

# The influence of plant sex on the performance of a detrimental herbivore and two biocontrol agents in the dioecious grey willow, *Salix cinerea*

*Md. Faisal Kabir*



Master's thesis • 30 hec • Second cycle, A2E

Ecology - Master's Programme

Uppsala 2012

Independent project/Degree project / SLU, Department of Ecology 2012:13

# **The influence of plant sex on the performance of a detrimental herbivore and two biocontrol agents in the dioecious grey willow, *Salix cinerea***

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**Credits:** 30 hec

**Level:** Second cycle, A2E

**Course title:** Independent Project in Biology

**Course code:** EX0565

**Programme/education:** Ecology - Master's Programme

**Place of publication:** Uppsala

**Year of publication:** 2012

**Picture Cover:** Md. Faisal Kabir (3,4,5,6); Walwyn (1); Wahlens (2)

**Title of series:** Independent project/Degree project / SLU, Department of Ecology

**Part no:** 2012:13

**Online publication:** <http://stud.epsilon.slu.se>

**Key Words:** Plant sex, *Salix*, herbivore, omnivore, biocontrol, dioecious, *Phratora vulgatissima*, *Anthocoris nemorum*, *Orthotylus marginalis*

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Plant-Insect Interactions

## Popular Science summery

Plants with separate male and female flowers (dioecious plants) can be used as model systems for studies on how variation in host plants affects insect-plant interactions. This thesis describes an investigation into the relationship between plant sex and plant-herbivore-omnivore interactions. The studied host plant, *Salix cinera*, was chosen because of the importance of *Salix* species in the production of biomass for bioenergy generation in Europe. The herbivorous insect pest *Phratora vulgatissima* can reduce *Salix* biomass production by up to 40%. And still it is not proved which plant sex is less affected by the herbivore and my aim was to find that plant sex. The results presented herein demonstrate that both the studied herbivore (*Phratora vulgatissima*) and two omnivorous biocontrol agents, *Anthocoris nemorum* and *Orthotylus marginalis*, prefer female plants. However, the survival rate for herbivore eggs was lower on female plants than on males. All three studied insect species were found to be abundant on female plants in the field, for reasons that are currently unclear. The observed difference may be related to the finding that female plants tend to have larger leaves than males or that sex-related differences in catkin quality might be an important factor. Overall, the results obtained indicate that there is a greater degree of top-down control (i.e. control exerted by organisms at higher trophic levels over the biomass production or abundance of those at lower trophic levels) over *P. vulgatissima* survival on female plants, with omnivores being the major cause of their eggs in the field. These findings suggest that it may be advantageous to use female clones exclusively in commercial *Salix* plantations as a method for controlling herbivore activity and thereby minimizing yield losses.

## **Abstract:**

Dioecious plants can be used as model systems for studying how variation in host plants affects plant-insect interactions. This thesis describes investigations into the impact of plant sex on plant-herbivore-predator interactions for the host plant *Salix cinerea*. Both herbivores and predators were found to prefer female plants, all other things being equal. As a result, top-down control had a more significant impact on the herbivore population on female plants than was the case for males. Despite this, *Phratora* beetles were more abundant on, and preferred, female plants. This may be due to bottom-up control, since female plants have longer leaves. Both herbivores and biocontrol agents were more abundant on female plants in the field. Because of predation by omnivores, survival rates for herbivore eggs on female plants were lower than those for male plants, and omnivore predation was the primary cause of death among herbivore eggs. These findings may provide novel opportunities for pest control in *Salix* short rotation coppices.

## Table of Contents

1. Introduction: .....	6
2. Materials and methods: .....	7
2.1 Study species: .....	7
<i>Salix cinerea</i> L. ( <i>Salicaceae</i> ): .....	7
<i>Phratora vulgatissima</i> L. ( <i>Coleoptera: Chrysomelidae</i> ): .....	7
<i>Cacopsylla pulchra</i> Zetterstedt ( <i>Hemiptera: Psyllidae</i> ): .....	8
<i>Anthocoris nemorum</i> L. ( <i>Hemiptera: Anthocoridae</i> ): .....	8
<i>Orthotylus marginalis</i> Reuter ( <i>Hemiptera: Miridae</i> ): .....	8
Insect obtainment .....	8
2.1.1 Study area: .....	8
2.1.2 Setup of the experiments: .....	9
2.2 Herbivore and omnivore performance on male and female plants: .....	10
2.2.1 <i>Anthocoris nemorum</i> : .....	10
2.2.2 <i>Orthotylus marginalis</i> : .....	11
2.2.3 <i>Phratora vulgatissima</i> : .....	11
2.3 Herbivore and omnivore preference for male and female plants: .....	11
2.4 Field survey/Natural abundance of the insects: .....	11
2.5 Biocontrol efficiency on male and female plants: .....	12
2.6 Leaf size measurements: .....	12
3. Results: .....	12
3.1 Insect performance on male and female twigs: .....	12
3.2 Herbivore and omnivore preference on male and female plants: .....	15
3.3 Natural insect abundance: .....	16
3.4 Biocontrol efficiency on male and female plants in the field: .....	17
3.5 Leaf phenology: .....	18
4. Discussion: .....	19
4.1 Influence of plant sex on herbivores: .....	19
4.2 Effects of plant sex on predators: .....	19
4.3 Effects of plant sex on herbivore and predator densities in the field: .....	20
4.4 Biocontrol efficiency: .....	21
5. Conclusions: .....	22
6. Acknowledgements: .....	22
7. References: .....	22

## 1. Introduction:

Willows (*Salix*) are an important source of bioenergy in Northern and central Europe. However, as dioecious plants, willows exhibit sex-related differences in growth rate and susceptibility to attacks by various herbivores, which can reduce their biomass production by up to 40 % (Björkman et al. 2000). Recent studies have shown that nectar and pollen feeding by herbivores can have a strong impact on plant-herbivore interactions (Wäckers et al. 2007). *Salix cinera* is a dioecious plant that is attacked by various insects, including the herbivorous beetle *Phratora vulgatissima*, which can cause severe damage when it feeds on the plant's leaves. To address this issue, there have been efforts to use *Anthocoris nemorum* and *Orthotylus marginalis* as biocontrol agents. Both of these species are natural predators of *P. vulgatissima* that feed on its eggs. Ashman (2002) has suggested that natural enemies such as predators, omnivores and pathogens may play an important role in sexual-system variation (Åhman 1997). This thesis describes an investigation into the effects of plant sex on herbivores and predators as well as the interactions between them. Sex affects both plant quality and growth in most dioecious plants (Boecklen et al. 1990). Previous studies have shown that females tend to invest more resources into reproduction than males (Lloyd and Webb 1977, Delph 1999) and might therefore exhibit lower vegetative growth (Cornelissen et al. 2005.). In addition, it has also been reported that male plants support higher densities of herbivores or sustain greater rates of herbivory than do females (reviewed by Boecklen et al. 1993). This suggests that females invest more resources into defense than do male plants.

