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# Fundamental and realized niches of two chrysomelid competitors on two Salix host species

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## Popular science summary:

The fundamental and realized niches of phytophagous insects constitute an important topic in contemporary ecology. Hutchinson (1965) formulized the concept of the fundamental and realized niche with regards to species responses to interspecific competition; and for the herbivore insects' community the consequence of interspecific competition is a debatable and questionable from the very beginning to till now. This study investigated the fundamental and the realized niche of two willow leaf beetles, *Galerucella lineola* and *Phratora vulgatissima*, on two different *Salix* host species, *S. viminalis* and *S. dasyclados;* and how plant species affect the fundamental and realized niches of these beetle species. To achieve the objective of this study, I focused on the egg-laying preference and placement of *Phratora* and *Galerucella* eggs in monospecific and mixed conditions. The results indicate that the fundamental niches of both beetle species are dissimilar to each other and that plant species affect their fundamental niches. The two leaf beetles affect each other's realized niche. One mechanism behind the results may be that the beetles induce changes in their shared host plants, leading to indirect interactions between the two beetles. Females could adjust their egg-laying behavior to different host plant species as well as to the presence of the opposite beetle species while ovipositing.

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# 1. Introduction:

In Ecology 'niche' is one of the most studied concepts. According to Gause (1934-1935), each species in a community or ecosystem occupies its own characteristic ecological niche. As a core principle the ecological niche predicts where a species can and will live in the physical world in relationship with the biotic and abiotic elements of its environment, in a simple word -what it needs to survive (Bruno et al. 2003). The niche concept was originally defined by Joseph Grinell (1914) and Chas. Elton (1927), but zoologist George Evelyn Hutchinson refined the niche as the interaction of all the range of tolerance under which an organism can live (Hutchinson 1957). He (1965) separated *fundamental niche* from the *realized niche* according to a species response to interspecific competition (Bruno et al. 2003). According to contemporary ecological textbooks, the *fundamental niche* is the species niche, where a species lives in the absence of negative interspecific interactions such as - competition, predation and parasitism (Bruno et al. 2003) and is determined by the physiological capabilities of the species. The *realized niche*, on the other hand, is a narrower area within the fundamental niche where a species lives in the presence of negative interspecific interactions with competitors and predators. The realized niche is thus the space where the species is competitively superior and also physically capable. Many experiments and models have been performed based on the fundamental and realized niches of folivorous insects. However, we should also gain knowledge that focuses on how host plant genotypes affect the realized niches of two competing herbivore species.

*Phratora vulgatissima* (L.) (the blue willow beetle) and *Galerucella lineola* (Fab.) (the brown willow beetle)(Coleoptera: Chrysomelidae) are two of the most common leaf feeding insects in *Salix* short rotation coppices in Sweden (Björkman *et al.*2000a). Epidemic levels of populations of these insects inflict severe leaf defoliation; as a result poor growth and occasional shoot death (Kendall *et all.* 1996), both in natural stands and in *Salix* plantations, can be observed every year. Since *Salix* spp. are cultivated to harvest biomass as a source of bioenergy production in Sweden and Europe as well, steps should be taken against these pest insects. Due to environmental concerns and commercial benefit, applying insecticides is not a suitable option.

Previous observations show that the biology of *Galerucella lineola* is very similar to that of *Phratora vulgatissima* (Kendall & Wiltshire, 1998). If these two beetles coexist and do not engage in interspecific competition with each other, the niche differentiation or resource-partitioning models will support that evolution has created different fundamental niches for the two species. However, as these species show regular outbreaks of population and significant exhaustions of their resource, interspecific competition exists between. Moreover Denno et al. (1995) conclude in their study that closely related species are more likely to compete as they share the same niche. Interspecific competition in a habitat for same resource may lead to competitive exclusion (Gause 1934, Hardin 1960, DeBach 1966, MacArthur and Levins1967, Denno et al. 1995), i.e. -the displacement of one or two ecological homology species (different species having the same niche) from the same habitat, which is known as Gause principle. If the two species coexist over time and then one may develop superior competitive abilities, we can assume that competitive displacement would eventually occur

(Reitz and trumble, 2002; Arthur 1991). For example, the Lantana hispid *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and the leaf mining fly *Ophiomyia camarae* Spencer (Dipetera: Agromyzidae), were released as bio-control agents against the weed *Lantana camara* L. (Verbenaceae) in South Africa in 1970s and 2001, respectively. In 2005, the population of *O. camarae* was increasing whereas the population of *U. girardi* was declining, which indicates a negative interaction between the two agents (April *et al*. 2011). Lawton and Strong (1981) and Strong *et al.* (1984) described the interspecific competition theory in their literature between plant feeding insects and criticize that- *"interspecific competition is too rare or impuissant to regularly structure communities of insects on plants"*. Similarly, in another paper Blossey (1995) described how two folivorous insects' species have the same ecological niche; he showed that *Galerucella calmariensis* and *Galerucella pusilla* have identical competitive abilities and frequently encounter interspecific competition for food on same habitat but none of them is superior.

Interspecific competition is one of the important ecological factors that can structure herbivore insect community (Kaplan and Denno 2007), distribution, and abundance (Lawton and Strong, 1981; Schoener, 1988). In this paper I study how two leaf beetles species - *Galerucella lineola* and *Phratora vulgatissima* - adjust their oviposition behavior when in the presence of interspecific competition and how plant genotypes interact with the competition to shape the oviposition pattern of these two outbreaking beetle species.

I presume that different characteristics of host plants, such as genotype, or physical traits could affect the insect's behaviors. *Salix* genotypes exhibit different suitability to phytophagous insects because different genotypes show different leaf chemistry. Not least the phenolic glycoside composition and its concentration within leaves differ between genotypes and species. Several reports have revealed that some *Salix* species and genotypes are less resistant to leaf beetles (Kendall et al. 1996; Lehrman et al. 2012) and this resistance is due to the phenolic compounds in the leaves (Rowell-Rahier, 1984; Tahvanainen et al., 1985; Kelly & Curry, 1991; Lehrman et al. 2012; Trop et al. 2013). *S. dasyclados* has high resistance, while *S. viminalis* is susceptible, and *S. cinerea* is medium resistant to *P. vulgatissima* (Lehrman et al. 2012). The resistance hierarchy to *G. lineola* is not known. For this study, I used two *Salix* species, *S. viminalis* (genotype: 78183) and *S. dasyclados* (genotype: Loden*)*, because of their different phenolic concentration in their leaves. Some studies have also showed that feeding damage by one or more insect herbivores can lead to induced chemical or mechanical defences in the plant, thereby altering the suitability of the plant for survival, growth, fecundity and development of other subsequent herbivores (Fowler and Lawton, 1985; Masters and Brown, 1992; Bezemer et al. 2003; Dam et al. 2005; Simelane 2006, Denno et al. 1995). For example, because of adult *P. vulgatissima* grazing on *S. cinerea,* the plant increased its induced defense by increasing the trichome density on the new leaves (Dalin & Björkman, 2003). Such induced changes in plant traits could potentially alter the realized niche of competing herbivores.

