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Laurence Jonkers

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Impact of wood ants on the spatial distribution and feeding by the pine weevil

Laurence Jonkers

Supervisor: Göran Nordlander, SLU, Department of Ecology

Examiner: Åke Lindelöw, SLU, Department of Ecology

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Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
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Abstract

The pine weevil (*Hylobius abietis* (L.)) is one of the most destructive pests in Swedish conifer plantations. Many efforts have been made to reduce its damage by the use of insecticides, planting site preparation, physical barriers and other management practices. A number of studies show the importance of ants in pest management in different systems. In this study I test the hypothesis that a high density of ants reduces damage on conifer seedlings by the pine weevil.

The study was conducted on two clearcut areas in the Lunsen forest S of Uppsala, Sweden. In total, 900 Norway spruce seedlings (*Picea abies* (L.) Karst.) were planted (divided into plots with 25 seedlings) and different measures of the weevil's damage were assessed, i.e. proportions of attacked and girdled seedlings, and mean feeding area. Densities of the ant species *Formica polyctena* and *Myrmica ruginodis* were obtained weekly by pitfall traps placed in each planting plot. No significant relationship between ant density and weevil damage was found when tested by means of the Spearman's correlation. Results elucidating the process of pine weevils damaging seedlings are reported.

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1 Introduction

The large pine weevil, *Hylobius abietis* (L.), is one of the most destructive pests in conifer plantations in the Nordic countries (Denmark, Sweden, Norway and Finland) (Day *et al.* 2004). Its effect on Scots pine (*Pinus sylvestris*, L.) and Norway spruce (*Picea abies* (L.) Karst.) clearcut plantations is devastating, affecting one of the keys in the forest economy in Sweden. The adult weevils feeding from stem bark of the seedlings, planted after final felling of conifer forest, may cause a mortality of up to 80-90% if no countermeasures are taken.

Many studies with the aim of finding methods to reduce the weevil damage have been conducted in Nordic countries. Delaying the planting for three years is a way of decreasing the pine weevil damage, since most pine weevils have left the clearcut at that time (Örlander and Nilsson 1999). However, the ground vegetation would represent a strong competitor, affecting the seedling establishment. Moreoover, a fallow period of three years would lead to an economical loss for the forest owners due to the loss of production (Petersson and Örlander, 2003).

In Sweden, relatively persistent insecticides (synthetic pyrethroids and the neonicotinoid imidacloprid) are widely used in regions where plantations are expected to be severely damaged by the pine weevil. However, the use of insecticides in Swedish forestry has long been questioned (Swedish Chemicals Inspectorate 2005). Therefore, many studies have been carried out in order to find alternatives to reduce pine weevil attacks. The alternatives are mostly based on physical barriers like shields or coatings. A recently developed coating, Conniflex, is now in large-scale practical use (Nordlander *et al.* 2009).

A combination of different measures appears to be the best strategy to manage the pine weevil problem (Petersson and Örlander 2003). These authors propose an integrated pest management, combining insecticides, scarification, physical protection and shelterwood, in order of effectiveness for each measure. Soil scarification, that removes the humus and vegetation cover, strongly decreases the risk for damage by pine weevil feeding (Christiansen and Bakke 1971; Örlander and Nordlander 2003). The use of shelterwood also significantly reduces pine weevil damage, probably because other food sources than the seedlings remain available for the weevils on the shaded ground (Nordlander *et al.* 2003).

The influence of ants on herbivore species has been widely studied. The herbivores sometimes possess chemical defenses (Heads 1986) or large body sizes (Tilman 1978), and hence can escape from ant predation (Ito and Higashi 1990). However, many studies have shown effect of ants in various systems. As an example, *Oecophylla* spp. are used in Africa against *Pseudotheraptus wayi* and *P. devastans* to reduce the damage that these pests cause in female flowers and young nuts in coconut palm plantations (Way and Khoo 1992). Other studies are more specific on wood ants of the genus *Formica*. Strong interactions were found between *F. polyctena* and different species of Carabidae in a Russian mixed pine-birch forest, as wood ants affect the carabid´s spatial distribution and behavioural patterns to avoid encounter with ants (Reznikova and Dorosheva 2004). In a Scots pine stand in England, increasing wood ant density reduced the abundance and species richness of carabids, resulting from predation, competition for food sources or interference competition (Hawes *et al.* 2002). Ito and Higashi present important changes in community structure of four species of weevils by the action of the red wood ant *Formica yessensis* in a *Quercus dentata* chaparral of northern Japan; while *Myllocerus griseus* and adult *Rhynchaenus japonicus* were rarely or never attacked by ants, *Phyllobius longicornis* and *Scytropus japonicas* were frequently attacked by ants, foraged for preys and even carried to ant nests.

The presence of colonies of wood ants (*Formica* spp.) might affect pine weevil feeding. Indeed, many studies show the influence of ants, and especially the *Formica* species, in controlling pests (Way and Khoo 1992). The ants' ability to react quickly to increasing prey density makes them effective in reducing the number of defoliating insects and hence ants may be a good pest management tool. The aggressive behaviour of ants against different generalist predators, like spiders, ground beetles, ladybirds etc. is often observed (Hawes *et al.* 2002; Reznikova and Dorosheva 2004; Oliver *et al.* 2008; Domisch *et al.* 2009). Mody and Linsemair (2004) found that herbivores such as some *Orthoptera*, *Thysanoptera*, *Heteroptera* and *Lepidoptera* larvae seem not to be affected by ant activity. On the other hand, a number of studies show an effective impact of ants against herbivores, and among them species of weevils such as *Phyllobius longicornis* and *Scythropus japonicus* in a *Quercus dentata* chaparral in Northern Japan (Ito and Higashi 1990).

Formica ants are the most abundant ant species in the study area, partly excluding other ant species from their surroundings. A large number of papers have been written about this complex of eight species. Their activity is low in the winter, and depends on temperature during the summer (Way and Khoo 1992), largely coinciding with the activity of the pine weevil. The presence and density of *Formica* ants is highest in medium-fertile or fine sandy soils, which present good aeration and water holding capacity. These conditions are usually found in birch and spruce stands, which provide shade to the ant nests. Moreover, occurrence of *Formica* ants is higher in older than in younger clearcut stands, due to changes in illumination and the removal of their main food source, the aphids (Kilpeläinen 2008).

Formica ants are likely to develop large permanent colonies generally made of Norway spruce needle litter and resin particles (Kilpeläinen 2008), which are aggressively defended against potential enemies (Punttila *et al.* 1996), in areas where shade provides them smooth temperatures and resources. It is known that *Formica* ants can prey on many defoliating pests in Europe, and in different phases of the preys' development. In fact, the response of these ants is generally highest at a rapid increase of density of epidemic pests , and "green islands" are often formed around colonies of *F. polyctena* during these pests' outbreaks, either because the ants kill larvae which would defoliate the trees around the ant nests (Laine and Niemelä 1980), or because the ants concentrate the nutrients in and around their nests (White 1985). Protective effect of ants is inversely related to distance from the nest.

