THE EFFECT OF FOREST SOIL PRODUCTIVITY AND VEGETATION TYPE ON DIPRIONID SAWFLIES COCOON PREDATION

Mar Ramos Sanz

MASTER´S THESIS OF SCIENCE IN AGRICULTURE AND FORESTRY
SPECIALIZATION IN FOREST ECOLOGY AND SILVICULTURE
Ramos Sanz, Mar. 2016. The effect of Forest soil productivity and vegetation type on Diprionid sawfly cocoon predation. University of Eastern Finland, Faculty of Science and Forestry, School of Forest Sciences, Master thesis of Science in Agriculture and Forestry, specialization in Forest Ecology and Silviculture, 52p.

ABSTRACT

The abundance of insect populations can change dramatically from generation to generation, and large increases are commonly known as "outbreaks". Insect outbreaks can be extremely destructive when the insect is considered as a crop or forest pest or it carries disease to humans, farm animals, or wildlife. Due to the economic losses caused by pests, it is very important to know and understand the processes (biotic or abiotic) that regulate insect populations. In Finland, pine sawflies (Hymenoptera, Diprionidae), consisting mainly in two species; *Diprion pini* (L.) and *Neodiprion sertifer* (Geoffroy) are one of the major defoliator groups of Scots pine forests. Several studies have recently estimated future outbreaks of pine sawflies, in the light of these future threats researchers considered that there is an increasing need in study their main controllers.

In this research, I studied one of the most important agents controlling pine sawflies populations, the cocoon predators, and how they are affected by the environment. Among other hypothesis and theoretical background, I took into account the Exploitation Ecosystem Hypothesis (EEH), which makes different predictions for predation depending on productivity levels. Hence, I focused my study on the different vegetation types of Scots pine forests. I used empirical models for the diprionid cocoon predation pressure, specifically generalized linear mixed model (GLMM) techniques. The mean percentage of predation was predicted as a function of forest vegetation type and season. The results of this research show that predation pressure is highly related with the type and structure of the forest where the pine sawflies predators live and predate. Rich forests (Mesic forests in this study) with higher vegetation diversity and structures supported the highest levels of pine sawflies cocoon predation.

Keywords: insect outbreaks, pine sawflies, predators, forest vegetation type and Exploitation Ecosystem hypothesis (EEH).
FOREWORD

This thesis is the final project for a M.Sc. of Agriculture and Forestry specialization in Forest Ecology and Silviculture, degree at the University of Eastern Finland. The thesis was conducted in the laboratories of Metla building, which form part of the Finnish Natural Resources Institute (Luke) and in Scots pine stands owned by Paihola Forest Common and UPM, located near Joensuu (Finland). The advisory committee has two members: Dr. Seppo Neuvonen (Luke) and Dr. Olli-Pekka Tikkanen from the University of Eastern Finland. I would also like to express my gratitude to all the people from Metla building for their help and technical support, and Antonio Rodríguez Olmo for his consultation and advice.
**TABLE OF CONTENTS**

1. **INTRODUCTION** .................................................................................................................. 6

2. **Background and objectives** .............................................................................................. 6
   1. 1. *Insect outbreaks and their importance in Forest ecosystems* ................................... 6
   1. 2. *Forest insect outbreaks and main hypotheses of herbivore control* .................. 7
   1. 3. *Pine sawflies and their control* ................................................................................. 9

2. **Hypothesis and specific objectives** ................................................................................. 12

2. **MATERIAL AND METHODS** ......................................................................................... 13
   2. 1. Insect cultures .............................................................................................................. 13
   2. 2. Study insect life cycles ............................................................................................... 15
   2. 3. Study sites .................................................................................................................. 16
   2. 4. Experimental design ................................................................................................... 17
   2. 5. Pupae/Cocoon analysis ............................................................................................. 19
   2. 6. Statistical analysis ...................................................................................................... 21
   2. 6. 1. *Data analysis and modeling* ............................................................................. 21

3. **RESULTS** ......................................................................................................................... 24
   3. 1. Total predation ............................................................................................................ 26
   3. 2. Vertebrate predation ................................................................................................. 29
   3. 3. Invertebrate predation .............................................................................................. 31

4. **DISCUSSION** .................................................................................................................... 33

5. **CONCLUSIONS** ............................................................................................................... 37

6. **REFERENCES** ..................................................................................................................... 38

7. **APPENDIX** ............................................................................................................................. 47
1. INTRODUCTION

Variations in space and time of host plant quality can affect the effectiveness and behavior of insect herbivores populations, ending in severe cases, to explosive increases in abundance (outbreaks) of those insect species (Berryman 1999). Herbivores during these periodic oscillations or outbreaks, can in return, influence their host resource availability (Krause & Raffa 1996). Such interactions may be complicated by the presence and interactions with natural enemies, which can in some cases mediate outcomes of plant–insect associations at the individual and population levels (Power 1992, Krause & Raffa 1996). In this context, and considering the ecological and economical importance of insect outbreaks, a better understanding of how these interaction on tri-trophic systems, would be a key issue in future forest pest management, through the development of more effective, and environmentally benign natural integrated pest-management strategies (Krause & Raffa 1996).

1. Background and objectives

1. 1. **Insect outbreaks and their importance in forest ecosystems.**

An outbreak can be defined as an explosive increase in the abundance of a particular species, commonly called *pests*, which occur over a relative short period of time (Berryman 2003, FAO 2010, Barbosa et al. 2012). In a broad sense, a pest is an insect that can damage or kill, during some stage of their development; agricultural crops, forest stands, ornamental plants, or native plants *in situ*, consume and/or damage harvested wood, cause illness or unproductivity in agricultural animals (i.e: cattle), or be vector of human diseases (Berryman 1987, Wallner 1987, Liebhold et al. 1995, Liebhold & McCullough 2011).

Temporal and spatial oscillations in abundance, or “outbreaks”, are one of the most significant characteristics of animal and insect population dynamics (Liebhold et al. 1995). These cycles affect the ecosystems in which insect populations live and develop and are common in many forest insect populations (Esper et al. 2007, Björkman et al. 2011). Impacts of forest insect outbreaks include extensive defoliation and/or tree mortality concluding in many cases with decreases in forest productivity and carbon storage, affecting forest structure, species composition and other ecosystem patterns (Niemelä et al. 2001, Jepsen et al. 2008). Outbreaks of insect herbivores causing a intermediate disturbances are known to act as important drivers of forest dynamics (Hunter 2001, Kouki et al. 2001, Behmer 2009).
There are many more factors affecting these cycles, however measuring and evaluating their influence is a difficult task (Coviella et al. 1999, Bale et al. 2002, Aukema et al. 2006, Tscharntke et al. 2007). In the case of climate, the intricacy of those ecological interactions are really difficult to quantify or predict especially changes in ecosystem processes (Mattson & Haack 1987, Volney & Flemming 2000, Logan et al. 2003). The problem in factors such as time and space is that detection and evaluation of those changes needs the measurement of long time series, and wide geographical ranges which are not commonly available for most natural systems (Ovaskainen & Hanski 2002, Hastings 2004, Koons et al. 2005, Yakamura et al. 2006).

