

Project title: Raspberry: A novel approach for ICM in fresh and processed crops

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Project leaders: Dr A Nick E Birch/ Mr Stuart C Gordon  
Scottish Crop Research Institute  
Invergowrie  
Dundee  
DD2 5DA

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Key workers: Carolyn Mitchell (PhD Student, SCRI)

Location: Scottish Crop Research Institute  
Invergowrie  
Dundee  
DD2 5DA

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The results and conclusion in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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# **GROWER SUMMARY**

## **Headline**

- A trapping system, based on the raspberry flower volatile, used to monitor and control the raspberry beetle works well but requires some optimisation for use under different conditions and population densities.
- There is a complex relationship between the resistance genes in existing raspberry varieties against the large raspberry aphid and the ability of aphid biotypes to overcome them.
- Raspberry varieties with A<sub>10</sub> resistance (eg Glen Rosa) can still be deployed as an effective part of an IPM for aphids.

## **Background and expected deliverables**

This report contains the results and conclusions from the second year of a three-year PhD project (2002-2005). The research involves studying two important insect pests of raspberries, the raspberry beetle and the large raspberry aphid.

The work concerning the raspberry beetle, involves the use of an enhanced trapping system in the raspberry plantations. The trapping system combines the use of white, non-UV reflective sticky traps and a highly active component of the raspberry flower volatile, which acts as an attractant to both sexes of the adult raspberry beetle. Experiments are in progress to optimise aspects of this trapping system to develop a monitoring system that will benefit raspberry growers. This year's work (2004) identified some aspects of the trapping system that still require optimisation.

The large raspberry aphid work is focussing on the effect of resistance genes found in UK raspberry varieties on the development and the number of young an individual can produce in the large raspberry aphid. This work will lead to a better understanding of the effect of resistance genes on the performance of the aphids and on the ability of parasitic wasps to attack the aphid on different raspberry varieties.

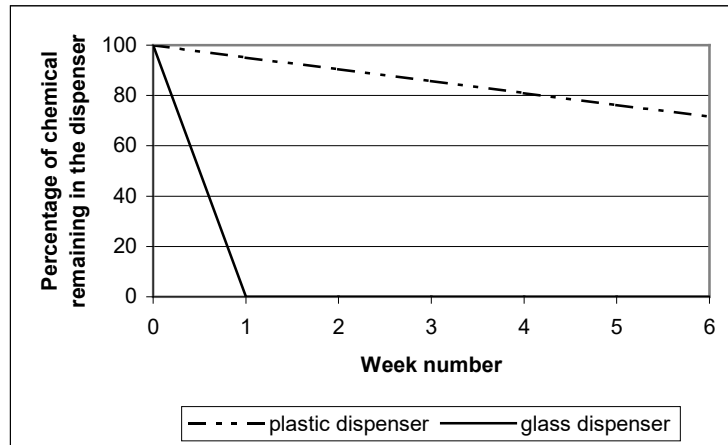
## **Summary of the project and main conclusions**

Using the experimental plot at the Scottish Crop Research Institute, Invergowrie, Dundee an experiment was set up to optimise the density of white, non-UV reflective sticky traps enhanced with a raspberry beetle attractant. One, two, three or four sticky traps were positioned in an area of 112m<sup>2</sup>. It was hoped that a balance could be reached, between the monetary cost of increasing the number of traps in an area and the number of beetles caught. These traps were positioned in the plantation at beetle emergence (10<sup>th</sup> May 2004) and were replaced with new traps once a week for six weeks.

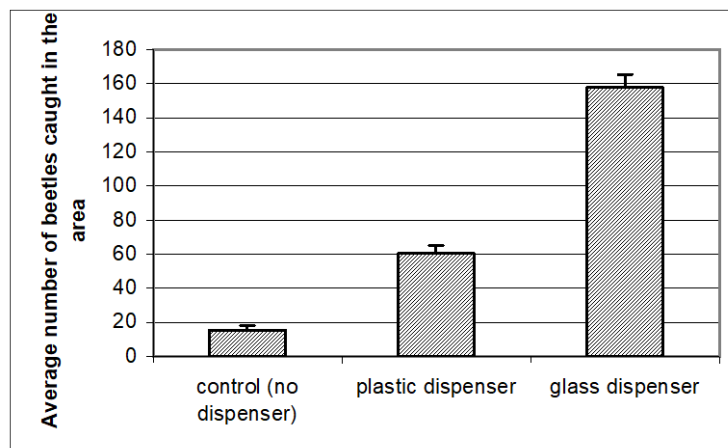
The data from the experiment showed that as the density of traps increased in the plantation, the number of beetles caught also increased. If the optimum density had been reached, the number of beetles caught would have stopped increasing as the density of traps in an area continued to increase. This suggests that there would have to be a higher density in the SCRI experimental plantation to optimise the trapping system. This plot has a very high beetle population in comparison to a standard growers' crops, as no insecticide sprays or alternative treatments have been used for several years.

An experiment was set up to compare the rate of evaporation of the attractant from two different types of dispenser. A glass dispenser with a small hole in the plastic cap was compared with a porous plastic dispenser. The plastic dispenser was the better dispenser as it is completely enclosed and therefore safer for workers to use.

The evaporation rate from the glass dispenser was much greater than the plastic dispenser:



The average number of beetles caught on sticky traps used in conjunction with a glass dispenser is much higher than the average number of beetles caught similarly in conjunction with a sticky trap using a plastic dispenser:

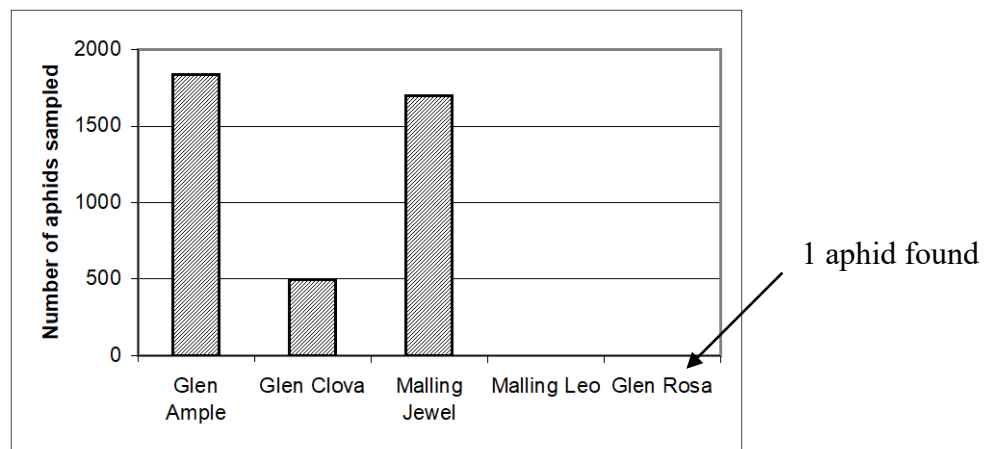


This suggests that there has to be further development of the dispenser system to obtain one that will last the required length of time out in the field, without the need to refill so that the growers would not have to come into contact with the chemical.

At the same experimental plot at SCRI, five raspberry cultivars, containing different resistance genes against the large raspberry aphid, were sampled to determine the level of infestation by the aphid. The cultivars used in the experiment were: Glen Ample ( $A_1$  resistance), Glen Clova (minor gene or multi-genic resistance), Glen Rosa ( $A_{10}$  resistance), Malling Jewel (susceptible) and Malling Leo ( $A_1$  and  $A_{10}$  resistance). Sampling began when the aphids were first observed in the plantation (17<sup>th</sup> June

2004) and the sampling was repeated once a week for nine weeks. The number of each development stage of the aphid was recorded along with the occurrence of any parasitized aphids.

At the end of the sampling period, no aphids had been found on Malling Leo and only one aphid was found on Glen Rosa. These results suggest that, in this plantation, the A<sub>10</sub> resistance gene is still successful at controlling the aphid population. Aphids were found on the cultivars with major and multi-genic resistance.



It would be expected the Malling Jewel would have a large number of aphids as this cultivar is completely susceptible. These results suggest that the aphid biotypes present at SCRI are better adapted to feeding on Glen Ample (single, major gene resistance) than on Glen Clova (multi-genic resistance).

The percentage parasitism of the large raspberry aphid varies between cultivars, but remains low in all cases. Aphids feeding on Glen Clova were more susceptible to parasitic wasp attack (1%) than Malling Jewel (0.5%) and Glen Ample had the lowest level of attack (0.05%). It is possible that aphids feeding on cultivars containing resistance genes require more of their resources to overcome the resistance mechanism and therefore become more susceptible to wasp attack at critical stages in aphid development. Alternatively, the quantity or quality of food may be restricted on aphid-resistant varieties. The results to date suggest that there is a complex interaction between the aphid resistance genes, the aphids' ability to overcome them and the effect on the rate of parasitism by wasps.

