PhD Thesis

MORPHOLOGICAL VARIATION IN CARNIVORES LADYBIRD BEETLES (COLEOPTERA: COCCINELLIDAE) AND THEIR HOST-PLANTS INTERACTION IN THE LOCAL ECOSYSTEM

By

SK. M. RAHAMAN

Abstract: From mid-May 2010 to mid-August 2013 coccinellid ladybird beetles were collected from five major localities: City center, Linnunlahti, Karsikko, Utra and Pilkko areas. In the family Coccinellidae, 16 species belonging to 10 genera were found in our experiment time. Most of ladybird's common host–plant was *Rosa rugosa* and also other different species of plants. All the host–plants were grown naturally in the local ecosystem of experimental area. Ladybird beetles abdomen consists 1st to 8th or 1st to 9th segments where is 1st, 2nd and 3rd segments are invisible. Only 4th to 8th in the male or 4th to 9th in the female ventral segments is clearly visible in the ladybird beetles abdomen. Sixteen experimental ladybird species first abdominal segment is well evaluated and significantly different, which was studied and resulted in the collection of total 365 specimens. Two-spots ladybird beetle (*Adalia bipunctata*) needs support from the plants species of legumes family *Phaseolus vulgaris* and *P. lunatus* for completing their life circle. This interaction serves the need in terms of food, shelter, host-plant suitability, and mutualism. This study researched the abdominal variation, interaction and different behavioural activity between the domestic ladybird and global plant species. Dualistic

eating habits and their behavioral activity with *Phaseolus* species plants are new finding in this research. Also, experimental data and results confirm that selected plant species are extremely important for *Adalia bipunctata* in context of their local ecosystems.

Key words: Coccinellidae, behaviour, ecosystem, interaction, legumes, mutualism, *Phaseolus vulgaris*, *Phaseolus lunatus*, morphology, mimicry, odors, ovipositor, segment.

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Chapter 1. INTRODUCTION

Ladybird beetles (Coleoptera: Coccinellidae) are insects commonly found across the globe and can be considered more or less worldwide in distribution. In the new world, humans appear directly or indirectly responsible for all or most ladybird invasions (Edward *et al.* 2011). They belong to family Coccinellidae which include insects living in warm habitats and regularly active on sunny days. Vandenberg (2000) reported that, about 6000 species of these beetles have been recorded worldwide while Linnaeus (1758) had described 36 beetle species from Europe from the genus Coccinella. In the old records Redtenbacher (1843) had divided ladybird beetles into two groups, one of which is aphidophagous and the other phytophagous. Almost 98% of ladybird beetles directly interaction with plants and they live in all terrestrial ecosystems like forest, tundra, grassland agroecosystems, and from the plains to mountains (Iperti 1999). Most of ladybird beetles are carnivorous and so few species of ladybird beetles are herbivorous.

Carnivores ladybird beetles larvae and adults feed on almost 39 species including aphids, scale insects, thrips, white–flies, leafhoppers, mealy–bugs, mites as well as other small soft-bodied species and their small eggs (Iperti and Paoletti, 1999; Gautam, 1989; Moreton, 1969). Shah (1985) recorded that, approximately 4200 species of coccinellids are considered beneficial because of their predatory activity, mainly against the homopterous insects (aphids and scale insects) and phytophagous mites, which are harmful to various forest and agricultural plants. Also, Abbas *et al.* (2013) reported that,

agricultural crops and their associated weeds comprising of a variety coccinellids species, which are better control agents of insect pests. There are many reasons for the growing popularity of the ladybird beetles for human beings. Most of the ladybird beetles possess bright colour patterns (Ullah, *et al.* 2012) and their black or other coloured spots are the main reasons for their attraction for people (Fig- 1). Therefore, my research study results are trying to establish the ladybird beetles morphological bodies shape and size; elytron; colour and spots; abdominal variation in ventral view; host-plants availability with selected host-plants interaction with two spots ladybird beetles for future research goal.

Figure 1. A ladybird beetles natural behavior in the host-plant.

1.1 Ladybird beetles local ecosystem

Ladybird beetles are briefly active in the Joensuu area between the middle of May and the middle of August, because of the short summer and warm sunny days. Some ladybird species are found at the end of August and until middle of September too (e.g. *Propylea quatuordecimpunctata* L.,) which depend on aphids for food or warm temperature. Adult ladybirds hibernate in the cold winters until the following summer. During the winter they shelter inside tree holes, natural hiding places (Majerus and Kearns, 1989), and under the tree barks, building corners, windows, or corners of verandas. Our research work was conducted in Joensuu city, located in the North–Karelia region. After 1975, no new record of morphology on the population of the ladybird beetle species and their host–plant varieties have been done earlier in these regions.

1.2 Host - Plants Interaction

One of the most important questions in biology and applied sciences is the relationship between insects and plants. These two groups of organisms are distributed on high scale in global ecosystem and their interactions have influence on surviving over generations in this ecosystem. Life of these organisms is related to their occurring in the same area and climatic conditions, and their developmental phases are crossed in similarity from the point of view of developmental success (Forister *et al*. 2012). The new developments in the evolution of ecological specialization are found more on genetic architecture of

insects and plants, and their multi-tropic interactions than only on simple genetic tradeoffs in last. However, ecological specialization is still fundamental concept characterized insect-plant interaction. Although insect-plant interactions are studied very actively during last two decades (1993-2013) and 5707 papers published with these key words are indicated by Web of Science, there is still a short knowledge on in vivo relationship, behaviour and possible communications between insects and plants. The relationship between ladybirds and plants is important and scientifically interesting work not only from the ecological and evolutionary points of view but also from the practical and economic significance. Some ladybirds are considered as a particularly useful insect in field productively practices (Banfield-Zanin *et al*. 2012). Ladybird-plants interactions are studied therefore actively also from this point of view.

There are 100 papers published in this subject, which is still to be scientifically challenged. Santos *et al.* (2012) studied coccinellid communities associated with olive, chestnut and almond crops in north-eastern Portugal and concluded that, Coccinelled species like *Coccinella septapunctata* L. and *Adalia decempunctata* L. can be used in divorcing pest management programmers that can encourage greater natural enemy biodiversity in agroecosystems. Moreover, the interactions between populations of insect species and plants make a problem of specialization and food preferring by ladybirds to be more complex and difficult to resolve. Especially in the cases of plants being not a part of the natural habitat for ladybirds this behavioral interaction is not known. In the case some of butterflies the colonization of exotic plants by herbivorous

insects is suggested to be effected on mate choice (Forister and Schall 2012). In ladybirds and legume behavioral interactions this problem is not known. Therefore, this study intends to broader knowledge on these areas by synthesizing new developments and collecting basic data and evidence from empirical experiments with ladybird and legume species. In the paper, the ladybird *Adalia bipunctata* L. and legumes are described and their formal interaction documented.

The synthesis of the data published before is provided for the experimental and theoretical understanding of the diverse relationship of two organisms. The choice of these organisms for the experiments was determined by previous studies suggested their importance (Schoonhoven 1999) and extending of these organisms in global scale (Kajita and Evans, 2010; Xue *et al*. 2012). Experiments were established with local populations of ladybirds and two beans known global. The following questions were addressed in our research: is there any real interaction between ladybird and beans and what kind of behavior of these organisms occurs and what is a reason of this behavior, what are the sources of their interaction between two kinds of organisms?

1.3 Morphological variations in abdomen

Ladybird beetles abdomen consists 1^{st} to 8^{th} or 1^{st} to 9th segments where is 1^{st}, 2^{nd} and 3^{rd} segments are invisible. Only 4^{th} to 8^{th} in the male or 4^{th} to 9^{th} in the female ventral segments is clearly visible in the ladybird beetles abdomen. Biranvand *et al.* (2015) said that, morphos of the ladybird beetles have different appearance, such as number, size,

color and pattern of spots on the elytra and pronotum; which can be effected for their abdomens or abdominal segments. According to Gordon (1985), Weise was apparently the first Coccinellid taxonomist to realize that male genitalia could be useful to distinguish species. The second half of the 20th Century was a 'Golden Age of Coccinellidae Taxonomy'. Their body is divided with three parts which call head, thorax, and abdomen.

The insect abdomen is the 3rd main segment of the body. Their abdomen is typically 11 segmented, where the eleventh segment is usually much reduced. Borror *et al.* (1981) described that the maximum number of insect abdominal segments rarely appears to be more than ten segments and of segments bear a pair of laterally located spiracles. A ladybird beetles body third part of the abdomen, which is segmented with eight to nine parts and last segment consists male or female sexual organ which are used for ovipositor. Seagraves (2009) described that, female ladybirds oviposition strategies which is turn determine the distribution of Coccinellid larvae in habits. Evans *et al.* (1986) identify in the host-plant aphids honeydew and odors are exposed Coccinellid ladybird oviposit very quickly.

Biology of ladybird beetle species that prey on armored scales (Hemiptera: Diaspididae) and feeding on soft scale insects (Drea and Gordon (1990); Ponsonby and Copland (1997) reviewed). Dixon's (2000) also described the ecology of predatory Coccinellidae and their interactions with various prey groups and discusses the consequences of

various ecological traits in ladybirds for their successful application in biological control. Darshana *et al.* (2013) described that, coccinellids ladybirds are important natural enemies of agricultural pests and it is important to know the diversity with some of the scientific evidence including morphometric specifications. The ladybird beetles morphological research study tries to stablish how abdominal segments are different with each other and how ladybirds abdomen shape and size are different? And also observing their abdominal segment having seasonal variation effect for oviposition and the role of the ladybirds abdomen for ecological behavior. The experimental data is discussed considering the theoretical syntheses and a set of reserved questions finding is presented to be immersing and useful for future research.

Chapter 2 MATERIAL AND METHODS

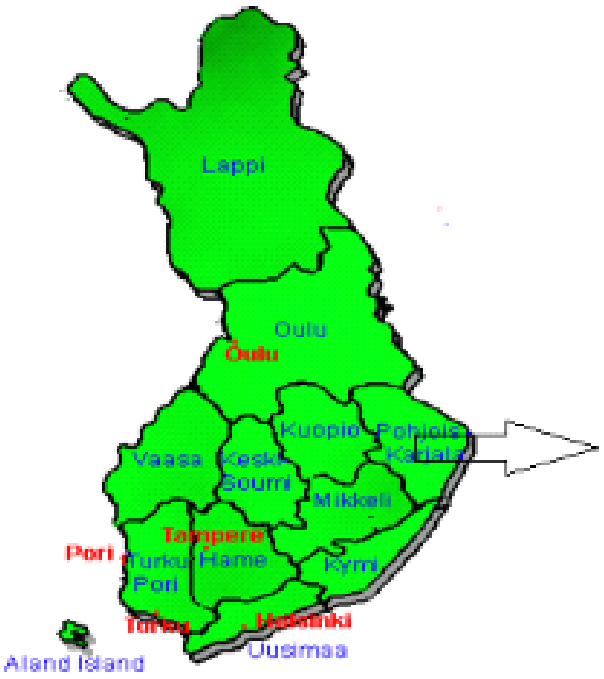
2.1 Field research study was conducted in the Joensuu city over the summer season in North Finland, which is located at 62º36ˈN and 029º45'E. The city covers almost 2,751.07 square kilometers in area which the land area is 2,381.76 square kilometers and the water area 369.31 is square kilometers. The River Pielisen flows through the city and many lakes are also present affording a suitable environment for the ladybirds. The city experiences extreme climates, with the temperature in summer reaching a maximum of (+35 ^{0}C) and a maximum winter temperature of (– 30 ^{0}C). Laboratory research was carried out at the Research and Teaching Laboratory of Applied Botany, Biological Interactions and Ecological Engineering of the Department of Biology, University of Eastern Finland during 2010 - 2015. Research plan was established after analyzing of old and new existing data published previously. Analysis of these data material was done and new experiment findings as well as synthesis of a main knowledge of the ladybird-legume relationship made. The data covered ladybird and legume species relationship was collected experimentally.

2.2 Before collecting ladybirds we have taken general preparation and collecting time was two–three days in a week. In this study period, we have collected 16 different species of ladybirds and total collection was 365 specimens. All ladybirds were caught in mosquito net, with hands and after collecting it was preserved proper way in the

laboratory. In the laboratory, we have done ladybird beetles abdomens anatomical experiment under the microscope. After experimental study or analysis of the ladybird beetles abdomens segmental variation, we stored their abdomens in the glass bottle for future experiment. A photograph was taken for documentary support with the high regulation camera CANON (ESO 500D and EOS 450D) and a microscopic camera Leica S8APO was used.

2.2.1. *Study areas of sample collections*

The study area was divided into five sampling regions: Joensuu city Centre, Linnunlahti, Karsikko, Utra and Pilkko areas for collection of the ladybird beetles (Fig- 2). Most of the collection was done three times in a week during sunny and warm weather where there was a possibility of finding the ladybird beetles. To collect the ladybird beetles we used the methods of hand–picking, sweeping hand nets, made with mosquito netting, glass bottles for the ladybirds thus collected, boots, sunglasses, plastic bags, cotton moistened with water, and cameras for natural photographs record the ladybird beetle activities. Samples were then collected three times in a week and each day involved three hours of collection time. Each specimen was properly labelled recording the date of collection, the place of collection and the specific host–plants.



