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Contents **Page Number**

Grower summary

CP 14

Raspberry: Novel approach for ICM in fresh and processed crops

Final report 2006

Headline

- White sticky traps have been effectively enhanced to trap large numbers of raspberry beetles, using a floral attractant identified at SCRI. These traps should enable growers to monitor for raspberry beetle flight activity and to detect 'hot spots' within plantations so that insecticide applications can be targeted more effectively.

Background and expected deliverables

Raspberries (*Rubus ideaus* L) are very susceptible to attack by the raspberry beetle (*Byturus tomentosus* Degeer) and the large raspberry aphid (*Amphorophora ideai* Börner). Currently the organophosphate chlorpyriphos (Equity, Lorsban WG, Alpha chlorpyrfios etc) is often applied to control raspberry beetle, but because of public pressure to reduce the use of organophosphates and subsequent detectable residues in fresh produce its use is undesirable and unsustainable. The future registration of chlorpyrifos beyond 2008 for controlling raspberry beetle is in doubt due to very low maximum residue level (MRL) restrictions. Currently, the other available chemical options for controlling raspberry beetle include synthetic pyrethroids which cannot be used with integrated pest management systems and thiacloprid (Calypso), which is limited to two applications in a season and is also used for controlling other insect pests. *A. ideai* is a major virus vector, transmitting raspberry leaf spot virus, raspberry leaf mottle virus, black raspberry necrosis virus and Rubus yellow net virus. This pest is partly controlled by genetic resistance bred into most current U.K. raspberry varieties and application of approved insecticides which target raspberry aphids and other raspberry pests. However, resistance-breaking aphid biotypes which have counter-adapted to resistance genes now threaten the continued use of aphid-resistant raspberry varieties in Integrated Pest Management strategies.

This project had two major aims:

1. To evaluate new options to reduce pesticide use for controlling raspberry beetle, using a prototype trap for monitoring the pest.
2. To investigate interactions of large raspberry aphid biotypes with different types of genetic resistance (based on single or multiple genes) in raspberry varieties and to assess the effect of a parasitic wasp species involved in biological control strategies.

The expected deliverables from this work included:

- An evaluation of the use of sticky traps enhanced with formulations of a volatile floral attractant for monitoring raspberry beetle and for future IPM based control strategies.

- An understanding of the effectiveness of different types of genetic resistance against resistance-breaking biotypes of the large raspberry aphid in UK raspberry varieties.

- An evaluation of the role of a parasitic wasp (parasitoid) species in reducing raspberry aphid populations on raspberry varieties containing different resistance genes.

Further research on the development and deployment of raspberry beetle monitoring and trapping systems would be needed before the results can be implemented by growers.

Summary of project and main conclusions

The catch rate of raspberry beetles on white sticky traps is enhanced by 10-65 fold with the addition of an SCRI floral attractant (Figures 1 and 2). The enhanced traps have the potential to be used in the pre-flowering period to monitor raspberry beetle populations. By implementing spray thresholds they can be used to reduce insecticide applications.

Raspberry beetle catches on enhanced sticky traps placed within open field plantations are influenced by local raspberry beetle population densities, time of raspberry flowering, crop type (e.g. open field raspberry or blackberry), threshold temperature for flight and by the location of alternate hosts in the surrounding habitat.

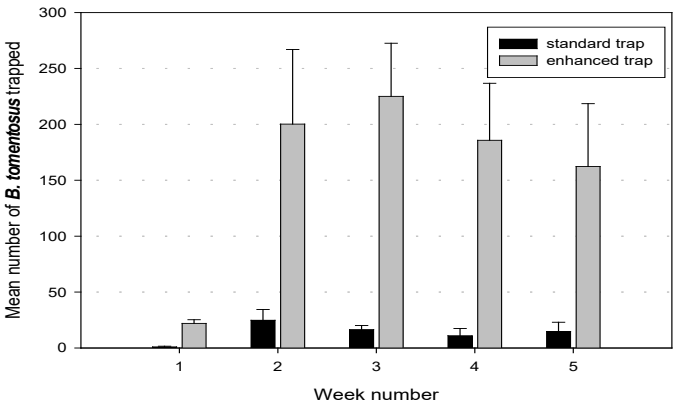

Figure 1: Effect of lure enhancement (attractant B) using white sticky traps on weekly raspberry beetle catches in commercially grown blackberries.

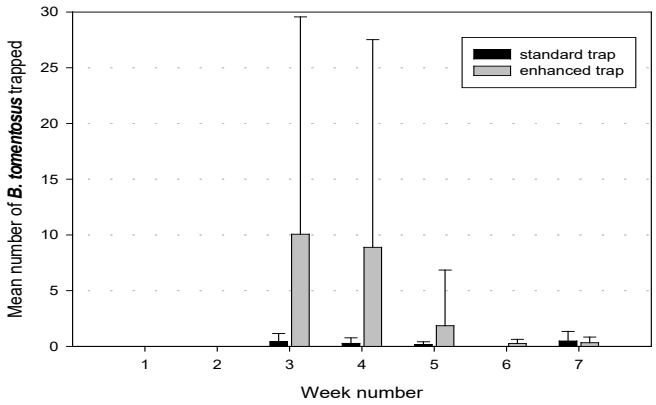

Figure 2: Effect of lure enhancement (attractant B) using white sticky traps on weekly raspberry beetle catches in young raspberry (Malling Leo) plantation. Note the effect of start of flowering on raspberry beetle at week 5.

Studies on trap density requirement indicates that more than 4 traps / 195 m^2 (5 rows of 17 plants) are required to reduce raspberry beetle numbers in heavily infested sites (e.g. SCRI experimental site) where the raspberry beetle population has not been previously controlled by insecticide sprays (Figure 3). Further research is needed to optimise trap densities on commercial growers' sites where raspberry beetle populations have been previously controlled by repeated insecticide applications or in newly established protected cropping plantings (new HortLINK HL0175).

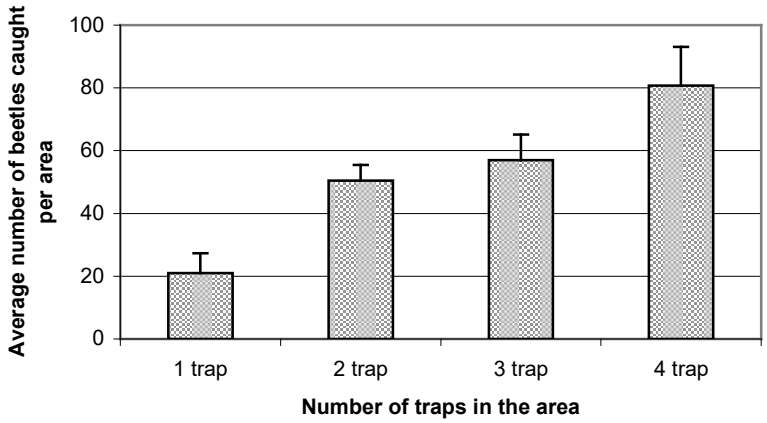

Figure 3: The effect of increased trap density on raspberry beetle catches in SCRI mixed variety plots (averaged for 4 raspberry varieties).

The release rate of the volatile attractant (B) affected raspberry beetle catch rate. A comparison of attractants A and B (Figure 4) in either a glass dispenser with wick (G; high evaporation rate system lasting one week) or a plastic vial (P; slow release system lasting several weeks) versus a control (C; no attractant) showed that for this type of floral attractant a relatively high release rate (up to 1700 µl/week) is required. Further work is needed to optimise dispenser design and attractant release rate so that attractant dispensers last the 4-6 week pre-flowering period when raspberry beetles are effectively trapped weeks and are easy to use (new HortLINK HL0175).

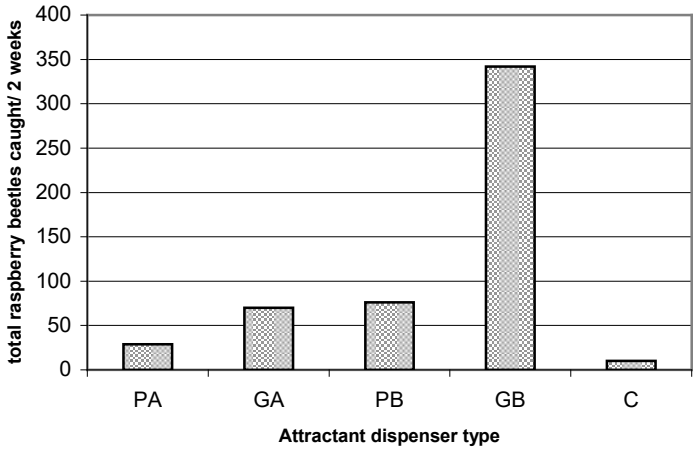

Figure 4: Comparison of raspberry beetle attractant A and B released either from glass (G) or plastic (P) dispensers

The effectiveness of a 'lure and kill' strategy to reduce or replace insecticide applications against raspberry beetle still needs to be validated under a range of growers' conditions. The trapping system offers most potential in situations where raspberry beetle populations in established plantations have previously been suppressed by regular insecticide applications or in newly planted areas (e.g. protected tunnels) where the initial pest population is low and can be controlled before it builds up.

Large raspberry aphid biotype 2 is able to overcome resistance gene A_1 (e.g. formerly resistant variety Glen Ample) and is prevalent in England and Scotland. Resistance gene A_{10} (e.g. in variety Glen Rosa) has been partially overcome by a recent biotype, common in England but not yet established in Scotland. The A_{10} resistance breaking biotype in Scotland is unable to survive or reproduce on some varieties containing gene A_1 (Glen Prosen, Malling Landmark, Glen Ample). Further research is required to develop molecular markers for raspberry aphid resistance genes so that breeders can develop more durable forms of resistance by combining aphid resistance genes.

The resistance genes present in a particular raspberry cultivar and the *A. idaei* biotype feeding on the cultivar had an effect on the ability of the parasitoid *Aphidius ervi* to successfully parasitise the aphid. The numbers of successful parasitism events were low in all treatments, suggesting that under the conditions tested *A. ervi* is not a suitable candidate for biological control of *A. idaei*.

Financial benefits

At this time there are no direct financial benefits to be gained by growers from this work, but a new Defra HortLINK project (HortLINK HL0175; 2006-2011) will further develop commercial monitoring and trapping systems for raspberry beetle and evaluate their use under standard agronomic practices.

Action points for growers

- The raspberry flower volatile attractant used in this experiment is not yet commercially available but growers could consider using white sticky traps available from AgriSense BCS Ltd (contact: info@ambiensis.com, Product

code BC2245) to monitor raspberry beetles and detect 'hot spots' within plantations so that insecticides can be targeted more effectively.

- Growers should continue to use a spray programme to control raspberry beetle but be aware that an enhanced monitoring and trapping system will be developed with commercial partners in HortLINK HL0175.

- In Scotland, where the A_{10}-breaking *A. ideai* biotype is still rare, consider planting raspberry varieties with A_{10} resistance (e.g. Glen Rosa, Glen Doll or Octavia) as part of an IPM strategy to minimise aphid outbreaks and subsequent virus problems. In England and Scotland, report any outbreaks of large raspberry aphids on A_{10} resistant raspberry plantations to ADAS advisors.

- Growers should monitor levels of raspberry aphids on any plantations with only the A_1 resistant gene (e.g. Glen Ample) and apply approved insecticides to prevent virus infection and spread.

Science section

Raspberry beetle

Introduction

The raspberry beetle, *Byturus tomentosus* Degeer, (Coleoptera: Byturidae) is the most important pest of commercial raspberry crops in the UK and in many parts of continental Europe (Gordon *et al.*, 1997). The adults emerge in the late Spring and feed on the developing flower buds. Once the flowers are open, the adults mate and the females lay their eggs in the flower. The main damage is caused by the larvae tunnelling into the developing fruit (Taylor & Gordon 1975). At present, control of this pest in commercial plantations involves applying one or more insecticides (e.g. Dursban, Decis, Calypso) to the late green bud and pink fruit stages before harvest which kills the newly emerged larvae.

Raspberry beetles are known to use visual and olfactory cues to locate raspberry flowers (Woodford *et al.* 2003). After a range of coloured sticky traps were tested, it was found that white, non-UV reflective traps were the most effective. Höhn *et al*. (1995) suggested that the numbers of beetles caught on the sticky traps was related to the amount of beetle damage observed in the plantation and that in some instances the use of sticky traps could help growers avoid the need for routine applications of insecticides.

A recent EU-funded project, 'Reduced Application of Chemicals in European Raspberry Production' (RACER) tested the use of these traps for monitoring raspberry beetles (Woodford *et al*., 2003). Adult raspberry beetle activity was monitored at twenty-three sites in Scotland, Switzerland and Finland and there was found to be a great variation in the numbers of beetles caught between sites and years. The extent of crop damage was not closely related to the number of beetles caught although there was very little damage at sites with fewer than 5 beetles caught per trap week before flowering.

The development of a beetle monitoring and trapping system was taken further at SCRI by Birch *et al*. (1996) with the identification of two flower volatiles, which are recognised by the beetles and involved in their attraction to flowers. This required the use of combined automated thermal desorption-gas chromatography-mass spectrometry (GC-MS) with an electro-antennogram (EAG) to identify volatiles emitted from raspberry flower (Robertson *et al*., 1993, 1994). EAG assays combined with behavioural studies in olfactometers and wind tunnels identified two floral attractants for B. *tomentosus* (coded chemical A and chemical B) for testing under field conditions in this project.

Materials and Methods

a) Raspberry beetle – optimising trap density in a commercial raspberry plantation (year 3).

Optimising trap density in a commercial raspberry plantation

Location of experiment

The location for the experiment was Wester Essendy, Blairgowrie, Perthshire, Scotland (NO 135 435). The experiment was undertaken on an early flowering raspberry variety, Glen Clova, which was planted in 2001. Directly to the east was a raspberry plantation of Glen Ample, a mid-season flowering variety, which was planted at a similar time.

An area within the Glen Clova plantation was chosen which could be spit up into four areas each containing one experimental block (Figure 5 shows one experimental block). A Latin Square design was chosen to distribute the treatments among the experimental area. Each block contained 5 rows, each row being 15 metres in length and containing approximately seventeen raspberry plants. Trap density treatments of either one, two, three or four white sticky traps were positioned in each block. The distribution of the traps within each block can be seen below. The traps were positioned on the bottom supporting wire, approximately 0.75 metres above the ground. Each of these traps was enhanced with attractant chemical B, which was released from a 1700 µl capacity porous plastic dispenser (AgriSense-BCS Ltd) and attached on the metal frame above the white sticky trap.

2 traps	1 trap	3 traps	4 traps
1 traps	2 trap	4 traps	3 traps

N

Figure 5; Diagramatic representation of the experimental set up to study optimal trap density at Blairgowrie, 2005.

Duration of experiment

The white sticky traps were put in position prior to emergence of *B. tomentusus* (2 May 2005) and the experiment continued until mid-flowering for Glen Clova (20 June 2005). The traps were changed once a week and the plastic dispensers containing attractant chemical B remained in place throughout the experiment.

Storage and assessment of the traps

On removal from the supporting wire, the traps were wrapped in Clingfilm® and stored at 4 ºC. To obtain the number of captured beetles, each sticky trap was inserted into a plastic bag marked with a grid.

Results and Discussion

An analysis of variance for *B. tomentosus* numbers showed that there was no significant difference between the four experimental areas at the Blairgowrie site, enabling the results to be pooled for statistical analysis. A two way analysis of variance showed that there was a significance difference (d.f. = 3; F = 3.552; P= 0.0020) in the number of *B. tomentosus* caught between areas containing different densities of traps, after allowing for the effects of differences in week of catch (Figure 6). Pairwise multiple comparison procedure (Holm-sidak method) showed that a significant difference in the number of *B. tomentosus* caught occurred between areas containing one trap and areas containing four traps. There was a significant

difference (d.f. = 6; F = 13.827; P < 0.001) in the number of *B. tomentosus* caught between weeks, after allowing for the effects of differences in the density of traps. Pairwise multiple comparison procedure (Holm-sidak method) showed that the significant difference in the number of *B. tomentosus* caught occurred between week 2 versus 1,3,4,5,6 and 7. Week 2 had higher levels of *B. tomentosus* caught than in the other weeks.