The purpose of my studies was to study the fundamental niche of both willow beetles species *G. lineola* and *P. vulgatissima* and how interspecific competition affects their realized niche on two different host plants species, *S. viminalis* and *S. dasyclados*. I hypothesized that:

1. The fundamental niches of *G. lineola* and *P. vulgatissima* are different from each other.
2. *G. lineola* and *P. vulgatissim* affect each other's realized niched when they coexist.
3. Host plant species (*Salix dasyclados* and *S. viminalis*) affect 1 and 2.

# 2. Materials and methods:

## *2.1 Study organisms:*

### a) The leaf beetles: *Phratora vulgatissima (L.)* (Coleoptera: Chrysomelidae):

*Phratora vulgatissima* (L.) is one of the most important and common univoltine defoliator beetles of Salix in Europe and also has an extensive distribution in Asia. This beetles is considered as a major economic insect pest of willows in short rotation coppice (SRC) (Kendall et al, 1996b, Björkman et al., 2003), which are a potential source of renewable energy production in northern Europe and can reduce the biomass production up to 40%(Björkman et al. 2003). As a generalist insect on *Salix* the adult beetle as well as the larvae of *P. vulgatissima* (L.) skeletonized the leaves of the plants (Stenberg et al., 2010; Peacock and Herrick, 2000). The adult *P. vulgatissima* (L.) hibernates away from the Salix plantation and concentrate in upright objects that can provide shelter, such as reeds or trees with ageing bark (Björkman and Eklund, 2006). According to Stenberg et al.2010, after the winter hibernation, adults emerge in April and feed on young Salix leaves for about two weeks before mating; and lay eggs from mid to late- May (Sage & Tucker 1998) till June. Beetles tend to feed at the top of the stems and oviposit on the underside leaves located near the bottom; size of egg clusters is between 2-50 (Kendall et al.; 1996). About 7days are required for egg hatching and the larvae feed on the leaves for about 18-30days, depending on leaf quality and temperature (Stenberg et al., 2010). Before pupating on or in the soil larvae develop through three instars (Kelly & Curry, 1991; Kendall et al., 1996). New adults emerge in July/August and feed on willow leaves until September and then they migrate to their overwintering locations (Peacock et al., 1999). Normally in Sweden, *P. vulgatissima* (L.) has only one generation per year (Stenberg et al. 2010).

Feeding habits of *P. vulgatissima* (L.) are negatively related to the concentration of phenylglucosides (mainly salicin and salicortin) in Salix leaves. Studies have revealed that Phratora does not consume the willow clones that contain high amount of phenolic (salicylate) glucosides (Denno et al., 1990; Kelly & Curry, 1991). The high yielding biomass clones *Salix viminalis* contain low level of salicortin resulting a preferred food source for this beetle (Kelly & Curry, 1991). Recent study showed that the inclusion of less favored variety in a willow plantation could motivate the distribution pattern of P. vulgatissima and postpone the beetles´ colonization and expansion on favorable varieties ((Peacock et al., 1999)).

### b) The leaf beetles: *Galerucella lineola L.* (Coleoptera: Chrysomelidae):

*Galerucella lineola L.* (Chrysomelidae: Galererucinae) is an oligophagous, generalist leaf beetle that feeds on several willow species (Salix spp.) and on alder (Alnus spp.) (in the coastal areas). Host plant records suggest that this beetle can be found on both species throughout its geographic range (Kendall & Wiltshire, 1998). This beetle is a univoltine in Sweden mostly prefering Salix viminalis as a host plant. The beetle overwinters as adults in bark crevice or in the leaf liter and colonizes its host plants in the spring at the time of budbreak (Kendall & Wiltshire, 1998). Adults become active in late April to early May and are present until late June; and then start mating and oviposition. Females usually lay eggs while feeding, and for that the food preference is strongly correlated with the oviposition preference (Sipura and Tahvanainen, 2000). The beetle lays eggs on all leaves at all the levels of shoots; and on both upper and lower sides of leaves, however lower sides seems to be preferred. (C. Björkman, K. Eklund, and S.Höglund, unpublished data). Eggs hatch after 2-3 weeks; larvae feed on the foliage, preferably on the lower leaf surface. Larval period is around 20days long and passes through three instars from May to early August. Larval mortality can be very high, presumably because of generalist predators such as pentatomids, chrysopids, and spiders (Häggström & Larsson, 1995). After the third instar, larvae move down the stem and pupate in soil. New generations emerge within few weeks and feed on foliage for several weeks before seeking out overwintering sites.

### c) Willow coppice:

In this experiment two host plant willow (Family: Salicaceae) species were used. *Salix viminalis* (genotype 78183) and *Salix dasyclados* (genotype Loden), are deciduous large shrubs and these two varieties are used for commercially production of willow biomass in Europe for many years. Although both species were introduced to Sweden, they have spread to surrounding areas and become part of the natural vegetation (Hylander 1971). The first record of introducing *S. viminalis* is in the 18th century, however *S. dacylados* did not appear until the 20th century (Hylander 1971).

The secondary compound, Phenolic group is significantly present in the Salicaceae family. Early studies show that, each willow species contain species-specific concentration and composition of phenolic glycosides. This compound performs a crucial part as a chemical defense against or stimuli for herbivory by insects (Tahvanainen et al.1985). European willows can be separated into two general groups on the base of the phenolic chemistry of their leaves. Some willows are rich in phenolic glycosides like salicin but lack of proanthocyanidins, a form of condensed tannin, however other willows contain few phenolic glycosides but higher concentration of proanthocyanidins (Tahvanainen et al., 1985).

