Czech University of Life Sciences Prague
Faculty of Forestry and Wood Sciences
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Influence of water deficit of Norway spruce (*Picea abies* /L./) on association of beetles (Coleoptera)

Diploma thesis

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE
Department of Forest Protection and Entomology
Faculty of Forestry and Wood Sciences

DIPLOMA THESIS ASSIGNMENT

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Thesis title

Influence of water deficit of Norway spruce (Picea abies /L./) on association of beetles (Coleoptera).

Objectives of thesis

1. Elaboration of review on the thesis with focusing on impact of volatile substances from the stressed Norway spruce on beetles.
2. Evaluation of host tree attraction for beetles on plots with trees stressed by water deficit in compare of trees in normal water regime.
3. Evaluation catches depending on the flight level.

Methodology

Master thesis research will be done in Kostelec nad Černými Lesy (Czech Republic) on 4 plots; the first two plots are under manipulated water regime by undercanopy roofs and the next two are in normal water regime for control. Inside each plot, there will be hanged up 10 window traps and those traps will be emptied every 14 days. All captured beetles will be indentified. Then, the number of trapped beetles will be counted at roofed and control plots will be compared to each other. The same methodology will be used for flight levels. ANOVA will be used for statistic evaluations of the differences among amount of beetles in all traps. Cluster analysis will be used for comparing of similarity of society of saproxylic beetles.

Schedule for processing

1. Start of the research: Traps installing from April 2013
2. Every two weeks checking the traps + change of fixation liquid in catching containers
3. End of the research: October 2013

The proposed extent of the thesis

55 stran včetně příloh

Keywords

water deficit, attraction, Ips typographus, Picea abies

Recommended information sources

Grambera W., Kreuzwieserb J., Wisthalera A., Cojocariub C., Grausa M., Rennenbergb H., Steignerc D., Steinbrecherc R., Hansela A. 2006: VOC emissions from Norway spruce (Picea abies L. [Karst]) twigs in the field – Results of a dynamic enclosure study. Atmospheric environment, 40: 128-137.

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Phillips M.A., Bohlmann J., Gershenzon 2006: Molecular regulation of induced terpenoid biosynthesis in conifers. Phytochemistry reviews, 5: 179-189.

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Zwenger S., Basu C. 2008: Plant terpenoids: applications and future potentials. Biotechnology and Molecular Biology Reviews Vol. 3 (1), pp. 1-7.

The Diploma Thesis Supervisor

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Electronic approval: December 11. 2013

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Dean

Declaration

I declare that this diploma thesis was written by me and me alone, merely using the cited sources. I agree with the loan of my work and its publication.

In Prague, 30.4.2014 Mladenović Strahinja

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Last, but not least, I would like to sincerely thank my family, my girlfriend and friends for their support during the last year.

Abstract: This diploma thesis deals with the drought stressed Norway spruce trees (*Picea abies* /L./) and its attraction for different families of beetles (Coleoptera). Beetles population dynamics is mainly affected by weather conditions, e.g. prolonged and frequent drought periods, windbreaks and the availability of reproductive material in the forest ecosystem. Bark beetles (Coleoptera: Curculionidae: Scolytinae) are considered as one of the most important disturbance factors in forest ecosystems which were not much observed in this experiment. Families such as Curculionidae, Elateridae and Carabidae were among the most abundant ones. Research was done at the spruce stand area in Central Bohemia (Czech Republic). It is an 80-year old spruce monoculture, at which were established four experimental plots. Two areas inside each plot of the size of 625 m^2 were covered with roof construction to prevent input of precipitation into the soil profile with the aim of manipulating with the water regime of the stand. Other two areas where marked as the control and they were of the same size. On the 16 trees were hung 32 window traps (passive flight interception traps) without bait, which means two window traps per tree. Traps were hung at the altitudes of 4 and 12 meters above the ground. Traps were exposed throughout the whole vegetative period and regularly collected each 7-21 days. The assumption was that all stressed trees will have a slightly higher attractiveness for the beetle associations compared with the control plot and that there will be difference in certain beetle families in their flight levels preference.

Keywords: Coleoptera, xylophagous, saproxylic, volatile compounds, water deficit, Picea abies, attraction, flight activity

i. Acronyms

ANOVA – Analysis of Variance
cca. – circa
CET – Central European Time
e.g. – for example
EU – European Union
i.e. – that is
IPCC – Intergovernmental Panel on Climate Change
IUCN - International Union for Conservation of Nature
PET - Polyethylene terephthalate
USA – United States of America
VOC – volatile organic compounds

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1. AIMS OF THE STUDY

- To elaborate of thesis review with focus on impact of volatile substances from the water stressed Norway spruce on the beetles associations;
- To evaluate of host tree attraction for beetles on plots with trees stressed by water deficit in comparison of the trees that were in the normal water regime;
- To evaluate catches depending on the flight level.

2. INTRODUCTION

Forest ecosystems are sensitive to climate change and that is why is so important to manage and improve their resilience and adaptive capacity. Forests can also reduce the influence of extreme weather events by modifying and decreasing temperatures, wind speed and water run-off. Forests have multifunctional purposes, such as economic, social and ecological. They are habitats for the variety of animal and plant species and have a major function in mitigating climate change. In European Union (EU), forest covers approximately 36 % of the total land area of the Union. EU's forest area have increased by cca. 0.3 % per year over few previous decades, but on the world level forest area continues to decrease (European Commission, 2003).

According to Intergovernmental Panel on Climate Change (IPCC, 2013), drought and water deficit, as one of the extreme events are likely to be caused by climate change which can be directed either by human activities or natural forces. Besides extreme droughts, high precipitation events can even be more catastrophic for the environment. IPCC (2007) warns that in the next 50-100 years, it is expected that mean annual temperature on the global level increases up to 4°C, where droughts will occur in many regions all over the world, including Central Europe.

Droughts can have huge influence on vitality of the trees and forest ecosystems in general. For example, water deficit in some tree species in Mediterranean area caused severe and large damages to the vegetation of that region, nevertheless different species responded differently to the water deficit (Peñuelas et al., 2001). Drought is an absence of precipitation for a certain period of time which long enough to result in reduction of soil moisture and damage to plants (Kramer & Boyer, 1995). Beetle species, such as *Ips* spp. and *Dendroctonus* spp. are forest pests and can cause even higher mortality in trees that were already damaged by drought (Allen & Breshears, 1998). Gartner et al. (2009) refers that spruce trees cannot keep their usual transpiration model during the day-time and had the highest sap flow values during the night-time and internal readjustment of sap flow inside the xylem and proves a higher sensitivity of Norway spruce to water stress.

Climate change and connection with temperature increasing will not only cause the shift of e.g. spruce bark beetle to higher elevation, but also change from semivoltinism to univoltinism reproduction behavior. Insect species usually migrate more rapidly than tree species therefore many temperate tree species are more likely to meet nonnative herbivore insects that were before restricted only to the forests of the subtropical regions (Logan et al. 2003, Dale et al. 2001). High temperatures and dry weather are usually followed by outbreaks of bark beetles and leaf eaters. There is also a positive connection between pest outbreaks and dry, nutrient-poor sites (Mattson & Haack, 1987).

Roof experiments in the forest stands can simulate water stress and the behavior of the trees inside the stand to show its relation towards soil reactions. Different types of roof construction can be created under the canopy in order to produce artificial drought (Lamersdorf et al. 1998). This is experiment is to express how man-made drought can affect relations between Norway spruce and beetle associations. Roof construction that was installed should stop rainfall input towards soil and therefore to simulate water stress in the research area.

3. LITERATURE REVIEW

3.1. Norway spruce – general information

According to Farjon (2013) on IUCN list, Norway spruce (*Picea Abies* /L. / [Karst]) is scientifically classified in the following order:

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class: Pinopsida
- Order: Pinales
- Family: Pinaceae
- Genus: Picea
- Species: *P. abies*

Norway spruce is a large, fast-growing coniferous tree, that can reach 35–55 meters height and trunk diameter usually makes of 1 to 1.5 meters. If conditions are good. it can grow very rapid when is young, in some cases up to 1 meter per year for the first 25 years, after it reaches 20 meters, growth is getting slower (Mitchell, 1974).

Branches have small-diameter and they sweep horizontally from the straight trunk which. Branchlets fall from the branches toward the ground and create a shape similar to a pyramid. Small diameter roots emerge from the base of the tree trunk and they can be generally found close to the soil surface. In general, the root system of Norway spruce is not deep, but usually dense, often very close to the tree trunk and because of it, grass grows much more difficult (Gilman & Watson, 1994).

Natural distribution of Norway spruce covers cca. 31 degrees of latitude from the Central Balkans (latitude 41°27’N) to the northeast, near the Chatanga River, Russia (latitude 72°15’N). Longitudinal range covers 5°27’E (French Alps) to 154°E at the Sea of Okhotsk in Russia (Eastern Siberia). Regarding its vertical distribution Norway spruce covers an area from sea level up to altitudes above 2300 meters (Italian

Alps). It is widely used in planting as a monoculture, especially in Central Europe and in Scandinavia (Skrøppa, 2003). Norway spruce is dominant in Boreal conifer forests of northern and northeastern Europe, where it replaces Scots pine, because of its shallow root system can avoid water table. It can be found from the mean sea level up to 2 kilometers in the mountain area. In the western mountain area of the Central Europe its ecotype has changed and adapted to sub-Atlantic weather when heavy and wet snowfall occurs in early winter. Expansion to the west Europe is limited by the fact that it cannot compete with shadow tolerant species such as Silver fir and European beech. It prefers mostly acidic soil, even though it can occur in almost all substrates. In boreal area, commonly growing with Norway spruce are birch trees and common aspen with willows next to the water bodies. In the Alpine mountain area, it occurs with larch, Swiss pine, Scots pine and Black pine or it can be found in pure stands. It is the species of the least concern in IUCN list (Farjon, 2013).