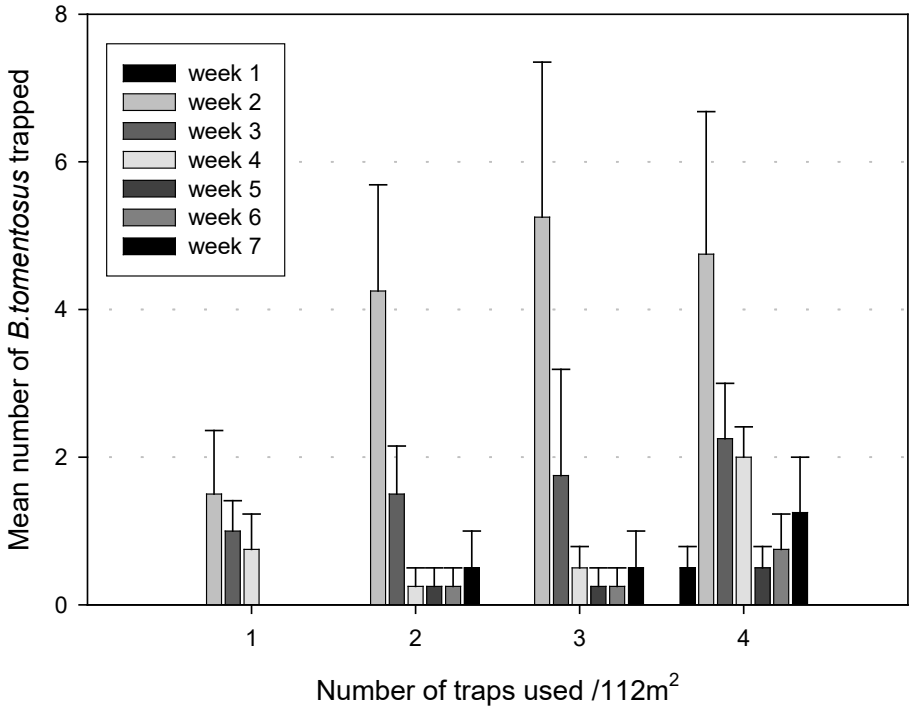

Figure 6; The mean number of *B. tomentosus* trapped per area each week containing either one, two, three or four enhanced traps (chemical B). Each treatment was replicated four times. The traps were put in position on 2nd May 2005 and changed once a week until 13th June 2005. Error bars represent standard errors. Flowering of Glen Clova commenced at the start of week 4.

Analysis of the effect of trap density (Figure 6) on *B. tomentosus* weekly catch numbers in the commercial plantation at Blairgowrie with a lower *B. tomentosus* population than SCRI's experimental site produced some interesting results. This site represented a typical commercial plantation where insecticidal control had been applied routinely in prior seasons to reduce *B. tomentosus* below economically damaging levels. There were a significantly greater total number of *B. tomentosus* trapped in area containing four traps when compared with areas having only one

trap, but there was no significant difference in the total numbers of *B. tomentosus* caught when comparing areas containing four traps with areas containing two traps or three traps. Although the numerical results indicated that there was an increase in the total number of *B. tomentosus* caught as the density of traps increased, this increase was not great enough to be statistically significant. Therefore the numbers of beetles caught started to plateau as the trap density increases. These results suggest that the optimum trap density was two traps per 112 m^2 for this site, which has a relatively low *B. tomentosus* population (i.e. weekly trap catches of 1-5 adults per trap week, compared with 20-500 adults per week in the insecticide-free experimental site at SCRI). The grower applied chlorpyrifos (Dursban®) insecticide against *R. tomentosus* over the entire plantation at the end of flowering, killing raspberry beetle adults and larvae to minimise the risk of damage. As a consequence no fruit damage data could be collected. Further work is therefore required to relate trap density to berry damage on a range of commercial plantations with insecticide-free experimental areas before establishing any relationship between trap density and fruit damage.

There were a significantly higher number of beetles caught in the second week than during the remainder of the experiment. This reduction after week two was not due to the start of flowering in the plantation, as this is does not occur until week four. This suggests that the introduction of the enhanced sticky traps in the plantation removed a proportion of the population of *B. tomentosus* from the plantation, therefore leading to the lower trap captures for the remaining weeks before the start of flowering. These results suggest that this trapping system may have potential to be used to reduce attack below economic thresholds in commercial plantations where the population of *B. tomentosus* is low (i.e. 1-5 adults/trap week). This is most likely to be the case in newly established raspberry plantations where there has not been time for *B. tomentosus* to build up to population levels which are beyond the limits for a mass trapping system to work effectively, or more established sites where *B. tomentosus* populations have been previously suppressed by routine insecticide control . This aspect needs to be further researched on growers' sites representing a range of conditions and raspberry beetle population densities.

Conclusions

- A floral attractant (compound A) was tested in a small pilot scale but did not improve *B. tomentosus* catch rate compared with compound B. Because of

limited field areas for replication all subsequent tests were performed with attractant compound B.

- Tests on two dispenser types showed that a fast release glass vial system was 2.7 times more effective in attracting *B. tomentosus* than a slow release plastic dispenser system. However, the glass vial system requires changing once or twice a week, so is too labour intensive for commercial use. The plastic dispenser loaded with compound B lasted longer (6-8 weeks) than the pre-flowering period when the traps are most effective. An alternative dispenser with an intermediate release rate needs to be developed so that it attracts large numbers of *B. tomentosus* but ideally lasts for 4-6 weeks (the pre-flowering active period for the pest). This will be addressed in HortLINK HL 0175.

- Attractant compound B effectively enhances the catch of *B. tomentosus* on white sticky traps by x10 (commercially grown raspberry) to x 65 (commercially grown blackberry) when compared with white sticky traps without the attractant lure.

- Based on a single site study, commercially grown blackberry is very attractive to adult *B. tomentosus*. Further research is needed to investigate the relationship between the high level of adult attraction and larval damage in this crop.

- The volatile enhanced trap is most effective in the period from adult *B. tomentosus* emergence to the start of raspberry flowering. Once raspberry commences flowering *B. tomentosus* is more attracted to raspberry flowers than enhanced traps, due to a competition effect for host volatile signals.

- Migration of *B. tomentosus* between wild hosts (e.g. hawthorn) and raspberry plantations can be monitored using volatile enhanced sticky traps, but this behaviour is variable between sites. At the SCRI site *B. tomentosus* were trapped adjacent to hawthorn up to 90m from the raspberry plantation, possibly suggesting movement by *B. tomentosus* between the crop and wild hosts. Further work is required to confirm whether movement between wild

hosts and the raspberry crop is important in designing a monitoring or trapping strategy for this pest.

- In plantations containing mixed raspberry varieties, *B. tomentosus* generally follows the phenology of flowering time, moving from early to later flowering raspberry varieties. *B. tomentosus* adults show a preference for feeding and oviposition in mixed variety plantations and is more attracted to Glen Clova than to Malling Leo and Glen Ample.

- In pre-flowering samples using enhanced sticky traps (compound B) from three out of four raspberry varieties the sex ratio of *B. tomentosus* was found to be 1:1 throughout the main six week flight and flower feeding period. On Malling Jewel more female than male *B. tomentosus* were trapped in the first two weeks of sampling but not in subsequent weeks. Thus the enhanced sticky traps are effective at catching both sexes of *B. tomentosus*, which fits with observations that both sexes of *B. tomentosus* use the raspberry flower as a feeding and mating site.

- Within commercial plantations of single raspberry varieties, variation in trap catches of *B. tomentosus* were detected, indicating 'hot spot' areas of pest activity. The reasons for this variation require further investigation but imply that optimal trap placement needs to take this local variation into account when designing a monitoring or trapping strategy. Identification of hot spots within plantations could enable growers to target insecticide sprays more effectively and reduce insecticide inputs where *B. tomentosus* populations are low.

- At the SCRI experimental site, which had an extremely high raspberry beetle population due to lack of insecticidal sprays for 7 years (up to 2500/per trap week at the peak), four enhanced traps per 195 m^2 were insufficient to reduce raspberry beetle numbers to a level needed for economic control. This was indicated in the high percentage of berries damaged by feeding raspberry beetle larvae (35% damage in control areas versus 22% in the best trap protected area). At commercial sites where raspberry beetle numbers are much lower due to prior insecticidal control or in situations of new plantings, a lower density of traps should be required. At a single study site on a

commercial farm (1-25 *B. tomentosus* adults/trap week in the four study areas during peak flight activity), both the Dursban protected and enhanced trap protected areas appeared to give good control of raspberry beetle damage (0% and 2% damaged fruit respectively). However, due to the low *B. tomentosus* population in the study area the numbers of raspberry fruit containing *B. tomentosus* larvae were too small to undertake statistical analysis. Further studies are required.

- Although there was insufficient time to quantitatively assess the impact of enhanced traps on non-target organisms (other pests and beneficial insects), visual assessments of traps were undertaken throughout the project. White sticky traps caught very few bees but did catch a small number of natural enemies, particularly 2- and 7-spot ladybird adults and larvae. Aphid parasitoids were trapped in very low numbers so should not be affected by this technology. The main non-target group trapped were dipteran species, which were not natural enemies of raspberry pests. The main disadvantage is that dipterans saturate the white traps and reduces their visual attraction to *B. tomentosus.* This problem will be overcome in the new HortLINK project by developing a non-sticky funnel trap which does not become saturated by raspberry beetles or non-target insects. The sticky traps were deployed before raspberry aphids were found on the crop and no raspberry sawflies were detected, so the use of enhanced sticky traps is unlikely to impact on other raspberry pests besides *B. tomentosus.*

Large raspberry aphid

Introduction

The large raspberry aphid, *Amphorophora idaei* Börn (Hemiptera: Aphididae), is an important pest of raspberries in the UK. In high numbers, this insect can cause direct damage through feeding, but more importantly, it is a vector of four viruses, which can cause serious diseases in the infected plant leading to loss of plant vigour and fruit yield and quality (Jones, 1986). The use of insecticides can control aphid numbers but they do not act quick enough to stop the spread of the viruses (Taylor and Chambers, 1969). There is no plant immunity in the *Rubus* germplasm, which works against any of the four plant viruses (Jones and Jennings, 1980; Jones, 1986), so the breeding of virus resistant plants is not an option. There is however,

resistance to the aphid, which has been identified in the *Rubus* germplasm, and through plant breeding a high percentage of raspberry cultivars used commercially contain one or more of the resistance genes. Unfortunately, continued use of these resistance genes has lead to the development of five biotypes of the large raspberry aphid which are able to overcome the resistance mechanisms (Birch & Jones 1988; Birch *et al.* 1994).

In the second year of the project (2004), the abundance of large raspberry aphid on cultivars containing resistance genes was investigated. It was hoped to observe a difference in the number of aphids on the various cultivars and therefore showing that some cultivars are more effective at keeping the population at a low level. The proportion of parasitized aphids was studied to investigate if there is any link between the resistance gene in the plant and the ability of the wasp to parasitize the aphid. It is thought that aphids feeding on cultivars containing resistance genes require more of their resources to overcome the resistance mechanism, taking longer to develop, and therefore become more susceptible to wasp attack.

In the laboratory, the research involved studying the fitness of two resistance gene breaking biotypes (biotype 2 = A_1 gene breaking biotype and A_{10} gene breaking biotype) of the large raspberry aphid, *A. ideai*, whilst feeding on cultivars with different resistance genes. Indicators of fitness included the length of time that the aphid required to develop into an adult and the number of young produced by the adult.

The effect of cultivar on the numbers of *Amphorphora idaei* present in an insecticide-free plot.

Materials and Methods

Five raspberry cultivars were sampled: Glen Ample, Glen Clova, Glen Rosa, Malling Jewel and Malling Leo. Glen Ample, Glen Clova, Glen Rosa and Malling Jewel were sampled in an experimental plot established in 2000. Malling Leo was sampled in an adjacent experimental plot, established in 1996.

Four plots of each cultivar were sampled. In each plot of cultivar, four plants were sampled by removal of two leaves from the top, two leaves from the middle and two leaves from the bottom of a primocane stem and a floricane stem. This was

repeated in the remaining three experimental plots, providing sixteen plants samples for each cultivar. Each pair of leaves was placed into a labelled bag which was tied shut leaving a pocket of air inside to avoid damaging the insects. These bags were stored at 4ºC until the aphids could be counted. For each leaf, the number of *A. idaei* and the stage of development were recorded using the method described in Dickson (1979). The cultivars were sampled weekly, with sampling starting 17 June 2004 and carried out for nine weeks.

The results were analysed using a generalized linear mixed model (GLMM). A Wald test was used to show any significant effect of the fixed effects of the model, cultivar and position of the leaf. The results from the two leaves in each plant height position, top, middle and bottom, were combined. The data from each plot, plant and cane type were also combined to reduce the number of zeros within the data. If the data contains too many zeros, the GLMM cannot estimate the parameters of the model. Therefore, when more than 90% of the observations for a cultivar in a single week were zero, the analysis was not conducted.

Results

The effect of cultivar on the numbers of *Amphorophora idaei* present in an insecticide-free plot

Nine weeks data was analysed, but Glen Clova was dropped from the analysis in week 9 as too few aphids were found. The results from Malling Leo were removed as no aphids were found over all 9 weeks. The results from Glen Rosa were also removed as there was only one aphid was detected, on one sampling date on a middle leaf of a primocane.

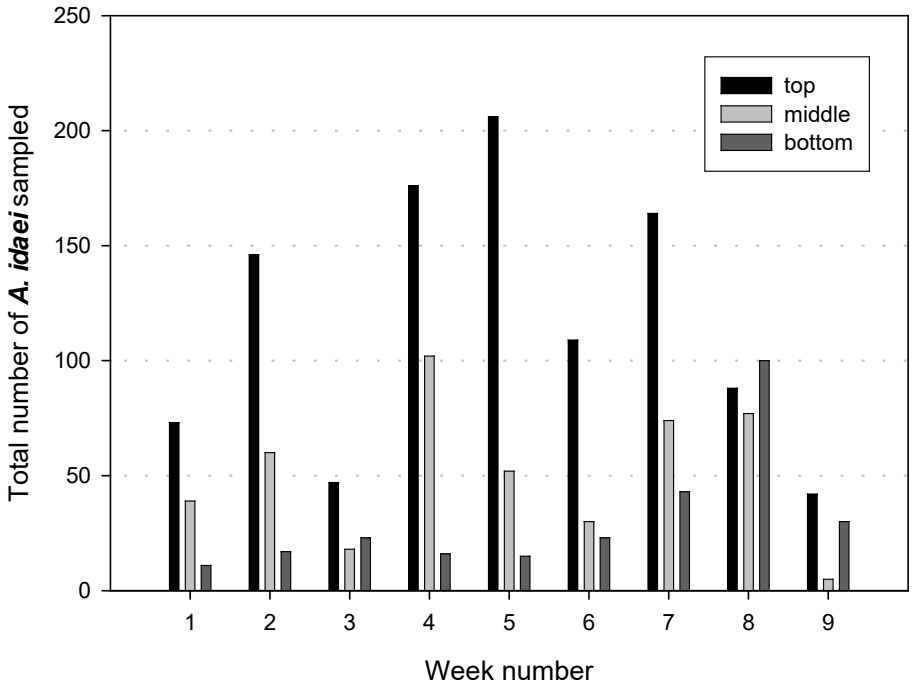

Figure 7: The total number of *A. idaei* sampled on 16 plants of Glen Ample in 2004. A total of 64 leaves were sampled at the top, middle and bottom of the raspberry plant.

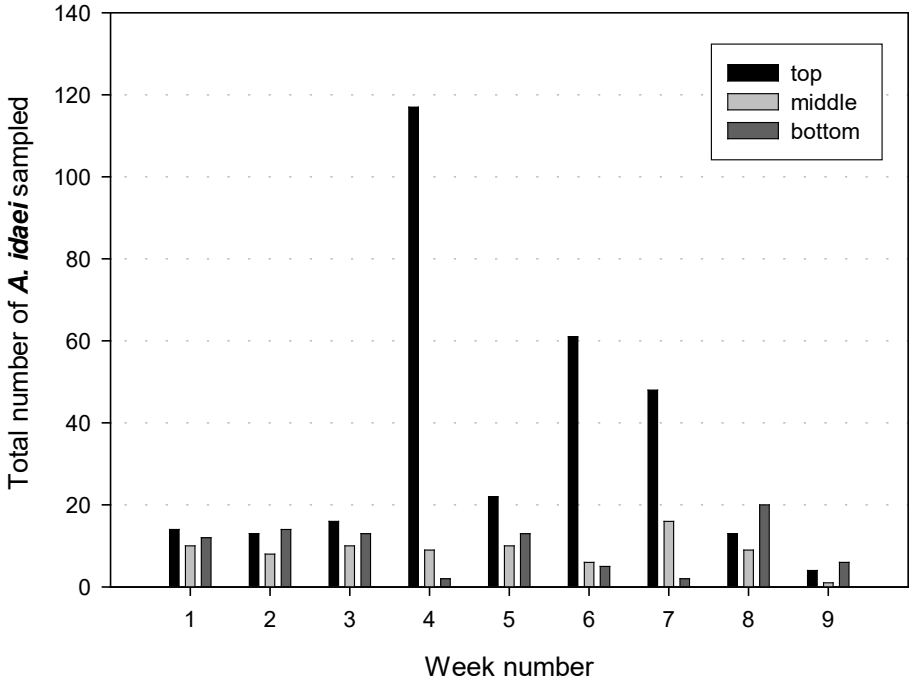

Figure 8: The total number of *A. idaei* sampled on 16 plants of Glen Clova in 2004. 64 leaves were sampled at the top, middle and bottom of the raspberry plant.