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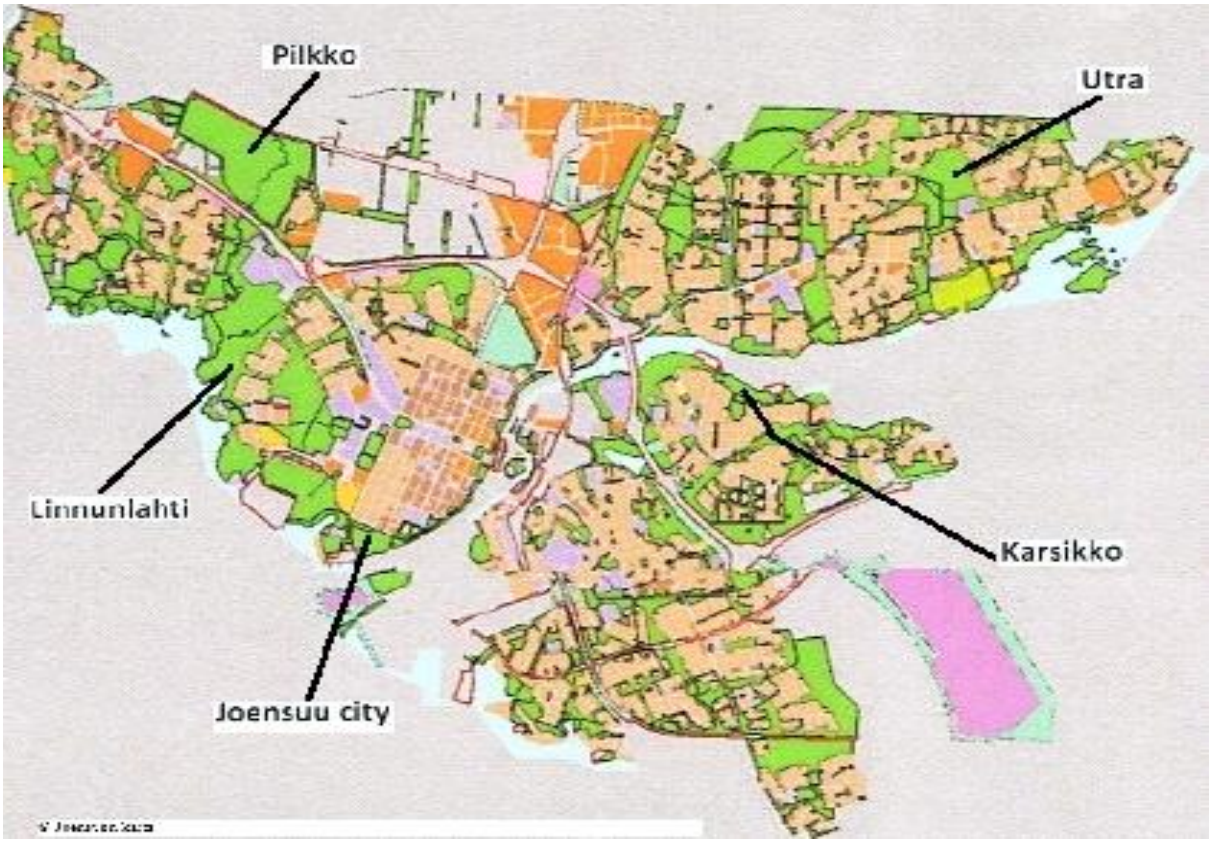

Figure 2. Ladybird beetles collection area in the Joensuu region.

2.2.2. *Ladybirds and plant materials*

Alive ladybirds were collected from area of Joensuu city by sweeping mosquito nets and storage in a glass bottle before using in the experiments. Each ladybird collected was morphologically studied and its taxon was identified. Only ladybird with abundant appearance (two-spotted ladybird, *Adalia bipunctata* L.) was used in the experiment. Ladybirds were trans-located back to their living host area after the experiment. Behavior of ladybirds was studied on two legume species (common bean, *Phaseolus vulgaris* L. and lima bean, *Phaseolus lunatus* L.) growing for this purpose. Seeds for the experiments were obtained from commercial market Oy Exotic Garden ab, Nämpnäs.

2.2.3. *Field experiments*

Field experiments were established 2010 in open sunny area of North-East part of the Botanical Garden (Botania) of the University of Eastern Finland covering the area of 16 m^2 dividing into four in size identical plots. The soil was tilled by the machine, taking out weeds out by hand and rolled for preparing the sowing. Twelve plants were showing on each plot and grow for six weeks, approx. 5-7 leaves at least. The growing of all plants was normal and typical. No damages or pathological phenomena of plants were observed before experimental contact with ladybirds. Experimental area was protected against rabbits and others animal by metal nets which height of 85 cm and diameter off holes 5.5 cm (Fig- 3).

Figure 3. *Phaseolus vulgaris* and *Phaseolus lunatus* in the field experiment established in Botania (botanical garden) with metal net and mosquito net.

2.2.4. *Laboratory experiments*

All the specimens were carefully handled, avoiding any kind of damage. The specimens collected were preserved in Petri dishes or glass vials and maintained in the Research and Teaching Laboratory of Applied Botany, Department of Biology, University of Eastern Finland. Laboratory experiments were established also by growing the plants (six individuals of each species studied) in the pots similarly as in field experiment. Plants were growing inside a big glass box illuminated and temperature of 21°C (Fig- 4). All the plants were growing well, and no pathological damage was observed before experimental contact with ladybirds. Newly collected ladybird beetles were stored in a glass bottle with legume plants leaves for the experiment in the laboratory. Young leaves, aphids and cotton with water were putted inside to glass bottle for ladybirds food (Fig- 5).

Figure 4. *Phaseolus vulgaris* and *Phaseolus lunatus* growing in the laboratory for behavioural experiments.

Figure 5. Feeding process are ladybirds for behavioural experiments.

2.2.5. *Measurements of plants and ladybirds*

The data collected were statistically analyzed using the Sigma Plot 11.0 statistical program as well as the general graph program. For photographs, we used the USB Microscope (20x–350x) Digital 'BRESSER' Germany; 'OLYMPUS' digital YYY Micro camera; Cannon 'DIGITAL IXUS' 8015 and for observation of the different parts of the ladybird beetles we used the laboratory microscope Carl Zeiss, Göttingen, Germany. The size of the ladybird beetles was measured with digital calipers 'RAWLINK' scale using the electronic reader with up to 0.00 mm accuracy. The specimens collected were identified referring to the latest literature available as well as the following books '*European Insect Guide'* by Chinery M. (1993); Biström *et al.,* (2001) journal and '*World Flowers*' by Polunin O and Polunin L. (1988).

All plants and ladybird were measured before interaction experiments. Plant heights were measured by the measure indicator. Leaves size and ladybird beetles were measured by the Dimitic with electronic reader with accuracy to 0.00 mm. Ladybird beetles were balanced by Scaltec with accuracy to 0.000 g. All parts of experimental ladybirds body (legs, wings, elytra, abdomen, antenna and pronotum) were taken photograph by the Zesis Stemi DV4 stereo microscope.

2.2.6. *Measurements of interactions*

Ladybirds were putted on plants especially covered by the mosquito net. Movement of ladybirds from base of the stem to top of the plants was measured by stopwatch. Other

behavior of ladybirds (resting, walking, flying, and trans-located from plant to mosquito net, changing places) was fixed. Searching and eating behavior (eating style, speed, and food selection) was measured by the appropriate equipment. Parts of plants and leaves vibration was measured when ladybirds were active. Moreover, plant locations of ladybird normal and different were fixed. Adult ladybirds were kept in the glass bottle with beans leaf and cotton-water as a food, and it was under 21^0C temperature in laboratory condition.

2.2.7. *Statistical analyses*

Data collected were statistically analyzed using Sigma Plot 11.0 statistical and graph program. Analysis of variance (ANOVA) was applied with (Shapiro-Wilk) One Way analysis of Variance was used. We used Descriptive statistics, experimental design, and multiple-comparison test with (Holm-Sidak method) for statistical analyses. For photograph data we used Canon (EOS 500D and EOS 450D) camera, microscope camera Leica S8APO and USB Microscope digital BRESSER.

Chapter 3 RESULTS

3.1 In total 365 specimens belonging to 10 genera of the Coccinellidae family, which represented 16 species of ladybird beetles were collected during the study period (Table-1). During the collection period several different ladybird species were found inhabiting different host–plants (Table-2). *Adalia bipunctata* and *Propylea quatuordecimpunctata* were collected from all the locations selected. *Adalia* was found to be most abundant genus (n=266) in the collection period. Genus *Propylea* was the second most abundant (n=42) ladybird beetle with a single species in the collection period. There were five species of ladybirds abundant from genus *Coccinellae* which were the third most abundant (n=20) species. On the other hand, eight genera was represented by a single species; *Anatis ocellata* (n=2), *Calvia quatuordecimguttata* (n=3), *Cryptolaemus montrouzieri* (n=7), *Hippodamia tredecimpunctata* (n=1), *Halyzia sedecimguttata* (n=7), *Psyllobora vigintiduopunctata* (n=12) and *Chilocorus stigma* [(n=1) new] as follows (Fig- 6). In the laboratory experiment, we found clear and theoretical evidence about the morphological character in each genus and species of the ladybird beetles collected (Table-3).

Table 1. Ladybird beetles (Coleoptera: Coccinellidae) found in the Joensuu fauna throughout sampling period.

Order	Family	Genus	Species	Total	Collecting months
Coleoptera	Coccinellidae	*Adalia*	*A. bipunctata* L.	266	May, June, July
		(melanic)	*A. sexpustulata* L.	3	June, July
		(melanic)	*A. quadrimaculata* L.	3	May, June, July
		Anatis	*A. ocellata* L.	2	June, July
		Coccinella	*C. septempunctata* L.	9	May, June, July
			C. trifasciatata L.	1	June
			C. undecimpunctata L.	6	June, July
			C. hieroglyphica L.	1	June
			C. quinquepunctata L.	3	June, July
		Calvia	*C. quatuordecimguttata* L.	3	June, July
		Cryptolaemus	*C. montrouzieri* L.	8	June, July, August
		Chilocorus	*C. stigma* L.	1	July, August
		Halyzia	*H. sedecimguttata* L.	7	June, July
		Hippodamia	*H. tredecimpunctata* L.	1	June, July
		Propylea	*P. quatuordecimpunctata* L.	42	May, June, July, August
		Psyllobora	*P. vigintiduopunctata* L.	11	June, July, August
		n=10	n=16	n=361	

Table 2. Observation of ladybird beetles and host–plants species in the Joensuu area.

Ladybirds	**Host–plants species**
Adalia bipunctata	*Rosa rugosa* L; *Salix caprea* L; *Salix triandra* L., *Phaseolus vulgaris* L.; *Phaseolus lunatus* L.
Adalia quadrimaculata	*Rosa rugosa* L
Adalia sexpustulata	*Rosa rugosa* L
Anatis ocellata	*Rosa rugosa* L
Coccinella septempunctata	*Rosa rugosa* L.; *Phaseolus vulgaris* L.; *Phaseolus lunatus* L.
Coccinella undecimpunctata	*Rosa rugosa* L
Coccinella quinquepunctata	*Rosa rugosa* L.; *Phaseolus lunatus* L
Coccinella trifasciatata	*Rosa rugosa* L
Calvia quatuordecimguttata	*Salix triandra* L; *Rosa rugosa* L.
Cryptolaemus montrouzieri	*Phaseolus vulgaris* L, *Stevia rebaudiana* L, *Amaranthus caudatus* L.
Hippodamia tredecimpunctata	*Solanum tuberosum* L
Halyzia sedecimguttata	*Rosa rugosa* L
Propylea quatuordecimpunctata	*Trollius europaeus* L; *Salix appendiculata* L.; *Rosa rugosa* L., *Vicia sepium* L. *Phaseolus vulgaris* L.; *Actaea erythrocarpa* L., *Tanacetum vulgare* L.
Psyllobora vigintiduopunctata	*Trollius europaeus* L; *Vicia sepium* L; *Clematis sibirica* L.
Chilocorus stigma	*Zea mays* L.

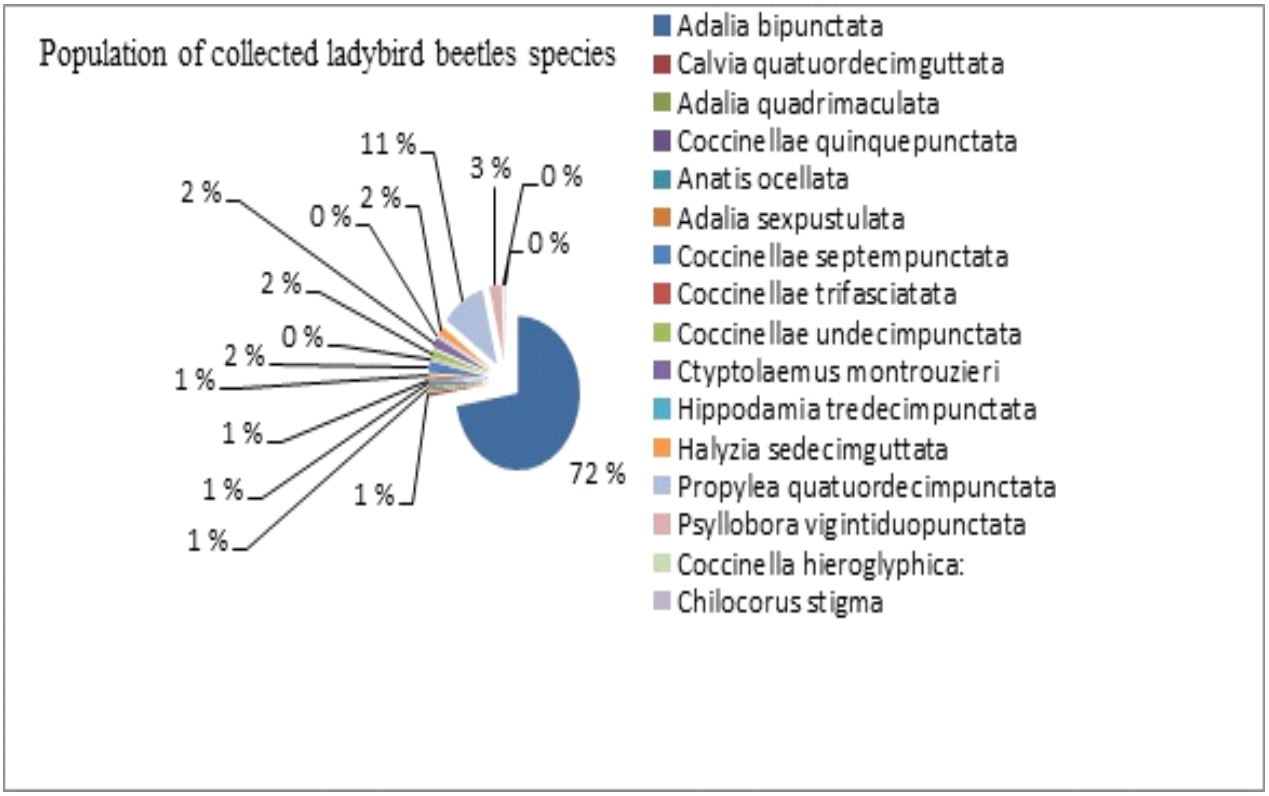

Figure 6. Total number of ladybirds during the period 2010 – 2015 in the Joensuu area.