Taking into account not only damages caused by defoliation, but also impacts of forest insects affecting nutrient cycle in the forest ecosystem, it can be considered that worldwide insect pests affect around 35 million hectares of forests each year, with their consequent economic losses (Allen et al. 2010, Alalouni et al 2013, Haynes et al. 2014). Economic losses caused by defoliating pest insects can be substantial (~300 - 1000 EUR ha\textsuperscript{-1} depending on intensity of needle loss and the length of outbreak period) (Latva-Käyrä 2011). Thus, enhancing the understanding of the biological controllers of these insects, and their potential success in controlling and regulating these herbivores population has become a significant topic in the field of forest research.

1. 1.2. Forest insect outbreaks and main hypotheses of herbivore control.

Along history, many insect species had caused severe losses related with their outbreaks, yet scientists are still trying to understand the outbreak phenomena (Berryman & Turchin 2001, Berryman 2003, Price et al. 2005). During decades, insect pests have provided to insect ecology researchers with many challenges (Kendall et al. 1998, Liebhold & Tobin 2008, Alalouni et al 2013).

Many hypotheses have been formulated in an effort to explain the causes of insect outbreaks (Berryman 2003, Kessler et al. 2012, Stenberg 2015). Multitude of negative feedbacks from lower (e.g. host plants, prey) and/or higher trophic levels (e.g. predators, diseases) are able to produce insect outbreaks, if the feedback is impacting after a time lag (Berryman 1996). However, in general, researchers agree that most oscillating cycles and outbreaks in forest pests, are the result of a combination of trophic interactions that contribute delaying negative

Along the history, two completely opposite approaches have been proposed to explain the population dynamics of herbivores (Moreau et al. 2006). Top-down approach point out that herbivore populations primarily by the trophic level above (i.e. natural enemies) (Hairston et al. 1960). This first approach was presented by Hairston et al. (1960) and although, they did not pose the question in their article it has been translated as “why the world is green?” till now (Hairston et al. 1960, Matson & Hunter 1992, Bond 2005).

In contrast to the top-down approach presented by Hairston et al. (1960), the bottom-up approach (Mattson & Addy 1975) suggested that the trophic level below (i.e. the plant resource) is the principal limitation of herbivore populations. Thus, bottom-up hypotheses, consider that herbivores and other organisms are resource limited (e.g., low host quality, presence of repellent or deterrent chemicals on their host plants etc.), even if their hosts plants appear to be abundant (Janzen 1988, Kessler & Baldwin 2002). Bottom-up hypotheses considered that herbivores do not have a regulating effect or any significant influence on the productivity of their host plants (Denno et al. 2002, Price et al. 2005, Turkington 2009).

Another view, consisted in considering that both, bottom-up and top-down processes can act together to influence the dynamics of herbivore populations (Hunter et al. 1997). This new joint hypothesis has become largely accepted recently (Moreau et al. 2006). According to this new concept, the instability on the herbivore population dynamic responses, could rely on several attributes of the community (Hunter 2001). Including both, bottom-up stimulus such as food quality of the host plant, and top-down stimulus such as the behaviour, abundance and success of natural enemies (Coupe & Cahill 2003). However, there are still remaining questions relating to the stability among these trophic forces, and how changes in the ecosystem can affect this stability (Rosenheim 1998, Terborgh et al. 2001, Turkington 2009, Kollberg et al. 2013).

Primary productivity has been postulated as one of the many factors modulating the relative strengths of bottom-up and top-down forces on herbivore population dynamics (Coupe & Cahill 2003). Related to this Oksanen et al. (1981) described the Exploitation Ecosystem hypothesis (EEH) that converges with Hairstorn´s hypotheses with respect to productive areas (forests and their successional stages, productive wetlands etc.). According to EEH, the control of herbivores by predators declines in unproductive ecosystems (tundras, high alpine
areas, steppes and semi deserts among others) which can be then characterized by their intense natural folivory (Oksanen et al. 1981, Oksanen & Oksanen 2000).

Thus, in low productivity environments plant biomass will be limited by nutrient availability and herbivore populations effect on biomass regulation will be low (Oksanen et al. 1981). In intermediate environments herbivore populations will be sustained by plants but not predator populations and the system will be dominated by plant-herbivore interaction (Oksanen & Oksanen 2000, Turkington 2009). Finally, in rich systems, plants can withstand both populations of herbivores and predators, and in those environments the system will be regulated by predator-prey interaction where predators will keep herbivore populations low enough to have little impact on the plant biomass (Oksanen & Oksanen 2000).

1. 1.3. Pine sawflies and their control.

Pine sawflies are among the most common defoliating insect species of pines forests all over Europe (Larsson & Tenow 1984, Lyytikäinen 1994, Virtanen et al. 1996, Augustaitis 2007, Dukes et al. 2008). Outbreaks are usually followed by long periods of low population densities for a number of decades or longer, although the species are still present in the forest ecosystem (Niemelä et al. 2001, De Somviele et al. 2004). Pine forests vary in their susceptibility to sawflies invasions, and outbreaks are more common in pine forests on low productivity soils (Niemelä et al. 2001, De Somviele 2004; 2007, Jepsen et al. 2008, Allen et al. 2010, Björkman et al. 2011, Nevalainen et al. 2015).

Pine sawfly larvae of Diprion pini (L.) and Neodiprion sertifer (Geoffroy) (Hymenoptera: Diprionidae), are the most common defoliating sawflies of Scots pine trees, Pinus sylvestris L., in Finland (Juutinen & Varama 1986, Niemelä et al. 1991, Virtanen et al. 1996, Lyytikäinen-Saarenmaa 1999). Outbreaks typically occur within 10-20 years intervals in central Europe (Hanski 1987, Pschorn-Walcher 1987, Larsson et al. 2000, Anderbrant 2003, Kurkela et al. 2005). According to Berryman (1987), the most common type of pine sawfly outbreak is sustained and eruptive, which is defined as; an outbreak that spreads from local epicentres to cover large areas and persists for several to many years (Larsson & Tenow 1984, Berryman 1987, Virtanen et al. 1996).

Among other damages, large sawfly infestations can cause growth loss and mortality, especially when followed by attacks from bark and wood-boring beetles (Coleoptera:
Buprestidae, Cerambycidae, Scolytidae,) (Kolomiets et al. 1979, Neuvonen & Niemelä 1991). Pine sawflies species, previously considered as harmless species, are now causing severe damage in Finnish forests (Lyytikäinen-Saarenmaa & Tomppo 2002, de Somviele et al. 2007), not only because of their rise and proliferation in affected areas, but also because of their high damage potential (Kouki et al. 2001, Niemelä et al. 2001, Barre et al. 2002, Kantola et al. 2011).


The main important research on diprionid cocoon predators was done by Hanski in the 90’s decade. According to his work the control of a prey at low density requires the operation of one or more directly density-dependent factors, for example predation (Hanski 1990b). In this context one may be attempted to dismiss predation by shrews and other omnivorous mammals as unimportant when no density dependence has been detected (Hanski 1990a).

However, considering the spatial element in outbreaks Hanski suggested that sawfly populations should had been examined in the context of metapopulation scenario in which small mammals might play a major role in the control of pine sawflies. This hypothesis is commonly known as the “generalist predator theory” or more generally the “metapopulation model of forest insects dynamics” (Hanski 1990a, Hanski & Hettonen 1996, Hanski et al. 2001).

Related with Hanski´s work Oksanen et al. (1981) proposed their Exploitation Ecosystem hypothesis (EEH), in which they pose that different predictions on predation and pest control could be obtained considering the dependence upon productivity levels on the ecosystem in which they operate (Oksanen et al. 1981, Oksanen & Oksanen 2000).
Both hypothesis pointed that predation pressure and effectiveness may be significantly affected by the habitat in which predators operate, the structure of the forest floor, the availability of food resources, affecting consequently on their success and increasing the probability risk of outbreaks in certain insect species (Oksanen et al 1981, Hanski 1987;1990a, Kollberg et al. 2013). I took into account these two hypotheses and their theoretical background to make my own hypothesis and tried to answer the ecological interactions studied in this thesis.