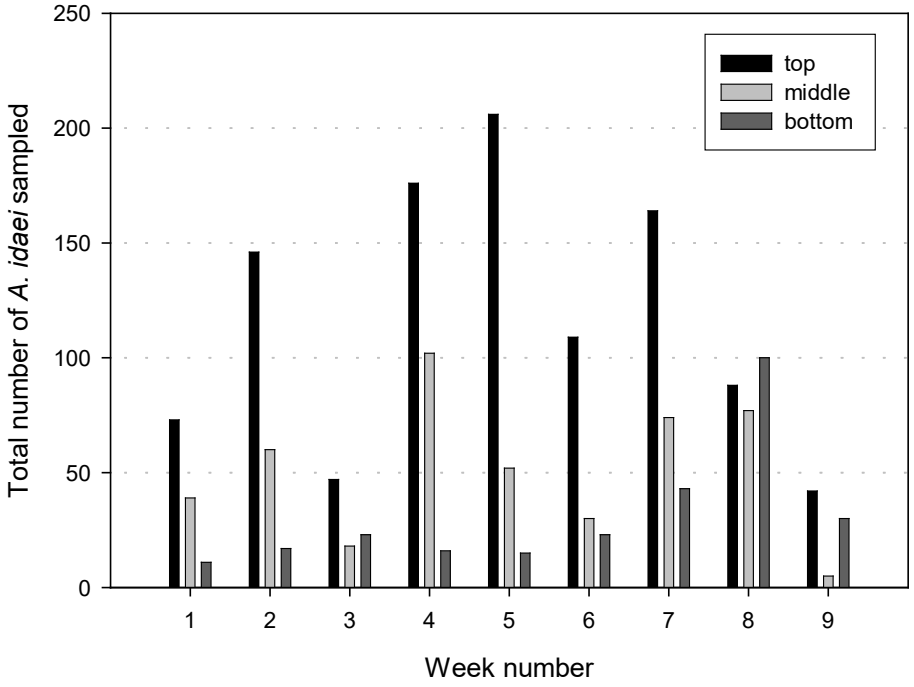

Figure 9: The total number of *A. idaei* sampled on 16 plants of Malling Jewel in 2004. 64 leaves were sampled at the top, middle and bottom of the raspberry plant.

Figures 7-9 show the total number of *A. idaei* sampled each week in the three positions on the plant. The results from the two leaves in each position, top, middle and bottom, were combined. The data from each plot, plant and cane type was also combined to reduce the number of zeros within the data. Within all cultivars the numbers of aphids sampled is greatest in weeks 4 and 5. However the numbers sampled on Glen Clova (Figure 8) are much lower than the numbers sampled on Glen Ample (Figure 7) and Malling Jewel (Figure 9). In all cultivars, between weeks 1 and 7, the numbers of aphids sampled at the top of the plant are much higher than the numbers sampled lower down the plant. Within all cultivars during week 8, the greatest number of aphids were found at the bottom of the plant. By week 9, the numbers of aphids in all the cultivars were much reduced.

	Predictive means						
Week number	**Cultivar**		**Position of leaf**		**Wald statistic**	**Degrees of freedom**	**Chi pr**
1	G. Ample	-0.242			18.72	2	<0.001
	G. Clova	-1.248					
	M. Jewel	-0.273					
			bottom	-0.851	2.02	2	0.364
			middle	-0.514			
			top	-0.399			
2	G. Ample	-0.408			48.02	2	<0.001
	G. Clova	-1.505					
	M. Jewel	0.038					
			bottom	-0.811	14.42	2	<0.001
			middle	-0.893			
			top	-0.171			
3	G. Ample	0.202			17.16	2	<0.001
	G. Clova	-0.124					
	M. Jewel	-0.379					
4			bottom	-0.906	11.48	2	0.003
			middle	-0.118			
			top	1.137			
5	G. Ample	0.641			35.42	2	<0.001
	G. Clova	-1.061					
	M. Jewel	0.282					
			bottom	-0.55	18.27	2	<0.001
			middle	-0.195			
			top	0.607			
6	G. Ample	0.417			24.31	2	<0.001
	G. Clova	-1.478					
	M. Jewel	-0.24					
			bottom	-1.11	74.08	2	<0.001
			middle	-0.87			
			top	0.679			
7	G. Ample	0.66			13.40	2	0.001
	G. Clova	-1.224					
	M. Jewel	0.719					
			bottom	-0.75	24.55	2	<0.001
			middle	-0.073			
			top	0.984			
8	G. Ample	0.025			47.84	2	<0.001
	G. Clova	-2.043					
	M. Jewel	-0.154					
			bottom	-0.511	10.61	2	0.005
			middle	-1.107			
			top	-0.554			
9			bottom	-0.208	19.95	2	<0.001
			middle	-1.518			
			top	0.192			

Table 1: Comparison of the number of aphids (predictive means) on Glen Ample, Glen Clova and Malling Jewel and a comparison of the number of

aphids sampled at the top, middle and bottom of the raspberry plant using the Wald tests for fixed effects. The results analysed consists of data sampled during weeks 1 to 9 during the 2004 season. In week 9, only data collected from Glen Ample and Malling Jewel were analysed. Only the significant results are shown.

	Predictive means					
	Cultivar	**Position of leaf**			**Wald statistic**	**Chi pr (d.f. = 4)**
Week		Bottom	Middle	Top		
1	G. Ample	0.0137	-0.0604	-0.6794	13.14	0.011
	G. Clova	-1.2390	-1.4213	-1.0849		
	M. Jewel	-1.3260	-0.0604	0.5665		
2	G. Ample	-0.0410	-0.9883	-0.1951	16.21	0.003
	G. Clova	-1.2937	-1.8533	-1.3678		
	M. Jewel	-1.0996	0.1616	1.0508		
4	G. Ample	1.061	0.067	0.722	29.35	<0.001
	G. Clova	-2.928	-1.424	1.141		
	M. Jewel	-0.849	1.004	1.549		
5	G. Ample	0.5053	0.5898	0.8291	13.08	0.011
	G. Clova	-1.1493	-1.4116	-0.6232		
	M. Jewel	-1.0032	0.2370	1.6136		

Table 2: The significant interactions between cultivar and leaf position tested using the Wald tests for fixed effects. The results analysed were collected during the 2004 season. Only the significant results are shown.

Effect of raspberry cultivar

Tables 1 and 2 show that in weeks 1, 2, 3, 5, 6, 7 and 8 the number of aphids sampled on Glen Ample was significantly greater than on Glen Clova. The number of aphids sampled on Malling Jewel was significantly greater than on Glen Ample in weeks 3 and 6 and this relationship is reversed in week 5.

Effect of leaf position

There was a significant effect of leaf position on the number of aphids sampled in weeks 1, 2, 4, 5, 6, 7, 8 and 9. In all significant weeks, the number of aphids sampled at the top of the plants was significantly greater than the number of the aphids sampled at the middle or the bottom of the plant. In week 8 there was also a

significantly greater number of aphids sampled in the middle of the plant when compared with the number of aphids sampled at the bottom of the plant.

Interaction between cultivar and leaf position

A significant interaction only occurred in weeks 1, 2, 4 and 5.

Week 1. A comparison between the same position of leaf on different cultivars, showed that there were significantly more aphids sampled on the bottom leaves of Glen Ample when compared with the number of aphids sampled on the bottom leaves of Glen Clova and Malling Jewel. There were also significantly more aphids sampled on the middle leaves of Glen Ample than the middle leaves of Glen Clova.

Week 2. A comparison between the same leaf position on different cultivars showed that there was significantly more aphids sampled on the bottom leaves of Glen Ample when compared with the bottom leaves of Glen Clova and Malling Jewel. There was a significantly greater number of aphids sampled on the middle and top leaves of Glen Ample when compared with Glen Clova. A comparison of the different position of the leaves within the same cultivar showed a significantly greater number of aphids sampled on the bottom leaves of Glen Ample when compared to the middle leaves of Glen Ample, and there was a significantly greater number of aphids sampled on the bottom leaves of Glen Clova when compared with the middle leaves.

Week 4. There were significantly greater number of aphids sampled on the bottom leaves of Malling Jewel and Glen Clova when compared with Glen Ample. There was also a significantly greater number of aphids found on the middle leaves of Glen Ample when compared with Glen Clova. There was a significantly greater number of aphids sampled on the top leaves of Glen Ample when compared with the number of aphids sampled on the bottom and the middle leaves of the same cultivar.

Week 5. There was a significantly greater number of aphids sampled on the bottom leaves of Glen Ample when compared with Glen Clova. There was also a greater number of aphids sampled on Malling Jewel when compared with Glen Ample. There was a significantly greater number of aphids sampled on the middle leaves of

Glen Ample when compared with Glen Clova and Malling Jewel. There was also a significantly greater number of aphids sampled on the top leaves of Glen Ample when compared with Glen Clova. Within the same cultivar, there was significantly greater number of aphids sampled on the bottom leaves of Glen Clova when compared with the middle leaves.

Identification of *Amphorphora idaei* biotypes present in an experimental plot

Materials and Methods

Eight plots of raspberry cultivars Glen Ample, Glen Clova and Malling Jewel were sampled in an experimental plantation which had been established in 2000. A leaf with *A. idaei* present was removed from each block of cultivar, therefore giving eight leaves from each cultivar. These leaves were bagged individually and brought back into the laboratory where one aphid from each leaf was placed into a Blackman box (Blackman, 1971). This Blackman box contained a leaf of the same cultivar from which the aphid was sampled. The sampled leaves were kept in plastic bags filled with air at 4 ºC until the aphid colony in the Blackman box became established. All insect rearing was done in a growth room at 15°C, 18 hours light: 6 hours dark.

Twice a week, the leaves were changed in the Blackman boxes and after the numbers of aphids in each Blackman box increased, two sub-samples of apterous (wingless) adult aphids were removed from the box into Perspex cages with a test plant of each of the following cultivars: Glen Ample, Malling Jewel, Glen Prosen, Glen Moy and Malling Landmark. Three adult aphids were also removed from the Blackman boxes of Glen Ample, Glen Prosen, Glen Moy and Malling Jewel respectively and positioned on plants of the same cultivar in a growth cabinet. The plants were watered every day and the density of aphids on each cultivar was recorded after fourteen days. The density of aphids on each plant was categorised semi-quantitatively ranging from 0 = no aphids to 4 = high density of aphids. An aphid found on a Glen Rosa plant during the season was also subjected to the same test.

A three way analysis of variance compared the effect of sample cultivar, plot and sample number on the density of *A. idaei* on the five test cultivars: Glen Ample (A_1), Glen Prosen (A_1), Glen Moy (A_1), Malling Landmark (A_1). Glen Rosa (A_{10}) was

removed from the analysis as no aphids were found on these plants in the insecticide-free experimental plots at SCRI. The aphids were collected from four plots, each containing three cultivars: Malling Jewel, Glen Clova and Glen Ample. Two samples were tested for each combination of sample cultivar and plot. The aphid found in the Glen Rosa plot was not used in the analysis. Due to the nature of the results no further analysis was possible.

Results

Test Cultivar	**Sample Cultivar**	**plot**	**Mean density score for *A. ideai* sample 1**	**Mean density score for *A. ideai* sample 2**
Glen Moy	Malling Jewel	1	2.75	3
		2	3.25	4
		3	3.5	1
		4	1.75	1.5
	Glen Clova	1	1.5	1.75
		2	2	1
		3	3	2
		4	1.25	1.75
	Glen Ample	1	1.75	1.5
		2	2.25	1.5
		3	1.5	1.5
		4	1.5	1.5
Glen Prosen	Malling Jewel	1	2.25	3
		2	3.75	3
		3	3.25	1.75
		4	2	1.25
	Glen Clova	1	1.75	2.5
		2	1.25	2
		3	1.25	1.5
		4	1.5	1.75
	Glen Ample	1	1.75	2
		2	1.75	1.75

		3	1.5	2
		4	1.75	1.5
Glen Ample	Malling Jewel	1	1.25	1
		2	1.75	1.5
		3	1.75	3.5
		4	3	2.75
	Glen Clova	1	3	3.25
		2	3.25	2.25
		3	3	3
		4	3.75	3.75
	Glen Ample	1	3.25	4
		2	3.75	2.75
		3	3	3.75
		4	3	0.75
Malling Landmark	Malling Jewel	1	1.75	1.75
		2	1.25	1.75
		3	1.75	2.75
		4	3.25	3
	Glen Clova	1	3	3.25
		2	3.25	3.5
		3	3.25	3.25
		4	3.25	3.5
	Glen Ample	1	3.25	3.5
		2	2.75	3.75
		3	4	3.25
		4	3.25	3.25
Glen Rosa	Malling Jewel	1	0	0
		2	0	0
		3	0	0
		4	0	0
	Glen Clova	1	0	0
		2	0	0
		3	0	0
		4	0	0
	Glen Ample	1	0	0
		2	0	0

		3	0	0
		4	0	0

Table 3: Mean aphid colony density score (categorised as 0 – no aphids, 4 – high density of aphids) of *A. idaei* found on five test cultivars after 14 days. The aphids were collected from three cultivars, Malling Jewel, Glen Clova and Glen Ample and tested on five cultivars, Malling Jewel, Glen Moy, Glen Prosen, Malling Landmark and Glen Rosa.

Sample cultivar	Density of aphids
Glen Ample	0.75
Glen Prosen	2.5
Glen Moy	3
Malling Landmark	1.25
Glen Rosa	1.25

Table 4: Mean aphid colony density score (categorised as 0 – no aphids, 4 – high density of aphids) of *A. idaei* found on five test cultivars after 14 days. The aphids originated from one individual found on Glen Rosa in the SCRI experimental plots.

Table 3 – 4 shows the mean density of *A. idaei* found on the five test cultivars after 14 days. The density of aphids was categorised as 0 – no aphids 4 – high density of aphids. None of the aphids collected were able to colonise Glen Rosa test plants. The other trials showed great variation in the density of aphids after 14 days.

Test Cultivar	Source of Variation	Degrees of freedom	F value	P value
Glen Ample	Cultivar	2	66.864	<0.001
	Plot	3	11.455	<0.001
	Sample	1	0.727	0.397
Malling Landmark	Cultivar	2	51.695	<0.001
	Plot	3	5.512	0.002
	Sample	1	3.659	0.06
Glen Moy	Cultivar	2	38.393	<0.001
	Plot	3	7.143	<0.001
	Sample	1	5.143	0.026
Glen Prosen	Cultivar	2	27.16	<0.001
	Plot	3	8.04	<0.001
	Sample	1	0.04	0.842

Table 5: A three way analysis of variance comparing the density of aphids on each test plant: Glen Ample, Malling Landmark, Glen Moy and Glen Prosen. The sample cultivars: Malling Jewel, Glen Clova and Glen Ample were collected from four plots and each combination of sample cultivar and plot was tested twice.

The three way analysis of variance (Table 5) showed for each test cultivar, the sample cultivar and the plot from which the *A. idaei* individuals were found to have a significant effect of the density of aphids found on the test cultivar after 4 weeks. There was a significant effect of sample number on the density of aphids found on Glen Moy. However, sample number was not significant for the other test cultivars.

