeting was held at the Game Conservancy Trust in Fordingbridge, Hampshire to discuss seed mix options and arrive at a consensus view. The 3D Farming project was represented by John Holland. The following is a copy of the minutes of that meeting.

PREAMBLE

Over the last 20 years there has been a great deal of research into developing seed mixes for agricultural margins with the aim of increasing biodiversity and controlling pests. The work done over this period has informed current farm-scale, countrywide experiments including 3D Farming, SAFFIE, Entomopathogenic fungi and BUZZ. These projects are differentiated by the detail of their objectives but address aspects of a common question; frequently both researchers and funding bodies are part of two or more projects. It would be valuable if those involved could arrive at a consensus, together with those who have worked on such projects in the past and those who are developing policy right now. The concern is that these projects will each arrive at a 'best' seed mix but that the knowledge gained remains fragmented and that those seeking guidance from the results of these projects will receive conflicting messages.

It is worth bearing in mind that at Cereals 2003, farmers were asking three questions of those presenting the 3D Farming and SAFFIE projects:

1. What seed mix shall I use?
2. Where can I buy it?
3. What will it do for me?

The aim of the meeting was not to formulate material which would suggest that expert advice is no longer necessary, but to discuss the extent to which a consensus on the critical components of seed mixes can be reached and how much flexibility there is in their design.

There were also concerns from HGCA, which funds several of these projects. HGCA would like to ensure that the guidelines being produced by HGCA funded projects (3D Farming, SAFFIE, Entomopathogenic

fungi) and projects funded by other organisations are pulled together so as to be practically applicable. They are also concerned that field margin guidelines are used to influence, and are not at odds to, policy, for example the Entry Level Scheme.

MEETING STRUCTURE

The following questions were circulated for discussion at the meeting:

What are the main objectives of field margin mixes?

What BAP, HAP and conservation targets are relevant?

Should we consider start points, end points and succession?

How can we balance prescription with diversity?

How many mixes are needed to achieve these objectives?

Can we arrive at a set of very basic seed mixes with key species for each objective?

What should be in these mixes?

Which species are performing well?

Which are failing to establish and why?

How important are species in these two categories (good and poor performers) in terms of the objective of the mixes?

Are there any species which are critical to all mixes?

Are there any cost-benefit issues?

Does the cost of any of the mixes, or their essential management, outweigh the benefit in terms of meeting the objectives of the field margin? (are they worth it?)

HGCA Concerns

Can the guidelines being produced by HGCA funded projects (3D Farming, SAFFIE, entomopathogenic fungi) and projects funded by other organisations be pulled together so as to be practically applicable?

Policy and Regulation

What are the current requirements of AE schemes and how do they sit with any guidelines?

Is it possible to ensure that field margin guidelines are used to influence, and are not at odds to, policy, for example the Entry Level Scheme?

OUTCOME FROM MEETING

Attendees

Chair: Jon Marshall (JM)

Organiser: Barbara Smith - GCT (BS)

Alex Ramsey - CAER (AR)

Andrew Sherrat - DEFRA (AS)

Clive Edwards - HGCA (CE)

David Sheppard - EN (DS)

David Smallshire - DEFRA (DSm)

Donald MacIntyre - Emorsgate (DM)

Duncan Westbury - CAER (DW)

John Holland - GCT (JH)

Judith Pell - Rothamsted Research (JP)

Marek Nowakowski - FE (MN)
 Paresh Shah - Rothamsted Research (PS)
 Peter Street - DEFRA (PSt)
 Peter Thompson - GCT (PT)
 Richard Brown - Emorsgate (RB)
 Richard Pywell - CEH (RP)
 Richard Snow - MOD (RS)
 Shona Campbell - HGCA (SC)
 Trevor West - Marshall Agro-ecology (TW)

Apologies from: Keith Walters, Mike Edwards, Paul Toynton, Simon Potts.

