Pine Weevil Feeding Behaviour in Relation to Conifer Plant Properties

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Cover: Pine weevil feeding on Norway spruce plant
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Abstract

The pine weevil (*Hylobius abietis* (L.)) is a forest insect distributed over the Palearctic region. The adults feed on the phloem of young conifer plants causing high economic losses for the European forest industry. Still, there is very little knowledge about the structure of its feeding behaviour. Feeding behaviour can be studied in several different temporal resolutions, from differences in feeding after several weeks to diel patterns and short-term feeding, i.e. feeding patterns at the level of feeding events and meals. The aim of this thesis was to increase the knowledge about the pine weevils' feeding patterns and the underlying behavioural mechanisms.

I studied the pine weevils' time budget and diel behaviour as well as its short-term feeding behaviour based on video recordings. In addition, I assessed how changes in plant properties due to girdling or induction of plant defences with a chemical elicitor, methyl jasmonate (MeJA), affect the feeding pattern and preferences of the pine weevil.

Pine weevils allocated only 6 % of the time to feeding. Most of the time was spent away from the plant (70 – 80 %). Damaged plants appeared to attract the weevils because they spent more time while not feeding on damaged plants than on undamaged plants. Feeding behaviour was mostly concentrated to the second half of the dark phase, after a peak of locomotion behaviour during the first part of the dark phase. During the light phase, pine weevils mostly rested. Analysis of the short-term feeding behaviour showed that pine weevils made 4-5 meals per day, removing about 13 mm^2 during about 24 minutes in each meal. Some of the feeding properties, such as how much time was spent not feeding during a meal, differed between male and female weevils. Girdling did not affect the time budget or feeding properties.

The induced plant defences with MeJA caused a reduction in meal duration. When meals consisting of only phloem, only needles or both were compared, the meal duration and the time until the initiation of a meal were more similar between the different meal contents on induced plants. In addition, the results from a no-choice and a choice experiment indicate that the protective effect of MeJA-induced defences is, besides an overall reduction of feeding, mainly due to the reduced amount that a pine weevil can feed at one place. Thus the risk of girdling and death of the plant is reduced.

Keywords: diurnal, feeding intervals, food intake, herbivore, meal criterion, *Picea abies*, *Pinus sylvestris*, plant-insect interactions, prandial correlation, sex differences

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Dedication

To my father

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Fedderwitz, F., Björklund, N., Ninkovic, V. & Nordlander, G. (2014). Diel behaviour and time budget of the adult pine weevil *Hylobius abietis*. *Physiological Entomology* 39, 103-110.

II Fedderwitz, F., Björklund, N., Ninkovic, V. & Nordlander, G. The structure of feeding behavior in a phytophagous insect (*Hylobius abietis*) (submitted manuscript).

III Lundborg, L., Fedderwitz, F., Björklund, N., Nordlander, G., Ohlsson, A.B. & Borg-Karlson, A.-K. Induced chemical defences of phloem and needles in Scots pine affecting pine weevil feeding (manuscript).

IV Fedderwitz, F., Nordlander, G., Ninkovic, V. & Björklund, N. Effects of the induced defence of conifer plants on the feeding behaviour of a bark-feeding insect (manuscript).

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The contribution of Frauke Fedderwitz to the papers included in this thesis was as follows:

I Main author, practical work and data analysis. Research question and design together with Göran Nordlander, Niklas Björklund and Velemir Ninkovic.

II Main author, practical work and data analysis. Research question and design together with Göran Nordlander, Niklas Björklund and Velemir Ninkovic.

III Second author with equal contribution as main author. Practical work and data analysis with Lina Lundborg and Anna B. Ohlsson. Research question and design with Lina Lundborg, Niklas Björklund, Göran Nordlander and Anna-Karin Borg-Karlson.

IV Main author, practical work and data analysis. Research question and design together with Niklas Björklund, Göran Nordlander and Velemir Ninkovic.

1 Introduction

The feeding damage on conifer plants caused by pine weevils has been a concern for a long time (Ratzeburg, 1839). It is therefore not unexpected that this is by far not the first study concerned with pine weevil feeding (e.g. Hesse *et al.*, 1955; Christiansen & Bakke, 1968; Christiansen & Bakke, 1971; Pohris, 1983; Manlove *et al.*, 1997; Nordlander *et al.*, 2003; Zas *et al.*, 2011). Unlike previous studies, this thesis focuses on how feeding is structured and the behavioural mechanisms behind it, rather than concentrating on the result of feeding after a certain time period.

1.1 Pine weevil

The pine weevil (*Hylobius abietis* (L.) (Coleoptera: Curculionidae)) is one of the economically most important forest pest insects in Europe (Fig. 1; Eidmann & Klingström, 1990). The adults feed on the bark of different conifer species such as Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) (Ratzeburg, 1839). Although the weevil feeds predominantly on roots and twigs of mature trees, the economically important feeding damages occur on young conifer plants (Örlander *et al.*, 2000; Bylund *et al.*, 2004; Wallertz *et al.*, 2006). Such damages can cause up to 90% plant mortality (Petersson & Örlander, 2003).

Figure 1. Pine weevil (*Hylobius abietis*) on Norway spruce (*Picea abies*) plant with feeding damage.

During their long-distance migration in spring and early summer adult weevils are guided by volatiles to suitable reproduction areas (Eidmann, 1974; Solbreck & Gyldberg, 1979). Their wing muscles degenerate soon after they settle (Nordenhem, 1989). Walking weevils take advantage of both visual and olfactory cues to locate conifer plants (Björklund *et al.*, 2005). After copulation female weevils lay their eggs near the roots of recently killed or dying conifer trees, which can be found at high density in man-made clear-cuts and after storm fellings or forest fires (Nordlander *et al.*, 1997). The larvae of the pine weevil feed by tunnelling in the bark of roots of conifer trees, but do not cause damages to plants as the adult pine weevils do (Ratzeburg, 1839).

1.2 Behaviour

1.2.1 Time budget

The proportion of time that an animal allocates to different behaviours is described by the time budget (Joern *et al.*, 1986). The proportion of time a certain behaviour is expressed, does not necessarily indicate its importance, but reflects the optimal solution to environmental challenges (Joern *et al.*, 1986). Many insects spend most of their time resting and only a small part of their time is spent feeding (Joern *et al.*, 1986; Mitchell & Low, 1994; Duan *et al.*, 1996; Wright *et al.*, 1999; Drees *et al.*, 2008).

1.2.2 Diel behaviour

Based on diel rhythms, organisms can prepare for periodic changes in the environment (e.g. end of darkness). For instance, the apple blossom weevil and the Warren rootcollar weevil rest mostly during the light phase and are active and feed during the dark phase (Cerezke, 1994; Duan *et al.*, 1996). Furthermore, the Warren rootcollar weevil and *Hylobius xiaoi* have distinct diel movement patterns, climbing up trees at dusk and down again at dawn (Cerezke, 1994; Wen *et al.*, 2004). Diel rhythms are also important for inter- and intra-species relationships (Danilevsky *et al.*, 1970; Harmsen *et al.*, 2011). For example, the females of different pyralid moth species release pheromones during different periods of the dark phase in accordance with high locomotion activity of the males of the respective species (Zavodska *et al.*, 2012).