Table 3. A brief glance of the morphological characteristics of the ladybird beetles collected.

Ladybird beetles	Elytra	Pronotum	Legs	Thorax	Abdomen	Spots	Scutellum
Anatis ocellata	Reddish brown	Dark black	Brownish black	Black	Black	Eight spots with eyed mark	Yes
Adalia bipunctata	Orange	Yellowish with black	Black	Black	Black	One black spot	No
Adalia quadrimaculata	Blackish red	Black	Dark brown	Dark brown	Dark brown	Four reddish spots	No
Adalia sexpustulata	Reddish black,	Black	Deep black	Deep black	Deep black	Six reddish spots	No
Coccinella hieroglyphica	Light orange	Dark brown, orange mark	Black	Black	Black	Three black spots	No
Coccinella septempunctata	Bright orange	Black with brown spot	Deep black	Deep black	Deep black	Seven black spots	Yes
Coccinella trifasciatata	Orange	Black with brown spot	Deep black	Deep black	Deep black	Six big bands	No
Coccinella undecimpunctata	Light yellow	Black with yellow line	Brown & black	Black	Black	Eleven black spots	Yes
Coccinella quinquepunctata	Red	Black with yellow mark	Deep black	Deep black	Deep black	Five black spots	Yes
Calvia quatuordecimguttata	Brownish, brown	Glossy with creamy spot	Brown	Black	Brownish black	Fourteen creamy spots	No
Cryptolaemus montrouzieri	Velvety black	Shining reddish	Brown	Black	Deep orange	Only reddish hairs	No
Hippodamia tredecimpunctata	Red, deep orange	Yellowish black, spot	Dark brown	Deep brown	Deep brown	Thirteen black spots	Yes
Halyzia sedecimguttata	Orange	Yellowish or orange	Orange	Yellow	Yellow	Sixteen creamy white spots	No
Propylea quantuordecimpunctata	Yellowish, orange	Pale yellow, black marks	Brown	Deep brown	Deep brown	Fourteen unmark black spots	No
Psyllobora vigintiduopunctata	Bright yellowish	Yellowish	Brownish	Glace black	Glace black	Twenty-two black spots	No
Chilocorus stigma	Shining black	Dark black	Black	Black	Reddish, yellowish	Two red spots	No

3.2 *Adalia bipunctata* is two-spots ladybird largely occurred and it is one of the sixteen different species found in Joensuu region. A total number of collected ladybird beetles, 93.3% of them were individuals belong to the species *Adalia bipunctata*. Other individual species were *Coccinella undecimpunctata, Coccinella septempunctata, Propylea quatuordecimpunctata, Hippodami tredecimpunctata, Coccinella quinquepunctata, Adalia sexpustulata, Adalia quadrimaculata, Calvia quatuordecimguttata, Anatis ocellate, Halyzia sedecimguttata, Cryptolaemus montrouzieri, Psyllobora vigintiduopuntata, Coccinella trifasciatata, Coccinella hieroglyphica* and *Chilocorus stigma* which remarkable smaller number was not used in the interaction experiments. The size and weight of *A. bipunctata* ladybird was measured individually.

Experimental ladybirds body length was in the field and laboratory from 3.56 mm to5.19 mm; wide from 1.20 mm to 2.16 mm. Same time used ladybirds weight was from 0.002 mg to 0.004 mg (Fig-7). Analytical results showing that ladybird beetles size are significantly different whereas *Adalia bipunctata* body length 4.43 ± 0.51 and wide 1.71 ± 0.27.

Adalia species of ladybird body length and wide statically significant difference value was P<0,001 in experiment. Experimental legume plants were grown well in both the field [height from 17.5 to 42.5 cm, (*Phaseolus vulgaris*) and from 9.5 to 41.5 cm, (*Phaseolus lunatus*)] and the laboratory [height from 35 to 57 cm, (*Phaseolus vulgaris*) and from 19 to 41.5 cm, (*Phaseolus lunatus*)] (Table 4). Leaf size was on the field

[length from 9.3 to 12 cm, (*Phaseolus vulgaris*) and from 6.8 to 9.3 cm, (*Phaseolus lunatus*)] and the laboratory [length from 5.6 to 10.2 cm, (*Phaseolus vulgaris*) and from 3.9 to 7.4 cm, (*Phaseolus lunatus*)]. Same time in the field leaf [wide from 5.3 to 6.9 cm, (*Phaseolus vulgaris*) and from 4.8 to 6.7 cm, (*Phaseolus lunatus*)] and the laboratory leaf [wide from 3.2 to 6.1 cm, (*Phaseolus vulgaris*) and from 3.5 to 5.4 cm, (*Phaseolus lunatus*)] (Table 5). Statically we analyses all the experimental plants in both places, and we found significant different with them. The result showed in ANOVA analysis in the field *P. vulgaris* height was 31.9 ± 9.6 whereas in the laboratory height 47.0 ± 9.5, same time in the *P. lunatus* height was 17.7 ± 13.4 whereas in the laboratory height 44.6 ± 20.03. Field and laboratory grown *Phaseolus* species plants statistically significant difference was P<0,015 (Fig- 8).

On the other hand, *Phaseolus* species of plants leaves size has significantly different with behavioral activities. Leaves size depending on aphid density and availability in both places' plants. Our analytical result showed that, in the field *P. vulgaris* leaf length was 10.42 ± 1.09 where as in the laboratory leaf length 8.20 ± 1.82, same time in the field *P. lunatus* leaf length was 7.78 ± 1.13 where as in the laboratory leaf length 5.58 ± 1.48. And the field grown *P. vulgaris* leaf wide was 6.24 ± 0.68 whereas in laboratory leaf wide was 4.62 ± 1.17, same time in the field grown *P. lunatus* leaf wide was 5.64 ± 0.89 where as in laboratory leaf wide was 4.20 ± 0.85. Both places grown *Phaseolus* plant leaves length were statistically significant difference value was P<0,001 and wide value was P<0,011(Fig- 9 & 10).

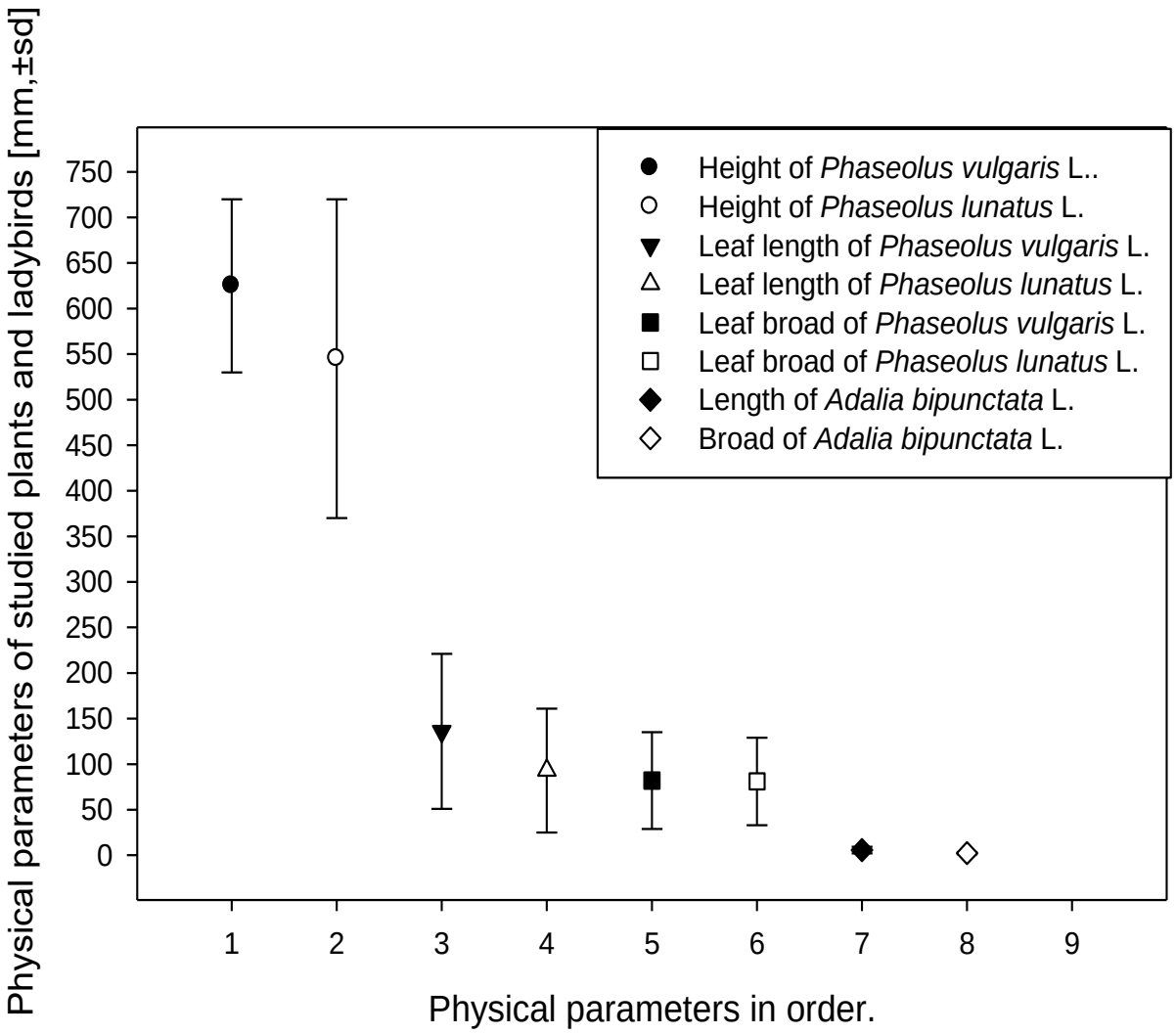

Figure 7. Selected plants and ladybird beetles measurements.

Table 4. Height of bean plants measured in both field and laboratory experiments.

Height (cm)			
Field		Laboratory	
Phaseolus vulgaris	*Phaseolus lunatus*	*Phaseolus vulgaris*.	*Phaseolus lunatus*
29.5	10.0	35.0	56.0
17.5	13.0	56.0	62.0
42.5	14.5	46.0	19.0
38.5	41.5	57.0	59.0
31.5	9.5	41.0	27.0

Table 5. Adult leaves parameters of experimental plants of *Phaseolus* spp.

Leaf size (cm)							
Field				Laboratory			
Phaseolus vulgaris		*Phaseolus lunatus*		*Phaseolus vulgaris*		*Phaseolus lunatus*	
Ll	Lw	Ll	Lw	Lw	Lw	Ll	Lw
12.0	6.8	9.3	6.5	9.5	3.7	7.4	4.7
10.4	5.8	8.7	6.7	10.2	4.8	5.7	3.1
9.5	6.4	7.1	5.0	7.3	6.1	4.3	5.1
9.3	5.3	6.8	4.8	5.6	5.3	6.6	3.5
10.9	6.9	7.0	5.2	8.4	3.2	3.9	4.6

(Abbreviations: Ll = Leaf length, Lw = Leaf wide)

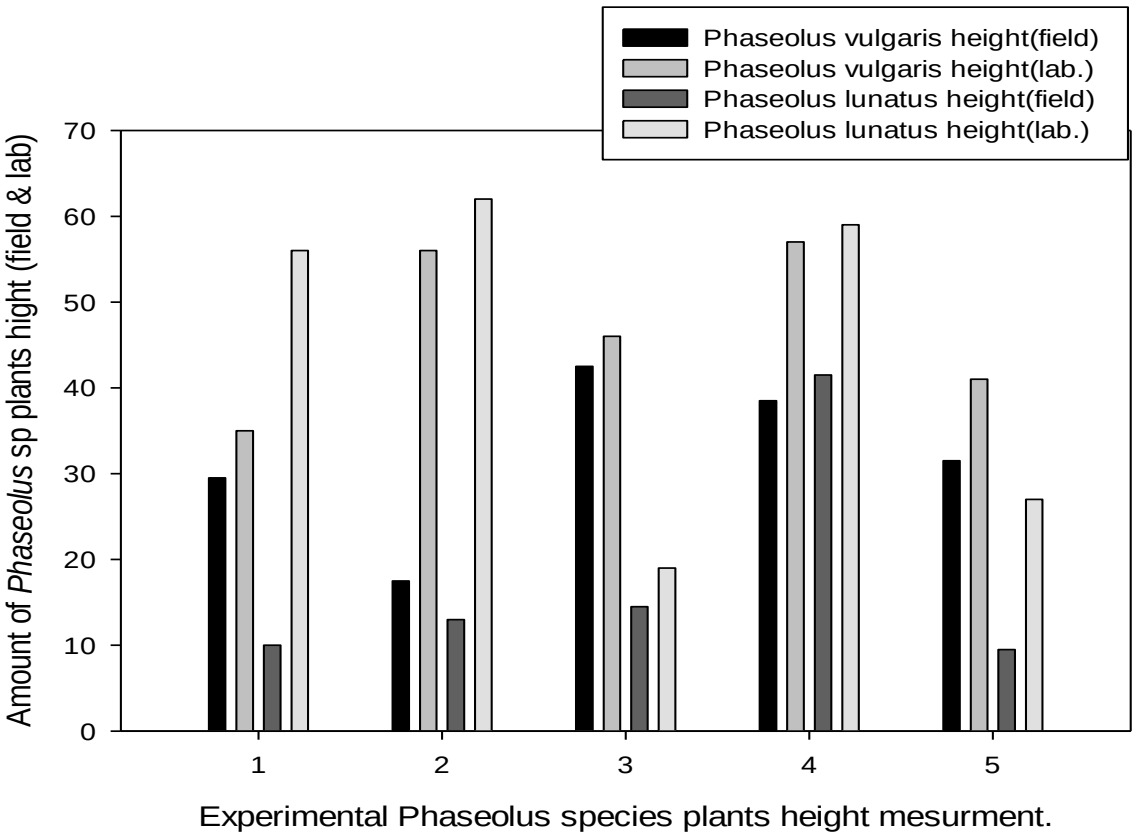

Figure 8. A measurement of the *Phaseolus* species plants height.

