ASSESSING THE POTENTIAL FOR SEVEN SPOT LADYBIRD (COCCINELLA SEPTEMPUNCTATA) TO CONTROL THE BLACKCURRANT APHID (CRYPTOMYZUS GALEOPSIDIS)

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TIIVISTELMÄ

Työssä selvitetään seitsenpistepirkon (*Coccinella septempunctata*) käytön mahdollisuuksia kirvojen torjuntaan mustaherukkaviljelmillä. Seitsenpistepirkon pääasiallista ravintoa ovat eri kirvalajit ja se esiintyy jo luontaisesti mustaherukan tuotantoalueilla. Kirvat eivät ole pahimpia mustaherukan tuholaisia, mutta ne aiheuttavat kuitenkin ongelmia säännöllisesti ja joskus pahojakin satotappioita. Biologinen torjunta tarjoaa uusia mahdollisuuksia kemiallisen torjunnan käytön vaikeutuessa. Työ tehtiin Scottish Crop Research – Instituutissa kesällä 2010.

Tavoitteena oli selvittää, mikä leppäpirkon kehitysasteista on tehokkain kirvojen saalistaja ja mitä kirvojen toukka-asteita ne suosivat. Erilaisilla peto – saalis -kokeilla tutkittiin leppäpirkkojen käyttäytymistä saaliseläinten valinnassa ja niiden tehokuuta kirvojen tuhoamisessa. Työhön oleellisena osana liittyi mustaherukkaviljelmien eliöstökartoitus, jonka avulla tutkittiin, mitä lajeja ja missä määrin kasvustoista löytyy.

Kokeet osoittavat, että kolmannen ja neljännen asteen toukat ovat aikuisten ohella tehokkaimpia kirvojen saalistajia. Ensimmäisen ja toisen asteen toukat eivät kokonsa puolesta voi syödä kolmannen ja neljännen asteen kirvoja ja aikuisia kirvoja, mikä luonnollisesti vähentää niiden tehokkuutta. Viljelmien eliöstökartoitus osoitti, että kirvojen esiintyminen vaihtelee suuresti eri vuosina, esimerkiksi säästä riippuen. Myös leppäpirkkojen esiintymisessä havaittiin vaihtelua.

Tutkimuksella todettiin seitsenpistepirkon soveltuvuus kirvojen biologiseen torjuntaan. Lisätutkimuksia kuitenkin tarvitaan leppäpirkkojen käyttäytymisestä ja todellisesta vaikutuksesta kirvakantoihin viljelyolosuhteissa jotta voidaan selvittää, onko biologisen torjunnan käyttö tässä tapauksessa tarpeeksi tehokasta ja taloudellista.

Avainsanat Biologinen torjunta, leppäpirkko, mustaherukka.

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ABSTRACT

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Assessing the potential for seven spot ladybird (*Coccinella septempunctata*) to control the blackcurrant aphid (*Cryptomyzus galeopsidis*)

ABSTRACT

The purpose of this study was to discover the possibilities to use seven spot ladybird (*Coccinella septempunctata*) to control the blackcurrant aphid (*Cryptomyzus galeopsidis*). Different species of aphids are natural prey for seven spot ladybirds and they occur in the blackcurrant fields already. Though the aphids are not the worst blackcurrant pests they do cause harm regularly and sometimes even major crop losses. Biological control may present new possibilities as the use of chemical control becomes more difficult. The research was done in Scottish Crop Research Institute in summer 2010.

The main object was to study which of the ladybird instar was the most efficient aphid predator and which aphid instars they favour. Different predator – prey tests were used to discover the behaviour of ladybird when selecting prey and consuming it. Important part of the work was the field survey of the different insect species including the aphids and ladybirds occurring in the blackcurrant fields.

The results shows the third and fourth instar larvae are among the adults the most effective aphid predators. First and second instar larvae could not consume the third and fourth instar aphids and the adult aphids because they were too small to capture them. This naturally decreases their efficiency. The main result of the field survey was the amount of aphids occurring in the fields varies a lot in different seasons depending for example the weather. There was a little variation of the ladybird's number also.

This thesis conduct the use of ladybirds to control the aphids is entirely possible. However more practical experiments are needed to discover ladybird's behaviour and actual effect on the aphid population in the blackcurrant fields to find out whether this biological control is effective enough to economical point of view.

Keywords Biological control, ladybird, blackcurrant.

Pages 32 p + appendices 1 p.

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APPENDIX 2/2 Ladybirds and aphids observed in different blackcurrant cultivars in 2010 (pcs)

1 INTRODUCTION

Blackcurrant has been an important and widely cultivated crop in Europe for hundreds of years. The blackcurrant aphid (*Cryptomyzus galeopsidis*) transmits at least two viruses and large populations can weaken the growth leading to crop losses. Pesticide restrictions limits their use and newer blackcurrant cultivars are planted more densely restricting the pesticide access to the plant providing pest refuges. Also general opinion of the public favours biological control.

The first time ladybird has been known to use as a biological control agent was in 1874 in New Zealand but the first successful use was in 1889 when Australian ladybird (*Rodolia cardinalis*) was used to control a scale insect (*kerya purchasi*) on citrus fruits in California, USA. This event was so successful it forms for the first time a great general interest for the possibilities of the biological control.

In history there is also examples of the introduction of new predators, which has seriously affect the abundance of natural endemic predators and reduce their number to even extinction. Therefore it is important to study if the predator is specific enough to the target insect and don't attack non-target insects or compete with the natural enemies of the target insects. Failure of these may cause side effects worse than the use of a pesticide would have caused.

Biological control has been successfully used for many years in the greenhouses to control multiple species of pest insects such as aphids. There is still less information and experiments of the use of biological control in the open field conditions but for example trips predator (*Amblyseius cucumeris)* have been used against the strawberry mite (*Phytonemus pallidus*) in strawberry fields.

The seven-spotted ladybird (*Coccinella septempunctata*) is a generalist predator of aphids, which naturally occur in Europe. This thesis studied if it could be used extensively to control the aphids on the blackcurrant and which of the four instars and the adult is more effective. Three different test sets were done to record the time of searching and consuming the aphid took and to study ladybird's behaviour of choosing the prey. Aphids weight was recorded to gain information of the aphid mass ladybirds may consume. Field survey was also done to discover the species naturally occur in blackcurrant fields.

2 BLACKCURRANT, APHIDS AND LADYBIRDS

2.1 Blackcurrant

Blackcurrant (*Ribes nigrum*) belongs to the genus *Ribes* generally considered to be the only genus in the family of *Grossulariaceae*. Genus *Ribes* consist about 150 species of small shrubs such as redcurrant and gooseberries. Blackcurrant is a perennial plant and it grows about one to 1,5 meter tall and two meter wide. The leaves are five to ten centimetres long and broad, the flowers are four to six millimetres diameter, with five reddish- or brownish-green petals. Flowers are produced in racemes, which are five to ten centimetres long. Berries are six to twelve centimetres diameter, deep purple to black in colour and with glossy surface. Blackcurrant favours moist, fertile and slightly acidic soil and sunny sites. (Matala 1999.)

Figure 1. Blackcurrant field block (SCRI)

Blackcurrants are founded and cultivated mainly in northern temperate regions of Europe and North America. It has been domestic crop in Northern Europe for last 400 years. Blackcurrants flower in UK usually in April or May and the length of flowering is about 3 to 4 weeks, depending on location, climates conditions and cultivar. Insects mostly pollinate blackcurrant, although self-pollination can also occur in some cultivars in certain weather conditions. (Brennan 1996.)