Types of margin were identified as :

Uncropped wildlife strip (natural regen)
Conservation headlands
Game cover
Wild bird seed
Pollen & nectar mixes
2m grass margin
6m grass margin
Tussocky grass strip
20m set-aside (sown)
Beetle Banks
20m set-aside (natural regen)
Flower-rich margin strips
Differential mowing on grass margins
Scrub margins
Riparian strips + willow strips
Hedge base flora, inc. re-creation
Poplar planted margins

Opportunities for maximising the value of margins

- Unsprayed margins can benefit species such as cornflower.
- A hedge-based margin can be harvested to increase margin value.
- The cropped area can also be useful, incorporating annual weeds into the crop.
- Restoration of existing, but derelict, margins by over-sowing.
- Diversity can be increased by introducing more than one margin type.
- Manipulating the margins of pasture may increase seed food and invertebrates for birds.
- Flower margins could be useful around silage fields.
- Reducing nitrogen leads to a more flowery sward.

The objectives for the uncropped margin

Rare arable plants.
 Pollen and nectar sources.
 Game cover and food.

Weediness can be seen as ‘good’ thing. It is possible to sow unsprayed margins and increase the weediness of the field in a managed fashion (e.g cornflower, corn marigold). However increased biodiversity may reduce yield and species such as cornflower may be difficult to eradicate. There is resistance by farmers to a weedy crop as they prefer to keep production and annual plants separate. Experience shows that even using a low seed rate, 8 years on annual plants are well established.

Arable weeds may be threatened by hedge bottom damage, if the hedge bottom is reinstated then arable weeds will recover. A 1m margin is appropriate for rare arable weeds, as they survive in and tend to ‘hug’ the edges; this may be because this is the only part of the field missed by the spray.

It is possible to sow arable species in a wild strip but winter crops are frequently sown and access to cultivate the wild strip is a problem.

Structure is important. For species such as grey partridge, cover is essential. For game species the strips are designed to provide winter seed for adult birds and in the summer, insects for chick food.

Diversity of approach is important.

The objectives for stewardship and flower mixes.

- Grass and flower margins
- Buffer zone – no inputs
- Soil conservation
- Bio-control
- Landscape connectivity

Pollen and nectar strips should be sown separately from the crop but managed as a crop. Inclusion of these species has huge environmental delivery. The strips should be managed for accessibility for the birds. The margins also serve as buffers - a no input zone – which benefits soil conservation. This will interest farmers who might be thinking of buffering watercourses.

The system may be steering farmers in the wrong direction because it is tempting to choose cheap and easy. Heterogeneity is important for biodiversity. From the farmers perspective, margins must be managed for each objective on a field by field basis. A farmer can have as many margins types as he can manage but good advice will be essential. One problem associated with the Entry level scheme is the lack of advice, although DEFRA feel confident about advice at the Higher Tier. European legislation is aimed at achieving: Full crop establishment, sustainable seed production, patches of bare ground with undesirable species controlled.

There is a general fear of fungal passage. A margin that isn't perceived as a fungal vector would be successful.

There is a trade-off between quality, quantity and dispersion, all of which are important.

Other types of margin

- Poplar planted margins
- 20m buffer strips, unmanaged for 20 years in stewardship.

Succession

Although beetle banks were planted with cocksfoot and yorkshire fog, over 15 years they became similar to the margin. Much depends on location, soil type and management. These are the most influential factors. Management is critical in the first two years; in different years there are different drivers of the community. The sown community also keeps developing over time; Terry Wells experimental plots are bringing up 'sown' species 20 years after sowing.

Nutrient management is very important. Many margins have high nutrients due to run-off. Difficult to reduce it.

It is not possible to re-create semi-natural communities. These are 'new' or gardened communities and should be treated as such. Management must be tailored to these communities.

Some endangered species are hedge bound, hedge garlic and red campion are examples of species that are reliable and will improve hedge bottoms. The improvement of degraded hedge bottoms is likely to be variable, depending on location.

General principles

- The location of a margin may influence its success.
- Aspect will influence colonising species.
- Even distribution of margins over a farm is important.
- Strategic placement is beneficial (near rare species for example)
- An internal margin may maximise refuges for bio-control

Farmers do tend to choose less profitable places to locate margins (e.g. watercourses, north facing slopes, shaded areas). Sometimes this may be useful as nutrient poor land supports diverse wild flowers and beneficial insect-pathogenic fungi like damper/shaded areas. Advisors are needed and DEFRA will be introducing guidelines in the future.

There are some benefits of an edge margin for within field treatments but these are difficult to maximise. An internal 'margin' may be an answer. Yield tends to be poor at the centre of a field and so it is possible to have a centre flower patch, buffered by grasses.

