# A study on *Byctiscus populi* (L. 1758) (Attelabidae) in Latvia and implications for conservation management in the UK

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**A study on *Byctiscus populi* (L. 1758) (Attelabidae) in Latvia and implications for conservation management in the UK**

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# Abstract

*Byctiscus populi*, (Attelabidae) has declined in the UK and is now classified as rare (JNCC Action Plan, Morris). This study examined the habitat preferences of this beetle in Latvia, where it is relatively common, in order to guide appropriate habitat management in the UK. The study also investigated the life history of the species, including mortality factors, dispersal ability and the process of leaf rolling.

This study validates the anecdotal reports that *B. populi* prefer young aspen trees.

A strong preference for young aspen under 2.80m tall in full sun was apparent. The species prefers to create leaf rolls on the tender young growth at branch tips, and young aspen tended to have more of this young growth than mature trees. 1-4 eggs can be laid in one roll, egg number is correlated with leaf length. The average time from laying to hatching was 3.7 days, larvae remain in the roll for about 15 days, then exit to burrow into the substrate. Pupation occurs in the soil in pupal chambers. Adults then emerge above ground, over-wintering sites are still unknown. Desiccation was a significant cause of mortality. Larvae inside rolls on the ground were subject to predation, possibly by shrews. Males tended to move further distances than females but more work is required in this area. The major factor preventing colonisation of suitable habitat in Latvia seemed to be isolation in an unfavourable matrix of pine forest. Female *B. populi* perform a stereotyped sequence of behaviour prior to rolling similar to that described for other attelabids.

Implications for conservation in the UK: Planting aspen in field margins and cutting on rotation every 4 years under agri-environment schemes near *B. populi* sites may facilitate dispersal to suitable habitat patches and create new habitat. Pruning aspen may stimulate young growth that can be utilised by *B. populi* on trees that otherwise would be unsuitable. Protection of rolls on the ground may be necessary in order to prevent predation and trampling.

# Contents

# 1. Introduction

Forest clearance and fragmentation resulting from agricultural intensification has caused large-scale losses of flora and fauna in the UK. Sound conservation and management techniques for threatened woodland invertebrates should be based on an adequate knowledge of the habitat requirements of target species. This study aims to determine the habitat needs of *Byctiscus populi*, an attelabid weevil that has declined in the UK and is now classified as Rare and is a BAP species. There are currently very few sites occupied by *B. populi* in the UK, with only one large population confirmed. The study was carried out in Latvia where the species is relatively common.

*Byctiscus populi* (L. 1758), formerly known as *Rhynchites populi* (L.) is associated with aspen (*Populus tremulae*) and white poplar (*Populus alba*). There is anecdotal evidence that the species prefers the young growth of suckering or regenerating aspen (2001, JNCC Action Plan). Females create leaf rolls, usually from one leaf, in which eggs are laid (Hymen and Parsons, 1992). The leaf stalk is incised, and as the leaf begins to wilt the female tightly rolls the leaf cigar-wise, usually laying one or two eggs in each. When the roll is complete, the female seals the end with a sticky anal secretion. These rolls fall to the ground at some point after creation.

*B. populi* can be distinguished from the similar species *Byctiscus betulae* (L. 1758, formerly *B. betuleti.* F. 1792) by a number of features, the easiest feature that allows the two species to be distinguished is that *B. betulae* has the same coloured upper and lower surfaces (golden-green or blue). *B. populi* has a dark blue underside and normally a greenish-red or coppery upper surface in contrast (Fowler 1891).

*B. populi* is also tends to be the smaller of the two species, measuring 4-5.5mm compared to *B. betulae* which are 4.8-7mm (Harde & Severa, 1998). In both species males are distinguished from females by the possession of spines on the lower margin of the pronotum on each side (Fowler, 1891).

*B. populi* is currently classified as rare in Great Britain (JNCC Action Plan, Morris). Historically the species was distributed across most of southern England and up into Norfolk, Worcestershire and east Gloucestershire. Post 1970 records exist for East Sussex, East Kent and Surrey and Worcestershire (JNCC, 2001). The decline is probably due in part to inappropriate woodland management, including removal of aspen, loss of broad rides and sunny glades, and the reduction in coppice management (JNCC Action Plan). *B. populi* is quite common and widely distributed throughout central-western Europe and is also present in the Mediterranean area , the Nordic countries, Asia Minor, central Asia, Siberia, Mongolia and Northern China (Action Plan). This study was carried out in Latvia, where *B. populi* is relatively common.

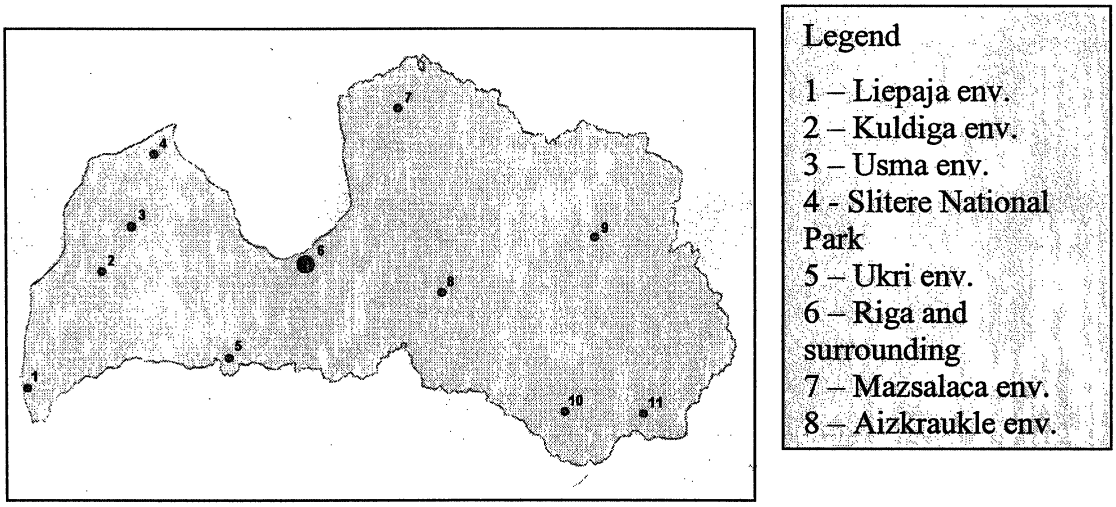


**Figure 1.1.** *B. populi* is widely distributed in Latvia. *B. populi* has been officially recorded at over 200 sites in Latvia. A selection of sites is listed in the legend. Data from D. Telnov, Entomological Society of Latvia.

Latvia, one of the Baltic States is situated in the western part of the East-European plain, on the eastern coast of the Baltic Sea, 55° 40' - 58° 05' N and 20° 58' - 28° 14' E. The total land area is 64,589 $km^2$. The highest point, the Vidzeme Upland is 312 m above sea level (Ministry of Agriculture, 1995).

Latvia has a temperate climate, which is influenced by Atlantic air masses but becomes increasingly continental further inland. Average annual precipitation is 500-800mm. The growing period (when the average temperature over a 24-hour period is over 5°C) commences in mid April and lasts roughly between 180-200 days depending on geographical location. The average maximum temperature (July) is 17°C, the minimum (January) is 2 to -7°C.

Currently 44.6% of Latvian territory is covered by forests. Prior to the second world war, this figure was just 25%. At present, the dominant tree species (comprising 39% of the whole forest area) is Scots pine *Pinus sylvestris,* followed by Norway spruce *Picea albies* (21% of forest area). The dominant deciduous species are the birches *Betula pubescens* and *B. pendula* (28%), Alder *Alnus incana* (6%), Aspen *Populus tremula* (3%), Black Alder *Alnus glutinosa* (2%), and hardwoods such as *Quercus robur* (penduculate oak) and Ash *Fraxinus excelsior* comprise 1 % of the total forest area.

38.8% of Latvian territory is currently used for agriculture. 69% of this is arable, 30% is semi-natural meadows and grasslands and 1% is orchards. Agricultural intensity has been low over the last 50 years and around 80% of privately owned farms use non-intensive methods (National Programme on Biological Diversity, 2000) (Figure 1.2). Large populations of the white stork *Cionia cionia* and the corncrake, *crex crex* are supported by Latvia's agricultural land. Over the previous 45 years about 2 million hectares of agricultural land has been abandoned.

Many species that are rare throughout Europe are common in Latvia. This is mainly due to the presence of many natural habitats that are extremely rare throughout most of western Europe, and the low-intensity land-use systems characteristic of Latvia's landscape.

However, utilisation of forest resources is currently increasing rapidly, and forestry has become one of the leading sectors of Latvia's economy. Between 1995 and 2000, the timber harvest doubled (National Programme on Biological Diversity, 2000).

Equivalent data on the abundance and distribution of aspen in the UK will be made available from the Forestry Commission Woodland Inventory Project in the following months.

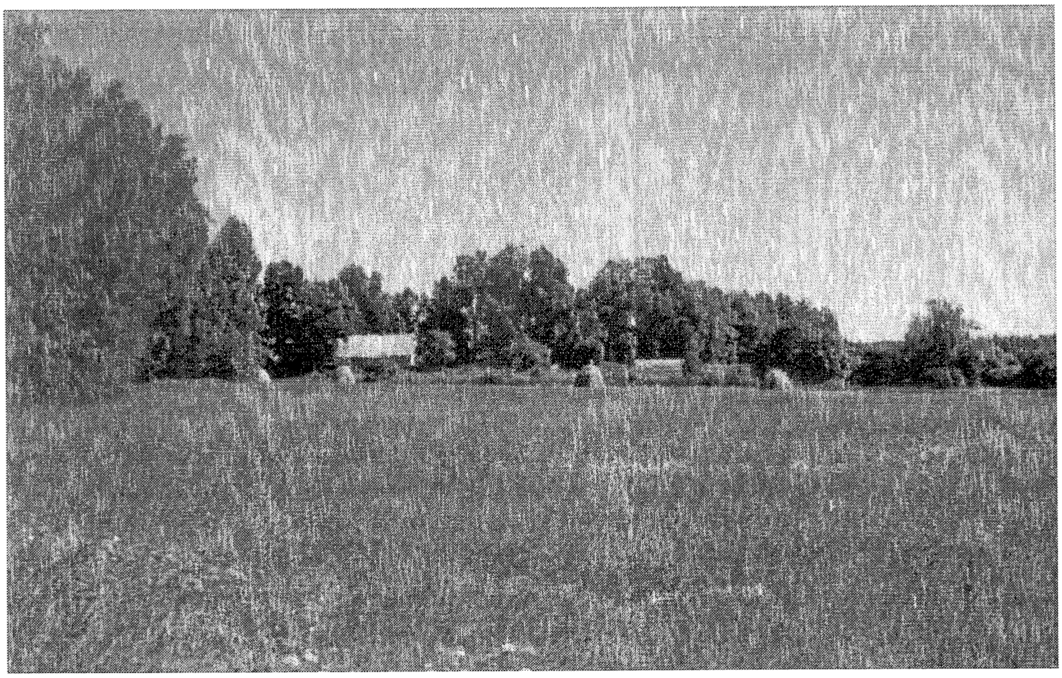

**Figure 1.2** Non intensive farming typical of Latvia.

**Objectives of the study**

1. **Habitat preferences**

What features of aspen trees and the surrounding habitat are important to *B. populi* adults when selecting aspen trees to create leaf-rolls on?

2. **Life history**

The details of the life history of this species is unknown, but it is generally thought that it over-winters as an adult. This study will aim to determine the number and duration of larval instars and whether pupation occurs within the leaf roll after it falls to the ground, or elsewhere, e.g. in the soil as in *B. betulae*. (Bily, 1990).

3. **Predation and other causes of mortality**

What species if any predate upon adults and larvae? What factors affect larval survival inside leaf rolls (e.g. desiccation, excess moisture).

### 4. Adult mobility

How well is this species able to locate and disperse to different suitable patches of habitat? What is the dispersal range of adults, and does this differ for males and females?

### 5. The sequence of behaviour exhibited by females during leaf rolling

The process of leaf rolling has been described in detail for a number of attelabid weevils, (Sakurai, 1989, 1990). The process of rolling will be observed for *B. populi*, and compared to that of other attelabid females.

# 2. Materials and methods

## 2.1 Study sites

### 2.1.1 Site 1

Site 1 was located at Cekule, in the Riga district, 12 Km East of Riga (56°55 N, 024°23 E). This site consists of secondary mixed forest over 40 years old, and is subject to recreational use by humans.

Three study plots measuring 7m by 7m were used at site 1. Plot 1 was selected by picking random points along x and y-axes formed from tape measures placed on the side of a railway embankment. This location is sheltered by the embankment to the south-east and another bank to the north-west. Young aspen (less than 3 years old) dominates this area (103 aspen in the plot) but young *Salix* and birch *Betula* spp. are also present. The site was in full sun after about 11am and the sward was very short consisting mainly of lichens and mosses.

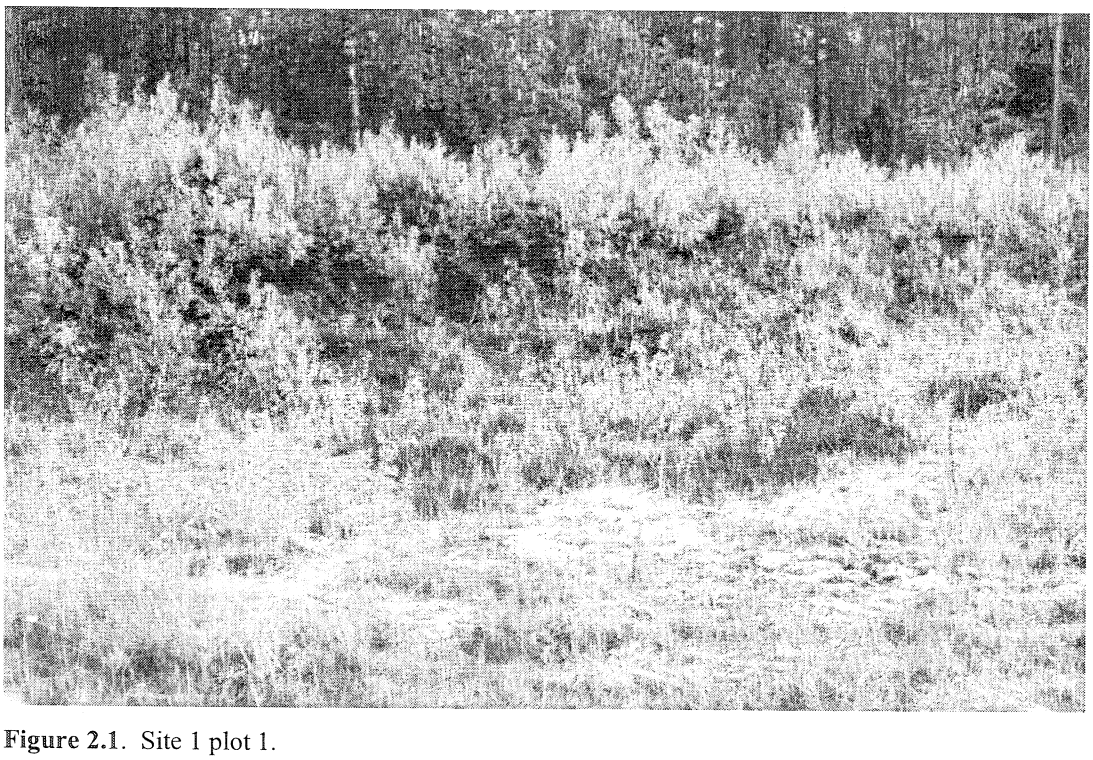

**Figure 2.1**. Site 1 plot 1.

Plot 2 (7 by 7m) was located at the edge of a broad ride cleared for electricity cables but was shaded by tall pines *Pinus sylvestris* and aspen (about 30-40m tall) and 5-7 m *Quercus* species. The average sward height was 25 cm and consisted mainly of various grass species. There were 36 aspen in the plot, the average height of which was 2.26m. A large ant nest (*Lasius niger*) was located in the south-east corner.