In southern Finland blackcurrant starts to flower usually in May and the flowering took about two to 2,5 weeks. In northern Finland flowering took place usually in first weeks of June. (Matala 1999.)

In Finland there is two blackcurrant cultivars commonly used in blackcurrant production. Öjebyn is widely used because it is resistant to Powdery Mildew (*Podosphaera mors-uvae*) and quite resistant to Blackcurrant Leaf Spot (*Mycosphaerella ribis*). It is also frost tolerance. Mortti (Öjebyn x Wellington XXX 1989) is also resistant to Powdery Mildew and quite resistant to Leaf Spot. It is frost tolerance though not as good as Öjebyn. It grows more erect so it is a better variety for machine harvesting than the Öjebyn. (Matala 1999.)

The main blackcurrant cultivar in Scotland is Ben Hope due to tolerance of gall mite (*Cecidophyopsis ribis*). Both Ben Gairn and Ben Tirran are used for extend the season. Ben Gairn is early cropping and Ben Tirran late. Some newer cultivars, which might become popular are for example Ben Dorain due to its high yields and high Brix levels and Ben Starav which is more adaptable in various environments, very high yielding and high colour cultivar. (Brennan, e-mail 10.12.2010.)

2.1.1 Cultivation

Generally in the Western Europe cultivars show erect growth habit where as in the Scandinavia cultivars more often have spreading habit, which is according to Matala (1999) due to their original growing habitat in windy places such as archipelagos. In both areas crop is almost entirely machine harvested and thus the compact and erect growing bushes with easy accessible berries have become chief objective for cultivation. Other objectivities in cultivation are obviously larger crops and due to those stronger but flexible branches. Also the pest and disease resistance maintain it significance in the cultivation. (Brennan, 2008.)

2.1.2 Distribution and crop statistics

Most of the crops of blackcurrant come from northern Europe. The biggest producers are Poland, Russia, United Kingdom and Scandinavia. There is less blackcurrant production in North America because blackcurrant is a secondary host of white pine blister rust (*Cronartium ribicola*). The crop area has decreased in UK and in Finland, as the Table 1 show.

Table 1. Blackcurrant crop area (ha)

	2005	2006	2007	2008
UK	2952	2670	2596	2553
Scotland	420	400	360	270
Finland	2113	2010	1951	1887

In the UK the major outlet in commercial production is for processing but the fresh market is also growing business. Most of the crop in commercial production is used for juice production. Crop is also used in jams, jellies, liqueurs, yoghurt and other dairy products and for conversion of white wines to rosé wines. In the UK the crop is harvested almost entirely mechanically and machine harvesting is increased also other countries producing blackcurrant. (Brennan 2008.)

According to Matala (1999) in Finland the crop is used mostly on commercial production same way as in UK although the fresh market is a bit stronger.

2.2 Aphids

The blackcurrant aphid (*Cryptomyzus galeopsidis*) is whitish on colour and fragile in appearance. They occurs the underside of mature leaves and they do not usually cause any deformation to leaves. However, sometimes the population crows so large in June that the foliage and fruits become contaminated by honeydew. Blackcurrant aphid is also transmitting the cucumber mosaic virus in blackcurrant and red currant. There are three forms of blackcurrant aphids. One lives in blackcurrant until in early summer when it moves to hemp nettle (*Galeopsis tetrahit*). Other one lives on blackcurrant through the entire year and the third one occurs throughout the year on red and white currants also. (Keep & Briggs 1971.)

Figure 2. Blackcurrant aphids (SCRI)

2.2.1 Defensive mechanisms

General opinion is that aphids are helpless and easy prey but several studies show that they do have some defensive mechanisms. First are the movements of the body. Aphids can kick their legs and try to pull free from the predator. They may also ”wax” the predator and just simply walk away or drop off to the plant while the ladybird cleans itself. Size also matters, if the aphid is large compared to the predator, the success of capturing aphid is lower. Aphid may also escape if their predator is a small larva which first reaction is fright response and attacks only after some time. Ants may also protect some aphid species against the predators. (Keep & Briggs 1971.)

2.2.2 Damage and managing to the blackcurrant

Large aphid populations can stop and at least weaken plant growth. Aphids can transmit veinbanding virus, which can infect also in redcurrant and gooseberry. (Keep & Briggs 1971.)

According to Dr Carolyn Mitchel (interview 22.06.2010) some newer cultivars such as Ben Connan are more resistant to the aphids than the others, for example Ben Gairn. The usual method of destroying the aphids is the use of pesticides. However the regulations of the pesticides are changing and more and more pesticides get withdrawn on the market. Pesticides do not also effect as well as before because the new blackcurrant cultivars are planted denser thus restricting the pesticides access to inside the bushes and providing aphids a place to breed. Also the general opinion of the public favours more environmental safe methods and demands residue free fruits. According to Cross (1985) minimum interval between pesticide application and harvesting may be as long as six weeks. (Johnson, e-mail, 24.05.2010.)

2.3 Ladybirds

Seven spot ladybird (*Coccinella septempunctata*) belongs to the family *Coccinellidae*, ladybirds that belong to the order *Coleoptera*, the beetles. There are nearly 6000 species of ladybirds in the world and most of them are predators preying other insects. Adult ladybirds have wings and they are oval shape formed and one millimetre to ten millimetres long. Some of the species are very bright coloured and females are usually larger than males. (Frank & Mizell 2000.)

According to Rotheray (1989) there are forty-two species of ladybirds in the Britain and most of them are aphid predators. *C. septempunctata* is aphidohagous species like most of the ladybirds in Europe are. It is also the most common ladybird in Europe. *C.septempunctata* lifecycle starts with cluster of eggs and after hatching follows four separate larval instars before pupate and metamorphoses into an adult ladybird. Adult *C.septempunctata* is about five to eight millimetres long. (Dixon 2000.)

Figure 3. Ladybird egg cluster (SCRI)

Adult *C. septempunctata* females produce of up to 40 yellow eggs. The eggs are in patches and often lay close to an aphid colony. The eggs hatch after two to three days and at the first new larvae feed on their eggshells and sometimes also any unhatched eggs. After few hours the larvae starts to search for prey. *C.septempunctata* has four larval stages, which can be identified on their different sizes. Length of fourth-stage larvae is about 11-17 mm. (Dixon 2000.)

Figure 4. Fourth instar larva (SCRI)

After growth is completed the larva attaches itself to the plant. Beneath the larval cuticle forms the rounded pupa, which is then shrugged off. The adults emerge one to two weeks later. At first they are pale yellow, but after few hours they start to develop their adult bright red coloration with seven spots. *C.septempunctata* may have two generations in a year with larvae present from May to September. (Dixon 2000.)

It is possible to breed several *C.septempunctata* generations in one year in the laboratory conditions under 18 hours of light and six hours of dark. This photoperiod will neglect the diapause for the majority of the females and they can produce at least nine generations in year without significant signs of degeneration. However the culture has to renew every summer by new individuals collected in the nature. (Hämäläinen, 1976.)

Figure 5. Ladybird pupa (SCRI)

Temperature has effect on the development time in different stages. The development is much faster in warmer temperature as the following diagram (Table 2) shows. (Hodek, 1973.)

Table 2. The affect of temperature in ladybird developing time

Temperature (°C)	Egg (days)	Larva (days)	Pupa (days)
15	10,3	35,5	15,0
20	5,0	18,6	8,4
25,6	2,6	8,7	4,0
30	1,9	6,7	2,9
35	1,8	5,4	2,5

Ladybirds spend the winter as adults. They often form large groups within overwintering sites such as under leaf litter or bark. Sometimes they enter to houses and spend the winter on window frames or in curtain folds. (Hodek 1973.)