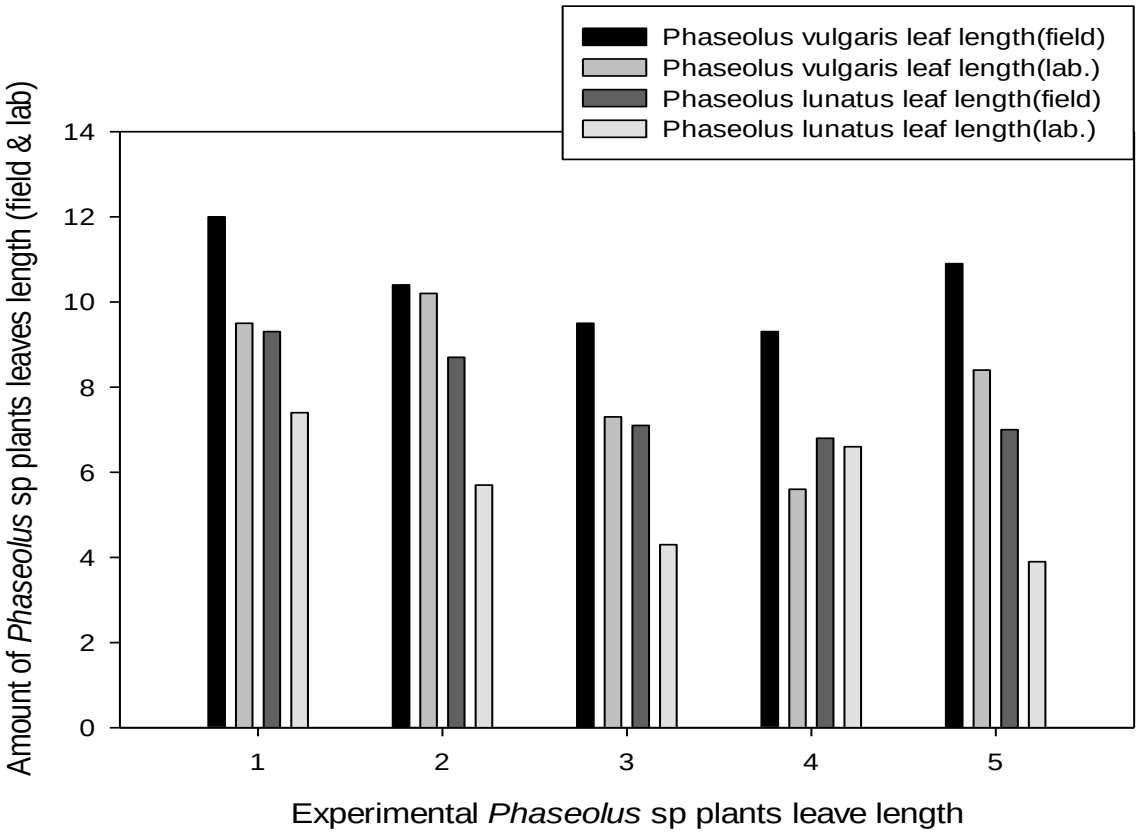

Figure 9. A measurement of the *Phaseolus* species plants leaves length.

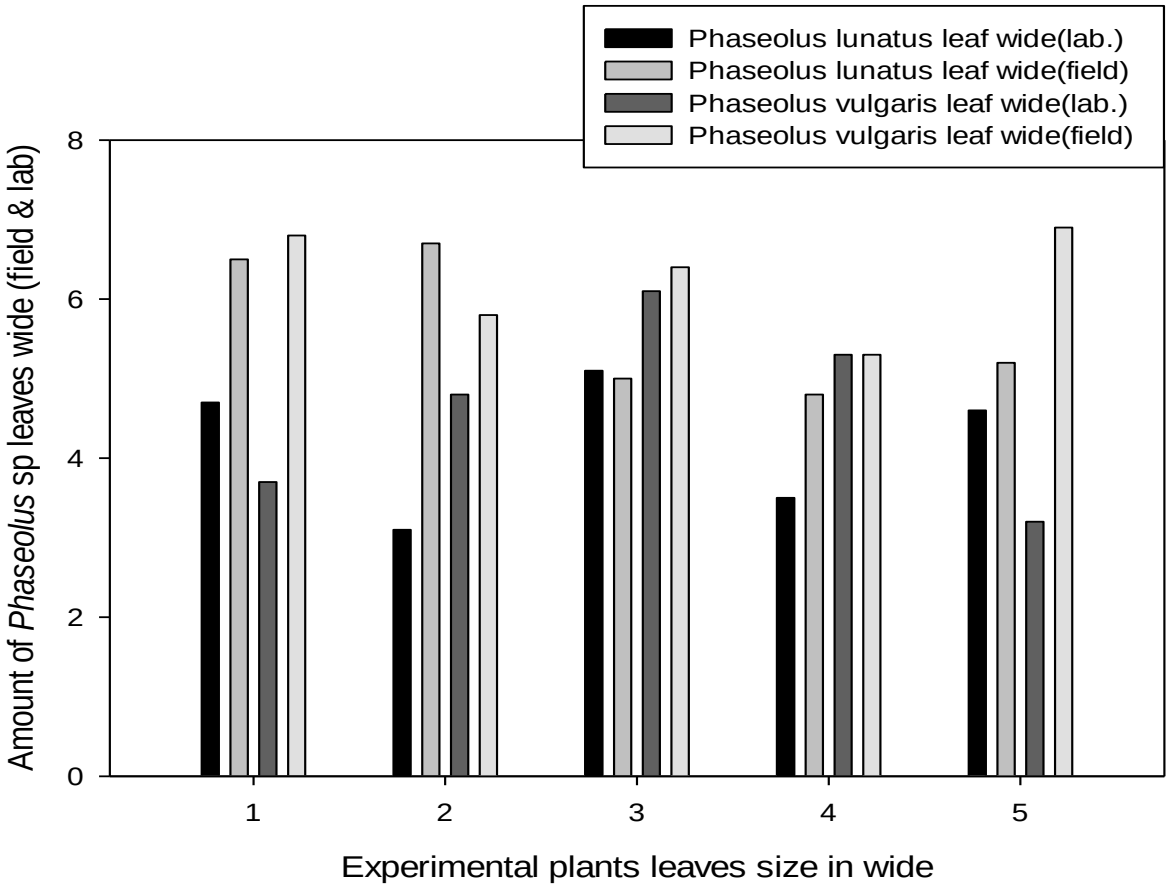

Figure 10. A measurement of the *Phaseolus* species plants leaves wide.

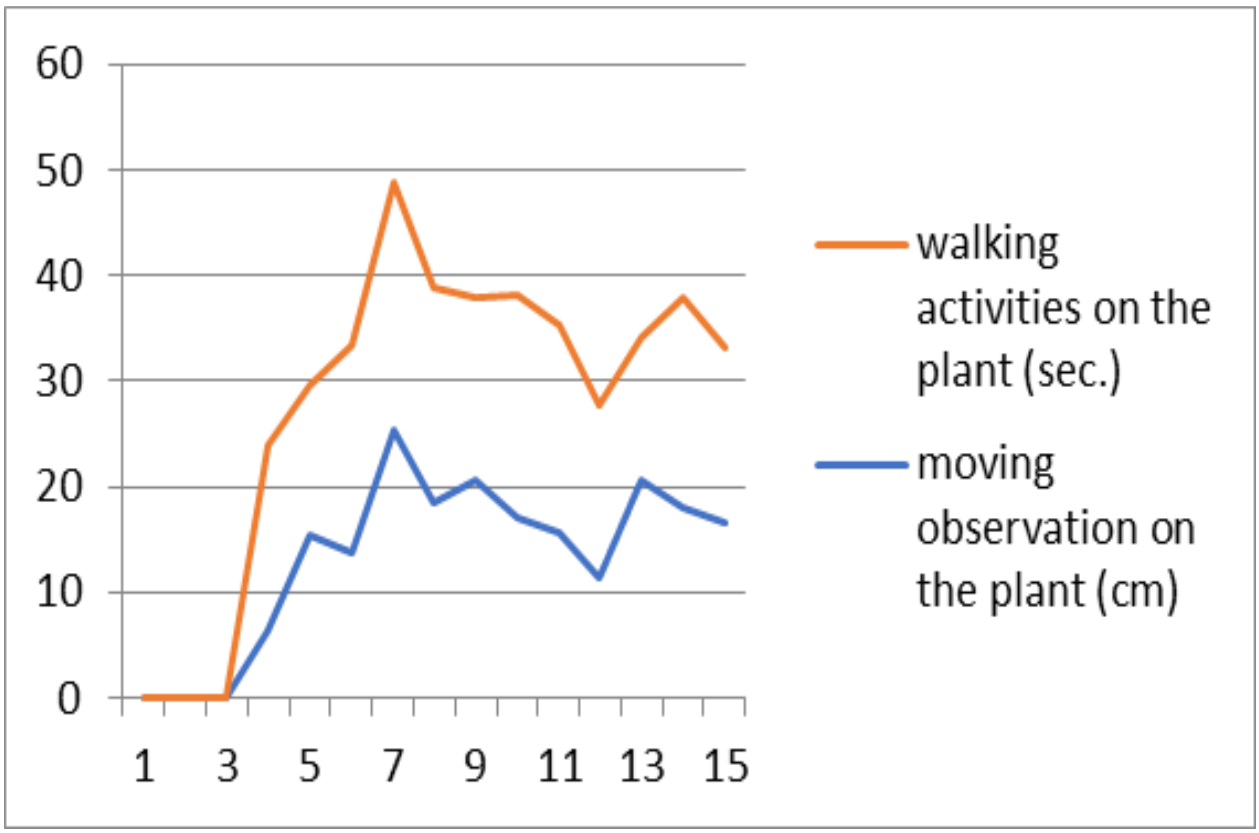

Figure 11. *A. bipunctata's* walking and moving activities in the experimental plants.

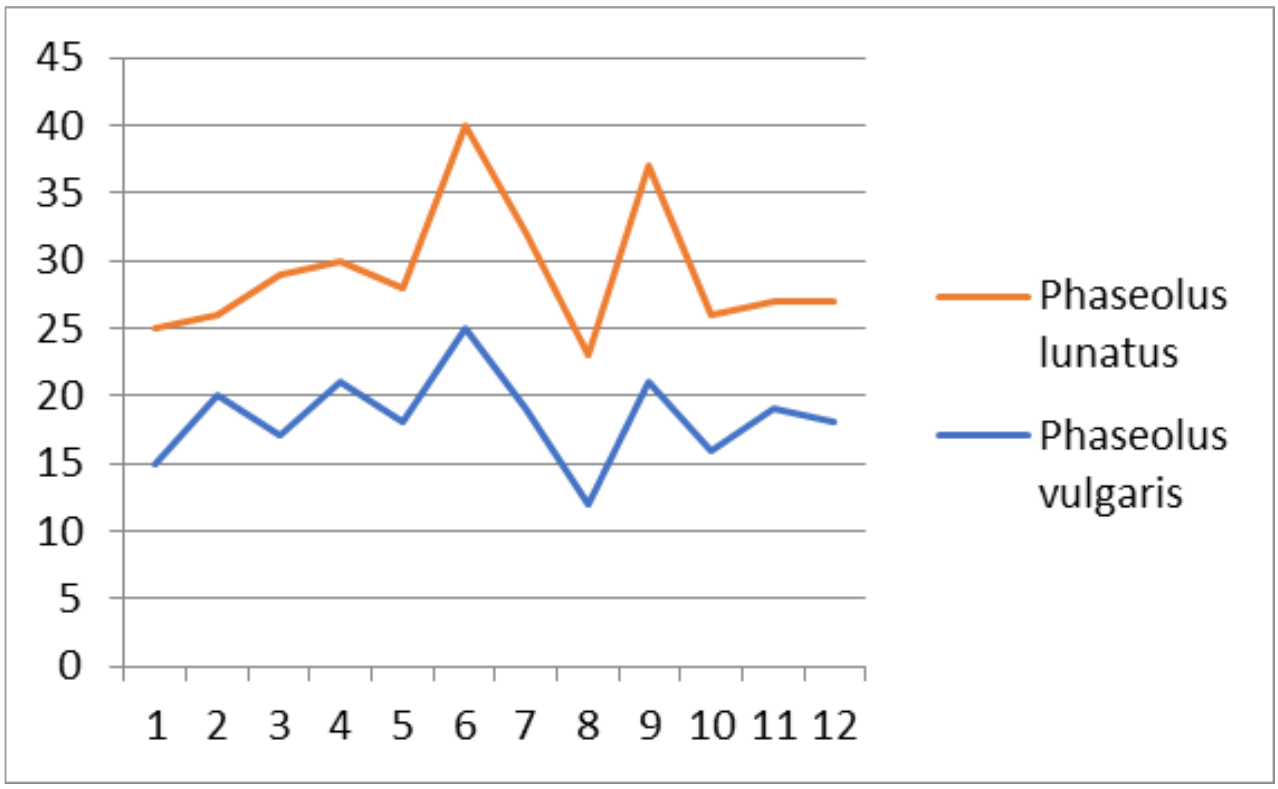

Figure 12. *A. bipunctata's* resting behaviors in the experimental plants (minute).