The two willow species used in this study vary in their phenolic profiles; *Salix dasyclados* (clone loden) leaves contain relatively higher concentration of anti-herbivore agent salicylates (Kelly & Curry, 1991) and smaller amount of condensed tannins; *Salix viminalis,* on the other hand, contains no salicin (Kelly & Curry, 1991) but high levels of condensed tannins (Julkunen-Tiitto, 1986). The variation of this phenolic level has been suggested that, the resistance of these two-plant varieties is different from each other for the herbivorous insects; so, it could be assume that egg laying behavior and the number of eggs of beetles are also varying according to the phenolic concentration in the leaves.

**d) *Willow plantation:***

About 20cm long Willow cuttings (winter cuttings) were raised in the green house. For each genotype 60 cuttings were used. All of the cuttings were planted individually in plastic pots in the green house six weeks earlier of the start of the experiment. Besides that another 40 plants were planted extra for the replacement of less vigor plants by the healthy plants. The cuttings were divided into two batches (80/80). The second batch of cuttings was planted one week later then the first batch. Willows were grown in a controlled environment room with at 20˚C temperature and photoperiod of L18:D8. The soil was kept moist by regular watering. After six weeks the willow plants were 60-65 cm tall and most of the plants had 3-4 branches.

## *2.2 Beetles collection and separate males/females for experiment:*

For this study both beetle species were collected from the same willow species - *Salix cinerea*. Adult *Phratora vulgatissima (L.)* were collected from the plants growing along the county road 255, the lanes between Uppsala and Märsta (N59° 39.039', E17° 46.894'. and *Galerucella lineola (L.)* adults were collect from Liljekonvaljeholmen, South of Uppsala (N59° 48.291', E17° 39.861') in Sweden (from 2/6/11 to 3/6/11). After collecting the healthy adults from the field, they were kept in 3liter plastic containers and stored in the climate chamber and provided with fresh leaves from S. cinerea. On the next day, male and female insects were separated from each other; and four beetles (3females: 1male) were placed separately in 30ml plastic vials. New beetles were added to the vials during the second study period. In total 160 male and 480 female beetles were used for this experiment.

## *2.3 The experiment:*

The aim of this experiment was to assess how plant genotypes and competition influence the oviposition behavior of two willow leaf beetles, *Phratora vulgatissima (L.)* and *Galerucella lineola (L.).*

On 6th June 2011, the first batch of plants were used for the first experiment in the green house. All the plants of this batch (60) were allocated into two groups – Loden (*Salix dasyclados*) and 78183 *(Salix viminalis)*. Before the starting of the experiment, only one shoot of each plant, which was healthier and straight, was selected and the other branches were cut down. So, all the plants had only one shoot. After that, the shoots were trimmed; keeping the height of each shoot was around 52-59cm from the soil surface of the pot. Then all the three treatments (adult beetles; 1.*Phratora, 2.Galerucella,* 3.*Phratora* + *Galerucella*) were introduced to the potted plants. When 1st (*Phratora*) & 2nd (*Galerucella*) treatments were placed on the plants, 1male with 3females from individual beetle species were separately put on the each plant. Whereas, when the 3rd treatment (*Phratora* + *Galerucella*) was run, combination of 1st & 2nd treatments was placed on each replication; resulting, 2 males (1 *Phratora* +1 *Galerucella*) along with 6females (3*Phratora* + 3*Galerucella*) were kept on every single plant at the same time. As males may stress the females and negatively affect the oviposition rate (Stenberg, personal communication), only one male was used against three females. Each plant was covered with a cylindrical (d=30cm, l=100cm) plastic cage. The top of the cages was covered with a polyester net and was fasted by a rubber band. The pots with the plants were placed on big plates with sand and then cover by the cylindrical cages separately. The bottoms of the cages were in the sand so that it stood straight and there was enough space between the cage walls and the leaves. Plants with the insects were then left for 7days to allowed oveposition of female that also feed on the leaves. After the 7th day beetles were removed from the potted plants. As the number of available beetles was limited some beetles were reused for the second batch of the experiment. The second batch of plants was treated in the same way, but the time duration was prolonged to ten days. The R software version 3.0.2 (supporting by –lattice, reshape, doBy, car, sciplot packages) was used in analyzing the data in all the experiments describe bellow.

### 2.3.1 *How plant genotypes and Competitions affect on clutch size of both beetles species:*

After removing cages and insects, total number of eggs per clutches, numbers of clutches per leaf were record. Each plant was given a unique id (n = 120) where each leaf of a single leaf also received distinct id. The interaction of plant genotypes and beetles competition on the egg clutch (nested within plant ID) was tested with one-way anova test. Eggs on per leaf and per plant affect by host plant genotypes and competition interaction also follows the same method.

### 2.3.2 *Plant genotypes and Competitions affect on Ovipositioning placement:*

To measure the ovipositioning placement on the leaf, egg clutch positions were identified in different ways, such as: – counting number of eggs on different side of leaf surface (upper /down leaf surface), vertically/parallel placement of egg clutch along the leaf blade (base, middle, tip), diagonal placement of egg clutch along to mid rib of leaf blade (edge, near to mid rib). In comparing the clutch positions within the leaf blade Exact Bionomial Test was used.

### 2.3.3 *Plant genotypes and Competitions affect on Ovipositioning placement on the shoots:*

For this experiment the length of all shoots was recorded in cm and the shoots were divided into three parts (upper, middle and lower). Then make a compare according to eggs placement.

# 3. RESULTS:

## *3.1 Effects of plant genotype and interspecific competition on clutch size of willow beetles:*

Plant genotypes and treatment significantly affect the clutch size of beetles. Phratora vulgatissima always laid larger clutches on Salix viminalis than Salix dasyclados, it does not matter weather interspecific or intraspecific competition had occurred. On the other hand, Galerucella lineola preferred to lay larger clutches on Salix dasyclados more when they are excluded form interspecific competition. The effect of competition on the clutch size was different for the treatments. There are no different between Galerucella lineola single (without interspecific competition with Phratora) and both (with interspecific competition) on Salix dasyclados as well Phratora vulgatissima single (with interspecific competition) and both (without interspecific competition) on Salix viminalis. However, all remaining treatments and plant species combinations had significant difference (Fig. 1, Table 1).