Experiments show that temperature affects development time and the number of offspring an individual can produce of the large raspberry aphid. Although development to an adult aphid is faster at 20 °C, there is a greater number of young produced at 15 °C. This suggests that there is a trade-off between faster development and the number of offspring produced in this species. The number of days required to develop into an adult, the number of young produced and the number of days producing nymphs is higher for aphids feeding on Malling Jewel than for those feeding on Glen Ample. This indicates that aphids feeding on cultivars containing resistance genes use some of their resources to overcome the resistance, or that the food source is in some way restricted, and therefore take longer to develop into adults and produce less young.

## **Financial benefits**

At this time, there are no direct financial benefits to be gained by growers from this work on raspberry beetle, but a new defra HortLINK project (2005-2010) will develop commercial monitoring and trapping systems and optimise their use under standard agronomic practices. Growers are already faced with tight restrictions on pesticides applications and minimum residue levels on raspberry and under EU regulations further pesticides will be withdrawn. In this scenario, growers will have to turn to IPM based solutions to continue production, so the financial benefits derived from this project will be influential within the next 3-5 years. Relative costs of using enhanced traps of various designs versus conventional pest control will also be addressed in the new HortLink project, so that growers can clearly see the relative advantages and disadvantages of new pest and disease control options.

One of the main constraints in developing and utilising natural plant attractant to manage pests is currently the high costs of registration of the products. Until regulatory bodies make changes to the registration process, this technology cannot be fully developed.



Current control of the large raspberry aphid is achieved largely by the application of insecticides, but as the future availability of these products become uncertain, there must be an increase in the use of resistant cultivars. At present, there is only a restricted number of cultivars recommended for high quality fresh food market and even fewer of these contain the A<sub>10</sub> resistance. However increased use of cultivars with A<sub>10</sub> resistance will inevitably lead to an increase in the numbers of aphids overcoming this last remaining resistance.

### **Action points for growers**

- The raspberry flower volatile attractant used in this experiment is not yet commercially available but the growers could consider using white sticky traps available from AgriSense BCS Ltd (contact: [info@ambiensis.com](mailto:info@ambiensis.com), Product code BC2245) to monitor raspberry beetles in crops and use existing thresholds to determine best options for raspberry beetle control in individual fields.
- Growers should continue to use a spray programme to control raspberry beetle but made aware that the enhanced trap will be commercially available as soon as possible for monitoring and later ‘lure and kill’ approaches to reduce pesticide use.
- Consider when selecting raspberry varieties for planting varieties with A<sub>10</sub> resistance as part of an IDM strategy to minimise aphid outbreaks and subsequent virus problems
- Report any outbreaks of aphids on A<sub>10</sub> resistant raspberry plantations
- Growers should monitor levels of aphids on any crops with only the A<sub>1</sub> resistant gene eg (Glen Ample)

## Science Section

### 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. 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 an insecticide to the ripening fruits a few weeks before harvest which kills the newly emerged larvae (Gordon *et al.* 1997).

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 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 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 attractants (coded chemical A and chemical B) for testing under field conditions.

In the first year of this project (2003), the white, non-UV reflective sticky traps (AgriSense-BCS Ltd) were used in conjunction with dispensers (Chromacol Ltd) filled with chemical B. These were used in two commercial sites, the first site was owned by R and JM Place Ltd, Church Farm, Tunstead, Norwich, Norfolk (OS TG 289 198). The second site was owned by Ewan McIntyre, Crauchan, Wester Essendy, Blairgowrie (OS NO 135 435). An experiment was set up to observe the effect of the chemical on the number of beetles caught on the sticky traps. The results indicated that there was a great amount of variation in the numbers of beetles caught between sites and also within the same site. The use of the attractant (chemical B) in conjunction with the trap, increased the numbers of beetles caught by 5-30 fold. However, the enhanced sticky traps were only effective at trapping the beetles before the start of flowering in the plantation, as the attractants released from the flowers masks, or competes with, the chemical attractant.

A restriction when using commercial sites, is that the amount of land offered is always limited as growers are not willing to reduce the quality of their fruit. Therefore, it was proposed that this years experiments (2004) would take place in the experimental plot at the Scottish Crop Research Institute, Invergowrie, Dundee (OS NO 333 342). There are a couple of drawbacks when using this experimental site. The site has not been treated with insecticides for a number of years so the numbers of beetles in the plantation is much greater than would be found in a commercial site. Also, some areas of the plantation have been effected by viruses and are not suitable for using in the experiments.

Experiments were designed to optimise trap density. A balance between the costs of increasing the number of traps in the area, with the number of beetles caught on the traps, had to be found. Included in this, was an assessment of the proportion of damaged berries in areas containing the different treatments. An experiment was set

up to compare the evaporation rate of the attractant chemical from two different dispensers and relate this to the proportion of damaged berries found. The two types of dispensers used were, an amber glass vial (Chromacol Ltd) with a small hole in the plastic lid and a porous plastic dispenser (AgriSense-BCS Ltd). An observation last year suggested that on occasions, the beetles were attracted to the trap but would sit in an area surrounding the trap. To investigate this phenomenon further, berries were picked in an area closely surrounding the sticky trap and the proportion of damage in this area was compared to the proportion of damage found in areas not located near to the traps.

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 led 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).

This year (2004), in the field, the numbers 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 also being studied to show 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 one biotype of the large raspberry aphid 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.

## **Raspberry Beetle**

### **Materials and Methods**

Optimising trap density using the white, non-UV reflective sticky traps in combination with the attractant (chemical B)

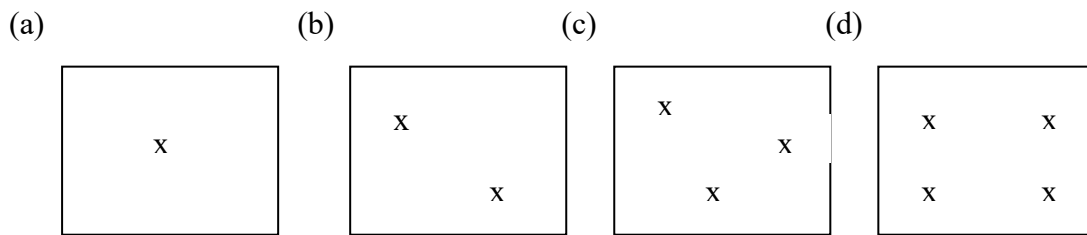
White, non-UV reflective sticky traps (AgriSense-BCS Ltd) were positioned in the 5 year old plantation at the beginning of beetle emergence (10<sup>th</sup> May 2004) and were changed twice a week for 6 weeks. Figure 2 shows the layout of the experimental site used in the experiment. Each block of cultivar was approximately 112 m<sup>2</sup> and consisted of 5 rows each containing 17 plants. The placement of the sticky traps in each plot, using 1, 2, 3 or 4 traps, can be seen in Figures 1a-d.

A slow release, porous plastic dispenser (AgriSense-BCS Ltd) containing chemical B, was attached to the frame of the trap and remained in position for the duration of the experiment. The sticky traps were positioned on the lower supporting wire, approximately 0.6 metres above the ground. On removal from the supporting wire, the sticky traps were wrapped in clingfilm and stored at 4 °C until analyses could be completed.

To count the number of captured beetles, each sticky trap was inserted in a clear plastic bag marked with a grid. This split the area of beetles to be counted into smaller more manageable quadrants.

After the commencement of fruit ripening, berries still containing their husks were picked in each of the experimental areas. A total of 50 berries were picked in the area immediately surrounding the sticky trap. This area was no more than one metre to each side of the sticky trap. As a contrast, 100 berries were picked from each experimental area, making sure to avoid the one metre area surrounding the trap.

The berries were labelled and frozen (-18 °C) in punnets until damage assessment could be completed. The berry assessment involved observing the berry and husk under a dissecting microscope and recording a presence or absence of beetle larva or damage.