Figure 13. Resting behaviours of *Adalia bipunctata* L. under the leaves in the *Phaseolus* spp.

Experimental ladybird beetles and legume plant behavior activities were fully significant. Behavioral observation results showed that, *Adalia bipunctata* ladybird moving activity in the *Phaseolus* species plants were 16.60 ± 4.80 and their walking activity was in plant or plant parts 18.26 ± 2.90. But ladybird beetles resting activity was different in same species plants. In the *P. vulgaris* plant *A. bipunctata* resting time was 18.41 ± 3.31, whereas *P. lunatus* plant resting time was 10.75 ± 2.86. They have statically behavioral significant with all above activities and their significant different value $P < 0{,}001$ (Fig 11 & 12).

Observation data showed that the ladybird's body sizes and weights was affected for food searching in the different stages on the host plant. Most of ladybirds were active in the *Phaseolus* spp. plants in search of aphids for food. In the field observation we found, most of ladybirds have eaten four to 10 pieces of aphid in each day from their host plants. The observation results showed that the medium and big adults of *A. bipunctata* were active in walking over the plants and took longer rests than the smaller *A. bipunctata* (Fig-13). The length, breadth, and height of the leaves of the experimented plants of *P. vulgaris* and *P. lunatus* were calculated for graphical structure.

Adalia bipunctata body lengths, breadth, and their activities with *Phaseolus* spp plants were calculated from expected results (Table 6). In the experiment, we found that the ladybirds were more interested in attacking and staying for longer periods in legume plant with aphids. The main reasons for this interaction behaviour with legumes are not only to for aphids as food, but also for resting, taking shelter, and obtaining alternative

food sources like juice, leaf buds, nectar, and young plant leaf parts. In the experiment, we measured of the moving speed of the ladybirds in their food searching behavioural activity. When foods were nearer or available in the host plant, they moved slowly with simultaneous movement of their antenna around those spaces (average moving speed 6.50 ± 16.60 $_{cm/min}$). The walking activity was also observed and measured for interpreting the behavioral interaction. A new finding in this observation was that they first walked in the plant or plant parts for food searching, for finding other food source from the host plants or to take shelter in the host plant. Their average walking speed was 13.50 ± 18.26 $_{cm/min}$. The results showed that, most of time walking speed activity depended on the plants height, because food or food source availability in the upper part of the plant varied in comparison to those in the lower parts. The eating style of *A. bipunctata* beetles was also observed in the experiment.

Hungry *Adalia* spp. ladybird ate aphids very actively and quickly, but their feeding activity was slower when they are eating leaf buds, juice or leaves of the plants. Resting inside the mosquito net of the *Phaseolus* spp plants, ladybird beetle took rest for different time in different parts of the plant like the steam, leaves, at the ground level of plants, in leaf buds and occasionally over the mosquito nets. Their average resting period was 12.00 ± 18.41 $_{min}$ with *P. vulgaris* and 6.00 ± 10.75 $_{min}$ with *P. lunatus*. The observation result showed that, the ladybird took more rest in *P. lunatus* than in *P. vulgaris* plants or plant parts; probably its happened cause of aphids was more available in *P. lunatus* plant. The common source of the interaction behaviour between the

ladybird beetles *A. bipunctata* and legume plants *P. vulgaris* and *P. lunatus* was food. However, at the same time, the ladybirds ate other food sources like young leaves, nectar, and plant juice when the common food aphid was unavailable (Fig-14). Aphids were available in the experimental time with *Phaseolus* plants in the field observation. Black leaf aphids *Aphis* spp were the pest on the *Phaseolus* plants. Most of aphids' body was wingless, but some had wings with black or green–brown coloration and wax pollination. They were mostly present under the plants' young leaves or leaf steam or buds. Experimental period we found aphids density was always higher under the young green leaves or leaf buds than the other parts of *Phaseolus* spp plants.

Table. 6. *Adalia bipunctata* L. size and weight measured for behavioural activity with *Phaseolus* sp. plants.

Adalia bipunctata length (mm)	*Adalia bipunctata* wide (mm)	*Adalia bipunctata* L weight (mg)	*Adalia bipunctata* moving on plants (cm)	*Adalia bipunctat* resting on *P. vulgaris* (min)	*Adalia bipunctata* resting on *P. lunatus* (min)	walking activities on *Phaseolus* sp. plants (sec.)
4.62	1.64	0.004	6.50	15.00	10.00	17.14
4.48	1.80	0.003	15.50	20.00	6.00	14.14
4.46	1.83	0.003	13.80	17.00	12.00	19.63
5.07	1.89	0.004	25.30	21.00	9.00	23.37
4.21	1.62	0.002	18.50	18.00	10.00	20.30
3.82	1.35	0.003	20.70	25.00	15.00	17.25
4.32	1.64	0.004	17.00	19.00	13.00	21.20
4.21	1.69	0.003	15.70	12.00	11.00	19.50
3.56	1.20	0.002	11.30	21.00	16.00	16.45
4.12	1.61	0.003	20.50	16.00	10.00	13.50
5.15	2.16	0.002	18.00	19.00	8.00	20.00
5.19	2.09	0.004	16.50	18.00	9.00	16.75

Figure 14. *Adalia bipunctata* L. eating nectar or leaf buds in plant when aphids or other foods are unavailable.

3.3 Morphological variation in the ladybird beetles abdomen segments in ventral view as upper one is 1st and lower one 6th or 7th segment (Fig-15). The dorsal segments are called tergite, the ventral segments sternite. The segments are more heavily chitinized and flexibly connected to each other, allowing the abdomen more flexibility than the head and thorax. The ladybird beetles female genitalia consist of an ovipositor which is formed by the appendages of abdominal segments 8 and 9. The external genitalia of the male is extremely variable in the ladybird beetles, but it is often quite complex and they are frequently of considerable taxonomic value of identification. Statically we analyses all experimental ladybird beetles species abdomen in their ventral view and it was significantly different with each other.

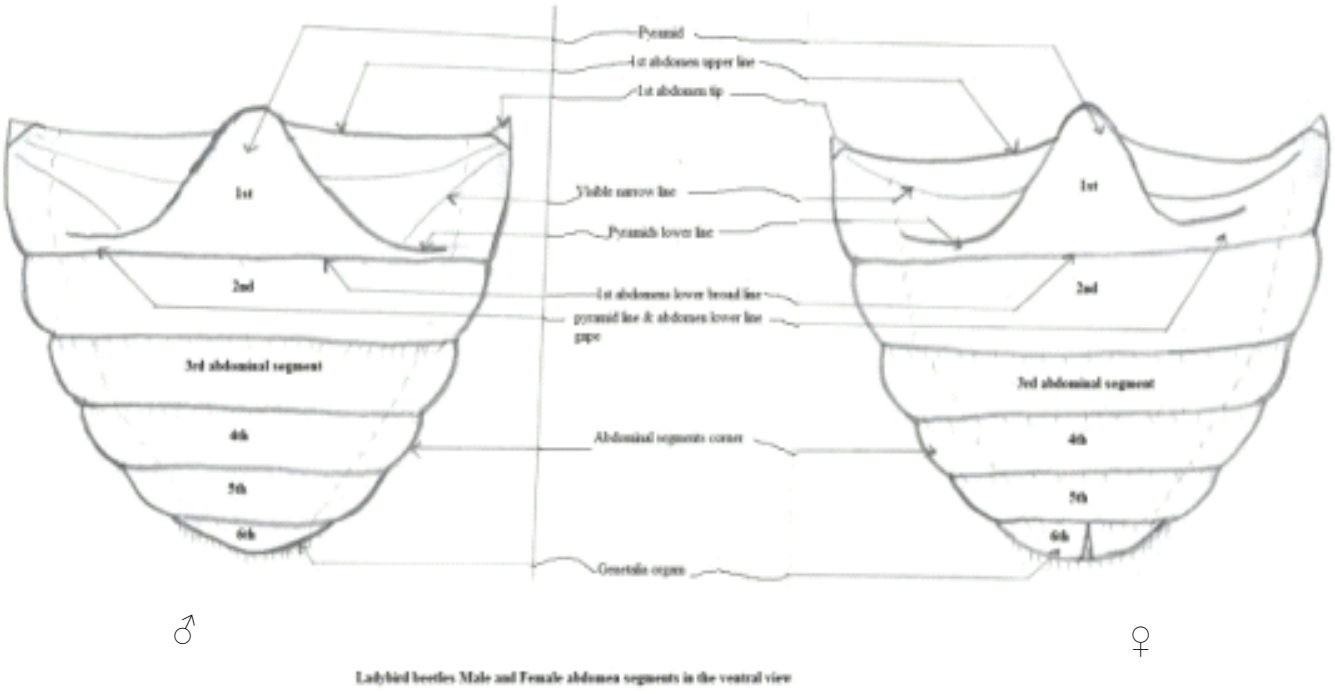

Figure 15. Abdominal segments of ladybird beetles in ventral view (male and female).

Chapter 4 DISCUSSION

In the local ecosystem we found that short summer and long winter are highly effected for establish our research results. After getting our research study (field and laboratory) introducing clear evidence for discussion.

4.1 *Coccinella quinquepunctata* adults are the commonly called the five spotted ladybird beetles. The adult body colours are red with five black spots on the elytra. The body is 4.88 mm long and 3.63 mm wide. Each elytron has one big black spot in the center and one small black spot on the lower side corner. Near the pronotum a ‘scutellary’ or a big black spot is seen on both elytra. The pronotum is fully black but near the head, both corners of the pronotum have small yellowish markings. The legs, thorax and abdomen are deep black (Fig. 16 A, 17 a).

Ecologically they are aphid feeding ladybird beetles. During the study period the following ladybirds were found in the wild rose host–plant in the Karsikko and city center areas**.** *Adalia sexpustulata* adults are melanic ladybird beetles with the *Adalia bipunctata* species. The adult body size is 5.38 mm long and 3.70 mm wide and red in colour. The elytra bear six reddish spots or marks. Each elytron carries three spots or marks, of which the first one is the largest in size, located in the corner; the middle one is bigger than the third one which at the end of the elytron.

The pronotum is fully black and without any marks. Legs, thorax, and abdomen are deep black (Fig. 16 B, 17 b). Ecologically they are aphidophagous ladybird beetles. During the study period they were found in the wild rose host–plant, small willow plants and their distribution was in the Karsikko and City centre areas. *Adalia quadrimaculata* adults are commonly known as the melanic forms (*A. bipunctata* L.) or shining ladybird beetle. Brakefield (1984a) described the melanic morphs as having black elytra and those with four red spots are the *Adalia quadrimaculata* ladybird beetle. The adult body is black in colour with red and is 4.63 mm long and 3.20 mm wide.

Their elytra are black bearing four reddish spots or marks. Each elytron carries two spots, with one small spot in the middle and the other large sized one in the upper side corner. The pronotum is black, while the legs, thorax and abdomen are dark brown or black in color (Fig. 16 C, 17 c). Ecologically they like to eat wild rose aphids and tender young willow aphids. During the study period they were found in two different collection areas, Joensuu city center and Karsikko. The *Calvia quatuordecimguttata* adult ladybird beetles commonly found in nature, have 14 cream spots. Biström *et al.* (2001) described the same genus, but a different species *C. quindecimguttata* (Fab.) which appears to be a declining species in Finland. The adult body length is 5.12 mm and wide is 3.74 mm. It is oval in form and weakly convex. Normally their body is brownish or brown in color.

Their elytra coloration varies with the natural habitat. The shape and size of the cream

spots also vary, whereas the upper spots are smaller than the lower spots and the spots arrangement normally include a straight line of six spots across both elytra. The pronotum is glossy and both corners of the pronotum have small creamy spots. The legs are brown, the thorax is black, and the abdomen is black on the upper side but brown on the lower side (Fig. 16 D, 17 d). Ecologically they are found on deciduous trees and bushes where they and their larvae feed on soft–bodied insects such as psyllids and aphids.