Effects of resistance genes on performance parameters of *Amphorophora idaei* biotype 2 and the A_{10} resistance breaking biotype

Materials and Methods

Individual 4th instar or adult *A. idaei* were placed on the underside of one leaf of an experimental plant. The plants used in this experiment were approximately 30 centimetres in height. A clip cage was used to keep the individual aphid confined to one leaf. Each plant was positioned in a growth cabinet set at 16 hours daylight (1000 lux light intensity). A total of ten plants could be positioned in one growth cabinet. All nymphs produced within the first 24 hours were removed and destroyed. The cages were then checked after a further 12 hours and one nymph was retained on the leaf. The adult aphid and any surplus nymphs were destroyed. The growth cabinets were set at a temperature of 15 ± 1 ºC.

Development of the nymphs was observed on a number of cultivars containing either A_1 or A_{10} resistance genes. Every twenty four hours, the nymphs were checked and the following factors were recorded: duration of pre-reproductive period, duration of reproductive period, the number of nymphs produced and longevity (days). Forty replicates were completed for both *A. ideai* biotypes on all test cultivars.

The difference in the pre-reproductive period (days), the reproductive period (days), the number of nymphs produced and longevity (days) was compared for the two biotypes and the cultivars used in the experiment. With two exceptions, comparisons between the A_{10} resistance breaking biotype and biotype 2 were performed using the Mann-Whitney rank sum test. Two comparisons were undertaken using a t-test as they past the test for normality. These comparisons were the number of days alive on Glen Moy and the number of nymphs produced on Malling Jewel. Comparisons between cultivars were done using the Kruskal-Wallis one way analysis of variance on ranks.

The population performance index r_m was calculated using the equation with c = 0.738, where c = mean regression slope of M_d over d for four aphid species (Wyatt & White, 1977). Antibiosis effects were assessed using parameters including development time (d), the fecundity in a period equal to d (M_d) and the intrinsic rate of population increase (r_m) Performance on cultivars for both *A. ideai* biotypes were compared used a Kruskal-Wallis one-way analysis of variance on ranks. Tukey Tests compared variation within the dependent variables.

Cultivar	**Resistance Gene**		
	No aphid resistance	**A_1**	**A_{10}**
Malling Jewel	X		
Malling Landmark		X	
Glen Ample		X	
Glen Prosen		X	
Glen Moy		X	
Glen Rosa			X

Table 6: Cultivars used in experiments, indicating whether they contain either no resistance genes (susceptible), the A_1 resistance gene or the A_{10} resistance gene.

Results

Individuals of the A_{10} resistance breaking *A. idaei* biotype reproduced on Glen Ample, Malling Landmark and Glen Prosen, but these nymphs failed to survive for any more than five days. These results were therefore removed from the analysis.

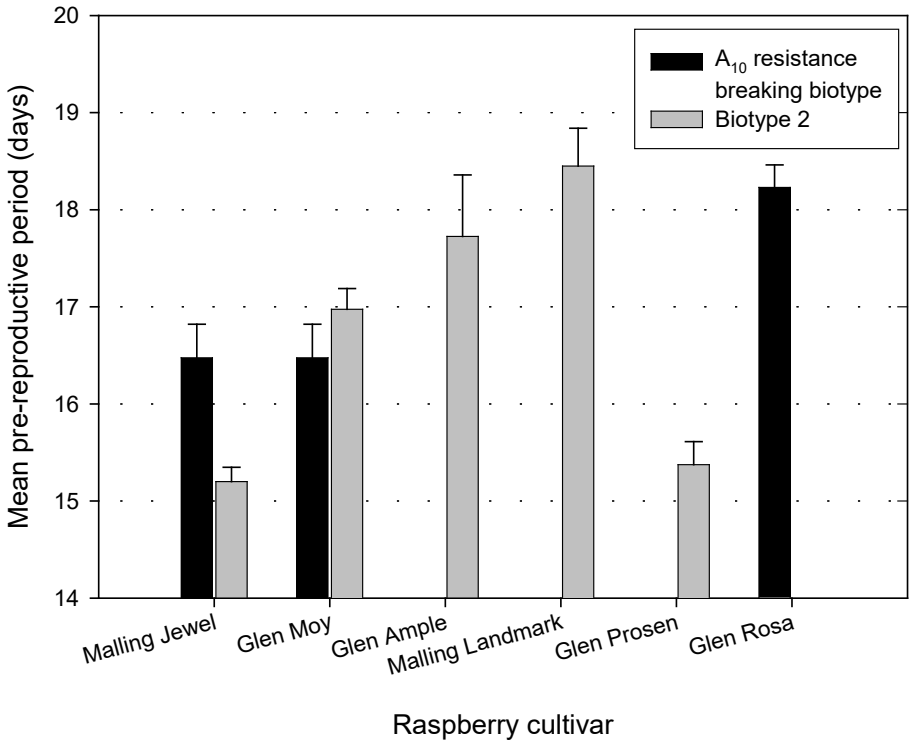

Figure 10: The mean pre-reproductive period (days) for *A. idaei* (A_{10} resistance breaking biotype and biotype 2) on cultivars containing different resistance genes: Malling Jewel (susceptible), Glen Moy, Glen Ample, Glen Prosen, Malling Landmark (all A_1 gene) and Glen Rosa (A_{10} gene). Error bars represent standard error.

Comparison of the pre-reproductive period of *A. idaei* between cultivars showed that there were significant differences (d.f = 4; H = 70.342; P < 0.001) between cultivars with different resistance genes and cultivars with the same resistance gene (Figure 10). *A. ideai* biotype 2 aphids developing on susceptible cultivar, Malling Jewel (15.2 days), took significantly less time to develop than the aphids developing on Glen Moy, Glen Ample and Malling Landmark, which all contain the A_1 gene. Comparisons between biotype 2 aphids developing on cultivars containing A_1 showed that aphids on Glen Prosen (15.4 days) took significantly less time than when feeding on Malling Landmark, Glen Ample and Glen Moy. Although aphids developing on Glen Moy (17.0 days), Glen Ample (17.7 days) and Malling Landmark (18.5 days) showed an increase in the number of days taken to develop, this was not statistically significant.

The pre-reproductive period for the A_{10} resistance breaking biotype also showed significant differences (d.f. = 2; H = 35.481; P < 0.001) between test cultivars. Significantly fewer days were needed to develop on the susceptible cultivar, Malling Jewel (16.5 days) than on Glen Rosa (18.2 days), which has A_{10} resistance. Significantly fewer days were required for aphids to develop on the cultivar with A_1 resistance, Glen Moy (16.5 days) than on Glen Rosa.

Comparisons between the two *A. ideai* biotypes showed that a significantly shorter pre-reproductive period was required for biotype 2 to develop on Malling Jewel (N = 40; T = 2087.5; P < 0.001) and Glen Moy (N = 40; T=1373.5; P = 0.018) than the A_{10} resistance breaking biotype.

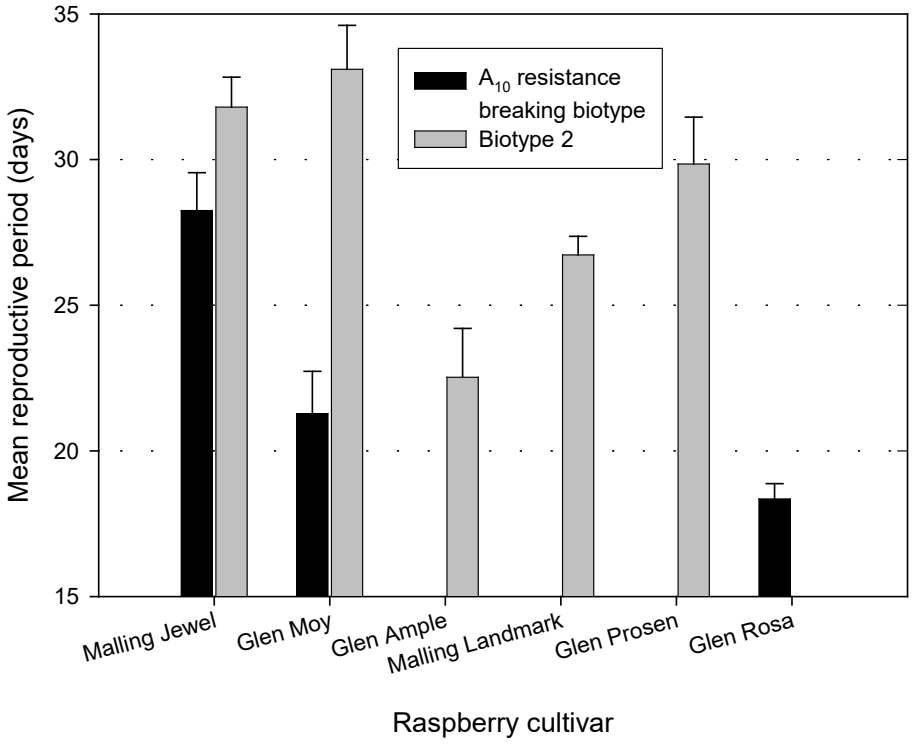

Figure 11: The mean reproductive period (days) for A. idaei (A_{10} resistance breaking biotype and biotype 2) on test cultivars containing different resistance genes: Malling Jewel (susceptible), Glen Moy, Glen Ample, Glen Prosen, Malling Landmark (all A_1 gene) and Glen Rosa (A_{10} gene). Error bars represent standard error.

Comparison of the reproductive period for *A. idaei* on various cultivars showed that there were significant differences (d.f = 4; H = 41.075; P < 0.001) between cultivars with different resistance genes and cultivars with the same resistance gene (Figure 11). Biotype 2 aphids developing on susceptible cultivar, Malling Jewel (31.8 days), produced nymphs over significantly more days than the aphids developing on Glen Ample (22.6 days) and Malling Landmark (26.7 days), which both contain the A_1 gene. Comparisons between *A. ideai* biotype 2 aphids producing nymphs on cultivars containing A_1 showed that aphids on Glen Ample produced nymphs over significantly fewer days than Glen Prosen (29.9) and Glen Moy (26.7). Biotype 2 aphids on Malling Landmark produced nymphs over significantly fewer days than on Glen Prosen and Glen Moy. Although *A. ideai* biotype 2 on Glen Moy produced nymphs over a greater number of days than on Glen Prosen, the difference was not significant.

The reproductive period for the A_{10} resistance breaking biotype to develop also showed significant (d.f. = 2; H = 32.461; P < 0.001) differences between test raspberry cultivars. Significantly fewer days producing nymphs were observed on Glen Rosa (18.4 nymphs) than on Malling Jewel (28.3 nymphs). For this *A. ideai* biotype there were also significantly fewer days producing nymphs on Glen Moy (21.3) compared with Malling Jewel. Comparisons between the two *A. ideai* biotypes showed that there were significantly less days producing nymphs by A_{10} resistance breaking aphids on Glen Moy (N = 40; T=1111.5; P < 0.001) and Malling Jewel (N = 40; T = 1405.0; P = 0.039).

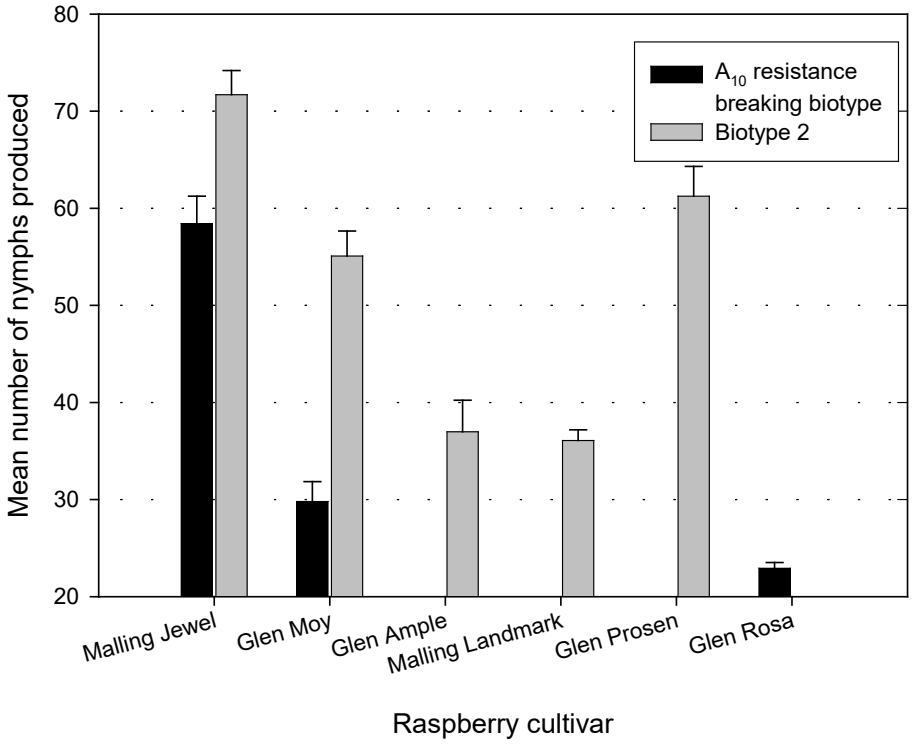

Figure 12: The total number of nymphs of A. idaei (A_{10} resistance breaking biotype and biotype 2) produced on test cultivars containing different resistance genes: Malling Jewel (susceptible), Glen Moy, Glen Ample, Glen Prosen, Malling Landmark (A_1 gene) and Glen Rosa (A_{10} gene). Error bars represent standard error.

Comparison of the number of nymphs produced on the various cultivars showed that there were significant differences (d.f = 4; H = 88.332; P < 0.001) between cultivars with different resistance genes and cultivars with the same resistance gene (Figure 12). As expected, *A. ideai* biotype 2 aphids produced significantly more nymphs on susceptible cultivar, Malling Jewel (71.7 nymphs), than the aphids developing on Malling Landmark (36.1), Glen Ample (37.0) and Glen Moy (55.1), which all contain the A_1 gene. Comparisons between *A. ideai* biotype 2 aphids producing nymphs on cultivars containing the resistance gene A_1 showed that significantly fewer nymphs were produced on Malling Landmark than on Glen Moy and Glen Prosen. *A. ideai* biotype 2 aphids reproducing on cultivars containing A_1 produced significantly fewer nymphs on Glen Ample than on Glen Moy and Glen Prosen.

The number of nymphs produced by the A_{10} resistance breaking aphid also showed significant differences (d.f. = 2; H = 65.526; P < 0.001) between cultivars. There were significantly fewer nymphs produced on Glen Rosa (2.9 nymphs) than on Glen Moy (29.8 nymphs) and Malling Jewel (58.4 nymphs). There were also significantly fewer nymphs produced on Glen Moy than on the susceptible standard cultivar, Malling Jewel.

Comparisons between the two *A. ideai* biotypes showed that there were significantly greater numbers of nymphs produced by biotype 2 on Glen Moy (N = 40; T=1005.5; P < 0.001) and on Malling Jewel (d.f. = 78; t = -3.534; P < 0.001) than by the A_{10} resistance breaking biotype.

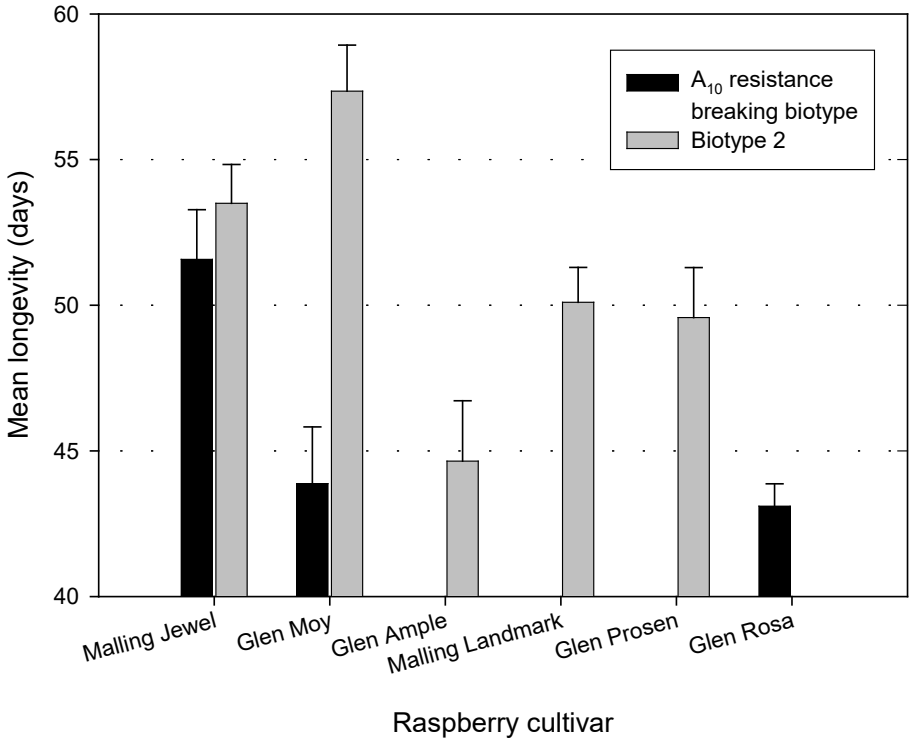

Figure 13: The mean longevity (days) for A. idaei (A_{10} resistance breaking biotype and A_1 gene breaking biotype 2) on test cultivars containing different resistance genes: Malling Jewel (susceptible), Glen Moy, Glen Ample, Glen Prosen, Malling Landmark (all A_1 gene) and Glen Rosa (A_{10} gene). Error bars represent standard error.