*Figure 1– Representation of the trap placement in each of the experimental areas. (a) 1 trap positioned in the centre of the plot, (b) 2 traps positioned in the 2<sup>nd</sup> row (3.75 metres from the end of the row) and 4<sup>th</sup> row (7.5 metres from the end of the row), (c) 3 traps positioned in the 2<sup>nd</sup> row (2.5 metres from the end of the row), the 3<sup>rd</sup> row (12.5 metres from the end of the row) and the 4<sup>th</sup> row (7.5 metres from the end of the row) and (d) 4 traps positioned in the 2<sup>nd</sup> row (3.75 metres and 7.5 metres from the end of the row) and the 4<sup>th</sup> row (3.75 metres and 7.5 metres from the end of the row).*

North



Glen Rosa <b>2 traps</b>	Glen Ample <b>3 traps</b>	Glen Ample <b>4 traps</b>	Malling Jewel <b>1 trap</b>
Glen Clova <b>1 trap</b>	Malling Jewel <b>4 traps</b>	Glen Rosa <b>3 traps</b>	Glen Clova <b>2 traps</b>

Glen Clova <b>3 traps</b>	Glen Rosa <b>4 traps</b>	Malling Jewel <b>3 traps</b>	Glen Rosa <b>1 traps</b>
Malling Jewel <b>2 traps</b>	Glen Ample <b>1 trap</b>	Glen Clova <b>4 traps</b>	Glen Ample <b>2 traps</b>

Glen Rosa <b>2 traps</b>	Glen Ample <b>3 traps</b>	Glen Rosa <b>3 traps</b>	Malling Jewel <b>1 trap</b>
Malling Jewel <b>4 traps</b>	Glen Clova <b>1 trap</b>	Glen Clova <b>2 traps</b>	Glen Ample <b>4 traps</b>

Glen Rosa <b>4 traps</b>	Glen Clova <b>3 traps</b>	Malling Jewel <b>3 traps</b>	Glen Ample <b>2 traps</b>
Glen Ample <b>1 trap</b>	Malling Jewel <b>2 traps</b>	Glen Rosa <b>1 trap</b>	Glen Clova <b>4 traps</b>

Figure 2 –Experimental layout of optimal trap density study. Each cultivar block is 112 m<sup>2</sup> and contains of 5 rows each containing 17 plants. The rows run north.

### Optimising release rate of the attractant (chemical B) from the dispensers

White, non-UV reflective sticky traps (AgriSense-BCS Ltd) were positioned in the plantation at the beginning of beetle emergence (10<sup>th</sup> May 2004) and were changed twice a week, for 6 weeks. Figure 3 shows the lay out of the experimental site used for the experiment. Each block of cultivar is approximately 195 m<sup>2</sup> consisting of 8 rows containing 20 plants. The blank areas were parts of the plantation that were not of a quality suitable for use in experiments. The treatment in each experimental plot consisted of either 1 plastic dispenser, 4 plastic dispensers, 1 glass dispenser or a



control, which was a plain sticky trap with no enhancement. Chemical B was used in every treatment.

The dispensers used in each treatment were attached to the frame of the trap in the middle of the experimental area and remained in position for the duration of the experiment. The glass dispenser required refilling twice a week. The sticky traps were positioned on the lower supporting wire, approximately 0.6 metres above the ground. On removal from the supporting wire, the sticky traps were wrapped in clingfilm and stored at 4 °C until analyses could be completed.

To attain the number of captured beetles each sticky trap was inserted in a clear plastic bag marked with a grid. This split the area of beetles to be counted into smaller more manageable sizes.

After the commencement of fruit ripening, berries still containing their husks were picked in each of the experimental areas. A total of 150 berries were picked in the row containing the sticky trap. The berries were randomly picked over the full length of the row therefore avoiding any effect of trap placement.

The berries were labelled and frozen in punnets until damage assessment could be completed. The berry assessment involved observing the berry and husk under a dissecting microscope and recording a presence or absence of beetle larva or damage.

To calculate the release rate, dispensers containing chemical B were positioned in the plantation, in a similar location to where they would hang if used in combination with the sticky traps (0.6 metres above the ground). Two glass dispensers and two plastic dispensers were hung in one area of the plantation and this was repeated in a total of five areas. The full dispensers were weighed at the beginning of the experiment, at various times during the two-week duration of the experiment and the dispensers were also weighed empty. This allowed the evaporation rate to be calculated.

Glen Rosa <i>2 plastic dispensers</i>	Glen Ample <i>4 plastic dispensers</i>	Glen Clova <i>1 plastic dispenser</i>	North ↑
		Malling Leo <i>Glass dispenser</i>	
Glen Ample <i>Glass dispenser</i>		Malling Leo <i>1 plastic dispenser</i>	
Glen Clova <i>2 plastic dispensers</i>		Glen Rosa <i>4 plastic dispensers</i>	
	Glen Clova <i>4 plastic dispensers</i>		
Glen Ample <i>1 plastic dispenser</i>	Glen Rosa <i>Glass dispenser</i>	Malling Leo <i>2 plastic dispensers</i>	
Glen Clova <i>Glass dispenser</i>	Glen Rosa <i>1 plastic dispenser</i>	Glen Ample <i>2 plastic dispensers</i>	
Malling Leo <i>4 plastic dispensers</i>			

Figure 3— Experimental layout of dispenser evaporation rate optimisation. Each cultivar block is 195 m<sup>2</sup> and consists of 8 rows containing 20 plants. The rows run north.

## Results and Discussion

### Optimising trap density using the white, non-UV reflective sticky traps in combination with the attractant (chemical B)

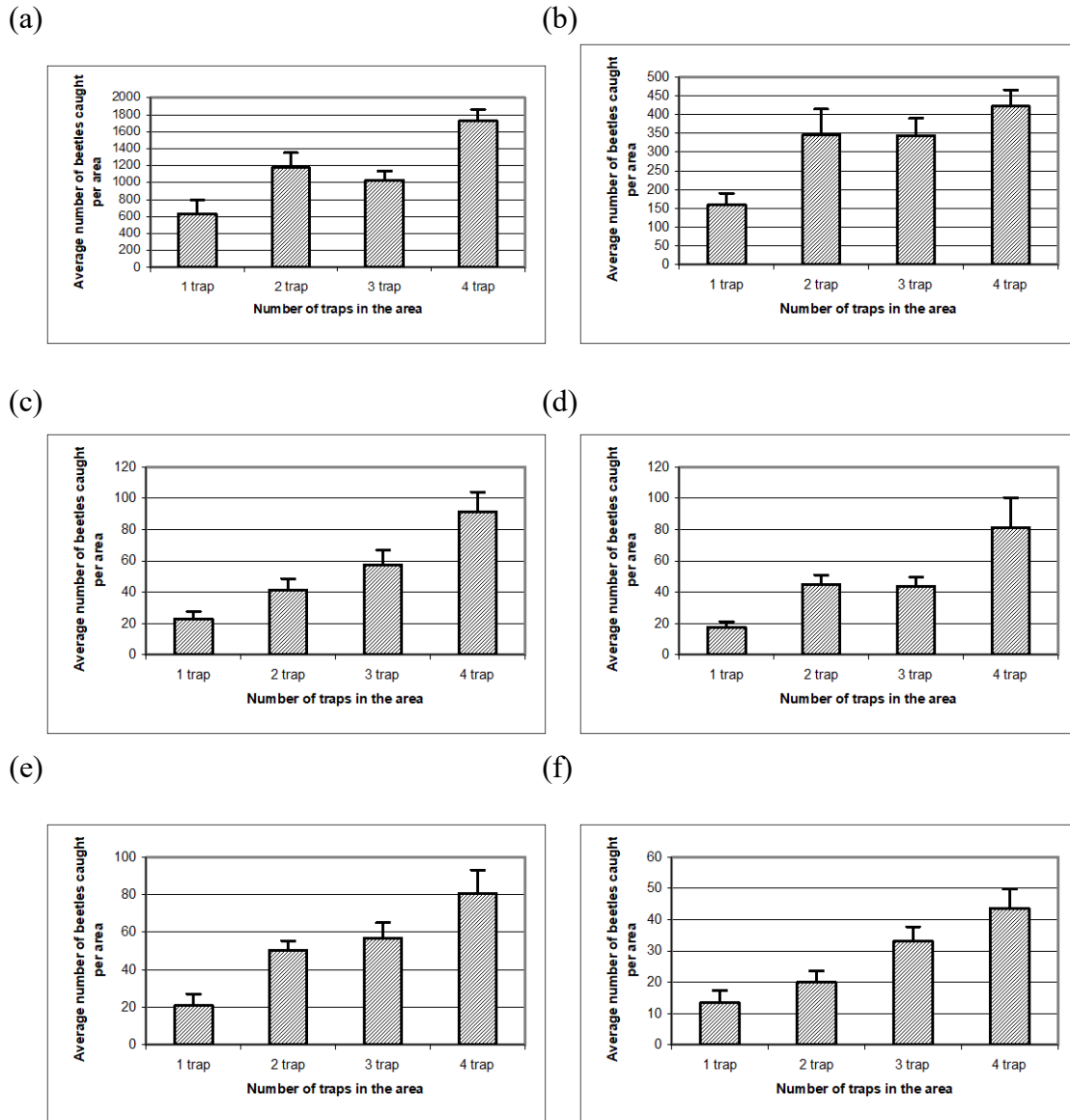
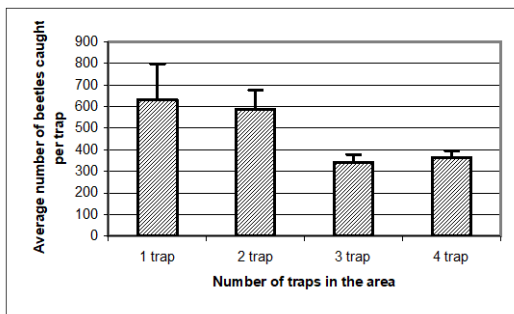