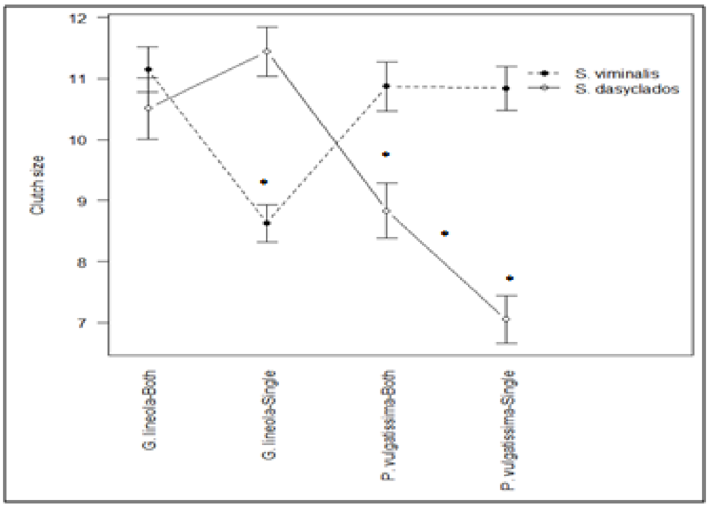

**Fig 1:** Clutch size (mean±SE) on a shoot in relation to treatments with either *P. vulgatissima* Singly, *G. lineola singly,* or both together in a cage for sevenen days. *P. vulgatissima* laid smaller clutches on S. dasyclados than on S. viminalis in both situation (involve in/or interspecific competition). *G. lineola* produced smaller clutch on *S. viminalis* when only intraspecific competition existed. *G. lineola* and *P. vulgatissima* showed opposite behaviour by diposite bigger and smaller clutch size respectively on *S. dasyclados* when they only involve in interspecific competition*.* *indicate significant differences.

### ANOVA Table:-

**Table 1:** Analyses of variance results estimating the effects of plant genotypes (S. dasyclados and S. viminalis) and beetles species combination (with or without interspecific competition) on the clutch size, number of eggs per leaf, and number of eggs per plant when beetles were allowed to colonize on the specific host genotypes.

| Variable | *df* | *MS* | *F* | *P* |
|---|---|---|---|---|
| **Clutch size depend on the genotype and/or treatment** | | | | |
| Genotype (Plant species) | 1 | 7.997 | 8.805 | <0.01 |
| Treatment Egg Id | 3 | 5.709 | 6.286 | <0.001 |
| Genotype:treat egg Id | 3 | 28.550 | 31.435 | <0.001 |
| Residuals | 1724 | 0.908 | - | - |
| **Number of eggs per leaf depend on the genotype and/or treatment** | | | | |
| Genotype | 1 | 18.656 | 15.166 | <0.001 |
| Treatment Egg Id | 3 | 20.086 | 16.329 | <0.001 |
| Genotype:treat egg Id | 3 | 7.727 | 6.281 | <0.001 |
| Residuals | 1158 | 1.230 | - | - |
| **Number of eggs on a plant depend on the genotype and/or treatment** | | | | |
| Genotype | 1 | 840.8 | 115.434 | <0.001 |
| Treatment Egg Id | 3 | 34.1 | 4.683 | <0.01 |
| Genotype: Treat Egg ID | 3 | 33.2 | 4.557 | <0.01 |
| Residuals | 347 | 7.3 | - | - |

### *3.2 Effects of plant genotype and treatment on eggs per leaf :*

Plant genotypes and treatments significantly influenced the eggs per leaf. On average *Phratora vulgatissima* laid more eggs on *S. viminalis* than *S. dasyclados* per leaf, but *Galerucella lineola* laid nearly same amount of eggs per leaf on both plant genotypes. *Galerucella lineola* laid more eggs than *Phratora vulgatissima* on *S. dasyclados* when they competed each other, whereas in case of *S. viminalis* the number of eggs per leaf seems close for these beetles species. It happened the same on both host plant genotypes when these two beetles species did not competed each other. Both species of with and without interspecific competition on both treatment *S. viminalis* and *S. dasyclados* are not differ significantly in producing egg clutch. However all remaining treatement and plant species combinations are significantly different.

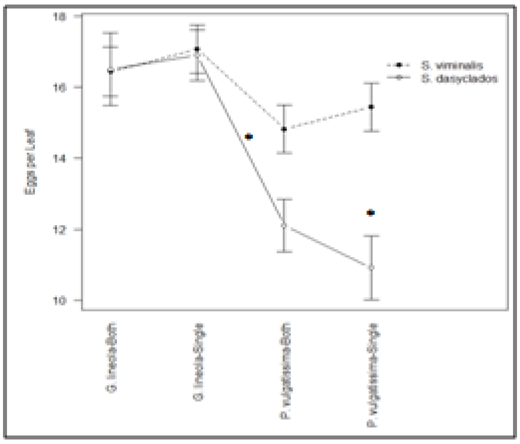

**Fig 2:** *Galerucella lineola* in single as well in mixed treatment laid almost same number of eggs per leaf on both treatment *S. viminalis* and *S. dasyclados* genotype. Whereas *Phratora vulgatissima* laid highest number of eggs in *S. viminalis* and lowest number of eggs per leaf on *S. dasyclados* when they are in single and mixed treatment too. *indicate significant differences.

### *3.3 Effects of plant genotype and treatment on eggs per plant:*

Both the beetle species laid more eggs on *S. viminalis* and competitions had no effects on number of eggs per plant. *Galerucella* laid less eggs per plant when they competed with *Phratora* on both host plant genotypes than they were not involved in interspecific competition; however *Phratora* laid nearly the same amount on *S. dasyclados* when they include or exclude to the competition, but less on *S. viminalis* when they competed with *Galerucella* rather than when they are not include in competition. The result shown that, *P. vulgatissima* both and *P. vulgatissima* single (with and without interspecific compition)on *S. dasyclados* are not different but significantly different from all remaining treatement and plant species combinations.