2.3.1 Enemies and threats in Britain

According to Hodek (1973) there are several parasitoids that are associated with ladybirds. One of them is a wasp-like braconids (Order *Hymenoptera*) belonging to the genera *Perilitus* and *Dinocampus*.

There are also two groups of *Diptera*: small yellowish and greyish scuttle flies (*Phoridae*) in the genus Phalacrotophora that favours the pupae, and housefly-like tachinids, which have been occurred on adult ladybirds. (Majerrus & Kearns 1989).

One of the worst parasites of *C. septempunctata* is *Dinocampus coccinellae.* Female inserts a single egg inside the ladybird, which hatch usually after five to seven days. Larva feeds on ladybirds organs and after 18 to 27 days having passed four larval stages the parasite immobilizes the ladybird, burrows out and makes a cocoon between it's hosts legs. It takes six to nine days for parasite to pupate and hour after pupatation the *D.coccinellae* is ready to lay eggs. One *D.coccinellae* can lay 100 eggs during it lifetime so they form a significant threat to *C. septempunctata.* According to the studies made by Irene Geoghegan as many as 70% of the seven spot ladybirds in Dundee area are parasitized by *D. coccinellae.* (Bruce 2010.)

Harlequin ladybird (*Harmonia axyridis*) invasion to Britain is considered to be threat to the abundance of *C. septempunctata. H. axyridis* was introduced to Europe as a biological control agent and now it has become one of the fastest spreading insects in Europe. It develops faster than *C. septempunctata* because it does not need a dormant period in order to breed and it feeds later in the autumn than any other ladybird species occurring in Britain. It also feeds broader range than *C. septempunctata,* consuming for example ladybird eggs and larvae, moth and butterfly eggs, caterpillars and juice of the soft fruits if there are not enough aphids available. It may displace *C. septempunctata* in some amount in the future. (Roy, Brown & Poland 2010.)

2.3.2 Searching behaviour

C.septempunctata has some basic searching methods. They usually search to the edge of the leaves or raised plants parts. This method directs the ladybirds to the tops of plants and veins of leaves, which aphids also are more likely to found. After they have encountered the prey they start to search more slowly and thoroughly by turning a lot more. Because the aphids usually occur in colonies this behaviour gives ladybirds advantage to devour the entire colony. (Helle, Minks & HarreWijn, 1988.)

These searching methods however are inefficient in human terms, although they nature is adaptive. Searching behaviour follows very simple pattern and at the most of the time adult ladybirds seems to do nothing. It has been also noticed that the edge of the leaves may be searched over and over again while the leaf on nearby is infested with aphids. The searching behaviour of adults and larvae is different only in terms of movement and rates of capture. If the adults do not find prey soon, they simply fly away. Ladybirds do not capture and consume all the aphids that they contact. Some aphids are just moved from their feeding sites, or they move themselves disturbed by the ladybird. This causes aphids to spread new areas and also the spread of the plant viruses they may transmit. (Helle et al. 1988.)

It seems that the searching activity is dependent upon the level of hunger. After being fed with one first instar aphid larva *C.septempunctata* larvae, which were starved for 25 hours, searched the arena more thoroughly and slowly than the larvae, which were starved for only five hours. Generally younger larvae are less efficient than the older ones and fourth instar larvae are more efficient than adults. In every study the youngest aphids are the most desirable prey to all mobile stages of ladybirds. In the field, which has a lot of ladybirds, this may lead to a situation where the aphid population is consisted of more mature aphids than the younger ones. (Helle et al. 1988.)

2.3.3 Catching the prey

In order to capture and consume aphids *C. septempunctata* have powerfully built mandibles. The tooth at the tip of each mandible is bidentate. Near of the mandibles are the maxillae and maxillary palps, which are essential in prey recognition. Without them the efficiency of capturing the prey decreases approximately 40 %. The aphidophagous species such as *C. septempunctata* the maxillary palps are bigger and contain more sensory receptors than phytophagous species. This improves the ability to catch aphids, which are less easily caught than coccids let alone the plants. (Dixon 2000.)

Figure 6. Ladybird feeding on aphid (SCRI)

2.3.4 Feeding the ladybirds

The daily feeding rate in adult aphidophagous *Coccinelliadae* is usually amounts to about 100 aphids. A female particularly ovipositing, feed more than male. Also females do not start to lay eggs until the aphid biomass consumed is more than they metabolically require. (Helle et al.1988.)

Different studies show that the number of aphids consumed by *C. septempunctata* averagely per day can change from 92 up to 243, however most authors have not specified how the feeding rate is measured. Several hundreds of aphids (mostly 200-600) are consumed by larvae during their development. Often about 60-70% of the total food intake is consumed during the fourth instar. (Sundby 1996.)

Table 3. Proportion of food intake in individual larval stages at C.septempunctata

	1st instar (%)	2nd instar (%)	3rd instar (%)	4th instar (%)
20°C /8hrs light	4,2	13,3	25,5	57,0
20°C /12hrs light	5,3	13,4	24,2	57,1
20°C /16hrs light	4,1	10,4	31,7	53,5

Studies show the larvae of *C.septempunctata* complete their development even if their total food consumption is reduced to 55 to 40 per cent by limiting their supply in the fourth larval instar to 30 to ten aphids (*Aphis fabae*) per day. The larval period of *C.septempunctata* is also possible to complete on only seven aphids (*Myzus persicae*) per day. This feeding rate was very low, only one-third compared to the normal value. Almost all larvae were pupated, but the emergence of adults was low (only three out of eight). Also the emerged adults were smaller and produced fewer eggs. (Sundby 1996.)

When the larvae are hatched, they need to find food within 24 to 36 hours after which point most of the larvae dies. The larvae usually search prey actively for about 25 to 35 hours. They can move only short distances for 75 cm to three meters. Distances are reduced if the leaves are covered with honeydew or hair. The random search method also reduces their chances to find the prey, because they may search same places several times. Therefore is essential that there are aphids nearby the eggpatcet. The chances of survival and capture more aphids are increased after the first aphid is consumed. Also cannibalism on unhatched eggs improves the larvae chances of survival. (Hodek 1973.)

General impression is that the temperature has only small affect to the total larval food consumption of *Coccinellidae*. While the total larval food consumption seems to be similar at different constant temperatures, it was found to be doubled when larvae of *C. septempunctata* were kept under naturally fluctuating temperatures in summer. Naturally, the daily feeding rate both of larvae and adults increases with temperature within favourable range. (Hodek 1973.)

Food consumption seems to grow along the prey density, within certain limits. More aphids were killed at high aphid density by adult *C. septempunctata*. There seems to be two reasons for this. Hungry ladybirds consumed the first few prey completely but after that the aphids are consumed at decreasing efficiency leading to the growing number of partially consumed prey. Secondly, when there is a lot of prey available, the larvae also consume considerably more than what is required for their development. This ability to adjust to variable food resources is very important if predator is to be used in bio control agents. (Hodek 1973.)

First and second instar larvae will often just sucked out the body fluids of the aphid and reject the solid parts, such as legs, antennae, etc. Larger larvae and adults usually consumed the whole aphid with few exceptions. If the aphid is very large or if the aphid population is dense the ladybird may just sucked the fluids. (Hodek 1973.)

3 MATERIALS AND METHODS

The experiments were done in Scottish Crop Research Institute in Dundee United Kingdom. The main tests were done in August 2010 but test preparations started in May 2010.

Figure 7. Scottish Crop Research Institute from the air (SCRI)

In order to carry out the experiments there need to have a lot of different stages of ladybirds and aphids. *C. septempunctata* adults were collected from natural populations on the blackcurrant field sites around SCRI in May of 2010. Best time to collect the ladybirds was on sunny afternoons when the ladybirds were at the top leaves, inactive and clearly visible.