The pine weevil has, under laboratory conditions, most locomotion activity during the beginning of the dark phase (Merivee *et al.*, 1998; Pszczolkowski & Dobrowolski, 1999). Under field conditions, ambient temperature and humidity influence locomotion activity, which are generally most favourable during twilight (Sibul *et al.*, 1999). Pine weevil feeding is mostly concentrated during the dark phase in field conditions, however no obvious pattern has been shown in shaded cages in semi-field conditions or under laboratory conditions (Christiansen & Bakke, 1971; Merivee *et al.*, 1998).

1.2.3 Short-term feeding behaviour

On a shorter time scale than diel behaviour, short-term feeding behaviour can be used to investigate feeding patterns at the level of feeding events and meals (Tolkamp *et al.*, 2000; Howie *et al.*, 2009). Bites into a food source (Fig. 2) or similar feeding events can be grouped into discrete bouts of ingestion. These bouts are called meals and are commonly used as a unit of short-term feeding behaviour (e.g. Simpson, 1981; Raubenheimer & Bernays, 1993; Mitchell & Low, 1994; Bernays & Singer, 1998; Tomlin *et al.*, 1998; Daoust *et al.*, 2010). Meals are independent of the unit they were originally measured in and thereby allow easier comparisons between different studies (Howie *et al.*, 2009).

Figure 2. Pine weevil feeding on Norway spruce plant

The non-feeding periods or intervals of chewing animals can be separated into intervals between meals (intermeal interval) and intervals within meals (intrameal intervals). Generally, chewing animals make a high number of intrameal intervals and a lower number of intermeal interval (Simpson, 1990). Intrameal intervals are, by definition, shorter than intermeal intervals. The shortest non-feeding interval considered to be between two meals is called meal criterion (Tolkamp *et al.*, 2011). The meal criterion is determined based on the distribution of the duration of non-feeding intervals, which results in separate populations for intrameal and intermeal intervals. Two distributions, generally normal or Weibull distributions, are fitted to the data and their intersection is the meal criterion. This statistical approach has been developed in recent years and takes into consideration that feeding is not started at random (Tolkamp *et al.*, 1998; Tolkamp *et al.*, 2011). The relationship between meal size and intermeal intervals before or after the meal, can be described with pre- and post-prandial correlations (Le Magnen & Tallon, 1966; Savory, 1981).

There are relatively few previous studies on insects' short-term feeding behaviour that use a meal criterion (Bowdan, 1988b; Simpson *et al.*, 1988;

Simpson *et al.*, 1989; Raubenheimer & Bernays, 1993; Bright *et al.*, 1994; Mitchell & Low, 1994; Wright *et al.*, 1999; Jones & Raubenheimer, 2002; Nagata & Nagasawa, 2006; Colasurdo *et al.*, 2007). In these studies the meal criteria were determined using a different statistical approach, which has an underlying assumption of events starting at random (Sibly *et al.*, 1990; Mitchell & Low, 1994). This thesis is the first to determine the pine weevil's meal criterion and analyse its short-term feeding behaviour. Additionally, Paper II is the first study on insects using the more recent statistical approach.

1.2.4 No-choice and choice feeding experiments

During choice experiments, animals have access to several different food sources, most commonly two but more are possible, while they only have one food source during no-choice experiments (e.g. Roa, 1992; Zas *et al.*, 2011; Meier *et al.*, 2014). In case of the pine weevil, no-choice and choice experiments have mostly been used to rank the preference of different tree species (e.g. Leather *et al.*, 1994; Manlove *et al.*, 1997; Månsson & Schlyter, 2004; Toivonen & Viiri, 2006; Wallertz *et al.*, 2014) or to test antifeedant and deterrent substances (e.g. Bratt *et al.*, 2001; Månsson *et al.*, 2005; Borg-Karlson *et al.*, 2006; Unelius *et al.*, 2006; Bohman *et al.*, 2008; Eriksson *et al.*, 2008). Additionally, the efficiency of treatments for plant protection has been tested (Heijari *et al.*, 2005; Moreira *et al.*, 2009; Sampedro *et al.*, 2011; Paper III).

No-choice and choice experiments focus on different aspects of the mechanisms behind feeding behaviour. In a no-choice experiment properties of different food sources can be compared without one influencing the other. Therewith assessments of the influence of food properties on feeding behaviour in no-choice experiments are more straightforward. However, having no choice also forces animals to adapt their feeding to the provided food source or not feed at all. Choice experiments, on the other hand, let animals choose on which food source to feed. Animals can switch between food sources and therewith compensate for e.g. nutritional unbalances. Nevertheless, a choice experiment can also result in an animal not feeding or feeding less on a food source that has been shown to be suitable in a no-choice experiment.

1.3 Plant treatments

Two methods were used to influence the properties of conifer plants: girdling (Paper I and II) and treatment with methyl jasmonate (Paper III and IV). Both methods have an impact on the plants' defence systems, which can in turn affect the behaviour of herbivorous insects towards their host (e.g. Miller *et al.*, 2005; Erbilgin *et al.*, 2006; Heijari *et al.*, 2008).

The defence systems of conifers consist of the constitutive defence, which is always present, and the induced defence, which is only expressed during an attack (Franceschi *et al.*, 2005; Eyles *et al.*, 2010). Conifer defences vary with age, chemotype and plant tissue (Kännaste *et al.*, 2013). According to the optimal defence theory, the most important or most often attacked tissues should have the best defences (Stamp, 2003). Nitrogen concentrations can be used as a proxy of the relative importance of different tissues (Moreira *et al.*, 2012). For example, in the stem tissue of *Pinus radiata* concentrations of nitrogen correlate with the concentration of non-volatile resin, which is part of the plant defences (Moreira *et al.*, 2012).

1.3.1 Girdling

Girdling means the complete severing of the phloem of a tree (Noel, 1970). There are, however, different approaches to girdling a tree. Girdling can refer to the removal of an entire ring of bark or a thin incision in the bark (Noel, 1970). If bark is manually removed, girdling can further be distinguished into whether or not tissues internal to the vascular cambium are removed (Noel, 1970). Depending on the tree species, the kind and extent of the girdling, trees can experience a range of negative effects from girdling, which might result in their death (Noel, 1970).

Figure 3. Norway spruce plant girdled by pine weevils.

Girdling of the stem is the most common cause of death in plants after pine weevil attacks (Fig. 3; Eidmann, 1974). Damages to plants generally change the chemical composition of the stem tissue as well as the emitted volatiles (Gref & Ericsson, 1985; Martin *et al.*, 2003). These chemical changes also influence pine weevil behaviour (Kännaste *et al.*, 2009). Damaged plants have been shown to be more attractive to pine weevils (Tilles *et al.*, 1986; Nordlander, 1991).

1.3.2 Methyl jasmonate

Methyl jasmonate (MeJA) is a naturally occurring plant hormone involved in the regulations of plant defences (Holopainen *et al.*, 2009). It can be used as an elicitor to induce the plant defence system, which can result in a more efficient defence reaction during subsequent stresses (Goellner & Conrath, 2008; Bruce, 2010; Delaunois *et al.*, 2014). The chemical response of plants to the exogenous application of MeJA can be similar to the response towards insect feeding (Rohwer & Erwin, 2008).