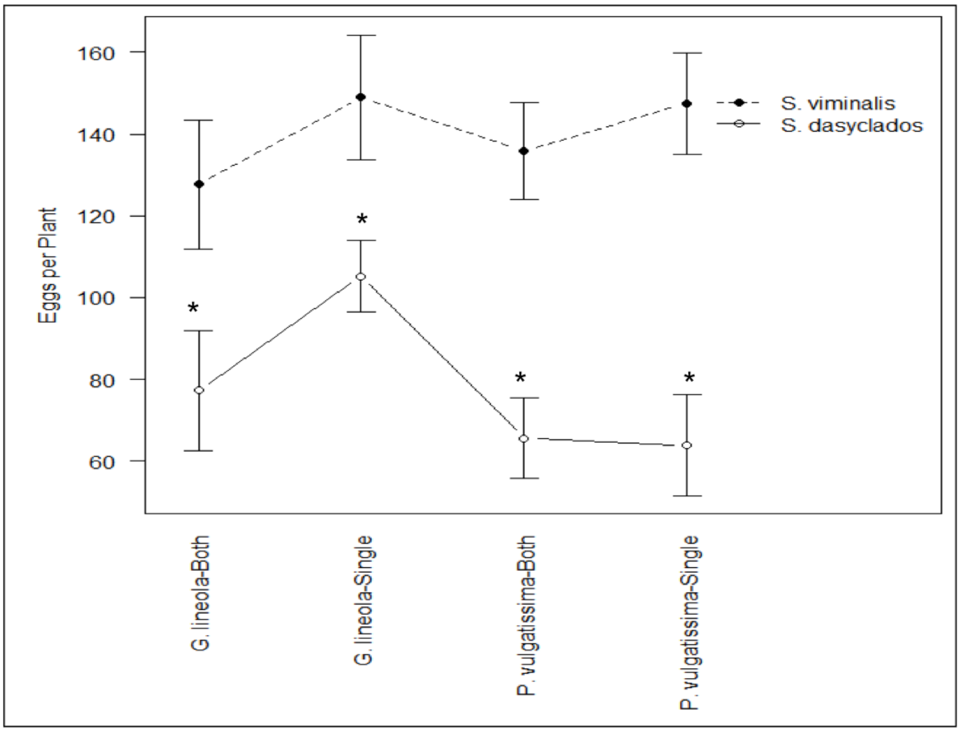

**Fig 3:** *G. lineola and* P. *vulgatissima* both laid highest number of eggs per plant on *S. viminalis* genotype . Whereas *P. vulgatissima* and *G.lineola laid* laid lowest number of eggs per plant on *S. dasyclados*. . *indicate significant differences.

## *3.4 Oviposition site on the leaf (Clutch diagonal to mid rib):*

On *S. dasyclados* almost the same amount of *Phratora vulgatissima* eggs were found on the edges as on the ribs of the leaves when they did not compete with *Galerucella lineola*; but in all other treatments (with or without interspecific compition) most eggs were oviposited near the edges of the leaves (about 70%) and are significantly different; however competition did not effect ovipositioning site significantly. In contrast, on *S. viminalis Phratora vulgatissima* preferred to oviposit near to the leaf rib (about 80%) and *Galerucella lineola* chose to oviposit near to the edge (about 60%) area of leaves. Here the interspecific competition did not affect the choice of oviposition site and all of these showed significantly difference.

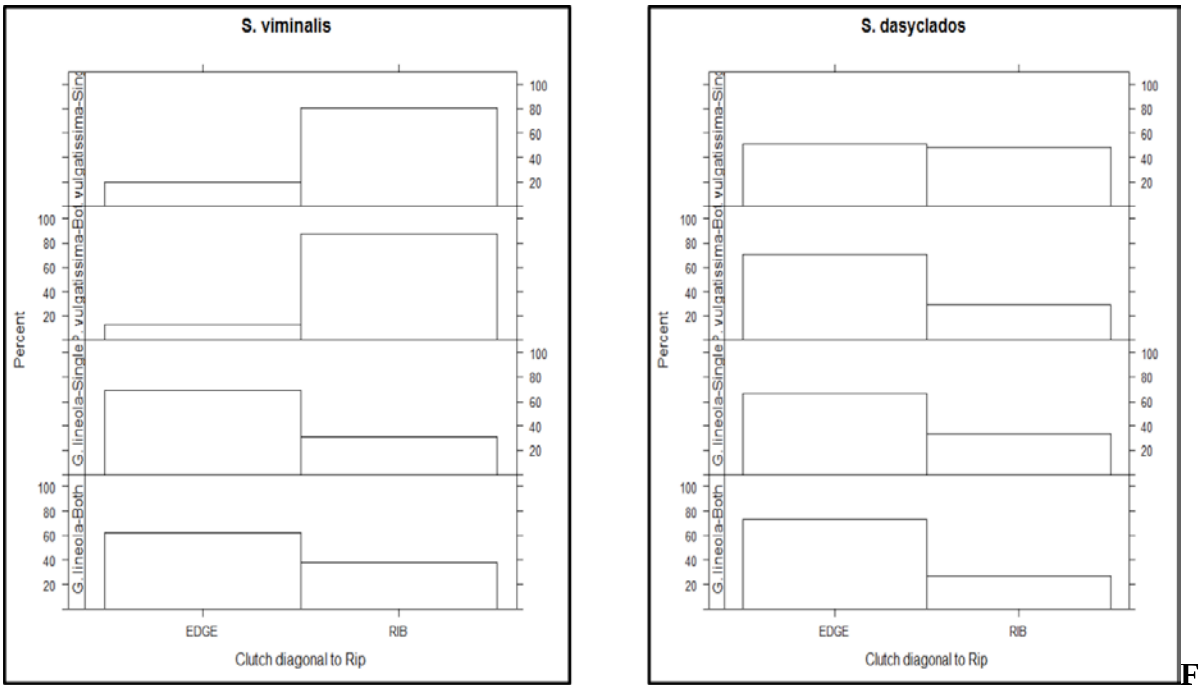

**Fig 4:** On *S. dasyclados* both *P. vulgatissima* and *G. lineola* preferred to oviposite near the edge of the margin or edge of the leaves, but on *S. viminalis* they preferred the opposite side to each other while ovipositing on the leaves.

### *3.5 Oviposition of beetles on the leaf (Clutch parallel to mid Rib):*

I found that, *Galerucella lineola* always tended to oviposit near the edge of leaf blade and along the periphery of apical half of leaf blade. *Phratora vulgatissima* tend to oviposit on the middle (about 90% in *S. viminalis* and about 80% in *S. dasyclados*) of the leaf blade for both *Salix* species, irrespective of the absence or presence of interspecific competition. Conversely, on *S. dasyclados, Galerucella lineola* laid more eggs (about 55%) on the tip when they were not incorporated with competition; but if they were included in the competition they produced eggs on the tip and middle of the leaf blade (about 40-45%). On the other hand; on *S. viminalis, Galerucella lineola* laid their eggs mostly in the middle of the leaves (interspecific competition 60% and intraspecific competition 55%), though on the tip percentage of the eggs were proximate to middle of the leaves (35% and 45% respectively). Besides that, it was found that beetles did not oviposit on the base of the leaves (less then 5%) on both plant species with all the treatment combinations.