Comparison of the longevity of *A. idaei* on the various test cultivars showed that there were significant differences (d.f = 4; H = 29.769; P < 0.001) between cultivars with different resistance genes and between cultivars with the same resistance gene (Figure 13). *A. ideai* biotype 2 aphids lived significantly longer on the susceptible cultivar, Malling Jewel (53.5 days), than when developing on Glen Ample (44.7 days), which contains the A_1 gene. The longevity of *A. ideai* biotype 2 was greater on Glen Moy (57.4 days), which contains the A_1 resistance gene than on Malling Jewel (53.5 days), although the difference was not significant. Comparisons between *A. ideai* biotype 2 aphids producing nymphs on different cultivars containing the A_1 gene showed significantly fewer days alive on Glen Ample than on Glen Moy. The number of days alive was significantly shorter on Malling Landmark (50.1 days) and Glen Prosen (49.6 days) than on Glen Moy (57.4 days).

The longevity of A_{10} resistance breaking aphids also showed significant differences (d.f. = 2; H = 22.047; P < 0.001) between cultivars. They number of days alive was significantly greater on Malling Jewel (51.6) when compared with Glen Moy (43.9) and Glen Rosa (43.1). Comparisons of the two biotypes showed that the number of days was significantly less for the A_{10} resistance breaking biotype on Glen Moy (d.f = 78; t = -10.640; P < 0.001) and Malling Jewel (d.f. = 78; t = -0.890; P = 0.376).

	Cultivar	***d***	M_d	r_m
A_{10} resistance breaking biotype	**Moy**	16.475(0.035)	22.625(1.525)	0.136(0.004)
	Jewel	16.45(0.273)	33.725(1.595)	0.158(0.004)
	Rosa	18.225(0.231)	22.3(0.67)	0.126(0.002)
Biotype 2	**Prosen**	15.375(0.237)	30.875(1.087)	0.165(0.003)
	Ample	17.725(0.643)	26.475(1.958)	0.138(0.007)
	Jewel	15.2(0.148)	35.075(1.17)	0.172(0.002)
	Moy	16.975(0.213)	29.25(1.285)	0.143(0.002)
	Landmark	18.45(0.389)	25.9(1.176)	0.13(0.002)

Table 7: Performance of A. idaei, biotype 2 and A_{10} resistance breaking biotype on raspberry cultivars. Parameters measured included development time (d), the fecundity in a period equal to d (M_d) and the intrinsic rate of population increase (r_m). Values indicate mean (standard error).

Kruskal-Wallis one way analysis of variance on ranks				**Tukey Test**		
Dependent variable	**d.f.**	**H**	**P**	**Comparison**	**q**	**P<0.05**
d	2	35.481	<0.001	Rosa vs Moy	7.761	Yes
				Rosa vs Jewel	6.305	Yes
				Jewel vs Moy	1.457	No
M_d	2	34.126	<0.001	Rosa vs Moy	0.641	No
				Rosa vs Jewel	7.445	Yes

				Jewel vs Moy	6.805	Yes
r_m	2	50.235	<0.001	Rosa vs Moy	4.836	Yes
				Rosa vs Jewel	10.020	Yes
				Jewel vs Moy	5.184	Yes

Table 8: Performance of A. idaei, (A_{10} resistance breaking biotype) on test raspberry cultivars. Parameters measured included development time (d), the fecundity in a period equal to d (M_d) and the intrinsic rate of population increase (r_m). Comparisons were made using a Kruskal-Wallis one-way analysis of variance on ranks, Tukey tests compared variation within the dependent variables. Rosa = Glen Rosa. Moy = Glen Moy

Table 7 shows the values obtained for *d*, M_d and r_m for *A. ideai* biotype 2 and for the A_{10} resistance breaking biotype on the various cultivars. There was a significant difference in the values for *d*, M_d and r_m for the A_{10} resistance breaking biotype developing on Glen Rosa, Glen Moy and Malling Jewel (Table 8). As previously described, the number of days taken to develop (d) was significantly greater on Glen Rosa (18.3 days) than on Glen Moy (16.5 days) and Malling Jewel (16.5 days). There was a significantly greater value for M_d (fecundity) for the A_{10}-breaking biotype when developing on Malling Jewel (33.735 nymphs) than on Glen Rosa (22.3 nymphs), and a significantly greater value for M_d on Glen Moy (22.625 nymphs) when compared with Glen Rosa (22.3 nymphs). There was a significantly greater r_m value for the A_{10}-breaking biotype when developing on Malling Jewel (0.158) than on Glen Moy (0.136) and Glen Rosa (0.126) and there was a significantly greater r_m value for this biotype when developing than when developing on Glen Moy than on Glen Rosa.

Kruskal-Wallis one way analysis of variance on ranks				**Tukey Test**		
Dependent variable	**d.f.**	**H**	**P**	**Comparison**	**q**	**P<0.05**
d	4	70.342	<0.001	Jewel vs Landmark	9.840	Yes
				Jewel vs Ample	6.106	Yes

				Jewel vs Moy	6.646	Yes
				Jewel vs Prosen	0.949	No
				Prosen vs Landmark	8.891	Yes
				Prosen vs Ample	5.156	Yes
				Prosen vs Moy	5.697	Yes
				Moy vs Landmark	3.193	No
				Moy vs Ample	0.541	No
				Ample vs Landmark	3.734	No
M_d	2	35.131	<0.001	Jewel vs Landmark	7.406	Yes
				Jewel vs Ample	6.541	Yes
				Jewel vs Moy	4.166	Yes
				Jewel vs Prosen	2.908	No
				Prosen vs Landmark	4.498	Yes
				Prosen vs Ample	3.633	No
				Prosen vs Moy	1.258	No
				Moy vs Landmark	3.240	No
				Moy vs Ample	2.375	No
				Ample vs Landmark	0.865	No
r_m	4	85.279	<0.001	Jewel vs Landmark	10.900	Yes

				Jewel vs Ample	8.407	Yes
				Jewel vs Moy	7.757	Yes
				Jewel vs Prosen	1.927	No
				Prosen vs Landmark	8.973	Yes
				Prosen vs Ample	6.480	Yes
				Prosen vs Moy	5.830	Yes
				Moy vs Landmark	3.143	No
				Moy vs Ample	0.650	No
				Ample vs Landmark	2.493	No

Table 9 Performance of A. idaei, biotype 2 on test raspberry cultivars. as Parameters measured included development time (d), the fecundity in a period equal to d (M_d) and the intrinsic rate of population increase (r_m). Comparisons were made using a Kruskal-Wallis one-way analysis of variance on ranks. Tukey tests compared variation within the dependent variables.

There was a significant difference in the values for *d*, M_d and r_m for *A. ideai* biotype 2 aphids developing on Malling Jewel, Glen Moy, Glen Ample, Glen Prosen and Malling Landmark. The values for d were discussed previously (Table 9). The values for M_d (fecundity) ranged from 25.9 nymphs (Malling Landmark) to 35.1 nymphs (Malling Jewel). There was a significantly greater value for M_d for aphids developing on Malling Jewel (35.1 nymphs) than on Malling Landmark (25.9 nymphs), Glen Ample (26.5 nymphs) and Glen Moy (29.3 nymphs). The value for M_d was greater on Malling Jewel (35.1 nymphs) than on Glen Prosen (30.9 nymphs), but the difference was not significant. There was a significantly greater value for M_d on aphids developing on Glen Prosen than on Malling Landmark.

The r_m values for *A. ideai* biotype 2 ranged from 0.13 (Malling Landmark, A_1) to 0.17 (Malling Jewel, susceptible). There was a significantly greater r_m value for *A. ideai*

biotype 2 developing on Malling Jewel than on Glen Ample (0.138), Glen Moy (0.143) and Malling Landmark (0.13). Biotype 2 aphids produced a significantly greater r_m value when developing on Glen Prosen (0.165) than on Glen Ample, Glen Moy and Malling Landmark.

Cultivar	Dependent variable	N	T	P
Moy	***d***	40	1373.5	0.018
	M_d	40	1307.0	0.003
	r_m	40	1515.0	0.315
Jewel	***d***	40	2087.5	<0.001
	M_d	40	1520.0	0.338
	r_m	40	1123.0	<0.001

Table 10 Mann-Whitney test comparing performance of two biotypes of A. idaei (A_{10} resistance breaking biotype and biotype 2) on Glen Moy and Malling Jewel. Parameters measured include development time (d), the fecundity in a period equal to d (M_d) and the intrinsic rate of population increase (r_m). Significant P values indicates a statistical difference in performance between the two A. ideai biotypes tested on each raspberry cultivar.

Comparisons of the two *A. ideai* biotypes developing on Glen Moy show significant differences in *d* and M_d, with biotype 2 aphids having a lower *d* and a higher M_d (Table 10). Biotype 2 aphids developing on Malling Jewel have a significantly lower d and higher r_m when compared with the A_{10} resistance breaking biotype developing on Malling Jewel.

Discussion

The number of *A. ideai* on the raspberry cultivars sampled in the experimental plots at SCRI confirms that there is varying degrees of efficacy for genetic resistance against the large raspberry aphid, caused by interactions with different *A. ideai* biotypes present in the SCRI field population. There was a significantly greater number of *A. ideai* sampled on Glen Ample (A_1 resistance gene) when compared

with Glen Clova (minor gene resistance). Malling Jewel (susceptible) also had high numbers of *A. ideai*. Only one *A. ideai* individual was found on Gen Rosa (A_{10} resistance gene). The sampling of Malling Leo, which contains both the A_1 and A_{10} resistance genes, failed to detect any aphids. The increased efficacy of the A_{10} gene compared with the A_1 gene in Scotland is in general agreement to previous research by Birch and Jones (1988). However, this earlier study showed that the raspberry cultivars containing A_1 resistance genes had a higher level of resistance than cultivars containing minor gene resistance. Since the study in 1988, there has been further selection pressure on *A. ideai* biotypes 2 and X (a biotype similar to biotype 2 but which performs better on Glen Ample, Prosen and Moy than on East Malling varieties containing gene A_1: Birch *et al*., 1994) resulting in them overcoming the A_1 resistance cultivars (Jones *et al*., 2000). In contrast, minor (multigenic) gene resistance has remained durable. In this study, we demonstrate that Glen Clova, which has minor gene resistance, has less *A. idaei* than Glen Ample with single gene based A_1 resistance.

There was shown to be a significant difference in the number of aphids sampled from the different leaf positions of the plant. Generally, there were a significantly greater number of aphids found at the top of the plant than at the middle and the bottom of the plant. The leaves at the top of the plant are younger and actively growing and may provide a better food source for the aphids. Shepherd *et al*. (1999) suggested that the young growing leaves in strongly resistant cultivars would be less favourable to the feeding on non-virulent *A. ideai* biotypes and that the older leaves would provide a better area for colonisation. They found that the young leaves had a greater coverage of wax. Senescing leaves at the bottom of the plant can have more aphids as they chemical protection of the leaf in resistant varieties breaks down during the growing season (Kronenberg and de Fluiter 1951; cited in Knight *et al*., 1959). The curling behaviour of the younger leaves may also provide some protection against predators, parasitoids and against changing weather conditions. Previous studies (Briggs, 1959; Jones, 1976; Birch and Jones 1988) have all shown that on susceptible plants lacking resistance genes or on resistant plants challenged by virulent *A. ideai* biotypes which have overcome the resistance gene, aphids tend to colonise the upper zones of the plants but there is a shift to colonising the bottom zone of resistant cultivars later in the growing season. It has also been observed that aphids tend to acquire and possible transmit viruses less readily from older leaves that younger ones (Cadman, 1954, cited in Birch and Jones, 1988). These results demonstrate that the *A. idaei* biotypes present in the SCRI plantation have

overcome the resistance mechanisms present in Glen Ample (A_1 resistance) and to a lesser extent in Glen Clova (minor gene resistance). This ability to overcome these resistance genes means that *A. ideai* will be able to transmit plant viruses and will require control using late season insecticide applications on formerly resistant cultivars until more effective resistance gene combinations can be bred into new raspberry cultivars.

Identification of the *A. ideai* biotypes present in the SCRI plantation proved to be difficult using the simplified method adopted: test varieties containing different resistance genes and a semi-quantitative scoring method for aphid numbers. There was variation in the density of aphids on the test plants after two weeks. This variation occurred between aphids sampled from different plots of the same cultivar and between aphids sampled from different cultivars. Results indicate that there is more than one *A. ideai* biotype present in the plantation and that biotypes 2 and X predominate (A_1 gene breaking).

Even though the four test cultivars, Glen Ample, Glen Prosen, Glen Moy and Malling Landmark all contain the A_1 resistance gene, these results suggests that they differ in the efficacy of resistance to *A. ideai* and therefore will vary in their ability to control the spread of viruses transmitted by this pest. This variation in A_1 gene containing cultivars is likely to result from differences in the genetic backgrounds in parental lines or cultivars used to create crosses in plant breeding. Birch and Jones, (1988) also demonstrated that there was differences between A_1 resistant cultivars bred at SCRI when compared to A_1 resistant cultivars that were bred at EMR. The SCRI bred cultivars, Glen Moy and Glen Prosen had greater number of *A. ideai* developing during trials than on the EMR cultivar Malling Landmark. Fruiting trials in England and Scotland had also shown similar findings (V. Knight, *pers comm*.). Possible reasons for these differences in efficacy of the A_1 gene have been speculated. Possibly the A_1 gene containing cultivars bred at SCRI have modifying genes which suppress the full expression of the A_1 gene. Alternatively, Malling Landmark contains additional minor genes that co-segregate with the A_1 gene and confer a more durable form of resistance than expressed in the SCRI cultivars.

The results indicate that the one *A. idaei* found on Glen Rosa was the A_{10} resistance breaking biotype, which has been observed in commercial plantations (Birch *et al*., 2003) and is now relatively widespread in England (J. Allen, *pers comm*.) but not yet established in Scotland. The A_{10}-breaking biotype was found to be very rare at the

SCRI experimental site, agreeing with observations of SCRI's selection trials at other Scottish sites. The selection pressure caused by cultivating A_{10} gene containing cultivars has been lower in Scotland than England. In addition, the number of A_{10} resistance breaking biotype has kept at low levels in the SCRI experimental plots due to the large number of other biotypes (2 and X) present in this mixed cultivar plantation. In commercial raspberry plantations, consisting of larger areas of single cultivars like Glen Rosa containing the A_{10} gene, the A_{10} resistance breaking *A. ideai* biotype could be selected relatively quickly (over several growing seasons) and spread to surrounding plantations. Our results indicate that biotype X is present in the SCRI experimental plantation, as in some cases the number of *A. ideai* on Glen Moy and Glen Prosen was higher than on Malling Landmark, as previously found by Jones *et al*. (2000).

This experiment highlights the disadvantage of using small scale "no choice" experiments under semi-natural conditions (growth rooms) to identify *A. ideai* biotypes. Small variations in experimental conditions can alter the result of the experiment. Plants grown in shady conditions or poor light quality have shown increased susceptibility (Jones *et al*., 2000). Variation in glasshouse light and temperature conditions when producing test plants over extended growing seasons conditions can also lead to variation in the expression of aphid resistance. Attempts have been made to find a molecular tool to identify individual *A. ideai* biotypes, which would provide a more rapid and effective method of recording the incidences of each biotype in commercial plantations (Birch *et al*., 1994). Although successful for some aphid species, molecular diagnostics have to date proved unsuccessful for *A. ideai* because this aphid reproduces sexually each autumn and as a result each resistance-breaking biotype consists of many different genotypes.