Norway spruce is tolerant towards most soil types. It tolerates dry conditions well, but it responds better if receives periodic precipitation or irrigation. (Gilman & Watson, 1994). Skrøppa (2003) describes Norway spruce is one of the most important tree species in aspects of economy and growth performances. It is mostly cultivated and planted in Central Europe since 19th century, mainly because its great performance on various site conditions and because it brings good yield. Skrøppa (2003) continues that this facts has changed many natural forests into monoculture forests and has led to the introduction of the Norway spruce far outside of its natural range, even in countries where it appears naturally (Germany and Norway), but many new countries started with artificial plantations of Norway spruce (Denmark, Belgium, Ireland and USA). Norway spruce produces high quality wood and it has long fibers which are significant in pulp and paper industry, but because it is key tree species in northern countries, this tree species is very important from the ecological point of view Skrøppa (2003).

3.2. Volatile substances in Norway spruce

According to Kesselmeier & Staudt (1999), volatile organic compounds (VOC) are all atmospheric gases with organic origin, but where carbon monoxide and dioxide are not included. In general, some main volatile organic substances include isoprenoids (most important are monoterpenes and isoprene) and many other such as ethers, acids, alkanes, alkenes, alcohols, esters and carbonyls. As Grabmer et al. (2006) refers, in the aspect of quantitative basis, most important volatile organic compounds are isoprene and monoterpenes.

3.2.1. Monoterpenes

The most significant volatile organic compound emitted by forests are monoterpenes ($C_{10}H_{16}$) and the level of its emission depends mostly of tree species, temperature and light (Sparcklen et al., 2008). Monoterpenes are part of the biochemical class of terpenoids and their carbon skeletons are usually composed of specific C_5 units (Mc-Garvey & Croteau, 1995). According to Kesselmeier & Staudt (1999), depending on the number of C_5 units, monoterpenes are divided in few groups. Monoterpenes belong to C_{10} units where it can found different substances, for example: α-pinene, camphor and menthol. It is known that these components are heavy smelling and hard to dissolve in water. Usually they are found in plants, animals and other microorganisms. Monoterpenes include acyclic, and mono-, bi-, or tricyclic structures. They can appear as hydrocarbons (with or without oxygen) in compounds such as linalool and geraniol.

In order to beetles find the new host tree, quantity and the spectrum of released volatile substances by the host is very important. Most common example would be interaction between Norway spruce and European spruce bark beetle, when monoterpenes released (including other volatile substances) by Norway spruce are significant odoriferous signals for the bark beetles, before settle on the new tree. (Rudinsky et al., 1971; Baier et al., 1999; Byers et al., 2000;). Regarding the temperature, monoterpenes exponentially increase with temperature and this process could be explained by increase of activity of monoterpenes emissions together with optimum temperature of 40°C (Fischbach et al., 2000). In their study, Guenther et al. (1993) prove that, the actual monoterpenes flux is not actually controlled by light, but it is in exponential dependence on the temperature of the leaf.

3.2.2. Isoprene

Isoprene emission from the plants is not only in dependence with current leaf temperature, but it is dependent on history of the temperatures of the plant as well (Guenther et al., 1999). According to Wagner et al., (1999) more than 90% of atmospheric isoprene is expected to come from terrestrial vegetation, but however there are also some other sources that are not distributed as much as vegetation (e.g. animals, aquatic organisms, microbes etc.). Kesselmeier & Staudt (1999) pointed out that after production of the isoprene, it is almost never stored in the plants, but it is quickly used by volatilization. Woody plants are those that more emitted isoprene than herbs or crops. From previous studies it is fairly known that emission rates from Norway spruce are low and increase very low together with temperature. Picea abies is known to be a low isoprene emitting species (Kesselmeier & Staudt, 1999; Grabmer et al., 2006).

3.2.3. Other volatile organic compounds

Other important volatile organic compounds that can be find in Norway spruce are: acetone, methanol, hexanal, hexenals, hexanol, methyl salicylate etc., which have their emission rates increasing exponentially with the temperature. Among all these compounds, hexanal and hexanol, showed the biggest temperature dependence. Hexenols, hexanal and hexenals (lipoxygenase-derived volatile compounds), were quite responsive to temperature changes too and it it is probably due the fact of induction of lipoxygenase activity (Filella et al., 2007; Heiden et al. 2003).

Methyl salicylate is a green leaf volatile substance that is emitted in higher levels by plants and trees, especially when they are under some kind of stress. Green leaf volatiles can then create certain chemical reactions with oxidants in the atmoshperes, yielding substances that can help to cause the formation of some secondary organic aerosols (Liyana-Arachchi et al., 2013). Filella et al. (2007) continue about methyl salicylate and its emission exponential dependence with the increasing temperature and emission levels of acetaldehyde and acetic acid were also increasing together with temperature until saturation appeared (cca. 30°C).

According to Lindelöw et al. (1991) in case for example that wood which was logged and stored, there was found an increasing of acetaldehyde and ethanol levels, comparing to due fresh standing wood. Ethanol for example, was found in slightly different relative levels in trees that were injured than in the trees that were healthy. Many scolytids are attracted to different volatile host substances together with ethanol, which suggests that ethanol and some other volatile compounds occurring in stored wood are from great importance when beetle search for appropriate place to breed.

3.3. Passive window traps

Window traps usually are made of a window-like vertical panel of glass or plastic with a funnel of preserving liquid which lays beneath it. Insect that fly and make a contact with the panel, drop towards the funnel and after it they are collected when fall into the fixation liquid. Traps can either be located near the ground (tree base), or they can be lifted up, above the ground. They can be placed up to the forest canopy and make their proper function even up there. (Gullan & Cranston, 2010). Even though most of the interception traps do not provide exact data about microhabitat, usually they are much more adequate method compared to the extraction models. Window traps have certain advantages such as: it is easy to make it standardized and to replace it, easy to design and build and big number of smaller flying taxon can be captured. Few disadvantages can be observed: higher expense per trap, there can be some complications with installation and improving of existing traps, only insects which are able to fly can be caught, in case of higher rainfall there is risk of flooding of preservation container and it is impossible to control which insects will be captured. In some cases, it can be seen by passers-by and it can be exposed to vandalism. (Bouget et al, 2008). As preservative is usually used the concentrated salt water or 2.5 % formaldehyde. Salt water does not attract insects and in case of beetles, salted water does not destroy them much and leaves them for further easy preparation and it better prevents the decomposition than formaldehyde (Schlaghamerský, 2000).

3.4. Saproxylic beetles

According to the Oxford dictionary (2014), the word „sapro” comes from the Greek relating to putrefaction, rotten, decaying substances and the word „xylo” is also derived from Greek, which means wood. Silvestri (1913) put together these two words in one and created a new word „Saproxylophiles“ which means „organisms decaying wood in the soil. Speight (1989) refers to saproxylic invertebrates as „those that are in some part of the life cycle in dependence of either dead or decaying wood, dying or dead trees (standing or fallen), or wood that is attacked by fungi“.

According to the Nieto & Alexander (2010), saproxylic beetles are those insects which are taking part in or dependent on wood decaying and their role is very significant in decomposition processes of living and dead trees and also for recycling nutrients in forest ecosystems. Saproxylic beetles choose dead and decaying wood according to tree species and what kind and position of the tissue is (spatial segregation). Temporal segregation occurs where many stages of wood decaying can be observed and each of the stage has a certain saproxylic fauna. Richness of the saproxylic beetles mainly depends on quality and quantity of the dead and decaying wood in the forest, and of course on forest area, management and fragmentation. Nieto & Alexander (2010) continues, there are important circumstances that relate to the host trees as well: 1) the total amount of the trees required to maintain viability of the population; 2) tree density (most of the beetles require open-grown trees, while others favor shadier situation; 3) age structure of the tree population; and 4) management history. Saproxylic beetles relate with other groups of organisms (mites, nematodes, bacteria and fungi) that are significant for the well-functioning of ecosystem. At least in Europe, the biology of many saproxylic beetle species is not yet well known and any further research on saproxylic beetles will improve our knowledge of the functioning of ecosystems in wooded landscapes. Csóka & Kovács (1999) refers that decomposition processes have certain steps and the first step of decomposition is done by saproxylic beetles when they establish on fresh dead trees (e.g. Cerambycidae) and their role is crucial for nutrients accumulation because without them nutrient cycle will be much slower since they are only ones who are able to feed on hard wood tissues. Csóka & Kovács (1999) continues that subfamily Cetoniinae feed on dead trees which are in successive stage, which means that those trees are in last decomposing stage and therefore assist in composting wood into soil.

In Central Europe there are more than 6000 species of beetles, of which cca. 1300 are saproxylic and currently significant number of them is protected or endangered species (Horák, 2008). Many species show long-term population decline and a large part also belongs among endemic species, including local and pan-European (Nieto & Alexander, 2010). According to Schlaghamerský (2000) some saproxylic beetle species are classified as extinct. Population decline and extinction is primarily caused by removal of fallen timber (Jonsell, 2012). Introducing monoculture forest management significantly reduces habitat biodiversity, which leads to the population decline of some saproxylic beetle species, because most of them in various stages of life development depend on different sites (Horák, 2008).

3.5. Xylophagous beetles

Oxford dictionary (2014) defines the Greek word “xylophagous” as organism feeding on or boring into wood (especially of an insect larva or mollusk). Xylophagous insects of many orders and different sizes (Coleoptera, Hymenoptera, Diptera etc.) are those that feed in the inner part of a tree trunk (hardest timber) of plants, but as well all insects that feed in stamps and branches as well (including shrubs), whether plant is dead or alive (Csóka & Kovács, 1999). Csóka & Kovács (1999) continue in their book that some of xylophagous insects communicate (acoustic and biochemical) in order to find host trees and some of them use it for reproduction purposes, but some control physiology of the plant to their last end. These insects are important for forest ecosystems and their role is multifunctional (for example, nutrient cycle in the forest). But some of xylophagous insects can devastate huge forest areas to extension that they present danger for the economy. Larvae of xylophagous insects demand high inputs of nitrogen (N) and therefore they search for a dead wood which offers them high nitrogen content, but most of the dead wood is low (under 1%) in nitrogen and larvae must eat up as much as woody tissue as it is their own body weight. (Csóka & Kovács, 1999; Csóka et al, 2006).