No significant effect of ant presence on pine weevil feeding was found by Fries (2006), who studied weevils enclosed in net cages accessible to ants through the mesh openings. However, when ants were attracted to seedlings with a sucrose bait the feeding by weevils tended to be lower on individual seedlings, although more seedlings were attacked. Fries (2006) suggested further research on this topic since she observed a behavioural reaction from the pine weevils when encountered by ants, i.e the weevil either "freezes" its movements or hides by burrowing into the soil. These protective behaviours should result in a loss of time for feeding, and maybe weevils frequently encountered by ants also will move out of areas with a high density of ants.

The aim of this study was to investigate whether the density of ants in plantations areas affects the feeding damage caused by pine weevil on conifer seedlings. If so, preservation of ant nests and surrounding habitat would contribute to an integrated pest management.

2 Pine weevil life cycle

The following terminology is used to denote the age of clearcuts related to the pine weevil life cycle (Bejer-Peterson *et al.* 1962, Nordenhem 1989): (A) is the growing season immediately after cutting, and the next years are called (A+1), (A+2), etc (Figure 1). In Scandinavia, the adult weevils leave overwintering sites in spring and undertake their flight to new clearcut areas in late May and early June year A (Solbreck and Gyldberg 1979). After arriving to the fresh clearcut areas, the oviposition period starts and last until August (Nordenhem 1989; Örlander *et al.* 1997). After overwintering hidden in the soil, oviposition may be resumed during year A+1.

Eggs are laid in the soil or in the bark of roots (Eidmann 1974; Nordlander *et al.* 1997). Newly hatched larvae in the soil migrate to suitable sites under the root bark of recently killed or dying conifer trees or fresh stumps (Nordenhem and Nordlander 1994; Nordlander *et al.* 1997). After pupation, the new generation of weevils emerge at the end of the summer year A+1. Most of them feed on the clearcut until hibernation, whereas some adults remain in the pupal chambers until spring year A+2. During late summer and autumn A+1 the pine weevil population on a clearcut may increase about tenfold, since the newly emerged weevils are added to the population of the previous generation (old adults). These old adults arrived to the clearcut in spring year A and overwintered on the clearcut.

The adults that stayed in the pupal chambers for overwintering (A+1) emerge in spring the next year (A+2). They feed on the clearcut area for several weeks until they migrate to fresh clearcuts, together with the weevils of the same generation that emerged already in autumn year A+1. Relatively few weevils remain on the clearcut after the main flight in spring or early summer year A+2. The pine weevils are hence most abundant in summer year A, summer and autumn year A+1, and in spring A+2. The damage risk is relatively low thereafter.

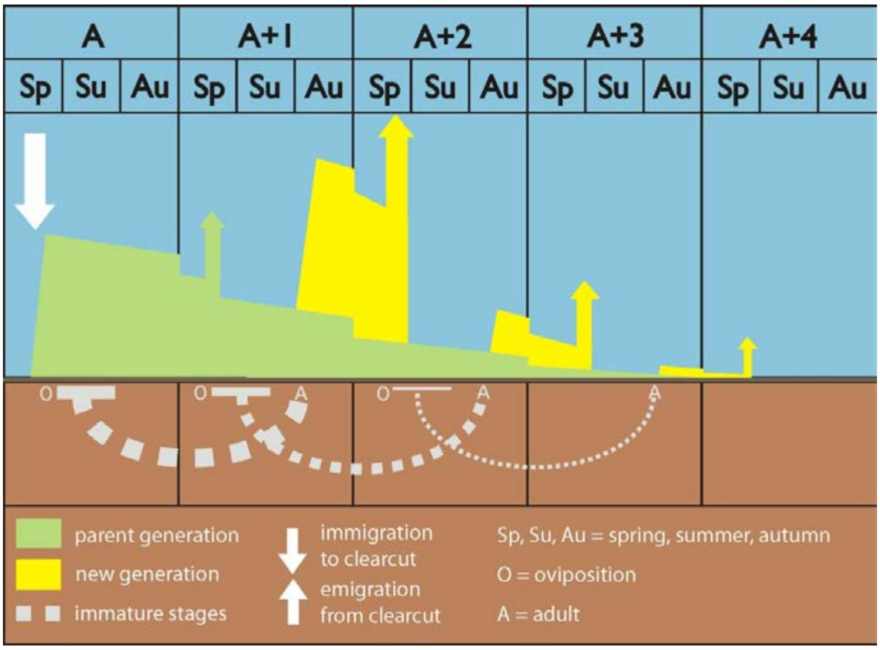


Figure 1. Pine weevil life cycle. After Nordlander et al., p. 7:
http://www2.ekol.slu.se/snytbagge/attachment/snytbaggehandbok_v1_3.pdf

3 Materials and Methods

3.1 Field work

3.1.1 Study areas

The study was conducted at two different fresh clearcut areas in the Lunsen forest, south of Uppsala. The clearcutting of the areas had been done in September 2008. We can thus say that the risk for damage by *Hylobius abietis* was at its peak at the time of the experiment since weevil colonization occurred in spring 2009 (A).

The study areas were called Area I and Area II (Figure 2). Experiments in Area I were terminated after the two first weeks because an extremely high damage level had already been reached. In Area II experiments were conducted from June to August 2009, and repeated in September 2009, when the temperatures were much lower. Area II was called Area II-1 for the first experiments, and Area II-2 for the second.

The minimum and maximum temperatures were measured using three temperature loggers between the 15^{th} of June and the 24^{th} of September 2009. The loggers were placed out on Area II at ground level and shaded by a cardboard roof, in order to measure the temperature most likely to be perceived by the pine weevils (Figure 2). Three loggers were used in case any technical problem would appear, and the means of the three simultaneous recordings were used.

3.1.2 Mapping of anthills

The entire area of both sites was carefully inspected for nests of wood ants. Each nest was given a number, its position was marked on a field map and the coordinates were registered with the GPS. The area investigated included the clearcut and the entire area 70 meters into the surrounding forest.

To determine which species inhabits each nest, at least 20 individuals were collected from each ant nest (Picture 1) and placed in a labeled jar with 80% ethanol. Species were determined in laboratory according to Collingwood (1979) and Douwes (1995).

Picture 1. Samples taken of *Formica* ants (pictures by L. Jonkers)

In September (Area II-2), a new anthill mapping was needed, since some of the anthills had been abandoned, and others had been rebuilt during the summer. Again, a sample of ants from each of the new anthills was taken to determine the species.

3.1.3 Planting design

Pine weevil feeding was assessed on containerized Norway spruce (*Picea abies* (L.) Karst.) seedlings that were planted on both clearcut areas. To obtain a large variation in ant density between the planting plots, a preliminary ant density measure by pitfall trapping was done before the actual experiment. Places with low, medium and high ant densities were observed on each area.

Taking the previous observations of ant densities into account, 300 seedlings were planted in each area, on the 17th and 18th June (Area I and Area II-1, respectively), and an additional 300 seedlings were planted in area II-2 on the 1st of September. In each area, the 300 seedlings were distributed as follows: 12 planting plots were established, spread out between low, medium and high ant density places. On each of the 12 plots, 25 seedlings were planted, forming a square of 5x5 seedlings. All seedlings were given an individual number within each plot. The week of planting was set to be week 0 and the following weeks were numbered accordingly.