The effects of MeJA-induced defence in conifers on the damages caused by the pine weevil have been studied in several publications during the past years (e.g. Heijari *et al.*, 2005; Sampedro *et al.*, 2011; Zas *et al.*, 2014). In general, the application of MeJA reduced the feeding on the bark of conifer plants, but the efficiency of the treatment is plant species and concentration dependent (Moreira *et al.*, 2009; Sampedro *et al.*, 2011; Zas *et al.*, 2014).

2 Thesis aims

The general aim of this thesis was to increase the knowledge about the feeding behaviour of the adult pine weevil and how it is affected by different plant properties. Research on pine weevil feeding has previously concentrated mainly on the "outcome" of feeding, meaning the damage that is caused after a certain time period. There was little information on how these damages are made and if the underlying mechanisms could be affected by plant treatments. This kind of knowledge is important to better understand the interactions between pine weevils and plants.

The specific aims of each paper were:

I Determining the diel feeding and locomotion behaviour as well as the time budget of the pine weevil on intact and girdled Norway spruce plants.

II Assessing the structure of short-term feeding behaviour of pine weevils on intact and girdled Norway spruce plants with an additional focus on differences between sexes.

III Linking the effects of MeJA-induced defence systems on the short-term feeding behaviour of pine weevils to the chemical changes in the plants.

IV Evaluating the effects of an induced defence in conifer plants (by MeJA treatment) on the feeding behaviour of pine weevils in a choice and no-choice situation.

3 Methods

In the first part of this thesis (Paper I and II) pine weevil behaviour is studied on undamaged and girdled plants, whereas the second part of the thesis (Paper III and IV) focuses on the effects of an artificially induced defence system. Paper I and II are based on video recordings of the same experiment, but focus on two different aspects: the time budget and diel behaviour in Paper I and the short-term feeding behaviour in Paper II. In Paper III, changes in short-term feeding behaviour due to induced plant defences were linked to effects of the induction and weevil feeding on plant chemistry. The no-choice and choice experiments in Paper IV investigated effects of induced plant defences, too. These last experiments were based on a larger number of plants compared to the other two experiments, but had a lower or no temporal resolution.

3.1 General experimental set-ups

Pine weevils were collected in central Sweden during spring migration in the year of each experiment. During storage in darkness at 10°C the weevils were fed with Scots pine branches. These storage conditions interrupt the reproductive development of the weevils, so that egg laying and the associated period of high feeding activity begins after the transfer to experimental conditions (Bylund *et al.*, 2004). Weevils were transferred to experimental conditions at least one week before they were used in the experiments. Directly before the experiments single pine weevils were kept for at least a day in Petri dishes. To make sure unhealthy weevils were excluded, only weevils that were feeding during this time were used in the experiments. Both sexes were equally represented in all behavioural experiments.

Norway spruce seedlings that were 1.5-years old were used for Paper I and II, 1-year old Scots pine seedlings were used for Paper III and Norway spruce cuttings that were rooted 3 years before the experiment were used for Paper IV.

The plants were kept outdoors and transferred to experimental conditions one to five days prior to the start of the experiments.

All experiments were conducted at room temperature (ca. 22°C) and with an artificial light-dark-cycle (L18:D6).

3.2 Time budget and diel behaviour

The time budget and diel behaviour were determined by video recording single pine weevils in a transparent plastic cylinder that was placed around the middle part of a Norway spruce plant (Fig. 4A; Paper I). Two video cameras (Sony Handycam HDR-XR550VE, Sony Corporation, Tokyo, Japan) were placed at a 45° angle to each other for best visibility of weevil activity close to the plant. Each weevil was recorded for two separate 24-hour observation sessions. During one observation session the weevil had access to a non-girdled plant and during the other observation session it had access to a previously manually girdled plant. The weevils' behaviour related to feeding and locomotion was each observed in mutually exclusive groups. Behaviours were recorded continuously (The Observer XT10, Noldus Information Technology, Wageningen, Netherlands).

3.2.1 Analysis

For the time budget analysis the following proportions of the time of an observation session were calculated (Paper I): locomotion and standing on the plant, locomotion and standing not on the plant, out of vision. The time on the plant was further divided into time when they were feeding or not feeding. The time spent in the above mentioned behaviours were compared between girdled and non-girdled plants as well as between male and female weevils (R, Version 2.12.1, The R Foundation for Statistical Computing, Austria).

For the diel behaviour analysis the time spent in each of the defined behaviours and the time spent on the plant during each hour of the observation sessions were calculated (Paper I). The duration of feeding, locomotion and the time spent on the plant before the first feeding were compared between girdled and non-girdled plants as well as male and female pine weevils.

3.3 Short-term feeding behaviour

3.3.1 Determination of the meal criterion

The meal criterion was based on the same data that were described in section 3.2 (Paper II). The start and end of every feeding event was measured continuously by visual examination of the video-recordings (The Observer

XT10). Feeding was defined as manipulation of bark or needles with the weevils' mouth part. An approach described by Tolkamp *et al.* (2011) was used to determine the meal criterion. The durations of all non-feeding intervals were $\log_e$-transformed and a model based on two Weibull distributions was fitted to the data.

3.3.2 Structure of short-term feeding behaviour

Short-term feeding behaviour on girdled and non-girdled plants was assessed based on the video files of the first experiment (see section 3.2; Paper II). Meals were separated based on the above described meal criterion, which allowed the calculation of meal properties, such as meal duration, number of meals and feeding rate. Each meal property was compared between girdled and non-girdled plants as well as male and female weevils. Furthermore pre- and post-prandial correlations were calculated to assess the influence of intermeals on meal duration and vice versa.

In addition, the average feeding scar size as well as locomotion behaviour after meals were measured.

3.3.3 Short-term feeding on MeJA-treated plants

Scots pine plants were sprayed twice with an aqueous solution of 25 mM MeJA (Paper III). Control plants were treated with the carrier solution. The plants had on average seven weeks from the second treatment for induction of chemical defences.

Pine weevils were filmed for a total of 20 days (Fig. 4B; Paper III). Each weevil had access to either a MeJA-treated plant or a control plant for four days, of which two non-consecutive days were video-recorded. Meals were identified with the meal criterion and separated according to the tissue weevils fed on (The Observer XT10): meals fed only on phloem, meals fed on phloem and needles (mixed meals) and those fed on only needles. The number of meals, meal duration and the time from the introduction of the plant to the start of the meal (latency) were calculated. The feeding rate of phloem meals was assessed, but could not be measured for mixed or needle meals. The influence of MeJA treatment, weevil sex and meal content on the feeding behaviour was assessed (R, Version 3.1.0).

Chemical analysis of MeJA-treated plants

On the same plant material as was used for the short-term feeding analysis chemical analysis were made (Paper III). The main aim of the chemical analysis was to evaluate changes in chemical defence compounds due to MeJA treatment and pine weevil feeding. Volatile fraction of the plant defences were extracted from both phloem and needles before and after weevil feeding. Terpenes were analysed by gas chromatography-mass spectrometry (GC-MS) and phenolics with spectrophotometric quantification. Selected monoterpenes were analysed for chiral enantiomers on a GC-GC-MS system. In addition volatile emissions were collected during a second feeding session to evaluate the temporal changes of the volatile profile. Volatile emissions were as well analysed by GC-MS. After this second feeding session extracts were also taken from phloem and needles and analysed as above.