Outbreaks of xylophagous beetles mainly happen in monoculture forests which are planted on nutrient poor sites, therefore outbreaks do not occur in forests which are healthy and where site is resembling natural conditions. However, this does not have to be taken into account in case there are long periods of drought or where air pollution is present. There are described cases of interaction between xylophagous insects and other organisms, whereas xylophagous insects are vectors of certain pathogens of trees. For example, between fungi *Ceratocystis ulmi* and elm bark beetle (*Scolytus scolytus*). Their relationship is mutualistic and fungi helps in killing the tree which is then attacked by bark beetle. After the attack and inserting larvae into the dying tree, fungi is dispersed to another tree by the bark beetle and another tree gets contaminated by fungi. Other examples can be found between some wood wasps and fungi and longhorn beetles and nematodes. Certain predators use larvae of xylophagous insects as food source and some of them are specialized and can only feed on those insects (Csóka & Kovács, 1999). Species richness of xylophagous beetles was much higher near the ground than under canopy probably due the sun exposed timber (ground level) and shadow under canopy (Vodka et al., 2009)

3.6. Selected families of saproxylic and xylophagous beetles

3.6.1. Scarab beetles (Scarabaeidae)

Family Scarabaeidae is one of the biggest families of beetles in the world and they are showing wide diversity of life history and shape and their size varies from 1.9 millimeters (*Pleurophorus longulus*) to 15 centimeters (spp. *Goliathus*). Certain groups of dung beetles mainly use live plants as food source (in adults) and rotting wood (in larvae), while others are mostly dung eaters. Some xylophagous beetles belong to this group as well. (McNamara, 1991a; Csóka & Kovács, 1999). Their antennae is 3-7 segmented, smooth and shiny which is probably the most characteristic feature of these beetles. Larvae are developed in decaying wood and they make pupa case from wood parts (e.g. Trichiinae, Cetoniinae and Dynastinae). Many of adults of these beetles does not like light and hide during the daylight and become active during nightfall, while other species prefer sunlight (Csóka & Kovács, 1999). During its flight, some scarab beetles swallow great amounts of air which will result in a swelled gut which will be uncomfortable for feeding (Resh & Cardé, 2003).

3.6.2. Jewel beetles (Buprestidae)

These beautifully colored and shiny beetles are mainly found in tropical regions. Some of them are used in production of jewelry and that is how they got their English name. They have hard body, elongated and flattened and narrow toward the rear, but in cross section it looks cylindrical and triangular. Prothorax is connected to the midthorax (Csóka & Kovács, 1999). Csóka & Kovács (1999) refer that most of the species are xylophagous and they usually settle in or under the tree bark or heartwood. Their favorite trees are those ones that are usually stressed by water deficit. Larvae are yellow or white, without legs and vision, antennae are 3 segmented and labial palps are missing (important in determination of longhorn beetles). Jewel beetles larvae make their tunnels flattened in cross section in the heartwood or sometimes under the tree bark where tunnels are wide and flat (similar to longhorn larvae). Larvae pupate either in the heartwood or under the bark. Adult beetles fly fast, prefer sunlight and warm weather conditions. Usually, they can be seen how they rest on flowers and piles of wood (Csóka & Kovács, 1999; Resh & Cardé, 2003).

3.6.3. Longhorn beetles (Cerambycidae)

These slender beetles with long antennae are one of the numerous families and approximately 35000 species have been described until today, but also there are known fossil remaining from the tertiary. They can run fast and fly good, but also they produce characteristic squeaking sound. Most of the beetles from this family feed under the tree bark, in the harder parts of the dead wood or dead branches of living trees. They are usually no treat for economy, even though some of longhorn beetles are considered pests (e.g. *Hylotrupes bajulus*). Larvae creates different kind of galleries, even though they are not as characteristic as those of bark beetles. Most of the beetles of this family choose sunny and warmer sites and adults can be found on various places: flowers, stems, branches etc. While some species prefer nighttime, others are active in the daylight. Adults feed on remaining of fresh and dead tree bark, but also on pollen and leaves and some of the species in order to become sexually mature, must feed regularly. Some of the longhorn beetles can be disease transmitters, e.g. genus *Monochamus* are carrying a nematode from genus *Bursaphelenchus*, which can cause line wilt disease on host pines (Csóka & Kovács, 1999; Resh & Cardé, 2003).

3.6.4. Weevils (Curculionidae)

This family has the most numerous family of beetles with ca. 50,000 species. The head is similar to snout and elongated into a rostrum, but some groups have the rostrum very short. All species are phytophagous with a very different range of appearance and life history and development. Their larvae are usually legless (Csóka & Kovács, 1999).

Subfamily of bark beetles (Scolytinae) has the biggest importance since they are xylophagous organisms. The bark beetles are usually small cylindrical beetles (1-10 millimeters). The family is very diverse, with more than 6,000 known species worldwide. In Europe there are approximately 300 species and ca. 150 in Central Europe. Tissues of different plants are most favorable food source of majority of the bark beetles. Adults of ambrosia beetles that belong to this family bring fungi spores to their galleries and larvae use fungi as a food source because it is easier to digest than hard wood (Csóka & Kovács, 1999; Eroğlu et al., 2005).

After the mating, females bore tunnels (egg galleries) into tissues of the specific tree where they will lay their eggs on the wall of the tunnel. Few egg galleries can diverge from just one mating chamber and from egg galleries radiate larvae galleries where at the end of each gallery is pupal chamber. Bark beetles only attack unhealthy and weak trees whose self-defense system cannot prevent the attack. In case they attack a healthy tree, tree can defend itself by flooding the tunnels with sap or resin after which beetles are usually killed (Csóka & Kovács, 1999).

Majority of the bark beetles is able to defeat tree defense mechanisms by aggregating the pheromone and colonization of the tree. Sometimes pheromone released by bark beetles can act as anti-aggregation pheromone towards other bark beetles. Therefore, some aggregation pheromones can be used in the control against pests. If the density of the attack is high, then there is a lower chance that larvae will fully develop since all the food source will be consumed in the tree. Some of the most famous bark beetles are *Ips typographus, Polygraphus poligraphus, Dendroctonus micans and Pityogenes calcographus* and all of them use Norway spruce as a main food source (from the thinnest stems up to the thick barked parts on the trunk. Norway spruce is most favorable tree species among bark beetles, but also it is one of the most economically important. In the last decade there have been reported massive outbreaks of the Ips typographus in the Czech republic (Kindlmann et al., 2012) causing damages in millions of cubic meters, which is few tens of thousands of hectares in few European countries as well such as for example Austria (Krehen, 2006). However it is known their damage in the southern USA where species *Anthonomus grandis* was important pest in the 20th century (Resh & Cardé, 2003).

3.6.5. Death watch beetles (Anobiidae)

These beetles are tiny with a cylindrical shape and a comb-like antennae. These beetles are interesting from the point of view that their larvae use dead wood, but already furnished to develop (Csóka & Kovács, 1999). Furniture beetles (*Anobium punctatum*) is famous for damaging furniture and other woodwork products. Some species of this family feed in the young stems of growing trees. For example, the drugstore beetle (*Stegobium paniceum*) and the cigarette beetle (*Lasioderma serricorne*) are dangerous pests of many stored products (drugs, seeds, cereal products, spices, tobacco, museum specimens, leather etc.) (McNamara, 1991b).

3.6.6. Click beetles (Elateridae)

Click beetles are elongated beetles, which have a „mobile-joint“ between the first and second thoracic segment. That mobile-joint helps them to flip over or flick into air and it is followed by sound that reminds of a click (Csóka & Kovács, 1999). Csóka & Kovács (1999) continue, larvae of the click beetles are so called „wireworms“, and they are usually hard and elongated. Larvae can either be phytophagous or saproxylic. Adults are short-lived, up to four weeks (Leibner, 2000).

Click beetles is connected with forest ecosystem or forest-stepe ecosystem, but however many of the species search for open fields, such as agricultural land. They can be found at almost any altitude. They usually live on herbs, shrubs, under tree bark, tree cavities and they feed on pollen, necter, flower parts, leaves and young plants (Leibner, 2000). Most of their larvae have various habits of feeding and many groups belonging to this family are saproxylic and phytophagous and usually these beetles consume their food which looks like “extraorally digested liquid” (Resh & Cardé, 2003).

4. MATERIAL AND METHODS

4.1. Experimental plot and methods

Main part of the master thesis experiment was done cca. three kilometers south from the town Kostelec nad Černými lesy, in the central Bohemian part of the Czech Republic (see Map 1 on the next page). It is a hilly area, where approximate elevation is 350 meters above sea level. According to Czech forestry typological map, forest stand belongs to acidic oak – beech stand. In the stand, there was no observable any shrub or herbaceous level.

There was created an experimented area inside of Norway spruce forest stand that belongs to Faculty of Forestry and Wood Sciences, Czech University of Life Sciences in Prague (49°58'7.309"N, 14°51'2.110"E). Norway spruce stand had an age of approximately 80 years and it belongs to the stands that have reached its felling maturity. Inside the stand were designated four plots; each plot had an area of cca. 625 m^2 (25 x 25 meters). On the Map 2 on the next page, it can be seen approximate size and position of the experimental plots. Two of the plots are under manipulated water regime by under canopy roofs and that roof construction was built from construction timber and industrial plastic foil, approximately two meters above the ground. Roof construction was built in order of to stop rainfall input towards the soil profile and with main intention to influence water regime in the stand. Two other areas were used as the control plots and they had the same size as the ones with the roof construction. Around the plots with the roof construction were constructed channels to improve water drainage from the trees roots and in total to make the water stress higher. In the Chapter 10 – Annexes can be seen how the experimental plot looked like where each photo was described.