The plantation was executed by inserting a planting tube into the soil, after lightly removing the grass vegetation with the foot. No soil scarification was done; therefore, by planting directly into the humus, the risk for pine weevil damage was maximized.

3.1.4 Damage checking

Registration of pine weevil feeding was checked weekly on seedlings in both areas. The following data related to weevil damage were registered weekly:

- Area of bark eaten (feeding area) in cm^2, measured with the aid of a millimeter paper or by calculating the damaged stem area from the stem diameter and the length of the area.
- Proportion of attacked seedlings; i.e. all seedlings showing feeding marks by pine weevils.
- Proportion of girdled seedlings, i.e. seedlings with the bark eaten all around the stem. This category included both girdled seedlings that were still alive and some dead seedlings with a large feeding area (which were most probably girdled, although this had not been recorded in the field).
- Proportion of dead seedlings.

1st period – Area I and Area II-1(June - August)

Week 1: In both Area I and Area II-1, all four damage parameters were registered.
Week 2: The same data were collected for Area II-1. For Area I, a high mortality among seedlings led to the decision to exclude the site from the study, although some observations were done later on this area.
Week 3: Due to unexpectedly high damage levels, the assessment process was changed into counting the dead and living seedlings instead of the feeding area (and hence proportion of attacked seedlings). The assessment of dead and girdled seedlings was also done in Area I in order to compare damages in both areas.
Week 11: The same data were collected for Area II-1.

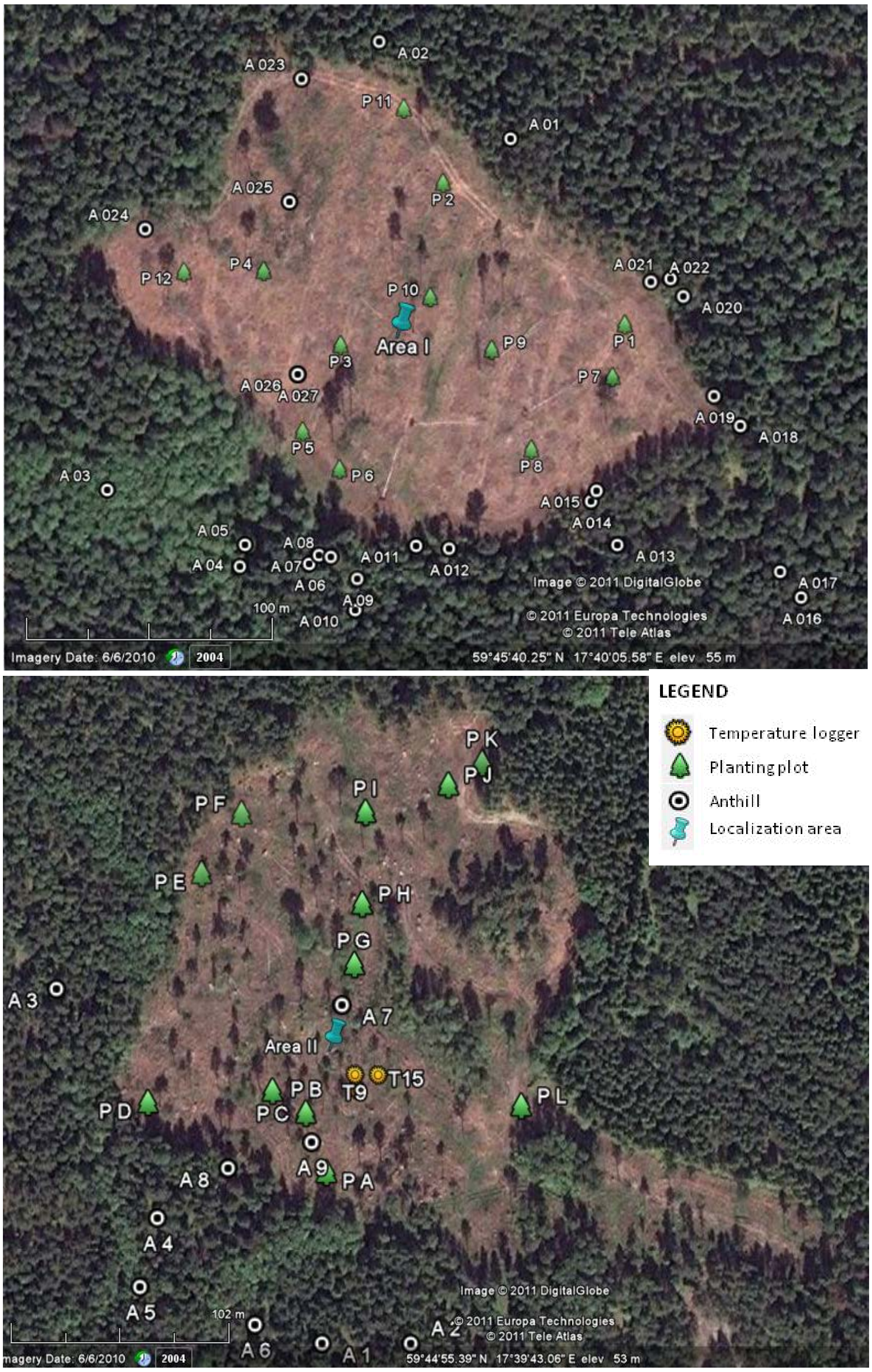


Figure 2. Position of anthills (A), planting plots (P) and temperature loggers (T) as registered by GPS marks for Area I (upper) and Area II-1 (lower).

After observing a high mortality (90% of the seedlings were dead in week 11 in Area II-1), the decision was taken to replace all seedlings and repeat the experiment with seedlings that were 3 months older, and should therefore be more resistant to pine weevil attack.

2nd period – Area II-2 (September)

The planting was done on the 1st of September (week 11), and observations on these seedlings and ant presence continued until week 16 (9th of October).

The time intervals between damage assessments were longer for the 2nd period because the level of damage was expected to increase slower at the beginning of September than in June due to lower temperature and thereby lower weevil activity. Two assessments were made, in week 14 and week 16 respectively, including all four damage parameters.

3.1.5 Ant trapping

In order to get an ant density map for both areas as a basis for selection of plot positions, a 24 h pitfall trap assessment of ant density was done before the actual experiment. To maximize the variation in ant density, pitfall traps were systematically placed next to the edge of the forest, in the center of the clearcut, next to or far away from an ant nest.

Also during the experiment, pitfall traps were used to assess the density of ants in the area. One pitfall trap was placed in each corner of each planted plot, i.e. between the four outermost seedlings, as shown in Figure 3.

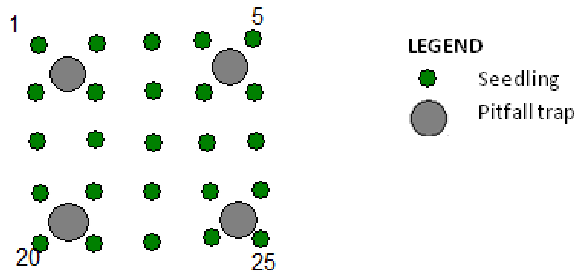


Figure 3. Experimental plot with 25 seedlings and four pitfall traps.