3.4 No-choice and choice feeding

Half of the Norway spruce plants were sprayed twice with an aqueous solution of 50 mM MeJA (Paper IV). The remaining plants were sprayed twice with the carrier solution as control. The no-choice experiment was conducted about four weeks and the choice experiment eight weeks after the second treatment.

For the no-choice experiment single plants were covered with transparent plastic cylinders and exposed to a single weevil (Fig. 4C). During the first five days of the experiment it was noted whether or not weevils had started to feed and the size and number of feeding scars at three time points per day. During the last five days, it was only recorded once per day whether or not weevils had started to feed on previously undamaged plants. At the end of the experiment, after 10 days, the number and size of feeding scars on all plants were recorded. Differences between MeJA-treated and control plants as well as male and female weevils were assessed (R, Version 3.1.1).

During the choice experiment each single weevil had access to a MeJA-treated and a control plant, which were together covered with a transparent plastic cylinder (Fig. 4D). To reduce the possibility of volatile interactions between the plants, the cylinders were connected to an air sucking system. The weevil was placed between the plants and removed after 10 days. The size and number of feeding scars were recorded at the end of the experiment. Differences between MeJA-treated and control plants as well as male and female weevils were assessed.

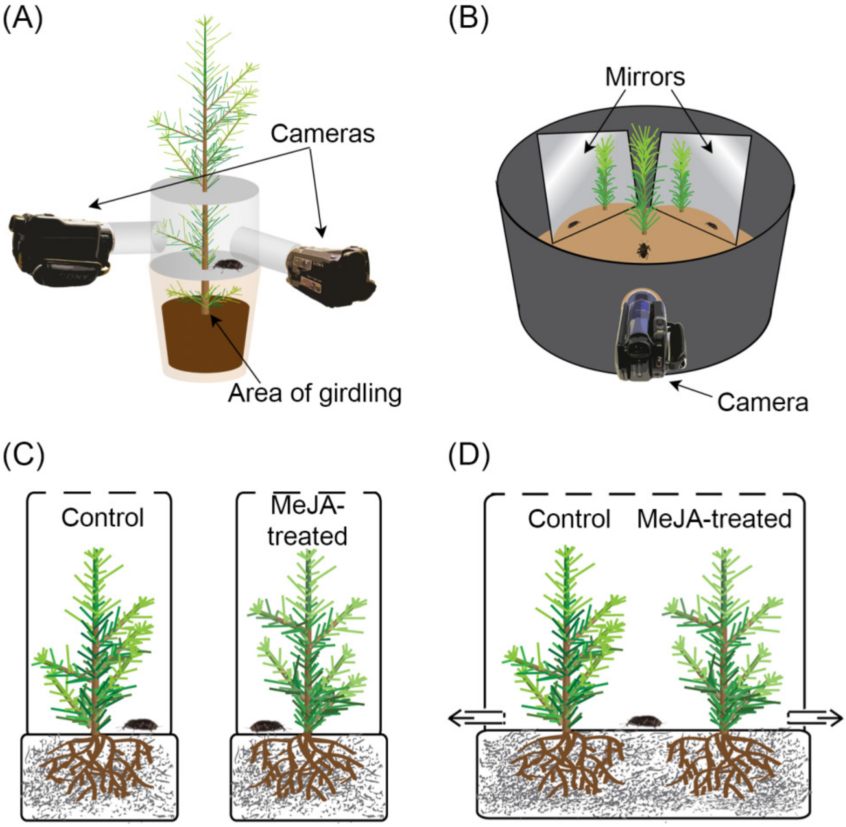

Figure 4. Experimental set-ups for experiments included in Paper I and II (A), the behavioural experiment of Paper III (B) and experiments of Paper IV (C and D). Video recording of a single pine weevil on either a girdled or non-girdled Norway spruce plant for analysis of time budget and diel behaviour (Paper I) and short-term feeding behaviour (Paper II) (A). Video recording of short-term feeding behaviour of single weevils on either a MeJA-treated or control Scots pine plant (B). No-choice experiment with single weevils on either a control or a MeJA-treated Norway spruce plant (Paper IV) (C). Choice-experiment with single weevils with access to both MeJA-treated and control Norway spruce plant; arrows indicate air flow (Paper IV) (D). Pine weevils had access to a water tube and a black hiding in all experiments (not shown). Figures are not to scale.

4 Results and Discussion

4.1 Time budget

Pine weevils were feeding during only 6 % of the time (Paper I). This is much less time than the apple blossom weevil spend feeding (up to 27 %), yet twice as much as Costa Rican weevils (Duan *et al.*, 1996; Wright *et al.*, 1999). These differences between species might be insect species specific, but might also be explained by the nutritional values or the defence reactions of the host plants (Harrison, 1987).

Regarding the location of pine weevils, they spent most of their time away from the plant (Paper I). During this time, female and male pine weevils spent similar amounts of time standing. However, if the time on the plant is included, females spent less time in locomotion. A higher locomotion activity for males has also been shown previously (Merivee *et al.*, 1998). In insects that do not produce long-range signals for attracting mates, as the pine weevil, it is common that males are more active in looking for mates (Tilles *et al.*, 1986; Brown *et al.*, 1997). Females spent more time on the plant although they are not necessarily feeding, which can also be seen in their diel behaviour (Fig. 5C). Girdling did not affect the time budget of 24 hour observation sessions.

Pine weevils, both males and females, spent, however, less time on non-girdled plants before the first feeding was recorded. This is also illustrated in the increase of time spent on the non-girdled plants while not feeding during the second half of the dark phase (Fig. 5B, Paper I). On girdled plants, there was no difference in time spent on plants before and after the first feeding (Paper I). After the first feeding, the time spent on girdled or non-girdled plants while not feeding is similar (Paper I). These results indicate that the undamaged plants are less attractive to the pine weevil, while there is no difference between girdling and recently made feeding scars. Damaged plants have also previously been found to be more attractive to pine weevils (Nordlander, 1991).

4.2 Diel behaviour

Locomotion activity of pine weevils was high during the dark phase, especially during the first part (Fig. 5A, Paper I). There was no difference in the diel distribution of locomotion behaviour between weevil sexes or girdled and non-girdled plants (Paper I). The high locomotion activity during the dark phase is in accordance with previous reports on the pine weevil (Merivee *et al.*, 1998; Pszczolkowski & Dobrowolski, 1999). In the field, pine weevil locomotion activity is especially high during dusk and dawn, when weather conditions are favourable in terms of temperature and moisture (Sibul *et al.*, 1999). In other weevil species, such as the Warren rootcollar weevil, pine root collar weevil, *Hylobius xiaoi* and the apple blossom weevil, high locomotion activity is common during the night as well (Wilson, 1968; Cerezke, 1994; Duan *et al.*, 1996; Wen *et al.*, 2004).