**Figure 2.2.** Site 1, plot 2.

Plot 3 was located in the ride, The height of the trees (mainly pine and birch) on either side of the ride was about 30-40m. There were 40 aspen in the plot, the average height of which was 0.62 m. The sward consisted mainly of grasses and *Melampyrum nemorosum* was a particularly common plant.

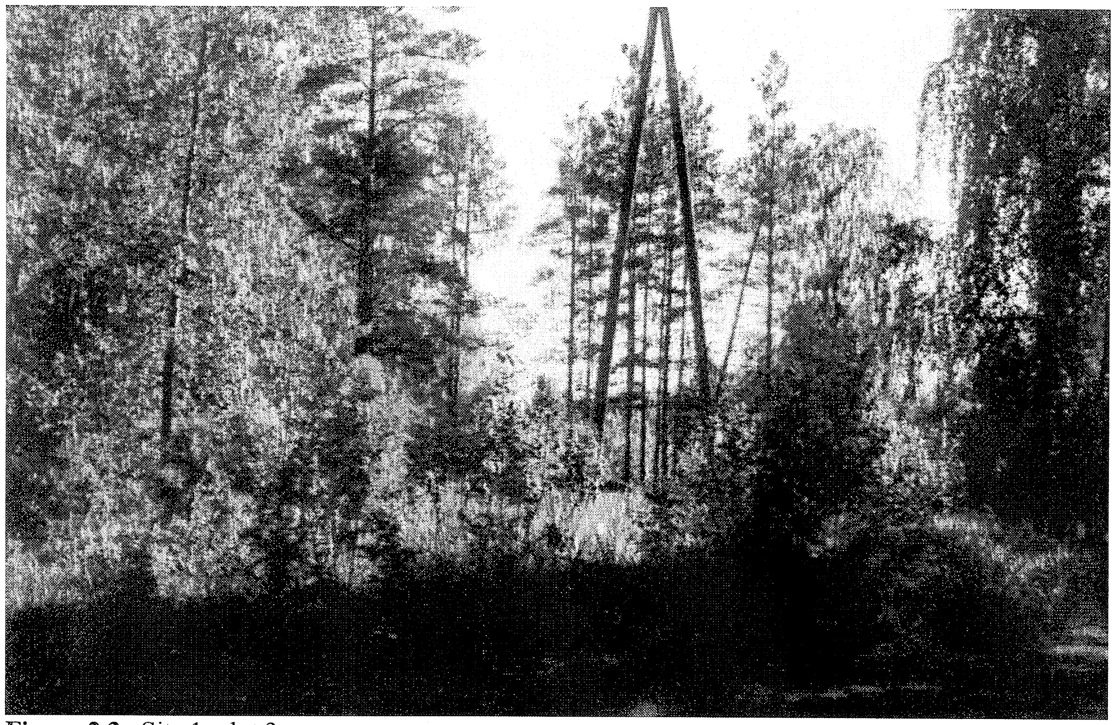

**Figure 2.3.** Site 1, plot 3.

### 2.1.2 Site 2

Site 2 was also located in the Riga district, on a highway between Riga and Ergli, 4.5 Km south of the village of Zakumuiza (N 56°56.759, N 024°27.742). The site, which is adjacent to the road was a clear-felled area, cut approximately 4 years ago. The site measures 110.3m by 108m. Young aspen (*P. tremula*) dominates but birch, *Salix* spp. and elder are also present. There were some areas of standing water present, but these mainly dried out towards the end of June. Large patches of felled branches were present at various locations on the site. The surrounding forest is dominated by pine (*Pinus sylvestris*) with birches (*Betula pubescens* and *B. pendula*) and aspen (*P. tremula*) as admixed species. The sward consisted of various grasses, other common species included *Juncus conglomeratus, Vaccinium myrtilus* and *Melampyrum nemorosum*.

**Figure 2.4.** Part of site 2.

### 2.1.3 Site 3

Site three was situated in the Ogre district, 3 Km north of Tinuzi (near Muizeni). (N 56°53.259, E 024°34.211). This site which measures 168.1 m by 220 m, is also a clear-felled area adjacent to a road. Very dense young aspen dominates the area (average height 0.98m), with *Salix*, birch and hazel also present. Some standing water was present, however this had dried out by the end of June. Large patches of felled branches were present on the site. *Juncus conglomeratus* and *Melampyrum nemorosum* were again common in the grassy sward. *M. nemorosum* was present in very large patches and was by far the commonest sward species. *M. silvaticum and Vacinnium myrtilus* were also abundant. Site 3 was surrounded by mainly pine forest (*Pinus sylvestris)* with some admixed birch and aspen. Low intensity farmland (mainly traditionally managed hay meadows) is located to the north and south of the surrounding forest.

**Figure 2.5**. Site 3.

### 2.1.4 Site 4

The transect study was carried out at Slitere National Park, in north-east Latvia. This reserve has been strictly protected since 1921. The study site was located on the edge of an extremely species rich traditionally managed hay meadow, with aspen at the meadow margin. The height of the aspen increased away from the meadow in a gradient towards the forest from about 60 cm to 30 m tall trees.

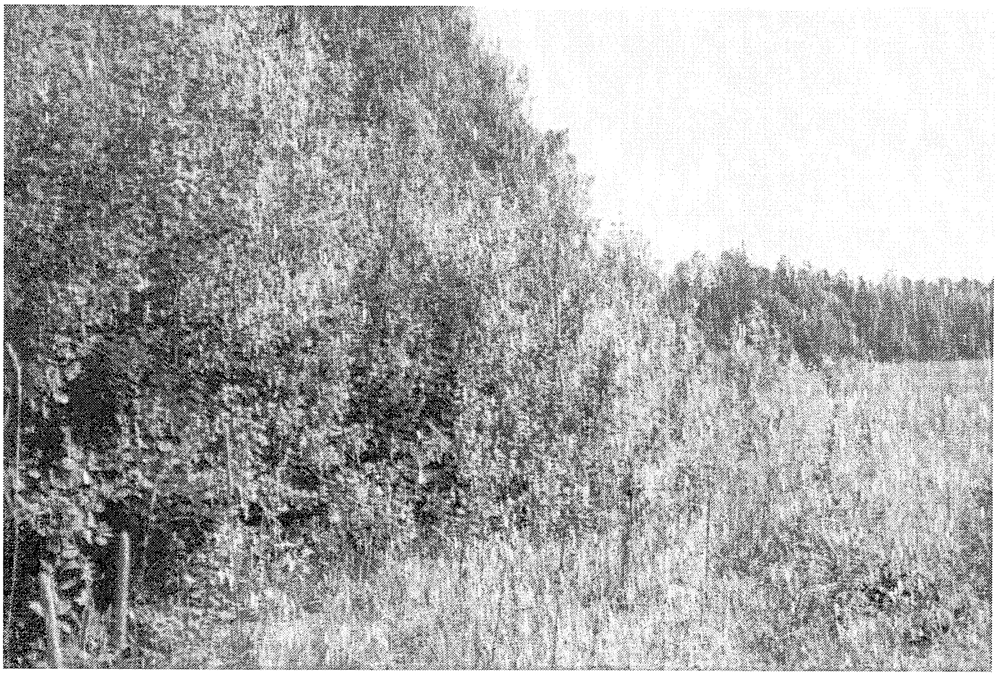

**Figure 2.6**. Transect site at edge of hay meadow in Slitere. The gradient of height of aspen is visible.

**Figure 2.7** Front view of transect site.

**Figure 2.8**. Hay meadow at Slitere. Transect study site was located at the edge of this meadow.

## 2.2 A note on leaf rolls and feeding damage

It soon became apparent that leaf rolls were present in much larger numbers than adults were. The leaf rolls of *B. populi* are easy to identify and easily distinguished from those of other species. They are usually constructed from a single leaf, but occasionally a second smaller leaf may be also be incorporated. The rolls hang vertically down from an incision point about 0.5mm above the leaf blade created by the puncturing of the leaf stalk with the rostrum (Figure 2.9). Rolls are neatly rolled 'cigar-wise' and could be described as 'slim-line' compared to the rolls of *B. betulae* which involve many leaves and are therefore much wider. *B. betulae* rolls are usually created from the entire tip of a shoot, which is cut horizontally across leaving a characteristic 'hinge-like' damage point on the shoot above the roll. In contrast *B. populi* rolls have a single small discrete puncture mark. When *B. populi* rolls fall to the ground, a characteristic 'scar' (Gruppe *et al.* 1999) is left on the leaf stalk when the roll breaks from the incision point.

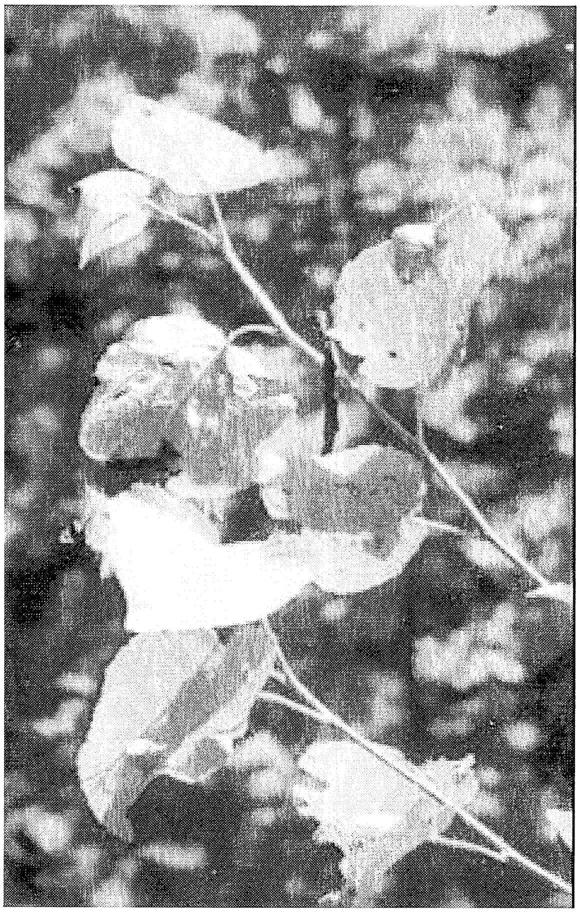

**Figure 2.9** Point a) highlights a dry *B. populi* leaf roll on an aspen tree.

*B. populi* feeding damage occurs on leaves in narrow track-ways typically measuring about 1 cm long and 1 mm wide. The leaf tissue is left turgescent and a 'window-pane' effect is created. As this feeding damage ages, the turgesence is lost and the damage passes through the entire leaf. *B. betulae* feeding damage is very similar, but is much wider and longer, about 2 cm long and 3mm wide. (See Figure 2.10)

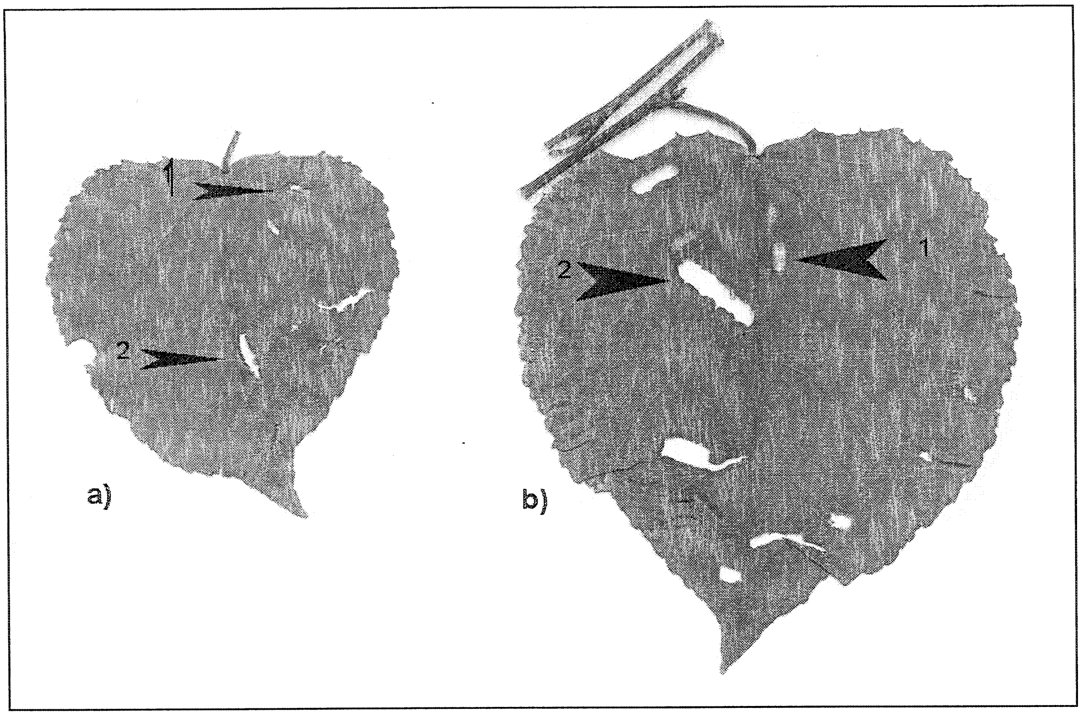


**Figure 2.10**. a) This aspen leaf shows *B. populi* feeding damage. The feeding damage at point 1 is still turgescent. At point 2 the feeding damage passes through the entire leaf tissue. b) Shows *B. betulae* feeding damage. At point 1 the damage is turgescent, at point 2 the turgescence has dried away.

## 2.3 Habitat preferences

In plots 1, 2 and 3 of site one, all bushes within the plots were tagged, numbered, and given individual co-ordinates by placing 2 tape measures at right angles to each other to form axes. Thirty bushes were selected using random number tables. For these bushes the following measurements were taken: bush height, circumference of trunk at base, nearest neighbouring aspen distance (nnd), the number of leaves per bush was counted when possible (for larger trees this was estimated using average number of leaves per branch), number of rolls and roll scars with aspect, number and location of adults and their activity, gender and time of observation. The height of the lowest and highest roll was recorded with temperature and humidity of the top of the bush and the middle and the bottom section taken at the trunk. Ground flora was recorded with sward height, and percentage cover of bare soil or leaf litter in a 1m radius around the trunk base.

For plot 3 of site 1, a measure of bush width was taken by measuring the longest horizontal branch. The distances of the rolls nearest to and furthest away from the trunk were recorded in this plot in addition to the above measurements.

At sites 2 and 3 the density of aspen in each plot was considerably higher so rather than numbering each bush, the 30 bushes in each plot were selected by picking random x and y co-ordinates along the axes formed by tape measures. The nearest aspen to these co-ordinates was then used. In addition to the measurements described above, for sites 2 and 3 all rolls and roll scars had the following measurements taken: height, aspect, distance to tip of branch or top of bush depending on position. If the roll or scar was located on a branch the branch length was also recorded.

The temperature and humidity of the trunk and the tip of the longest radial branch at the middle of the tree was recorded, and the temperature and humidity of the top of the tree was also noted in order to determine whether this influenced roll location.

A transect study was carried out at the 4th study site at Slitere on the 18th July 2001. A tape measure was placed along the south-western facing edge of the hay meadow and 4 points were selected at random along its length. A second tape measure was then placed at right angles to this tape measure at these points, and each aspen tree along this gradient was analysed. Tree height was measured with the number of rolls and roll scars on the tree. Adults were not present at this site at the time of study, possibly because one generation had recently ended.