The pitfall traps consisted of 20 cl plastic cups buried in the ground with their rims at level with the ground and half filled with water with two drops of detergent added to reduce the water surface tension. For each trap, the number of specimens of different ant genera was recorded.

Picture 2. Pitfall trap with water and detergent.

1st period – Area I and Area II-1(June – August)

Trap catches of ants were made during weeks 2 and 3. The weather was colder and rainier during week 3 than in week 2, when it was warm and sunny. To obtain samples that were both large enough and of approximately similar size in both weeks, the pitfall traps were in operation for 24h week 2 and 48h week 3.

2nd period – Area II-2

Ant densities (24 hour trap catches) were recorded for weeks 15 and 16.

3.2 Data treatment and statistics

Damage data collected weeks 1 to 3 for Area I and weeks 1, 2, 3 and 11 for Area II-1 were included in the statistical analysis, which was made in Minitab.

Before investigating the relationship between the ant density and the weevil feeding data, both datasets were checked for consistency over time:

- To check if ant numbers varied between weeks, the data for each ant species were compared in successive weeks.

- Feeding data consistency was checked by correlating different measures which should logically present a positive linear relationship (3.2.1).

Since data did not present a normal distribution, and log-transformation did not improve it, all associations between ant density data and weevil feeding data were tested by using the non-parametric Spearman rank correlation test (3.2.2).

3.2.1 Feeding data treatment

In the data analyses three ways of measuring the actual damage of the weevil feeding were used:

- Proportion of attacked seedlings in each plot.
- Mean feeding area (surface (cm^2/seedling) of bark consumed over time). The mean was calculated dividing the total feeding area in each plot by the total number of living seedlings in the same plot, i.e. including non-attacked seedlings too (some very few seedlings were ripped out by some animal in the first days, and were completely dry and useless for the pine weevil during the assessment).
- Proportion of girdled seedlings in each plot. This measure is closely related to seedling mortality caused by weevil feeding, since girdling always leads to death.

These parameters were considered the most adequate to evaluate the damage caused by the pine weevil feeding, and the relationships between them provide thus a good assessment of consistency in data collection.

The following first two relationships were assessed by using ranked data in a Pearson rank correlation in Minitab, and by that a non parametric test was performed equivalent to a Spearman rank correlation. The third one was a simple analysis over time done in Excel.

- Correlation between the proportion of girdled seedlings and mean feeding area.
- Correlation between the proportion of attacked seedlings and mean feeding area.
- Progress over time of the proportion of girdled seedlings (or seedlings killed by *H. abietis*).

3.2.2 Relationship ants/damage

To test whether the weevil feeding on seedlings was affected by the presence of ants (both species separately), the following relationships were investigated by means of the Spearman's correlation:

- Association between the proportion of attacked seedlings and ant density (number of ants per plot).
- Association between the mean feeding area and ant density.
- Association between the proportion of girdled seedlings and ant density.

4 Results

4.1 Ant species

The dominating species in all anthills and also trapped on the clearcut was *Formica polyctena*. In addition, some seedlings were frequently visited by *Myrmica ruginodis*.

4.2 Weather

The high temperature registered over the summer (Figure 4) on the clearcut should have affected the activity of both pine weevils and ants.

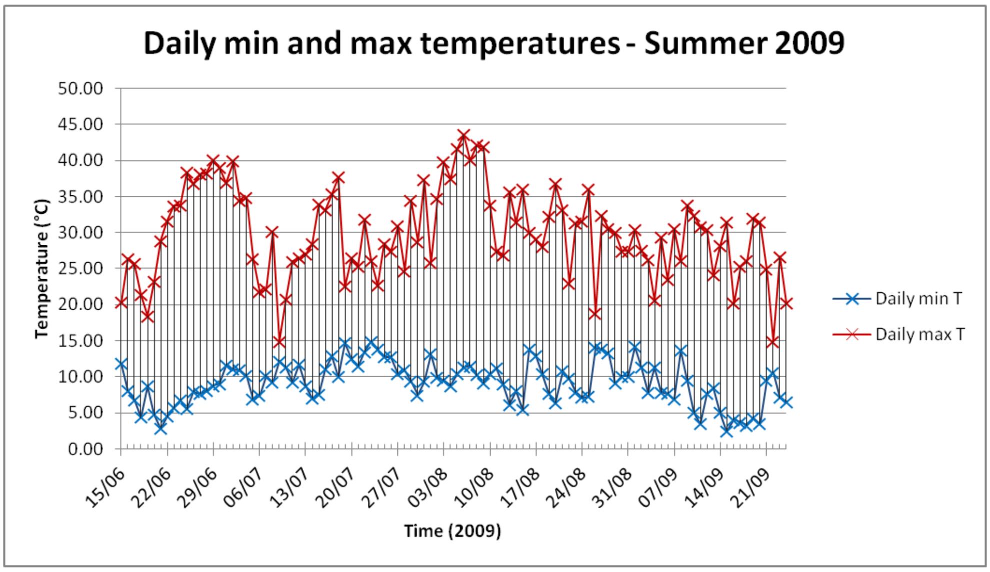


Figure 4. Daily minimum and maximum temperatures in the shade at ground level during the experiment.

4.3 Ant density

In total during the whole experiment, 27 anthills were found on Area I, and 8 on Area II (Figure 2).

Ant catches did not show a homogeneous pattern at any time during the experiments, and hence were not normally distributed. In the case of *Formica* spp., the range of ant catches in week 2 varied between 0 and 567 from one plot to another. In Area II-1, the number of *Myrmica* spp caught per trap varied a lot between weeks (Table 1).

Table 1. Summarized data of ant catches per plot (sum is for all 12 plots in one Area).

	Formica					
	Area I		Area II-1		Area II-2	
	Week 2	Week 3	Week 2	Week 3	Week 15	Week 16
Sum	747	794	1111	295	34	8
Mean	62	66	93	25	3	1
Minimum	1	0	0	0	0	0
Maximum	285	628	567	191	21	4
Median	20	9	11	2	1	0
	Myrmica					
	Area I		Area II-1		Area II-2	
	Week 2	Week 3	Week 2	Week 3	W15	W16
Sum	16	24	240	120	3	5
Mean	1	2	20	10	0	0
Minimum	0	0	0	1	0	0
Maximum	5	8	73	37	1	1
Median	0	1	20	7	0	0

The Spearman rank correlations of densities of ants (number of ants per trap) within site between the second and the third week were positive and significant for *Formica* in both areas and both study periods. In the case of *Myrmica* ants, there was a significant positive correlation between weeks 2 and 3 in Area II-1 (Table 2). These significant positive correlations between the ants trapped in two following weeks shows that the number of ants per trap were correlated and hence consistent between weeks: high densities in traps of *Formica* or *Myrmica* in these specific cases would be consistently associated to higher densities in traps catches in neighboring periods of time (Figure 5).

Table 2. Results of Spearman rank correlation, r_s, for densities of Formica and Myrmica ants, respectively, between the second and third week for both areas and both study periods (N = 12).