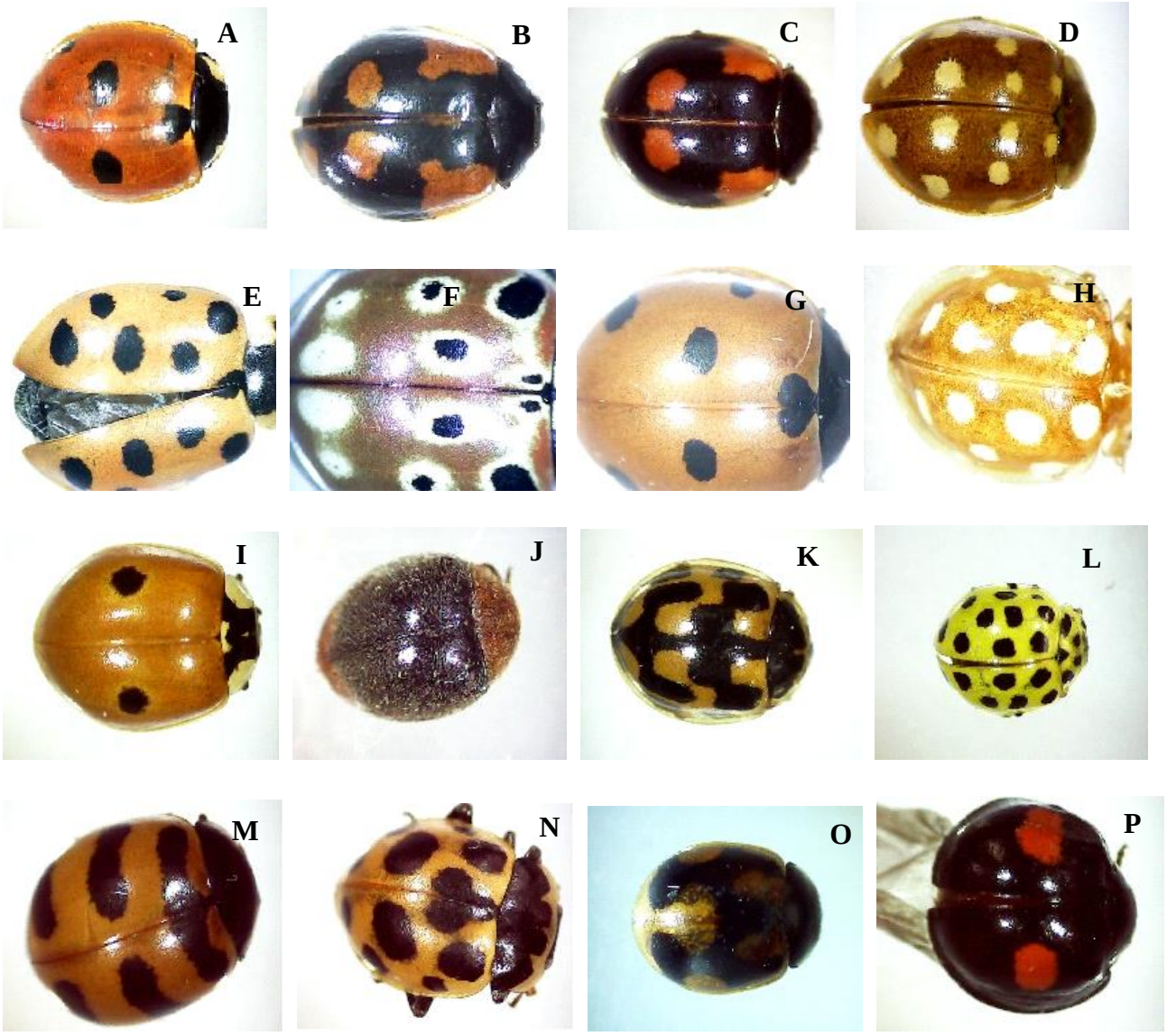

Figure 16. Collected ladybird beetles in a dorsal view: (A) *Coccinella quinquepunctata* (B) *Adalia sexpustulata* (C) *Adalia quadrimaculata* (D) *Calvia quatuordecimguttata* (E) *Hippodamia tredecimpunctata* (F) *Anatis ocellata* (G) *Coccinella septempunctata* (H) *Halyzia sedecimguttata* (I) *Adalia bipunctata* (J) *Cryptolaemus montrouzieri* (K) *Propylea quatuordecimpunctata* (L) *Psyllobora vigintiduopunctata* (M) *Coccinella trifasciatata* (N) *Coccinella undecimpunctata* (O) *Coccinella hieroglyphica* (P) *Chilocorus stigma*.

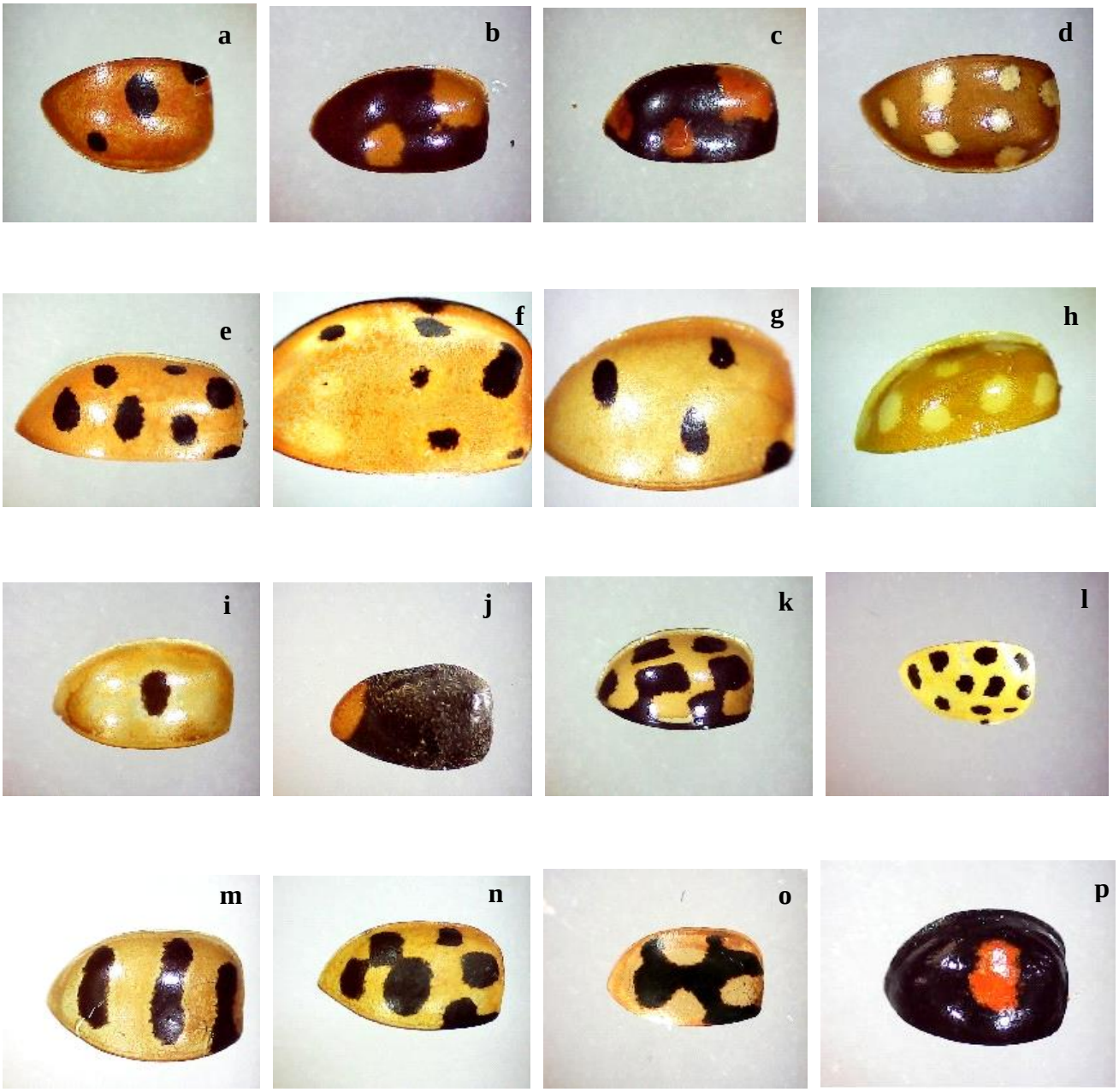

Figure 17. Collected ladybird beetles elytron in dorsal view: (a) *Coccinella quinquepunctata* (b) *Adalia sexpustulata* (c) *Adalia quadrimaculata* (d) *Calvia quatuordecimguttata* (e) *Hippodamia tredecimpunctata* (f) *Anatis ocellata* (g) *Coccinella septempunctata* (h) *Halyzia sedecimguttata* (i) *Adalia bipunctata* (j) *Cryptolaemus montrouzieri* (k) *Propylea quatuordecimpunctata* (l) *Psyllobora vigintiduopunctata* (m) *Coccinella trifasciatata* (n) *Coccinella undecimpunctata* (o) *Coccinella hieroglyphica* (p) *Chilocorus stigma*.

They are feed on psyllids to promote faster larval development, especially *Cacopsylla mali* (Ijaz, D. 2013). There are many Aphididae species that have been identified as an essential food for *Calvia quatuordecimguttata*: *Chaitophorus tremulae*, *Cavariella konoi, Aphis farinose, Eucalipterus tiliae, Euceraphis betulae, and Macrosiphoniella artemisiae* (Gordon, 1985; Kalushkov and Hodek, 2001). During the study period they were found in the Utra area with their preferred prey, the aphid's host–plants wild rose and small willow plants. *Hippodamia tredecimpunctata* are commonly known as 13-spotted ladybirds, with deep orange to red colored elytra. Adult body length is 6.75 mm and wide is 4.38 mm. They are mainly oval in shape as adults with dome-like backs. Sometimes they are shiny with short legs and with antennae. Each elytron has six dark black spots and one small sized black 'scutellum spot' near the pronotum, which is shared on both elytra. Three black spots are larger in size than the other three spots. One-third of the pronotum 1/3 space is covered with black marks but both corners have two small black spots, light yellow in color.

The legs are dark brown, whereas the thorax and abdomen are deep brown (Fig. 16 E, 17 e). Ecologically they were found in the areas of wild vegetation where green grass and legume plant species abounded. During the study period they were found only in one area selected for the study. The ladybirds were found in Pilkko where the inhabitants cultivate small gardens. The adults were found to eat the potato infesting aphids (Vaundell, and Storch, 1972). The *Anatis ocellata* ladybird beetles were largest in size among the spotted ladybird species collected in all. The adult body length 8.5 mm and

wide is 6.3 mm. The elytra are reddish brown in colour with black-eyed spotted marking. Each elytron has eight eyespots whereas others have 2–6 black spots, the other spots being only eye marking. The first and last black spots are bigger in size than all the other spots and each spot is surrounded by a yellowish halo ring. One black spot on the scutellum called ‘scutellary spot’ is also surrounded by an eye mark. The pronotum is dark black with five white spots. The legs are light brown or black in colour, thorax and abdomen are fully deep black (Fig. 16 F, 17 f). Ecologically they prefer the pine aphids (Family, Adelgidae), and are attracted to the scent of the pine (Kalushkov and Hodek, 2001).

Adults also eat other aphid species like the wild rose aphids. During the study period they were found only in the Utra area where the wild rose host–plant is available. *Coccinella septempunctata* is one of the big sized spotted coccinellid ladybird beetles. The body colour is bright orange and oval in shape and is 7.24 mm long and 5.50 mm wide. They have three large sized black spots on each elytron, where one black spot is in the middle of the elytron and the other two are in the side corners of the elytra. The middle black spots are bigger than the other two black spots. The ‘scutellary spot’ is shared between both elytra near the pronotum. The pronotum is big and black in colour and both sides of the pronotum near eyes have brown spots. The legs, thorax and abdomen are fully deep black in colour (Fig. 16 G, 17 g). Ecologically they were found on their common host plants wherever aphids are available. Usually they feed on the pea aphids but also eat other aphids and scale insects. During the study period they were

found in all the five collection areas mentioned on the wild rose host–plant. *Halyzia sedecimguttata* are common 16 creamy–white spotted ladybird beetles. The adult has an oval body shape and every part is bright orange in colour. Their body length is 5.89 mm and wide is 4.50 mm. Each elytron has four creamy-white spots which are bigger in size than the other spots. They are normally found in cool habitats and commonly active on the available food source like aphids. The pronotum is yellow or orange in colour without marks. The legs, thorax and abdomen are fully orange or yellowish and their whole body colour is orange or yellowish (Fig. 16 H, 17 h). Ecologically they are found on the wild rose aphid host–plant. During the study period they were found in the Utra and Joensuu city areas with their host-plants. *Adalia bipunctata* adult is a two–spotted ladybird beetle common in Finland. The body length on average is 5.13 mm and wide is 4.35 mm with an ovoid body shape that shines body in the light. The elytron is orange in colour with one black spot in the middle.

They are also a polymorphic species, Palearctic and Nearctic in distribution, occurring in many forms, ranging from red to black in colour (Omkar and Pervez, 2005). The pronotum has black marks in the middle but both the side corners and areas near the scutellum are yellowish. The legs, thorax and abdomen are black in colour (Fig. 16 I, 17 i). Zakharov (2003) reported that, the European and North Asian populations of *A. bipunctata* contain 5–10% melanics (black individuals) with high percentages (60–80%) in Rome (Italy), Marseilles (France), St. Petersburg, Vologda (Russia), Yalta (Ukraine) and Yerevan (Armenia). Ecologically *A. bipunctata* is a polyphagous species with a

wide range of aphids prey. During the period of study, it was found that their preferred aphid prays included wild rose leaf aphids, legume bean aphids and a different species of willow aphids. Therefore, *A. bipunctata* is of very great economic importance in agricultural biology. The *Cryptolaemus montrouzieri* ladybird species was a new collection during this period, because of the velvety black hair on the body. The adult ladybird beetles have a body length of 4.7 mm and wide is 3.3 mm. The body is shining and completely different from the normal spotted ladybird beetles. They have a narrow reddish hair band at the end of both elytra. The pronotum is fully covered with reddish hair and is shining in nature. The prosternum is light orange in colour, while the eyes are black, antennae light brown, legs deep brown and tarsus has a narrow reddish mark. The thorax is black, but the abdomen is deep orange in colour (Fig. 16 J, 17 j).