## 2.4 Life history

Rolls were collected from bushes at site 1. These rolls were examined and the contents were recorded. Rolls were opened by sliding the tip of a retractable pencil under the sealed part of the leaf edge, all rolls were opened over a black surface in order to find eggs and larvae easily if they fell out of the roll. All rolls were then checked each day, contents were recorded and the size of larvae (if present) was measured. 79 rolls were followed in this way. In addition, 51 rolls created in captivity were also followed. The advantage of using these rolls was that the date of laying was known precisely. This allowed the time from laying to hatching to be elucidated. Measuring larvae allowed the larvae to be assigned to various size classes, which may reflect the larval instars. A number of advanced rolls were taken from the ground at site 1 in order to observe the later stages of larval development.

Rolls were placed in a variety of conditions in an attempt to find optimal rearing conditions. 15 rolls were placed in sterile sealed tubes measuring 8cm with a 1cm diameter. 20 were placed on leaf litter in ventilated plastic containers, 20 were placed in airtight sealed plastic containers with moist nylon beneath them to buffer the moisture level and keep the moisture of the rolls lower. 40 rolls were placed on a layer of soil and leaf litter and sealed in 12cm by 8cm plastic containers. In all cases if the rolls became excessively dry, water was sprayed into the container. Rolls were always checked between 7pm and 11.30pm.

## 2.5 Causes of mortality

Thirty five rolls were collected from trees on the 8th June 2001 from site 1. Only 1 roll was collected from each tree. All the rolls were opened, their contents recorded and the length of the roll was measured. These were then sealed inside a large airtight glass jar for 2 weeks, no moisture was added and the rolls were subjected to very dry conditions. After 1 week all rolls were re-opened and contents recorded. If no egg or larvae was present, then evidence of larval activity such as feeding damage or frass was looked for.

Thirty six rolls collected on the 26th June 2001 were subjected to the same procedure, but were placed in very humid conditions, on a layer of wet tissue paper.

A further 30 rolls were also collected on the 26th June 2001 and used to control for the effects of opening the rolls. These were initially kept in dry conditions for 4 days then placed in a ventilated plastic container on leaf litter, if these rolls became dry they were sprayed with water in the evening.

## 2.6 Predation

On 18 June, 30 rolls collected from site one on the previous day were marked with coloured tip-ex, and placed on the ground in groups of 10 at the base of the nearest aspen bush to randomly picked co-ordinates in each of the study plots of site 1. Previous field trials had shown that coloured tip-ex remained on the rolls for an extended period even in very wet conditions. Co-ordinates were obtained by placing measuring tapes at right angles to each other to form x and y co-ordinates. The location of each group of leaf rolls was marked with a large labelled stick and the ground flora was recorded, with maximum sward height and % bare ground and leaf litter. All rolls had been collected from the ground in an attempt to place advanced rolls containing larvae in the plots. After one week the rolls were counted and checked for visible signs of damage. The same procedure was carried out at site 2 on the 27 June.

During the study, spider's webs were examined for any remains of *B. populi*, and observations of any fauna interacting with leaf rolls or feeding in the vicinity of leaf rolls were recorded.

## 2.7 Adult mobility

Forty one (19 males, 22 females) adults were collected from site 3 on the 3 July. These were marked on the elytra with a small amount of coloured correction fluid with a dab of clear nail varnish over the top to seal. All marked individuals were kept overnight in a large well-ventilated cage with aspen leaves. The following day the cage was placed at the base of a centrally located aspen bush at site 3 and left until all beetles had vacated. On the 6$^{th}$ July the site was searched and marked adults were caught, sex and activity noted with time of recapture and distance to release bush taken. All captured individuals were retained whilst the site was searched. Only 13 individuals were recaptured, 7 males and 6 females. This may be due to marks being lost (unmarked individuals were present) or to individuals leaving the site.

## 2.8 The sequence of behaviour exhibited by females during leaf rolling

The process of leaf rolling was observed in captivity in 3 cases from before the initial incision of the leaf stalk to the female vacating the finished roll. In 2 cases the end of rolling was observed, when the female seals the leaf roll. Females were kept in well-ventilated cages with young aspen shoots. In the field, leaf rolling was observed completely twice and the final stages were observed once. For rolls created in captivity the route of the female across the leaf surface was recorded and the timing and sequence of each stage noted. This was then compared to similar information on other leaf-rolling species (*Chonostropheus chujoi, Apoderus balteatus*, and *Deporaus* spp., Sakurai, 1988,1990)

## 2.9 Statistical procedures

All statistical tests were carried out on SPSS version 9. In all cases data were tested for normality using the Kolmogorov-Smirnov test and for equality of variances using the Levene's test (Dytham, 1999). If the assumptions of parametric tests were not met even after transformation, the appropriate non-parametric equivalent was employed. Tests used include Spearman's rank correlation, one-way ANOVA, t-tests, paired sample t-tests, the $\chi^2$ test, and the Kruskal-Wallis test with Tamhane's T2 (assumes non-equal variances) as a post-hoc. Multivariate analyses used were multiple regression using the enter method, and binary logistic regression using the forward stepwise (conditional) method.

# 3. Results

## 3.1 Habitat preferences

### Site 1

Data from all 3 sub-plots of site 1 were combined for analysis.

### Tree and habitat characteristics

At site 1, the average height of the 90 aspen trees selected at random across the 3 plots was 1.11m, ranging from 0.20m to 14.50m tall (Table 3.1).

**Table 3.1**. Descriptive Statistics for tree and habitat characteristics measured at site 1.

| | N | Range | | Mean | Std. Deviation |
|---|---|---|---|---|---|
| | | Min | Max | | |
| Aspen height (m) | 90 | 0.20 | 14.50 | 1.11 | 2.09 |
| Number of leaves | 90 | 8.00 | 3300.0 | 129.23 | 396.33 |
| Circumference of the trunk (cm) | 90 | 0.80 | 59.00 | 3.48 | 7.37 |
| Nearest aspen distance (m) | 90 | 0.00 | 2.26 | 0.41 | 0.41 |
| % bare soil | 90 | 0.00 | 41.00 | 2.61 | 6.00 |
| Sward height (cm) | 90 | 0.30 | 40.00 | 18.86 | 11.28 |

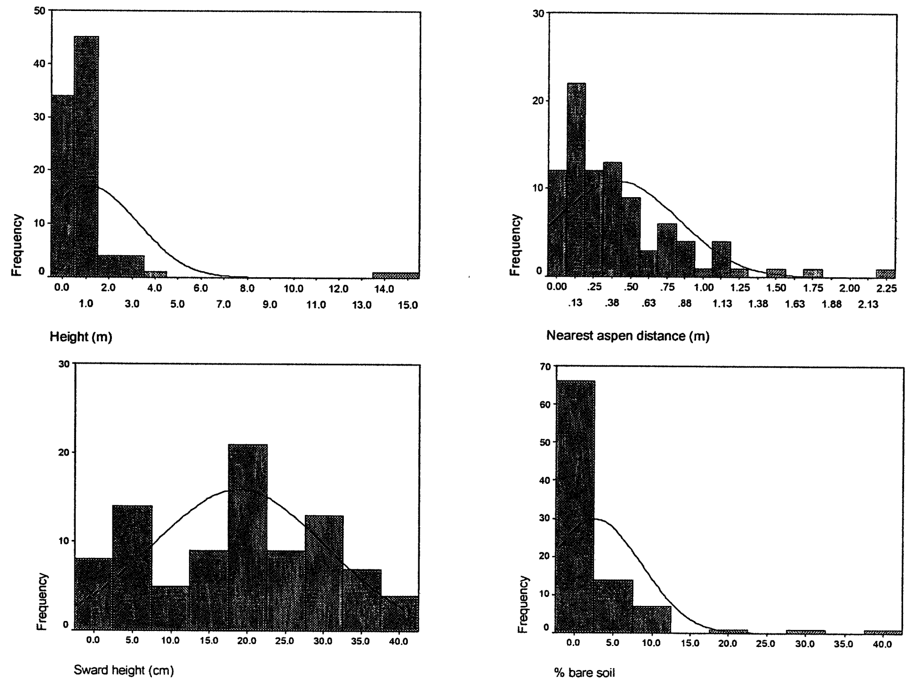


**Figure 3.1**. Frequency histograms of aspen height, nearest aspen distance, sward height and % bare soil.

Mann Whitney U tests indicated that there was significantly more bare soil surrounding trees occupied by adults, rolls and roll scars than there is surrounding unoccupied trees (Table 3.3 and Figure 3.2). None of the other variables measured were significantly different for occupied and unoccupied trees.

**Table 3.2.** Descriptive statistics for occupied and unoccupied aspen trees in site 1.

| | Occupied trees | | | | | Unoccupied trees | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min | Max | Mean | Std. Dev. | N | Min | Max | Mean | Std. dev. |
| Height | 53 | 0.30 | 3.0 | 0.77 | 0.49 | 37 | 0.20 | 14.5 | 1.59 | 3.18 |
| Leaves | 53 | 11.0 | 407.0 | 73.94 | 70.33 | 37 | 8.00 | 3300.0 | 208.43 | 608.5 |
| Nearest aspen dist. | 53 | 0.00 | 2.3 | 0.35 | 0.39 | 37 | 0.00 | 1.7 | 0.50 | 0.44 |
| Circumference | 53 | 1.00 | 10.0 | 2.54 | 1.60 | 37 | 0.80 | 59.0 | 4.83 | 11.28 |
| % bare soil | 53 | 0.00 | 98.0 | 16.57 | 23.11 | 37 | 0.00 | 100.0 | 9.46 | 22.10 |
| Sward height | 48 | 0.34 | 40.0 | 16.95 | 10.49 | 35 | 0.30 | 40.0 | 20.39 | 12.14 |

**Table 3.3.** Mann-Whitney U test results comparing occupied and unoccupied trees at site 1.** = significant at the 0.01 level.

| | Aspen height | Number of Leaves | Nearest aspen distance | Circ. of trunk | % bare soil | Sward height |
|---|---|---|---|---|---|---|
| Mann-Whitney U | 870.0 | 901.5 | 769.0 | 810.0 | 688.5 | 693.0 |
| P | 0.365 | 0.517 | 0.083 | 0.162 | 0.008** | 0.172 |

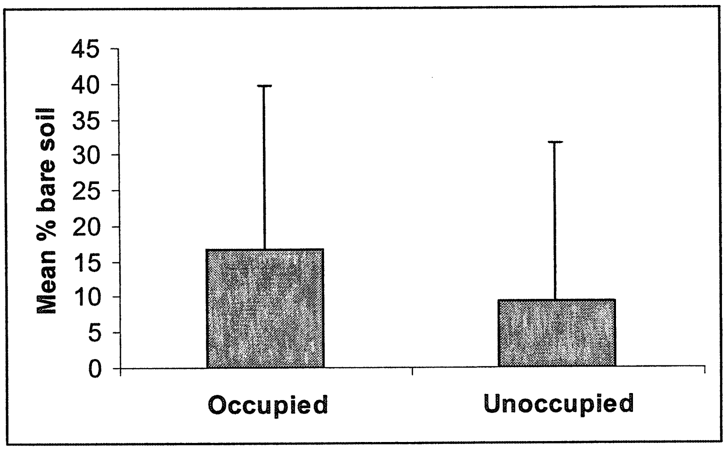


**Figure 3.2.** Aspen trees occupied by adult *B. populi*, rolls and roll scars are surrounded by a significantly greater percentage of bare soil than are unoccupied trees (Mann Whitney U = 688.5, P = 0.008). Error bars show standard error.

As one would expect, the height of the aspen trees was positively correlated with both the number of leaves on the tree and with the circumference of the tree. The number of leaves on a tree was also positively correlated with the circumference of the trunk (Table 3.4). Taller trees had both more leaves and trunks with bigger circumferences. Aspen height was not correlated with the distance to the nearest neighbouring aspen nor with the percentage of bare soil surrounding the tree base. It was, however correlated to the sward height; taller aspens tended to be surrounded by a taller sward (Table 3.4). This may be because the taller trees at site 1 tended to be in the shade, a longer sward was found in these areas.

The distance to the nearest neighbouring aspen was not correlated with the % of bare soil surrounding the trees, but was correlated with the sward height; the greater the distance between aspen trees, the taller the sward (Table 3.4). The percentage of bare soil was not correlated with the sward height.

**Table 3.4**. Spearman rank correlations between tree and habitat features at site 1. ** significant at 0.01 level, *** significant at 0.001 level.

| | rs | P | N |
|---|---|---|---|
| Height vs No. of leaves | 0.755 | P< 0.001*** | 90 |
| Height vs. circumference of trunk | 0.801 | P< 0.001*** | 90 |
| No. of leaves vs. trunk circumference | 0.789 | P< 0.001*** | 90 |
| Height vs. nearest aspen distance | 0.179 | 0.091 | 90 |
| Height vs. % bare soil | -0.185 | 0.082 | 90 |
| Height vs. sward height | 0.464 | P< 0.001*** | 90 |
| Nearest aspen distance vs. % bare soil | -0.021 | 0.846 | 90 |
| Nearest aspen dist. vs. Sward height. | 0.316 | 0.002** | 90 |
| % bare soil vs. sward height | -0.056 | 0.603 | 90 |

**Presence of adult *B. populi* and leaf rolls**

Spearman rank correlations were carried out on aspen and environmental variables measured in site 1 with both adults and (rolls + scars) as dependent variables. The number of rolls on a tree was positively correlated with the number of adults present (Table 3.6). Both the number of adults and the number of rolls and scars on a bush were significantly correlated with aspen height and the circumference of the trunk (Table 3.6), so generally taller trees (with larger circumferences) had more rolls and more adults on them. The number of rolls and roll scars was also significantly correlated with the number of leaves on a tree. The numbers of rolls or adults were not correlated with any of the environmental features such as sward height or % bare soil.

**Table 3.5**. Descriptive statistics for of adults, rolls and roll scars at site 1.

| | N | Range | | Mean | Std. Deviation |
|---|---|---|---|---|---|
| No. of adults | 88 | 0 | 3 | 0.13 | 0.48 |
| No. of rolls | 90 | 0 | 11 | 1.10 | 2.24 |
| No. of roll scars | 90 | 0 | 39 | 2.49 | 5.82 |
| No. of (rolls + scars) | 90 | 0 | 44 | 3.59 | 7.06 |

**Table 3.6.** Spearman rank correlation results for adult *B. populi* and leaf rolls vs. habitat and aspen features.* = significant at the 0.05 level. ** = significant at the 0.01 level.

| | **Adults** | | | **(Rolls + Scars)** | | |
|---|---|---|---|---|---|---|
| | **rs** | **P** | **N** | **rs** | **P** | **N** |
| Number of rolls | 0.305 | 0.004** | 88 | - | - | - |
| Height (m) | 0.273 | 0.010* | 88 | 0.251 | 0.017* | 90 |
| Nearest aspen distance (m) | 0.014 | 0.895 | 88 | -0.179 | 0.091 | 90 |
| Leaves | 0.095 | 0.380 | 88 | 0.279 | 0.008** | 90 |
| Circumference (cm) | 0.272 | 0.010* | 88 | 0.291 | 0.005** | 90 |
| % bare soil | 0.109 | 0.313 | 88 | 0.197 | 0.062 | 90 |
| Sward height (cm) | 0.167 | 0.120 | 88 | 0.038 | 0.725 | 90 |

**Multivariate analyses**

Using the enter method a significant multiple regression model emerged with adults as the dependent variable. ($F_{6,81}$ =1.973, P = 0.079, adjusted R square = 0.063). The percentage of bare soil was the only significant predictor (B =0.226, P = 0.041), despite this being a non-significant factor when analysed with a Spearman rank correlation.