	Area I		Area II-1		Area II-2	
	Formica	*Myrmica*	*Formica*	*Myrmica*	*Formica*	*Myrmica*
Spearman r_s	0.655	0.393	0.815	0.677	0.782	-0.008
p-value	0.021	0.206	0.001	0.015	0.003	0.763

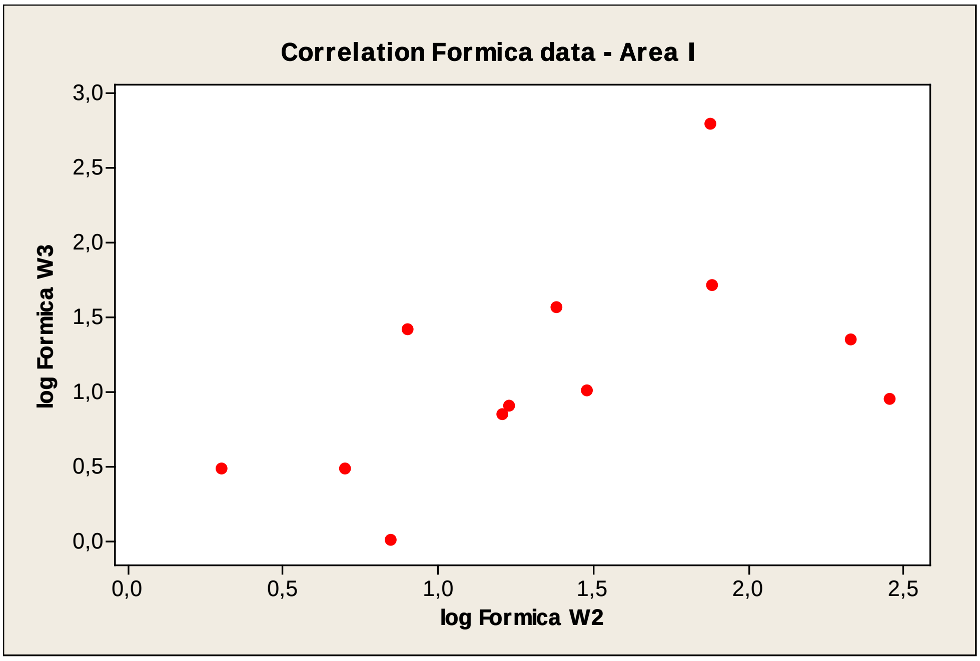


Figure 5. Correlation between log (X+1) number of *Formica* per trap in week 2 and week 3 for Area I. For test statistics confer Table 2.

4.4 Feeding by pine weevil

A general observation was that the seedling damage in the second period (Area II-2) was lower than for the first one (Area II-1). However, most seedlings were rapidly attacked and girdled already in the beginning of the experiment.

A significant positive correlation between proportion of attacked or girdled seedlings and feeding area could be observed, especially for Area II-1.

4.4.1 Relationship between proportion of girdled seedlings and feeding area

In both areas and both study periods a significant positive relationship between the proportion of girdled seedlings and the weevil feeding area was found especially for Area II-1 (Table 3).

Table 3. Spearman rank correlation coefficient, r_s, and significance level for correlation between mean feeding area (cm^2) and the proportion of girdled seedlings in both areas and both study periods (N = 12).

	Area I	Area II-1		Area II-2	
	Week 1	Week 1	Week 2	Week 14	Week 16
Spearman r_s	0.724	0.910	0.968	0.805	0.963
p-value	0.008	0.000	0.000	0.002	0.000

In the first week, up to 55% of the seedlings were girdled in Area II-1, even though the mean feeding area was low. During the next week, the proportion of girdled seedlings reached 70%. However, although the feeding area can continue to increase for some time, the increase in proportion of girdled seedlings must gradually cease, when fewer ungirdled seedlings remain (Figure 6).

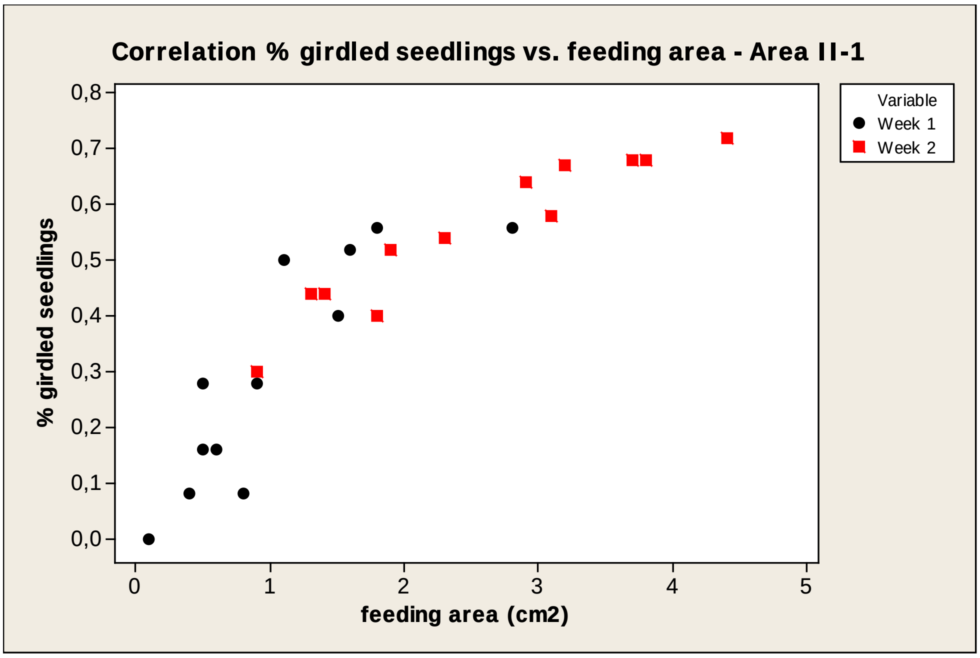


Figure 6. Correlation between the proportion of girdled seedlings and the mean feeding area (cm^2) in Area II-1. For test statistics confer Table 3.

4.4.2 Relationship between proportion of attacked seedlings and feeding area

A high attack rate was observed in Area II-1, where a proportion of 70 to 80% of the seedlings in some plots were attacked in the first week. Again, during the following week when few seedlings remained undamaged, the weevils generally continued feeding from the same seedlings, increasing the mean feeding area for each plot.

Table 4. Spearman rank correlation coefficient, r_s, and level of significance for correlations of pine weevil mean feeding area (cm^2/seedling) and the proportion of attacked seedlings for both areas and both study periods (N = 12).

	Area I	Area II-1	Area II-2
Spearman r_s	0.599	0.840	0.760
p-value	0.040	0.001	0.004

There was a significant positive correlation between the proportion of attacked seedlings and the mean feeding area, in both areas and study periods, especially in Area II (Figure 7).