*Salix* is one of the most widely used systems for studying the impact of plant sex on plant-insect interactions. Boecklen et al. (1994) showed that male *S. lasiolepis* plants were more likely to be attacked by herbivores than female plants. Similarly, a review by Cornelissen and Stiling (2005) noted that male plants tended to be less well-defended and to harbor more herbivores than female plants. Åhman (1997) showed that male plants were less well-defended than females against herbivores but also found that sex had a relatively minor impact on herbivore populations for *S. viminalis*. However, it should be noted that Åhman (1997) did not consider the detrimental blue willow beetle (*Phratora vulgatissima*) in her study. In contrast to the results of many other studies, Banuelos et al. (2004) showed that male plants allocate more resources to defense than female plants in the dioecious shrub *Rhamnus alpinus*.

Dormann and Skarpe (2002) suggested that female *S. polaris* plants have higher reproductive costs for producing gametes and these costs imply a retarded growth; they should therefore be more well-defended than males (Dormann and Skarpe 2002). However, in relative terms, males invest more resources into vegetative growth than females (Putwain and Harper 1972, Harper 1977). Similar suggestions and findings were put forward by Hjältén & Palo (1992).

Most previous studies in this area have focused on plant-herbivore interactions and ignored the third trophic level. However, analyses of the third trophic level such as those reported in this thesis are needed in order to understand how top-down control of herbivores is affected by plant sex. The studies reported herein were conducted to determine the impact of plant sex on herbivores feeding on *Salix cinerea* and their omnivorous predators.

The studies presented herein were conducted to answer the following questions concerning the influence of plant sex on plant-herbivore-predator interactions in *Salix cinerea*:

- i) Do the predators and herbivores prefer one plant sex over the other (all else being equal)?
- ii) Do the predators and the herbivores perform better on one plant sex compared to the other (all else being equal)
- iii) Does the abundance of herbivores and predators differ between the two sexes in the field?
- iv) Does herbivore egg mortality (due to predation) differ between the two sexes in the field?

## **2. Materials and methods:**

### **2.1 Study species:**

#### ***Salix cinerea* L. (Salicaceae):**

*Salix cinerea* is a deciduous shrub/small tree that grows to heights of 4-15 m. Its leaves are spirally arranged and are usually 2–9 cm long and 1–3 cm wide, with a crenate margin. The flowers (which are known as catkins) are 2–5 cm long and are produced in early spring. It is dioecious with male and female catkins on separate plants. The male catkins are silvery at first, turning yellow when the pollen is released; the female catkins are greenish-grey, maturing in early summer to release their numerous tiny seeds, which are embedded in a white cottony down that facilitates wind dispersal. It usually grows in wetlands.

#### ***Phratora vulgatissima* L. (Coleoptera: Chrysomelidae):**

*Phratora vulgatissima*, also known as the blue willow beetle is blackish-blue in colour. It belongs to the Chrysomelidae family and is frequently found on various willow species. *P. vulgatissima* is most likely to be present and abundant at older Willow sites on clay soils and at sites that have certain free-living willow species growing nearby (Peacock et al. 2004) Egg production and larva proliferation varies depending on the surrounding vegetation and the weather (Sage & Tucker 1998). *Phratora* overwinters as an adult, emerging shortly after *Salix* leaves become available. It lays its eggs in April and May. Larvae

emerge in May-June and pupate in late summer. *Phratora* is univoltine in Sweden (Dalin 2011)

***Cacopsylla pulchra* Zetterstedt (Hemiptera: Psyllidae):**

The adult insects are grayish to yellowish in color; the male grows to lengths of 2.86-3.33mm and the female reaches 3.14-3.67mm (Ossiannilsson 1992). It has five instars. The 5<sup>th</sup> instar nymph is whitish-green, with the wing-pads and sclerites being concolorous or pale yellow. The species is widely distributed in Denmark, Sweden, and Norway. *Salix cinera*, *S. caprea*, and *S. repens* are important host plants for this insect. They overwinter as adults on conifers and their larvae are found in female catkins. (Ossiannilsson 1992).

***Anthocoris nemorum* L. (Hemiptera: Anthocoridae):**

The insect *Anthocoris nemorum* belongs to the flower bug (*Anthocoridae*) family. Usually they occur on willows, fruit trees and stinging nettles. They overwinter as an adult and become active when the surrounding temperature increases in the spring. First instar nymphs have been observed to feed on fluids from plants' leaves and twig bases (Stenberg 2010). They feed on aphids, thrips, mites and the eggs of various insects. However, their young feed primarily on the eggs of *Phratora vulgatissima* beetles. They also feed on plant sap but cannot normally survive on a strict vegetarian diet. In Sweden, they overwinter as adults and are often found feeding in willow flowers during the spring. The first instar larvae appear at the end of May (Liman 2003).

***Orthotylus marginalis* Reuter (Hemiptera: Miridae):**

*Orthotylus* is a genus of stinkbug from the miridae family. *O. marginalis* is one of the most abundant species from this family and is commonly found on willows. Adults of this species are around 6 mm long on average and their upper surface is covered with dense pale hairs. Their membrane veins are green and they have brown antennae. They overwinter as eggs on *Salix* stems and the nymphs start to appear in May. Both the nymphs and the adults feed upon the eggs and small larvae of *Phratora vulgatissima*, as well as larvae of *Galerucella lineola* and *Lochmea caprea*. It also feeds on mites, aphids and other small insects.