Using the same method but with rolls + scars as the dependent variable, the model produced was non-significant ($F_{6,83}$ = 0.750, P = 0.611). For both models, all of the variables listed in table 3.1 were entered into the model as independent variables.

A binary logistic regression model (forward stepwise conditional method) was produced to test for differences between occupied (adults and rolls + scars) and unoccupied aspen trees. The model had an overall correct group membership of 57.83%, none of the variables of interest were significant and were excluded from the model.

**Site 2.**

**Tree and habitat characteristics**

At site 2 the average height of the 90 aspen trees selected at random across the 3 plots was 1.05 m, ranging from 0.26m to 4.20m (Table 3.7). This site was a fairly homogeneous clear-felled area, the 3 study plots were broadly similar in character.

There were no significant differences between the variables measured for occupied and unoccupied trees at site 2. (Table 3.9).

**Table 3.7.** Descriptive Statistics for tree and habitat characteristics at site 2

| | N | Range | | Mean | Std. deviation |
|---|---|---|---|---|---|
| | | Min | Max | | |
| Height (m) | 89 | 0.26 | 4.20 | 1.05 | 0.78 |
| No. of leaves | 89 | 11.00 | 1100.0 | 116.92 | 161.79 |
| Circumference of trunk | 89 | 0.80 | 12.00 | 3.96 | 2.16 |
| Radius/ length of longest branch (cm) | 89 | 4.00 | 84.00 | 26.92 | 21.93 |
| % bare soil | 89 | 0.00 | 100.00 | 66.22 | 36.56 |
| Sward height (cm) | 89 | 1.00 | 80.00 | 19.95 | 17.61 |
| Nearest aspen distance (m) | 89 | 0.01 | 1.74 | 0.32 | 0.29 |

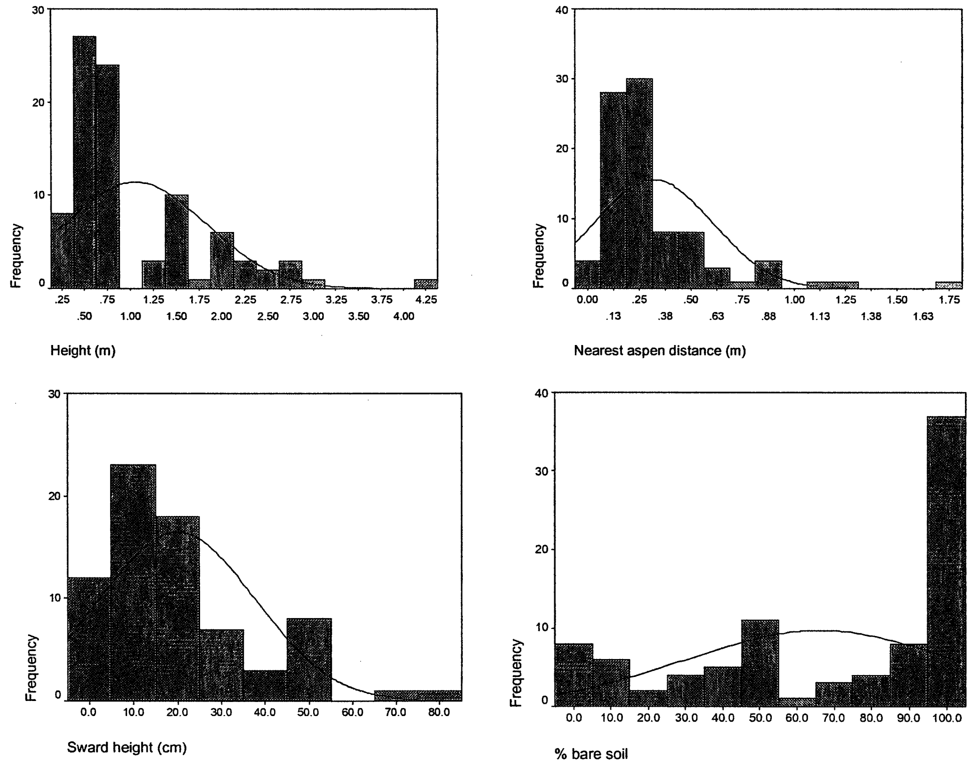


**Figure 3.3**. Frequency histograms of aspen height, nearest aspen distance, sward height and % bare soil.

**Table 3.8.** Descriptive statistics for occupied and unoccupied aspen trees in site 1.

| | Occupied trees | | | | | Unoccupied trees | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **Min** | **Max** | **Mean** | **Std. Dev.** | **N** | **Min** | **Max** | **Mean** | **St. dev.** |
| Height | 28 | 0.43 | 2.68 | 0.850 | 0.52 | 61 | 0.26 | 31.60 | 1.66 | 3.99 |
| Leaves | 28 | 16.0 | 1100.0 | 120.2 | 204.4 | 61 | 11.0 | 620.00 | 115.39 | 139.90 |
| Nearest aspen dist. | 28 | 0.04 | 1.23 | 0.31 | 0.26 | 61 | 0.01 | 1.74 | 0.32 | 0.29 |
| Circumference | 28 | 1.70 | 7.70 | 3.80 | 1.82 | 61 | 0.80 | 12.00 | 4.03 | 2.31 |
| Radius | 27 | 4.00 | 82.00 | 24.45 | 19.77 | 61 | 5.00 | 84.00 | 28.02 | 22.90 |
| % bare soil | 28 | 0.00 | 100.0 | 70.38 | 34.36 | 61 | 0.00 | 100.00 | 64.31 | 37.64 |
| Sward height | 22 | 3.00 | 53.00 | 18.77 | 13.78 | 50 | 1.00 | 80.00 | 20.76 | 19.20 |

**Table 3.9**. Mann Whitney U test results for site 2. Non of the measured variables differ significantly for occupied and unoccupied trees.

| | **Aspen height** | **Number of Leaves** | **Nearest aspen distance** | **Circ. of trunk** | **% bare soil** | **Sward height** | **Radius (longest branch)** |
|---|---|---|---|---|---|---|---|
| Mann-Whitney U | 784.000 | 817.500 | 795.500 | 795.500 | 539.000 | 798.000 | 771.500 |
| P | 0.536 | 0.747 | 0.605 | 0.605 | 0.892 | 0.617 | 0.638 |

As in site 1, sampled aspen height was again correlated with the number of leaves and the circumference of the trunk (Table 3.10). At this site the radius of the tree, as measured by the length of the longest branch was measured for all trees in addition to the other variables. This was positively correlated with tree height, the number of leaves on the tree and with the circumference of the trunk (Table 3.10). Circumference of the tree trunk is positively correlated with the number of leaves (Table 3.10). Taller trees therefore have more leaves, thicker trunks and longer branches than smaller trees.

As in site 1 there are no correlations between aspen height and the distance to the nearest aspen or the percentage of bare soil. Aspen height was correlated with sward height at site 1 but this was not the case at site 2. There are no correlations between distance to the nearest aspen and the percentage of bare soil or with the sward height. There is however a negative correlation between the percentage of bare soil and the maximum height of the sward; the more bare soil there is surrounding a tree, the taller the sward (Table 3.10).

**Table 3.10**. Spearman rank correlation results for site 2. ** = Significant at the 0.01 level.

| | rs | P | N |
|---|---|---|---|
| Height vs. number of leaves | 0.779 | 0.000** | 89 |
| Height vs. circumference of the trunk | 0.703 | 0.000** | 89 |
| Height vs. radius (longest branch) | 0.739 | 0.000** | 88 |
| Radius (longest branch) vs. number of leaves | 0.863 | 0.000** | 88 |
| Radius (longest branch) vs. circumference of trunk | 0.780 | 0.000** | 88 |
| Height vs. nearest aspen distance | 0.112 | 0.295 | 89 |
| Height vs. % Bare soil | -0.124 | 0.245 | 89 |
| Height vs. Sward height | -0.19 | 0.87 | 72 |
| Radius (longest branch) vs. nearest aspen distance | 0.197 | 0.066 | 88 |
| Radius vs. sward height | -0.221 | 0.064 | 71 |
| Radius vs. % bare soil | 0.031 | 0.777 | 88 |
| Nearest aspen distance vs. % bare soil | -0.046 | 0.671 | 89 |
| Nearest aspen distance vs. sward height | -0.002 | 0.988 | 72 |
| % bare soil vs. sward height | -0.563 | 0.000** | 72 |

### Adults and rolls

Spearman rank correlations were carried out on all aspen and environmental variables measured, with both adults and (rolls + scars) as dependent variables. As in site 1 the number of adults on a tree was positively correlated to the number of rolls and scars on the tree. The number of adults on a tree was negatively correlated with the nearest neighbouring aspen distance; the greater the distance to the nearest aspen the fewer adults there were. See table 3.12 for Spearman rank results.

**Table 3.11**. Descriptive statistics for adults and rolls + roll scars at site 2.

| | N | Range | | Mean | Std. deviation |
|---|---|---|---|---|---|
| | | Max | Min | | |
| No. of adults | 89 | 0 | 3 | 4.49E-02 | 0.33 |
| No. of (rolls + scars) | 89 | 0 | 14 | 0.96 | 2.43 |

**Table 3.12.** Spearman rank correlation results for site 2. *= Significant at the 0.05 level. ** = Significant at the 0.01 level.

| | Adults | | | (Rolls + Scars) | | |
|---|---|---|---|---|---|---|
| | rs | P | N | rs | P | N |
| Number of rolls | 0.269 | 0.01** | 89 | - | - | - |
| Height (m) | 0.011 | 0.92 | 89 | -0.025 | 0.81 | 89 |
| Nearest aspen distance (m) | -0.221 | 0.04* | 89 | 0.001 | 0.99 | 89 |
| Leaves | 0.020 | 0.85 | 89 | 0.115 | 0.28 | 89 |
| Circumference (cm) | -0.076 | 0.48 | 89 | -0.017 | 0.88 | 89 |
| % bare soil | 0.194 | 0.69 | 89 | 0.081 | 0.45 | 89 |
| R. longest branch length (cm) | -0.037 | 0.73 | 88 | 0.012 | 0.91 | 89 |
| Sward height (cm) | -0.046 | 0.70 | 77 | -0.030 | 0.80 | 72 |

### Multivariate analyses

Using the enter method, a non-significant multiple regression model emerged with adults as the dependent variable ($F_{6,82} = 0.381$, P = 0.910, adjusted R square = -0.066). Using the same method but with rolls and scars as the dependent variable, a significant model emerged ($F_{6,82} = 4.365$, P = 0.000, adjusted r square =0.278) height (B = -0.611, P = 0.002) and the number of leaves was a significant predictor (B = 0.796, P = 0.000). A binary logistic regression model (forward stepwise conditional method) was produced to test for differences between occupied (adults and rolls + scars) and unoccupied aspen trees. The model had an overall correct group membership of 70.42%, none of the variables of interest were significant and all were excluded from the model.

## Site 3

### Tree and habitat characteristics

The average height of the 90 aspen trees selected at random across the 3 plots was 1.04m at site 3, ranging from 0.20 to 2.01m tall (Table 3.13). This site, like site 2, was a fairly homogeneous clear-felled area was characterised by very dense, young aspen. The average nearest aspen distance at this site was 0.23m compared to 0.32 for site 2 and 0.41cm at site 1 (Table 3.13).

Mann Whitney U tests showed that at this site, occupied trees were significantly taller, had greater trunk circumferences and longer branches than unoccupied trees (Table 3.15, Figures 3.5, 3.6, 3.7)

**Table 3.13**. Descriptive Statistics for tree and habitat characteristics at site 3.

| | N | Range | | Mean | Std. Deviation |
|---|---|---|---|---|---|
| | | Min | Max | | |
| Height (m) | 90 | 0.20 | 2.01 | 1.04 | 0.44 |
| Number of leaves | 90 | 10.00 | 316.0 | 123.63 | 73.91 |
| Circumference of trunk (cm) | 90 | 0.80 | 6.20 | 3.15 | 1.24 |
| Nearest aspen distance (m) | 90 | 0.01 | 1.08 | 0.23 | 0.25 |
| % Bare soil | 90 | 0.00 | 100.0 | 57.97 | 37.45 |
| Sward height (cm) | 90 | 0.00 | 87.00 | 25.70 | 23.93 |
| Radius (longest branch) (cm) | 90 | 2.00 | 64.00 | 23.59 | 13.24 |

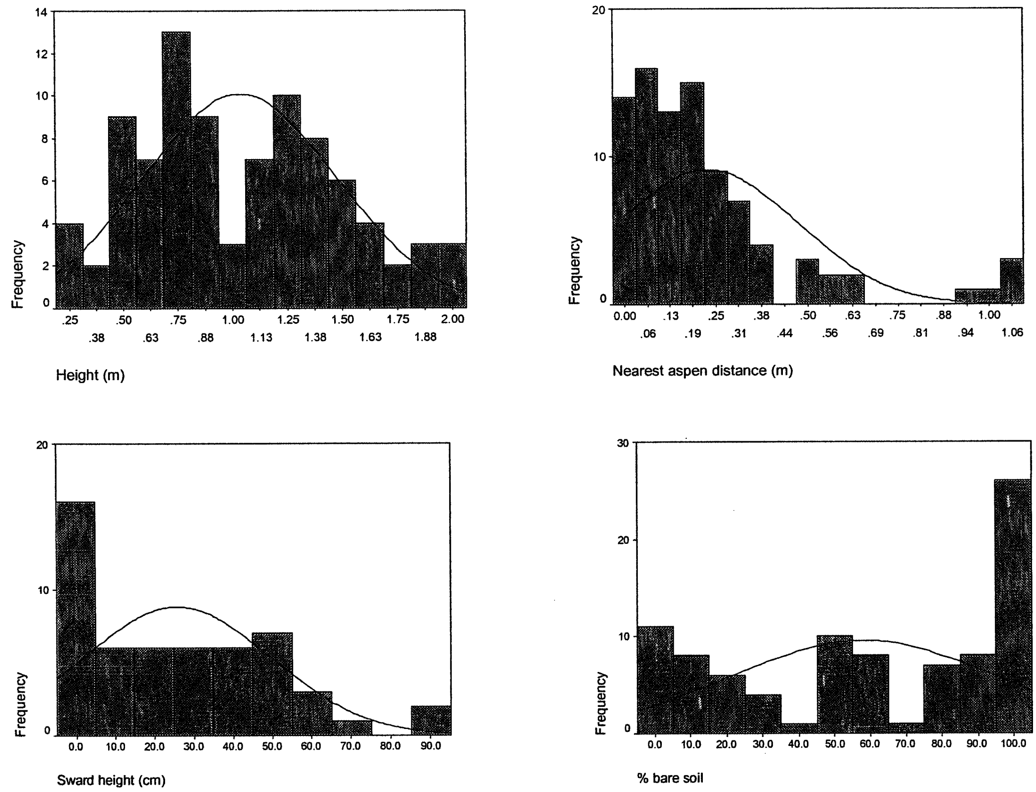


**Figure 3.4**. Frequency histograms for aspen height, nearest aspen distance, sward height and % bare soil.

**Table 3.14.** Descriptive statistics for occupied and unoccupied aspen trees in site 1.

| | Occupied trees | | | | | Unoccupied trees | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **Min** | **Max** | **Mean** | **Std. Dev.** | **N** | **Min** | **Max** | **Mean** | **St. dev.** |
| **Height** | 25 | 0.64 | 2.01 | 1.37 | 0.36 | 65 | 0.20 | 1.94 | 0.91 | 0.41 |
| **Leaves** | 25 | 27.0 | 316.0 | 168.72 | 72.45 | 65 | 10.0 | 292.00 | 106.29 | 67.31 |
| **Nearest aspen dist.** | 25 | 0.01 | 0.57 | 0.17 | 0.17 | 65 | 10.0 | 292.00 | 106.29 | 67.31 |
| **Circumference** | 25 | 1.10 | 5.30 | 3.82 | 0.99 | 65 | 0.80 | 6.20 | 2.90 | 1.24 |
| **Radius** | 25 | 6.00 | 64.00 | 30.70 | 14.01 | 60 | 2.00 | 51.00 | 20.62 | 11.80 |
| **% bare soil** | 25 | 0.00 | 100.0 | 56.32 | 38.60 | 65 | 0.00 | 100.00 | 58.60 | 37.28 |
| **Sward height** | 23 | 0.00 | 86.00 | 29.22 | 22.67 | 61 | 0.00 | 87.00 | 28.05 | 23.05 |