Map 1 - Approximate position of the research area, south of the Kostelec nad Černými lesy, marked with red rectangular. Map edited by Mladenović Strahinja (Source: www.mapy.cz)

Map 2 - North experimental plot 2 (S2 and K2) presented by blue color and south experimental 1 (S1 and K1) presented by red colot. Map edited by Mladenović Strahinja (Source: www.mapy.cz)

Inside each plot, there was hanged up eight passive window traps (without any baits). On the each tree, inside the each plot, were installed two window traps. It means that inside each plot, there were four trees and each of those trees had installed two window traps. One window trap was hanged up at four and the other one at twelve meters above the ground. Window traps were made of two transparent thermoplastic panels (Plexiglas) with dimensions of 70x40 centimeters (see Photo 4 and 14, Chapter 10 – Annexes). Both of the Plexiglas were connected with wires or ropes, so that from the top view they look like a cross. As a roof of window traps was put plastic bowl that is used under flowerpots with a diameter of 45 cm and also connected with wires or ropes. Under the Plexiglas, there was installed a plastic funnel (made from industrial foil), so it makes a circle around the Plexiglas cross. They were connected with a harder wire. Under the funnel, there was attached a catching container, made from PET bottle which was cut in half. Traps were exposed through the whole vegetative period and regularly checked. Every seven up to fourteen days traps were emptied, depending on the weather conditions in the stand. After each time when traps were emptied, change of the fixation liquid in the catching containers was done too. As a fixation liquid was used a mixture of the table salt (NaCl) and water.

Traps were hanged up using twelve meters high ladders (see Photo 11 – Chapter 10 – Annexes), even though some of the lower traps could be reached without any ladder assistance due the fact that on same places was possible to stand on the roof construction. Upper traps were connected with a rope and pulley and by pulling the rope up and down, window traps could be easily manipulated, and therefore ladders were not necessary. Collection of window traps was done in the way that catching containers were taken down together with caught insects and fixation liquid. Then, it was filtered through the sieve and filtered material was stored into a separate bag for each trap. Each bag with material was then marked with: name of the research area, date, number of the window trap (see Photo 10 – Chapter 10 – Annexes).

After the field work and collection of the window traps material, bags were stored in the freezer where temperature was approximately -20°C. Therefore, before process of insect preparation started, bags had to be defrosted and then materials from it was added into Petri dish. Then only insects from the order of Coleoptera were determined and dried, which was followed by gluing beetles for the small labels of the mounting paper. After it, beetles glued on the label, were pinned in entomological box in order from the first up to the last trap. Under each label with glued insect, there was put another label with certain identification details such as, number of the trap and position of the trap (upper or down), date and location. Position of the traps is important in order to realize flight levels of the beetles in the stand. Entomological boxes were then marked with the name of the research area location and the dates.

All beetles that were caught were determined to the family level and labeled with names. Determination was assisted by doc. Ing. Oto Nakládal, Ph.D. and Ing. Petr Šenfeld from the Department of Forest Protection and Entomology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague. After it, a database was created with all beetles, including date when they were caught and number and position of the trap.

During 2013, eleven times experimental field was visited and material from the window traps was collected, emptied and again fixed on the tree for the next visit. Experiment started on 7th of May at 8:00 CET when traps were installed on selected trees. After it every seven, fourteen or twenty-one days (in dependence of weather conditions in Central Bohemian region), experimental plot near Kostelec nad Černými lesy was visited. Other dates of research are sorted by the date and time:

- 15th of May; 8:00–15:00 CET;
- 22nd of May; 8:00–15:00 CET;
- 12th of June; 8:00–15:00 CET;
- 19th of June; 8:00–15:00 CET;
- 2nd of July; 8:00–15:00 CET;
- 16th of July; 8:00–15:00 CET;
- 30th of July; 8:00–15:00 CET;
- 13th of August; 8:00–15:00 CET;
- 27th of August; 8:00–15:00 CET;
- 24th of September; 8:00–15:00 CET.

During this period following families and subfamilies of order Coleoptera were caught in window traps, and later prepared, glued or pinned into the entomological boxes. As it was mentioned, research started in April 2013, when roof construction was built and window traps hanged up. Research ended in October 2013 when all window traps were taken down to protect them from the snow damage. For the same reason, plastic foil was taken down from the roof construction and stored nearby the experimental area.

In order to understand better, meaning of the following codes of trees where traps where hanged is explained, for example:

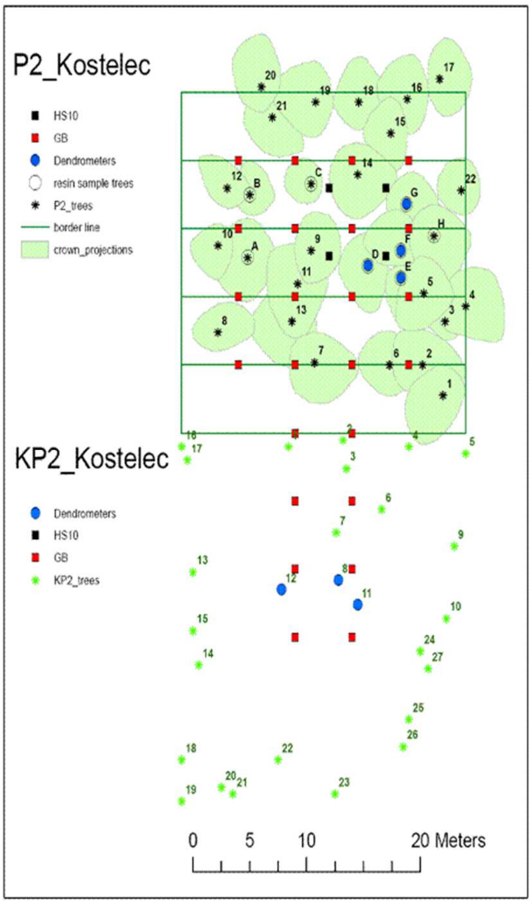
- S4H – stressed tree number 4, upper window trap;
- K8D – control tree number 8, down window trap,

where S stays for Stressed and K stays for Control, H stays for Upper and D stays for Down.

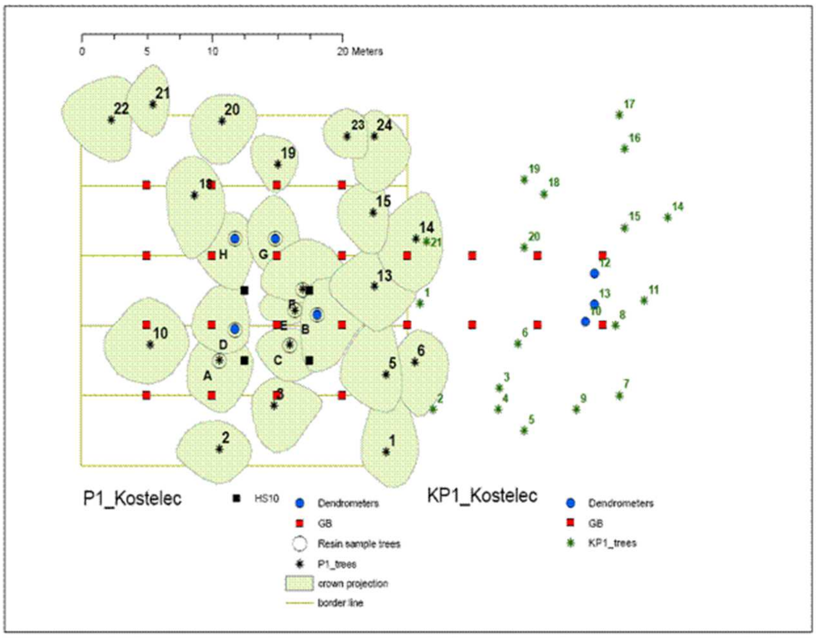
Trees and window traps positions in the research area, can be seen on the next page on Map 3 and 4 and legend for those maps can be seen below in Table 1.

Table 1 - Legend of the trees and traps numbers on stressed (S) and control (K) plot 1 (S1 and K1) and experimental plot 2 (S2 and K1)

Experimental plots	Window traps	Tree numbers
S1	1	B
S1	2	D
S1	4	G
S1	5	H
K1	6	6
K1	7	11
K1	8	19
K1	9	19
S2	11	F
S2	12	D
S2	14	A
S2	15	B
K2	16	12
K2	18	9
K2	19	24
K2	20	23



Map 3 - Positions of certain trees and window traps in the experimental plot 2. Upper map represents area that was roofed (stressed trees) and map under it presents control plot.



Map 4 - Positions of trees and window traps in the experimental plot 1. On the right side it is represented area where trees were streesed, on the left, trees in the control regime.

4.2. Statistical analyses

4.2.1. Cluster analysis

In order to analyze collected data and to explain the dependence between stressed trees and non-stressed trees in relation with beetles associations, software STATISTICA was used. Cluster analysis was a method which was applied in order to see if there was any significant difference in beetles' preference between trees in control (trees which had received regular amount of precipitation) and trees with water deficit (trees that were in regime of not receiving any precipitation in the soil profile around them).

Cluster analysis is an exploratory tool that include various methods and algorithms for gathering similar data (values) into corresponding classes. Most common problem among the scientists is how to categorize those data into specific classes that will create rational, valid and significant answer. Its main objective is to categorize different values into classes in that manner that degree of relation between two values is at its maximum when they are part of the same class and at its minimum when is vice versa. Algorithm used was so called "Joining (Tree Clustering), whereas it provides joining together similar values into bigger clusters using measure of distance. Typical output is the hierarchical tree. Euclidian distance method was applied because Euclidian method is referred as a "geometric distance in the multidimensional space". Euclidian method has its advantages and disadvantages. As an advantage, it is worth to mention that in case of adding a new value into the analysis, it will not affect the distance between any other two. But, distances can be highly influences by different scale between the dimensions, so it is recommended to transform dimensions to same or similar scales. (StatSoft, 2013).

4.2.2. Pearson's chi-squared test (χ^2)

In order to find if there is any significant difference between flying activity among different families and subfamilies, regardless if experimental plot was stressed by water deficit or if it was in normal (control) regime, software Microsoft Office Excel was used where certain formula was applied.

Since in some families, there was low number of caught individuals, both experimental plots 1 and 2, were summed together and total number was used for further calculations. Even though it was summed together, some subfamilies and families did not fulfill all conditions to be statistically analyzed. Therefore, for all beetles families and subfamilies, chi-squared test (χ^2) was used. Yates' chi-squared test (Yates's correction for continuity) was tried to be applied but as it was mentioned before, some families did not have all conditions for that test to be applied due to low number of caught individuals.

Chi-squared test is a statistical hypothesis test, which is generally used to see make a comparison between data there were observed and data that were expected to be obtained during the research in accordance to a certain hypothesis. The chi-squared test is actually testing what scientists refer as the null hypothesis, which defines that there is not any significant difference between the observed and expected result.