I studied the cocoon predation pressure and effectivity, taking into account not only the presence and abundance of prey but also the quality of the environment in which the predators and preys are located. In my knowledge, there are no previous studies where, the effect of all cocoon predators (vertebrates and invertebrates), that potentially regulate pine sawflies populations of both species, are considered, and the effect of the environment in their predation success is quantified. In this study the vegetation type that describes the productivity of the forest stands was hypothesized as a key factor in the success of the control of pine sawfly populations by cocoon predation.
1. 2. Hypothesis and specific objectives.

The main hypothesis of this study is:

“High productivity forest soils (mesic vegetation type) are expected to have higher predation pressure on pine sawfly cocoons than lower productivity forest soils (xeric and sub-xeric vegetation types)”

Specific objectives:

1) Analyse the cocoon predation response considering all types of predators in three different forest vegetation types.

2) Analyse the cocoon predation response of vertebrate predators in three different forest vegetation types.

3) Analyse the cocoon predation response of invertebrate predators in three different forest vegetation types.

4) Analyse the different predation responses of vertebrate and invertebrate predators.
2. MATERIAL AND METHODS

2.1. Insect cultures

For conducting this experiment approximately 3000 larvae of Neodiprion sertifer (Geoffroy) (Hymenoptera: Diprionidae), were reared during the summer of 2014. The larvae were from different locations near Joensuu and Puumala (this last location was chosen because it maintains a permanent population of the insects). The insect were disposed in groups of 20-30 larvae of same instar of development in plastic boxes with food ad libitum (Scots pine shoots and cut branches) until they reached the pupae instar (cocoon) (see Figure 1; cf. Baldassari et al. 2003). The rearing took place in the laboratories of Metla building (Joensuu) in a period of two months, from 26 of May to 31 of July.

The plastic boxes for rearing were divided in three layers from bottom to top (see Figure 1):

1. The bottom layer consisted on Sphagnum and other mosses previously treated in order to avoid soil predators and other biotic agents (Sphagnum and soil were boiled and then dried).

2. The second layer was a wet filter paper with a threefold purpose; keeping a moist environment inside the box, preventing contamination of the Sphagnum layer by the larval faeces, and setting an easy to clean division between the larvae and the Sphagnum layer, where larvae can move but no their faeces (some larvae prefer to pupae inside the Sphagnum layer) and because the cleaning of faeces was easier.

3. The third layer contained the larvae and the plant material (Scots pine shoots and cut branches) which was previously washed and checked to avoid the presence of predators such as spiders, beetles or others.

4. Finally the top layer consisted of net cloth that allowed the entrance of air in the box while preventing the escape of larvae.

Every second day during two months boxes were checked and faeces were removed from the middle layer, the filter paper was changed, dead larvae were removed and fresh new plant material was changed and added. The rearing boxes were maintained clean in order to avoid contamination and larval mortality (Olofsson 1987, Heliövaara et al. 1991, Björkman & Gref 1993, Larsson et al. 2000, Giertych et al. 2007).
The boxes were placed in the laboratory at room temperature (18±2°C) with a natural photoperiod and their position in the room were changed randomly every day (Barre et al. 2002, Baldassari et al. 2003, Kollberg et al. 2013) (see Figure 1).

Figure 1. Neodiprion sertifer larvae (a) and pupae (b), a rearing box presenting the three first layers and (c) and a rearing box with all the layers (d) (Source: Mar Ramos Sanz).

At the end of the rearing season (June-July 2014) approximately 2,600 pupae (cocoons) of N. sertifer were collected. These cocoons were divided to conduct different experiments and placed in a dark cold room (5°C±1 °C) to prevent the hatching of the adults (Baldassari et al. 2003, Kollberg et al. 2013). Considering the information obtained in previous studies, a high probability of larval mortality was expected during the rearing period due to baculovirus and fungi (Kolomiets et al. 1979, Olofsson 1989, Morimoto & Nakamura 1989, Heliövaara et al. 1991, Saikkonen & Neuvonen 1993, Lyytikäinen-Saarenmaa 1999).
Thus, larvae were carefully treated while rearing. Whenever one or few disease larvae were detected they were discarded in order to avoid a quick spread of the disease. The final result shows the importance of a careful rearing: at the end of the rearing period the mortality rate of larvae due to fungi and baculovirus was only 8.3±2%.

2. Study insects life cycles

I used the previously collected *N. sertifer* cocoons (1200 cocoons) for studying the predation pressure of both species (see Figure 2.). I could not use cocoons of *D. pini* because I did not have access to larvae of this species. However both species have similarities in their life cycles, and morphologies of their developmental stages (specially their larvae and pupae). Because of that, I took advantage of those similarities, and thereby obtain results about predation pressure on both species.

*N. sertifer* lays eggs on Scots pine needles at the beginning of spring, their larvae fed on groups in Scots pine needles during spring time and in early summer they begin to pupate, the pupation period takes around one month and on later summer adults begin laying eggs which overwinter in this state of development (see Figure 2.) (Kolomietz et al. 1979, Hanski 1987, Lyytikäinen-Saarenmaa 1999, Pasquier-Barre et al. 2000).

On the other hand, *D. pini*, lays eggs during mid and late summer, their larvae fed on Scots pine needles during summer till early autumn when it develops into pupae overwintering in pupal stage (see Figure 2.) (Sharov 1993, Neuvonen & Niemelä 1991). Furthermore, *D. pini* spend the main part of its cycle in the cocoon stage not only hibernating but sometimes even diapausing up to several years (Herz & Heitland 2003, De Somviele et al. 2007). However both cycles can be variable in time (1-1.5 months) in Finland due to environmental factors (especially temperature).

There were a high possibility that many *N. sertifer* adults hatch approximately a month before *D. pini* entered in pupal stage (see Figure 2.) (Dahlsten 1967, Hanski 1987, Pschorn-Walcher 1987, Neuvonen & Niemelä 1991, Sharov 1993, Kouki et al. 1998, Larsson et al. 2000). In order to avoid this option, I decided to submit half of the pupae to a heating treatment (48 hours at 90ºC) and thus kill the individual inside pupal. Consequently, the insect inside the cocoon was dead but the nutritional properties for predators were the same (Hanski 1990a).
Study sites

Study sites were primarily even-aged Scots pine forests representing different vegetation types on relatively mesic to dry soils. The majority of forests in the area were young or middle-aged (approximately 40-50 years). To conduct the predation experiment two different locations near Joensuu were used; Kruununkangas (62° 36’ 55”N, 29° 55’E) and Jaamankangas (62° 42’N, 29° 43’E) with three stands per locality dominated by *Pinus sylvestris* of approximately the same age class per locality (see Figure 3 and Appendix 2).

In both locations, the stands of the following three soil/vegetation types were selected; Forest growing on poor productivity soils (*Xeric* or *Calluna* type), forest growing on medium productivity soils (*Sub-xeric* or *Vaccinium* type), and forest growing on high productivity soils (*Mesic* or *Myrtillus* type) (see Figure 3, and Appendix 2). A complete description of the stands is provided in the Appendix section (Appendix 2). I followed Cajander’s (1949), vegetation units and forest vegetation types, to describe the study sites of this research (Cajander 1949, Kuusipalo, 1983, Tonteri et al. 1990; 1990b, Tonteri 1994, EEA 2006).