**Table 3.15.** Mann Whitney U test results for site 3.

| | **Aspen height** | **Number of Leaves** | **Nearest aspen distance** | **Circ. of trunk** | **Radius (longest branch)** | **Sward height** | **% bare soil** |
|---|---|---|---|---|---|---|---|
| Mann-Whitney U | 316.0 | 408.0 | 620 | 437.5 | 421.5 | 676.0 | 794.5 |
| P | <0.001*** | <0.001*** | 0.084 | 0.001** | 0.002** | 0.798 | 0.870 |

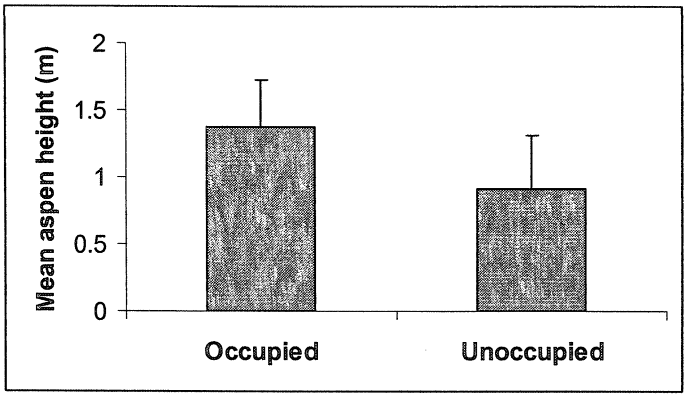


**Figure 3.5**. Occupied aspen trees are taller than unoccupied trees at site 3. Error bars show standard error.

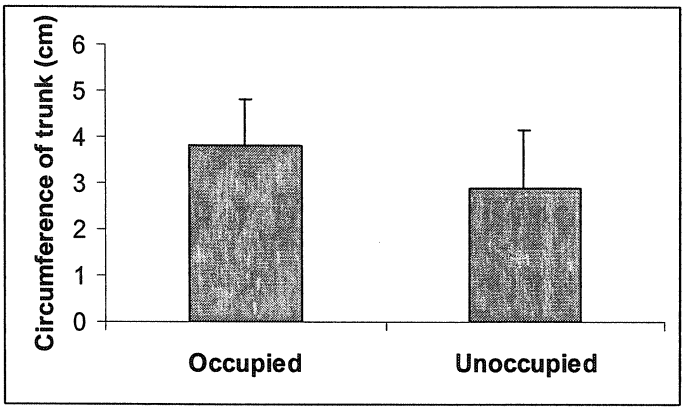


**Figure 3.6** Occupied trees have greater circumferences than unoccupied trees at site 3. Error bars show standard error.

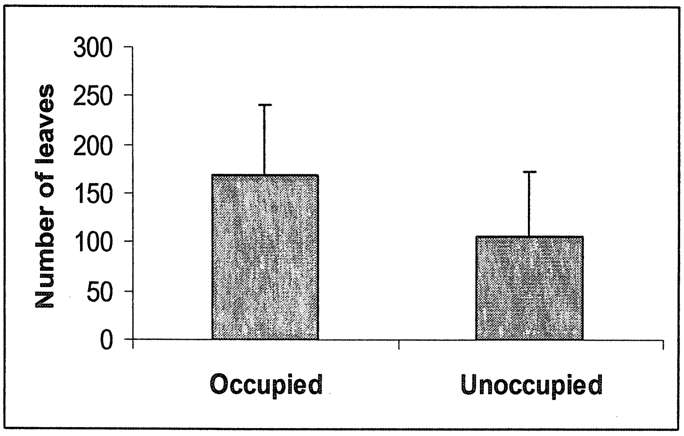


**Figure 3.7**. Occupied trees have more leaves than unoccupied trees at site 3. Error bars show standard error.

Height was again correlated with the number of leaves, the trunk circumference, and radius (longest branch length) (Table 3.16). The tree radius was also correlated with the circumference of the tree, and with the number of leaves. Circumference of the tree trunk is again correlated with the number of leaves. There was a negative correlation between height and distance to the nearest aspen at this site; taller trees tended to have shorter distances to the nearest aspen tree than shorter trees (Table 3.16). Again there was no correlation between aspen height and the percentage of bare soil, or with sward height. There are no correlations between distance to the nearest aspen and the percentage of bare soil or with the sward height. There is a negative correlation between the percentage of bare soil and the height of the sward; the more bare soil there is surrounding a tree, the taller the sward, this was also the case at site 2 (Table 3.16).

**Table 3.16**. Spearman rank correlations between tree and habitat features at site 3. * = Significant at the 0.05 level. ** = Significant at the 0.01 level. ***= Significant at 0.001 level

| | rs | P | N |
|---|---|---|---|
| Height vs. number of leaves | 0.862 | P<0.000*** | 90 |
| Height vs. circumference of the trunk | 0.760 | P<0.000*** | 90 |
| Height vs. radius (longest branch) | 0.663 | P<0.000*** | 85 |
| Radius (longest branch) vs. number of leaves | 0.651 | P<0.000*** | 85 |
| Radius (longest branch) vs. circumference of trunk | 0.742 | P<0.000*** | 90 |
| Height vs. nearest aspen distance | -0.269 | 0.010* | 90 |
| Height vs. % Bare soil | -0.136 | 0.202 | 90 |
| Height vs. Sward height | 0.090 | 0.524 | 53 |
| Radius (longest branch) vs. nearest aspen distance | 0.123 | 0.263 | 85 |
| Radius vs. sward height | -0.017 | 0.905 | 50 |
| Radius vs. % bare soil | -0.176 | 0.106 | 85 |
| Nearest aspen distance vs. % bare soil | -0.070 | 0.515 | 90 |
| Nearest aspen distance vs. sward height | -0.036 | 0.799 | 53 |
| % bare soil vs. sward height | -0.713 | P<0.000*** | 53 |

## Rolls

Only one adult was recorded in the sample in site 3. Due to this small sample size no further analysis was carried out on adults for site 3.

The number of rolls was positively correlated with height, the number of leaves, the circumference and the radius of the tree (Table 3.18).

**Table 3.17**. Descriptive statistics for adults and rolls at site 3.

| | N | Range | | Mean | Std. Deviation |
|---|---|---|---|---|---|
| | | Min | Max | | |
| No. of adults | 90 | 0 | 1 | 1.111E-02 | 0.1054 |
| No. of (rolls + scars) | 90 | 0 | 5 | 0.5444 | 1.0930 |

**Table 3.18**. Spearman rank correlations for rolls vs. tree and habitat features at site 3. ** = Significant at the 0.01 level. *** Significant at 0.001 level. Adults were not analysed due to small sample size.

| | rs | P | N |
|---|---|---|---|
| Rolls vs. height | 0.471 | P<0.000*** | 90 |
| Rolls vs. NND | -0.107 | 0.315 | 90 |
| Rolls vs. leaves | 0.380 | P<0.000*** | 90 |
| Rolls vs, circumference | 0.376 | P<0.000*** | 90 |
| Rolls vs. % bare soil | -0.053 | 0.623 | 90 |
| Rolls vs. Radius | 0.369 | 0.001** | 90 |
| Rolls vs. sward height | -0.009 | 0.947 | 53 |

**Multivariate analyses**

Using the enter method a significant multiple regression model emerged with rolls as the dependent variable ($F_{6,78}$ = 12.339, P = 0.040, adjusted R square = 0.087). None of the variables entered into the model were significant, however.

A binary logistic regression model (forward stepwise conditional method) was produced to test for differences between occupied (adults and rolls + scars) and unoccupied aspen trees. The model had an overall correct group membership of 71.25%, none of the variables of interest were significant and all were excluded from the model.

**Anecdotal notes**

Four very large aspens fell in a storm at site three between the $9^{th}$ and $19^{th}$ of July. All were around 30 m tall. Only one of these trees had new growth at the tips of branches (29.2m tall). There was a dried *B. populi* roll at 21.5m up the tree at the tip of a branch on new growth. The contents were examined in order to determine the age of the roll. It contained 2 large 5mm larvae, which indicated (from comparing to the life history data) that the roll had been on the tree for at least 2 weeks, it was therefore rolled at a height of 21.5m, and not after the tree had fallen. Another roll at 25.2m contained 2 eggs and the roll was still green, this roll was probably rolled after the tree fell down and was at a height from the ground of 97cm. Another *B. populi* roll was at 20.7m. The roll was old and dry and contained a live 6mm long larvae and two orange nematodes. This roll was also therefore rolled when the tree was standing. An old *B. betulae* roll was also present on this tree at 21m up. *B. populi* feeding damage was visible in the vicinity of the rolls also on the younger leaves at branch tips.

**Consistency between sites**

At all three sites, aspen height was positively correlated with the number of leaves and the trunk circumference (see table 3.19 for comparisons across sites). At sites 2 and 3 height was correlated with the radius of the tree as measured by the longest horizontal branch. This was not measured at site 1. Radius was correlated with the number of leaves and with trunk circumference. As one would expect, taller trees therefore have thicker trunks, more leaves and longer branches than shorter trees.

Sward height was positively correlated with aspen height and the nearest aspen distance at site1 only. Aspen height was correlated with nearest aspen distance at site 3 but not at the other sites. The percentage of bare ground was positively correlated with sward height at sites 1 and 2 (Table 3.19).

At sites one and three there were more rolls on taller trees. There were positive correlations between number of rolls and tree height, and between the number of leaves and the circumference of the trunk, factors which, as we have seen are associated. Roll number was also correlated with the tree radius at site 3 (Table 3.20). However it must be borne in mind that at site 3 the maximum tree height was just 2.01 m.

There was no consistency between sites in the results of the multiple regression analysis. With adults as the dependent variable, a significant model was produced only for site 1. The percentage of bare soil was a significant predictor, despite not being a significant factor when analysed using a Spearman rank correlation.

Significant models emerged with rolls as the dependent variable for sites 2 and 3, however non of the predictor variables were significant at site 3. At site 2 height and the number of leaves were significant predictor variables, however these were not significant when analysed with the Spearman correlation. It is interesting to note, however that these variables were significantly positively correlated with the number of rolls at sites 1 and 3.

**Table 3.19**. Spearman correlations for tree and environmental characteristics across sites.

| | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| Height vs. number of leaves | P<0.001 | P<0.001 | P<0.001 |
| Height vs. circumference of the trunk | P<0.001 | P<0.001 | P<0.001 |
| Height vs. radius (longest branch) | N/A | P<0.001 | P<0.001 |
| Radius (longest branch) vs. number of leaves | N/A | P<0.001 | P<0.001 |
| Radius (longest branch) vs. circumference of trunk | N/A | P<0.001 | P<0.001 |
| Height vs. nearest aspen distance | NS | NS | P=0.010 |
| Height vs. % Bare soil | NS | NS | NS |
| Height vs. Sward height | P<0.001 | NS | NS |
| Radius (longest branch) vs. nearest aspen distance | N/A | NS | NS |
| Radius vs. sward height | N/A | NS | NS |
| Radius vs. % bare soil | N/A | NS | NS |
| Nearest aspen distance vs. % bare soil | NS | NS | NS |
| Nearest aspen distance vs. sward height | P=0.002 | NS | NS |
| % bare soil vs. sward height | NS | P<0.001 | P<0.001 |

**Table 3.20**. Spearman rank correlation significance for adults and (rolls + scars) across sites.

| | Site 1 | | Site 2 | | Site 3 |
|---|---|---|---|---|---|
| | **Adults** | **Rolls** | **Adults** | **Rolls** | **Rolls** |
| number of rolls | P=0.004** | - | P=0.01** | - | - |
| height | P=0.010* | P=0.017* | NS | NS | P<0.001 |
| nearest aspen distance | NS | NS | P=0.04* | NS | NS |
| leaves | NS | P=0.008** | NS | NS | P<0.001 |
| circumference | 0.010* | P=0.005** | NS | NS | P<0.001 |
| % bare soil | NS | NS | NS | NS | NS |
| radius (longest branch length) | - | - | NS | NS | P<0.001 |
| Sward height | NS | NS | NS | NS | NS |

## Combined data for sites 1, 2 and 3

Data on tree characteristics from sites 1, 2 and 3 were combined. Mann-Whitney U tests showed that non of the tree or environmental variables measured were significantly different for occupied and unoccupied trees (Tables 3.22 & 3.23).

**Table 3.21**. Descriptive statistics for tree and environmental features for sites 1, 2 and 3 combined.

| | N | Range | | Mean | Std. Deviation |
|---|---|---|---|---|---|
| | | Min | Max | | |
| Aspen height (m) | 269 | 0.20 | 14.50 | 1.07 | 1.31 |
| No. of leaves | 269 | 8.00 | 3300.0 | 123.29 | 250.20 |
| Circumference | 269 | 0.80 | 59.00 | 3.53 | 4.49 |
| Nearest aspen dist. (m) | 269 | 0.00 | 2.26 | 0.32 | 0.33 |
| % bare soil | 269 | 0.00 | 100.00 | 45.87 | 40.17 |
| Radius (longest branch) | 173 | 2.00 | 84.00 | 25.28 | 18.22 |