Figure 8. Capturing the ladybirds

When collecting the ladybirds in the field they have to check if they were parasitized because carrying a parasite changes the way ladybirds behave. There were several parasitized ladybirds among the ones I collected. They were easy to recognize after a few days and move away from the others. The *C. galeopsidis* were collected on blackcurrant cultivars, such as Ben Cairn, Ben Hope, Big Ben and Ben Connan in June of 2010.

3.1 Breeding the ladybirds

Total of 54 *C.septempunctata* were collected and placed on a Petri dishes four to five ladybird per one dish with pieces of leaf. The dishes were cleaned every day and leaf replaced to a new one. Ladybirds were fed about ten to 18 aphids per day. After two weeks this method was proved not very successful for breeding ladybirds. The amount of ladybirds was reduced to 32 and no eggs were laid. There was clearly a need of a bigger place and more food.

Figure 9. Breeding ladybirds in Petri dishes

Ladybirds were then divided to five boxes. There were two kind of boxes, two boxes measurements were 20 cm x 14 cm x 10 cm and other three boxes were 22 cm x 15,5 cm x 7 cm. Several blackcurrant leaves were placed in the boxes and the amount of aphids per day was raised for 100 to 150. Boxes were cleaned and new leaves changed every day.

Figure 10. Breeding ladybirds in the box

After a few days first eggs were laid. At first, the number of eggs in patches was small only ten to 15. After two weeks of increased supply of food the numbers of eggs in the patches rise for 40 to 60. Eggs were collected in the boxes as soon as they were noticed to avoid cannibalism. Eggs were placed on a Petri dish either separately or whole cluster with leave if they were laid on a leave.

Larvae were hatched after a few days and were transformed to a new dish, at ten to 20 larvae together. Amount of food was increased when the larvae grow and the number of larvae in a same dish was reduced gradually until there was only single fourth instar larva per dish. Ladybirds were fed in different species of aphids depending of their availability. Most often the aphid used on feeding was the pea aphid (*Acyrthosiphon pisum*) but green peach aphid (*Myzys persicae*), cereal aphid (*Sitobion avenae*) blackcurrant aphid (*Cryptomyzus galeopsidis*) and potato aphid (*Macrosiphum euphorbiae*) was used also.

3.2 Breeding the aphids

As stated before, pea aphids were the primary food for the ladybirds. They were placed in two pea plants, which were inside the plastic box. Aphid population expand rapidly and plants were changed when the aphids had destroyed them. These aphids were used to feed the growing population of ladybirds. There were several occasions when the pea aphids simply run out. Fortunately there were other projects involving aphids and those aphids could also used to feed the ladybirds.

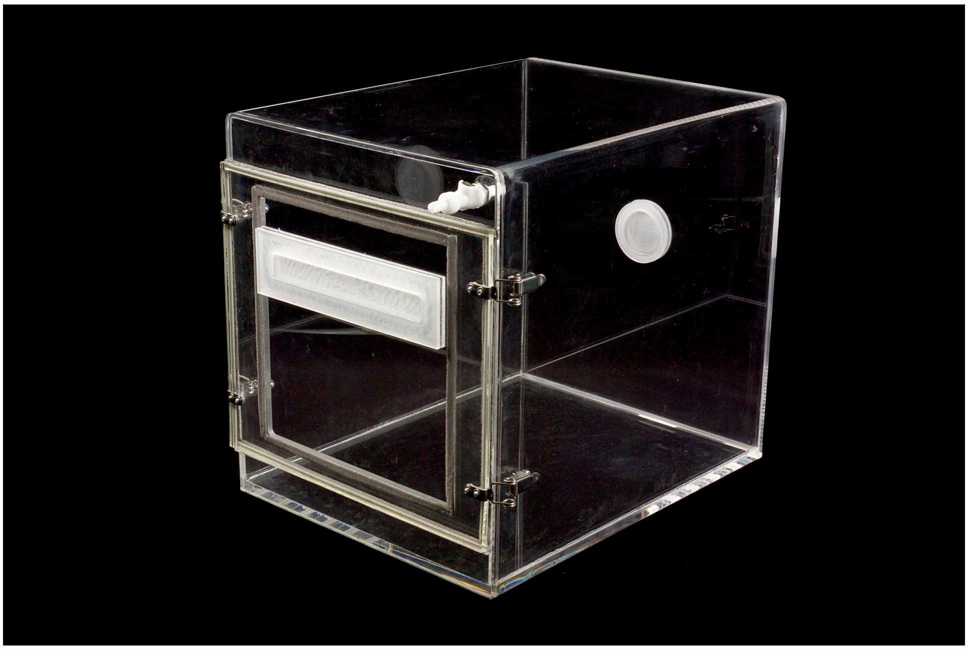

Figure 11. Box used to breed the pea aphids (SCRI)

Blackcurrant aphids used on the tests were breed in double dish. Blackcurrant stem were placed inside the dish providing food and growing habitat. Dish diameter was 7,3 cm and height 13 cm. Dish was sealed in a net cover, which allows the ventilation. First breeding experiments were not very successful possibly for using the leaves of Ben Connan cultivar, which is supposed to have resistance to aphids. After a few weeks blackcurrant stem was replaced to Ben Cairn cultivar, which is more susceptible to the aphids. This proved out to be the right solution since the aphids start to reproduce rapidly. There were eight to ten cultivation in operation all the time and during the tests at least one cultivation was usually renewed every day to guarantee steady supply of the aphids and to prevent creation of mould in the dish.

Figure 12. Dish used on breeding the blackcurrant aphids (SCRI)

3.3 General methods of the experiments

Since the hunger is known to affect on ladybirds voracity all experiments were carried out using the same feeding method. Every individual have plenty of aphids day before the experiments until it was 16 hours left to the experiment. At that point all the aphids were removed from the dish. This was done to rule out any differences of the behaviour caused by hunger.

Ladybirds were transferred to the experimental arena very gently to avoid any stress that might affect on their behaviour. According to G. E. Rotheray (1989) ”disturbed predators will try to escape rather than feed or search for prey. Signs of disturbance are animals which move to the top or sides of the cage or hide underneath leaves and remain still”. Transferring the larvae was easy and no sign of stress were observed. The adults were much more difficult to handle because of their mobility and two experiments had to be done again due to obvious signs of stress behaviour.

Every individual was used only once in same test. This was to eliminate any experience individual might have gained and could affect on it performance. Also everyone was handled in a same manner so any variation could not alter the result.

3.4 Experiment 1: Predator preferences for different aphid stages

Fifty blackcurrant aphids (*Cryptomyzus galeopsidis*) were placed in to arena which diameter were 18,5 cm and height 2,5 cm. Aphids consist of ten of each larval instars and ten apterous adults. After a single first-instar *C.septempunctata* was then placed in the centre of arena and arena were closed on a glass cover. After one hour the ladybird was removed from the arena and the arena were placed in the fridge for half an hour in order to slow down the aphids movements. The remaining aphids were then counted to find out how many and which aphid stages had been predated. Each bioassay was then repeated for second, third and fourth instars and adult *C.septempunctata* in ten arenas each.

Figure 13. Experiment 1. Predator preferences for different aphid stages

3.5 Experiment 2: Activity test

A single first-instar aphid larva were placed to a small Petri dish which diameter was 8,7 cm and height 1,6 cm. After the aphid a single first instar *C.septempunctata* were introduced in to the same dish. After that the time were taken how long it takes ladybird to locate the aphid. This was then repeated for each ladybird larval instar and adult for each of the aphid development stages and adult in ten dishes for each.