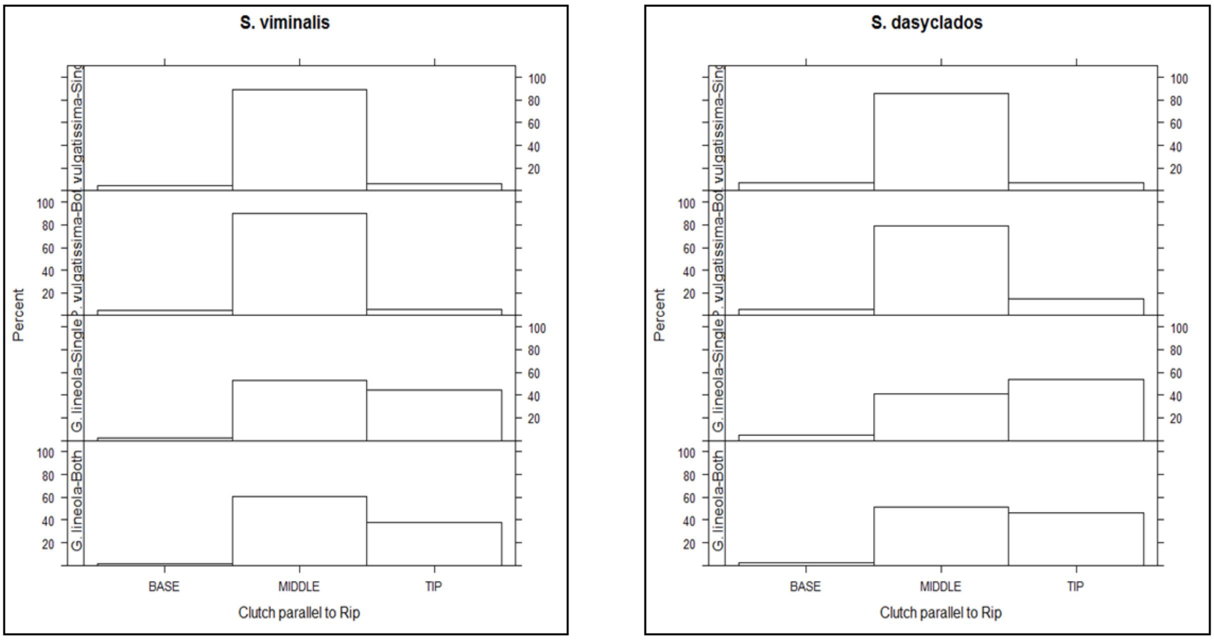

**Fig 5:** *Phratora vulgatissima* prefer the middle of the leaf blade on both Salix species. However *Galerucella lineola* more frequently oviposit on the middle and the tip of the leaves. Treatment did not change these behaviors.

### *3.6 Oveiposition side of beetles on the leaf:*

Significant difference were found between two oviposition´s side (lower and upper sides of leaves) of both beetle and plant species combimations. Both species significantly preferred the abaxial leaf surface for oviposition  (fig. 6). *Phratora vulgatissima* chose to oviposit only on the lower side (100%) of leaves on both *Salix* species. Though the ovipositioning percentage of *Galerucella lineola* was same (80-90%), but they tended to lay eggs on the upper side of the leaves. These patterns were not affected by competition.

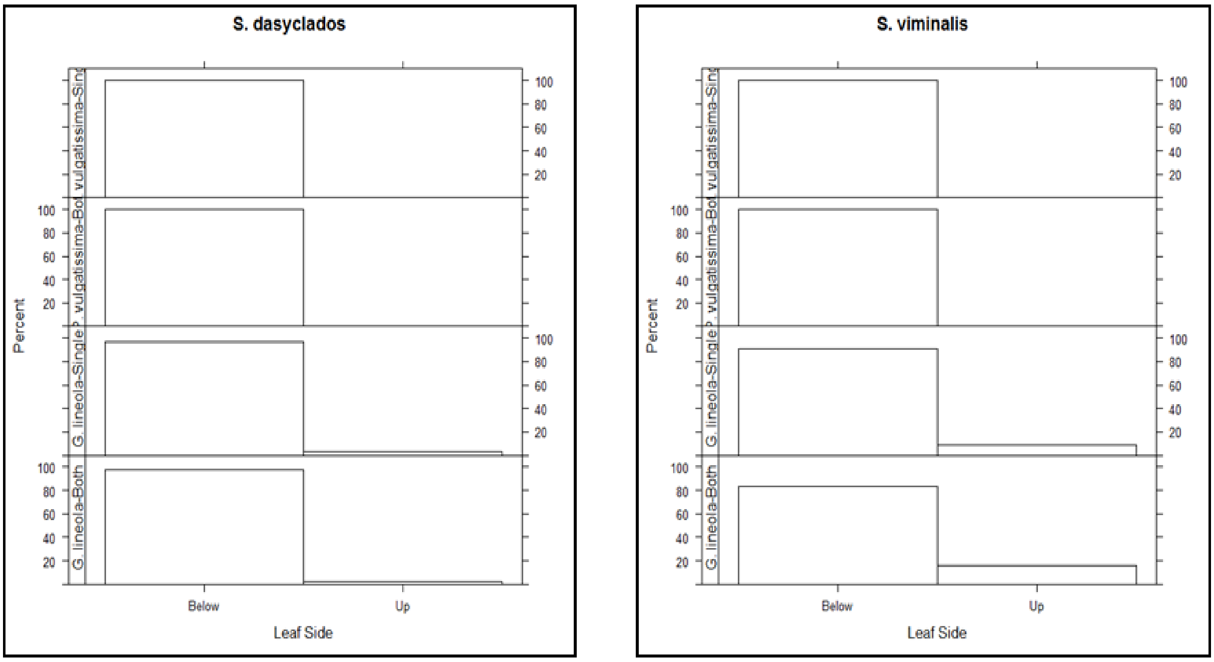

**Fig 6:** *Galerucella lineola* oviposited on both sides of the leaves in both treatments, however *Phratora vulgatissima* oviposited only on the below side of the leaves.

## *3.7 Oviposition of beetles at the plant level (shoot):*

Both beetle species preferred to oviposit on the middle part of the plant shoots and; and plant irrespective if plant species.