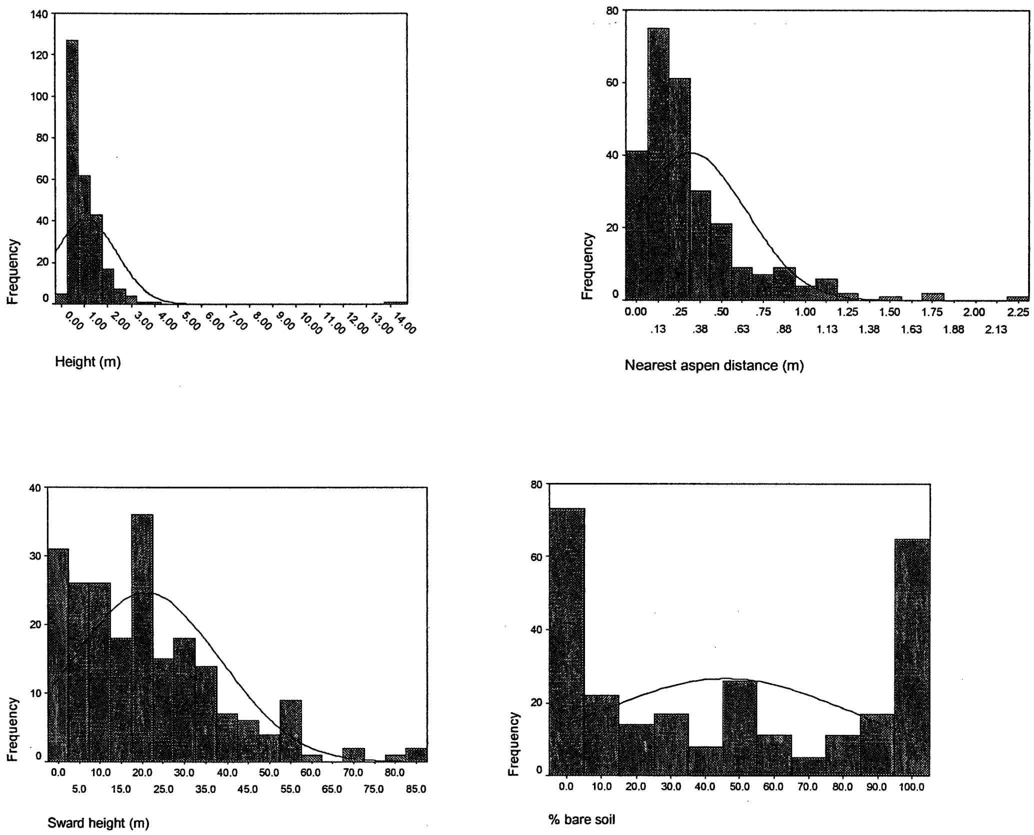


**Figure 3.8** . Frequency histograms of height, nearest aspen distance, sward height and % bare soil.

**Table 3.22.** Descriptive statistics for occupied and unoccupied trees. Sites 1,2 and 3 combined.

| | Occupied trees | | | | | Unoccupied trees | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min | Max | Mean | Std. Dev. | N | Min | Max | Mean | St. dev. |
| Height | 106 | 0.30 | 3.00 | 0.93 | 0.53 | 163 | 0.20 | 31.60 | 1.34 | 2.89 |
| Leaves | 106 | 11.0 | 1100 | 108.53 | 126.1 | 163 | 8.00 | 3300 | 132.88 | 304.9 |
| Nearest aspen dist. | 106 | 0.00 | 2.26 | 0.29 | 0.32 | 163 | 0.00 | 1.74 | 0.33 | 0.34 |
| Circumference | 106 | 1.00 | 10.00 | 3.17 | 1.66 | 163 | 0.80 | 59.0 | 3.76 | 5.61 |
| Radius | 88 | 0.03 | 82.00 | 19.27 | 17.37 | 121 | 2.00 | 84.0 | 24.35 | 18.56 |
| % bare soil | 106 | 0.00 | 100.0 | 40.17 | 38.67 | 163 | 0.00 | 100.0 | 49.58 | 40.80 |
| Sward height | 93 | 0.00 | 86.00 | 20.41 | 15.77 | 146 | 0.00 | 87.0 | 23.72 | 19.81 |

**Table 3.23.** Mann Whitney U test results comparing tree and environmental features for occupied and unoccupied trees.

| | Aspen height | Number of Leaves | Nearest aspen distance | Circ. of trunk | % bare soil | Sward height | Radius (longest branch) |
|---|---|---|---|---|---|---|---|
| Mann-Whitney U | 8587.00 | 7955.00 | 8240.50 | 8508.50 | 6275.00 | 7595.50 | 5906.50 |
| P | 0.934 | 0.273 | 0.523 | 0.834 | 0.519 | 0.090 | 0.486 |

As one would expect, there were highly significant positive correlations between tree height and the circumference of the trunk, between tree height and number of leaves, and between tree height and tree radius as measured by length of longest horizontal branch. See table 3.24 below and Figure 3.9.

**Table 3.24.** Spearman rank correlations for tree and environmental features. Sites 1, 2 and 3 combined. * = significant at the 0.05 level,**= significant at the 0.01 level, *** = significant at the 0.001 level.

| | rs | P | N |
|---|---|---|---|
| Height vs. number of leaves | 0.829 | < 0.001*** | 269 |
| Height vs. circumference of the trunk | 0.770 | < 0.001*** | 269 |
| Height vs. radius (longest branch) | 0.735 | < 0.001*** | 173 |
| Radius (longest branch) vs. number of leaves | 0.769 | < 0.001*** | 173 |
| Radius (longest branch) vs. circumference of trunk | 0.751 | < 0.001*** | 173 |
| Height vs. nearest aspen distance | -0.010 | 0.876 | 269 |
| Height vs. % Bare soil | -0.010 | 0.876 | 269 |
| Height vs. Sward height | 0.228 | 0.001** | 216 |
| Radius (longest branch) vs. nearest aspen distance | 0.135 | 0.077 | 173 |
| Radius vs. sward height | -0.145 | 0.113 | 121 |
| Radius vs. % bare soil | -0.054 | 0.479 | 173 |
| Nearest aspen distance vs. % bare soil | -0.100 | 0.103 | 269 |
| Nearest aspen distance vs. sward height | -0.006 | 0.932 | 216 |
| % bare soil vs. sward height | 0.135 | 0.048* | 216 |

**Table 3.25.** Spearman correlation results. ***All results are significant at the 0.001 level.

| | rs | P | N |
|---|---|---|---|
| Height vs. circumference of the trunk at base | 0.770 | P= <0.001*** | 269 |
| Height vs. number of leaves | 0.829 | P= <0.001*** | 269 |
| Height vs. tree radius (length of longest branch) | 0.735 | P= <0.001*** | 173 |

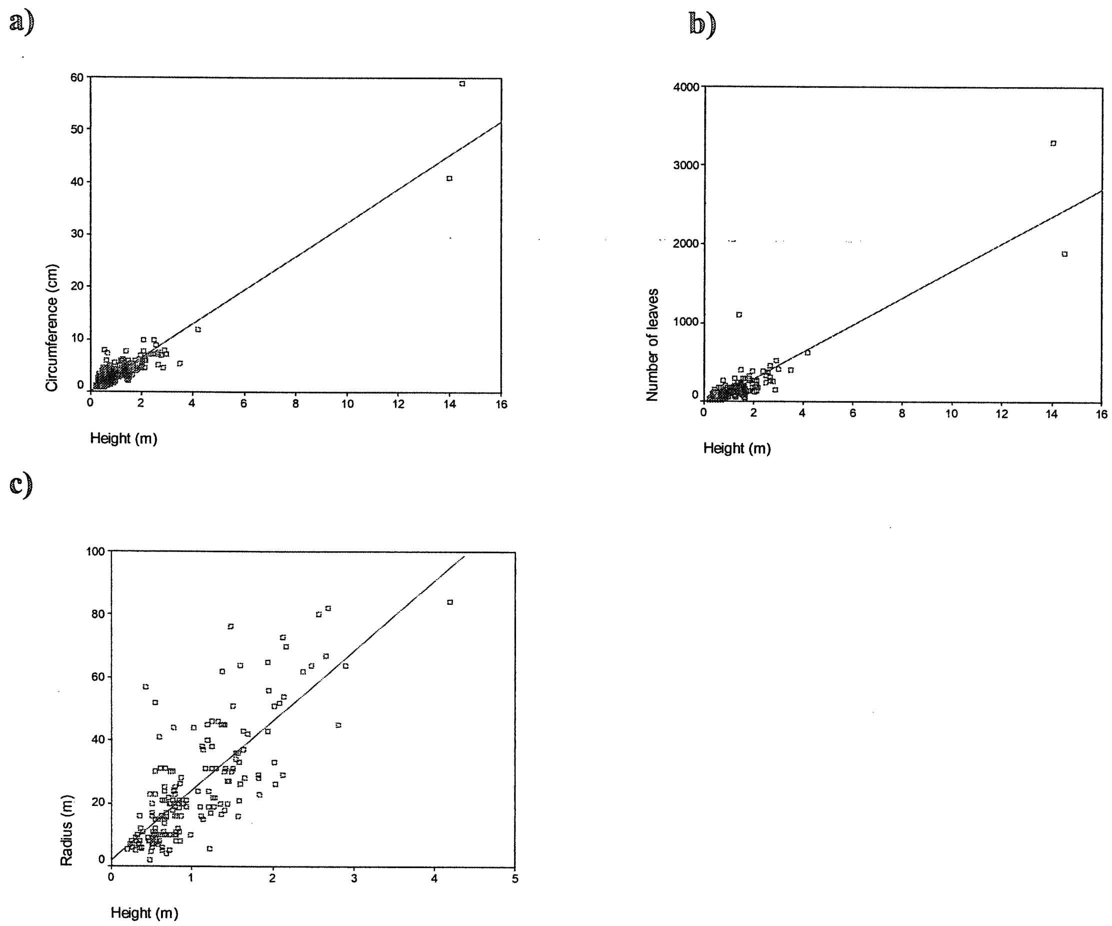


**Figure 3.9.** Tree height is positively correlated with a) the circumference of the trunk (rs = 0.770, P = 0.000, N = 269) b) the number of leaves on the tree leaves (rs = 0.829, P = 0.000, N = 269) and c) the radius of the tree.

There is a highly significant positive correlation between the number of rolls on a tree and the number of adults present (rs = 0.281, P = 0.000, N = 267, Figure 3.10).

The number of rolls and scars on a tree is weakly negatively correlated with the % bare soil cover, (there are significantly more rolls on trees which have 0% bare soil) table 3.26and 3.27, Figures 3.11 and 3.12. The number of rolls is positively correlated with the radius of the tree (Table 3.27, Figure 3.13, and Figure 3.14). height is not significantly correlated with the number of (rolls + scars) but a ‘humped’ relationship exists (Figure 3.15).

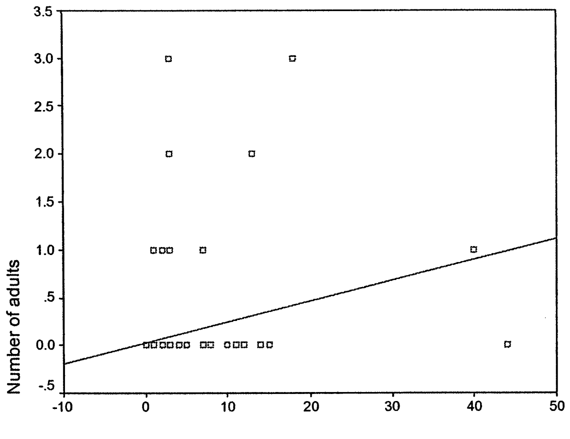


**Figure 3.10.** There is a significant positive correlation between the number of rolls on a tree and the number of adults present (rs = 0.281, P = 0.000, N = 267)

### Adults and rolls

**Table 3.26.** Descriptive statistics for adults and (rolls + scars) for sites 1, 2 and 3.

| | N | Range | | Mean | Std. deviation |
|---|---|---|---|---|---|
| | | Min | Max | | |
| Adults | 269 | 0 | 3 | 5.99 E-02 | 0.34 |
| (Rolls + scars) | 269 | 0 | 44 | 1.70 | 4.55 |

**Table 3.27.** Spearman rank correlation results, sites 1, 2 and 3. Site 3 was excluded from the analysis for 'adults' due to small sample size. * = significant at the 0.05 level, *** = significant at the 0.001 level.

| | Adults | | | (Rolls + Scars) | | |
|---|---|---|---|---|---|---|
| | rs | P | N | rs | P | N |
| Number of rolls | 0.31 | P <0.001*** | 177 | - | - | - |
| Height (m) | 0.15 | 0.05 | 177 | 0.06 | 0.36 | 270 |
| Nearest aspen distance (m) | -0.05 | 0.48 | 177 | -0.02 | 0.69 | 269 |
| Leaves | 0.04 | 0.57 | 177 | 0.09 | 0.14 | 269 |
| Circumference (cm) | 0.08 | 0.29 | 177 | 0.46 | 0.45 | 269 |
| % bare soil | 0.01 | 0.86 | 177 | -0.20 | P<0.001*** | 269 |
| R. longest branch length (cm) | 0.03 | 0.74 | 177 | 0.16 | 0.037* | 173 |
| Sward height (cm) | -0.03 | 0.72 | 126 | 0.047 | 0.490 | 216 |

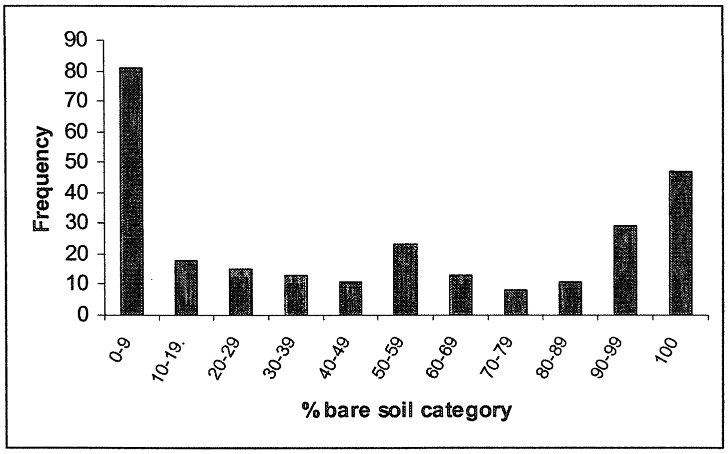


**Figure 3.11.** Frequency histogram for % bare soil.

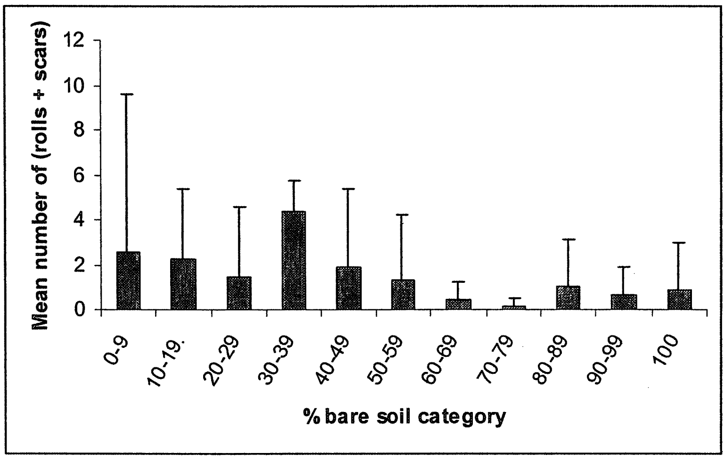


**Figure 3.12.** There are significantly more (rolls + scars) at lower values of % bare soil than higher values. Error bars show + standard error.

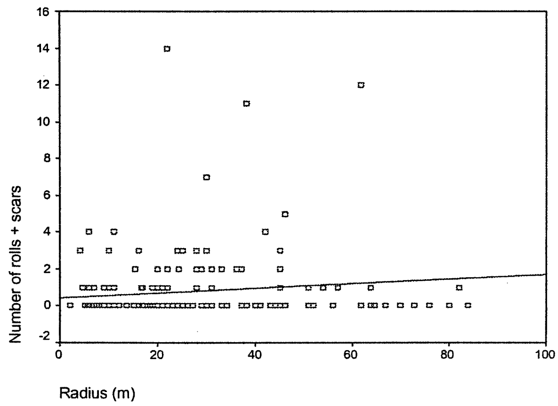


**Figure 3.13.** Significant positive correlation between radius of the tree and the number of rolls and roll scars on the tree. Rs = 0.159 P = 0.037 N =173.