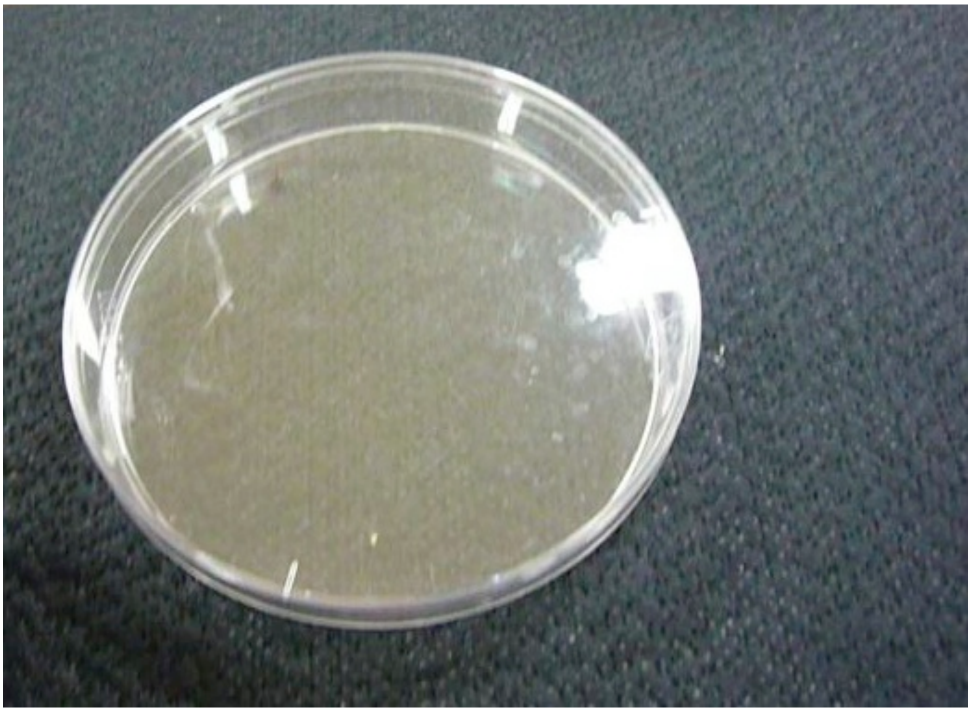

Figure 14. Experiment 2. Activity test.

3.6 Experiment 3: Handling time

A single first instar aphid larva was placed to a similar Petri dish used in a previous experiment. After the aphid a single adult *C.septempunctata* was introduced in to a same dish and time was taken how long it takes to lady-bird to consume the aphid. This was then repeated for second, third and fourth instars and adult aphid in ten dishes each.

3.7 Aphid mass

Ten of each *C.galeopsidis* instars and ten adults were placed in small plas-tic tubes and then stored in the freezer for several hours. After that the aphids were freeze-dried. Freeze drying works when the material is frozen, the pressure surrounding the material is reduced and the heat is added only enough to allow the frozen water in the material to sublime directly to the air. After drying the aphids were weight individually to the microbalance and their mass was recorded.

3.8 Field survey

Aim of the survey was to collect information of the species and quantities that exist in blackcurrants. Survey was done in the summers of 2009 and 2010 in four field blocks around SCRI. In 2009 there were four cultivars, that where recorded, Big Ben, Ben Gairn, Ben Alder and Ben Hope. In

2010 there were five cultivars, Big Ben, Ben Gairn, Ben Alder, Ben Hope and Ben Connan. All of these varieties are produced in SCRI. For each field block at each cultivar six plants were selected randomly on each occasion. Survey was done at same day and same time every second week, if the weather was suitable.

Figure 15. Collecting invertebrates on the tray. (SCRI)

Insect collection was done in pairs, white beating trays were placed on both sides of the plant, which then were shake for 30 seconds. Every invertebrate were then collected on the tray and stored into plastic vial containing alcohol. After collection was done the species were analyzed and results recorded.

4 RESULTS

4.1 Aphid mass

Aphid mass was recorded at end of the tests, but will be presented first (Figure 16) because it will provide information further needed on the experiments results presentation.

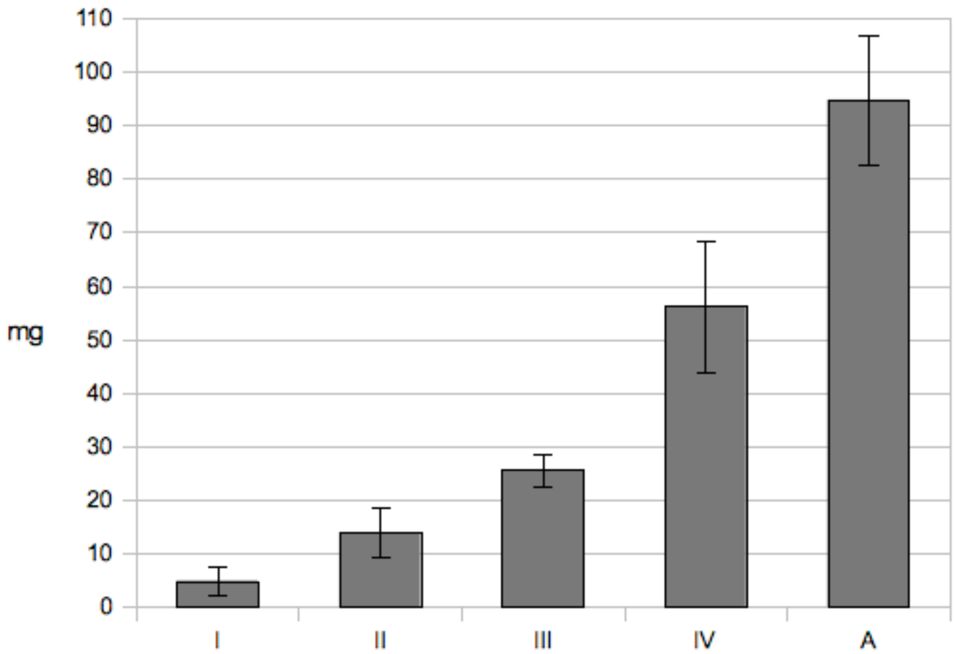

Figure 16. Mean weight of the different stage aphids (I = 1st instar aphid, II = 2nd instar aphid, III = 3rd instar aphid, IV = 4th instar aphid and A = adult aphid)

4.2 Experiment 1: Predator preferences for different aphid stages

The following diagram (Figure 17) shows the first and second instar larvae will not be able to eat fourth instar and adult aphids. They may try to but cant capture them long enough to actually eat them.

Total mean number of the aphids eaten is for first instar larvae 6,3, second instar larvae 5,4 and the third 7,9. The fourth instar larva was most effective predator with 22,5 aphids. They favour second, third and fourth instar aphid larvae but only by little, as do the third instar and adult ladybirds. The previous studies have shown that given the choice of freedom all ladybird stages including adult tend to eat younger stages of aphids. In this test results were different, because only first, second and third instar larvae favours the younger stages of aphids.

The adults were a complicated group. Their total mean number was 14,6 but there was a lot more variation between the individuals than the larval stages. Some of the tested individuals were as effective as the fourth instar larvae but some of them eat hardly any aphids. The best individual ate 39 aphids and the worst only one. As stated before, adult ladybirds were much more difficult to handle without they get stressed, which can alter their behaviour to run away or to freeze. At least four individuals were obviously stressed consuming only few aphids. If these individuals are ruled out from the results, the adult ladybirds become as effective as the fourth instar larvae with the total mean number of 22,7 aphids eaten. This would also cause error bars to be smaller on the adult part. Error bars are quite high and probably due to dealing with live individuals, some specimen behaving differently than the other.