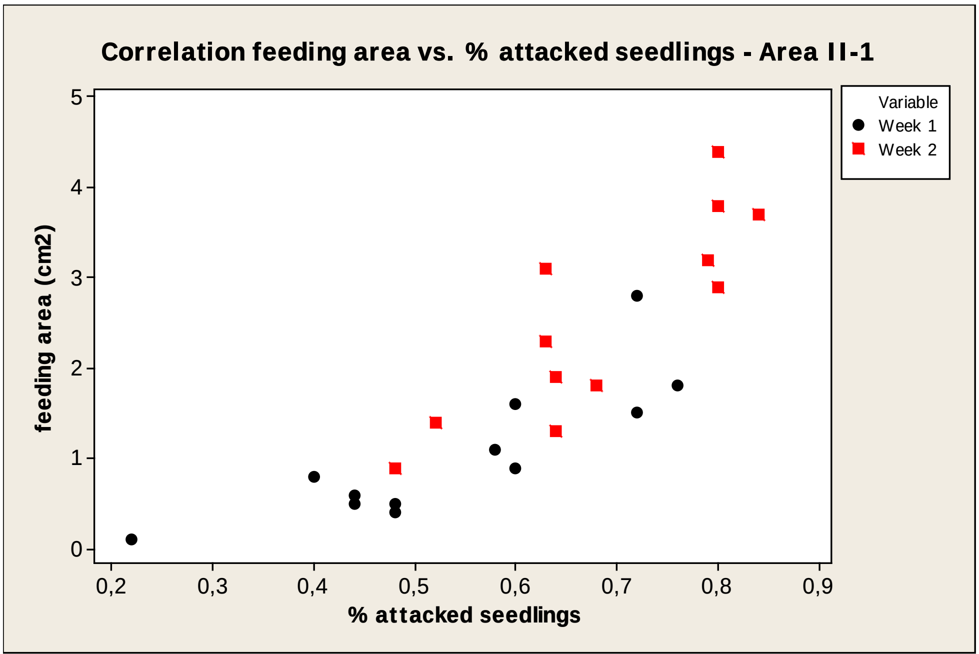


Figure 7. Correlation between mean feeding area (cm^2/seedling) in each plot, and proportion of attacked seedlings per plot in Area II-1. For test statistics confer Table 4.

4.4.3 Progress of proportion of girdled seedlings

The progress of the proportion of girdled seedlings (Figure 8) during the three first weeks is very fast and quite similar for all plots, although the increase in damage is much larger at plots with low damage in the first week. At the end of the experiment, all plots for Area I (week 3 – 9th of July) and most for Area II-1 (week 11 – 31st of August) had more than 80% girdled seedlings, which most probably were going to die in the next few weeks. Regardless of whether the initial damage was low (less than 30% girdled) or high (more than 80%), all plots reached a high damage level at the end of the 11 weeks. In Area II-2, most seedlings had no damage, and only two plots showed an increase in damage between week 14 and week 16 (not shown).

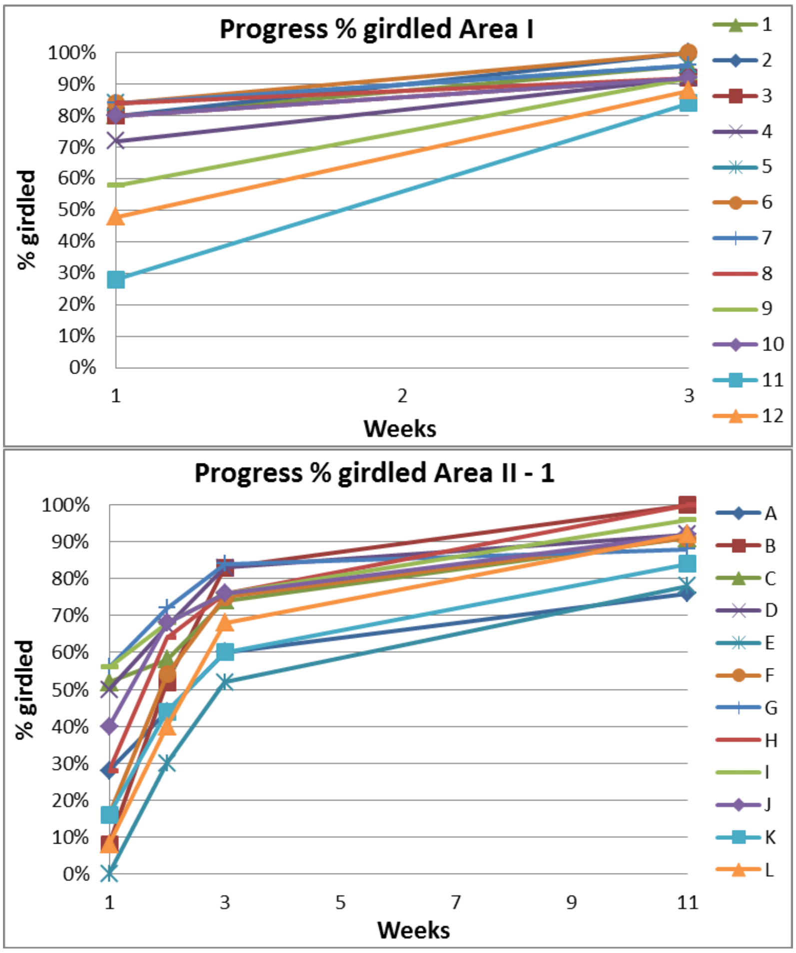


Figure 8. Progress of proportion of girdled seedlings in Area I and II-1.

4.5 Relationship between the number of ants and weevil damage

Considering the high seedling damage levels found already in the beginning of the study in Area I and the low damage and activity noted in Area II-2, the results presented in this part of the study are essentially those of Area II-1. Results for Area I and Area II-2 were generally not analyzed further.

4.5.1 Proportion of attacked seedlings vs. mean number of ants per trap

There was no significant relationship between the number of *Formica* or *Myrmica* and the number of attacked seedlings in Area II-1 during the first three weeks (Figure 9).

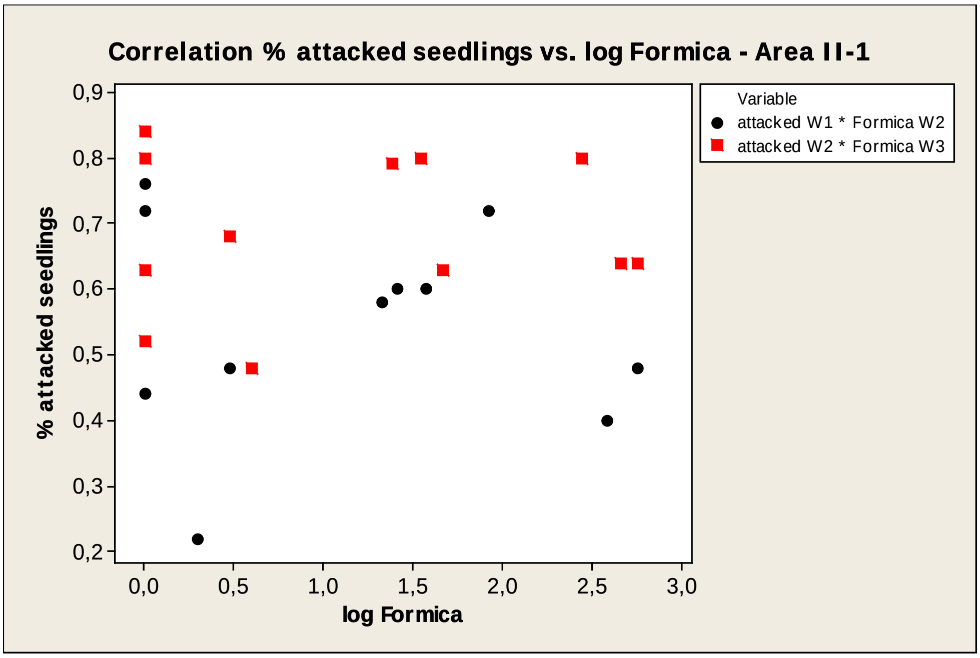


Figure 9. Relationship between log ant density and proportion of seedlings attacked by pine weevils.