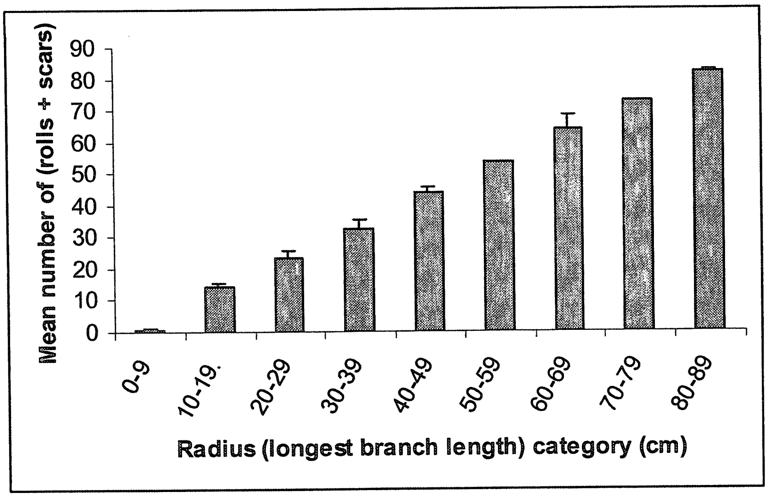


**Figure 3.14.** There are more (rolls + scars) on trees with longer branches. Error bare show + standard error.

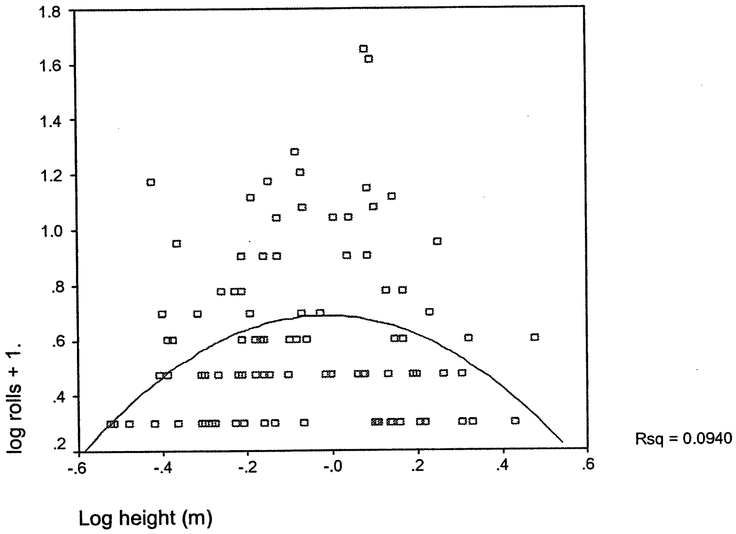


**Figure 3.15.** Relationship between height and number of rolls, with zero values removed. Line = quadratic regression.

## Multivariate analyses

Using the enter method a significant multiple regression model emerged with rolls as the dependent variable ($F_{5,263} = 2.407$, $P = 0.037$, adjusted R square = 0.026) The percentage of bare soil was a significant predictor ($B = -0.179$, $P = 0.004$). Using the same method but with adults as the dependent variable, a non- significant model emerged ($F_{6,170} = 0.439$, $P = 0.820$, adjusted r square = -0.016). This analysis only used data from site 1 and 2 as there were so few adults at site 3.

A binary logistic regression model (forward stepwise conditional method) was produced to test for differences between occupied (adults and rolls + scars) and unoccupied aspen trees. The model had an overall correct group membership of 62.50%, none of the variables of interest were significant and all were excluded from the model.

**Transect data**

Data from the 4 transects were combined for analysis. 55 aspen trees were analysed in total. Tree height ranged from 0.62 m to a maximum of 30 m tall. The maximum number of (rolls + scars) per tree was 9 (Table 3.28). As distance along the transect line increased, there was a trend towards increased aspen height (Figure 3.17). However it is apparent that there are short trees amongst the taller trees which had no signs of *B. populi* activity despite being of an appropriate height. It is apparent from Figure 3.18 that there is a cut-off point (6m) along the transect gradient, above which rolls or scars are not seen. Below 6m along the transect, 21 trees were analysed. 17 of these were below 2.80 m tall, just 4 were taller than this. At distances over 6m along the transect, of the 18 trees analysed, 14 were taller than 2.80 m, but 4 were shorter. Despite these 4 trees being the same height as those in the open that supported rolls, no rolls were present. These trees were shaded by the taller trees, and had very little or no new growth compared to trees of a similar height that were in the open.

**Table 3.28**. Descriptive statistics for transect data.

| | N | Range | | Mean | Std. deviation |
|---|---|---|---|---|---|
| | | Min | Max | | |
| Aspen height | 55 | 0.62 | 30 | 4.05 | 4.70 |
| (Rolls + Scars) per tree | 55 | 0.00 | 9.00 | 0.78 | 1.64 |

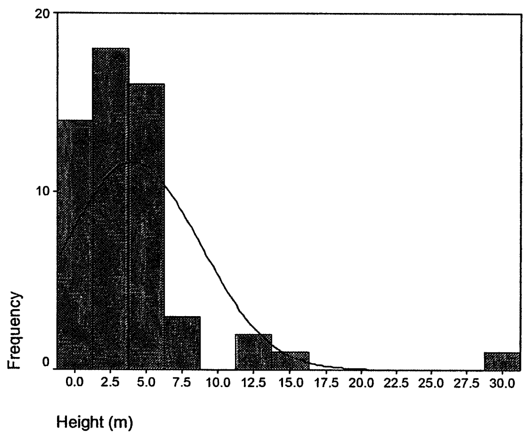


**Figure 3.16.** Frequency histogram of height for transect data.

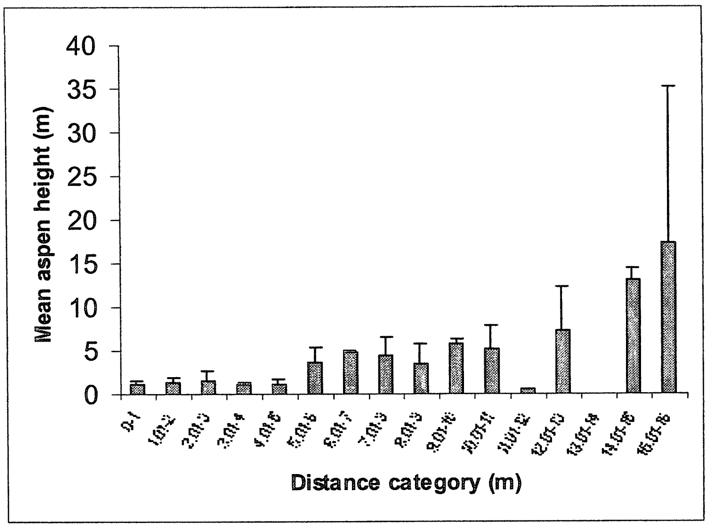


**Figure 3.17.** As distance along the transect increases, the mean height of the trees increases. Error bars show + standard error.

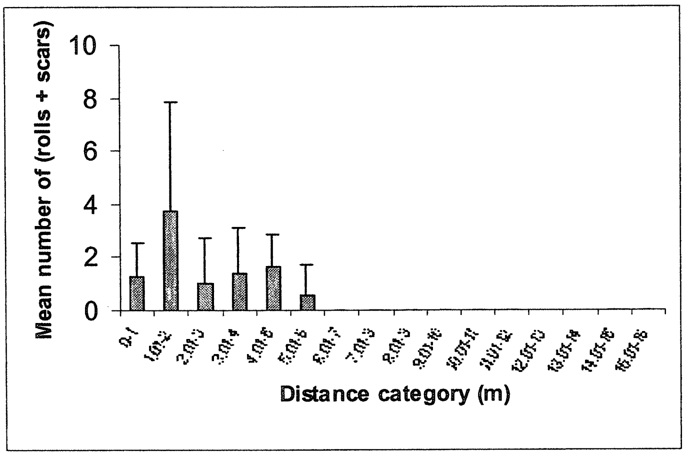


**Figure 3.18.** As distance along the transect increases (and aspen height increases) the number of rolls drops off. Error bars represent + standard error.

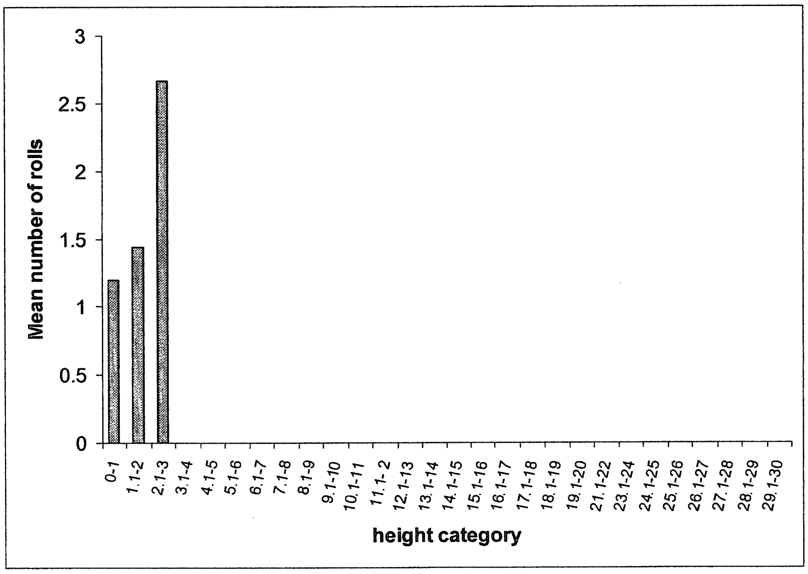


**Figure 3.19.** Rolls are only found on short aspens below 3 m tall that are in the open.

A Spearman rank correlation showed that there was a significant negative correlation between height and the number of rolls and roll scars on trees ($r_S$= -0.435, P = 0.001, N = 55, Figure 3.20); there were more rolls and scars on shorter trees. The tallest tree to have rolls or scars present was 2.80m tall.

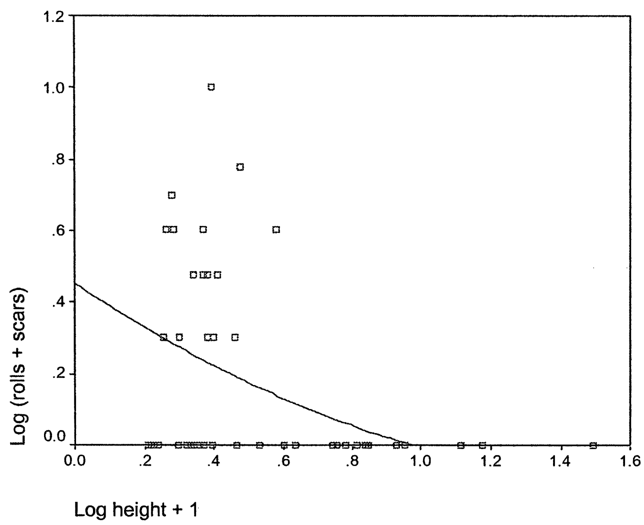


**Figure 3.20.** There are more rolls on shorter trees that are in the open.

In a number of cases, areas of apparently suitable *B. populi* habitat were found with no signs of adults or rolls. All such sites were isolated in large expanses of pine forest. It seems that the isolation of these habitat patches in an unfavourable matrix (pine forest) was the main factor preventing colonisation by *B. populi.*

## 3.2 Roll position

At sites 2 and 3 measurements concerning all rolls and roll scars on trees included in the analysis showed that the height of rolls and scars is positively correlated with the height of the tree on which they are located ($R_S$ = 0.880, P = 0.000, N = 96. Table 3.29. Figure 3.21). A chi squared test showed that the distribution of rolls along branches differs significantly from random ($\chi^2_{22}$ =272.27, P <0.01), with more rolls being located at the very tips of shoots than expected (Table 3.36, Figure 3.22).

A paired sample t-test was used to compare temperature and humidity at different points on the trees. This test showed that there were significant differences in temperature between the trunk and branch tip (the trunk was warmer), the trunk at mid point and the top of the tree (the trunk was warmer), and between the branch tip at mid point and the top of the tree (The tip of the branch was warmer). There were no significant differences in humidity. See table 3.31. When the data were analysed using a one-way ANOVA, there were no significant differences in temperature or humidity between trunk and branch tip at the mid-point, or between the very top of the tree and the trunk or branch tip at mid-point (1 way ANOVA. $F_2$ = 0.660, P = 0.518, NS.). Although there are within tree differences in temperature at different points of the tree, when different trees are analysed no differences were apparent.

**Table 3.29.** Descriptive statistics for aspen height and roll and scar height

| | N | Min | Max | Mean | Std. Dev. |
|---|---|---|---|---|---|
| Aspen height | 270 | 0.20 | 14.50 | 1.07 | 1.31 |
| Height of rolls and scars | 270 | 0.00 | 44.00 | 1.70 | 4.55 |

**Table 3.30** Descriptive statistics for distance from branch tip.

| | N | Min | Max | Mean | Std. Dev. |
|---|---|---|---|---|---|
| Distance to branch tip (cm) | 31 | 0.00 | 0.23 | 0.11 | 5.86E-02 |

**Table 3.31**. Descriptive statistics. Temperature and humidity at different points on the sampled aspens.

| | Temperature | | | Humidity | | |
|---|---|---|---|---|---|---|
| | Trunk | Branch tip | Tree top | Trunk | Branch tip | Tree top |
| N | 74 | 74 | 74 | 74 | 74 | 74 |
| Min | 22.70 | 22.60 | 22.60 | 29.90 | 32.00 | 32.30 |
| Max | 35.50 | 35.20 | 34.00 | 76.70 | 76.70 | 80.90 |
| Mean | 27.99 | 27.67 | 27.42 | 51.62 | 52.48 | 51.83 |
| Std. Dev. | 3.18 | 3.01 | 2.88 | 11.73 | 11.81 | 11.39 |

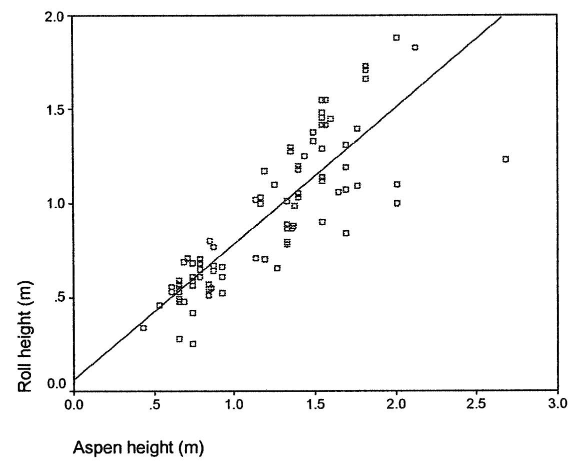


**Figure 3.21** Height of rolls is positively correlated with the height of the tree on which they are located ($R_S$ = 0.880, p = 0.000, N = 96)

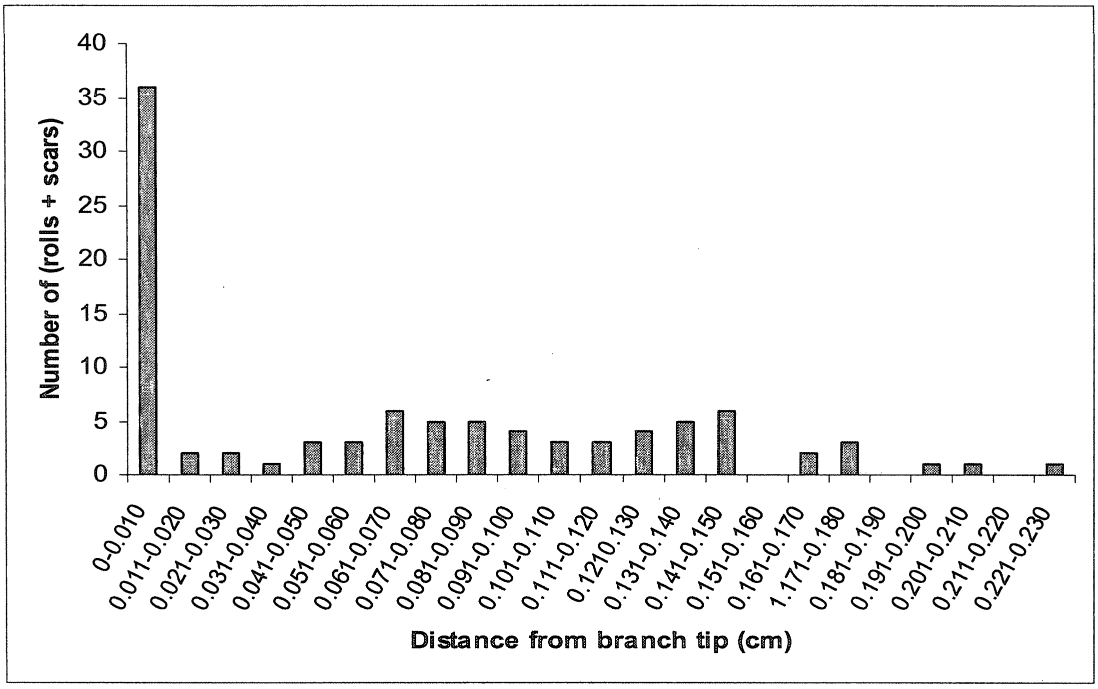


**Figure 3.22.** More rolls and scars are found at or very near to the tip of the branch or the top of the trunk than expected by random.