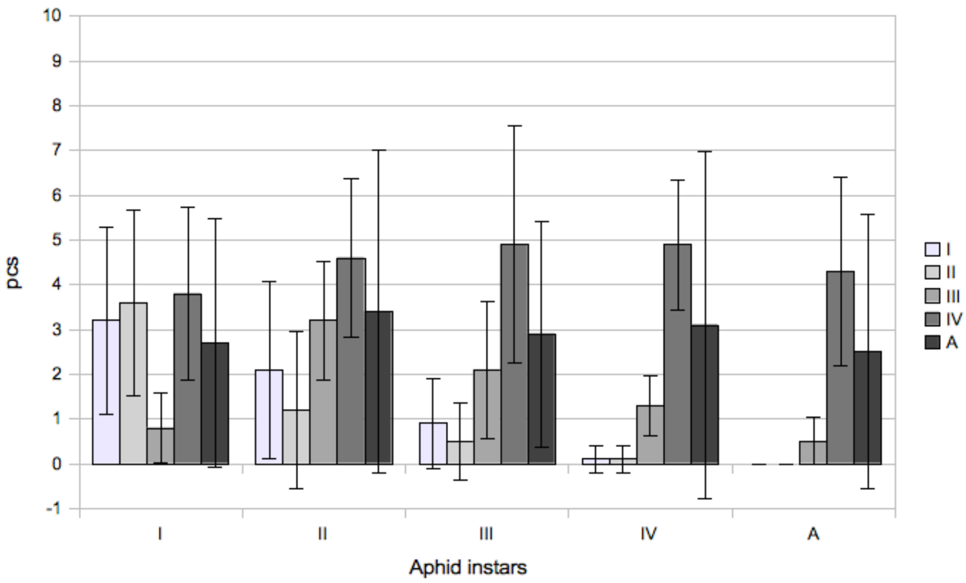

Figure 17. The amount of different aphid instars fed by different ladybird instars. (I = 1st instar, II = 2nd instar, III = 3rd instar, IV = 4th instar and A = adult ladybird.)

Following diagram (Figure 18) is a combination of the aphid mass test and experiment 1. It shows even more clearly the fourth instar supremacy compared to the other instars. Total mean mass eaten by fourth instar is over two times more than first, second, and third instar together.

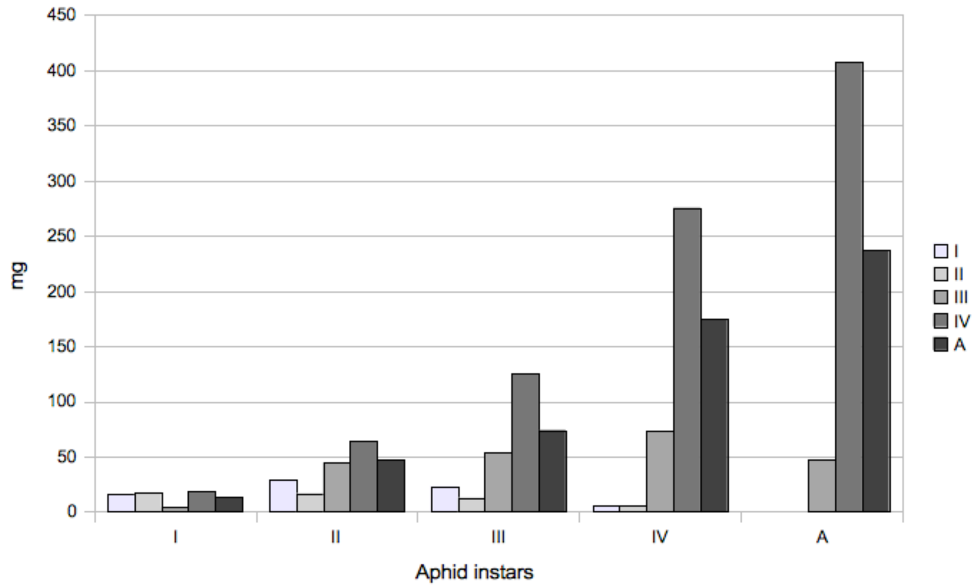

Figure 18. The amount of different aphid instars mass fed by different ladybird instars. (I = 1st instar, II = 2nd instar, III = 3rd instar, IV = 4th instar and A = adult ladybird.)

4.3 Experiment 2: Activity test

The diagrams in the Figure 19 have two values, mean time, which is the average of the times of the ten ladybird larvae and the adult ladybirds excluding the ones that did not found their aphid in one hour. The number of ladybirds did not found their prey in one hour is marked on a point.

The fourth instar larvae were most active founding nearly all aphids in given time save for a few first instar aphids. Their behaviour was also significantly different than others. They were searching and moving around the dish constantly. As stated before on the text, over 50% of the total food intake is consumed during the 4th instar. This could explain their activity.

First and second instar larvae have disadvantage of their smaller size compared to the other instars and adult. They have to move more to cover the same area than the others on same time. Of course this will effect on their result but it will be the same in the nature also. They could not search the same area in a same time as to the older stages.

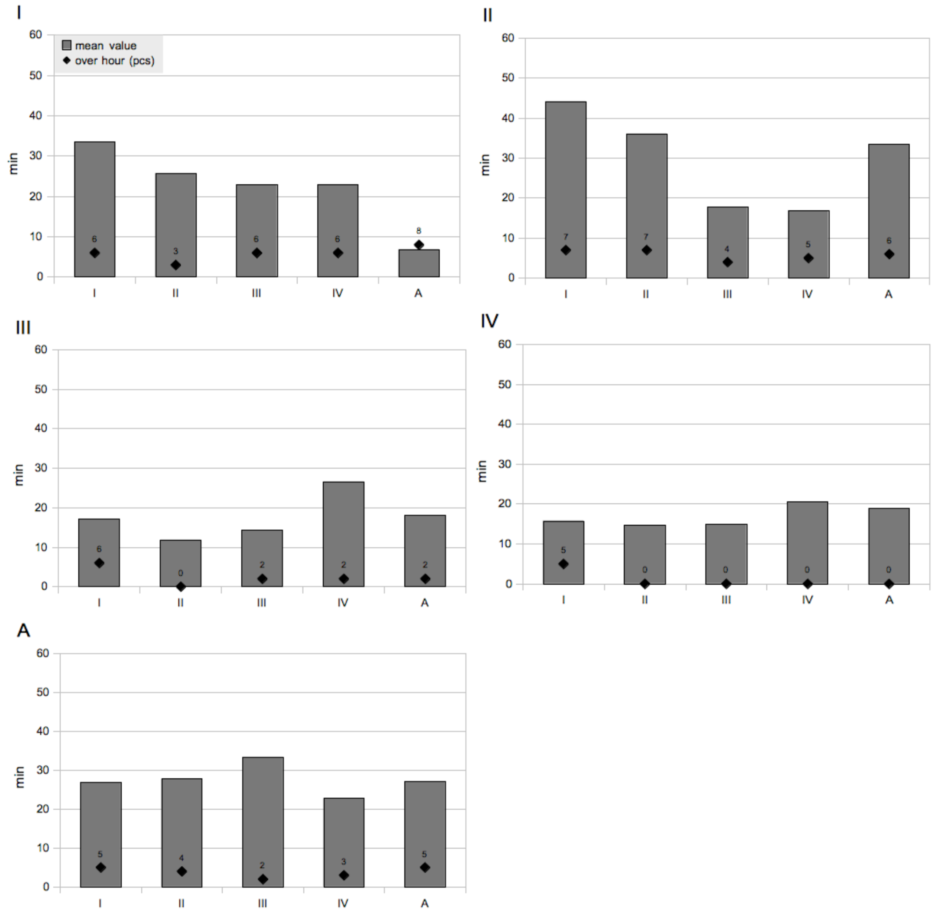

Figure 19. Mean time the ladybird found the aphid. Points marked how many did not found the aphid on time. I = 1st instar, II = 2nd instar, III = 3rd instar, IV = 4th instar and A = adult aphid. (Marks on the x-axis: I = 1st instar, II = 2nd instar, III = 3rd instar, IV = 4th instar and A = adult ladybird.)