Two rain gauges were established per locality and they were checked every week during the time when the experiment was set, in order to consider the effect of precipitation in the experiment (Kollberg et al. 2014). The summer and autumn temperatures and rain were normal compared to previous years, and also the two rain gauges located close to both areas follow this trend of precipitation (see Appendix 3, Figure 1).
Figure 3. Location of the study sites (Source: © Maanmittauslaitos (National Land Survey of Finland 2010).)

2. 4. Experimental design

The aim of this experiment, consisted of measuring the cocoon predation pressure per site while considering the effect of productivity and vegetation type of the chosen stands. In this case, the chosen prey were two of the major defoliators of Scots pine forests, the sawflies Neodiprion sertifer and Diprion pini (Hymenoptera, Diprionidae), however as I explained above the cocoons used in the experiment were from N. sertifer previous rearing.
The cocoons/pupae collected during the rearing period were used as baits for all kind of predators (vertebrate and invertebrate). These baits enclosed a 1 m long line of string in which, every 20 cm a thread of around 15 cm with a glued pupae in the end was attached (©Loctite super glue precision) (see Figure 4).

Simultaneously, a total of 50 cocoons were glued, reared and observed separately in order to measure a possible side effect of the glue treatment. All of them developed normally. All the pupae used in the experiment were randomly chosen (both sexes randomly mixed), glued to thread ends, and attached to string lines. Every string had 5 threads attached with 5 cocoons glued in their extremes (see Figure 4). Those strings were kept under laboratory conditions (room temperature (18±2 °C) with a natural photoperiod before the experiment was set in the field.

As it was explained in the previous section, I decided to use half (600) of the obtained cocoons for simulating *N. sertifer* with natural cocoons and half (600) for simulating *D. pini* populations (see Figure 2 and Appendix 3). The first 600 live cocoons were set at the end of June simulating the pupal stage of *N. sertifer*, and 600 heat treated cocoons at the end of August simulating the pupal stage of *D. pini* (see Figure 2). The exposure of the cocoons were 4 weeks for each period. The length of the experiment was selected to permit the detection and following activity of natural enemies (Niemelä et al. 1991, De Somviele et al. 2007). Every cocoon was placed in the organic soil layer at approximately 3-5cm deep and cover by the surrounding vegetation (Björkman & Gref 1993, Nageleisen & Bouget 2009).

In every Scots pine stand a 50m x 50m square grid was set and strings with attached cocoons were placed in the four corners of the grid. Five strings (a total of 25 cocoons) were placed in every corner at approximately 10 meters of the edge (see Figure 4 and Appendix 4). The distance among strings was 20 cm (see Figure 4) and they covered an area of 1m². Altogether, a total of 100 pupae of *N. sertifer* were placed per stand and area, thus the final number of pupae was 600 (200 per vegetation type) (see Appendix 4).
Figure 4. Diagram of the experiment placed in each Scots pine stand (left), example of a set of 5 strings set in the field forming a 1m² square (middle) and one string with its five threads and pupae baits in detail (right).

After 4 weeks (at the end of June and in middle of September for *N. sertifer* and *D. pini* experiments respectively), the cocoons were collected from the field and the remaining cocoons of a single group of 5 strings with or without damages, were put separately in labelled plastic bags, and placed in a dark cold room (5°C±1°C) for a later analysis (Nageleisen & Bouget 2009).

2. 5. Pupae/Cocoon analysis

Once all the material was collected and placed in the laboratory, damages and signs of predation in cocoons were classified in the following categories (see Figure 5):

- **Intact pupae**: Pupae without any visible damage, were considered non-predated.

- **Disappeared pupae**: Pupae disappeared from their thread were considered as predated (Kouki et al. 1998, Denno et al. 2003).

- **Regular hatching**: The regular holes on one of the cocoon edges represented the hatching of adult sawflies and they were considered as not predated (Kolomiets et al. 1979, Nageleisen & Bouget 2009).

- **Vertebrate predation**: The damages included in this section were made by birds or small mammals (voles and rodents). These damages involved big size irregular holes in the external part of the cocoons, chewed cocoons, etc. (Kolomiets et al. 1979).

- **Invertebrate predation**: The main damages observed were produced by beetles. These damages consisted in irregular or regular small size holes in the ventral
middle part of the cocoon (damages produced by the families Carabidae or Elateridae) or irregular small size holes in one of the two edges of the cocoon (mainly produced by family Staphylinidae) (Kolomiets et al. 1979, Nageleisen & Bouget 2009).

- Parasitism: The damages produced by the hatching of parasitoids (small regular holes or single regular holes in one of the cocoon extremes) were excluded from the analysis and those cocoons were considered non-predated. I took this decision considering that I did not know the exact location of the larvae reared and due to the fact that they could be already parasitized adding noise to the results. (Herz & Heitland 1999; 2003).

**Figure 5.** Different types of damages and predation; a=Intact pupae, b= Hatched parasitoids (considered as non-predated), c=Vertebrate damage (chewed cocoons), d= Adult sawfly hatch hole, e=Small holes produced by hatched parasitoids, and f=Different types of invertebrate damages in the middle-ventral side of the cocoons (mainly produced by beetles). (Source: Mar Ramos Sanz).
2. 6. Statistical analysis

2.6.1. Data analysis and modelling

The collected general data is shown in Appendix 1. The number of total observations was 48 (each observation was obtained considering 4 square (with 25 pupae) x 3 vegetation type x 2 seasons), as a result the data had a hierarchical structure (Miina et al. 2009). The final data is given in Table 1 (see also Appendix 1) in the following order; obs (number of observations), forest vegetation type indexed as M (mesic), Sx (sub-xeric) and X (xeric), area (J=Jaamankangas and K=Kruununkangas), site (this variable was included to avoid overdispersion and included the effect of four 1m² edges per stand as a random factor), season (summer and autumn), total predation, vertebrate predation and invertebrate predation (see Appendix 1).

The first general exploration of the data was done using descriptive statistical analysis. The average and standard error of the percentage of the three response variables; intact pupae, total predation, vertebrate predation and invertebrate predation were calculated for the three vegetation types (Mesic, Sub-xeric and Xeric) and for the two seasons (summer and autumn). I used these averages to represent graphically the distribution of the data of those three response variables (divided in seasons and forest vegetation type). The mean percentage of predation of both types of predation (vertebrate and invertebrate) was plotted together in order to obtain a better representation of the results and to compare them. This first exploration gave me an idea of the general responses of the treatments and allowed me to consider the best way of analysis of the data (Quinn & Keough 2002).

Models were considered for total predation (Model 1), vertebrate predation (Model 2) and invertebrate predation (Model 3). The percentage of predation values for these three continuous variables were used as response variables (calculated by extracting the number of cocoons consumed from the number of cocoons in each stand edge (25 cocoons, 1m² square subplots)). After that, I observed the distribution of those variables using their histograms (see Appendix 5). None of these variables followed a normal distribution, instead of that they followed a binomial distribution (Crawley 2005). Due to the binomial distribution of the data set, I used statistical approaches that better match with the data obtained (Bolker et al. 2009), instead of trying to fit the variables into classical statistical methods (using transformations to obtain a normal distribution). Generalized linear mixed models (GLMMs) combine the
properties of two statistical frameworks that are widely used in ecology, linear mixed models (which incorporate random effects) and generalized linear models (which handle non-normal data by using link functions and exponential family e.g. normal, Poisson or binomial distributions) (Venables & Ripley 2002, Bolker et al. 2009).