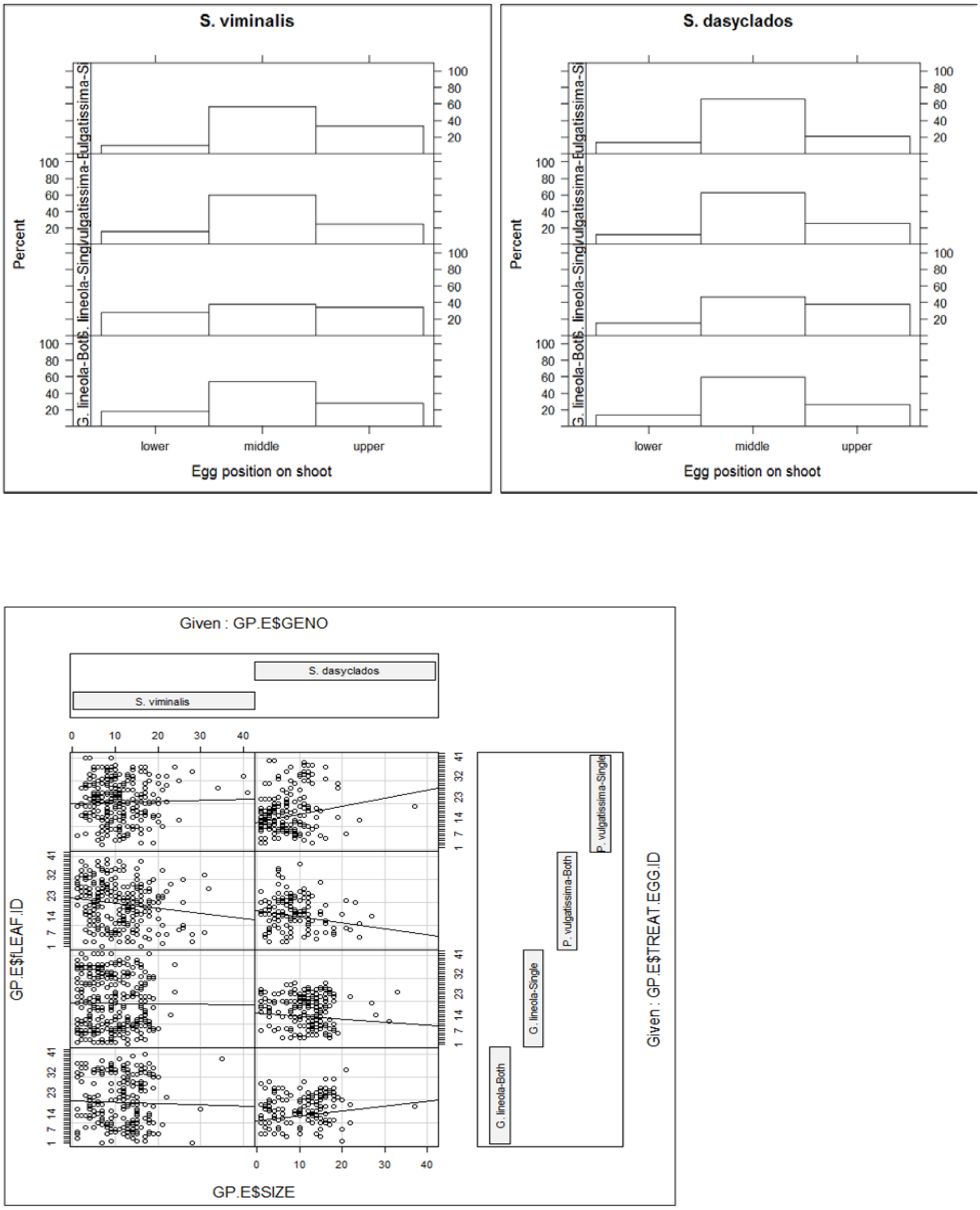

Fig 7: Both *Galerucella* and *Phratora* beetles tended to lay their eggs all over of the shoot on both *S. dasyclados* and *S. viminalis*. There was no change to preference when they stayed together. The lower part of the figure shows a great variation in the vertical position effect.

# 4. Discussion:

## *4.1 How does plant species and competition affect the ecological niche:*

*Phratora vulgatissima* and *Galerucella lineola* lay their eggs in clusters and females probably decide on oviposition site and clutch size according to host plant qualities. The two host plants used in this study, *Salix viminalis* and *Salix dasyclados*, have previously been shown to differ in some aspects of quality (especially when it comes to secondary metabolites); these differences may have affected the beetles' oviposition patterns in the current study. The beetles' oviposition choices, especially clutch size, could also be motivated the existence of intraspecific and interspecific competition. Effects of competition and plant genotype may also show significant interactions on clutch size. The study result presented here show that females determine their clutch size based on the presence or absence of the other beetle species that uses the same resource. The result also show that the two beetles species responsed differently to competetion while ovipositing.

### *4.1.1 Fundamental niches: Effect of plant species on egg clutch distribution in the absence of interspecific competition:*

Ovipositing females should adjust their distribution of eggs according to variation in resources among their host plants. So female phytophagous insect should select the oviposition site where the availability of food is high and in that way ensure the maximum survival of their larvae. It has previously been shown that *Salix* quality is dissimilar between species and genotypes, especially when it comes to phenolic compounds, and tannins; and these substances undoubtedly affect the oviposition behavior as well as the offspring's development of leaf beetles. My results show that host plant species affect the egg distribution of the two beetles differently. Therefore I show that the fundamental niches of the two leaf beetles species differ from each other, even though they overlap. The results also show that the fundamental niches of both leaf beetles are affected by the host plant species. *S. dasyclados* contains high amounts of salicylates, and this may be the reason why I found a positive effect on the clutch size of *Galerucella* and a negative effect on *Phratora* and vice versa on *S. viminalis*. Clutch size may affect risk of parasitisms, and predation. The different clutch sizes on *S. viminalis* and *S. dasyclados* may therefore have important implications for the beetles trophic interactions. The patterns indicate that females are able to adapt the clutch size according to their host plant.