During the second half of the dark phase, locomotion activity decreased and feeding activity increased (Fig. 5A; Paper I). Feeding and locomotion are mutually exclusive behaviours and it is therefore difficult to determine whether locomotion is reduced towards the end of the dark phase because of the increased feeding or vice versa. Pohris (1983) has shown that pine weevils consume more food during constant darkness compared to constant light, which is in accordance with our results. Unlike the pine weevil, the apple blossom weevil has a sharp increase in feeding activity directly after the onset of the dark phase, which could be explained by the more evenly distributed locomotion activity during the dark phase (Duan *et al.*, 1996). Weevil sex did not influence the initiation of feeding, but females spent more time feeding throughout the day than males (Paper I). The diel feeding pattern was more distinct in male weevils compared to female weevils (Merivee *et al.*, 1998; Paper I). This difference in the feeding pattern could be explained by a higher nutritional requirement of females for e.g. egg production. Females need to digest larger amounts of food than males, but both sexes have similar digestion times (Nuorteva, 1972), which could make it necessary for females to spread their feeding behaviour over longer time periods and consequently affect the diel behaviour.

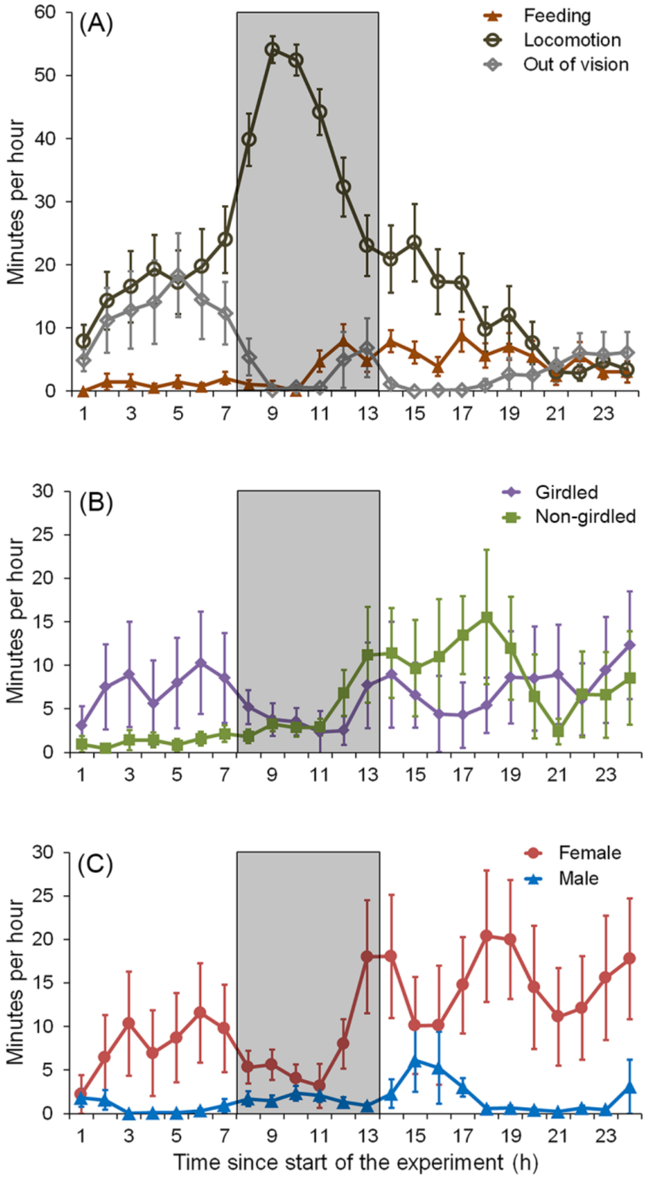

Figure 5. Diel behaviour pattern of the pine weevil based on both non-girdled and girdled plants (N = 12; Paper I) (A). Time spent on plant while not feeding for non-girdled (N = 12) and previously girdled (N=12) seedlings (B) as well as female (N = 6) and male (N = 6) weevils on both non-girdled and girdled plants (C). Shaded areas indicate the dark phase. Values are given as mean ± standard error.

4.3 Short-term feeding behaviour

The pine weevil's meal criterion is 4 minutes and 8 seconds. Therefore non-feeding intervals with at least this duration separate two meals (Fig 6; Paper II). The meal criterion was determined with a recent statistical approach that takes the non-random initiation of feeding into consideration (Tolkamp *et* *al.*, 2011). This method has previously only been implemented on vertebrates. Insect meal criteria have so far been determined based on log-survivorship or log-frequency analysis (e.g. Sibly *et al.*, 1990; Mitchell & Low, 1994; Tolkamp *et al.*, 1998; Tolkamp *et al.*, 2011).

The meal criterion of the pine weevil is in the lower range of meal criteria of other insects, ranging from under 2 minutes for the larvae of the silkworm to about 16 minutes for the forest tent caterpillar (Nagata & Nagasawa, 2006; Colasurdo *et al.*, 2007). However, these criteria have been estimated with a different method, which generally results in shorter criteria. For example, when the meal criterion for cows was estimated with the more recent approach it increased from about 8 to 35 minutes (Tolkamp & Kyriazakis, 1999). Thus the meal criterion of the pine weevil seems to be rather short.

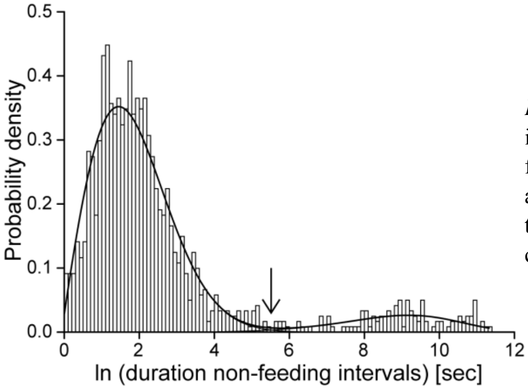

Figure 6. Distribution of non-feeding intervals ($N = 1205$) and fitted model for two Weibull distributions. The arrow indicates the intersection and therewith the location of the meal criterion (Fig. 2 in Paper II).

Some properties of the feeding pattern of the pine weevil differed between sexes (Fig. 7; Paper II). Male weevils had a shorter total meal duration per day, a longer total intermeal interval duration, fewer feeding occurrences within a meal as well as shorter average and total duration of intrameal intervals. The shorter total feeding time of male pine weevils has also been observed previously (Merivee *et al.*, 1998; Bylund *et al.*, 2004; Toivonen & Viiri, 2006). In previous studies of the pine weevil, feeding rate for male weevils was lower (Wainhouse *et al.*, 2004) and males made smaller feeding scars (Bylund *et al.*, 2004; Toivonen & Viiri, 2006). Neither could be observed in Paper II, and there were rather indications of a higher feeding rate in males.

Pine weevils made on average about four meals per day (Paper II). This is a rather low frequency compared to other insect species, for example the Costa Rican weevil (5 meals in 10 hours), Colorado potato beetle (9 meals in 7 hours) and the tobacco hornworm caterpillar (2 meals per hour, based on meal and intermeal durations) (Reynolds *et al.*, 1986; Mitchell & Low, 1994; Wright *et al.*, 1999).

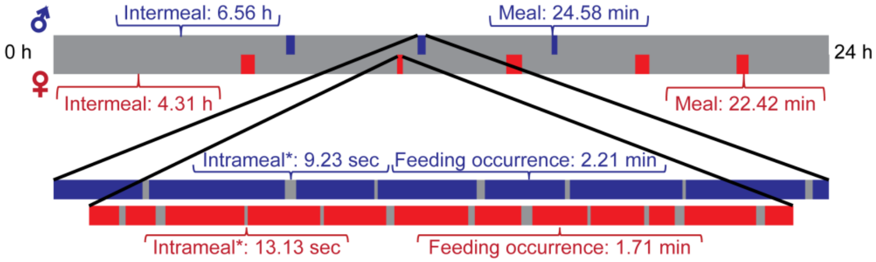

Figure 7. Schematic feeding pattern of male (N = 6) and female (N = 6) pine weevils during 24 hour observation sessions. Colored sections denote feeding activity (blue for males and red for females). The lower part of the figure shows median meals with single feeding occurrences and intrameal non-feeding intervals. (shown values are medians based on mean values for individual weevils; * p<0.05; Fig. 3 in Paper II).