The response variables were modelled using generalized linear mixed models (GLMMs) with a binomial response (logit-link function) because the data were collected in multiple levels of grouping, and the predictors were both fixed (variables forest vegetation type and seasons), and random (areas and sites) effects (McCulloch & Searle 2001). The multilevel hierarchy of the data (edge, site, forest vegetation, area), and its subsequently correlated observations, was taken into account by including random effects at different levels in the variance component models, and by allowing the intercept to vary randomly across the levels (Miina et al. 2009).

Overdispersion of the data in the models was noted by adding a random term as being at the bottom level (“pseudo” level), which in this case is the variable “site” (see Appendix 1, Table 1) (Venables & Ripley 2002, Miina et al. 2009, Rodríguez & Kouki 2015). The GLMMs were estimated with maximum likelihood (Laplace approximation) using the cbind function of the lme4 package for the R statistical programming language (R-3.2.2 version) (R development Core Team 2013, Bates et al. 2015) to conduct binomial errors GLMMs.

For a better interpretation of the results and the possible interaction among predictors (forest vegetation type and season). I checked their effects calculating the odds-ratio of the fixed effects for the three models (using the formula: $1 - \exp(fixed(model))$). With the inclusion of odds-ratio I could had a clearer interpretation of the fixed effects for all the levels for the three models. Odds ratio were used to compare the differences among levels for both predictors considering that they were calculated using the highest percentage as a baseline (Miina 2009). Thus, odds-ratio showed the percentage of decrease between levels compared with the highest level of predation detected. I also used the graphical representation to analyse and interpret the results given by those odds ratio (Miina 2009, Bates et al. 2015, Rodríguez & Kouki 2015).
I used the mean percentages of the observed data to plot two bar plots and show the differences in percentage of predation (total, vertebrate and invertebrate) between forest vegetation type and seasons. For doing this graphics I used MASS package in R for R 3.2.2 version (Crawley 2005, R development Core Team 2013). For representing graphically the results of the models and the possible interaction among predictors (season and forest vegetation type) I utilized sciplot package for R (R-3.2.2 version) (R development Core Team 2013, Morales 2015).

**General model:**

The general multi-level binomial model was utilized for the three models (total predation, vertebrate predation and invertebrate predation) and was represented as follows (see Model 1):

\[
y_{ijk} \sim \text{Binomial}(n_{ij}, p_{ij})
\]

\[
\ln(p_{ij} | 1 - p_{ij}) = f(x_{ij}, \beta) + u_i + u_{ij}
\]

Model (1).

Where \(y\) is the observed predation in the edge (i.e. percentage of consumption calculated by extracting the number of cocoons consumed from the number of cocoons in each stand edge (25 cocoons, 1m\(^2\) square subplots)); \textit{Binomial} (n, p) represents the binomial distribution with its parameters \(n\) (binomial sample size, in this case \(n_{ij}\) are all equal to 25) and \(p\) (proportion of successes i.e. the number of cocoons consumed/predated); \(\ln(p | 1 - p)\) is a logit-link function; and \(f(.)\) is a linear function with arguments \(x_{ij}\) (i.e. fixed predictors) and \(\beta\) (i.e. fixed parameters). Subscripts \(i\) and \(j\) refer to type of forest vegetation and season respectively. \(u_i, u_{ij}\) are random, normally distributed between-forest vegetation type and between seasons with a mean of 0 and constant variances. Random terms at different hierarchical levels were assumed to be uncorrelated (Bolker et al. 2009, Miina et al 2009).
3. RESULTS.

The calculated average and standard error for the general data is represented in Table 1. This table shows the data separated in four subclasses; intact pupae (cocoons with no damage or with signs of hatched adults from both parasitoids and sawflies), total predation (cocoons predated by vertebrate and invertebrate), vertebrate predation (damages and signs of predation produced by birds and small mammals) and invertebrate predation (damages and signs of predation produced by insects mainly beetles). Thus, Table 1 represents the mean percentage of pupae and their predation per study site, (xeric, sub-xeric and mesic) and season (summer and autumn).

General trend of data shows an increase in predation in mesic forest vegetation sites, the maximum percentage (mean ± S.E) of total predation being in summer (51±7.6). Also the maximum mean percentage of vertebrate predation appeared in mesic sites during summer (35.5±11.5). However this trend cannot be applied to the mean percentage of invertebrate predation that seems to be higher in autumn and mesic sites (23±4.6) (see Table 1).

**Table 1.** Proportions (%) of intact and total of predated pupae (mean ± S.E) for the two seasons, summer (June) and autumn (September) and the three forest vegetation types (Xeric, Sub-xeric and Mesic). Predation is given as total predation, vertebrate predation and invertebrate predation.

<table>
<thead>
<tr>
<th>Season</th>
<th>site</th>
<th>Intact</th>
<th>Total predation</th>
<th>Vertebrate predation</th>
<th>Invertebrate predation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xeric</td>
<td>89.5±4.6</td>
<td>10.5±4.7</td>
<td>6±3.1</td>
<td>3.5±3</td>
</tr>
<tr>
<td>Summer</td>
<td>Sub-xeric</td>
<td>82±4.5</td>
<td>18±4.5</td>
<td>13.5±4.8</td>
<td>4.5±1.2</td>
</tr>
<tr>
<td></td>
<td>Mesic</td>
<td>49±7.6</td>
<td><strong>51±7.6</strong></td>
<td><strong>35.5±11.5</strong></td>
<td><strong>15.5±4.4</strong></td>
</tr>
<tr>
<td>Autumn</td>
<td>Xeric</td>
<td>81±1.4</td>
<td>19±1.4</td>
<td>4.5±1.59</td>
<td>14.5±1.8</td>
</tr>
<tr>
<td></td>
<td>Sub-xeric</td>
<td>68.5±5</td>
<td>31.5±4.5</td>
<td>15.5±6</td>
<td>16±4.47</td>
</tr>
<tr>
<td></td>
<td>Mesic</td>
<td>56±7.6</td>
<td><strong>44±6.7</strong></td>
<td><strong>21±4.1</strong></td>
<td><strong>23±4.6</strong></td>
</tr>
</tbody>
</table>
I analysed first the mean percentage results in summer, and the observed trend was consistent with the main hypothesis of this study where mesic forest vegetation type had the highest cocoon predation for all three types of predation (Total: 51±7.6; Vertebrate: 35.5±11.5; Invertebrate: 15.5±4.4). In contrast, mean percentages of predation for Sub-xeric (Total: 18±4.5; Vertebrate: 13.5±4.8; Invertebrate: 4.5±1.2) and Xeric (Total: 10.5±4.7; Vertebrate: 6±3.1; Invertebrate: 3.5±3), forest vegetation types were much lower showing a lineal decrease (see Figure 6).