### *4.1.2* ***Realized nices: Effect of plant species on egg clutch distribution in the presense of interspecific competition****:*

I found that both beetle species modify their ovipositio behaviour in the presence of the other beetle species. This finding shows that the beetles utilize a narrower, or at least different, realized niche, in the presense of other competing beetle species. The clutch size seems to be more affected by host plant species  in the presense of interspecific competition than in the monospecific situations. In their realized niche, the two species also seems to adjust their clutch sizes by increasing or decreasing the clutch as compared to monospecific situations. The result shows that, though *Phratora* and *Galerucella* deposited their smallest clutch sizes on *S. dasyclados* and *S. viminalis* respectively in their monospecific condition, but they increase their clutch size according to the clutch size of opposite species while they involved in interspesific competition.  As a result intraspecific competetion among the neonate offspring should also increase when a second leaf beetle species is present. Intraspecific competition is normally more dominant and frequently powerful than than interspecific competition. Consequently, coexistence between the two competing species will occur very simply because total larval populations will prohibit to promote the specific beetle species populations growth before reaching the levels at which they exhibit their superiority to other. As was pointed out by MacArthur (1972), increasing intraspecific competition relative to interspecific competition promotes the coexistance of species. So it could be suggested that on competitive displacement do not necissarly have to occur.

The effect of the presence of a second leaf beetle species, on the egg distribution of a first species, could have to do with direct interspecific competition. However, another intriguing possiility is they indirectly affect each other by inducing changes in the leaf chemistry of the shared host plant. Recently Kaplan & Denno (2007), suggested that interspecific compitition between phytophagous insects is intensely affected by indirect competition mediated by plant responses to herbivry; and in competing herbivores one species could indirectly affect the other by inducing secondary compounds in the plant (Denno et al. 1995; Karban and baldwin1997).

### *4.2 Effects of plant species and interspecific competetion on eggs per leaf  and per plant:*

The presence of less secoundary phenolic compounds, and higher amount of leaves per plant, may be two reasons why both leaf beeetle species diposited more eggs per plant on  *S. viminalis* than on *S. dasyclados.* The results further show that when the two leaf beetle species coexist, the presence of one species negatively affect the number of eggs laid per plant by the other leaf beetle species on both host plant species. In this experiment, the affect of competition and genotypes interactions on number of eggs per leaf and per plant shows different competitive abilities of both species in their different niche, but eggs per plant of each beetle species shows that they seem to possess same competitive abilities in their realized niche, none of them are superior or inferior by eggs deposition. Ågren and Fagerstrom (1984) argued that under this conditions species have the abilities to occupy the same resource niche without exhibiting any competitive exclusion, though they demonstrated this argument for plants. Thus the beetles species coexist together and affect their total number of eggs in their realized niche.

## 4.3 Oviposition at the leaf area:

To select an optimal egg-laying site is crutial for the larval development and survival of herbivores. Ovipositioning placement of *Galerucella* and *Phratora* are always specific on the leaves of both host species (fig. 4 & 5); and in their realized niche the condition is well-off rather than their monospecific condition. In their realized niche none of them negatively influenced the opposite species ovipositioning place or did not mixed up their eggs cluster with each other. Egg aggregation of these beetle species also promote coexistence in the same way as resource partitioning. Both species females´ oviposite on specific places on same leaf area, reasulting in the conspecific larvae would be crowded into small patches. So increasing intraspecific competition relative to interspecific competition, that facilitating coexistence. Even, in their realized niche they tried to avoid the more vurnarable place though they faced difficulties for limited place for deposite eggs, such as – leaf base (great risk for predation) and upper surface (unplesent condition for the egg development because of sunlight, wind and rainfall as well as predator) of the leaves; they follow their usual ovipositioning placement behavior.. However, few eggs also find on these exceptional area could be explained by avoiding larval competitions on less spacious leaf or because of competition, sufficient time was unable to extended oviposition behavior by finding the identical place for egg release. So it could be suggest that, because of their different nature of placing eggs on the leaf area beetle species did not effect on the opposite species ovipositioning placement. Thus they share the same leaf area without any intense competition.

## 4.4 Beetles oviposition on shoot level:

Females place eggs normally on most nutritious and safest parts of plants. Both leaf beetle species, on each plant genotype maximum number of eggs had tend to gather middle portion of shoots (younger leaves of this part have suitable microclimate with nutrition availabilities, that is most important for the egg development) rather than lower or upper part, and this ovipositional behavior is same in their nonspecific condition as well interspecific competition (fig: 7). To avoide the intense interspecific competition and successfuly coexistance in their realized niche, among the species the most acceptable one could be that species, those utilize the resources by resources partitioning and also adopted the habitat by the another species and so they participate less in interspecific competition, though the effect and cost of the interspecific competition normally unequal and could be vary in different species. Selecting the ovipositioning place middle of soots, clearly disply the coexistance in same microhabitate, though host plant genotypes did not seems to effect directly the egg deposition behavior of both species. Beetles are tend to share the same ovipositional place without showing any superiority to each other because they select different specific place on the leaves of specific shoot area for ovipositioning. So, both beetle species use their partitioning habit while ovipositioning on the same leaf area and adjust their egg deposition behavior by properly utilized the resources in the realized niche.

## 5. Conclusions:

In line with my hypotheses I conclude that the fundamental niches of both beetle species (*G. lineola* and *P. vulgatissima*) are different from each other. As for their realized niches, it is clear that they adjust their egg laying behavior when they coexist on the same plant and thus they affect each other's realized niches. The host plant genotypes affect beetles fundamental and realized niche by their chemical profile in the leaves. Some phenological traits such as large leaf area, or high amount of leaves, also affect beetles egg laying behavior. However the behavior of beetles' egg placement on specific area (on the leaf level or on the shoot level) was not mediated by the host plant genotypes.

The present investigation only addresses to check the competitive abilities of two willow beetles species through the deposition of egg clutch and how the ovipositioning placement and egg aggregation intensifies the interspecific competetion on their realized niche. I only tried to focus on fundamental and realized niches of both species during their ovipositioning period. Nevertheless, it could be more authentic if the interspecific and intraspecific competition were also demonstrated between the larvae for food to growth and their development. Moreover, presence of other ecological factors (such as temperature, humidity) and natural enemies and how they affect the coexistence of beetles should also be interesting to study further since theoretical models revealed that density dependent mortality because of natural enemies can play a vital role in stable coexistence of competitors (Blossey, 1995; Holt 1987). Several important aspects for recognizing the realized niche of willow beetles such as, food consumption related to the egg clutch sizes, competition between the larval stages along with or without humidity, effect of plant genotypes and competition interactions on larval development stages will emphasized in an subsequent paper.

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