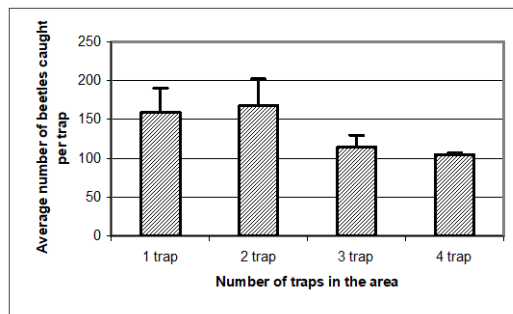
Figure 4 - The average number of raspberry beetles caught in each experimental area each week. An area ( $195 \text{ m}^2$ ) contained one, two, three or four sticky traps enhanced with chemical B. Error bars represents standard error. (a) week 1, (b) week 2, (c) week 3 (start of flowering), (d) week 4, (e) week 5, and (f) week 6.

Figures 4(a) – (f) show the average number of beetles caught in each experimental area each week. An experimental area contained one, two, three or four sticky traps enhanced with chemical B. A general trend evident, is the reduction in the number of beetles caught in all treatments as the experiment progressed from week to week. The number of beetles caught in week 1 (Figure 4a) is much greater than in week 2 (Figure 4b) and there is another decline in numbers caught observed in week 3 (Figure 4c). This decline in week 3 coincides with the start of raspberry plant flowering within the plantation. The numbers of beetles caught remains low for the remainder of the experiment (Figures 4d-f). Within week 1 (Figure 4a), it is seen that there is a greater number of beetles caught in areas containing four traps than in the other treatments. An analysis of variance reveals that in week 1, there is a significant difference (d.f. 31,  $F < 0.001$ ) in the numbers of beetles caught in the four treatments. The same trend is observed in the following five weeks and an analysis of variance shows that there continues to be a significant difference in the numbers of beetles caught in the four treatments. Week 2 (d.f. 31,  $F = 0.009$ ), week 3 (d.f. 31,  $F < 0.001$ ), week 4 (d.f. 31,  $F = 0.004$ ), week 5 (d.f. 31,  $F < 0.001$ ) and week 6 (d.f. 31,  $F < 0.001$ ).

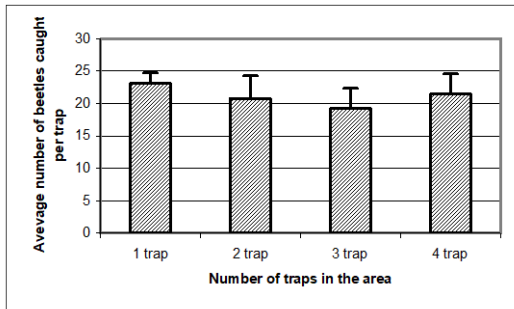
(a)



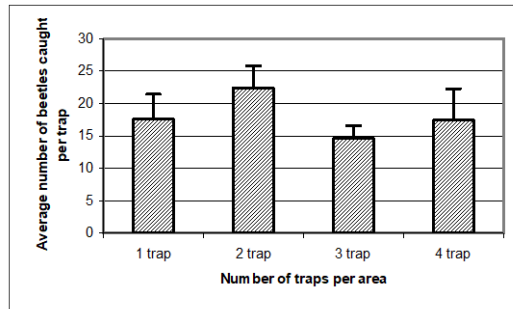
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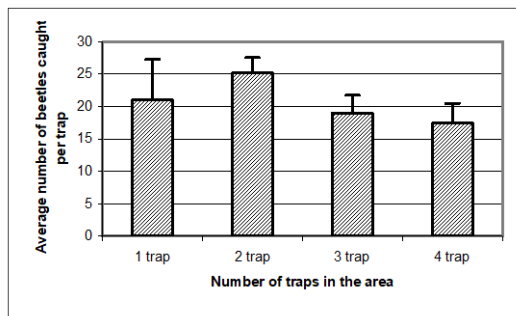
(c)



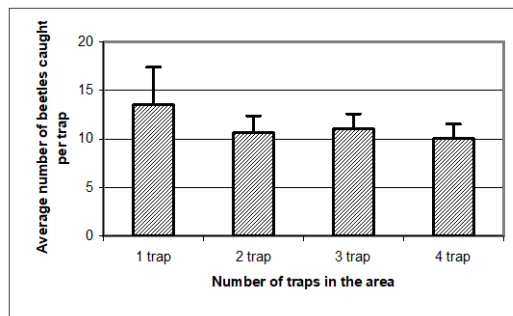
(d)



(e)

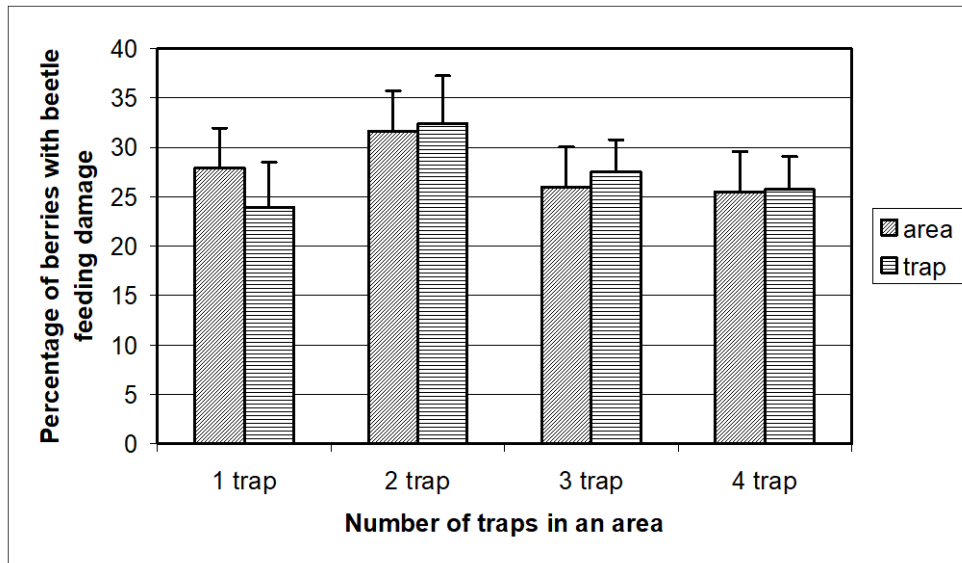


(f)



*Figure 5 - The average number of raspberry beetles caught per trap in each experimental area each week. Each experimental area contained one, two, three or four sticky traps enhanced with chemical B. Error bars represents standard error. (a) week 1, (b) week 2, (c) week 3, (d) week 4, (e) week 5, and (f) week 6.*

Figures 5(a)-(f) show the average number of raspberry beetles caught per trap in each of the experimental areas containing one, two, three or four sticky traps each week. As in Figure 4(a)-(f) it shows the reduction in the numbers of beetles caught between weeks 1 (Figure 5a) and week 2 (Figure 5b), and another reduction in week 3 (Figure 5c). The graphs show variation in the numbers of beetles caught per trap in each treatment but there is no obvious treatment that catches the most beetles per trap. This is verified by an analysis of variance, which finds no significant differences between the four treatments in all six weeks.



*Figure 6 - Percentage of berries with raspberry feeding damage. The berries were collected in a small section, approximately one metre to each side of the trap (trap) and in the total experimental area (area). Each experimental area consisted of one, two, three or four sticky traps enhanced with chemical B. Error bars represent standard error.*

Figure 6 shows the proportion of damaged berries as a result of feeding by the raspberry beetle larvae. Berries were picked in a small section of the experimental area, approximately one metre to each side of the trap (trap) and randomly throughout the entire experimental area (area). Each experimental area consists of one, two, three or four sticky traps enhanced with chemical B. There is a slight variation in the proportion of damaged berries between the four treatments and between the two different areas sampled within the one treatment. An analysis of variance indicates that there is no significant difference between the proportions of damaged berries collected in the four treatments. There is also no significant difference in the proportion of damaged berries sampled in the area, one metre to each side of the trap and the proportion damaged berries sampled in the entire area.