**Insect obtainment**

For all laboratory tests and experiments, adult insects were collected from the 60 different *Salix cinera* bushes located around the Ultuna campus and along county road 255, which runs between Uppsala and Märsta. Only healthy adult insects were used in these studies. All the insects were collected between the 3<sup>rd</sup> of April and the 10<sup>th</sup> of May, 2011.

**2.1.1 Study area:**

All insects and plant material used in this study were collected from 60 randomly selected *Salix cinerea* bushes (30 male and 30 female) located along a transect (county road 255) running between Uppsala (N 59° 80.611', E 17° 66.438') and Märsta (N59° 16.555', E17° 43.223'). This transect is approximately 30 km long.



Material from these 60 bushes was used in both the field experiments and the laboratory studies.



**Fig.1:** Map showing the locations of the sampled *Salix cinera* bushes (black symbols) along county road 255 between Ultuna and Märsta.

### **2.1.2 Setup of the experiments:**

The laboratory-based preference and performance tests were conducted using twigs that had been detached from the bushes. The experimental arena consisted of two plastic containers stacked on top of one-another (see Fig. 2a) and separated by a perforated partition. Water was placed in the lower container and the bottoms of the twigs were placed in the water. The insects were released into the top containers, which housed the upper sections of the twigs. The upper container was covered with perforated polythene in order to allow for gas exchange without permitting the insects to escape.



**Fig.2:** (a) Cafeteria experiment in the laboratory; (b) Performance tests **using male and female twigs** in the climate chamber; (c) **Testing egg survival** in the field.

## ***2.2 Herbivore and omnivore performance on male and female plants:***

The performance of *Phratora vulgatissima*, *Orthotylus marginalis* and *Anthocoris nemorum* on male and female plants was tested on detached male and female twigs in a climate chamber. Statistical analyses of the resulting data were conducted using Student's T-test in the R environment for statistical computing.

### ***2.2.1 Anthocoris nemorum:***

Adult Anthocorid bugs were collected on the 5<sup>th</sup> of May, 2011 from the same locations as the twigs, and stored in the laboratory at room temperature in a small box until they give birth to new nymphs. Green beans (*Phalesolus vulgaris*) were supplied as food while waiting for the nymphs to be born, and the bugs were inspected regularly to see whether the nymphs had yet been born. As soon as nymphs were observed in a box, the 1-2 day old nymphs were collected and individually placed in a rearing container (10cm × 8.5cm × 2 cm) with either one male or one female *Salix* twig (see Fig 2b).

All 30 male and female boxes were kept in a climate chamber at a temperature of 18.5°C during the day and 12.5°C at night until the insects within reached adulthood. The relative humidity within the climate chamber was 80%. The boxes were checked regularly to ensure that the experimental setup had not been disturbed. A piece of wet cotton was placed in each of the upper containers to ensure that the insects had access to water. The chamber was inspected every day and the point at which each insect reached adulthood was recorded. Once an

insect reached adulthood, it was dried and its total body length (excluding wings and antennae) was measured.

### **2.2.2 *Orthotylus marginalis*:**

Nymphs were collected in the field between the 1<sup>st</sup> and 12<sup>th</sup> of May in 2011 and grown according to the procedures described in the section 3.2.1 until they reached adulthood. Each nymph was provided with fresh leaf-bearing *Salix* twigs as a food source.

### **2.2.3. *Phratora vulgatissima*:**

The performance of *Phratora* on male and female plants was evaluated using the method of Lehrman *et al.* (2012). 30 adult female beetles were divided into two groups. Each insect was placed in a separate plastic rearing container (5cm × 1.5cm) containing a single male or female leaf of *Salix cinera* on 15th June 2011; 15 of the insects were given male leaves and 15 female. The insects were kept in a climate chamber at 18.5°C constantly to lay eggs. The number of eggs was counted every day until the first larva was observed, which typically occurred on the 8<sup>th</sup> day after insertion.

### **2.3 Herbivore and omnivore preference for male and female plants:**

Cafeteria experiments were performed with the three insect species (*Phratora*, *Anthocoris* and *Orthotylus*) to determine whether they have any preference for male or female *Salix cinerea*. This was done by putting one male (15cm) and one female twig (15cm) into an experimental arena (see illustration, Fig. 1) For each case, one individual insect was inserted into the arena and after 30 minutes the apparatus was inspected to see whether the insect was sitting on the male or the female twig. In some cases, the insect was moving around the container rather than resting on one of the twigs; when this occurred, the insect was removed and replaced with another. 50 replicates were used for each insect species and the data so obtained were evaluated using Chi<sup>2</sup> tests.

### **2.4 Field survey/Natural abundance of the insects:**

A field survey was conducted on the 19<sup>th</sup> of June, 2011, to investigate the abundances of the three insects on the two plant sexes. Insects were collected by knockdown sampling (*sensu* Björkman *et al.* 2003) from a 35-cm section at the top of a shoot. The following insect species were sampled: *Phratora vulgatissima*, *Anthocoris nemorum*, *Orthotylus marginalis*, and *Cacopsylla pulchra*. 22 male and 22 female plants along county road 255 between Uppsala and Märsta were sampled. The statistical significance of the differences between the observed abundances was evaluated using t-tests.

## **2.5 Biocontrol efficiency on male and female plants:**

The survival of *Phratora vulgatissima* eggs in the field was tested to study the predation pressure imposed by omnivores on the *Phratora* eggs on each plant sex. Female *Phratora* individuals were collected from the field and kept in the laboratory until they laid eggs on detached *Salix cinera* leaves in rearing containers. The containers were checked every day and the number of eggs per leaf was counted. Eggs that were less than 24h old were collected for the field experiment. 21 male and 21 female *Salix cinera* plants growing in the wild along the transect linking Uppsala and Märsta were selected for field studies and a clutch of ten eggs was attached to each one using insect pins. The number of eggs consumed by predators was counted every day until the first larva was observed, which typically occurred six days after the start of the experiment. The statistical significance of the differences between the observed egg survival rates was evaluated using t-tests.