The formula that was used for calculating chi-squared (χ^2) is:

$$\chi^2 = \sum_{i=1}^{n} \frac{(|p_i - o_i|)^2}{o_i}$$

In order to get the most accurate results, Pearson's chi-squared test (goodness-of-fit test) was used. It means that chi-squared is the sum of the squared difference between observed (p) and the expected (o) data, divided by the expected data in all possible categories (StatSoft, 2013).

4.2.3. ANOVA method

In order to verify Pearson’s squared test, flying activity was analyzed once again in STATISTICA software, using one-way ANOVA method. According to Electronic Statistics Textbook StatSoft (2013), aim of ANOVA is to test for any significant differences among means. If two means are compared, then method ANOVA will create same result as it would create the t-test for independent or dependent samples (if two various groups of observations are compared or if two variables in one set of observations are compared).

First it was checked if values of all caught individuals had normal distribution. In case they had, one-way ANOVA method was tested right away. In case they showed no normal distribution, data of total number of all caught individuals were transformed using either logarithm (y=ln(x+1)) or cubed values. In case that did not show any normal distribution, then ANOVA method could not be used in that particular case and it was not tested.

5. RESULTS

Families and subfamilies that were collected from the window traps appear in the alphabetical order:

- Anobiidae (Death watch beetles)
- Anthribidae (Fungus weevils)
- Buprestidae (Jewel beetles)
- Byturidae (Fruitworm beetles)
- Cantharidae (Soldier beetles)
- Carabidae (Ground beetles)
- Cerambycidae (Longhorn beetles)
- Cleridae (Checkered beetles)
- Coccinellidae (Ladybugs)
- Colydiidae (Cylindrical bark beetles)
- Curculionidae (True weevils)
- Curculionidae: Scolytinae (Bark beetles)
- Dermestidae (Skin beetles)
- Elateridae (Click beetles)
- Eucnemidae (False click beetles)
- Chrysomelidae (Leaf beetles)
- Lampyridae (Fireflies)
- Leiodidae: Cholevinae (Small carrion beetles)
- Lymexylidae (Wood-boring beetles)
- Melandrydae (False darkling beetles)
- Nitidulidae (Sap beetles)
- Oedemeridae (False blister beetles)
- Scarabaeidae (Dung, scarab beetles)
- Scraptiidae (False flower beetles)
- Staphylinidae (Rove beetles)
- Tenebrionidae (Darkling beetles)

In the Table 2 below, it can be seen subfamilies and families with highest number of caught individuals for each day when experiment was undertaken (from May until September). Certain families and subfamilies showed higher total amount of caught individuals during the period of five months, while others had less than 10 individuals during the same period and the total number of all caught individuals can be seen in Table 3 on the next page. During the experimental period, families such as Anobiidae were presented by 32 individuals of various genera, Anthribidae (19), Carabidae (57), Cerambycidae (44), Cleridae (17), Coccinellidae (19), Curculionidae (96), Curculionidae: Scolytinae (25), Elateridae (502), Chrysomelidae (15), Melandrydae (36). Other families were presented by one up to seven individuals, e.g. Nitidulidae (7), Cantharidae (4), Staphylinidae (3) etc. and therefore it was statistically insignificant to include them into Results.

Full data regarding full number of caught individuals, families, date and location where they were caught is available in Chapter 10 – Annexes, Table 7.

Table 2 – Selected beetle families and number of caught individuals from May until September 2013. Other families were not represented due low number of caught individuals. ANOVA and Pearson chi-squared p-values

Family/Date	7.5.	15.5.	22.5.	12.6.	19.6.	2.7.	16.7.	30.7.	13.8.	27.8.	24.9.	Total
Anobiidae	0	2	3	1	8	8	9	0	1	0	0	32
Anthribidae	1	1	2	2	9	0	2	1	1	0	0	19
Carabidae	2	9	2	1	15	3	4	10	7	4	0	57
Cerambycidae	0	5	6	10	4	9	4	4	2	0	0	44
Coccinellidae	2	3	3	2	1	0	0	2	1	5	0	19
Curculionidae	10	5	8	5	21	8	7	15	3	4	10	96
Scolytinae	5	7	2	5	3	0	1	1	0	1	0	25
Elateridae	40	128	65	74	146	34	7	6	2	0	0	502
Melandrydae	0	0	0	3	7	11	5	8	0	2	0	36

Table 3 - Total number of all caught individual beetles of all subfamilies and families and on both experimental plots from May to September 2013.

Families	Total number of caught individuals on both experimental plots
Anobiidae	32
Anthribidae	19
Buprestidae	2
Byturidae	2
Cantharidae	4
Carabidae	57
Cerambycidae	44
Cleridae	17
Coccinellidae	19
Colydiidae	1
Curculionidae	96
Curculionidae: Scolytinae	25
Dermestidae	1
Elateridae	502
Eucnemidae	1
Chrysomeloidea	15
Lampyridae	1
Leiodidae:Cholevinae	1
Lymexylidae	1
Melandrydae	36
Nitidulidae	7
Oedemeridae	1
Scarabaeidae	1
Scraptiidae	3
Staphylinidae	3
Tenebrionidae	3

5.1. Relation between stressed and non-stressed trees with beetles associations tested by cluster analysis

At the experimental surface area 1, there is visible different preference of beetles between roofed plots (water stress) and control plots (non-water stress). In the Figure 1 below, it can be seen that certain trees and position of the window traps (upper or down) show similar attraction for beetles and they create similar groups. For example at the roofed plot, down window trap at the stressed tree number 4 make a group with upper window trap on trees number 1 and 2. Then, this new group connects with down window trap on tree number 2. New group (which includes all previous mentioned window traps and trees) connects with the group that is formed by S4H and S5H (upper window traps on trees 4 and 5). Down window trap at the tree 5 then groups with the previous group which includes all previous mentioned values.

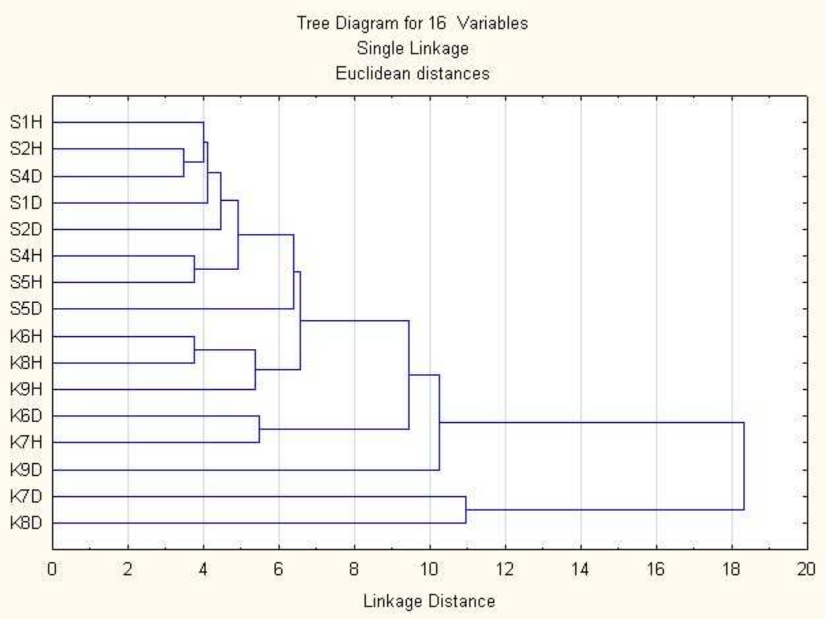


Figure 1 – Results of cluster analysis- tree diagram (Single linkage; Euclidian distances) for stressed and control at experimental plot 1.

Regarding the control plot, for example it can be seen that trees K7D and K8D cluster into one group which is then connected with big group that contains other remaining trees in the control plot which then clusters together with plot where trees where stressed. It is interesting that, even though it is same tree, there is significant difference for example between tree K6 and its window traps. K6H has more similarity with K8H, then with K6D. This can be explained by different flying activity among various families, which will be explained later. On the same graph can be clearly seen that beetles' association preferred trees that were not stressed by water deficit i.e. trees that were receiving precipitation with no limiting.

However, regarding experimental plot 2, there are some differences in comparison with experimental plot 1. For example, on the Figure 2 below, it is seen that tree K18H make cluster with the big group that contains almost all trees from the stressed plot (except tree S14D), but it also includes tree K18D from the control plot. Trees S11D, S12H and S15D make a group which is then connected in new one with tree S11H. This new group cluster with S15H into new one, which is done connected with the group that contains previously mentioned K18H, S14H and S12D. Also already mentioned, tree K18D then cluster with this new big group. Tree K20D cluster with the group which includes K20H and K19D, and then this new group connects with other remaining trees.

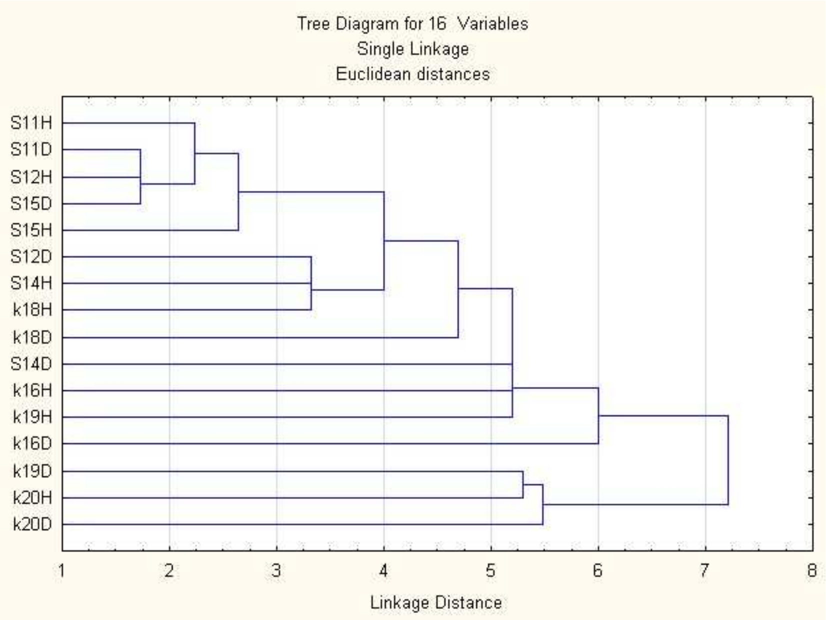


Figure 2 – Results of cluster analysis - tree diagram (Single linkage; Euclidian distances) for stressed and control at experimental plot 2.