Figures 4(a)-(f) show that as the number of traps in the area increases, the number of beetles caught in the area also increases. If the optimum trap density had been found, the number of beetles caught as the number of traps in the area continued to increase, would start to plateau. This suggests that the density of traps in the area would have

to be increased further in order to observe any additional increase and finally a plateau in the number of beetles caught in the area. Although these results suggest that there will have to be a higher density of traps in the area it must be noted that this experiment was undertaken in a very artificial environment. The raspberry plantation used in the experiment has not been treated with insecticides for a number of years and the population of beetles is much higher than would be found in a commercial plantation. If this experiment was repeated in a commercial plantation it may show that the number of beetles caught in the area as the density of traps increases would plateau at a lower density of traps than found here.

Figure 5(a)-(f) show as the density of traps in an area increases, there is no significant difference in the number of beetles caught per trap. This is to be expected as Figure 4(a)-(f) suggests that the optimum density of traps in the area has not been reached. When the optimum density of traps in the area has been reached, the number of beetles caught on each trap would start to decrease as the density of traps continues to increase.

The analyses of the berry damage (Figure 6) also suggests that the traps densities used were not great enough to removed enough beetles from the plantation to have any effect on the damage observed. In previous experiments, observations suggested that the position of the sticky trap might influence the distribution of the beetles, resulting in an area surrounding the trap with a higher density of beetles. The berry analysis suggests that even although this phenomenon is observed, the levels of damage in the area surrounding the traps is the same as is found in the whole experimental area. Again, these results are affected by the large population of beetles in the plantation, which may mask any effect the treatment may be having on the proportion of damaged berries.

## Optimising release rate of the attractant (chemical B) from the dispensers

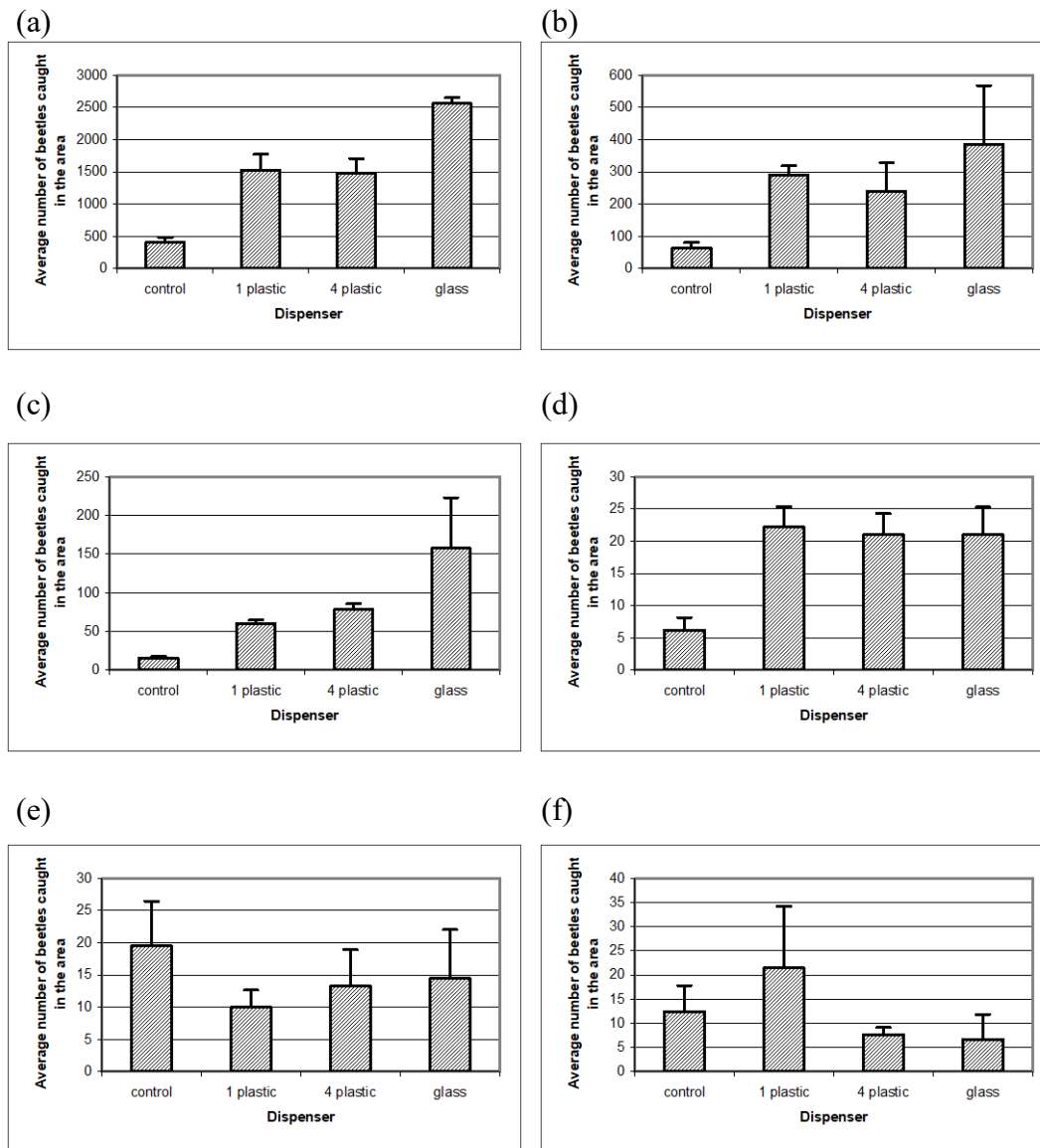
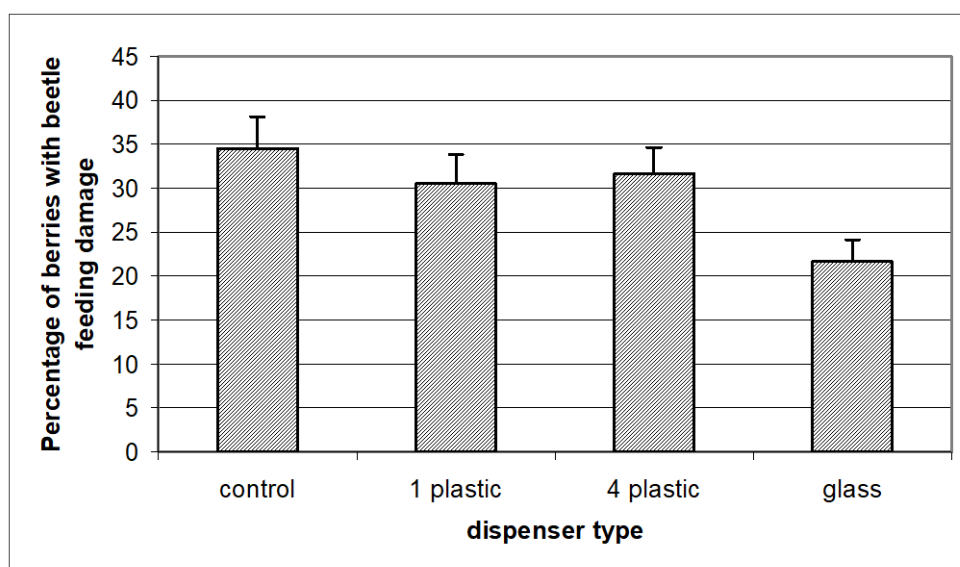


Figure 7 - Average number of raspberry beetles caught in the four experimental areas each week. Each experimental area contains one sticky trap and either one plastic dispenser, four plastic dispensers or one glass dispenser. The control was an area with a sticky trap but with no enhancement. Error bars represent standard error. (a) week 1, (b) week 2, (c) week 3, (d) week 4, (e) week 5, and (f) week 6.