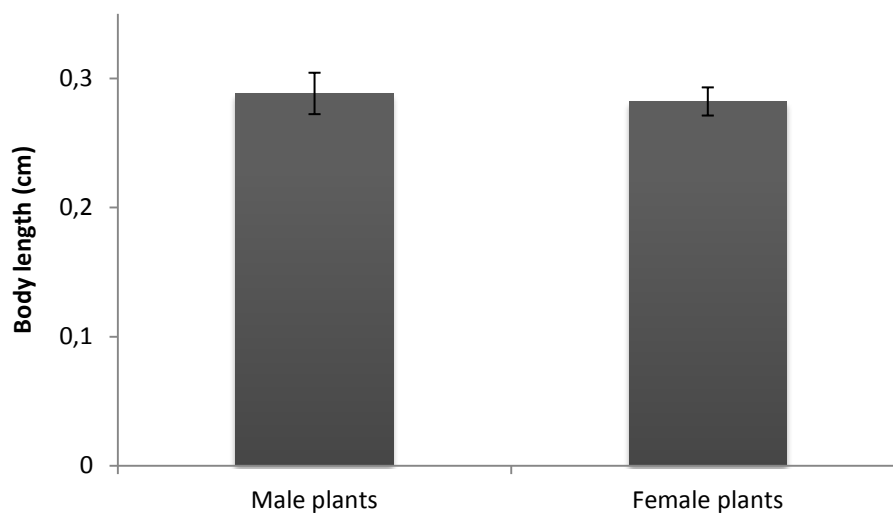
## **2.6 Leaf size measurements:**

The lengths of leaves on the uppermost 20 cm of randomly selected twigs of *S. cinera* were measured for both plant sexes (22 replicates). The number of leaves per twig within these 20 cm sections was also recorded.

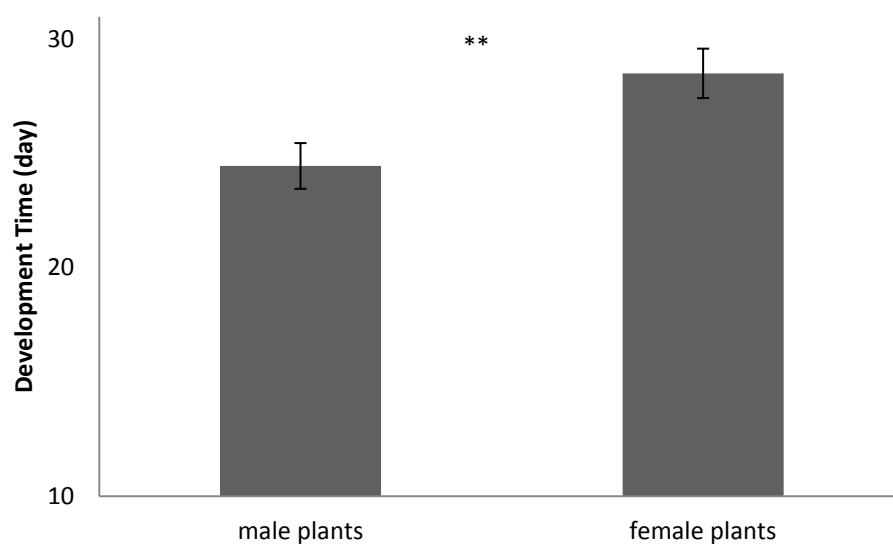
# **3. Results:**

## **3.1 Insect performance on male and female twigs:**

Performance tests for *Anthocoris nemorum* and *Orthotylus marginalis* were performed to determine how well they do on plants of different sexes in the absence of animal food. As shown in Fig. 3, there was no significant difference in the body lengths of *Anthocoris nemorum* individuals grown on male and female twigs ( $t = -0.320$ ,  $df = 49.335$ ,  $p\text{-value} = 0.750$ ). However, there was a significant difference between the two groups in terms of development time ( $t = 2.917$ ,  $df = 54.454$ ,  $p\text{-value} = 0.005$ ) as shown in Fig. 4. Individuals feeding on female twigs took longer to develop.

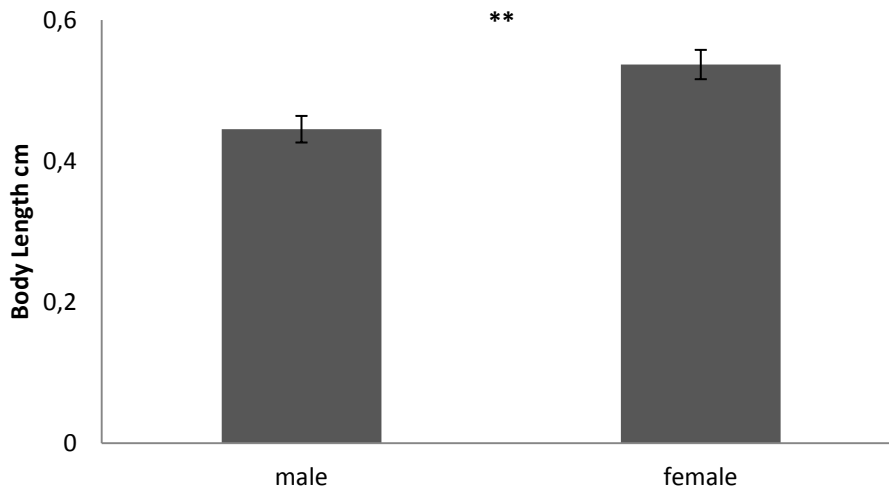


**Fig. 3:** Body lengths for adult *Anthocoris nemorum* individuals grown in the presence of male or female *Salix cinera* twigs in the laboratory without animal food (n=29). Error bars indicate standard errors.