4.4 Experiment 3: Handling time

Adult ladybird consume the first and second instar larva under ten seconds where as the adult aphids took over two minutes to consume (Figure 20). Therefore wheatear it measured by number of pieces or in weight it is more effective to adult ladybird to eat large amounts of small aphid instars than larger and older instars.

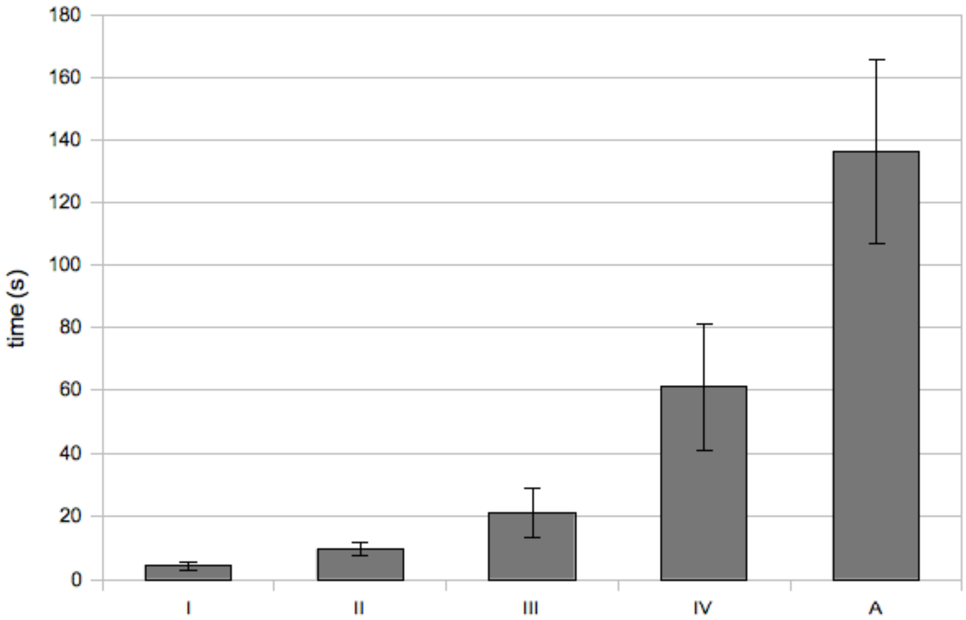

Figure 20. Mean time adult ladybird consume aphid. (I = 1st instar, II = 2nd instar, III = 3rd instar, IV = 4th instar and A = adult aphid.)

The following diagram (Figure 21) presents the amount of aphids and the consumed aphids mass that ladybird could theoretically eat in one hour. The actual amount and number is of course smaller, because it took some time the ladybird to search and locate the aphid.

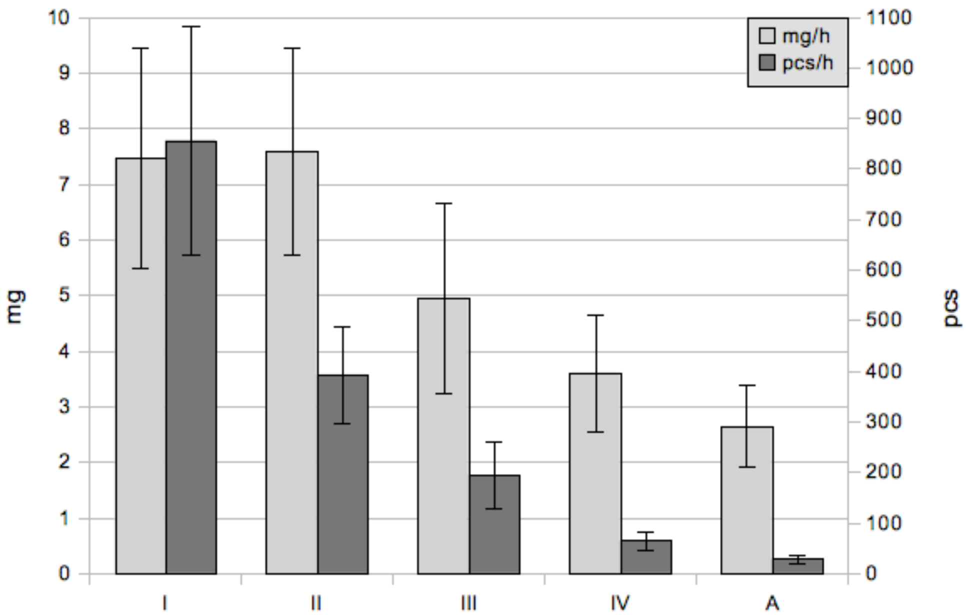

Figure 21. Mean theoretical number and mass of aphids adult ladybird could eat in one hour. (I = 1st instar, II = 2nd instar, III = 3rd instar, IV = 4th instar and A = adult aphid.)

4.5 Field survey

There was significant difference in aphids number in years 2009 (Figure 22) and 2010 (Figure 23), more than normally occurs. One reason for this might have been the unusually cold and snowy winter. This may cause the aphids late appearance and low numbers. The aphid species observed in the survey have also other host plants than blackcurrant which they might have preferred in summer of 2010 because the season was shorter. Out of this survey the season 2010 was rather good year for some aphid species, there were record numbers of them at the end of the summer.

Last season there was no physical damage on blackcurrant caused by aphids. Only couple of the bushes have a few curled leaves in their growing tips. These were noticed during the collection of aphids, not during the field survey. In season 2009 there was some physical damage mainly on Ben Gairn variety. According to Carolyn Mitchell (Mitchel, e-mail 16.11.2010), some growing tips were quite distorted, leaves curled and there was some discolouration.

