Pine Weevil (*Hylobius abietis*) Feeding Pattern on Conifer Seedlings

Frauke Fedderwitz[1], Niklas Björklund, Velemir Ninkovic, Göran Nordlander

Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
[1]*frauke.fedderwitz@slu.se*

Abstract

The pine weevil (*Hylobius abietis*) is one of the most important forest pests in Europe, yet there is very little known about its detailed feeding behaviour. We study the temporal feeding pattern of individual pine weevils of both sexes for 24 hours with two treatments, intact and girdled seedlings. Properties of a meal, such as feeding duration, size and ingestion rate are of particular interest. The shortest interval considered to separate one feeding bout from another, the meal criterion, has never been published and it is only available for a few other insect species. Video recordings are analysed for feeding behaviour (e.g. duration of feeding activity, interval length between feeding activities, movements between and within feeding scars). We measured general activity patterns as there is insufficient knowledge on the daily behavioural patterns. We thereby got an in-depth view of the pine weevil feeding activity that would otherwise be difficult to assess.

Introduction

Herbivorous insects reduce the fitness of plants directly and indirectly by feeding despite inducing plant defence systems against herbivory [1]. Trees have especially low defence and tolerance as seedlings in comparison to other phases in plant ontogeny except for over-mature and senile plants [2].

The pine weevil (*Hylobius abietis*) is economically one of the most important forest pests in Europe [3]. Adults feed on the stem bark of conifer seedlings [4] and can cause seedling mortality of up to 90 % in the first three years [5]. Economic losses tend to be especially high in conifer forests that are managed by clear-cutting and replanting [3]. Despite the importance of behavioural components of insect feeding for understanding bases of host plant resistance [6-9], a lot of aspects of pine weevil feeding behaviour have not yet been studied. Studies on feeding behaviour in this species have mostly been concentrating on the total amount of consumed bark after a specific time period in relation to different aspects, for example, presence of other weevils, weevil size, microclimate, chemical composition of the bark or treatments with light and/or nitrogen [10-13] or as comparisons between different tree species [14-16].

In this ongoing project we are assessing the detailed pine weevil feeding behaviour and time budget on Norway spruce (*Picea abies*) seedlings during a 24-hour period. Generally feeding over longer durations, i.e. several days, often shows little variation in total intake of nutrition between individual insects, whereas the detailed feeding pattern of individuals varies greatly even under controlled environmental conditions [17]. The detailed feeding behaviour can be described by groups of feeding events, i.e. meals. These can be determined by the bout criterion, the shortest non-feeding interval considered to be between meals. The bout criterion is based on the distribution of non-feeding intervals since it can be expected that animals make a large number of short non-feeding intervals within a meal and relatively few longer intervals between meals [e.g. 18]. The bout criterion itself has an indirect importance for understanding feeding behaviour as it allows separating meals on an ethological meaningful basis. Thereby meal properties (size, duration and ingestion rate) and potentially other indicators can be related to initiation and termination of meals and thus may have important implications for insect-plant interactions. By understanding these properties it might be possible to influence the length of a meal. Shorter meals might reduce the risk of large feeding scars that can endanger the seedling's survival. For testing the effect of previous damage caused by larger feeding scars we are also measuring the effect of girdling. Girdling represents a type of pine weevil damage that is often observed on planted seedlings [19]. Furthermore wounding of plants can induce different effects of defence mechanisms as shown in experiments on several conifer species [20-22].

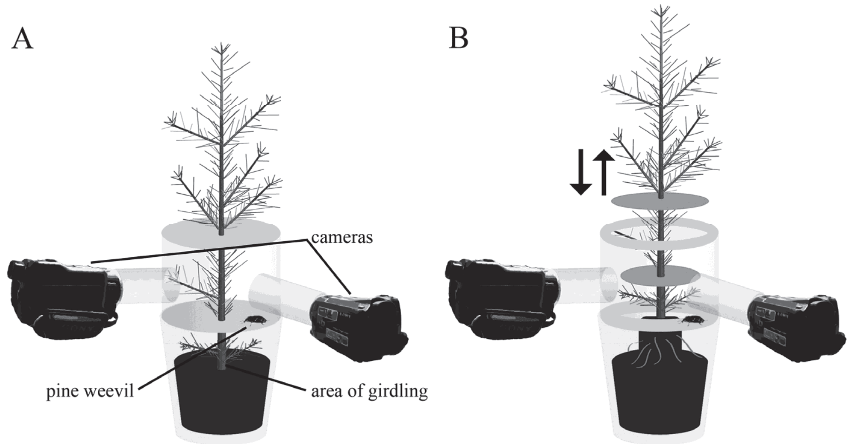

Figure 1. Experimental set-up during observation (A). A small water tube and a dark coloured shelter for weevils to hide under inside the cylinder are not shown. Changing of seedling after a 24 hour acclimatization period (B). The cylinder had a hole in the bottom and the lid which was closed by two plastic discs placed around the plant. Thereby it was possible to replace the plant with a fresh seedling with a minimum disturbance of the weevil that was inside the cylinder.