When I explored the mean percentages for autumn period the results were less clear than for the summer period, with almost no differences among mesic (Total: 44±6.7; Vertebrate: 21±4.1; Invertebrate: 23±4.6) and sub-xeric (Total: 31.5±4.5; Vertebrate: 15.5±6; Invertebrate: 16±4.47) vegetation types, and showing differences between mesic and xeric (Total: 19±1.4; Vertebrate: 4.5±1.59; Invertebrate: 14.5±1.8) but almost no differences between sub-xeric and xeric (see Table 1 and Figure 6). One interesting result was that invertebrate predation appeared higher in autumn than vertebrate predation for the three types of vegetation (see Figure 6). In both cases (summer and autumn) vertebrates and invertebrates presented a similar pattern with a higher predation in mesic forest vegetation type and lower for sub-xeric and xeric (see Table 1 and Figure 6).
3. 1. Total predation:

After fitting this first model for total predation, I firstly analysed the fixed factors (forest vegetation type and season) and their interaction separately without analysing their levels. This first approach was done using the estimated values of the model and analysing them using a Chi-squared test. The results showed a significant effect of forest vegetation type (p-value < 0.0001) and an interaction among both fixed factors (p-value = 0.0284) (see Table 2).

Table 2. General results of the Chi-squared analysis for estimated values for fixed factors (Veg. type = Forest vegetation type and season) and their respective interactions, the df for those values, the Sum of squares of the test, the Mean of squares and the corresponding p-value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg. type</td>
<td>2</td>
<td>36.74</td>
<td>18.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>3.17</td>
<td>3.17</td>
<td>0.39</td>
</tr>
<tr>
<td>FxSeason</td>
<td>2</td>
<td>6.32</td>
<td>3.15</td>
<td>0.0284</td>
</tr>
</tbody>
</table>

I decided to use the season levels (summer and autumn) to contrast and obtain a better interpretation of the results. In both cases, forest vegetation type appeared significantly
different, where the highest mean predation is presented in mesic forest vegetation level (the reference level) (see Table 3). Sub-xeric forest vegetation type appeared significant different (p-value<0.0001) compared with the other two types of forest vegetation, xeric and mesic (see Table 3). Xeric vegetation type did not present significant differences compared with sub-xeric vegetation type and mesic (p-value=0.081), as it is showed in Figure 7 and Table 3 (see Table 3 and Figure 7).

The fixed factor season did not present significant differences among levels, where summer appeared to have the highest percentage of total predation and was used as a reference level (autumn p-value=0.40). However, season presented interaction with forest vegetation levels sub-xeric (Sx:Season p-value=0.042) and xeric (X:Season p-value=0.028) (see Table 3).

**Table 3.** Statistical results for the first multilevel binomial model using mean percentage of total predation as response variable with the estimated values for the fixed factors; Forest vegetation type (M; Mesic, Sx; Sub-xeric, X; Xeric) and season (summer and autumn) and their respective interactions (vegetation type and season), the standard error for those values, the z-value of the test, the calculated odds-ratio and the corresponding p-value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std Error</th>
<th>z-value</th>
<th>Odds ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Predation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest vegetation type</td>
<td>M (reference level)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sx</td>
<td>-0.57</td>
<td>0.41</td>
<td>-1.37</td>
<td>0.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>X</td>
<td>-1.24</td>
<td>0.42</td>
<td>-2.92</td>
<td>0.93</td>
<td>0.081</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer (ref. level)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>-0.27</td>
<td>0.35</td>
<td>-0.82</td>
<td>0.3</td>
<td>0.40</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M;Season (ref.level)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sx:Season</td>
<td>-1.21</td>
<td>0.56</td>
<td>-2.03</td>
<td>0.7</td>
<td>0.042</td>
</tr>
<tr>
<td>X:Season</td>
<td>-1.40</td>
<td>0.63</td>
<td>-2.19</td>
<td>0.75</td>
<td>0.028</td>
</tr>
</tbody>
</table>
The highest predation was found in mesic forest vegetation types for both season summer and autumn (see Figure 7). Compared to mesic forests vegetation type total predation was 83 % lower in sub-xeric forests (odds-ratio=0.83) and 93 % lower in xeric forests (odds-ratio=0.93) on average.

In autumn, compared to summer total predation was 30% higher (odds-ratio= 0.3) for all forest vegetation levels on average (see Table 3 and Figure 7). Interaction showed a significant decreased of total predation in sub-xeric forest vegetation sites 70% (odds-ratio=0.7) and of 75% in xeric forest vegetation sites (odds-ratio=0.75) compared with mesic forest vegetation type sites (see Table 3 and Figure 7).

**Figure 7.** The modelled mean percentage of total predation among seasons (summer and autumn) and forest vegetation type (M=Mesic; Sx=Sub-xeric and X=xeric).
3. 2. Vertebrate predation:

In this second model for vertebrate predation, forest vegetation type predictor was significantly different (p-value <0.0001) while season did not present significant differences (p-value= 0.383) and there were not interaction between predictors (see Table 4).

Table 4. General results of the Chi-squared analysis for estimated values for fixed factors (Veg. type= Forest vegetation type and season) and their respective interactions, the df for those values, the Sum of squares of the test, the Mean of squares and the corresponding p-value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg. type</td>
<td>2</td>
<td>20.38</td>
<td>10.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>0.46</td>
<td>0.47</td>
<td>0.383</td>
</tr>
<tr>
<td>FxSeason</td>
<td>2</td>
<td>0.79</td>
<td>0.4</td>
<td>0.602</td>
</tr>
</tbody>
</table>

Forest vegetation type presented significant differences among all levels. Thus, all the levels showed different mean percentage values when they were compare to mesic forest vegetation type (reference level) (sub-xeric p-value=0.042 and xeric p-value<0.0001) (see Table 5).

Table 5. Statistical results for the first multilevel binomial model using mean percentage of vertebrate predation as response variable with the estimated values for the fixed factors; Forest vegetation type(M: Mesic, Sx: Sub-xeric, X: Xeric) and season (Summer and Autumn), the standard error for those values, the z-value of the test, the calculated odds-ratio and the corresponding p-value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std Error</th>
<th>z-value</th>
<th>Odds ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebrate predation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest vegetation type</td>
<td>M (reference level)</td>
<td>-0.91</td>
<td>0.45</td>
<td>-2.03</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Sx</td>
<td>-2.21</td>
<td>0.5</td>
<td>-4.43</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>-0.26</td>
<td>0.56</td>
<td>-0.65</td>
<td>0.22</td>
</tr>
<tr>
<td>Season</td>
<td>Summer (ref. level)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>-0.26</td>
<td>0.56</td>
<td>-0.65</td>
<td>0.22</td>
</tr>
</tbody>
</table>
The highest vertebrate predation appeared in mesic forest vegetation sites for both seasons. Compared to mesic forests there was a mean percentage decrease of 60% in sub-xeric (odds-ratio=0.6) and 89% in xeric vegetation types (odds-ratio=0.89) (see Table 5 and Figure 8).

There were no significant differences among season levels (autumn p-value= 0.575) and no interaction between levels of both predictors. Comparing summer (reference level) with autumn the mean percentage of decrease was of 22% (odds-ratio=0.22) (see Table 5 and Figure 8).

**Figure 8.** The modelled mean percentage of vertebrate predation among seasons (summer and autumn) and forest vegetation type (M=Mesic; Sx=Sub-xeric and X=xeric).
3. Invertebrate predation:

Finally, the third model showed significant differences for both predictor factors, forest vegetation type and season with non-significant interaction among predictors (see Table 6).