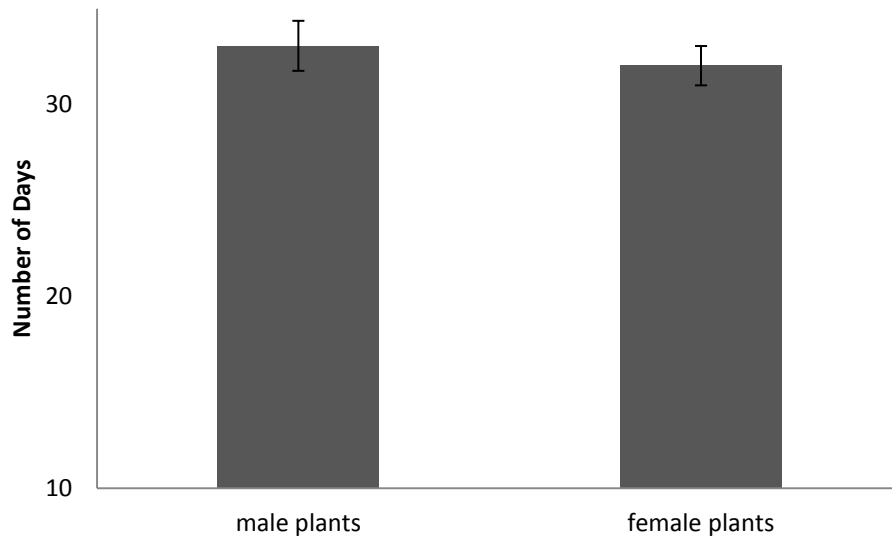


**Fig.4:** Development times for *Anthocoris nemorum* on male and female *Salix cinerea* twigs in the laboratory without animal food (n=29). Error bars indicate standard errors. \*\* indicates  $p < 0.01$ .

For *Orthotylus marginalis*, the t-test showed that individuals feeding on female twigs grew significantly larger than those feeding on male twigs ( $df = 55.473$ ;  $t = 3.2699$ ;  $p = 0.001852$ ; fig.5). There was no significant difference between male and female twigs in terms of the development time for *Orthotylus marginalis* ( $df = 53.46$ ;  $t = -0.623$ ;  $P = 0.535$ ; fig. 6).

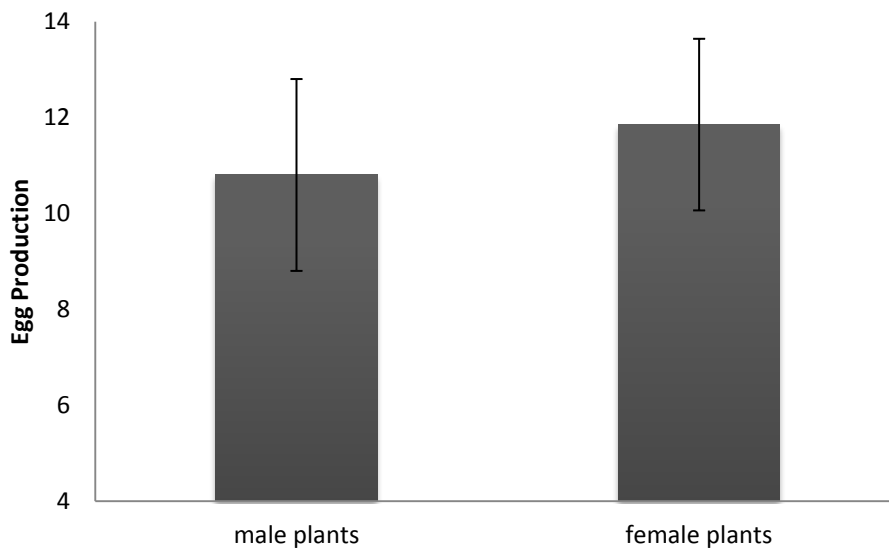


**Fig.5:** Body lengths for adult *Orthotylus marginalis* individuals fed on male vs. female *Salix cinerea* twigs in the laboratory without animal food (n=29). Error bars indicate standard errors. \*\* indicates  $p < 0.01$ .



**Fig. 6:** Development times for *Orthotylus marginalis* on male and female *Salix cinerea* twigs in the laboratory without animal food (n=29). Error bars indicate standard errors.

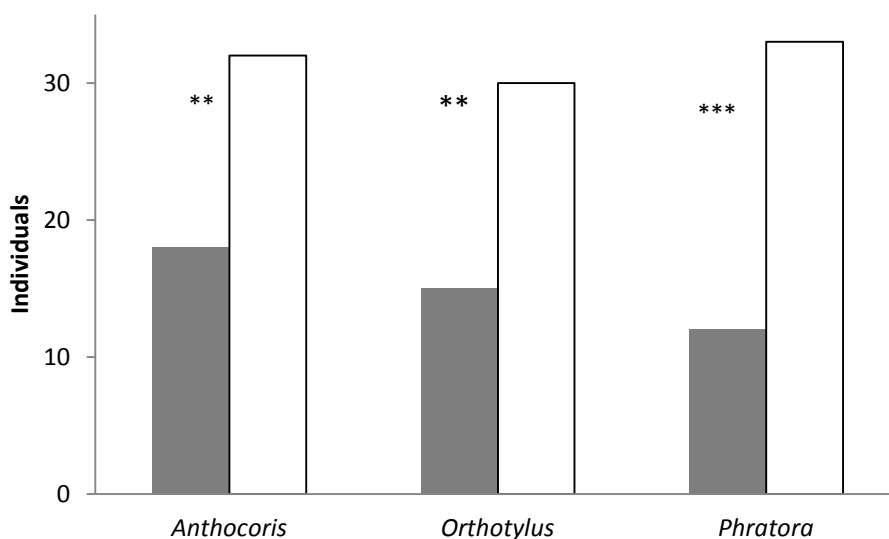
The average number of eggs laid by *Phratona vulgatissima* on male twigs was not significantly different from that for female twigs (df = 10.977;  $t = 0.866$ ;  $P = 0.404$ ; Fig 7).



**Fig. 7:** Average number of eggs laid by *Phratora vulgatissima* females on *Salix cinerea* in the absence of enemies in the laboratory. Egg laying period = 7 days; n= 20.

### **3.2 Herbivore and omnivore preference on male and female plants:**

In the cafeteria experiment using *Anthocoris nemorum*, 18 individuals preferred male twigs and 32 preferred female twigs ( $\chi^2= 6.76$  ; df = 1; p =0.009322; Fig. 8).



**Fig.8:** Number of insects that preferred male (black) vs. female (grey) *Salix cinerea* twigs in cafeteria experiments in the laboratory. n=50 for *Anthocoris nemorum*; n=45 for *Orthotylus marginalis* and *Phratora vulgatissima*. \*\* indicates  $P < 0.01$ ; \*\*\* indicates  $P < 0.001$ .