Figure 7 shows the average number of raspberry beetles caught in the four experimental areas each week. Each experimental area consists of one sticky trap and either one plastic dispenser, four plastic dispensers or one glass dispenser. The control represents an area with a sticky trap but with no enhancement. In the first four



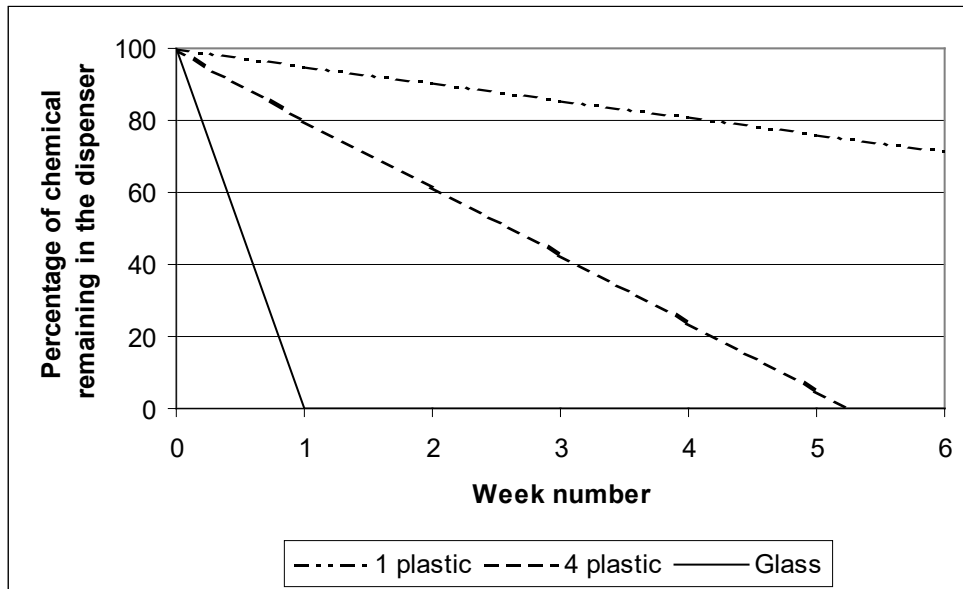
weeks (Figure 7a-d) the control sticky trap catches fewer beetles than the sticky traps with enhancement but this is not evident in week 5 (Figure 7e) and week 6 (Figure 7f). There is a reduction in the number of beetles caught in week two (Figure 7b) when compared with week one (Figure 7a) and then a further reduction in week three (Figure 7c). The decline in the number of beetles caught after week 3 coincides with the start of flowering. An analysis of variance shows a significant difference in the numbers of beetles caught between treatments in week 1 (d.f. 15,  $F < 0.001$ ), week 3 (d.f. 15,  $F < 0.001$ ) and week 4 (d.f. 15,  $F = 0.01$ ).



*Figure 8 – Percentage of berries with raspberry beetle feeding damage in the four experimental areas. Each experimental area contains one sticky trap and either one plastic dispenser, four plastic dispensers or one glass dispenser. The control represents an area with a sticky trap but with no enhancement. Error bars represent standard error.*

Figure 8 shows the proportion of berries with raspberry beetle larvae feeding damage in the four experimental areas. Each experimental area contains one sticky trap and one plastic dispenser, four plastic dispensers or one glass dispenser. The control represents an area with a sticky trap but with no enhancement. All the dispensers contain chemical B. Berries picked in the control area had the highest proportion of damage (34.5) and area with the glass dispenser had the lowest proportion of damage

(22). An analysis of variance showed that there is a significant difference (d.f. 35,  $F=0.028$ ) between the amounts of damage occurring in the different treatments.



*Figure 9 - Percentage of chemical remaining in the two types of dispenser, one plastic and glass, during the six weeks of the experiment. The results shown for four plastic dispensers is a simulation of what would be expected if the plastic dispenser had four times the evaporation rate as the plastic dispenser used in the experiment. Error bars removed for clarity.*

Figure 9 shows the percentage of chemical remaining in the two types of dispenser during the six weeks of the experiment. The results for four plastic dispensers is a simulation of what would be expected if the plastic dispenser had four times the evaporation rate as the one used in the experiment. From the results, it is clear that the chemical in the glass dispenser only lasts one week whilst there is still 76% of the chemical in the plastic dispenser at the end of the six weeks. The simulated plastic dispenser has an evaporation rate, which falls in between the other two dispensers and the chemical last for just over five weeks.

In the first three weeks (Figure 7a-c), the control sticky trap, which does not have any chemical enhancement, has fewer beetles than any of the other traps. In weeks 1-3, the sticky trap with the glass dispenser has more beetles trapped than the other traps. This pattern is lost after week 3 and is associated with the beginning of flowering in the plantation. Overall, the sticky trap with the glass dispenser is attracting more beetles than either the one plastic dispenser or the four plastic dispensers. The results from the berry assessment (Figure 8) show that there is a significant difference in the amount of damage found in berries picked in the four experimental areas. The most damage was found in the control areas, which shows that because less beetles were being trapped in the area there was more opportunity for beetle damage. There was not much difference in the amount of berry damage in areas with one plastic dispenser and four plastic dispensers. This suggests that the difference in the evaporation rate between the two dispenser types was not enough to affect the amount of beetles attracted to the traps and the amount of damage observed. The least amount of beetle damage observed, was in areas with the glass dispenser. This indicates that in the areas with the glass dispenser, the numbers of beetles caught was reducing the amount of damaged berries observed.

The timescale over which the enhanced traps would be required out in the plantation is an important factor to consider. The enhanced traps are only effective after beetle emergence and before flowering and therefore are required for approximately four weeks. Although the evaporation rate from the glass dispenser appears to be the most successful at attracting beetles, the chemical only lasts for one week. Refilling the dispenser is an option, but the chemical has to be handled with care and this would also be time consuming. Comparing the number of beetles caught using the one plastic dispenser with that caught using four plastic dispensers, shows that there is not much of a difference. The simulation of a plastic dispenser with four times the evaporation rate lasts the correct length of time, but the numbers of beetles caught on that enhanced trap is much less than using the enhanced trap with the glass dispenser. These results suggest that the appropriate dispenser has to have the evaporation rate similar or greater than that of the glass dispenser but must be much larger so it can last the four weeks that is required.

## Large Raspberry Aphid

### Materials and Methods

Effect of plant resistance genes on the number of large raspberry aphid present and the proportion of parasitized aphids on five raspberry cultivars.

Five raspberry cultivars were used in the experiment: Glen Rosa ( $A_{10}$  resistance), Glen Ample ( $A_1$  resistance), Glen Clova (minor gene or multi-genic resistance), Malling Leo ( $A_{10}$  and  $A_1$  resistance) and Malling Jewel (susceptible). Each week, 16 plants from each cultivar were sampled by removing two leaves from the top, middle and bottom of both the primocane and floricanes and placing them into labelled bags. The leaves were brought into the laboratory for analyses. The experiment started when the first large raspberry aphids were observed in the 5 year old field plantation (17<sup>th</sup> June) and ran for 9 weeks. The leaves were sampled once every week.

The aphids were identified and broken down into instar stage using the method described in (Dickson 1979). Any parasitized aphids (mummies) observed were also recorded. They were not identified to species. The leaves harbouring aphids were then repositioned into the sample bag and the sample bag was tied shut leaving a pocket of air within the bag. These bags were kept at room temperature for approximately 1 week to record any further development of parasitoid mummies.

The effect of temperature and cultivar on the development of the large raspberry aphid (biotype 2)

#### Temperature experiment

Individual adult aphids were introduced onto leaves of raspberry cv. Glen Ample plants and prevented from leaving the leaf by using clip cages. These plants were situated in growth cabinets with 16 hours daylight (1000 lux). The growth cabinets were set at a temperature of 10°C, 15 °C and 20°C ± 1 °C. On production of a nymph, the adult aphid was removed and the nymph remained contained on the leaf by use of the clip cage. These nymphs were checked every day and the following factors were

recorded: duration of pre-reproductive period, duration of reproductive period, number of days alive and fecundity. Forty replicates were completed at each temperature.

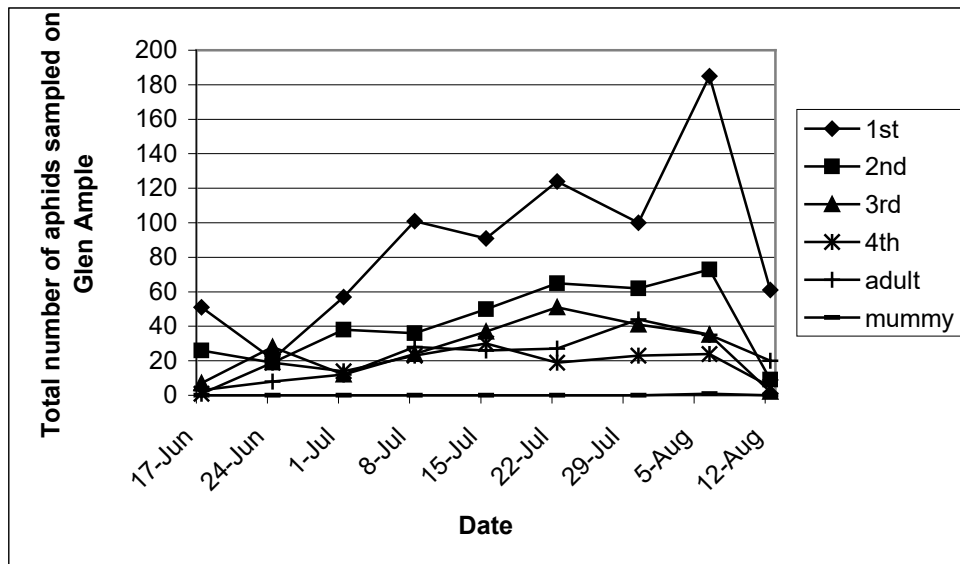
#### Cultivar experiment

Glen Ample (A<sub>1</sub> resistance) and Malling Jewel (susceptible) were used in the experiment. The experimental protocol is the same as the previous experiment. All replicates were observed at 15 °C.