Resistant raspberry cultivars had differential effects on the development of the biotype 2 and the A_{10} resistance breaking biotype. The cultivars used in the experiment had a range of resistance genes: Malling Jewel (susceptible control), Malling Landmark, Glen Ample, Glen Moy and Glen Prosen (all A_1 gene) and Glen Rosa (A_{10} gene). Results indicate that there are differences in the performance of both *A. ideai* biotypes on cultivars containing the same resistance gene. For example, the A_{10} resistance breaking aphids failed to develop on Malling Landmark, Glen Ample and Glen Prosen but successfully developed on Glen Moy. All of these cultivars contain the A_1 gene. This indicates that the A_1 gene acts differently in cultivars with dissimilar genetic backgrounds. Evidence suggests that the

effectiveness of the major resistance genes is influenced by inherited genetic background of resistance and susceptibility to *A. idaei* (Jones *et al*., 2000). If fully understood, the difference in the genetic backgrounds could provide an opportunity to breed new raspberry cultivars that can combat the spread of the A_{10} resistance breaking biotype. The performance of the A_{10} resistance breaking biotype on the susceptible cultivar, Malling Jewel, was better than on the cultivar Glen Moy (A_1 gene) and Glen Rosa (A_{10} gene). A comparison of the development of the two *A. ideai* biotypes on the three raspberry cultivars indicates that there has been a high fitness cost of overcoming the A_{10} resistance gene. Despite this fitness cost, raspberry cultivar prevalence and/or growing conditions in England have now selected for the A_{10} breaking biotype.

Comparisons of the development of *A. idaei* biotype 2 on the test raspberry cultivars produced some interesting findings. Although development was faster and the number of nymphs produced was greater on the susceptible cultivar, Malling Jewel, the reproductive period and longevity was greater on cultivars containing the A_1 gene. This result indicates a fitness cost for biotype 2 in overcoming A_1 gene based resistance. *A. idaei* biotype 2 development on the A_1 resistance gene cultivars showed cultivar differences. The four development traits studied can be split into two groups. The pre-reproduction period was shorter and the greatest number of nymphs are produced on Glen Prosen, whilst aphids on Malling Landmark are slowest to develop and produce less young. Malling Landmark is EMR bred cultivar and Glen Prosen is a SCRI bred cultivar. The other two development traits studied, the reproductive period and longevity, were longest on Glen Moy and shortest on Glen Ample. Both these cultivars are bred at SCRI and are members of the same breeding series, although have some differences in parentage and genetic background.

Studies indicate that the leaf surface of *Rubus*, particularly the cuticular wax components, plays a significant role in determining resistance to infestation to *A. ideai* (Robertson *et al*., 1991; Shepherd *et al*., 1999). This resistance mechanism to aphids has been suggested for several other plant species. Other morphological characteristics and chemicals can also be involved in aphid resistance resistance. Deterrence by presence of a waxy lamina, (Mote & Shahane, 1994), dense cover of very short hairs attracting fewer aphids (Lage *et al*., 2004) and nitrogen levels effecting the host suitability of various phloem feeding Homoptera (Singh *et al*., 2004) have all been suggested as modes of action against other aphid species.

The r_m value describes the growth potential of a population of an aphid biotype on any given cultivar. The r_m values obtained show that there is significant differences in the growth potential of populations of both Biotype 2 and the A_{10} resistance breaking biotypes on the test raspberry cultivars. As expected for both biotypes, the r_m value was greatest on the susceptible control cultivar, Malling Jewel. Results indicated that the resistance mechanisms in cultivars containing the A_1, and the A_{10} resistance genes have a fitness cost, reflected by an effect on the overall growth potential of the population. Comparison of the r_m values for *A. ideai* biotype 2 on the cultivars containing the A_1 gene show significant differences. This increases the evidence for the genes working differently in various genetic backgrounds. The fecundity (M_d) was less affected by performance on resistant cultivars, indicating that the differences in r_m found were mainly due to differences in the development time (d). Morgan *et al*. (2001) also noted that the differences observed in the r_m values for *Acyrthosiphon pisum* could be explained by differences in development time. The index r_m has been shown to be particularly sensitive to changes in the duration of the development period (van Rijn *et al*., 1995).

Overall from the results it can be seen that the A_1 and the A_{10} resistance genes found in *Rubus* are having an effect on all stages of development of both biotype 2 and the A_{10} resistance breaking biotype of *A. idaei*. Information gathered has suggested that the cultivars containing the A_1 gene vary in their levels of resistance. This variation does not only occur between cultivars bred at different breeding centres but also between more closely related cultivars from the same breeding programme. More work needs to be done to understand the genetics behind the resistance and to understand the methods of resistance found in *Rubus*. This will help future breeding of cultivars with resistance against *A. idaei* and will help to combat the emergence of further resistant-breaking *A. ideai* biotypes.

Conclusions

- Aphid counts on Malling Jewel (susceptible), Glen Clova (minor gene resistance), Glen Ample (A_1 resistance gene), Glen Rosa (A_{10} resistance gene) and Malling Leo (A_{10} and A_1 resistance genes) in an insecticide free plantation, showed differences in the numbers of aphids present. Malling Jewel and Glen Ample had higher numbers that Glen Clova, demonstrating the durability of minor gene (multigenic) resistance.

- Biotype testing of aphids from the insecticide-free experimental plots indicated that there was more than one *A. ideai* biotype present in the experimental plots. The testing also highlighted a need to develop a better system to identify *A. ideai* biotypes.

- Laboratory testing of *A. ideai* biotype 2 development at 10, 15 and 20 °C show that the optimum development of the aphid is 15 °C.

- Laboratory experiments testing the development of *A .ideai* biotype 2 and the A_{10} resistance breaking aphids showed that there were significant differences between the two biotypes and also significant differences in the development of each biotype on different cultivars. Most importantly, it showed differences in the development both *A. ideai* biotypes on cultivars containing the A_1 resistance gene. This highlights the need to understand the effects of genetic background of the cultivars on efficacy of major resistance genes for future breeding for resistance against *A. idaei*.

Effect of *Amphorophora idaei* biotype and raspberry cultivar on the parasitism by *Aphidius ervi*

Introduction

The use of parasitoids in biological control of aphids has been well documented (Hoelmer & Kirk, 2005). An understanding of the biology of both the aphid species and the parasitoid species and the interaction of the two under different conditions is essential to ensure the success of the system under usual crop growing conditions in an enclosed glasshouse.

Previous observations (N Birch, personal communication) suggested that the number of parasitoid mummies varied between raspberry cultivars. During 2000, the number of large raspberry aphids and parasitized aphids were counted on five raspberry cultivars with varying resistance genes against the large raspberry aphid: Glen Clova (minor gene resistance), Glen Ample, Glen Prosen, Malling Landmark (A_1 resistance), Glen Rosa (A_{10} resistance) and Malling Leo (A_1 and A_{10} resistance). It was found that there were a significantly greater proportion of parasitized aphids on

Glen Rosa when compared with the other cultivars. It was proposed that this might be due to the large raspberry aphid having a slower development rate on Glen Rosa and therefore aphids being available for parasitism for a longer period. A beneficial interaction between resistant cultivars and biological control agents has been observed in several studies (e.g. Kalule and Wright, 2002).

During field sampling during 2003 and 2004 the number of parasitized aphids was shown to vary between raspberry cultivars. These surveys identified Glen Clova as the cultivar with the larger proportion of parasitized aphids when compared with Glen Rosa, Glen Ample and Malling Jewel (susceptible). However the numbers of parasitized aphids were very low and the results were not suitable for analysis.

In an attempt to identify a possible candidate for biological control of the large raspberry aphid, examples of the wasps emerging from mummies were collected from the 2003 sample. A sample of the emerged parasitoids was identified by Prof Wilf Powell of Rothamsted Research. Present in the sample was *Dendrocerus* sp, which is a hyperparasitoid, *Aphidius* sp., possibly *A. urticae*, *Aphidius picipes, Praon volucre* and an *Aloxysta* sp. Overall, with the small number of parasitoids collected and the variety of species present, so it was not possible to identify a dominant species.

To investigate a suitable commercially parasitoid to develop a biological control system for the large raspberry aphid, three parasitoid species were obtained from Koppert Biological Systems Ltd: *Aphidius ervi*, *Aphidius colemani* and *Aphelinus abdominalis*, to identify a parasitoid that was capable of parasitizing *A. idaei*. *Aphidius colemani* Viereck is thought to be indigenous to India, but has been found in many other parts of the world (Jones *et al*., 2003). This parasitoid is produced commercially for biological control of *Myzus persicae* Sulzer and *Aphis gossypii* Glover in contained cropping systems. *Aphelinus abdominalis* a non specific parasitoid of cereal aphids (Honek *et al*. 1998) and is a promising candidate for biological control (Couty & Poppy, 2001). It is already used in against potato aphid *Macrosiphum euphorbiae* and the glasshouse potato aphid *Aulacorthum solani*. This parasitoid is smaller than the other parasitoids tested and is not very mobile so must be released near to the site of infestation. *Aphidius ervi* (Haliday) is widely distributed in Europe and has a wide host range (Takada & Tada, 2000). In biological control systems it is used against the potato aphid *Macrosiphum euphorbiae* and the glasshouse potato aphid *Aulacorthum solani*.

The three parasitoid species were supplied as mummies mixed with woodchips (*A. abdominalis* and *A. colemani*) or buckwheat (*A. ervi*). To identify a possible candidate the three parasitoid mummies were allowed emerge, mate and feed on 70 % honey for 24 hours. They were then released into a cage containing a raspberry plant colonised with *A. idaei*. This was a large colony with a range of available instars. The wasps were allowed to continue to parasitise until they died. The cages were checked regularly for evidence of mummies and second generation adults. *A. abdominalis* and *A. colemani* were unsuccessful at parasitizing *A. idaei*, but a large number of *A. ervi* adults emerged. As *A. ervi* was easy to purchase it was chosen as the parasitoid to use in investigating possible biological control of *A. idaei* using a parasitoid wasp.

Results from previous fieldwork indicated that there was possibly an interaction between the large raspberry aphid resistance gene in the cultivar and its effect on the ability of the parasitoids to successfully parasitise the aphids. The use of an aphid parasitoid to control *A. idaei* numbers in combination with the resistance genes already available in raspberry cultivars could be used to develop a biological control system. Development of a successful system requires the understanding of the behaviour of *A. ervi* when presented with *A. idaei*. An experiment was designed to identify whether *A. ervi* preferred a certain development stage of *A. idaei*. This would have profound effects on the population dynamics of both the aphid and parasitoid communities. A second experiment explored the effect of the different resistance (A_1 and A_{10} resistance genes) in the raspberry cultivars on the success of *A. ervi* at parasitising biotype 2 and A10 resistance breaking biotypes of *A. idaei*. Varing the density of aphids available for oviposition would help understand the interaction between host and parasitoid. If a link between the resistance gene and elevated levels of parasitism could be found, this could provide the basis for a biological control method for *A. idaei* in protected raspberry cultivation.

The effect of developmental stage of *Amphorophora idaei* on the ability of *Aphidius ervi* to attack

Materials and Methods

An individual *A. ideai* biotype 2 adult was positioned on two leaves of a Malling Jewel plant and left for twelve hours. Any nymphs produced were removed and discarded

and the plant was rechecked every twelve hours. Any further nymphs produced were removed and positioned on a leaf of a fresh Malling Jewel plant. The *A. ideai* nymphs were left to feed on the plant until they were the correct age for the experiment (see Table 11). Twenty four hours before an experiment, twenty *A. ideai* nymphs of the same, known age were removed from the plant and positioned on a Malling Jewel leaf contained in a Blackman box.

Instar	Number of days development
2nd	2
3rd	5
4th	7
Adult	11

Table 11 The number of days development on Malling Jewel of biotype 2 aphids required to reach 1st, 2nd, 3rd, 4th instars and adult stages.

After allowing the aphids of each age group to feed for twenty four hours, the leaf was removed from the Blackman box and positioned aphid side up in a Petri dish. A mated female wasp was released into the Petri dish and the lid was replaced. The wasps' behaviour was observed and the number of ovipositions was recorded. After an aphid had been attacked, it was removed from the leaf by using a fine moist paintbrush. When the wasp remained inactive for five minutes the experiment was terminated. 1st, 2nd, 3rd, 4th instars and adults aphids were tested and each experiment was replicated five times using new aphids and female wasps on each occasion.

Results

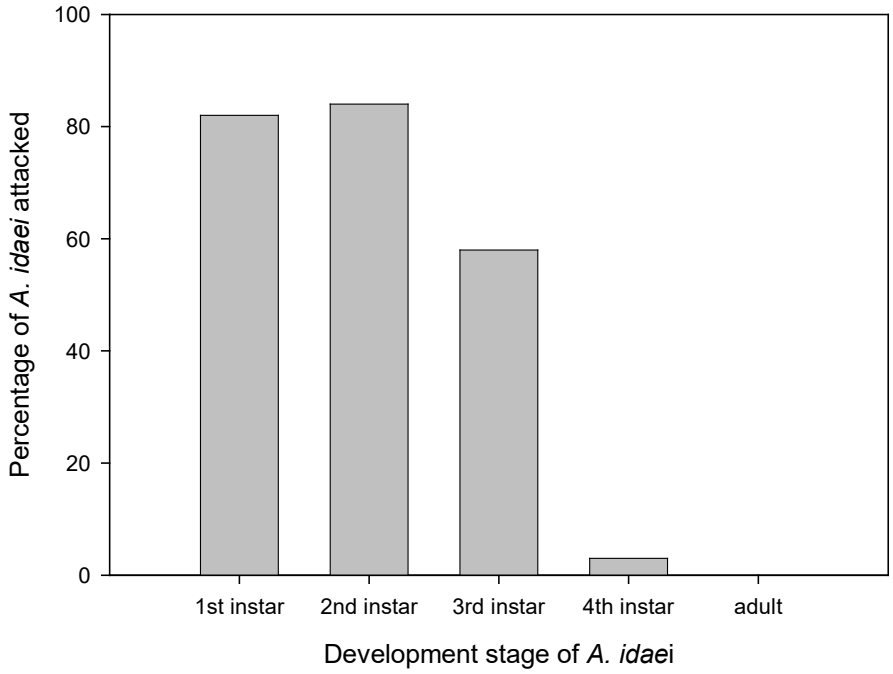

Figure 14 The percentage of *A. idaei* attacked by *A. ervi* at the different development stages of *A. ideai*

Figure 14 shows that there was variation in the percentage of *A. idaei* parasitized at the different development stages. There was a decline in the percentage parasitized with increasing size of aphid. There was a greater percentage of 1st and 2nd instars parasitized, 82 % and 84 % respectively, when compared with the larger instars. The percentage of 3rd instars parasitized was 58 %. The percentage of 4th instar and adults was very low, 3 % and 0 % respectively.

Effect of *Amphorophora idaei* density, biotype and raspberry cultivar on the number of aphids attacked by *Aphidius ervi*

Materials and Methods

Three cultivars were used in the experiment: Malling Jewel (susceptible), Malling Landmark (A_1 resistance gene) and Glen Rosa (A_{10} resistance gene). The

experiment used biotype 2 aphids and A_{10} resistance breaking aphids at densities of 5, 10, 20 and 50 aphids on one leaf of a plant.

Adult aphids were positioned on Malling Jewel, Malling Landmark and Glen Rosa plants and left for twelve hours. Any nymphs produced were removed and discarded. The plants were rechecked every twelve hours and any nymphs produced were removed and positioned on a new plant of the same cultivar. Aphids were positioned on the new plants at the density required for the experiment. They were allowed to feed on this plant until they were at 2nd instar stage.

A plant with feeding 2nd instar aphids present was placed into a perspex cage (900 x 450 x 350 mm). The plant was rotated so that the observer could see the underside of the leaf housing the aphids. A mated female wasp was introduced onto the leaf. The wasp was observed for 30 minutes and the number of attacks was recorded. If the wasp had not started to attack the aphids within five minutes of the start of the experiment, it was replaced with another wasp. The number of aphids observed falling from the leaf was also recorded.

The effect of cultivar, biotype and density on the mean number of ovipositions and the mean number of aphids falling off the leaf during the experiment was analysed using Kruskal-Wallis one way analysis of variance on ranks.

Results

Dependent variable	Factor	H value	Degrees of freedom	P value
No. of drops	Cultivar	31.054	2	<0.001
	Biotype	11.148	1	<0.001
	Density	4.276	3	0.233
No. of ovipositions	Cultivar	13.478	2	0.001
	Biotype	3.854	1	0.05
	Density	81.663	3	<0.001

Table 12: The effect of cultivar (Malling Jewel, Malling Landmark and Glen Rosa), biotype (A10 resistance breaking and biotype 2) and density (5, 10,20 and 50 aphids per leaf) on the number of ovipostions by *A. ervi* and the number of *A. idaei* dropping from the plant.