*Orthotylus marginalis* also exhibited a significant preference for female twigs: of 45 insects, 15 chose male twigs and 30 chose female ( $\chi^2= 8.7111$ ; df = 1;  $P = 0.003163$  ; Fig. 8). The herbivore *Phratora vulgatissima* also exhibited a significant

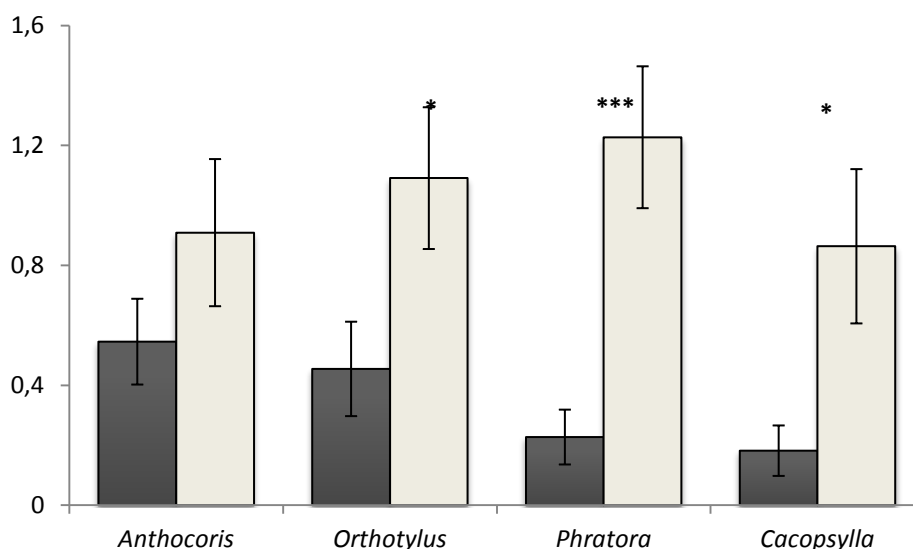
preference for female twigs ( $\chi^2 = 12.8508$ ;  $df = 1$ ;  $P = 0.0003373$ ; Fig. 7): of 45 beetles, 33 chose female twigs.

### 3.3 Natural insect abundance:

Twenty-two individual male and female plants were studied to investigate the natural abundance of the studied omnivores and herbivores on the two plant sexes in the field. The four insect species that were studied were all more abundant on female than on male plants (Fig 9 and Table 1).

**Table 1:** T-test results for the effects of plant sex on the natural abundances of the insects.

Insects	df	t-value	p-value
<i>Anthocoris nemorum</i>	33.78	0.7997	0.4295
<i>Orthotylus marginalis</i>	36.56	2.2404	0.0312
<i>Phratora vulgatissima</i>	3.9422	27.137	0.0005
<i>Cacopsylla pulchra</i>	2.51842	25.442	0.0104

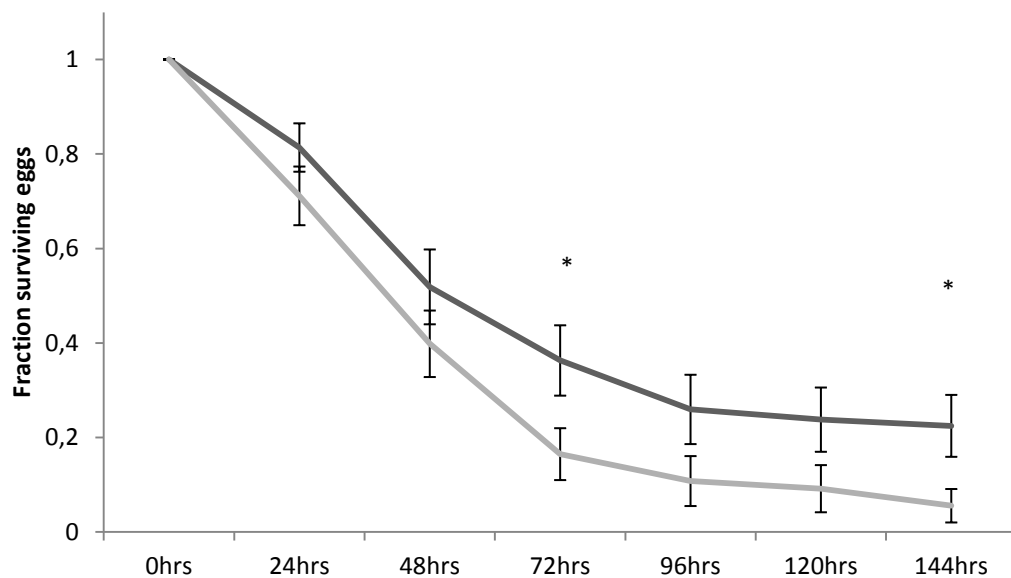


**Fig.9:** Natural abundance data for predatory bugs (*Anthocoris nemorum* and *Orthotylus marginalis*) and herbivores (*Phratora vulgatissima* and *Cacopsylla pulchra*) on male (black bars) and female (Grey bars) *Salix cinerea* in the field. Data were collected by knock-down sampling (n=22 bushes). \* indicates  $P < 0.05$ ; \*\* indicates  $P < 0.01$ ; \*\*\* indicates  $P < 0.001$ .



### 3.4 Biocontrol efficiency on male and female plants in the field:

Clutches of ten *P. vulgatissima* eggs each were attached to male and female *S. cinerea* trees growing in the field and the eggs' survival rates were monitored. It was found that survival rates on female plants were lower than those on male plants due to higher predation. Predation was the major cause of death for herbivore eggs in the field. The same number of eggs were initially present on both male and female twigs and the initial survival rate was similar for both sexes. However, after 72 hours, there were significantly fewer eggs remaining on the female plants than on the males (Table 2; Fig 10). After 144 hours, almost all of the eggs initially placed on the female plants had been consumed.