Selecting plants species

- Rare annual species can be included but rare perennials should be avoided.
- Rare species should be restricted to special projects as they are localised.

Provenance

It is hard to generalise sufficiently to create localised mixes. Generally, using seeds of local provenance is not a priority in agricultural margins, especially as agricultural varieties are often used. These are a good pollen source and don't tend to persist. Broad regional seed resources are currently being developed.

Both grass and clover have been widely traded so provenance may be irrelevant. The native range of plants is being changed.

- A native mix should only be used within the native range.

An alternative is to use green hay which can be very high quality although the quantity is likely to be limited. Best practice should be implemented in buffer zones around SSSI etc. Other agricultural areas are less important.

Performance of plants

- Generalists do well
- Predictive traits for good performers: colonisation ability, vegetative growth and seed bank persistence.
- Stress tolerators perform poorly
- Grass/flowers = 80/20 split is a good ratio
- Management is critical to maintain diversity

Successful species

Achillea millefolium

Anthyllis vulneraria

Centaurea nigra

Cynosurus cristatus

Echium vulgare

Festuca rubra

Leucanthemum vulgare

Lotus corniculatus

Prunella vulgaris

Rumex acetosa.

Poor performers

Sanguisorba officinalis

Thymus vulgaris

Rhinanthus may be a useful management tool and could be tested at a field scale. Introduction should be cautious as it has an effect on other species and establishment is inconsistent. 1000 seeds/m² is a useful rate for controlled productivity. The majority of failure is associated with mowing or mowing at the wrong time.

In general, it is necessary to have enough representation in the seed mix to allow species development. The proportions are not really important the sward will find a natural balance over time.

As long as there is a nearby seed source to provide good colonising species, a simple mix can be used.

Festuca rubra can form dense swards which lodge, preventing native forbs to colonise; this can be prevented by adding *Cynosurus* and mowing correctly.

Legumes

Legumes should not be added to wildflower mixes

When sown with ryegrass, legumes will dominate in the initial years but become less dominant in time . It has been observed that areas which are low in N but high in P and K will have a pulse of legumes first followed by grasses.

Legumes must be used with caution, for example, a mixture of black medic and vetch will be dense and unlikely to recruit other species.

Legume species can be important for encouraging beneficial organisms such as parasitoids and aphid-pathogenic fungi.

Grasses

A tussocky grass mix should include *Holcus*, *Dactylis* and *Deschampsia*. The management is very important, for example, tussock mixes are good for spiders, so a low cut will not be beneficial. It is the structure of the tussock that is important rather than the species of plant, a tussock and an understory are necessary for cover to benefit many invertebrate species.

It is not necessary to have a big block of tussock grass, hedge bound grass may be sufficient.

Research has shown:

- Predatory beetle diversity can be greater in fine grass plots when compared to tussocky plots.
- In comparison: Grass by wire fences = greater abundance of predatory beetles while Grass in hedge bottoms = greater predatory beetle diversity.
- Including a nectar source nearby will be beneficial.
- *Holcus* and *Dactylis* is good for aphids and also a reservoir for their natural enemies, including fungus.
- *Sitobion* spp. (e.g. *S. avenae* a cereal pest and *S. fragariae*, a non-pest) use grasses in the summer.
- Aphids are not necessarily moving out from the margin – some species exist only on the wild plants in the margin.
- Pest effects are difficult to predict.

Flower mixes

- A grassless flower mix would need so much seed it is impractical.
- What ever is done, the proportion of grasses and flowers will end up the same. Proportions will be controlled by soil type and management.
- It is important to have a long period of nectar production for all bee species.

Standardizing is dangerous, as species are not reliable in all regions.

Some species should be included for specific invertebrates or mammal species. For example, long tongued bees need plants with deep corollas and these may also provide food for bats. 3D Farming has shown that umbellifers are useful for beneficials such as the hoverfly and should be included. Umbelliferae, compositae and rosacea are useful species for bees

Diversity is the best option and serendipity will adjust the mix.

Cost

The costs of some of these mixes may not be tolerated by farmers. The aim of the Entry level scheme is to draw in farmers to Higher Tier where they could recoup costs. It is important to discourage farmers using very low sowing rate as this may lead to disappointment and discouragement. Linking economic benefits directly to plants (e.g. by showing that beneficial fungi are supported by legumes for example) may help encourage farmers to invest as they get added benefits.