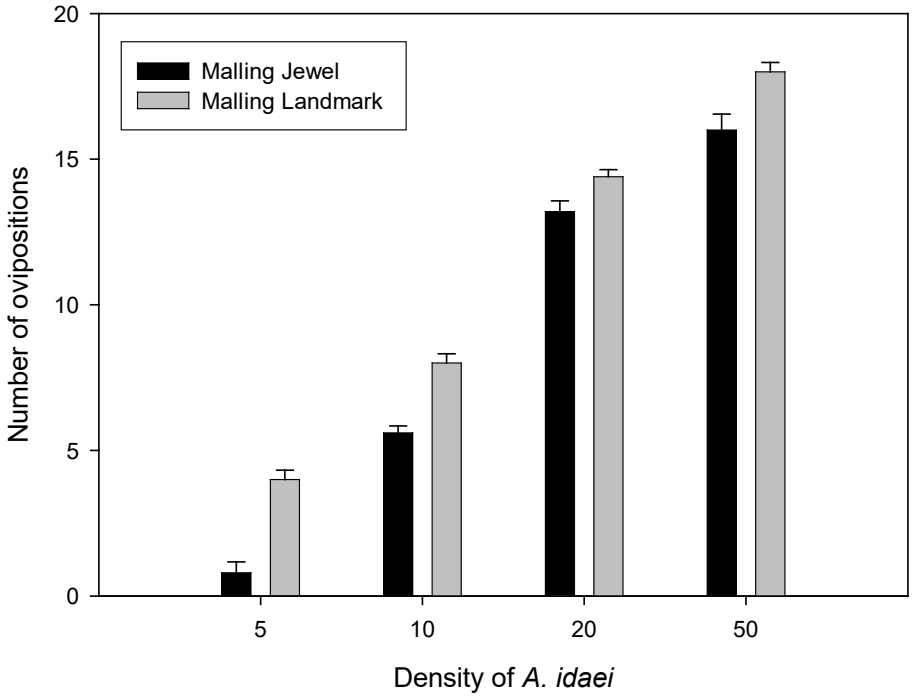

Figure 15: The number of ovipositions by *A. ervi* at four densities of 2nd *instar A. idaei* (5, 10, 20 and 50 aphids per leaf), biotype 2. Two raspberry cultivars were used: Malling Jewel and Malling Landmark). Error bars represents standard error.

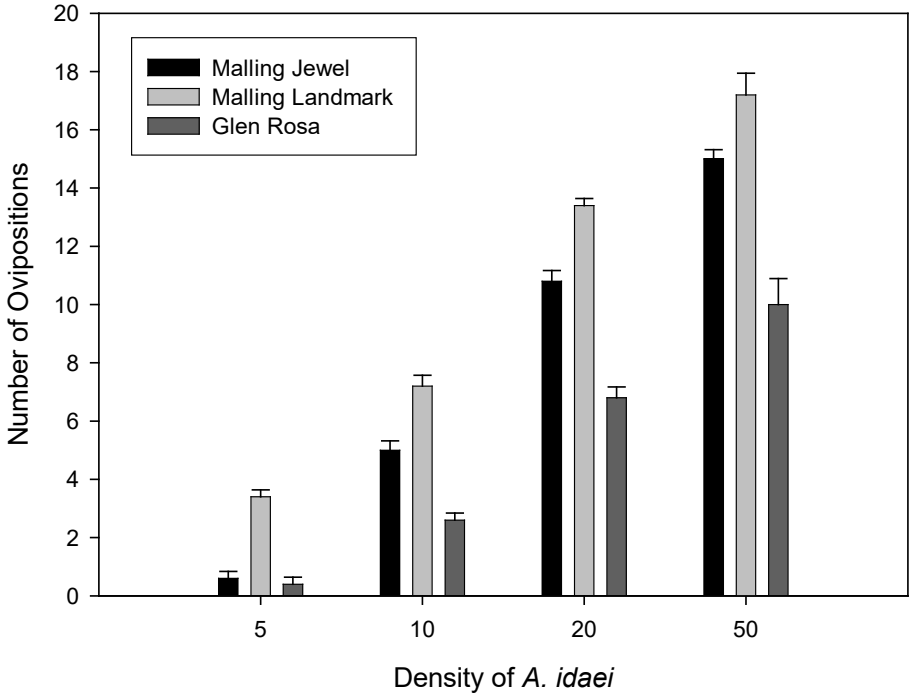

Figure 16: The number of ovipositions by *A. ervi* at four densities of 2nd instar A. idaei (5, 10, 20 and 50 aphids per leaf), A10 resistance breaking biotype. Three raspberry cultivars were used: Malling Jewel, Malling Landmark and Glen Rosa). Error bars represents standard error.

There was a significant effect of raspberry cultivar, *A. idaei* biotype and density on the number of oviposition events (Table 12). Pairwise multiple comparison procedures (Dunn's method) showed there were significantly more oviposition events on Malling Landmark when compared with Glen Rosa. There were significantly more oviposition events in the treatments using A_{10} resistant breaking biotype than in the treatments using biotype 2. Figures 15 and 16 show as the density of aphids on a plant increased, the numbers of oviposition events also increased. Pairwise multiple comparison procedures showed that there was a significant difference between all *A. ide*ai densities apart from 50 versus 20.

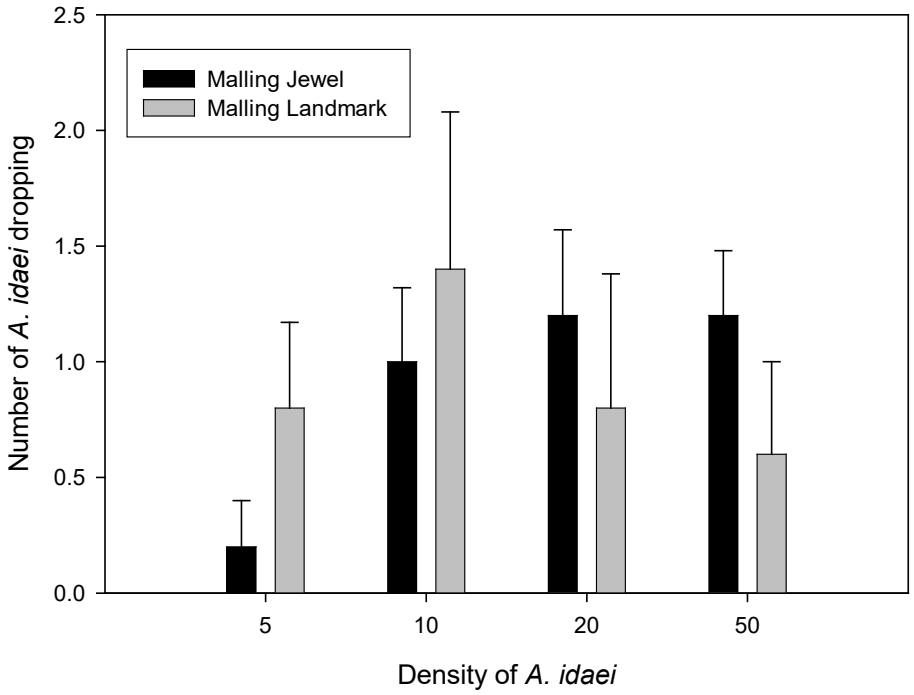

Figure 17: The number of 2nd instar *A. idaei*, biotype 2, dropping from the plant during foraging by *A. ervi* at 4 densities of *A. idaei*: 5, 10, 20 or 50 2nd instars on one leaf of a plant. Two cultivars were used: Malling Jewel and Malling Landmark. Error bars represents standard error.

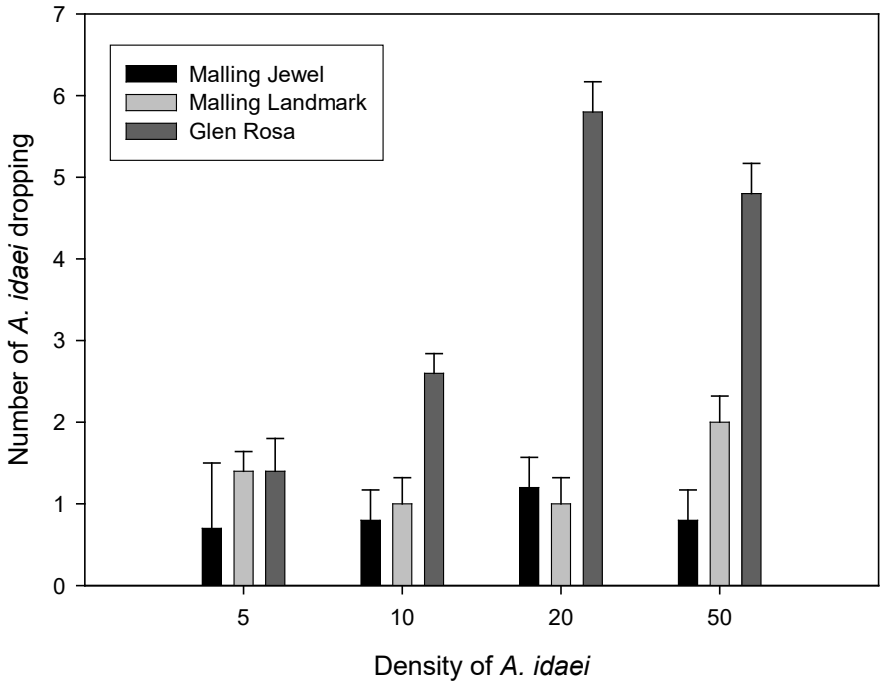

Figure 18: The number of 2nd instar *A. idaei*, A_{10} resistance breaking biotype, dropping from the plant during foraging by *A. ervi* at 4 densities of *A. idaei*: 5, 10, 20 or 50 2nd instars on one leaf of a plant. Three cultivars were used: Malling Jewel, Malling Landmark and Glen Ample). Error bars represents standard error.

There was a significant effect of cultivar and biotype on the mean number of A_{10} breaking biotype of *A. idaei* dropping of the plant during each treatment. Pairwise multiple comparison procedures (Dunn's method) showed there were significantly more aphids dropping from plants in treatments using Glen Rosa when compared to Malling Jewel and Malling Landmark. There were significantly more aphids dropping from plants in treatments using the A_{10} resistance breaking biotype (Figure 17) than in treatments using *A. ideai* biotype 2 (Figure 18). There was no significant effect of density on the number of aphids dropping of the plants between treatments.

Due to the unbalanced nature of the experimental design it was not possible to do a three- way analysis of variance. The results from A_{10} resistance breaking biotype feeding on Glen Rosa were removed from the analysis to test for any interaction between raspberry cultivar, *A. ideai* biotype and density of aphids.

Factor	Degrees of freedom	F value	P value
Cultivar	1	157.255	<0.001
Biotype	1	24.891	<0.001
Density	3	1204.285	<0.001

Table 13: The effect of cultivar (Malling Jewel, Malling Landmark and Glen Rosa), biotype (A_{10} resistance breaking and biotype 2) and density (5, 10,20 and 50 aphids per leaf) on the number of ovipostions by *A. ervi*.

A three-way analysis of variance showed that there were no significant interactions between cultivar, biotype and density of aphids (Table 13). There were significantly more ovipositon events by *A. ervi* on aphids feeding on Malling Landmark than on Malling Jewel and significantly more oviposition events by *A. ervi* on biotype 2 aphids than on A_{10} resistance breaking aphid. There was a significant difference in the number of oviposition events by *A. ervi* between different densities of aphids. A pairwise multiple comparison procedure (Holm-sidak method) showed that there was a significant difference between all densities, with the greatest number of oviposition events at a density of 50 aphids and the least number of oviposition events at a density of 5 aphids. There was no significant effect of cultivar, biotype and density of *A. idaei* on the number of aphids dropping off during the experiments.

Discussion

The results show that the size of the aphid has an effect on the ability of the wasp to successfully parasitise its host. In this system, *A. ervi* favours the smaller 1st and 2nd instars. During the experiments it was observed that the larger aphids displayed many anti-predator tactics associated with responses to predators and parasitoids which had been displayed by aphids in previous studies. *A. idaei* was observed to kick out in response to the searching wasp (Villagra *et al*., 2002) and raised their abdomen high off the leaf surface to prevent the wasp from inserting her ovipositor. It was also noted that the larger aphids moved quickly away from the leaf (Villagra *et al*., 2002) and when an attempt at oviposition on aphid has been made the other aphids started to move from the leaf. This suggests that the larger *A. idaei* may release an alarm pheromone (trans-β farnesene) from the cornicle (Villagra *et al*., 2002), and alerted the other aphids to the danger and they too moved away from the leaf surface.

The results from this study suggest that there are tri-trophic interactions between the resistance genes within the plant, the 2 biotypes of *A. idaei* and *A. ervi*. There was a significantly greater number of oviposition events of *A. ideai* biotype 2 and the A_{10} resistance breaking biotype on Malling Landmark (A_1 resistance gene) than on Malling Jewel (susceptible). Although Kalule & Wright (2002) suggested that the number of attacks was greater on aphid biotypes with a longer development time, this experiment was only run over 30 minutes, so that information was not available to the wasp. These findings suggest that the aphid must be providing some information that can be used by the wasp. Powell *et al*. (1998) noted that *A. ervi* could distinguish between plants damaged by host and non host aphids. Chemical analysis of volatiles from both damaged plants showed differences in the components of the plant volatiles. There may be differences in the plant volatiles released from the different raspberry cultivars in response to the two *A. ideai* biotypes feeding. *A. ervi* may have a differential response to the plant volatiles.

There were significantly less oviposition events of the A_{10} resistance breaking biotype feeding on Glen Rosa (A_{10} resistance). Unsettled behaviour of the A_{10} resistance breaking aphid on Glen Rosa was observed, although they had received the same pre-experimental treatment as the other aphids. This unsettled behaviour resulted in a higher number of aphids dropping off the Glen Rosa plants when compared with other treatments. Dropping from plants is a common defence tactic for aphids avoiding parasitism and the effect can be enhanced by using partially resistant plant varieties (van Emdem, 1995; Villagra *et al*., 2002) but there are fitness costs. It is thought that dropping from the leaf is energetically costly as the aphid has removed itself from its feeding site. It will now have to re-locate to a new host plant or plant tissue and will expose itself to predators on the ground (Losey & Denno, 1998; Villagra *et al*., 2002). It would be expected that the unsettled aphids feeding on Glen Rosa, which have been shown to take longer to develop into adults and produce less young, would react with a less energetically costly anti-predator response, as seen in Villagra *et al*. (2002). That study showed that starved aphids were less likely to drop from the plant but rather kick using their hind legs which is less energetically costly.

The number of ovipositions by *A. ervi* is affected by the density of available aphids. As the density of aphids increases the number of ovipositions also increases. In most cases the density of aphids had no effect on the number of aphids falling off the

plants. The only exception was A_{10} resistance breaking biotype feeding on Glen Rosa. As previously explained the aphids on the cultivar were less settled than in other treatments and more aphids fell from the plant.

The number of successful parasitism events in all cultivar treatments was very limited. The parasitoid was reared on *A. idaei* for two generations before use in experiments to avoid any effect of enzyme compatibility which has been blamed for the reduced reproductive performance when transferred between hosts (Powell and Wright, 1988; Henry *et al*., 2005). Dissection of a sample of aphids removed from plants revealed no eggs. The low number of successful parasitism suggests that *A. idaei* physiological defence mechanisms have overcome the parasitoid eggs. A physiological mechanism of resistance by aphids is the non-development of the egg inside inappropriate hosts with no encapsulation visible and the egg disappeared within 72 hours (Henter & Via, 1995).

The low numbers of successful parasitisms and the restriction of size of instar suitable for parasitism, demonstrates limitations for the use of *A. ervi* to control *A. idaei* in commercial protected raspberry cultivations. Unless the wasp is introduced when the number of aphids is very low within the plantation and attack of smaller nymphs is very successful, the larger aphids are protected from this wasp species and are able to produce young at a very rapid rate. This would result in a sharp increase in population size and as in other systems, where wasps are used as biological control, the success is limited when the pest population become established.