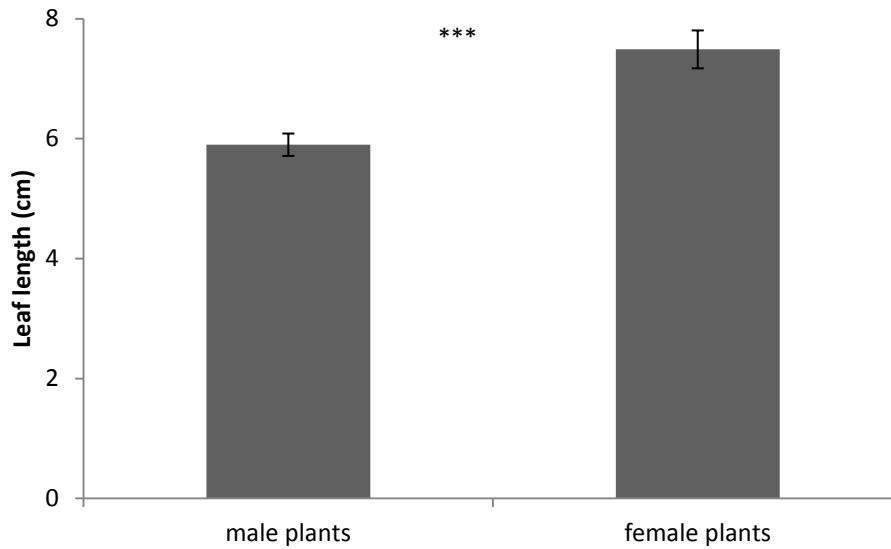
**Fig.10:** Fraction of surviving *Phratora vulgatissima* eggs in egg clutches attached to male (black solid line) and female (grey line) *Salix cinerea* trees in the field. Eggs that died were consumed by *Orthotylus marginalis* and *Anthocoris nemorum*. n=21. Error bars indicate standard errors; \* indicates p<0.05.

**Table 2:** T-test results for the effects of plant sex on the survival of *Phratora vulgatissima* eggs in the field after 24, 48, 72, 96, 120, and 144 hours.

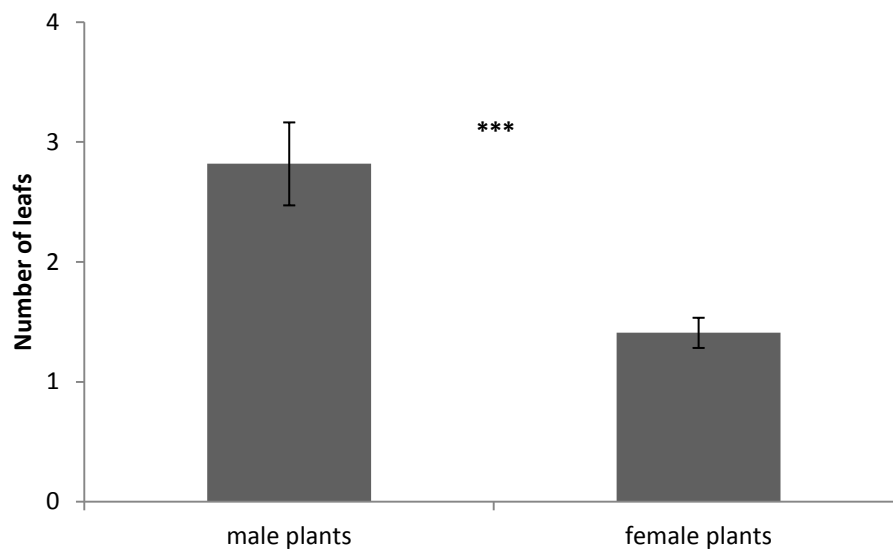
fraction	df	t-value	p-value
24hrs	38.604	-1.2714	0.2112
48hrs	39.454	-1.1369	0.2624
72hrs	36.791	-2.1432	0.03878*
96hrs	36.353	-1.677	0.1021
120hrs	36.699	-1.7319	0.09168
144hrs	30.765	-2.2693	0.03041*

### 3.5 Leaf phenology:

Based on 22 observations, the leaves of female plants were significantly longer than male leaves ( $df = 30.916$ ;  $t = 4.1174$ ;  $p = 0.0002$  where  $p < 0.001$ ; Fig. 11).



**Fig.11:** Leaf lengths in male and female *Salix cinera* plants ( $n=22$ ). \*\*\* indicates  $P < 0.001$ . Error bars denote standard error.



**Fig.12:** Numbers of leaves on a 20 cm section of a single twig in male and female *Salix cinera* plants ( $n=22$ ). \*\*\* indicates  $P < 0.001$ . Error bars indicate standard errors.

Fig.12 shows that male plants tended to have significantly more leaves than females ( $t = -3.8283$ ,  $df = 26.465$ ,  $p\text{-value} = 0.0007$ )

## 4. Discussion:

### 4.1 Influence of plant sex on herbivores:

The results presented herein show that female *Salix cinerea* attract and support herbivores (the defoliating leaf beetle *Phratora vulgatissima* and the sap-sucking psyllid *Cacopsylla pulchra*) to a greater extent than male plants. This stands in contrast to previous findings that male plants are often more susceptible to herbivory than females in other plant species (Boecklen et al., 1990, 1994; Hjalten, 1992; Fritz, 1995; see review by Cornelissen and Stiling, 2005). Previous investigations into the effects of plant sex have suggested that female plants invest comparatively more resources into reproduction, and should therefore be more well-defended than males (Cornelissen and Stiling 2005, and references therein). This has also been suggested for *Salix* (e.g. Boecklen et al. 1994; Dormann and Skarpe 2002; Hendricks et al. 2003) but based on the results presented herein, it does not seem to be the case for *S. cinerea*. It is possible that female *S. cinerea* is more susceptible to *Phratora* than the male because the female leaves are comparatively larger than the male leaves (at least during the early summer when the phenology study was undertaken; see Fig. 11). Larger leaves may provide more feeding resources and shelter to the beetle.

It is also possible that sex-related differences in the chemical compositions of the leaves may account for some of the results obtained in this work, but time constraints made it impractical to investigate this hypothesis.

The greater abundance of psyllids on female *S. cinerea* is readily explained. According to Ossiannilsson (1992), *Cacopsylla pulchra* lay their eggs in female catkins only, which would explain why many more *Cacopsylla pulchra* individuals were found on female plants (see fig.9).

Natural predators of *Phratora* and *Cacopsylla* were also more abundant on female twigs (see section 5.2). While this might have been expected to reduce the herbivores' willingness to settle on female plants, the opposite was observed. It thus seems that predators do not exert top-down control over the feeding behavior of the herbivores in this case; instead, it seems that the herbivores' feeding behavior is dictated by plant phenology, i.e. from the bottom up (Figs. 11 and 12).