The Spearman rank correlation between the density of *Formica* ants and the proportion of attacked seedlings was not significant (p-value = 0.846 for week 2; p-value = 0.794 for week 3; r_s = -0.063 for week 2; r_s = 0.085 for week 3; N = 12). In neither of the study periods the attack rate of seedlings was related to *Formica* ant densities (Figure 9).

No association was found between the number of *Myrmica* ants per trap and the proportion of attacked seedlings for Area II-1. In trap catches from Area II-2, *Myrmica* ants were mostly absent (no association graph shown).

4.5.2 Mean feeding area vs. number of ants

None of the analyses revealed significant correlations between mean feeding area per plot and ant trap catches. Neither trap catches of *Formica* ants (r_s = -0.006 and p-value = 0.853 for week 2; r_s =0.186 and p-value= 0.563 for week 3; N = 12), nor *Myrmica* trap catches (r_s = 0.032 and p-value = 0.922 for week 2; r_s = -0.218 and p-value = 0.496 for week 3; N = 12) were correlated with mean feeding area (Figure 10).

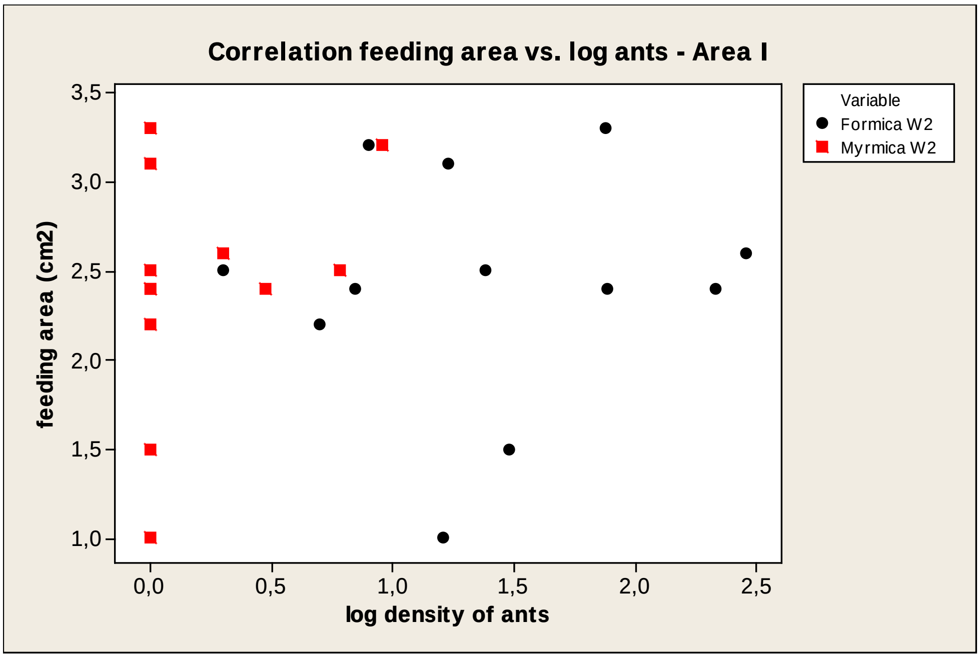
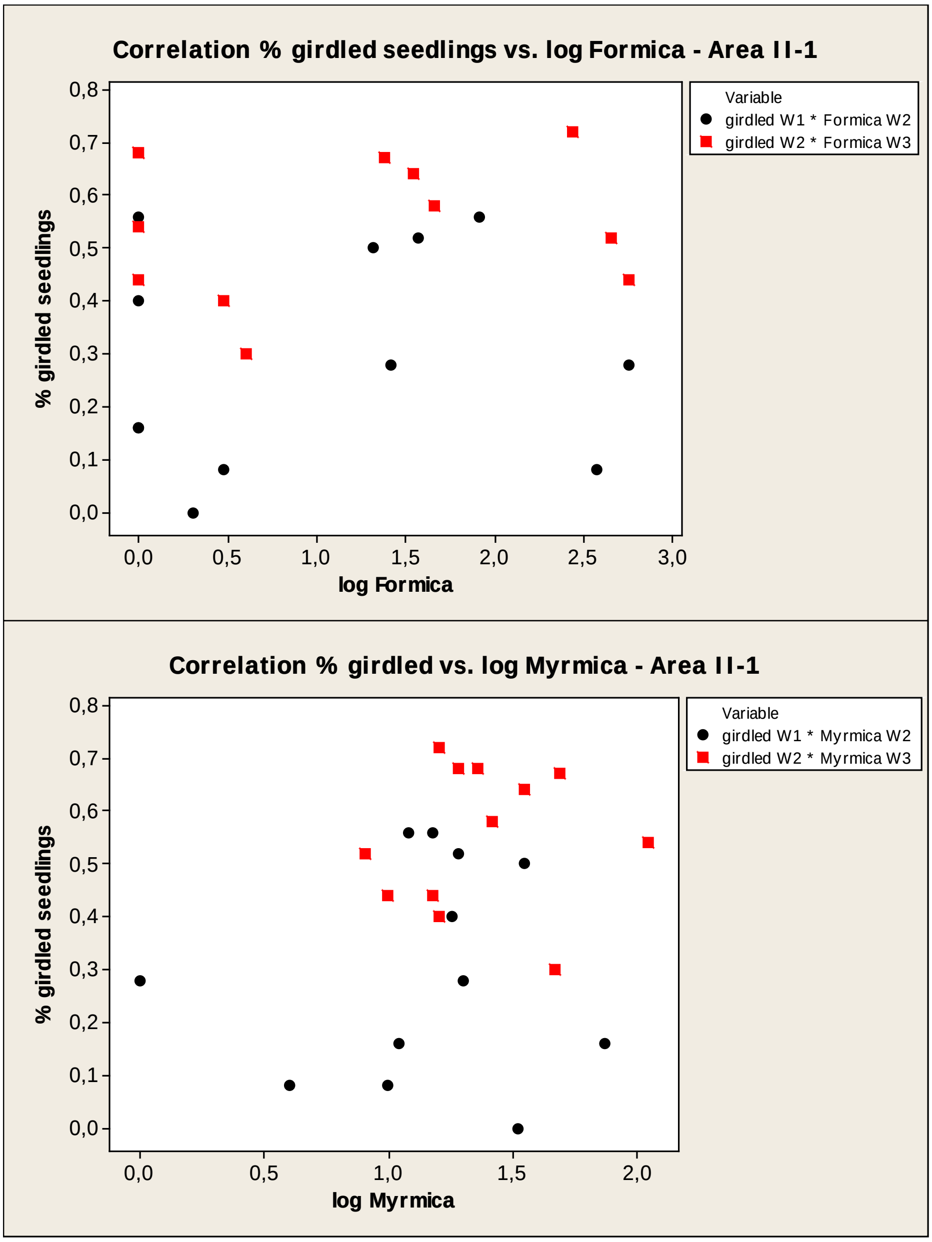


Figure 10. Correlation between the feeding area (cm^2) and density of ants in Area I.