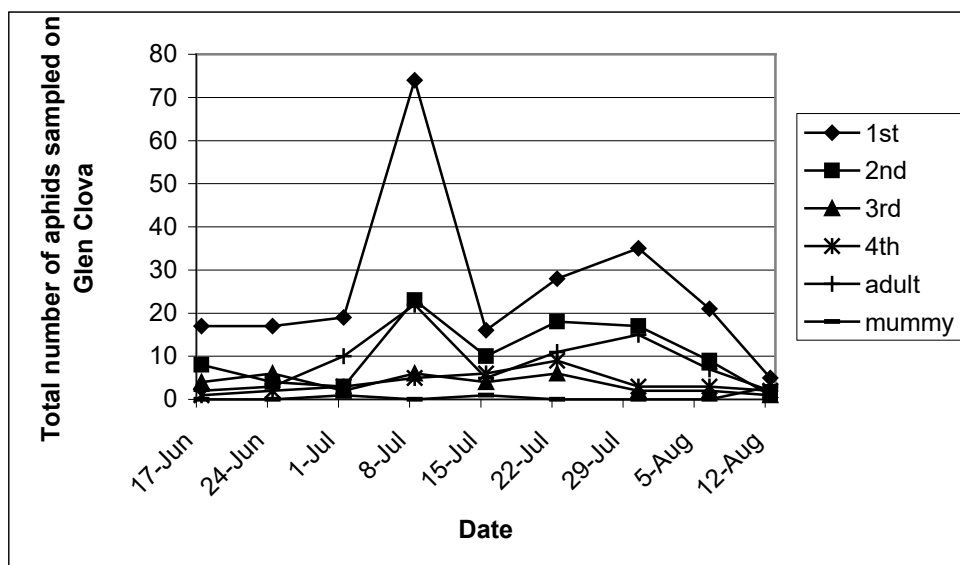
## Results and Discussion

Effect of plant resistance genes on the number of large raspberry aphid present and the proportion of parasitized aphids on five raspberry cultivars.

(a)



(b)



(c)

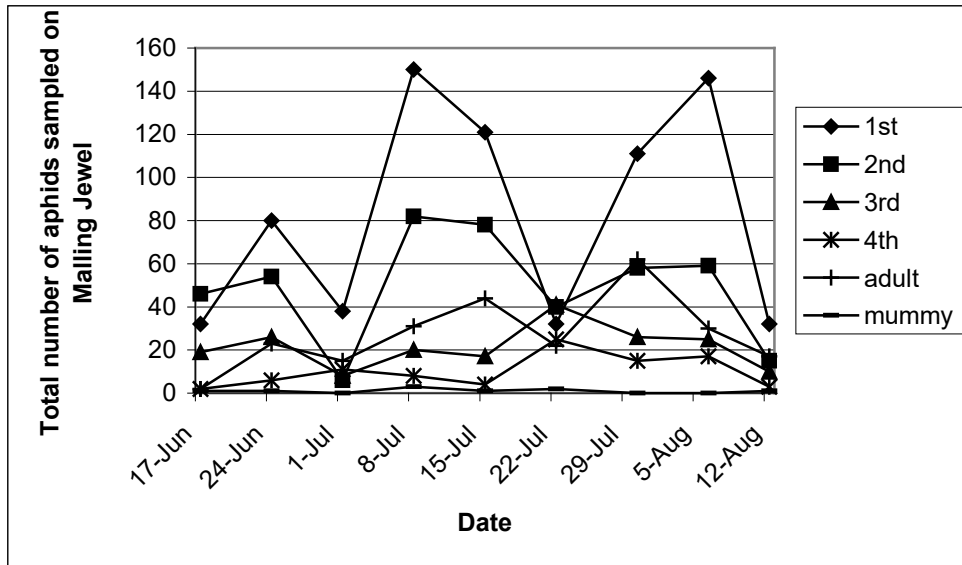


Figure 10 - Total number of large raspberry aphid sampled on (a) Glen Ample, (b) Glen Clova and (c) Malling Jewel between the 17<sup>th</sup> June and the 12<sup>th</sup> August. The sampled aphids are sorted into development stage. Number of alates removed for clarity.

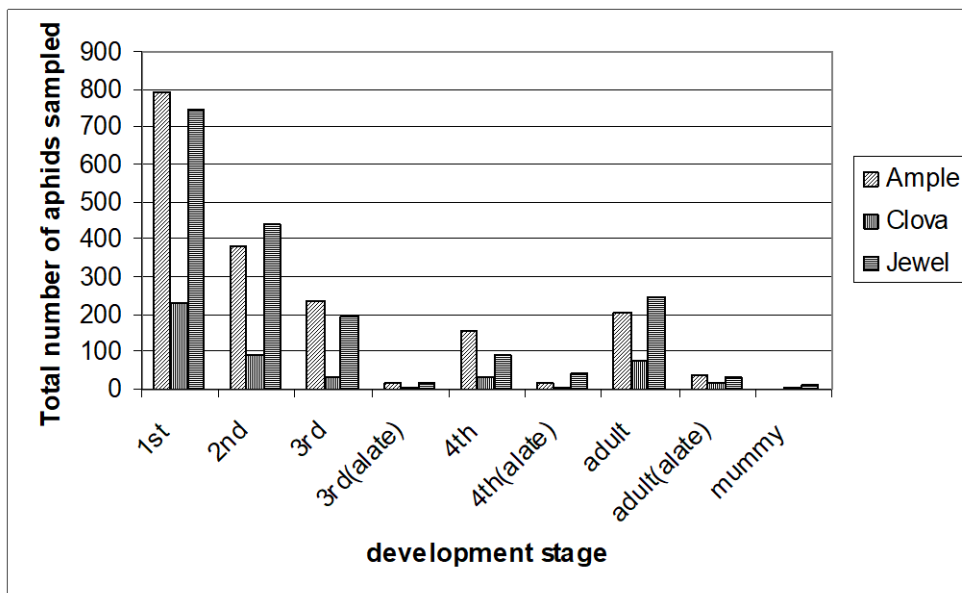


Figure 11 - Total number of large raspberry aphid collected during the sampling period (17<sup>th</sup> June – 12<sup>th</sup> August ) on Glen Ample, Glen Clova and Malling Jewel. The sampled aphids are sorted into development stage.

<b>Cultivar</b>	<b>Total number of aphids</b>	<b>Total number of parasitized aphids (mummies)</b>	<b>Percentage parasitism</b>
Glen Ample	1836	1	0.05
Glen Clova	496	5	1
Malling Jewel	1700	9	0.5

*Table 1 - Total number of large raspberry aphids sampled on Glen Ample, Glen Clova and Malling Jewel. The total number of parasitized aphids and the percentage parasitism for each cultivar.*

Figures 10 a-c and 11 show that there is a great amount of variation in the numbers of each aphid development stage on the same cultivar between weeks and also a great amount of variation in the numbers of each aphid development stage on the three different cultivars. These figures only show the results from Glen Ample, Glen Clova and Malling Jewel. There was only one individual aphid found on Glen Rosa and no aphids found on Malling Leo. Table 1 shows that on the three other cultivars, Glen Clova had the least amount of aphids (496) with less than a quarter of the number of aphids found on Malling Jewel (1700) or Glen Ample (1836). It is expected that Malling Jewel would have a high number of aphids present as it has no resistance genes against the aphid. Glen Clova has multigenic resistance which means that it is more durable against the mixture of aphid biotypes that were present in the plantation and therefore the number of aphids was quite low. It was expected that the number of aphids on Glen Ample would be much lower as it has A<sub>1</sub> resistance but this resistance works specifically against certain biotypes. The high numbers found on Glen Ample suggest that this resistance had no effect against the biotypes of aphids present on the site at SCRI.