Ecologically they found on Red-tail and legume as hostplants. They normally feed on scale insects and aphids. Raupp *et al.*, (1993) described the *C. montrouzieri* adult as also eating mealy bugs. During the study period they were found on the garden *Phaseolus* spp. host plant in the Utra and *Amaranthus* spp. in the Linnunlahti areas. *Propylea quatuordecimpunctata* species include the 14–spotted medium sized ladybird beetles. Adults are 3.8–4.5 mm long and 2.8–3.9 mm wide. They are the most aphidophagous ladybirds in ecology and active in nature entire summer. The elytron is light yellow to light orange in colour with 14 unmarked black spots, which almost look like rectangular spots. Usually the several black spots show larger markings in the midline elytron and they also resemble the shape of anchor. The pronotum is pale yellow in colour with a big

black band mark. They also sometimes have 4–6 small black spots fused together on the black marks. The legs are brown while the thorax and abdomen are deep brown (Fig. 16 K, 17 k). Ecologically they are aggressive aphid feeding polyphagous ladybird beetles. The adults and larvae ate their own eggs in the laboratory conditions. However, during the study period they were found in all the five collecting points in the Joensuu area in the different hostplants. The *Psyllobora vigintiduopunctata* adults were the brightest small yellowish ladybirds found in natural habitats. Their body length was on average 3.9 mm and the wide was 3.52 mm. Both elytra always bear 22 black spots and almost all the spots are of the same size. The pronotum is very thin, small, and fully yellow in colour. In the pronotum, there are five black spots, of which three spots are bigger than the other two. The legs are brownish in colour with black marks; the thorax and abdomen are light black in colour (Fig. 16 L, 17 l). Ecologically they are aphid and scale insect eating ladybird beetles. Adults actively find aphids on their host–plant on worm or sunny days. They always move slowly on their host–plants from one side to other side. During the study period they have found them on small wild host–plants in the Utra and Linnunlahti areas.

The *Coccinella trifasciatata* ladybird looks cross–banded orange in colour and oval in shape. The body length is 4.9 mm and wide is 3.8 mm. They have three big black bands on both elytra where the first band joints the shape to look like one band. The second and third black bands are clearly separate in both elytra. The pronotum is big with a big black band and small brown spots near the head portion. The legs, thorax and abdomen

are completely deep black in colour (Fig. 16 M, 17 m). Ecologically they are in the wild rose host–plants with aphids. During the study period they were found in the city center area. The *Coccinella undecimpunctata* is also called 11–spotted ladybird beetle with a yellowish body. The adult body length is 5.3 mm and the wide is 4.7 mm. Wheeler, *et al.* (1981) reported that, *C. undecimpunctata* is an Old-World aphid predator that occurs throughout much of Europe, Iceland, central Asia, and Northern Africa. Both elytra are yellowish or light orange and carry ten black spots. Each elytron has three bigger black spots in the middle, middle corner, and upper corner.

One big black spot occurs on both elytra in the upper position and is funnel shaped, called the 'scutellary spot'. The pronotum is covered with black marks but with a very narrow yellow line on the upper portion. The legs are brown with black, while the thorax and abdomen are black in colour (Fig. 16 N, 17 n). Ecologically they are found on the wild rose aphid host–plants. During the study period they were found on the small willow and wild rose plants in the Karsikko and city center areas. The *Coccinella hieroglyphica* adult was variably marked and light orange in colour. The adult body length is 4.6 mm and the wide is 3.8 mm. The elytra color is light orange in colour on both sides and each elytron carries three big black spots of which one is the 'scutellary spot'. Those black spots have often fused each other, together making a big black mark on both elytra. The pronotum is dark brown with an orange mark in both corners. The legs, thorax and abdomen are black in colour (Fig. 16 O, 17 o). Ecologically they are aphid feeding ladybirds. During our observation period only one specimen was found on

the host–plant in the Karsikko area. The *Chilocorus stigma* adult is oval shaped and shining black with two spots. The body length is 4.43 mm and wide is 3.47 mm. The colour of the elytra is shining black or deep royal blue. Each elytron has one big reddish or deep orange spot on the middle position. The pronotum is black or deep blue without any marks. The legs are black with a small yellowish mark on the tarsus area; the thorax is black; the abdomen is reddish or deep orange in colour and the first abdominal segments have a big black mark (Fig 16 P, 17 p). Ecologically they are found living in area of vegetation where green plants and scale insects are found. Gordon (1985) reported that, nearly all the species of the genus *Chilocorus* are predaceous on scale insects, although some will accept aphids or adelgids as prey. During the study period they were found on corn host–plant and their area of distribution was the Linnunlahti collection spot.

4.2 The results showed that ladybird beetle *A. bipunctata* are relatively abundant in the study locations and they interaction with *Phaseolus* plant species. Other ladybird species occur also occasionally but with a small population made impossible to their use in the experiment. We found that legume plants and *Adalia bipunctata* ladybird has significantly interaction relationship for ecological life. Our experimental data also fully indicate that, *Adalia* species of ladybird beetles are interacting with *Phaseolus* species plants for their life needs. Legume plant species of *Phaseolus vulgaris* and *Phaseolus lunatus* have mutualism relation with *Adalia bipunctata*. In the life stage *of Adalia* spp ladybird beetles need interaction relation with *Phaseolus* spp plants for food security and

search of a host plant for completion of the life circle. In this case, the experimental behaviour indicate that plants height depends on the available aphids density, which is inviting ladybird for food source, and field experiment indicates that aphids density was more in the higher level of plants' leaves than in the lower level of plants leaves. Previously published studies also reported the importance of interaction behavior of two-spots *A. bipunctata* ladybird with different plants. The results indicate that, selected ladybirds (*A. bipunctata*) and legume plants (*Phaseolus* spp) has closely interaction relation. These behaviours are depended on the different characteristics of plants and ladybirds such as sizes and interests. The interest for the ladybird may include food security and searching for a potential host, and for plants, possible reductions of herbivore bio-agents as food for the ladybirds and leave vibration and cleaning of dust particle. Our results indicate that, plants height is important for ladybird invitation for food sources.

Especially, *Phaseolus* spp plant leaves at higher level were more attractive to the ladybirds than those at the lower stage leaves. Moreover, physical parameter of the plants and the ladybird studied are important for interaction. The gradual prey suitability and specialization on one aphid species by *A. bipunctata* for generations has an evolutionary significance in its establishment in different zoogeographical habitats. It is also known that food have significant influence on *A. bipunctata's* searching behaviour (Kalushkov 1999). Ladybirds' *A. bipunctata* are known in some countries as one of the most common coccinellid predators, for example, in an Iranian study on fruit and nut

crops, this ladybird was regularly active in the pistachio gardens (Mehrnejad *et al.* 2010). The low nutritional value or food limitation often expedites their development, resulting in smaller size of an adult ladybird (Frances *et al.* 2000). After all investigation, the experimental and the results indicate that, *A. bipunctata* and legume plants species of *P. vulgaris* and *P. lunatus* have strong interaction relation in the ecology. Finally, ladybird beetles need internally or externally supports from the legume plants in the natural condition for complete their successful life circle.

4.3 In our morphological research, analytic results showed that collected ladybird beetles abdomen long mean was 6.27±1.30 and wide mean 7.51±1.47 in the experimental condition. In the ventral view of the ladybird beetles abdomen size (long and wide) statistically significant difference was P<0,017 (Fig- 18). For our experimental results, we have examined ladybird beetles abdomens analysis and color variation in the different species (Table -7).

Coccinella trifasciatata L abdomen was a round shape with deep brown. It was long 2.90 mm and broad is 3.70 mm. First abdominal segments both upper tip was light brown; upper narrow-curve line was attached with pyramid shape in the middle; pyramids lower area was long and straight with 1^{st} segment line and without joint; both sides of pyramids lower area was visible narrow with convex line; 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} segments were parallel shapely; and 5^{th} & 6^{th} segments were brownish black (Fig- 19A).

Coccinella quinquepunctata L abdomen shape was not rounded and colorfully black.

First segment upper tip was yellow; abdomen size was long 2.30 mm and broad 2.40 mm. The pyramid was a short and a lower area shortly closed with the 1st abdominal segment. Two narrow visible lines on both sides of the 1st abdominal segment were concave, long; upper side was joining with abdominal tip and lower side was same time joint with pyramid ground and 1st abdominal lining (Fig- 19B).

Halyzia sedecimguttata L abdomen shape was long and broad, not round; color was fully creamy-yellow with light brown shade. It was 3.00 mm long and 3.10 mm wide; pyramid shape was broadly short. In the 1st abdomen pyramids lower area was short and slightly curved; not joint with a 1st abdominal line; there had been a narrow line always invisible (Fig- 19C).

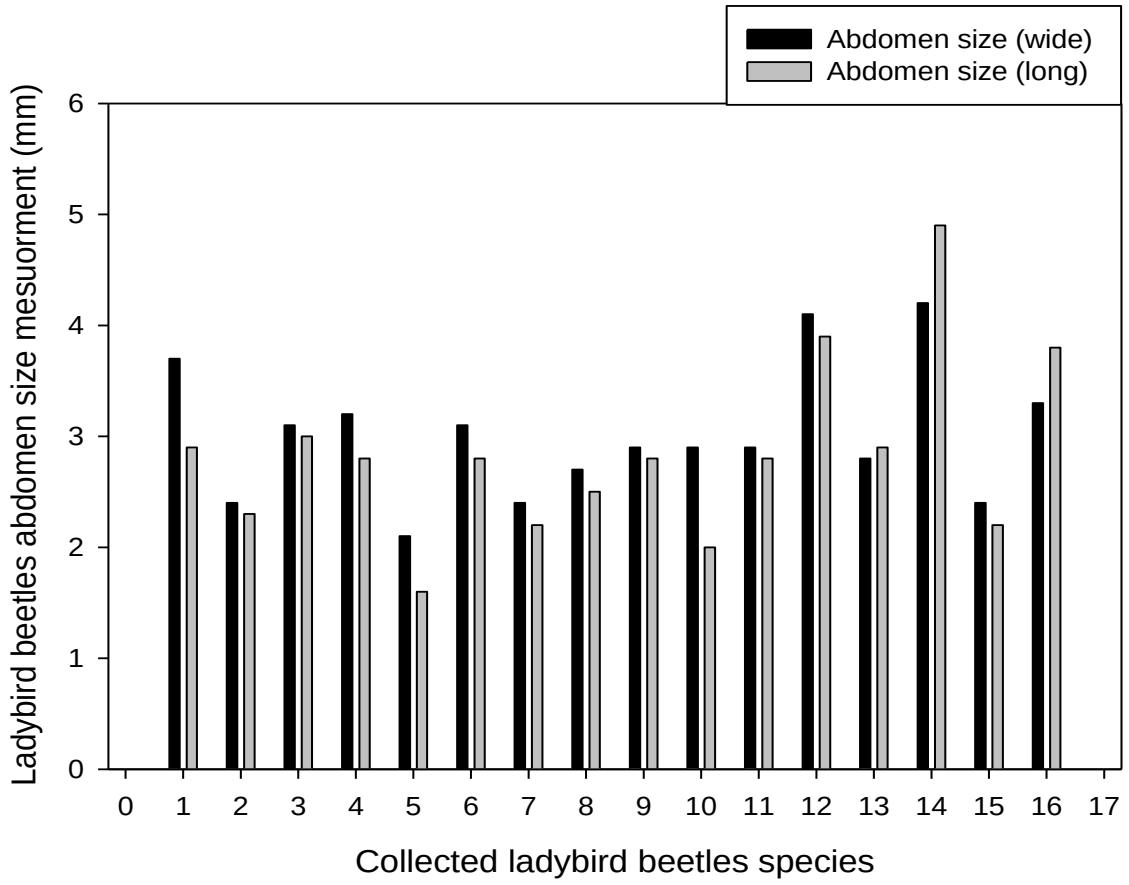

Figure 18. Collected ladybird beetles abdominal variation with shape and size.

Table 7. Experimental ladybird beetles abdominal size and colours measurement.