One meal by pine weevils took about 24 minutes on average (Paper II), which is in the range of meal lengths of the Colorado potato beetle and the forest tent caterpillar, whose meals are about 20 and 30 minutes long, respectively (Mitchell & Low, 1994; Colasurdo *et al.*, 2007). Meals of other insects are, however, shorter ranging from about 2 to 18 minutes in duration (Simpson, 1982; Wright *et al.*, 1999).

Meal size depends on both meal duration and feeding rate. In the absence of external interruptions such as predators, meals end either due to the amount ingested or the time spent feeding. An analysis of the variation in feeding rates and meal sizes can give indications about which mechanism is most important for the termination of meals. Meals of the migratory locust, for example, rather end after a certain time than after a certain amount is ingested (Simpson, 1982). In Paper II it was only possible to determine meal size directly for 17 out of 117 meals. Based on these data, meal sizes tend to be linearly correlated to feeding durations. Thus feeding rate is approximately constant. Also for the Colorado potato beetle feeding rate is more or less constant for small meals, but during larger meals it becomes more variable (Mitchell & Low, 1994). Feeding rates are also variable for migratory locust, the first instar of the American bird grasshopper and the Australian sheep blowfly while their meal durations are constant; unless meals are very large (Simpson *et al.*, 1988; Simpson *et al.*, 1989; Chapman & Beerling, 1990). The feeding rate of these insects does however not change within a feeding occurrence, except for very large meals of the migratory locust (Simpson *et al.*, 1988; Chapman & Beerling, 1990). The increase in feeding rate per meal is accomplished by the increase of the proportion of time spent feeding during the meal, which can be achieved by reducing the number and/or duration of intrameals.

Within 16 out of 117 recorded meals of the pine weevil no intrameals were observed (Paper II). Colorado potato beetles had no intrameals in about half of the observed meals (Mitchell & Low, 1994), whereas no meals without intrameals were observed for the first instar of the American bird grasshopper (Chapman & Beerling, 1990). In contrast, only meals without intrameals were observed for the Costa Rican weevil (Wright *et al.*, 1999). Data from more insect species are necessary to be able to draw conclusions about the significance of meals without intrameals.

The interaction between feeding rate, meal duration and the resulting meal size as well as patterns within a meal can be influenced by a multitude of factors. The insect's physiology determines how fast it can obtain and process food, while the composition of the food including but not limited to deterrents and toxins affect meal durations or feeding rate (Szentesi & Bernays, 1984; Usher *et al.*, 1988; Glendinning & Slansky, 1995; Jones & Raubenheimer, 2002).

Prandial correlations describe how the meal size or duration influences the following intermeal (post-prandial) and how this intermeal influences the duration or size of the next meal (pre-prandial). Thereby prandial correlations indicate mechanisms that underlie initiation and termination of meals. They also allow the prediction of meal sizes from the duration of the preceding intermeal interval or the duration of the following intermeal interval based on meal size. Both the pre-prandial and post-prandial correlations were significant for the pine weevil (Paper II). Thus meals after short pre-prandial intervals were shorter than meals after medium or long pre-prandial intervals. Additionally, post-prandial intervals were shorter after small and medium-sized meals than intervals after large meals. Other insects have been shown to have either both a significant pre- and post-prandial correlation (Colorado potato beetle and migratory locust), a significant pre- but no post-prandial correlation (Australian sheep blowfly, tobacco hornworm caterpillar, American bird grasshopper) or a significant post- but no pre-prandial correlation (Costa Rican weevil) (Simpson, 1982; Reynolds *et al.*, 1986; Simpson & Ludlow, 1986; Bowdan, 1988b; Bowdan, 1988a; Simpson *et al.*, 1989; Chapman & Beerling, 1990; Mitchell & Low, 1994; Wright *et al.*, 1999).

Pine weevils created one or several feeding scars during one meal and sometimes returned to old scars during meals. Feeding scar sizes were positively correlated to meal duration, for those meals where both parameters could be directly estimated. In order to judge whether the number of feeding scars could be used as an indicator for the number of meals, different measures of feeding scar sizes and how they relate to meal sizes were compared (Paper II). The amount of bark removed during one meal by male pine weevils was about equivalent to the size of one feeding scar (22 mm^2). For females, however, the median feeding scar size (31 mm^2) was the result of about 2.2 meals.

Most often pine weevils had contact to the plant and left again without feeding (83 % of all contacts to the plant). These contacts had a median duration of 44 seconds. The median contact to the plant before feeding was shorter (37 seconds). Some pine weevils fed up to four consecutive meals without leaving the plant in between (Fig. 8). Yet after the majority of meals (83 %) pine weevils left the plant before eating another meal (Paper II). After those meals pine weevils left the plant in median within 49 seconds. A reason for leaving could be that during such circumstances the positive effects of leaving the plant, e.g. decreased risk of predation, outweigh the negative effects, e.g. reduced opportunities to meet a mate and higher energy use. The time between two meals was shorter when the plant was not left between the meals compared to the time when pine weevils left the plant (Paper II). This may have been due to either that the pine weevils stayed on the plant because they were going to have another meal soon or that the close proximity to the food triggered another meal sooner.

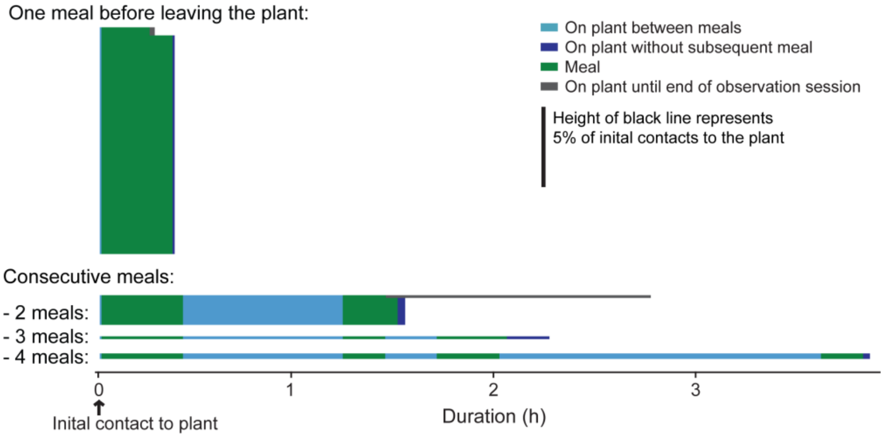

Figure 8. Median durations of time spent on the plant during meals (green), between meals (light blue) and after meals (dark blue). After the initial contact to the plant (on the far left of the figure), pine weevils have one meal (proceeding to the right of the figure) after which they either leave the plant (upper part of the figure) or feed consecutive meals without leaving the plant (lower part of the figure). Up to four consecutive meals without leaving the plant between meals were observed. The height of the different blocks represent the proportion of all initial contacts to the plant (N = 758; including those that are not followed by feeding).