5.2. Flight activity between upper and down window traps in both experimental plots tested by Pearson’s chi-squared test

In the table 4 on the next page, obtained results can be seen. As it was mentioned, only certain families satisfied all conditions for Pearson’s chi-squared test to be used. Some families, even though they had enough individuals to be manipulated with, did not show any significant difference in flying activity. Families such as: Anobiidae, Anthribidae, Carabidae, Cerambycidae, Cleridae, Cleridae, subfamily Scolytinae and Chrysomelidae were among those families and they did not show any preference between flying on lower or higher levels. While, majority of families such as: Buprestidae, Byturidae, Cantharidae, Colydiidae, Dermestidae, Eucnemidae, Lampyridae, subfamily Cholevinae, Lymexylidae, Nitidulidae, Oedemeridae, Scarabaeidae, Scraptiidae, Staphylinidae and Tenebrionidae did not satisfy all conditions for statistical analysis due to low number of caught individuals. For these families not even Yates's correction for continuity could not be used. However, as it can be seen in the Table 4, families such as: Coccinellidae, Curculionidae, Elateridae and Melandrydae showed significant difference in flying activity. Coccinellidae, Curculionidae and Elateridae mostly preferred higher levels to fly and most of their individuals were caught in the upper window traps. Only one family Nitidulidae was showing higher preference in lower levels for flying and most of their individuals was caught in down window traps.

Table 4 - Observed and expected data of beetles flying activity for both experimental plots, results of chi-squared test (χ^2) and its p-values (p).

Observed		Expected				Families
Upper	Down	Upper	Down	χ^2	p-value	
13	19	16	16	1.13	0.29	Anobiidae
9	10	9.5	9.5	0.05	0.82	Anthribidae
0	2	1	1	2	-	Buprestidae
0	2	1	1	2	-	Byturidae
0	4	2	2	4	-	Cantharidae
24	33	28.5	28.5	1.42	0.23	Carabidae
18	26	22	22	1.46	0.23	Cerambycidae
7	10	8.5	8.5	0.53	0.47	Cleridae
5	14	9.5	9.5	4.26	0.04	Coccinellidae
0	1	0.5	0.5	1	-	Colydiidae
33	63	48	48	9.38	0.002	Curculionidae
12	13	12.5	12.5	0.04	0.84	Curculionidae: Scolytinae
0	1	0.5	0.5	1	-	Dermestidae
189	313	251	251	30.63	3.12E-08	Elateridae
0	1	0.5	0.5	1	-	Eucnemidae
7	8	7.5	7.5	0.07	0.80	Chrysomelidae
0	1	0,5	0,5	1	-	Lampyridae
1	0	0,5	0,5	1	-	Leiodidae:Cholevinae
0	1	0,5	0,5	1	-	Lymexylidae
24	12	18	18	4	0.046	Melandrydae
1	6	3.5	3.5	3.57	-	Nitidulidae
0	1	0.5	0.5	1	-	Oedemeridae
0	1	0.5	0.5	1	-	Scarabaeidae
2	1	1.5	1.5	0.33	-	Scraptiidae
2	1	1.5	1.5	0.33	-	Staphylinidae
2	1	1.5	1.5	0.33	-	Tenebrionidae

5.3. Flight activity between upper and down window traps in both experimental plots tested by ANOVA method

Results that were obtained, were quite different than in the previous statistical method. Most of the families and subfamilies did not have normal distribution, not even after data were transformed. Only three of all caught families showed normal distribution. Carabidae and Elateridae did not show normal distribution tested from the values of all caught individuals, but after data were transformed using the logarithm transformation function y=ln(x+1). Only one family, Cerambycidae, showed normal distribution tested from the values of all caught individuals and therefore any transformation function didn’t have to be used. Results obtained for all families can be seen in Table 5 on the next page. However, none of the families had their p-values lower than 0,05 when one-way ANOVA was applied, therefore, it can be concluded that there was not any significant difference in flight activity preference between upper and down. Only Elateridae had its p-values of 0,17746 obtained by one-way ANOVA method close to the 0.05, but not enough to prove if there is any important difference in flight activity (see Figure 3 below). Families Carabidae and Cerambycidae had their p-values obtained by one-way ANOVA method much higher than Elateridae and therefore it can be concluded that there is no significant difference in flight levels preference. In Table 6 (see page 43) are represented p-values of both statistical methods ANOVA and Pearson chi-square test.

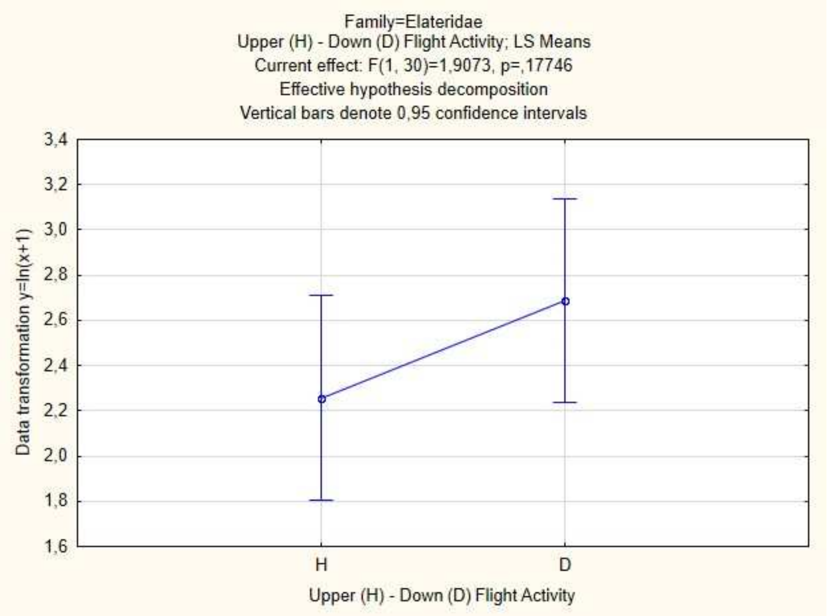


Figure 3 - Elateridae flight activity between upper (H) and down (D) levels;

Table 5 – P-values that show if there is significant difference in flying activity obtained by one-way ANOVA method in STATISTICA software

Families	Total number of caught individuals	Normal distribution	Transformation function	Normal distribution *p-value*	ANOVA *p-value*
Anobiidae	32	NO	y=ln(x+1)	0,00002	*not tested*
Anthribidae	19	NO	y=ln(x+1)	0,00000	*not tested*
Buprestidae	2	NO	y=ln(x+1)	0,00000	*not tested*
Byturidae	2	NO	y=ln(x+1)	-	*not tested*
Cantharidae	4	NO	y=ln(x+1)	0,00000	*not tested*
Carabidae	57	NO	y=ln(x+1)	0,11607	0,48005
Cerambycidae	44	YES	-	0,28867	0,25990
Cleridae	17	NO	y=ln(x+1)	0,00000	*not tested*
Coccinellidae	19	NO	y=ln(x+1)	0,00000	*not tested*
Colydiidae	1	NO	y=ln(x+1)	-	*not tested*
Curculionidae	96	NO	$y=x^3$	0,00014	*not tested*
Curculionidae: Scolytinae	25	NO	y=ln(x+1)	0,00000	*not tested*
Dermestidae	1	NO	y=ln(x+1)	-	*not tested*
Elateridae	502	NO	y=ln(x+1)	0,41129	0,17746
Eucnemidae	1	NO	y=ln(x+1)	-	*not tested*
Chrysomelidae	15	NO	y=ln(x+1)	0,00000	*not tested*
Lampyridae	1	NO	y=ln(x+1)	-	*not tested*
Leiodidae:Cholevinae	1	NO	y=ln(x+1)	-	*not tested*
Lymexylidae	1	NO	y=ln(x+1)	-	*not tested*
Melandrydae	36	NO	y=ln(x+1)	0,00000	*not tested*
Nitidulidae	7	NO	y=ln(x+1)	-	*not tested*
Oedemeridae	1	NO	y=ln(x+1)	-	*not tested*
Scarabaeidae	1	NO	y=ln(x+1)	-	*not tested*
Scraptiidae	3	NO	y=ln(x+1)	0,00000	*not tested*
Staphylinidae	3	NO	y=ln(x+1)	0,00000	*not tested*
Tenebrionidae	3	NO	y=ln(x+1)	0,00000	*not tested*

Table 6 - ANOVA and Pearson chi-squared p-values

Family	*ANOVA p-value*	Pearson’s chi-square p-value
Anobiidae	*not tested*	0.29
Anthribidae	*not tested*	0.82
Buprestidae	*not tested*	-
Byturidae	*not tested*	-
Cantharidae	*not tested*	-
Carabidae	0,48005	0.23
Cerambycidae	0,25990	0.23
Cleridae	*not tested*	0.47
Coccinellidae	*not tested*	0.04
Colydiidae	*not tested*	-
Curculionidae	*not tested*	0.002
Curculionidae: Scolytinae	*not tested*	0.84
Dermestidae	*not tested*	-
Elateridae	0,17746	3.12E-08
Eucnemidae	*not tested*	-
Chrysomelidae	*not tested*	0.80
Lampyridae	*not tested*	-
Leiodidae:Cholevinae	*not tested*	-
Lymexylidae	*not tested*	-
Melandrydae	*not tested*	0.046
Nitidulidae	*not tested*	-
Oedemeridae	*not tested*	-
Scarabaeidae	*not tested*	-
Scraptiidae	*not tested*	-
Staphylinidae	*not tested*	-
Tenebrionidae	*not tested*	-

5.4. Flight activity from May until September for selected beetle families

In the Figures 4 and 5 below, and Table 2 (see page 35) is visible that selected families of Anobiidae, Anthribidae, Carabidae, Cerambycidae, Curculionidae, Curculionidae: Scolytinae, Coccinellidae, Elateridae and Melandrydae were flying more during the warmer months (June-July) of 2013. However there is a big difference in period of flight activity between for example, Elateridae and Anobiidae.