Ladybird beetles species	**Abdomens**				
	Long (mm)	Wide (mm)	Colour	Segments	
				Tip colour	Corner colour
Coccinella trifasciatata L	2.9	3.7	Deep brown	Light brown	No colour
Coccinella quinquepunctata L	2.3	2.4	Black	Yellow	No colour
Halyzia sedecimguttata L	3.0	3.1	Creamy-yellow	No colour	No colour
Adalia bipunctata L	2.8	3.2	Brown	No colour	No colour
Psyllobora vigintiduopunctata L	1.6	2.1	Brown	Dark brown	Light brown
Calvia quatuordecimguttata L	2.8	3.1	Brown	No colour	No colour
Coccinella hieroglyphica L	2.2	2.4	Dark brown	Light orange	No colour
Adalia sexpustulata L	2.5	2.7	Deep black	No colour	Light orange
Propylea quatuordecimpunctata L	2.8	2.9	Black	No colour	Deep orange
Chilocorus stigma L	2.0	2.9	Bright orange	Black	No colour
Coccinella undecimpunctata L	2.8	2.9	Orange	Light orange	No colour
Coccinella septempunctata L	3.9	4.1	Deep black	No colour	No colour
Adalia quadrimaculata L	2.9	2.8	Creamy brown	Brown	No colour
Anatis ocellata L	4.9	4.2	Dark brown	Orange	Light orange
Cryptolaemus montrozieri L	2.2	2.4	Orange	Brownish orange	Light orange
Hippodamia tredecimpunctata L	3.8	3.3	Deep black	Creamy brown	Creamy-spot

Figure 19 A. *Coccinella trifasciatata*

Figure 19 B. *Coccinella quinquepunctata*

Figure 19 C. *Halyzia sedecimguttata*

Figure 19 D. *Adalia bipunctata*

Figure 19 E. *Psyllobora vigintiduopunctata*

Figure 19 F. *Calvia quatuordecimguttata*

Figure 19 G. *Coccinella hieroglyphica.*

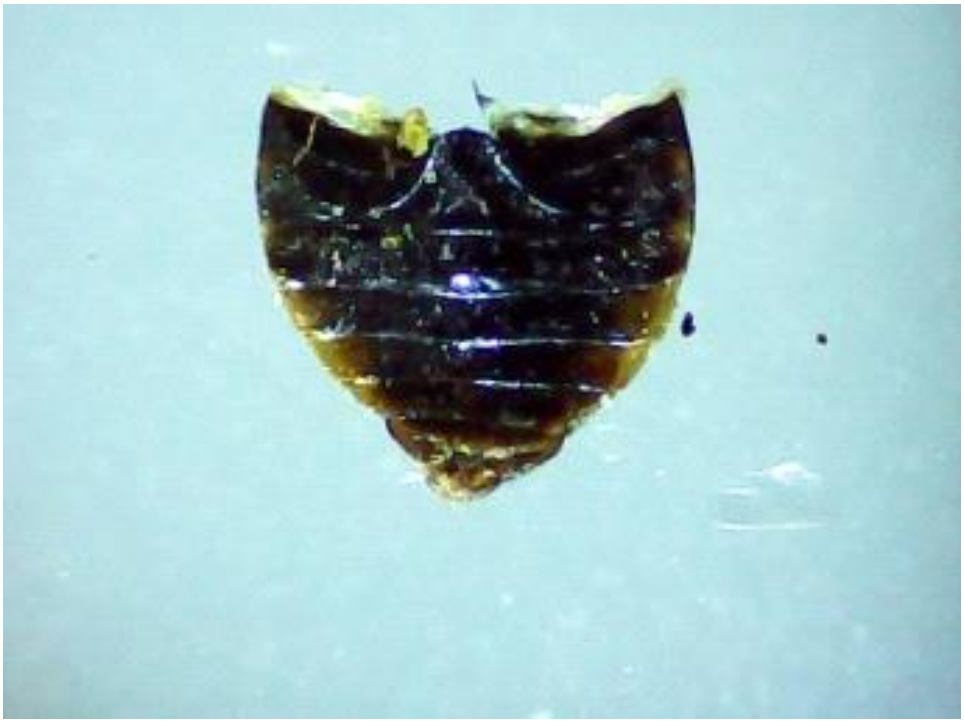

Figure 19 H. *Adalia sexpustulata*

Figure 19 I. *Propylea quatuordecimpunctata*

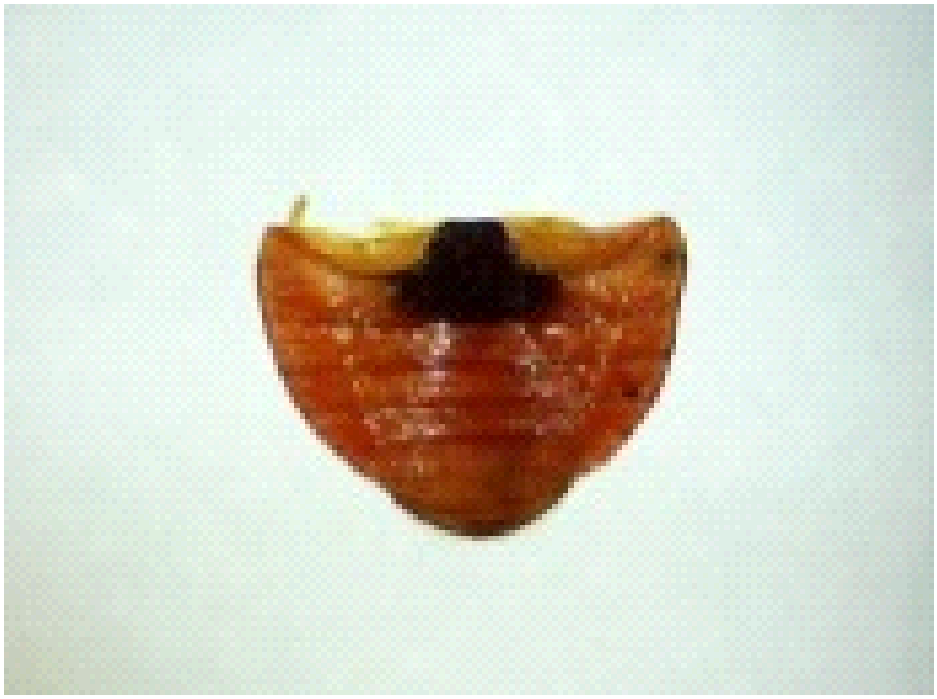

Figure 19 J. *Chilocorus stigma*

Figure 19 K. *Coccinella undecimpunctata*

Figure 19 L. *Coccinella septempunctata*

Figure 19 M. *Adalia quadrimaculata*

Figure 19 N. *Anatis ocellata*

Figure 19 O. *Cryptolaemus montrozieri*

Figure 19 P. *Hippodamia tredecimpunctata*.

Adalia bipunctata L abdomen both sides of segments corner were a light brown color but middle area always deep brown. Their abdominal size was long 2.80 mm and broad 3.20 mm; slightly rounded with short size. The pyramid was short with broad; lower line was nicely curve; not joint with 1st abdominal line. Narrow line was clearly visible and jointly with pyramid line, which makes a deep convex line. Narrow visible line tip was nearly joined with 1st abdominal tip (Fig- 19D).

Psyllobora vigintiduopunctata L abdomen nearly rounded short and brown color. Both sides of the abdominal corner area were light brown. It is 1.60 mm long and 2.10 mm broad in size. The pyramid was short, and tip was dark brown and their lower line was straight long, and parallel with the 1st abdominal line; their narrow visible line was absent; 1st abdominal segments upper line was deep convex; 2nd abdominal segment lines middle area was little curve. All others abdominal segment lines were parallel and straight (Fig- 19E).

Calvia quatuordecimguttata L abdomen was small and broadly round shape. Their abdomens middle area was a dark brown color, but both side corner areas were broadly light brown. The abdomen size was 2.80 mm long and wide was 3.10 mm. Pyramid size was shorter, lower, narrow line deeply curved, not join with a 1st abdominal line; both side narrow lines were visible and join with the pyramid narrow line, which make a deep curved. First abdominal segments, upper line curved, parallel shortly near pyramid tip; line and tip were at the same level; the other segments line was straight and parallel

shape (Fig- 19F).

Coccinella hieroglyphica L abdominal size was 2.20 mm long and 2.40 mm wide; nicely rounded; color was dark brown or black. 1st abdominal segments, upper tip was a light orange color; pyramid shape was short, and the lower line was shortly parallel with 1st abdominal lines. The narrow visible line was broad, long, slightly curved, and nearly closed with 1st abdominal tip. Forth abdominal segment lines, both corners were shortly concave; the others all segmental lines were parallel and straight (Fig- 19G).

Adalia sexpustulata L abdominal size was 2.50 mm long and 2.70 mm wide with short rounded. Abdomens color was deep black, pyramid short and lower area close to the 1st abdominal line. The narrow visible line was joint with lower pyramid line and with upper tip; segments 1st and 2nd both side corner was a light orange narrow band; segments 3rd, 4th and 5th each side was broadly creamy-orange color; last segment was fully creamy-orange. All abdominal segment lines were parallel (Fig- 19H).

Propylea quatuordecimpunctata L abdominal size was 2.80 mm long and 2.90 mm wide; broadly rounded with black color. Pyramid size was so short and lower area long gap with the 1st abdominal segment; the narrow visible line was long and jointly with the lower line of pyramid with nicely curved. Abdominal segments 1st and 2nd both side corner was a deep orange narrow band; 3rd and 4th segments both corners was broadly orange band; and 5th segment were shortly orange band in both side corners. Abdomen last segment was a light orange color (Fig- 19I).

Chilocorus stigma L abdomen size was 2.00 mm long and 2.90 mm wide with widely rounded. Color was deep bright orange; both sides were a light orange big band in the corner area; pyramid was so short, black, and lower line was joining with the 1st abdominal line. The narrow visible line was absent, and 1st abdominal segment tip was blocked. Upper side of 1st abdominal segment narrow lines corner was shortly concave, and all segments line was parallel with the straight (Fig- 19J).

Coccinella undecimpunctata L abdomen size was 2.80 mm long and 2.90 mm wide with short rounded. Abdomen color was dark brown or orange; but both sides of corner area were light orange. The pyramid was broadly short and the lower line was big gape with a 1st abdominal line; the narrow visible line was long and joining with the lower area of pyramid which making a long, smooth curve; the narrow line tip was joining with the 1st abdominal segments upper line tip. Other lines were parallel and clear visible (Fig- 19K).

Coccinella septempunctata L abdomen was the second biggest size then other collected ladybird beetles. It was 3.90 mm long and 4.10 mm wide, almost rounded with a deep black color. The pyramid was long, but not broad; lower line was long, curve and parallels with the 1st abdominal segments line but not joining. The narrow visible line was long, broad and lower are curved, joining with the pyramids lower line middle area. Abdominal all other lines were parallel and long straight (Fig- 19L).

Adalia quadrimaculata L abdomen was broadly rounded and small size. It was 2.90 mm long and 2.80 mm wide; middle area of the abdomen was brownish color, but both sides of the corner creamy-brown color. The pyramid was a short and lower line not gone, big gap with a 1st abdominal, lower line; the narrow visible line was broad, shortly convex and join with the pyramids lower line, but upper area was not closed with a 1st abdominal tip or line. First abdominal upper tip was brown color, parallel with pyramid tip and corner area was concave. The other abdominal segments line was narrow, straight, and parallel (Fig- 19M).

Anatis ocellata L ladybird beetles abdomen was the biggest size of the total collected ladybird species. It was 4.90 mm long and 4.20 mm wide in size with round shape and black or dark brown color. The pyramid was short, broad, and lower area was broadly curved, remarkably close with a 1st abdomen lower line. The narrow visible line was not clearly visible; a 1st abdominal line upper tip was orange color; the upper line tip area was concave and broadly parallel with the pyramid tip area. Second, 3rd and 4th abdominal segments lower lines middle area was orange band; another segment line was straight with parallel. Both sides of the abdomen corner area were light orange (Fig- 19N).

Cryptolaemus montrozieri L abdomen was the orange color medium size and semi rounded. Their abdomen size was 2.20 mm long and 2.40 mm wide. The pyramid was broadly short, lower lines were deeply curved, but not join with the 1st abdomens lower

line. The narrow visible line was long with broadly visible, the lower part was joined by lower pyramid line and the upper part was joining with 1st abdomens tip. First abdominal upper line tip area was shortly concave, and the tip was brownish-orange color. Abdomens both sides of the corner area were light orange; the other segments line was straight and parallel (Fig- 19O).

Hippodamia tredecimpunctata L ladybirds abdomen was deep black color. It was 3.80 mm long and 3.30 mm wide in size with nearly rounded. Their pyramid was long, broad and the lower line was shortly joined with the 1st abdominal lower line. The narrow visible line was absent; 1st abdomens upper line tip was not concave but middle area was shortly convex and both side corner areas where narrow creamy-brown straps. Abdomens 2nd and 3rd segments both side corner areas where finger- nail shape where creamy-brown spots were; the last or the 6th segment was fully dark black color (Fig-19P). Abdomen has a great role for oviposition and mimicry behavior for predator. Experiment result indicates that, ladybirds abdomen shape and size are affected in their behavioral characters significantly. Different color and structural variation of abdominal segments may be the evolutionary effects, or it can be for seasonal variation, which should need more study. Different species of ladybirds abdomen structure may have a big role in reproduction and also for host–plant selection to lay eggs. The present study is an attempt to identify different species of ladybirds ventral view abdominal segments and color in North-Karelia province, Finland. Ladybirds abdomen studies can strongly provide important information for identifying the different species for future study.

Chapter 5 CONCLUTION

Coccinellidae family members of ladybird beetles are particularly important in agricultural ecosystems. Coccinellidae species of ladybird beetles are commonly considered as a beneficial for their predatory activities with aphids and mites. Most of the Ladybird beetle species are ecologically important to the agro-ecosystem for their role as effective biological pest control. About 90% of the ladybirds are insects beneficial for agriculture, as they act as effective biological pest controls. Therefore, the ladybird beetles are best known as beneficial insects in biological ecology. It is known that every year's many crops or crop related plants are damaged by different aphid species. This necessitates the increased use of pesticides in the crop fields which destroys the animal food chain, causes pollution and several other hazards. After further experimentation with aphidophagous ladybird beetles they can easily be used in crop protection. If we succeed, then the economy and environment will develop. Also, by the artificial culture of the ladybird beetles and larvae with their attractive colours, beautiful shapes, different coloured spots and their natural importance, it is possible to set up indoor 'Ladybird Beetles Eco-Parks' to gain basic knowledge, as well as for public study and research. Different species of ladybird beetles have own unique shape and size which are their morphological character.

Abdominal segments can use for ladybird beetles species identification points. Same time their abdominal segments and color variation also has a big role of different species characters. So, it is a clear evidence of morphological variation in the different species of ladybird beetle's abdomen. Ladybird beetle abdomens different color may help for mimicry behavior in their biological life. Abdomen tarsi or hairs (which present in the last two segments than other segment) have a big role in finding a right host–plant for oviposition. The agricultural sector is also proved that, ladybird beetle is a beneficial insect for most of the year; it has contributed to a decrease in pesticide use on many crops and in orchards.

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