Table 6. General results of the Chi-squared analysis for estimated values for fixed factors (Veg. type = Forest vegetation type and season) and their respective interactions, the df for those values, the Sum of squares of the test, the Mean of squares and the corresponding p-value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg. type</td>
<td>2</td>
<td>7.71</td>
<td>3.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>16.2</td>
<td>16.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FxSeason</td>
<td>2</td>
<td>4.31</td>
<td>2.15</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Forest vegetation type was a significant predictor with significant differences between all levels compared with mesic forest vegetation type (sub-xeric p-value < 0.0001 and xeric p-value < 0.0001) (see Table 7). Season was also a significant predictor, showing significant differences among summer and autumn, where autumn was the reference level (summer p-value < 0.0001) (see Table 7). For this third model differences were clearer than for the previous two (see Figure 9).

Table 7. Statistical results for the first multilevel binomial model using mean percentage of invertebrate predation as response variable with the estimated values for the fixed factors; Forest (M; Mesic, Sx; Sub-xeric, X; Xeric) and season (Summer and Autumn), the standard error for those values, the z-value of the test, the calculated odds-ratio and the corresponding p-value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std Error</th>
<th>z-value</th>
<th>Odds ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrate predation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest vegetation type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M (reference level)</td>
<td>-1.04</td>
<td>0.36</td>
<td>-2.85</td>
<td>0.64</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sx</td>
<td>-1.06</td>
<td>0.36</td>
<td>-2.92</td>
<td>0.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn (ref. level)</td>
<td>-1.28</td>
<td>-0.31</td>
<td>-4.02</td>
<td>0.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The highest mean percentage of cocoons predated by invertebrates appeared in mesic vegetation forest sites and during autumn (see Figure 9). When forest vegetation types levels sub-xeric and xeric were compared with mesic, their odds-ratio calculated showed a decrease in predation of 64% in sub-xeric forest vegetation (odds-ratio=0.64) and of 65% in xeric forest vegetation type (odds-ratio=0.65) (see Table 7 and Figure 9). Cocoon predation by invertebrates was significantly lower during summer (odds-ratio=0.72) for all forest vegetation levels compared with autumn (see Table 7 and Figure 9).

Figure 9. The modelled mean percentage of invertebrate predation among seasons (summer and autumn) and forest vegetation type (M=Mesic; Sx=Sub-xeric and X=xeric).
4. DISCUSSION.

This research considers three forest vegetation types ranging from poor sites (xeric or *Calluna* type) to medium (sub-xeric or *Vaccinum* type) and rich sites (Mesic or (*Myrtillus* type) and two main predator guilds (vertebrate and invertebrate). The predation pressure was considered by summing both kind of predator types and separating them to observe which one was more efficient and in which environment their effect was higher. The three applied models gave similar results, all indicating that there were a significant difference in predation among the three forest vegetation types especially between the extreme; the richer (Mesic) and the poorer (Xeric).

Results obtained from this study strongly support the posed hypothesis and confirm the high potential of cocoon predation as control agent of pine herbivores. This hypothesis is based in the work of “generalist predator theory” of Hanski and the Ecosystem Exploitation hypothesis (EEH) of Oksanen, and as expected forest on nutrient rich sites were populated by larger populations of small mammals and other kind of predators and due to that predation pressure was higher in those sites than in poor forest sites (Oksanen et al. 1981, Hanski 1990a). However, this potential control can be threatened by the fact that there are no many natural Scots pine forests growing in mesic forest vegetation types (Kuusipalo 1983, Tonteri 1994, Fuller & Quine 2015).

The highest predation pressure was caused by vertebrate predators. However, since direct observations of the predators affecting the cocoon population were not measured, I can only speculate on the reasons behind these results. Previous studies pointed that small mammals are the main vertebrate predators of this system, more specifically shrews and voles (Herz & Heitland 2005). Shrews (*Sorex* spp.) and voles (*Myodes* spp. and *Microtus* spp.) are known to find their food in places where it would be risky to stay for long periods, in example the open forest floor (Hanski 1990a). Due to that, the food item is picked and transported to a spot where the small mammal is safe from its predators and competitors (Hanski et al. 2001, Sullivan et al. 2004). In this sense, when the gathering benefits fall below the energetic costs and the risks of being killed are high, small mammals should stop foraging (Functional response type 3) (Terborgh et al. 2001, Verdolin 2006). Opened and chewed cocoons were mainly consumed by small mammals according to the specific feeding signs (Kolomiets et al. 1979, Raymond et al. 2002).
Both types of predation were observed in this study (cocoon disappeared or with feeding signs). Due to the higher vertebrate predation pressure in mesic sites it can be considered that these particular ecosystems provided small mammals enough shelter and food to developed their activities reducing the risks of starvation and been predated, than in sub-xeric and xeric sites (Hanski & Parviainen 1985, Hanski 1990a; 1990b, Larsson et al. 2001, Korpimäki et al. 2005), the number and quality of food captures have several important functions in the ecology of shrews and other omnivorous small mammals (Hanski 1990b) promoting a numerical response to a prey type that is otherwise effectively seasonal (Hanski 1990a).

Another important vertebrate predator of insect pests are birds (Morrison et al. 1990, Barbaro & Battisti 2011). Kouki et al. (1998) pointed out in their discussion that birds could play a very significant role in the functioning of forest ecosystems, and especially their importance as insect defoliator regulators (Kouki et al. 1998). Birds prey on all sawfly stages, including the egg, larval, cocoon and adult (Morrison et al. 1990, Barbaro & Battisti 2011). However the actual effect of avian predation is not clear. In some habitats, most avian species highly feed on sawflies but in other cases such predation has been viewed as insignificant (Morrison et al. 1990). Avian predation may be particularly important at low prey population densities, birds may also affect sawflies populations indirectly through the dispersal of pathogens (Hanski & Parviainen 1985, Kouki et al. 1998, Barbaro & Battisti 2011, Kollberg et al. 2014). Due to their likely possible relevance in this system I think birds should be considered in future studies.

Despite that the main predation effect in this study was produced by vertebrates, invertebrate predation was also significantly higher during autumn especially in mesic stands. According with previous studies, invertebrate predation is mainly caused by beetles (Carabids and Staphylinids among others) (Codella & Raffa 1993, Tanhuanpää et al. 1999 Raymond et al. 2002). Beetle predation leaves considerable fragments of the cocoon and make especial marks such as irregular holes, whereas mammals remove the entire pupa from the cocoon or makes different marks (Elkinton et al. 2004). In this research invertebrate predation was lower than vertebrate predation but differed significantly among the three vegetation types especially for
the richer forest vegetation type (mesic), which had the highest invertebrate predation pressure.

Although this experiment was not designed to measure the competition among predator guilds, the general results gave a pattern in which both predators seem to have an association ranging from no relationship to a negative relationship, having vertebrate predators a negative impact over the potential cocoon predation of invertebrates (Larsson et al. 2000, Kollberg et al. 2014). This association also could be explained as an inverse relationship between vertebrate (mainly small mammals) and invertebrate predation (Tanhuanpää et al. 1999, Raymond et al 2002, Elkinton et al. 2004). In that sense, small mammals would be more effective in finding and foraging cocoons, and as a consequence invertebrate predators would act attacking the remaining cocoons left by vertebrates, hence invertebrate predation probably will be relatively higher when densities of small mammals are low (Lee et al. 2001, Finke & Denno 2002; Hastings et al. 2002). Despite I cannot make a general statement, it is possible that competition play an important role in this system and the relationship among both types of predator guilds should be consider in future research.