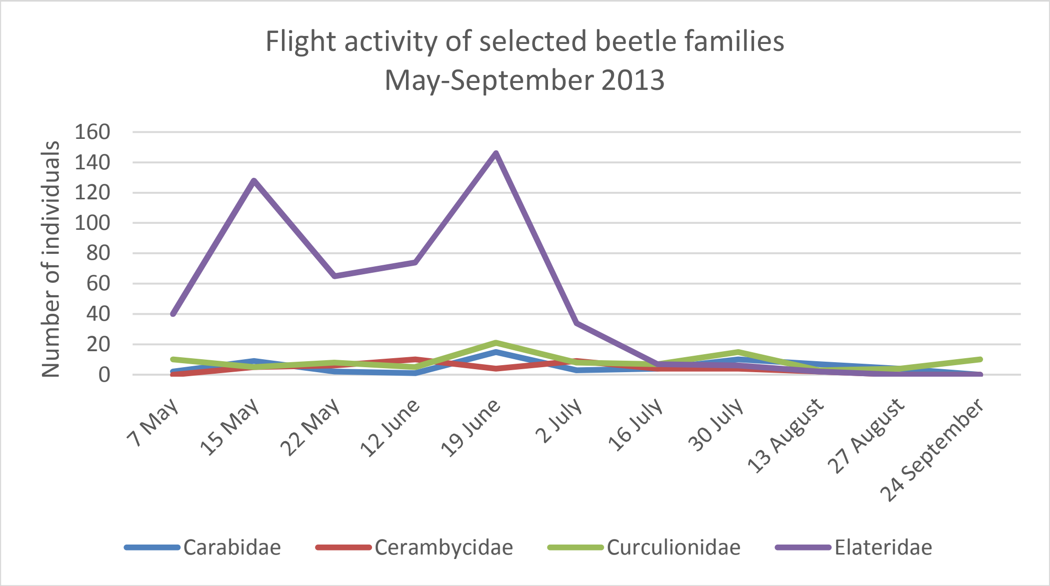


Figure 4- Flight activity from May until September for selected beetle families.

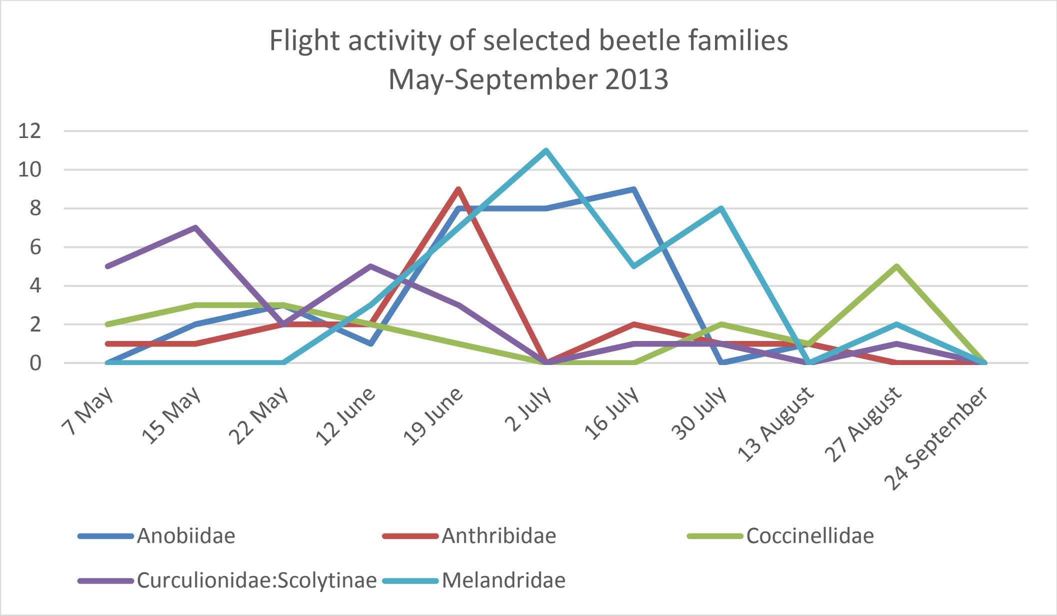


Figure 5 - Flight activity from May until September for selected beetle families.

6. DISCUSSION

6.1. Passive window traps

Different types of window traps can be used for catching various groups of beetles such as: a) attractive traps, b) interception traps or c) attractive interception traps (Bouget et al., 2008). Many kinds of window traps exist, such as passive window traps that were used in this experiment with two plastic panels that were creating cross (from the top view) with a container half filled with salty water. In order to catch certain saproxylic beetles Olsson et al. (2012) mention that in the forest stand with no fresh dead wood, using of tube-shaped window traps baited with different substrate types (into mesh bags, stapled inside so it still allows odors to be released while stopping beetles to feed on the bait). Olsson et al. (2012) continue, traps that were used for flight interception were made of thin sheet of transparent plastic (25x15 cm) which were bended and connected with for a pole made of wood from both sides – creating a tube of 5 centimeters diameter. From the top was closed with one more piece of plastic to prevent beetles entering the trap from above. Under it was installed tray made of aluminum, with a fixation liquid (changed every ten days) made of detergent and 50% propylene glycol were insects were collected.

However, like in this research, some scientists discuss that cross-vane window traps show better results when collecting saproxylic groups of beetles, even though sometimes they do not present very accurate data regarding micro-habitats. Approximately 60% of the all flying beetles can be caught in window traps, therefore it can also shows a representative status of saproxylic beetle fauna (Siitonen, 1994; Similä, 2002). Different models of traps were compared in Økland (1996) and regular window traps were the ones with highest efficiency where the most beetles was collected (60.2% of all collected specimens) in comparison with trunk-window traps (41.6%) and extraction cylinders (1.6%). Økland (1996) mentions that these window traps were able to catch flight active beetles from a quite large area of the forest.

6.2. Effects of VOC in Norway spruce – beetle association interactions

In this experiment, there was no further detailed research of the emission of volatile organic compounds released by Norway spruce trees in the experimental plot. However, many researchers were experimenting about this topic. Duduman & Vasian (2012), for example in their research about bark beetle, *Ips duplicatus* (Curculionidae: Scolytinae), mention that large number of this species was captured in areas where fresh host material tree was occurring and it proves that this beetles species population was increasing and directed by natural volatile organic substances released from Norway spruce, not only attracted by pheromones released by other beetles. Wide range of volatiles substances released by Norway spruce debris is somehow resembling to spectrum of volatile compounds released by weakened trees chosen for colonization by beetles.

Volatile substances depend on temperature in the surrounding area or of the temperature of the tree which is emitting it. Fillela et al., (2007) showed that, for example monoterpenes emissions rates, including other volatile substances such as methanol, hexanal, hexanol and MeSa depend more on temperature of other emitted substances when rate of emission is marked against the temperature of the leaf

Zhao (2011) mentions that defensive role of terpenes in conifers against bark beetles is indirect, even though those phytochemicals were generally considered to be very important in conifers defense mechanisms against beetles. Zhao (2001) continues, accumulation of terpenes activated when fungi were by inoculated which resulted in colonization of *Ips typographus* in dose – dependent way. This was one of the pioneer studies which showed a dose – dependent interactions among induced therpenes and resistance of the tree against bark beetle.

In order to understand the importance of physicochemical features of various biogenic volatile organic compounds, it is necessary to determine the dynamics of their emission (Fillela et al., 2007).

6.3. Influence of droughts in Norway spruce

Regarding the drought in this experiment, during the experimental period in the research area there was regular precipitation (for a conditions of the Central Bohemia), which means there was no extreme drought in the control plots. Slopes of the spruce stand in the experimental area was facing south-west which might had had influence on potential attack of saproxylic beetles.

In central Europe, Norway spruce is one of the most important tree species in the matter of economics, rather than in forest ecology; but since it was very sensitive on water stress, it might be affected by intense and frequent droughts which are going to be more often happen in the near future due the climatic changes (Gaul et al., 2008).

However, as Berg at al., (2006) mention, if hot temperatures occur in longer periods (5-6 years) it will probably result in the outbreaks of spruce bark beetle, whereas their reproduction will increase together with stress where drought was introduced. Bark beetles mainly choose to attack at the end of the spring, mostly mature trees which struggle with short seasonal droughts because of cold soil, irregular water uptake or when precipitation is still very low. Spruces that grow in north – facing slopes have better chance to survive the drought, due that fact that soil is much warmer on that side. (Hard, 1987).

In the research of Nikolova et al., (2009), even though mean annual temperature during 2003 was very similar, drought factors reduced soil respiration in European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*), soil respiration was decreased much more in soil profile under spruce than beech. Regarding exhausted capacities of available soil water took much more time in stands of spruce than stands of beech, i.e. 75 days was in spruce and 45 days was in case of beech. Same was concluded by Borken & Beese (2005) in stands of beech and spruce where higher probability of water stress was observed in organic soil profile in spruce than in beech. In their research with experimental drought Gaul et al. (2008) found that effects of the artificial drought appeared only in the organic layer in fine roots of the Norway spruce, where loss of the fine roots was two times higher than in the control plots and production of the fine roots was much higher in the control plots which received precipitation on the regular basis during the drought period of six weeks.

It is expected that visible abiotic factors of climate change in European forest ecosystems will be damages of trees caused by hail, water deficit and wind throws, since mostly soil chemistry will be affected too. Dryness of the soil can start to release some organic and inorganic solutes. It was observed that usually water stress affect the microbial biomass of the soil (Lamersdorf et al., 1998).

Even though, this issue has huge influence on tree suffering during the drought periods, no further research was done in this experiment regarding soil chemistry in water stressed area and interaction between tree root systems and microbial flora and fauna. Tree functioning when is missing the water input via soil and its root system induce further reactions with saproxylic and xylophagous beetles in the forest ecosystem.