4.3.1 Short-term feeding behaviour with induced defences

In Paper II half of the plants were manually girdled 24 hours before use to simulate the most common reason of plant mortality caused by pine weevils. By this, not only were the defences induced but also the nutrition and water flow in the plants affected (Noel, 1970). Nonetheless none of the examined meal or other feeding properties were significantly different between girdled and non-girdled plants. This can be related to the short time between first and second wounding chosen in Paper II. It might not have been enough time to reach peak levels in defence responses (Lewinsohn *et al.*, 1991), although conifers can respond to damages within a few hours (Ralph *et al.*, 2006; Sampedro *et al.*, 2011). On the other hand, the short time between girdling and weevil exposer chosen in the current study also represents the naturally occurring situation for plants established on clear cuts (Nordlander, 1991; Björklund *et al.*, 2005). Nevertheless short-term feeding behaviour might only be influenced by a stronger induction of plant defences.

In Paper III plants were treated with methyl jasmonate (MeJA) to estimate effects of an induced defence system on pine weevil short-term feeding behaviour. MeJA influenced the chemical profile of the treated plants compared to control plants and therewith had also an effect on the feeding behaviour of pine weevils (Paper III). For example, monoterpenes, such as (+)-3-carene and (+)-α-pinene, and sesquiterpenes, such as germacrene D4-ol, were reduced in the phloem (Paper III).

Pine weevil reacted to the MeJA-induced plant defences by discriminating less between phloem and needles in regards to initiation of feeding and meal duration (Paper III). They also reduced the meal duration and female weevils reduced their feeding rate. Furthermore, in a situation where weevils could choose between MeJA-induced and control plants, the feeding scars made by both sexes were smaller and males reduced the number of feeding scars and the total amount fed (see 4.4; Paper IV).

The time from which both plant and weevil were present in the experimental set-up until the initiation of a meal is referred to as latency. In Paper III the latency on induced plants was longer compared to control plants (MeJA: 68 ± 4 h; control: 56 ± 7 h), which could be due to the increased concentration of a number of volatile aromatics, which are potentially antifeedant. This analysis included pine weevil feeding on phloem as well as on needles. Pine weevils feed during one meal either only on phloem, only on needles or on both tissues (mixed meals). When feeding damages solely on the phloem were assessed at three time points each day, there was no delay in the initiation of feeding (MeJA: 45.6 ± 2.1 h, control: 47.8 ± 8.7 h, two-sample t-

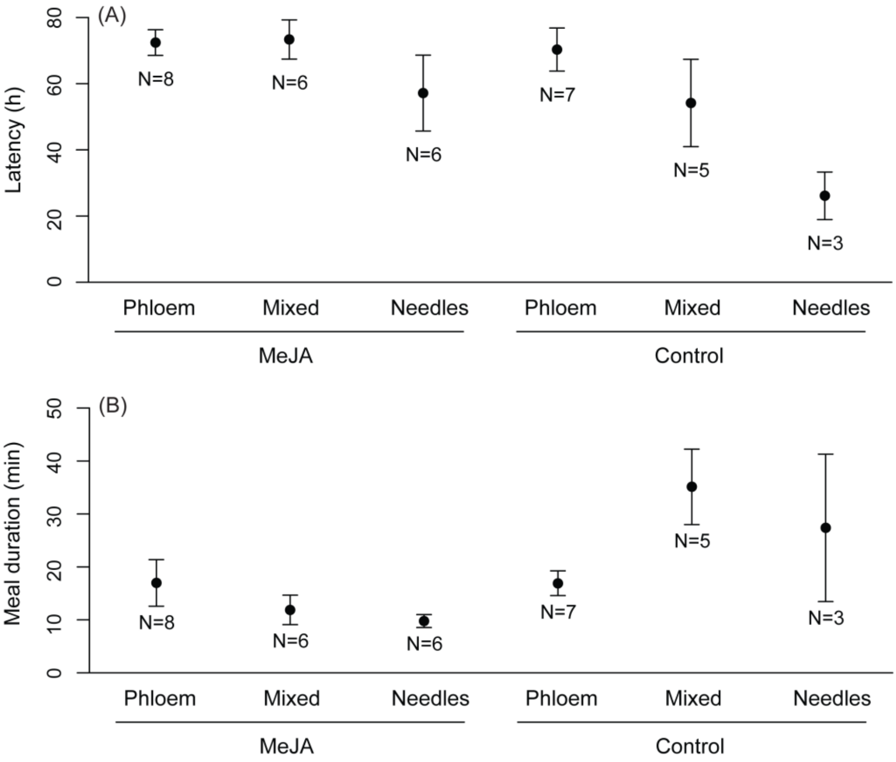

Figure 9. Elapsed time between offering a MeJA-treated or control plant to pine weevils and the start of meals (A) as well as meal duration (B) of meals fed on only phloem, phloem and needles (mixed) and only needles (mean ± standard error). N-values are number of meals observed. (Fig. 11C and 10C in Paper III).

test unequal variance p = 0.81). This calculation corresponds to the analyses done in Paper III where no effect of MeJA-treatment on the initiation of feeding was either found. As mentioned above, the differences in latency for different meal contents (phloem, needles or both) were less distinct on induced plants (Fig. 9A; Paper III). On average, meals fed only on needles were initiated after the shortest latency and meals fed only on phloem after the longest latency (Fig. 9A; Paper III).

Furthermore, meals were shorter on induced plants (MeJA: 13 ± 9 min; control: 25 ± 16 min) and pine weevils seemed to discriminate less between tissues on induced plants (Fig. 9B; Paper III). Especially meals that were fed on both phloem and needles were shorter on induced plants. In accordance with the behavioural results, the chemical profile of the two investigated tissues became more similar after the induction of defences, i.e. the MeJA treatment (Paper III). Even after pine weevil feeding, the tissues of the MeJA-treated

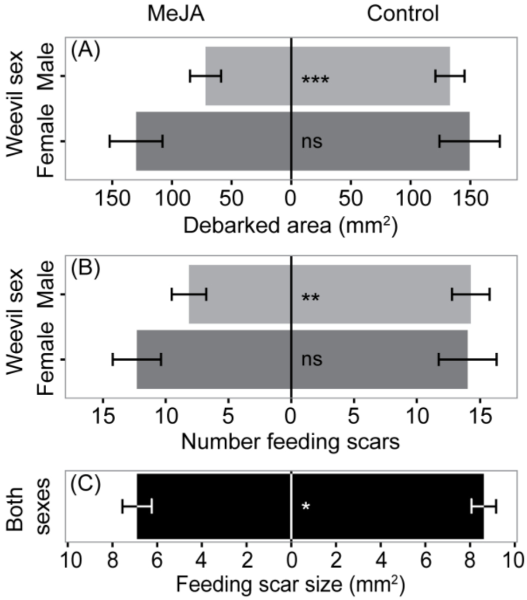

Figure 10. Effect of MeJA and the therewith induced plant defences on debarked area (A), average number of feeding scars (B) and average feeding scar size (C) in the choice experiment (Paper IV). Female (N = 20) and male (N = 20) weevils reacted differently to the MeJA treatment with respect to debarked area and number feeding scars, but not for feeding scar size. Feeding scar size is based on the median feeding scar size of each plant. (mean ± standard error; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant; Fig. 5 in Paper IV).

plants were more similar to each other than those of control plants were to each other.