Materials and Methods

We video recorded the behaviour of 6 male and 6 female pine weevils on one-year old Norway spruce seedlings under 24 hour periods for assessing the bout criterion and time budget. Two cameras were placed at a 45 degree angle for high visibility of weevil activity regardless of the feeding position of the weevil. We limited the weevil's access to the middle part of the seedlings by placing a transparent plastic cylinder around the stem (see Figure 1A). This area can be assumed to represent a rather homogenous feeding source with regard to the distribution of resin ducts and growth factors. The experiment was conducted at room temperature under an artificial day-night cycle of 7 h light – 6 h darkness – 11 h light. During the day the light was provided by several light sources with a combined intensity of 59 µmol m^{-2} s^{-1} (Skye Instruments LTD, Sky 200 SKP). A light bulb at low intensity (0.1 µmol m^{-2} s^{-1}) as used to imitate a Swedish summer night with an additional red light (0.9 µmol $m^{-2}s^{-1}$) to increase the visibility during the night.

A single weevil was placed with a seedling in the cylinder 24 hours before filming so that the weevil had time to acclimatize to its new surroundings. Pre-test weevils were rarely feeding during this first day although they were eating before and afterwards when placed in Petri dishes with pieces of Scots pine (*Pinus sylvestris*). After the acclimatization phase the plant was replaced with a new seedling to avoid effects of possible feeding scars (see Figure 1B). The weevil was disturbed as little as possible. Video recording was started when the new seedling was installed and ended after 24 hours. The area eaten (mm^2) was measured on both plants and the location of feeding scars on the second seedlings. Each weevil was tested with two treatments: an intact seedling and a seedling that had been manually girdled under the first node 24 hours prior to filming to test effects of induced defence. These two treatments were started alternating between the weevils. Between the treatments the weevils were placed for two days in Petri dishes with Scots pine pieces.

Feeding behaviour was defined as manipulation of bark, needles and eggs with the mouth parts. It was not possible to distinguish periods of bark removal and ingestion. Consequently it cannot be completely asserted that the weevil was feeding when it showed feeding behaviour as defined above (from here on only feeding).

The duration of each feeding occurrence and the length of intervals between them were measured (The Observer XT 10, Noldus Information Technology, Wageningen, The Netherlands). In addition locomotion behaviours were noted. All behaviours were measured continuously. The length of non-feeding intervals was assigned to bins of the next full second. The distribution of the frequency of all non-feeding intervals was used to assess the bout criterion. The bout criterion was then used to determine average meal length and sizes as well as feeding rate. For the time budget the day was split up into one hour bins in which the additive time spent feeding or in locomotion was calculated. From these values relations to the dark/light cycle as well as between different activities and individuals were made. Additionally we measured the feeding scar distribution on the seedling and in which order they were made. We will thereby be able to assess if there is a general pattern in location of feeding on the plant. We also evaluated how often a pine weevil returned to an old scar in order to continue feeding there. Furthermore we noted direction in which they continue to feed after each non-feeding interval. That means we determined if the first bite after a non-feeding interval is extending the feeding scar upwards, downwards or horizontally. In connection with the meal determination we will also be able to see whether there is a link between meals and feeding scars, e.g. one meal is one feeding scar. This kind of information might indicate avoidance of locally induced defence systems or large resin ducts. The differences between treatments were assessed for all measurements.

Results and Discussion

So far only a part of the data has been analysed. This preliminary analysis indicates that the bout criterion lies close to 16 seconds and does not show a difference for the two different treatments. This duration is relatively short as the bout criterions established for other insects are in the range of minutes; examples include the Colorado potato beetle (*Leptinotarsa decemlineata*) with 4.8 minutes [8] and different grasshopper species such as *Taeniopoda eques* with 8 minutes [7] or *Locusta migratoria* with 2-6 minutes [9]. The short bout criterion could indicate a larger difference between intrameal and intermeal intervals in pine weevils. The longer non-feeding intervals recorded during this experiment range several hours and were probably mostly incomplete due to the cut off points before and after 24 hours.

Some weevils showed locomotion and feeding behaviour influenced by the dark/light rhythm whereas others expressed a similar activity pattern during both phases. In some cases there might even be an influence of the lighting on either behaviour but not on the other. Individuals expressing a daily pattern in locomotion showed more activity during the dark phase. Similar results have been shown previously [23]. For feeding activity Merivee and co-workers [23] found a significant pattern for male weevils. Our preliminary data points however towards an effect of individuals, meaning that there are both females and males that seem (not) to be influenced. In individuals showing a temporal pattern, feeding occurred mainly towards the end of the night or shortly afterwards. Other weevil species have been found to be predominantly nocturnal in relation to feeding behaviour [24; 25].

Based on these results it seems that the presented method is suitable for assessing the bout criterion and a detailed time budget of pine weevils. The detailed behaviour that is measured in this study is partly in the range of seconds and a lot of aspects need continuous observation. Therefore it would be very difficult to assess this type of data with a more automated or less precise method, e.g. instantaneous or one-zero sampling, at this stage. It should be possible to transfer the set up to other herbivorous insects for comparable studies.

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