There was substantial variation in the weather during the sampling period, which may affect the results obtained. Although when possible sampling was avoided on very windy days or wet days, this was not always feasible and the numbers of aphids found



on these days was reduced. The season finished earlier than expected due to very heavy rain showers over a two day period, which killed off any remaining aphids. The numbers are very low but there is evidence of an interaction between the cultivar and the ability of the wasp to parasitize the aphid. Aphids on Glen Clova are more susceptible to wasp attack (1%) than Malling Jewel (0.5%). This may be a result of the aphids feeding on Glen Clova, which has minor gene resistance, being more susceptible to the wasp as it needs to use more of its resources to overcome the resistance genes and survive on the cultivar. However, this is not the case with aphids feeding on Glen Ample, which contains the A<sub>1</sub> resistance genes, as the percentage parasitism of aphids on this cultivar (0.05%) is less than both Glen Clova and Malling Jewel. This suggests that there is a complex interaction between the resistance genes and the aphid's ability to overcome them, with different resistance genes having varying effects on the aphid's biology.

The effect of temperature and cultivar on the development of the large raspberry aphid (biotype 2)

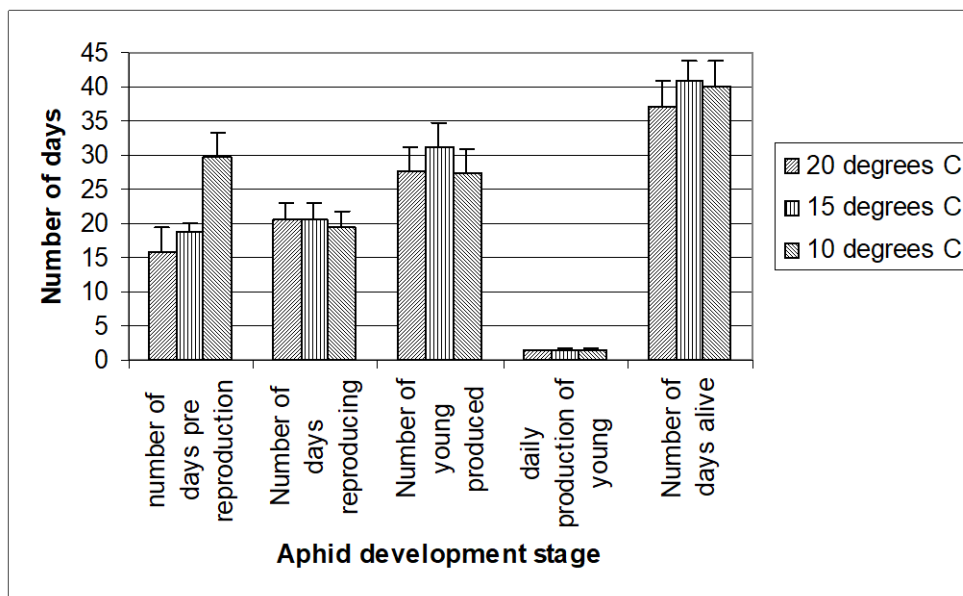
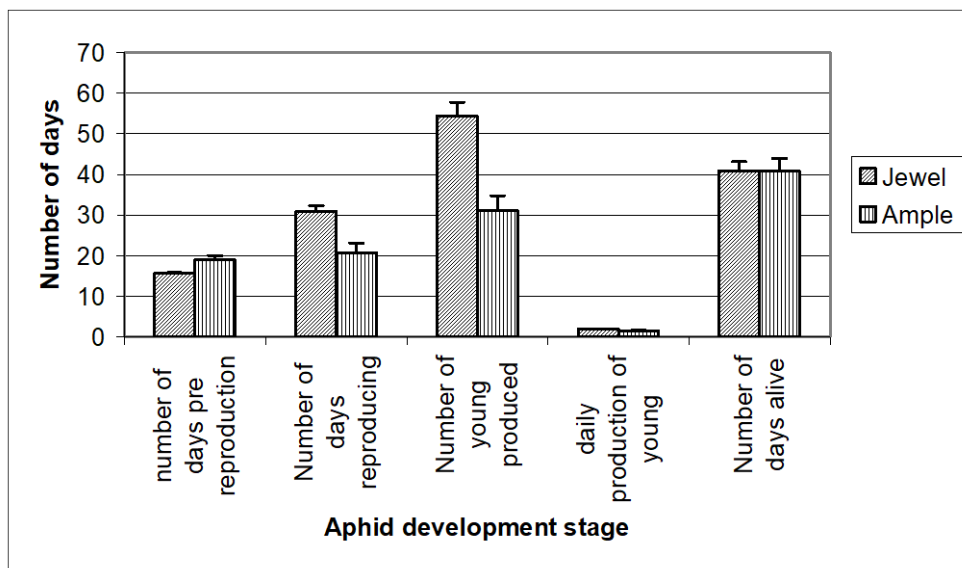


Figure 12- Average number of days pre reproduction, number of days reproducing, number of young produced, daily production of young and number of days alive for the large raspberry aphid (biotype 2) feeding on Glen Ample at 20, 15 and 10 °C. Error bars represents standard error.

Figure 12 shows that there was a difference in all the factors observed between 10, 15 and 20 °C. The number of days reproducing, the number of young produced, the daily production of young and the number of days alive were all greater at 15 °C than at the other two temperatures but an analysis of variance did not show any significant difference between the temperatures. This may be caused by the small number of replicates, which made it difficult to analyse accurately. There was however a significant difference (d.f. 80,  $F < 0.001$ ) in the number of days pre reproduction between the three temperatures, with aphids feeding at 20 °C requiring the least number of days and aphids feeding at 10 °C requiring the most. The results suggests that even although aphids feeding at 20 °C develop faster, the number of young produced is less than at 15 °C. This implies that there may be a payoff between quicker development and fecundity.



*Figure 13 - Average number of days pre reproduction, number of days reproducing, number of young produced, daily production of young and number of days alive for the large raspberry aphid (biotype 2) feeding on Malling Jewel and Glen Ample at 15 °C. Error bars represent standard error.*

Figure 13 shows that for all factors studied, except the number of days alive, there was a difference between the two cultivars. An analysis of variance shows that there

was a significant difference in number of days pre-reproduction (d.f. 61,  $F < 0.001$ ), the number of days reproducing (d.f. 50,  $F < 0.001$ ), the number of young produced (d.f. 50,  $F < 0.001$ ) and daily production of young (d.f. 52,  $F < 0.001$ ). These results suggest that aphids feeding on Glen Ample, which contains the  $A_1$  resistance gene, have to use more resources to overcome the effects of the resistance mechanism. They therefore take longer to develop into reproducing adults and produce less young over a shorter length of time than aphids feeding on Malling Jewel, which contains no resistance genes.

## Conclusions

- The results suggest that the densities of the enhanced (chemical B), white, no-UV reflective traps used in the experiment were not great enough to show a reduction in the number of beetles in the SCRI plantation and therefore could not reduce the quantity of berry damage to an acceptable level. However, this experiment was carried out in a plantation where the number of beetles was much greater than what would be found in a commercial plantation and therefore the results cannot be extrapolated for use commercial use.
- Studies to optimise the evaporation rate from the dispenser have suggested that the evaporation rate from the plastic dispenser (AgriSense-BCS Ltd) is not great enough to attract the same number of beetles as the glass dispenser (Chromacol Ltd). The plastic dispenser is much safer and easier to use than the glass dispenser and therefore more work has to be undertaken to optimise the evaporation from the plastic dispenser.
- The numbers of large raspberry aphid found on the different cultivars of raspberry was varied. There was only one aphid found on Glen Rosa and no aphids found on Malling Leo. This is expected as they contain A<sub>10</sub> resistance which is the only resistance mechanism still giving a good level of protection against the large raspberry aphid (however, there are reports that the A<sub>10</sub> resistance gene has been overcome in England). The susceptible cultivar, Malling Jewel, had a large number of aphids present and Glen Ample, which has A<sub>1</sub> resistance, also had a large number of aphids. The cultivar with minor gene resistance, Glen Clova, has a much lower level of infestation. This suggests that there was a mixture of biotypes in the plantation and the more durable multigenic resistance found in Glen Clova was more successful at keeping the numbers of aphids low than the more specific A<sub>1</sub> resistance found in Glen Ample.
- The proportion of parasitized aphids on the cultivars containing the different resistance genes showed that there is a complex interaction between the aphid resistance genes and the ability of the aphids to overcome them, therefore affecting the wasp's ability to parasitize.

- The optimum temperature for large raspberry aphid production in the laboratory was found to be 15 °C. Comparison of the time to develop and the number of young produced, suggests that aphids feeding on Glen Ample, which has A<sub>1</sub> resistance must overcome resistance present in the plant. They therefore take longer to develop into adults and produce less young than aphids feeding on Malling Jewel, which has no resistance.

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