The feeding rate of female pine weevils was reduced on MeJA-treated plants, probably as a result of the induced plant defence (MeJA: 0.33 ± 0.63 mm^2/min; control: 0.99 ± 0.14 mm^2/min; Paper III).

4.4 No-choice and choice feeding with induced defences

The influence of an induced defence system by MeJA-treatment on the decisions regarding when, how long and how much to feed were analysed in Paper IV. Firstly, the initiation of feeding was not influenced by the induced defences in the no-choice experiment (Paper IV). In the choice experiment data on the initiation of feeding were not obtained.

Secondly, the pattern of feeding, indicated by number and size of feeding scars, was affected by the induced defence in the choice experiment, but not in the no-choice experiment. Males made fewer feeding scars and both sexes made smaller feeding scars on induced plants compared to control plants (Fig. 10). Thus, the induced defences influenced the feeding pattern in the choice experiment, whereas pine weevils without a choice fed with similar patterns on both induced and control plants.

Thirdly, at the end of the 10 day experiments, the debarked area was smaller on induced plants in the choice (MeJA: 101 ± 85 mm^2; control:

141 ± 88 mm^2), but not the no-choice (MeJA: 177 ± 94 mm^2; control: 161 ± 80 mm^2) experiment.

Thus, the induced plant defences do not deter pine weevils from feeding on plants. However, if another food source is available, pine weevils change their feeding pattern and the amount that they can feed at the same place is reduced. On the other hand, in a no-choice situation feeding amounts on induced and control plants are similar, which might be because pine weevils are not unaccustomed to feeding on induced plants. Similarly, thrips larvae were deterred from settling on MeJA-treated bean leave discs and reduced their feeding on them in a choice experiment, whereas in a no-choice situation feeding was not reduced on treated leave discs but more larvae tried to leave treated potted plants (Egger & Koschier, 2014).

4.5 Linking diel, short-term and choice feeding

Pine weevil feeding was mostly concentrated to the second half of the dark phase (Paper I). Until the first feeding, girdled plants were more attractive than non-girdled plants, which was indicated by more time spent on the girdled plants (Paper I). It has previously been shown that undamaged plants are less attractive to pine weevils (Nordlander, 1991). This is probably due to a changed chemical profile of volatiles (Paper III). Interestingly, MeJA-induced plants that were attacked by pine weevils have higher concentrations of (-)-β-pinene and a similar concentration of (+/-)-α-pinene in the phloem compared to undamaged control plants (Paper III). (-)-β-pinene is deterrent to pine weevils (Klepzig & Schlyter, 1999). Whereas (-)-α-pinene is a pine weevil attractant and pine weevil antennal receptor responses are even stronger for the (+)-enantiomer (Nordlander, 1990; Wibe *et al.*, 1997). This indicates that pine weevils are probably less attracted to MeJA-induced plants and would therewith also spend less time on them when they are not feeding.

As a consequence of this comparably lower attraction, pine weevils should sooner leave MeJA-treated plants compared to untreated plants. Pine weevils leave plants soon after they have finished their meal unless they feed a consecutive meal without leaving the plant (Fig. 8; Paper I). The time between two meals is larger when the pine weevil leaves the plant (Paper I). Thus their chances of finding a different food source, i.e. roots of mature trees, are higher and damages on the plants would be reduced, when they are treated with MeJA. This should be especially true for males that have higher locomotion activities than females (Paper I). In accordance, male pine weevil made fewer and smaller feeding scars on MeJA-induced plants in a choice experiment, leading to an overall reduction in debarked area (Paper IV). Female weevils also caused smaller feeding scars, yet the total amount of damage was the same on MeJA-induced and control plants (Paper IV). Besides effects on scar size, also meal duration of both sexes was shorter on MeJA-induced plants (Paper III), which could explain the reduction in feeding scar size. Feeding scar size is, at least in males, a good indicator for number of meals (Paper II). In addition, females had a lower feeding rate on MeJA-induced plants (Paper III), which is either due to a slower food intake or an increase in the time spent in intrameal intervals (Simpson *et al.*, 1989; Colasurdo *et al.*, 2007). Intrameal intervals were not measured in Paper III and therefore no conclusions regarding the mechanism behind how the feeding rate was reduced can be drawn.

In this thesis pine weevil feeding on both Norway spruce (Papers I, II and IV) and Scots pine (Paper III) plants was studied but due to that different set-ups were used the following comparisons should be interpreted with caution. On average pine weevils made four meals per day on Norway spruce, but only 2.5 meals on Scots pine (Paper I and III). Nevertheless meal durations were similar on both plant species (Paper I and III). However, on Norway spruce plants pine weevils spent 4 % of the feeding time on needles (Paper I), whereas they spent about 30 % of the feeding time on needles on Scots pine (Paper III). This may indicate that the nutritional value and chemical composition of the different tissues could vary in the two plant species.

5 Conclusions and Future research

In this thesis several aspects of pine weevil feeding behaviour that were previously unknown are presented. Paper I and II increase the basic knowledge about pine weevil behaviour, whereas Paper III and IV use this basic knowledge to interpret and understand pine weevil behaviour on plants with their defence system induced by an exogenously applied elicitor (MeJA).

Since the meal criterion of the pine weevil is now known (Paper II), more studies can benefit from exploiting possibilities of short-term feeding behaviour experiments. Studies of short-term feeding behaviour do not only provide a high temporal resolution, but since they are often based on video recordings detailed analysis are possible and behaviours are seldom missed. For example, the initiation of feeding on phloem was not significantly different between MeJA-induced and control plants in Paper III or the no-choice experiment of Paper IV. However, feeding on needles could also be analysed based on the video recordings of Paper III, which is otherwise very difficult. Thereby it was possible to measure a delay in feeding on induced plants. Patterns of pine weevil feeding have been shown to be affected in a choice experiment (Paper IV). Therefore an additional experiment studying the short-term feeding behaviour on induced and control plants would be interesting. Such an experiment could help to understand the behavioural mechanisms behind the different patterns and answer questions such as: Do pine weevils switch regularly between plants after they have finished their meal? Or do they even change plants within a meal?

The behaviour of male and female weevils differs in several aspects, but especially in the amount of time spent in locomotion and the time on the plant while not feeding. Males spent more time in locomotion, which should be considered for instance when results from trappings in the field are interpreted. The data of Paper II on the time spend on the plant should be complemented by further studies taking different proximities to the plant into consideration.

The diel behaviour shows an increase in feeding activity during the second half of the dark phase after a peak in locomotion. The concentration of feeding behaviour during the dark phase can be of interest when designing future experiments. If the experimental design requires a rather short exposure of plants to pine weevils, a dark period should be included to increase chances of high feeding activity.

In addition, comparisons between feeding behaviour on different plant species could be interesting, especially if differences in feeding patterns can be linked to chemical properties of the plant species.

This thesis increases overall the knowledge about pine weevil feeding behaviour as well as linking it to a more applied context by investigating the behavioural mechanisms leading to a reduced feeding damage on MeJA-induced plants. The results also highlight the advantages of studying short-term behaviour along with long-term behaviour.

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