### 4.2 Effects of plant sex on predators:

To the author's knowledge, only a few papers dealing with the effects of plant food on sap-feeding predators have been published (Agrawal et al. 1999; Eubanks and Denno 1999; 2000; Stenberg et al. 2010; 2011), and none of these studies examined the effects of plant sex on the predators. Like the psyllid, the two predators investigated in this study also suck sap from the plant and are should thus respond to sex-related differences in plant properties and defenses. In addition to sap, the twigs used in these experiments also carried catkins, which

provided pollen or nectar to the predators. Like the herbivores, the two omnivorous predators also preferred female plants. One of the predators (the mirid, *Orthotylus*) performed better on female plants, while the other (the anthocorid, *Anthocoris*) performed better on males in the absence of animal food. These differences coincide with differences in leaf phenology (see fig. 11 and fig.12), but identifying the mechanisms responsible for these differences in performance is beyond the scope of this thesis. Stenberg et al. (2010; 2011) reared *Anthocoris nemorum* and *Phratora vulgatissima* individually on a number of different *Salix* genotypes and found that genotypes which the beetle preferred and performed well on were unsuitable for the anthocorid and vice versa. In my case, the anthocorids developed more slowly on female plants, which were more suitable for the beetles.

The sex of the host plant was not found to have any significant effect on body length in the omnivore *Anthocoris nemorum* (see fig.3) but there were significant sex-related differences in development time for this species (see fig.4). Individuals that fed on female plants took longer to develop, which is consistent with the findings of Wolfe (1997), who noted that male flowers are more abundant and longer than those of the female, and so attract more florivores. In addition, Hendricks et al. (2003) suggested that the male plants might offer higher quality food, which would account for the longer development times seen on female plants.

Interestingly, the results obtained for *Orthotylus marginalis* were directly opposed to those seen with *A. nemorum* (see fig.5): adult *O. marginalis* individuals that had been reared on female twigs (in the absence of animal food) were larger than those reared on male plants. This could be due to sex-related differences in food quality. However, this observation and hypothesis contradict the results of Ashman (1994) and Roulston et al. (2000), both of whom proposed that male flowers might be better food sources due to their pollen, which is rich in nitrogen.

It should be noted that there were some technical problems relating to the operation of the climate chamber used in these experiments, which may have affected the reliability of the results obtained. However, the apparent contradiction between the results presented herein and those from previous studies remains to be explained.

In contrast to the situation observed for *A. nemorum*, *O. marginalis* individuals fed on male plants took longer to develop than those feeding on female plants (see fig.6) This result is hard to explain and is not consistent with the findings of Ashman (1994) and Roulston et al. (2000).

### **4.3 Effects of plant sex on herbivore and predator densities in the field:**

In general, the lab experiments indicated that female *S. cinerea* trees were more suitable food source for herbivores and omnivores than the males, from a

bottom-up perspective. To complement the lab experiments, field studies were conducted to determine the actual abundances of the insects. These also indicated that female plants harbored higher densities of *A. nemorum*, *O. marginalis*, *P. vulgatissima* and *C. pulchra* (see Fig. 9). As discussed in section 5.1, herbivores' preferences and performance are probably subject to bottom-up control, which would explain their greater abundance on female plants. The mechanisms responsible for the observed trends in predator performance on female plants are less clear. Females seem to attract both anthocorids and mirids, but only mirids perform better on females (in the absence of animal food). It is thus possible that the mired at least is subject to bottom-up control as well. However, the relatively high densities of herbivores on the female plants would also tend to support greater predator densities, and this may have a greater impact than plant quality per se.

#### **4.4 Biocontrol efficiency:**

In the field, *Phratora* eggs had lower survival rates on female plants than on males (Fig. 10). This is probably the most important finding of those reported in this thesis. Egg clutches were attached to selected plants in order to expose them to field conditions and observed daily. The effects of direct predation by omnivores were readily detected by considering the fraction of eggs consumed each day. Overall, herbivore eggs on female plants were significantly less likely to survive than those on male plants, indicating that they are subject to top-down control stemming from predation. This is consistent with the high abundance of omnivores on female plants.

**Table 3:** Fraction surviving *Phratora vulgatissima* eggs on male vs. female eggs in *Salix cinerea* trees in the field.

sex	hrs	24hrs	48hrs	72hrs	96hrs	120hrs	158hrs
male	1	0.813	0.518	0.362	0.259	0.237	0.224
female	1	0.711	0.398	0.164	0.107	0.0916	0.055

These results suggest that female plants are more resistant to insect herbivores due to their tendency to attract omnivores that can function as potent biocontrol agents. It may be advantageous for commercial *Salix* growers to use female clones in order to minimize the impact of herbivore outbreaks. However, the impact of the damage caused by herbivores in the field should be investigated in future studies before implementing any such measures.

## 5. Conclusions:

The results presented in this thesis indicate that omnivores such as *Anthocoris nemorum* and *Orthotylus marginalis* significantly reduce the size of herbivorous *Phratora vulgatissima* populations on *Salix cinerea*. Because the density of omnivores is higher on female plants, there is more severe predation of herbivore eggs, which reduces the size of the plant's herbivore population. As such, it may be useful to use only female clones in commercial *Salix* plantations. This should reduce the size of the herbivore population within the plantation, which should in turn reduce yield losses. However, female plants also supported a greater density of herbivores than male plants, probably as a consequence of bottom-up mechanisms. It might therefore be tempting to include male plants in short rotation coppices. However, since male plants attract relatively few omnivores, a severe herbivore outbreak in a male-heavy coppice could be very difficult to control and might have devastating effects. Thus, while female plants host greater densities of herbivores, they are likely to be more resistant to major outbreaks. While it may be undesirable to have males in direct contact with commercial *Salix* plantations, it may be advantageous to have some male plants nearby to serve as a source of pollen for the omnivores. The precise reason for the omnivores' preference for female *Salix* trees remains unknown, and more research will be needed to properly understand the physical and chemical factors responsible and their effects on insect population dynamic aspects.

## 6. Acknowledgements:

I am deeply grateful to my supervisor Dr. Johan A. Stenberg. Without his close supervision I would have been unable to complete my project successfully. I would also like to thank my examiner Richard Hopkins, as well as Kwabena O. Baffoe and Peter Dalin for their help and suggestions.

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