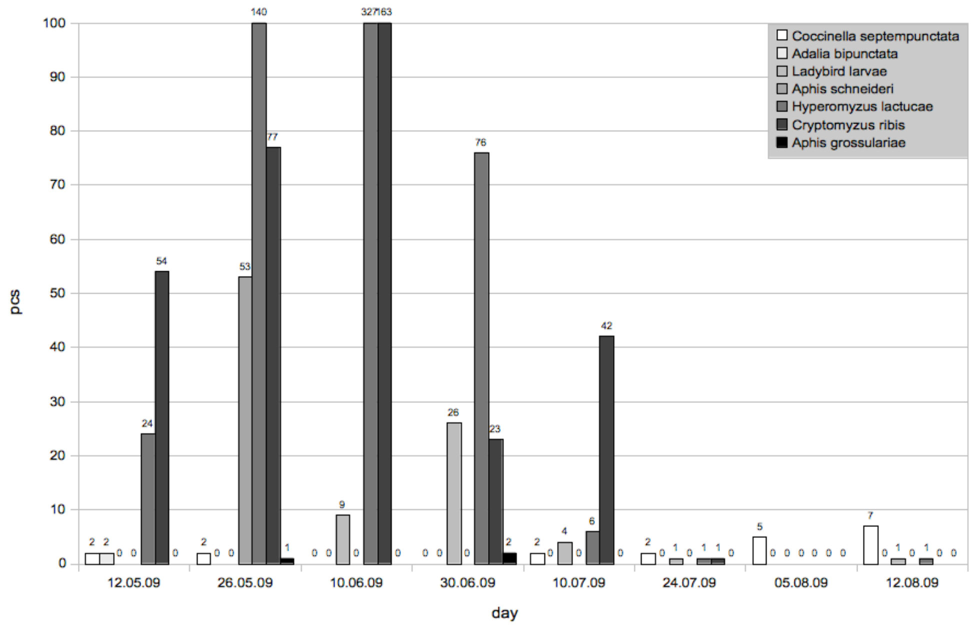

Figure 22. Number of the aphids and ladybirds observed in the fields on 2009

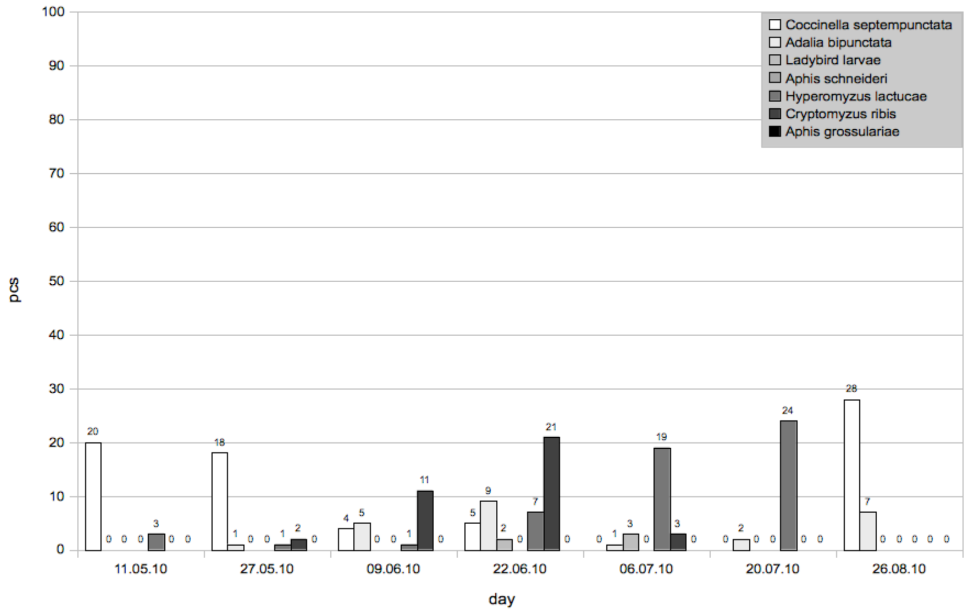

Figure 23. Number of the aphids and ladybirds observed in the fields on 2010

5 DISCUSSION

Lack of the time was a little problem during the experiment. Founding the right method to breed the ladybirds took more time than was expected. After a long and cold winter there were hardly any blackcurrant aphids on the fields and there were problems to collect enough aphids to breed them successfully. Breeding them was also difficult at first and took some additional time. When there was enough aphids to carry out the tests, ladybirds breeding season was almost over and the newly hatch ladybirds would need a diapause before able to breed.

Experiment 3, the handling test was not meant to done only with adult ladybirds but to repeat with all ladybird instars. Unfortunately this test was done at last and by that time there simply was not enough ladybird instars to complete the handling test. It was then completed only by newly hatched adult ladybirds, which were plenty. Even for this unfortunate event the other tests were done correctly and they give some image of the ladybird's possibilities in the biological control. It is also possible the other student of the SCRI might complete the handling test in near future.

The results clearly show that the *C. septempunctata* fourth instar larva is the most effective to search and consume any size of aphids. The adults are probably almost as good to consume the aphids given the variations in the tests but they seem to be a little less active to search them at least in close range.

Previous studies which have been referred in the literature part have shown the adult ladybirds will not search aphids very long in one plant if they do not find an aphid but move to another plant or even another field rather quickly. They also do not search thoroughly but might search on the same locations over and over again and not notice the aphids nearby. Use of adult ladybirds for prevent control on aphids might not work very well and would need further studying in the field conditions. However in the protected production, for example in raspberry production coverings would probably reduce this movement causing ladybird's better concentration on crop plants.

Use of larvae for prevent control is not likely to success very well because smaller instars could not move long before they have to eat and so they will need existing aphid population to survive. Third and fourth instars do not seek aphids for wide range but might even stay on only one plant. If there is not aphid to be found near they will starve. The time larvae could search aphids is relatively short for use of a biological control agent. Some form of an aphid bank might be a worth of experiment. They could provide food to the ladybirds while there are not enough aphids in the blackcurrants. Plants used for aphid banks could be planted for example in between the blackcurrant lines.

In the nature ladybirds lay their eggs on all ready existing aphid population. Due to their development, they are always little late on the growing aphid population which guarantees lots of prey to ladybird instars but causes also more damage to the plant. Existing aphid populations are maybe destroyed rapidly but only by third and fourth instars. First and second instar larvae might not have serious affect on the aphid population because they need relatively small amount of aphids for their development. Third and fourth instar larvae do consume a lot of aphids and they actively search them, but they do that only a relatively short period of time, before they pupated and stop consuming. Also small problem for the use of larvae to biological control is they have short dormancies between the instars developments, which takes some time and gives aphids chance to reproduce.

Aphid population have some variation in every year, some year there are hardly any aphids on the blackcurrant fields and the other year there might be masses of them. Since the ladybirds have to breed in advance the prediction of aphid's amount in coming season is important. However such predictions might be hard to make and since the aphids do not usually cause much damage to blackcurrant even in larger numbers one might question if it is profitable or even needed to use biological control in this occasion. Maybe a better solution to handle the problems caused by aphids would be to improve the aphid resistance on the blackcurrant cultivars.

It is still certainly possible to use *C. septempunctata* as a biological agent against the aphids in the blackcurrant. They do that in the field all ready in some scale and adding them in the field by breeding them is relatively easy though takes a lot of work. *C. septempunctata* can be bred in the laboratory through the year so it is possible to keep a large stock of ladybirds on reserve if they are needed to control the aphids. They adapt their eating with the prey density and eat a lot more aphids when there are plenty of them.

More study and testing is certainly needed to discover whether it is economical and effective enough to use *C. septempunctata* against the aphids and how, when, in what amount and in what stage of growth they should distributed in the fields to gain the best result.

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APPENDIX 1/2

LADYBIRDS AND APHIDS OBSERVED IN DIFFERENT BLACKCURRANT CULTIVARS IN 2009 (PCS)

	Big Ben	Ben Gairn	Ben Alder	Ben Hope
Coccinella septempunctata	5	7	4	3
Adalia bipunctata	0	0	0	0
Ladybird larvae	12	18	7	4
Aphis schneideri	53	0	0	0
Hyperomyzus lactucae	55	354	140	28
Cryptomyzus ribis	103	154	40	56
Aphis grossulariae	2	0	0	0

APPENDIX 2/2

LADYBIRDS AND APHIDS OBSERVED IN DIFFERENT BLACKCURRANT CULTIVARS IN 2010 (PCS)

	Big Ben	Ben Gairn	Ben Alder	Ben Hope	Ben Connan
Coccinella septempunctata	13	14	15	22	8
Adalia bipunctata	9	5	1	6	4
Ladybird larvae	2	2	0	0	1
Aphis schneideri	0	0	0	0	0
Hyperomyzus lactucae	8	0	7	11	29
Cryptomyzus ribis	6	6	1	11	13
Aphis grossulariae	0	0	0	0	0