In this study mortality caused by pupal parasitoids was not measured. The main reason was that the larvae utilized in the experiment were originally sampled from different areas, outside the study area. Because it was not controlled whether the larvae were previously parasitized or they were parasitized as pupal stage during the experiment finally, it was decided not to include parasitism in this study, and the pupae with marks of parasitism were counted as non-predated. However based on the information found from previous studies, it is probable that the influence of parasitism in the overall control of pine sawflies cocoons is not higher enough compared with the control made by vertebrate predators (Hanski 1987, Herz & Heitland 1999; 2003; 2005, DeSomviele et al. 2007).
This study results showed a strong response in predation pressure especially in rich forests (Mesic stands). Considering that both kind of predators (vertebrate and invertebrate) are generalist, and usually do not present a strong numerical response to specific preys, but their population densities are reasonably influenced by the abundance of all prey in the habitat (Berryman 1994), this finding support the general knowledge of their ability to control low-density insect populations (Hanski & Hettonen 1996). If this functional response can be extend in time, those generalist predators have the potential of control and regulate this low-density pine sawflies populations (Hanski 1990a, Kollberg et al. 2014).
5. CONCLUSIONS

Due to the potential economical, ecological and environmental damage that pine sawflies can produce, during the last decades lot of research has been done in order to explore the specific system that includes, pine sawflies and specially their predators and natural controllers. The results of this master thesis study supports previous research suggesting, that apart from the connections to weather, sawfly outbreaks often occur in forests growing on nutrient-poor soils. This work demonstrates that predators and hence the pupal predation pressure is significantly influenced by site productivity. The main explanation for this results is that food resources are generally more abundant in rich forest habitats and this has a direct positive influence on predator’s populations for several reasons, included that a rich forest usually contains more sheltering structures which are used to escape from top predators. The most important result of this research was the significant effect of forest vegetation type on both kind of predators (vertebrate and invertebrate). As a result of this effect predation was higher in mesic or richer forest vegetation types, than in sub-xeric or xeric poorer forest vegetation types. Despite that the most important and effective type of predation was exerted by vertebrate predators, it is relevant to notice that this is the first study in which both types of predators (vertebrate and invertebrate) were included. Finally, it is important to consider that this system is more intricate than it seems and whenever more variables and actors are included (for example. in this study the weather, competition among predators and top predators were not included) the more complex the results will be. Future research will be focus on the effect of new factors (weather, competition, etc.) affecting the effectiveness of the total predation.
6. REFERENCES


### 7. APPENDIX.

**Appendix 1.** Table with all the data used in the analysis coded by: Obs (observations) Forest (forest vegetation type M=Mesic, Sx=Sub-xeric and X=xeric), Area (J=Jaamankangas and K=Kruununkangas), Site (J1, J2, J3 and K1, K2, K3), Season (summer and autumn), Total predation, Vertebrate predation and Invertebrate predation.

<table>
<thead>
<tr>
<th>Obs</th>
<th>Forest</th>
<th>Area</th>
<th>Site</th>
<th>Season</th>
<th>Total predation</th>
<th>Vertebrate predation</th>
<th>Invertebrate predation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>autumn</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>autumn</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>autumn</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>autumn</td>
<td>15</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>autumn</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>autumn</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>autumn</td>
<td>13</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>autumn</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>summer</td>
<td>22</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>summer</td>
<td>10</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>summer</td>
<td>19</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>J</td>
<td>J3</td>
<td>summer</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>summer</td>
<td>9</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>summer</td>
<td>16</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>summer</td>
<td>11</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>K</td>
<td>K3</td>
<td>summer</td>
<td>7</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>autumn</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>autumn</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>autumn</td>
<td>7</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>autumn</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>21</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>autumn</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>autumn</td>
<td>14</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>autumn</td>
<td>9</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>24</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>autumn</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>25</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>summer</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>26</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>summer</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>summer</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>28</td>
<td>Sx</td>
<td>J</td>
<td>J2</td>
<td>summer</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>29</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>summer</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>----</td>
<td>---</td>
<td>----</td>
<td>---------</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>30</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>summer</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>31</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>summer</td>
<td>10</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>32</td>
<td>Sx</td>
<td>K</td>
<td>K2</td>
<td>summer</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>autumn</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>34</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>autumn</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>35</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>autumn</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>36</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>autumn</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>37</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>autumn</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>38</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>autumn</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>39</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>autumn</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>40</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>autumn</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>41</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>summer</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>summer</td>
<td>9</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>43</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>summer</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td>X</td>
<td>J</td>
<td>J1</td>
<td>summer</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>summer</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>46</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>summer</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>47</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>summer</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>X</td>
<td>K</td>
<td>K1</td>
<td>summer</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
**Appendix 2.** More detail description of the three types of forest vegetation used; Xeric (Fig 1.), Sub-xeric (Fig 2.) and Mesic (Fig 3.)

<table>
<thead>
<tr>
<th>Xeric or <em>Calluna</em> type:</th>
<th><img src="image" alt="Figure 1. Xeric forest vegetation type (Calluna type) (Source: Mar Ramos Sanz.)" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>This forest type corresponds to dry soils and canopy layer mainly composed by <em>Pinus sylvestris</em> (Scots pine forests), rarely mixed with other tree species. Considered by Cajander as dry and dryish land forest class. The ground vegetation is dominated by <em>Calluna vulgaris</em>. Lichen vegetation is nearly always present forming a continuous cover depending on the driest heath. Mosses are almost absent, while herbs and grasses are scarce. Dwarf shrubs are abundant in general and xerophilous species.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Sub-xeric or <em>Vaccinum</em> type:</strong></th>
<th><img src="image" alt="Figure 2. Sub-xeric forest vegetation type (Vaccinum type) (Source: Mar Ramos Sanz.)" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>This type is special because the understorey vegetation of these forests has mixed attributes from two forest vegetation classes. Thus, although in general the understorey vegetation of these forests is mesophilous it has some species that corresponds to dry and dryish forest class. These forests hold a mixture of lichens and mosses. Herbs and grasses can be present at some degree. Dwarf shrub vegetation mainly consists of <em>Vaccinum vitis-idaea</em>. Despite this class can hold other types of tree species the most abundant specie was <em>P. sylvestris</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Mesic or <em>Myrtillus</em> type:</strong></th>
<th><img src="image" alt="Figure 3. Mesic forest vegetation type (Oxalys-Myrtillus type) (Source: Mar Ramos Sanz.)" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>In general the understorey vegetation of these forests is mesophilous. These forests hold an abundant cover of mosses (<em>Hylocomium, Dicranum</em> etc). Lichens are scarce with the exception of epiphytes. Herbs and grasses can be present at some degree. In this case there were moderate to abundant. Dwarf shrub vegetation mainly consisted of <em>Vaccinum myrtillus</em>. The humus layer is well developed consisted in dry peat. In this study, these forests were dominated by Scots pine mixed with different broadleaved trees species with almost no presence of Spruce.</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Graphical representation of the rain (mm) for the previous years (2012, 2013) and the year of the experiment (2014) including the two rain gadgets data (for Kulho and Jaamankangas) (Above) and mean temperature (°C) for these five months for 2012, 2013 and 2014 (bellow).
Appendix 4. Graphical representation of the experimental design set in the field for both areas Kruununkangas (62° 36' 55"N, 29° 55'E) and Jaamankangas (62° 42'N, 29° 43'E).

Mesic = *Oxalis-Myrtillus*

Subxeric = *Vaccinium*

Xeric = *Calluna*
Appendix 5. Graphical representation of the histograms of the response variables.

**Figure 2.** Histograms for the response variables: Total predation (above left), vertebrate predation (above right) and invertebrate predation (below left).