6.4. Flight activity in selected beetle families

Due to low number of caught individuals in many families, it is very hard to conclude if there is any difference among them and their preference between higher or lower elevations in the experimental area. However families such as: Anobiidae, Carabidae and Cerambycidae were presented with quite large number and more abundant than others, but statistically tested, there was not important difference between flight level preference. Their p-values were generally that high, that is almost sure that these beetles were flying in same manner at the higher and lower elevations. Anobiidae, according to Vodka & Čížek (2013), preferred both, understory and canopy for flying activities, it was depending on the species itself, where it was occurring mostly. Family Coccinellidae was presented with lower number of caught individuals compared to three previously mentioned above, but statistically tested, these beetles showed difference in preference between elevations, since more individuals were caught in the lower window traps. However, in their research in the USA in a temperate deciduous forest, Ulyshen & Hanula (2007), caught more individuals and species of the ladybugs family in the canopy (flight-intercept traps were suspended in the canopy on more than 15 meters) than at the half meters above the ground. Similar results were shown for family Cerambycidae which were also quite abundant but more prefer higher elevations. Carabidae were also one of the abundant species, but they have in comparison to Cerambycidae preferred lower (ground) elevations.

The most interesting family among those that were caught were click beetles (Elateridae) were highest number of individual beetles was caught and also with lowest p-value it can be concluded that these beetles highly preferred lower elevations than upper. Similar results were achieved by Vodka & Čížek (2013), where they showed that higher percentage of certain click beetle species was observed at the edge of the understory other than at the edge of the canopy. They discuss that it might be due the fact that there is higher amount of the dead wood material in the understory than in the canopy.

7. CONCLUSION

Due the fact that climate change and slightly temperature increase can be one of the factors that disturb forest ecosystems, it is expected also a change in ecology of some insect pests. According to Hlásny & Turčáni (2009), in the case of the for example, *Ips typographus* and its distribution in the Europe – it is expected that the area where it is possible to develop its second generation in the spruce range will be doubled by the year 2075 and 20% of the current spruce range will be potential area for the third generation development up to 2045. Trees in this area are much more sensitive, since they are not quite adapted to more than two generation of *Ips typographus*. Hlásny & Turčáni (2009) continue that moisture is also one of the most important factors when is about physiological condition of the host tree, because vital trees are more resistant to stop the attack of bark beetles.

Also, further research about volatile organic substances in Norway spruce and correlation with various saproxylic and xylophagous beetle species during climate changes should be done – especially regarding insects behavior, which are considered to be pests and that can damage the host tree.

In this experiment, there were obtained quite interesting results and data. It can be concluded that majority of families that were caught during the research preferred to associate with the spruce trees that were not stressed during five months of 2013. Even though, there were some individual trees that showed similar characteristics as trees that were not stressed by water deficit, brings the idea that anatomy and genetics of these trees can be further examined due the fact that spruce might getting adapted on more dry climate, i.e. drought in general.

However, it cannot be generally concluded that most of the families were flight active in higher elevations of the experimental area, but there are certain varieties among them. As it was mentioned before, due the fact that was caught low amount of individual beetles, it is hard to make a conclusion for a major part of families. But, those that showed higher abundance, gives a picture about their flight activity in the experimental area.

In the future, it will be important to understand how these above mentioned abiotic factors (especially the drought) influence insects' behavior towards vegetation. It is also very important to understand how insect pests and economically important trees interact, due the fact that demand for wood in the near future will be increasing.

8. RECOMMENDATONS

In case of further research or repeating the experiment, it is recommended to include some of the following advises:

- Design of the used window traps was satisfactory in the matter of size, shape and materials used for the building of the traps.
- In case of climbing the ladder it is recommended to use forester climbing equipment.
- Since, some of the families showed low number of caught individuals, it is recommended to extend the experiment two or more seasons.
- Install the equipment for following the data about volatile organic substances in the stressed and non-stressed trees and compare it with families of beetles which were attracted to those trees.
- It was clear that stressed trees had different beetle associations. It is recommended in order to follow better their flight activity to reduce the elevation of the window traps and to get better knowledge (e.g. lower the window traps to get more knowledge about certain species from the families of Coccinellidae and Nitidulidae)
- Apply results obtained in different fields of study, e.g. correlation between stressed trees in the areas with dry soils in general, no matter of the precipitation.

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10. ANNEXES

10.1. Photos

Photo 1 – Experimental plot covered by roof system. There can be seen gutter, through with rainfall was taken out from the plot. Ladder was used to climb on the roof and empty and change window traps.
(Mladenović Strahinja, 2013)

Photo 2 – Experimental plot covered by roof system.
(Mladenović Strahinja, 2013)

Photo 3 – Control experimental plot and one of the window traps which was put down in order to take insect material and change fixation liquid. (Mladenović Strahinja, 2013)

Photo 4 – Window trap without catching container.
(Mladenović Strahinja, 2013)

Photo 5 – System of roofs in experimental plot and the window traps hanging. (Mladenović Strahinja, 2013)

Photo 6 – System of roofs in experimental plot. (Mladenović Strahinja, 2013)

Photo 7 – Complex of gutters was taking water input far away from experimental plot. (Mladenović Strahinja, 2013)

Photo 8 – Roof system in the experimental plot. (Mladenović Strahinja, 2013)

Photo 9 – Roof system in the experimental plot. (Mladenović Strahinja, 2013)

Photo 10 – Plastic bags where insect material was stored after emptying the containers. Each bag was marked with: Trap position and number of the tree, Location and Date. (Mladenović Strahinja, 2013)

Photo 11 – Equipment that was used to climb higher window traps.
(Mladenović Strahinja, 2013)

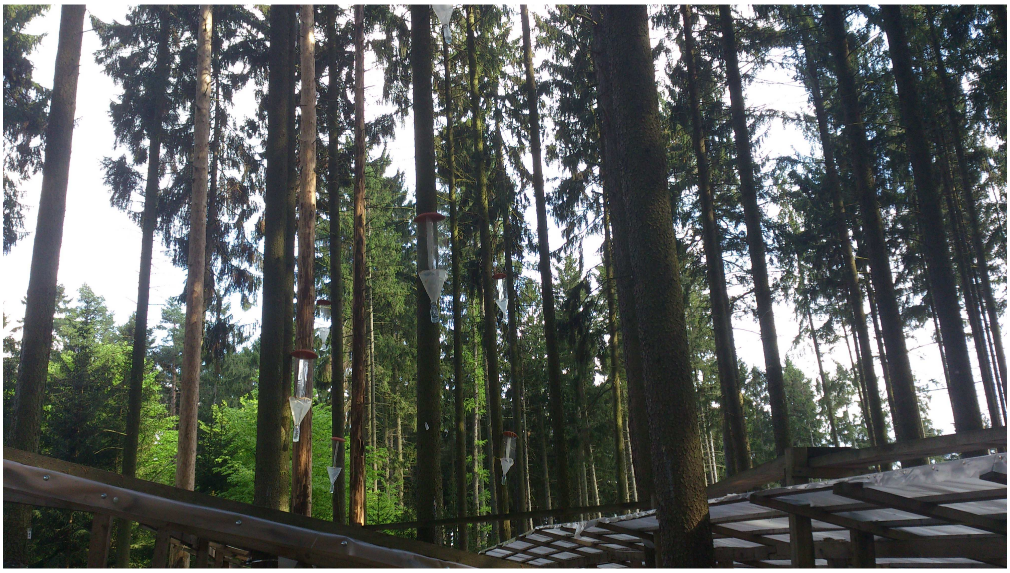

Photo 12 – Window traps above the roofed experimental plot.
(Mladenović Strahinja, 2013)

Photo 13 – Canopy of the control spruce stand (Mladenović Strahinja, 2013)

Photo 14 – Window trap hanging with the container of fixation liquid (Mladenović Strahinja, 2013

Table 1 - Total number of all caught individuals in each family, represented by stressed and control plots, tree numbers and positions of the the trap (H - upper, D - down).

Stressed / Control plot	Stressed								Control								Stressed								Control							
Tree number / Trap	1		2		4		5		6		7		8		9		11		12		14		15		16		18		19		20	
Family	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Anobiidae	2	3	1	0	0	0	1	1	1	1	5	2	0	2	1	2	0	0	0	1	1	2	0	0	0	1	0	2	1	1	0	1
Anthribidae	0	0	0	0	0	0	0	0	0	2	0	2	1	2	1	1	0	0	0	0	0	0	0	0	3	0	0	0	2	2	2	1
Buprestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Byturidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Cantharidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1
Carabidae	1	3	0	0	0	0	2	2	1	6	5	5	2	2	1	1	1	1	1	1	2	2	1	1	1	3	3	4	1	2	2	0
Cerambycidae	0	2	2	1	2	0	3	1	3	1	1	5	2	4	0	2	0	1	0	0	0	1	2	1	1	3	1	1	1	2	0	1
Cleridae	0	0	0	0	0	1	0	2	0	0	1	0	0	2	0	1	1	0	1	0	0	0	0	0	0	1	0	2	2	1	2	0
Coccinellidae	1	0	0	0	0	1	0	0	0	1	2	1	1	0	0	0	0	0	0	2	0	2	0	0	0	0	0	1	0	3	1	3
Colydiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae	1	4	4	7	2	3	2	8	3	3	5	10	3	10	3	2	0	2	1	1	1	1	0	3	4	2	0	1	3	4	1	2
Curculionidae:Scolytinae	0	1	0	0	0	0	1	0	0	3	3	1	1	1	0	0	0	0	0	2	1	3	1	0	0	0	2	0	1	1	2	1
Dermestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Elateridae	6	8	7	9	12	8	14	14	21	30	29	52	22	61	19	37	0	2	2	6	5	11	3	3	7	16	7	9	12	21	23	26
Eucnemidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Chrysomelidae	0	0	0	2	2	1	1	0	1	1	1	2	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lampyridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Leiodidae:Cholevinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymexylidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melandridae	0	0	1	1	0	0	1	0	6	4	3	4	4	0	2	0	0	0	0	1	2	0	0	0	2	0	1	1	1	0	1	1
Nitidulidae	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
Oedemeridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Scarabaeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Scraptiidae	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylinidae	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tenebrionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0