Although *A. ervi* is possibly not the best parasitoid to use in this system, the results show that the resistance genes within the raspberry have positive effects on the ability of *A. ervi* to parasitise *A. idaei*. A model developed by Van Emden & Wearing (1965) indicated that better aphid control would be achieved by using a partially resistant variety and biological control rather than using a resistant variety on its own.

Conclusions

- *A. ervi* can only parasitise 1st and 2nd instars of *A. idaei* as larger instars mount a physical defence which include kicking and moving away from the parasitoid.

- Less *A. idaei* biotype 2 were parasitized on Malling Landmark (A_1 resistance gene, which is ineffective against this biotype) than on Malling Jewel (susceptible). Less *A. idaei* A_{10} resistance breaking biotype were parasitized on Glen Rosa (A_{10} resistance gene, effective against this biotype) than on Malling Landmark (A_1 resistance; ineffective resistance gene) and Malling Jewel (susceptible). This suggests that an interaction between the host plant resistance and the aphid biotype can make the aphid more susceptible to parasitoid attack, but requires further investigation.

- Low numbers of *A. idaei* were parasitized by *A.ervi* in all treatments, suggesting that *A. ervi* is not a suitable candidate for biological control of this aphid. However it does highlight the complex relationship between aphid and plant host and the possibility to study other potential parasitoid species for biological control.

Technology transfer:

Evaluation of prototype SCRI enhanced raspberry beetle traps by collaborators under MRS IP agreements:

1. Norway: Dr Nina Trandem Planteforsk, Høgskolveien 7, Ås 1432 Norway.
2. USA: Craig MacConnell, Washington State University Extension Service, Washington State, USA.

Communications / dissemination:

2003

Oral presentation: Cross, J.V. & Gordon, S.C. 2003. Raspberry beetle and cane midge. *Ashford Fruit Conference,* Ashford, Kent.

Oral presentation: Gordon, S.C., Birch, A.N.E., Woodford, J.A.T. & Mitchell, C. 2003. Managing pests of raspberry using IPM techniques - European Experience (abstract). *I Symposium on Raspberry,* Cacak, Serbia and Montenegro, 28-30 October 2003

Oral presentation: Gordon, S.C., Birch, A.N.E., Brennan, R.M. & Graham, J. 2003. Alternative control strategies for managing pests of cane and bush fruits in Europe (Abstract). *Advances in European Crop Protection,* John Innes Centre, Norwich, 19-20 May 2003.

Advisory IT input: Gordon, S.C. 2003. Raspberry Beetle (*Byturus tomentosus*). *CABI Crop Protection.* CD.

Poster presentation: Birch, A.N.E., Gordon, S.C., Fenton, B., Malloch, G., Mitchell, C., Jones, A.T., Griffiths, D.W., Brennan, R.M., Graham, J. & Woodford, J.A.T. 2003. Developing a sustainable IPM system for high value Rubus crops (raspberry, blackberry) for Europe (Poster). *COST 836 Final Workshop,* Ancona, 9-11 October 2003.

2004

Proceedings: Birch, A.N.E., Gordon, S.C., Fenton, B., Malloch, G., Mitchell, C., Jones, A.T., Griffiths, D.W., Brennan, R., Graham, J. & Woodford, J.A.T. 2004. Developing a sustainable IPM system for high value *Rubus* crops (raspberry, blackberry) for Europe. *Acta Horticulturae,* **649,** 289-292.

2005

Oral presentation: Gordon, S.C. 2005. Raspberry and Currant Entomology - The Drivers for Change in Europe (abstract). *9th International Rubus/Ribes Symposium, Acta Horticulturae,* Chile, December 2005

Oral presentation: Gordon, S.C., Mitchell, C., Birch, A.N.E., Griffiths, D.W., Casagrande, E. & Jones, O. 2005. Use of natural plant derived chemicals to monitor and the prospects of managing raspberry beetles (*Byturus tomentosus*) in open field production. *Workshop Pest and Weed Control in Sustainable Fruit Production,* Poland.

Oral presentation: Gordon, S.C., Barker, H., Graham, J., Brennan, R.M., Dolan, A., Birch, A.N.E., Mitchell, C., Cross, J., Berrie, A., Fitzgerald, J. & Allen, J. 2005. Integrated crop management related cane and bush fruit research in the UK. *WG3 1st Meeting Sustainable Berry Production (COST 863 Euroberry) (abstract),* Wageningen, 7-9 July 2005.

Oral presentation and proceedings: Gordon, S.C., Birch, A.N.E. & Mitchell, C. 2006. Integrated Pest Management of pests of raspberry (*Rubus idaeus)* - Possible developments in Europe by 2015. *IOBC/wpr Bulletin,* Stavanger, Norway, 5-8 October 2005.

Oral presentation and proceedings: MacConnell, C.B., Murray, T.A., Burrows, C.L., Gordon, S.C., Birch, A.N.E. & Tanigoshi, L.K. 2005. Threshold Development and Monitoring of the Raspberry Beetle, *Byturus unicolor*. *Acta Horticulturae (in press).*

Round robin oral presentation: ADAS/East Malling Soft Fruit Conference, 23-26 November 2005. 'The LEAF Innovation Centre at SCRI – new IPDM strategies for raspberry'.

2006

Oral presentation: Field demonstration of prototype raspberry beetle trapping system. SCRI LEAF Innovation Centre Open Day, 23 May 2006.

2007

HDC Factsheet: Allen, J. & Gordon, S.C. 2007. Sucking insect pests of cane fruit crops. *Horticultural Development Council*.

Norwegian collaborators' publications related to SCRI raspberry beetle trap evaluation.

Bøthun & Heiberg, 2004. Satsing på økologisk bringebær (Aiming for organic raspberry production). Norsk Frukt og Bær 7(4):16-18.

Ekeland 2005. Limfeller forsterket med duftstoff fra bringebær som alternativ bekjempelse av bringebærbille. (Sticky traps enhanced with volatiles from raspberry as alternative control measure for raspberry beetle. MSc Thesis, Norwegian University of Life Sciences, 115 pp.

Heiberg & Trandem, 2005. Utfordringer ved økologisk dyrking av bringebær. (Challenges in organic raspberry production) Grønn kunnskap 9(2): 607-610

Trandem, N. 2002. Norske bringebærbiller gjør det annenhvert år - om billas biologi og muligheter for alternativ bekjempelse (Norwegian raspberry beetles do it every second year – beetle biology and possibilities for control). Norsk Frukt og Bær 5 (2): 18-19.

Trandem, N. 2006. Nye tiltak mot bringebærbille og bladmidd (New control measures against raspberry beetle and raspberry leaf and bud mite). Bioforsk FOKUS 1(1):101

Trandem, 2006. Skadedyr i bringebær (Raspberry pests). I: Plantevern og plantehelse i økologisk landbruk, Bind 4 (Frukt og bær) (Plant protection and plant health in organic production, volume 4 (fruits and berries). In press.

References

Birch, A.N.E., Fenton, B., Malloch, G., Jones, A.T., Phillips, M.S., Harrower, B., Woodford, J.A.T., & Catley, M.A. (1994): Ribosomal spacer length variability in the large raspberry aphid, *Amphorophora idaei* (Aphidinae: Macrosiphini). Insect Molecular Biology 3:239-249.

Birch, A.N.E., Gordon, S.C., Griffiths, D.W., Harrison, R.E., McNicol, R.J., Robertson, G.W., Spencer, B., Wishart, J. & Woodford, J.A.T. (1996): The role of flower volatiles in host attraction and recognition by the raspberry beetle, *Byturus tomentosus*. IOBC/WRPS Bulletin 19(4):117-122.

Birch, A.N.E. & Jones, A.T. (1988): Levels and components of resistance to *Amphorophora idaei* in raspberry cultivars containing different resistance genes. Annals of Applied Biology 113:567-578.

Birch, A.N.E., Jones, A.T., Malloch, G., Fenton, B. & Gordon, S.C. 2003. Resistance-breaking raspberry aphid biotypes: a challenge for plant breeding. Integrated Protection of Fruit Crops (Soft Fruits), IOBC/wprs Bulletin 26 (2), pp 51-54.

Blackman, R.L. (1971). Variation in the photoperiodic response within natural populations of *Myzus persicae* (Sulzer). Bulletin of Entomological Research 60:, 533-546.

Briggs, J B (1959). Three new strains of *Amphorophora rubi* (Kalt.) on cultivated raspberries in England. Bulletin of Entomological Research 50:81-87.

Dickson, A. T. (1979): The population dynamics of raspberry aphids in Eastern Scotland. PhD Thesis, University of Dundee pp.1-238.

Couty, A. and Poppy, G. M. (2001). Does host-feeding on GNA-intoxicated aphids by *Aphelinus abdominalis* affect their longevity and/or fecundity? Entomologia Experimentalis et Applicata 100: 331-337.

Gordon, S.C., Woodford, J.A.T., & Birch, A.N.E. (1997): Arthropod pests of *Rubus* in Europe: Pest status, current and future control strategies. Journal of Horticultural Science 72:831-862.

Henry, L.M., Gillespie, D.R., Roitberg, B.D., (2005). Does mother really know best? Oviposition preference reduces reproductive performance in the generalist parasitoid *Aphidius ervi*. *Null.* Entomologia Experimentalis et applicata 116:167-74.

Henter, H. J. and Via, S. (1995). The Potential for Coevolution in a Host-parasitoid system .1. Genetic variation within an Aphid Population in susceptibility to a parasitic wasp. Evolution 49: 427-438.

Hoelmer, K.A., Kirk, A.A., (2005). Selecting arthropod biological control agents against arthropod pests: Can the science be improved to decrease the risk of releasing ineffective agents*? Biological Control* 34:255-64.

Höhn, H., Neuweiler, R. & Höpli, H. U. (1995): Integrierte Schädlingsregulierung bei Himbeeren. Schweiz. Z. Obst- und Weinbau 131:308-3110.

Honek, A., Jarosik, V., Lapchin, L., and Rabasse, J. M. (1998). Host choice and offspring sex allocation in the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera : Aphelinidae). Journal of Agricultural Entomology 15: 209-221.

Jones, A.T. (1986): Advances in the study, detection and control of viruses and virus diseases of *Rubus*, with particular reference to the United Kingdom. Crop Research 26:127-171.

Jones, A.T. (1976). The effect of resistance to Amphorophora rubi in reaspberry (Rubus idaeus) on the spread of aphid-borne viruses. Annals of Applied Biology 82:503-510.

Jones, A.T. (1986). Advances in the Study, Detection and Control of Viruses and Virus Diseases of Rubus, with Particular Reference to the United Kingdom. Crop Research 26:127-71.

Jones, A.T, Jennings, D.L. (1980). Genetic-Control of the Reactions of Raspberry to Black Raspberry Necrosis, Raspberry Leaf Mottle and Raspberry Leaf-Spot Viruses. Annals of Applied Biology 96:59-65.

Jones, A.T., McGavin, W.J, Birch, A.N.E. (2000). Effectiveness of resistance genes to the large raspberry aphid, *Amphorophora idaei* Borner, in different raspberry (*Rubus idaeus* L.) genotypes and under different environmental conditions. Annals of Applied Biology 136:107-13.

Jones, D. B., Giles, K. L., Berberet, R. C., Royer, T. A., Elliott, N. C., and Payton, M. E. (2003). Functional responses of an introduced parasitoid and an indigenous parasitoid on greenbug at four temperatures. Environmental Entomology 32: 425-432.

Kalule,T. & Wright,D.J. (2002) Effect of cabbage cultivars with varying levels of resistance to aphids on the performance of the parasitoid, *Aphidius colemani* (Hymenoptera : Braconidae). *Bulletin of Entomological Research*, **92,** 53-59.

Knight, R.L., Keep, E, Briggs, J.B. (1959). Genetic resistance to *Amphorophora rubi* (Kalt.) in the raspberry. 1 The gene A1 from Baumforth A. Journal of Genetics 56:261-280.

Lage, J., Skovmand, B., Andersen, S.B., Lage, J., Warburton, M.L., Crossa, J., Skovmand, B., Andersen, S.B., (2004). Resistance categories of synthetic hexaploid wheats resistant to the Russian wheat aphid (*Diuraphis noxia*). Assessment of genetic diversity in synthetic hexaploid wheats and their *Triticum dicoccum* and *Aegilops tauschii* parents using AFLPs and agronomic traits. Euphytica 136:291-6.

Losey, J. E. and Denno, R.F., (1998). Inerspecific variation in the escape responses of aphids: effect on risk of predation from foliar-foraging and ground-foraging predators. Oecologia 115:245-252.

Morgan, D., Walters, K.F.A., Aegerter, J.N., (2001). Effect of temperature and cultivar on pea aphid, Acyrthosiphon pisum (Hemiptera : Aphididae) life history. Bulletin of Entomological Research 91:47-52.

Mote, U.n. & Shahane, A.K., (1988). Studies on cultivar reaction of sorghum to delphacid, aphid and leaf sugary exudation. Sorghum newsletter 27:86.

Powell, W., Pennacchio, F., Poppy, G.M., Tremblay, E., (1998). Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). Biological Control 11:104-12.

Powell, W. and Wright, A. F. (1988). The abilities of the aphid parasitoids *Aphidius ervi* Haliday and *A. rhopalosiphi* de Stefani Perez (Hymenoptera, Braconidae) to transfer between different known host species and the implications for the use of alternative hosts in pest control strategies. Bulletin of Entomological Research 78: 683-693.

Robertson, G.W., Griffiths, D.W., Birch, A.N.E., Jones, A.T., McNichol, J.W. & Hall, J.E. (1991). Further evidence that resistance in raspberry to the virus vector aphid, Amphorophora idaei is related to the chemical composition of the leaf surface. Annals of Applied Biology 119, 443-449.

Robertson, G.W., Griffiths, D.W., Woodford, J.A.T., Birch, A.N.E., Pickett, J.A. & Wadhams, L.J. (1993): A comparison of the flower volatiles from hawthorn and four raspberry cultivars. Phytochemistry. 33:1047-1053.

Robertson, G.W., Griffiths, D.W., Woodford, J.A.T. & Birch, A.N.E. (1994): Changes in the chemical composition of volatiles released by the flowers and fruit of the raspberry cv. Glen Prosen. Phytochemistry. 38:1175-1179.

Shepherd, T., Robertson, G.W., Griffiths, D.W., Birch, A.N.E., (1999). Epicuticular wax composition in relation to aphid infestation and resistance in red raspberry (Rubus idaeus L.). Phytochemistry 52:1239-54.

Singh, B.U., Padmaja, P.G., Seetharama, N., (2004). Biology and management of the sugarcane aphid, Melanaphis sacchari (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop Protection 23:739-55.

Shepherd, T., Robertson, G.W., Griffiths, D.W., Birch, A.N.E., (1999). Epicuticular wax ester and triacylglycerol composition in relation to aphid infestation and resistance in red raspberry (Rubus idaeus L.). Phytochemistry 52:1255-67.

Takada, H., Tada, E., (2000). A comparison between two strains from Japan and Europe of *Aphidius ervi*. Null. Entomologia Experimentalis et Applicata 97(1):11-20.

Taylor, C.E. & Chamber, J. (1969): Effects of insecticide treatments on aphid populations and on spread of latent viruses in raspberry in cane nurseries. Horticultural Science 9:37-43.

Taylor, C.E. & Gordon, S.C. (1975): Further observations on the biology and control of the raspberry beetle (*Byturus tomentosus* (Deg.)) in eastern Scotland. Journal of Horticultural Science 50:105-112.

Van Emden, H.F., (1995) Host plant – Aphidophaga interactions. Agriculture, Ecosystems and Environment 52:3-11.

Van Emden,H.F & Wearing,C.H. (1965) The role of host plant in delaying economic damage levels in crops. *Annals of Applied Biology*, **56,** 323-324.

Van Rijn, P.C.J., Mollema, C. & Steenhuis-Broers, G.M., (1995). Comparative life history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera; Thripidae) on cucumber. Bulletin of Entomological Research 85:285-297.

Villagra,C.A., Ramirez,C.C. & Niemeyer,H.M. (2002) Antipredator responses of aphids to parasitoids change as a function of aphid physiological state. *Animal Behaviour*, **64,** 677-683.

Woodford, J.A.T., Birch, A.N.E., Gordon, S.C., Griffiths, D.W., & McNicol, J.W. (2003). Controlling raspberry beetle without insecticides. IOBC/WRPS Bulletin 26:87-92.

Wyatt,I.J. & White,P.F. (1977) Simple Estimation of Intrinsic Increase Rates for Aphids and Tetranychid Mites. *Journal of Applied Ecology*, **14,** 757-766.