4.5.3 Proportion of girdled seedlings vs. number of ants

No significant pattern could be found either when correlating the proportion of girdled seedlings and the number of ants per trap. Figure 11 show the lack of effect of the number of *Formica* (r_s = -0.048 and p-value = 0.882 for week 2; r_s = 0.502 and p-value= 0.096 for week 3; N = 12) and *Myrmica* ants (r_s = 0.225 and p-value = 0.483 for week 2; r_s = -0.261 and p-value = 0.412 for week 3; N = 12), respectively, on the proportion of girdled seedlings in Area II-1.



Figures 11. Correlation between proportion of girdled seedlings and density of ants *Formica* and *Myrmica* ants in Area II-1.

5 Discussion

5.1 General field observations

Some observations made during the field work were quite unexpected and might be relevant to consider for further studies.

Many seedlings which had been planted exactly at the soil level, neither too deep nor too shallow, presented no or very low damage. The caring in the planting phase should be maximized in order to have more consistent results. Seedlings planted on wet soils generally appeared more vital and less damaged than seedlings planted on drier soils, probably as a consequence of that seedlings require good water supply and because the pine weevil avoid moist areas. The ant density was also lower on wet soils than on drier soils, which shows that the soil type might be more important than the absence or presence of ants.

When *Myrmica* ants were present on the ground around a seedling, they were observed to climb it and react aggressively if the seedling was touched. However, the results did not show any effect on feeding damage, probably because *Myrmica* ants were only present in very few spots on the area. In a way, the seedlings on which *Myrmica* ants were found resembled the myrmecophytic plants described by Buckley (1982), Beattie (1985), and Davidson and MacKey (1993). These plants attract specialized ant colonies, offering them a nesting place as well as food. The attracted ants hence permanently inhabit their hosts, performing the function of protecting them mainly against herbivores. This behavior was actually observed in the field for *Myrmica* ants on most seedlings where these ants were sitting.

Another unexpected observation was that at very high *Formica* ant density (several hundred ants per plot), very near to anthills or on ant paths, the damage was highest. In these plots and these days, all seedlings were girdled in Area I, and 64% of the seedlings were attacked and 44% girdled in Area II-1. Even if no relationship was found between the damage and the ant presence, it is possible that *Formica* ants in these high density spots are more focused on other activities rather than attacking other insects, such as pine weevils.

5.2 Effect of ants on weevil damage

The original hypothesis of this study, that ant species of the genera *Formica* and *Myrmica* relieve conifer seedlings from pine weevil feeding was not verified. No significant relationship was found between the presence of ants and any of the variables defining damage by pine weevil.

According to Oliver *et al.* (2008), ants are much more effective against a pest when defending common resources. Indeed, ants often protect Homoptera (e.g. aphids, scales and mealy-bugs) against their predators, such as ladybirds. The fact that the pine weevil is not a predator of any homopteran might be one explanation of the absent effect seen in this study. Furthermore, Whay and Khoo (1992) only describe evidences of the role of *Formica* ants in protection of forest trees by preying on defoliating pests, such as the lepidopteran *Panolis flammea* but not against debarking pests.

Considerable information about the pine weevil attack process was obtained in this study. In the first weeks after plantation, feeding area increased with the proportion of seedlings attacked, i.e. the increase in feeding area was primarily due to that more seedlings are attacked. At the end of the experiment, however, the proportion of attacked seedlings remained practically constant, while the feeding area continued increasing, due to the decreasing likelihood of finding additional unattacked seedlings (Nordlander 1991). This study does not allow an explanation about the pine weevil's behaviour with time, but the results show that as long as unattacked seedlings are easily found, the feeding area increases about linearly with attack rate, but when few unattacked seedlings remain, the total feeding area keeps increasing because of continued feeding on the same seedlings.

The proportion of girdled seedlings at the end of the experiment was very high, regardless of the proportion at the beginning of the experiment. This indicates that although the pine weevil pressure was high, it took some time for the weevils in some plots to locate the seedlings and eventually girdle them.

The high weevil pressure found in this study might partly be explained by the very hot weather during the summer months. Also the absence of soil scarification on the clearcut before planting is known to greatly increase the risk of pine weevil attack (Petersson and Örlander 2003). While the weevils still could feed on the stem base of seedlings in shelter of the humus, the warm weather probably resulted in low activity of ants during the day, when they were sheltering in their colonies. Finally, the plantation of spruce seedlings during spring year A, i.e. the first growing season after clearcutting, also increased the risk of pine weevil damage. The most common practice in central Sweden to reduce this risk is to plant one year later.

5.3 Study planning

Planning decisions like using small seedlings and planting them directly in humus without soil scarification, and during a very hot period, resulted in a very high damage which made it difficult to detect significant differences. However, the opposite situation (using larger seedlings, planting them in scarified soil and later in the season) could also lead to difficulties to obtain clear results, because of the risk of too little damage by the pine weevil.

The distinction made between Area I, Area II-1 and Area II-2 is interesting because the data obtained in two different clearcuts (i.e. two different soil humidity and sun exposure) and two different periods of the year (i.e. the first one, June, sunny and warm, and the second one, September, cloudy, colder and rainier) provide similar results, i.e. no significant relationship between the abundance of ants and the weevil feeding.

6 Conclusion and future research needs

In this study no correlation between ant presence and pine weevil damage was observed.

Further research will be needed, partly because weevils are likely to benefit from the higher temperatures and later autumns coming with the predicted climate change (Tan *et al.* 2010), assumed to lead to increasing damage to the conifer plantations.

Way and Khoo (1992) mention that effects of small and apparently inoffensive species may be stronger than of large and more aggressive ant species. This could possibly have been the case for *Myrmica* spp. in this study area if their number had been greater. Although no results were obtained regarding effects of *Myrmica* spp. on the pine weevil, further studies should specifically focus on areas where the dominant species is *Myrmica* spp.

If some interactions between *Hylobius abietis* and *Formica* or *Myrmica* ants will be found in future studies, it should become important to focus on preserving ants in Swedish conifer plantations to reduce the feeding damage by pine weevils. Vegetation removal produces changes in nest microclimate (bigger range of temperatures and less moisture content) and over-drying of nests (Palladini *et al.* 2007; Véle and Holuša 2008). Preservation of *Formica* ant nests after clearcutting would improve the integrated pest management, especially when there are newly established bud-nests (Sorvari and Hakkarainen 2007). Ant nest preservation practices should be focused on reducing the size of clearcut areas and leaving dead and living trees on the clearcut or using shelterwood instead of clearcutting, in order to provide food, shelter and building material for the ants (Punttila *et al.* 1994).

It is not known yet under which circumstances an effect could be shown in the ant-pine weevil system, but further research could start with a comparison of data from different weather conditions, since the extremely hot period observed in June might have reduced the impact of ants. Another interesting aspect to study is that the weevil damage might be reduced by attracting ants to the seedlings with aphids present on the stems.

7 Literature

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