**Table 3.32**. Results of paired sample t test for temperature and humidity at different points on sampled aspens. ** = significant at the 0.01 level. ***= significant at the 0.001 level.

| Pair | T | df | P | Greatest value |
|---|---|---|---|---|
| Temp. Trunk: branch tip | 3.23 | 73 | 0.002** | Trunk |
| Temp. Trunk: tree top | 4.51 | 73 | P<0.001*** | Trunk |
| Temp. Branch tip: tree top | 4.10 | 73 | P<0.001*** | Branch tip |
| Humidity. Trunk: branch tip | -1.91 | 73 | 0.060 | Branch tip |
| Humidity. Trunk: tree top | -0.52 | 73 | 0.606 | Tree top |
| Humidity. Branch tip: tree top | 1.55 | 73 | 0.125 | Branch tip |

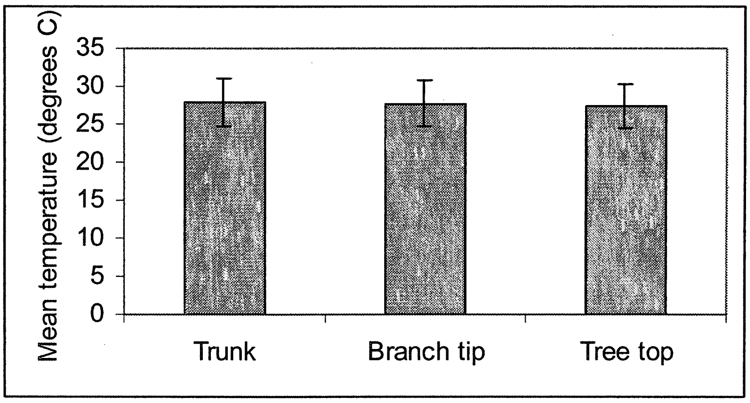


**Figure 3.23**. Mean temperature at the trunk of the tree (mid point), the tip of the longest branch (mid point) and the very top of the tree. Error bars show ± standard error.

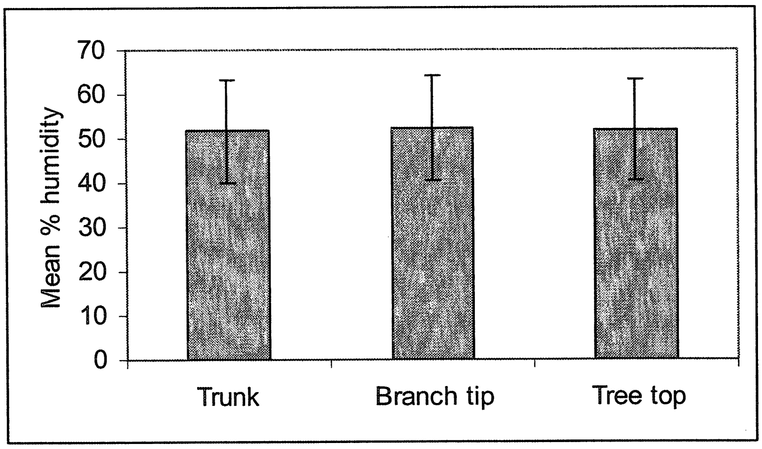


**Figure 3.24.** Mean humidity at the trunk of the tree (mid point), the tip of the longest branch (mid point) and the very top of the tree. Error bars show ± standard error. There is no significant difference.

## 3.3 Life history

The until a leaf roll falls from the tree varies from immediately following roll creation to after the larvae have vacated. In 6 cases, immediately after a roll was completed, female *B. populi* were observed incising the leaf stalk until the roll fell to the ground. In all cases, upon inspection the rolls were found to contain eggs. About 10 very dry, brown rolls were found still attached to the tree. These rolls contained large larvae, up to 6mm long. This indicated that these rolls had been attached for at least 2 weeks. The average time from laying to hatching was 3.7 days (N = 30). Larvae are white-cream in colour with no legs. The average time that larvae spent inside the was 15.57 days (N = 21). However, it seems that if conditions are unsuitable where the roll falls larvae are able to exit the roll earlier. In 3 cases when rolls were kept in sealed glass tubes, rolls became quite dry and larvae exited when only 2mm long, about 3-4 days after hatching, and were found at the bottom of the tube. Six broad size classes of larvae were observed (1mm long, 2mm, 3mm, 4mm, 5 mm and 6mm). Upon exiting the roll, usually when 5 or 6mm long, larvae burrow into the substrate and from 'pupal chambers' as do *B. betulae* (Bily, 1990). They seem to favour clumps of soil in which to form these chambers. They remain inside these chambers for up to 3 or 4 weeks and do not grow any larger. Pupation occurs within the chamber. Pupae are white in colour and slightly smaller than the last larval instar, measuring between 4 and 5 mm in length. Pupae are active and can move their posterior section . The pupal stage is relatively short compared to the larval stage, adults typically emerged above ground less than 1 week after pupation. Upon emerging from the substrate, adults feed but do not mate or roll leaves (Mellings, J. *Pers com.*). They over-winter as adults, possibly under bark (Telnov, D. *pers. com.*). There are at least 2 generations in Latvia, possibly 3 as adults are seen between May and September (Telnov, D. *Pers. com*). One generation reached an end near the beginning of June, numbers dropped off and adults were very difficult to find. About a week later numbers began to increase as the next generation emerged. Numbers of adults fell again towards the end of July. It seems that this generation lasted for roughly 6-7 weeks.

## 3.4 Mortality and predation

### Mortality

A Kruskall Wallis test showed that there was a significant difference in mortality between the 3 different treatments ($\chi^2_2 = 12.08$, P = 0.002). Tamhane's T2 post-hoc test (equal variances not assumed) showed that this difference arose between the dry and control treatment (Table 3.34.). The control treatment had lower mortality than both dry and humid treatments (Figure 3.33.)

**Table 3.33.** Descriptive statistics for mortality study

| No. larvae or eggs dead per roll. | N | Min | Max | Mean | Std. dev. |
|---|---|---|---|---|---|
| **Dry** | 28 | 0 | 3 | 1.21 | 0.83 |
| **Humid** | 28 | 0 | 3 | 1.0 | 0.87 |
| **Control** | 28 | 0 | 3 | 0.5 | 0.84 |

**Table 3.34.** Tamhane's T2 results following ANOVA.

| Pair | Mean difference | P |
|---|---|---|
| Dry and control | 0.7143 | 0.007** |
| Dry and humid | -0.2143 | 0.699 |
| Humid and control | -0.5000 | 0.076 |

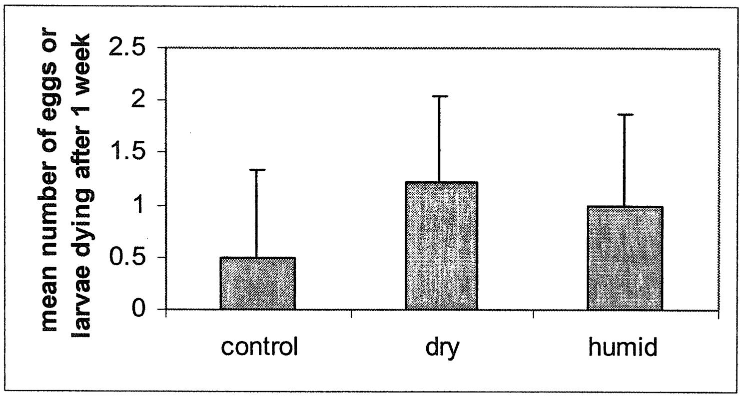


**Figure 3.25.** Mean number of eggs or larvae dying per roll after 1 week in various conditions. There is a significant difference only between the control and dry treatments.
Error bars show + standard error.

When rolls were being opened to examine contents it was noted which surface of the leaf formed the exterior of the leaf roll. Leaves were rolled so that the upper leaf surface formed the exterior of the roll significantly more frequently than expected by random, ($\chi^2_1 = 41.66$, $P < 0.01$, N = 60). (Figure 3.26)

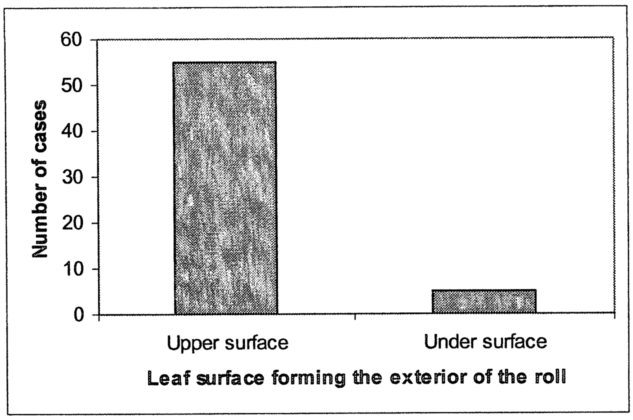


**Figure 3.26.** The upper leaf surface forms the exterior of the leaf roll significantly more often than expected, ($\chi^2_1 = 41.66$, $P < 0.01$, $N = 60$).

Leaf rolls were rolled with equal frequency from the left and right side of the leaf (30 cases from the left, 30 from the right.)

### Predation

Inspection of marked leaf rolls one week after being placed in sites 1 and 2 revealed that leaf rolls were being entirely removed from the area and others were fragmented or extensively damaged with 'chewing' marks. Examination of contents revealed that these damaged rolls were empty.

A Mann-Whitney U test indicated that site 1 had a higher level of predation than did site 2 (site 1 had fewer intact rolls than site 2) . ( U= 8.500, P = 0.003) (Table 3.35 & Figure 3.27) There was no significant difference in sward height between the two sites (U = 25.0, P = 0.190).At sites 1 and 2 there was no significant correlation between the sward height and the number of rolls found intact (rs = -0.263, P = 0.494, N = 9 NS; rs = -0.60, P = 0.879, N = 9, NS, respectively).

**Table 3.35**. Descriptive statistics. No. of rolls intact out of 10 in each plot.

| | Site 1 | | Site 2 | |
|---|---|---|---|---|
| | **Sward Height** | **Rolls intact** | **Sward Height** | **Rolls intact** |
| N | 9 | 9 | 9 | 9 |
| Min | 2 | 0 | 2 | 7 |
| Max | 35 | 10 | 80 | 10 |
| Mean | 16.44 | 2.22 | 30.78 | 9.33 |
| Std. Dev. | 12.02 | 4.15 | 25.65 | 1.12 |

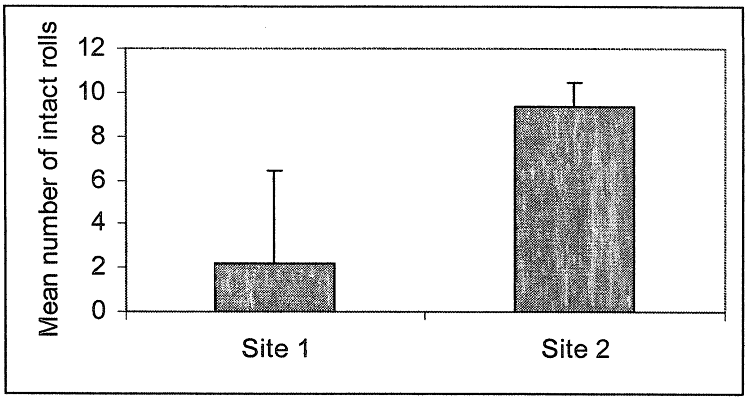


**Figure 3.27** Mean number of intact rolls at sites 1 and 2. Site 1 had significantly fewer intact rolls after 1 week than did site 2. Error bars show + standard error.

**Anecdotal**

During the course of the study It was observed that ants Lasius niger were often present on young aspen in aggregations around aphids. The presence of ants near leaf rolls may provide the eggs or larvae within the roll with a degree of indirect protection. Ants often postured aggressively when leaf rolls were disturbed and attacked on a number of occasions.

An individual *Glischhochilus quadripunctatus* (Nitidulidae) was observed biting into a fallen *B. populi* leaf roll. This is a predatory species. On two occasions adult *B. populi* were seen in spider's webs.

At sites 2 and 3 there was a large amount of standing water in June which dried out towards July. *B. populi* adults fall to the ground when threatened so the presence of standing water below inhabited aspen may be a significant mortality factor. For this reason a number of adults, 2 male and 2 females were individually dropped into water from a height of 1m in order to investigate their behaviour in water. Initially all individuals landed 'head first' into the water with rostrum, head and part of the pronotum submerged. Using their legs and pulling backwards all individuals managed to manoeuvre so that they were fully clear of the meniscus and standing on the water surface. They then moved their legs backward and forwards occasionally. One of the males reached a blade of grass and clung to it. However the other individuals remained roughly in their original position.

## 3.5 Leaf length and number of eggs

Rolls were found to contain between one and four eggs in the wild and in captive situations. One egg per roll was more common than 2 eggs. Three and four eggs in a roll were relatively rare, but did occur (Figure 3.28). The number of eggs in a leaf roll is positively correlated with the length of the roll (measured as length of mid-rib) in both captive and wild situations, (Table 3.36, Figure 3.29). There is a stronger correlation in captive populations than in the wild.

**Table 3.36**. Spearman rank correlations for leaf length vs number of eggs in roll. * = significant at the 0.05 level, ** = significant at the 0.01 level.

| | rs | P | N |
|---|---|---|---|
| Wild | 0.232 | 0.025* | 93 |
| Captive | 0.451 | 0.040* | 21 |
| Wild + captive | 0.284 | 0.002** | 114 |

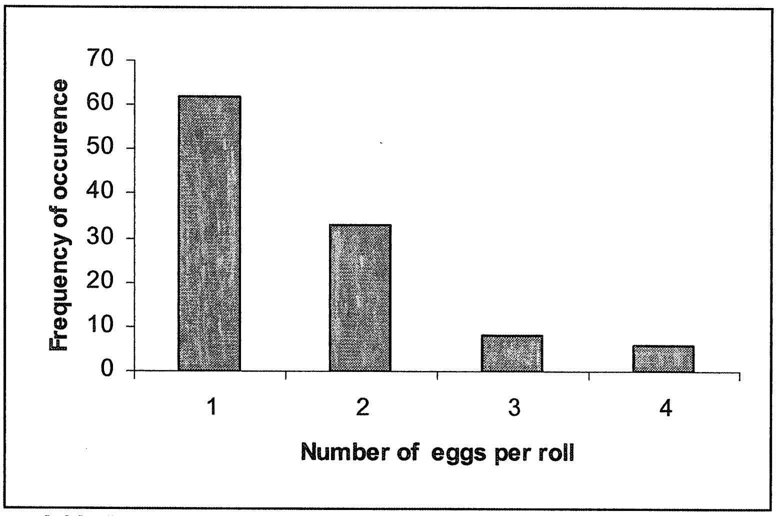


**Figure 3.28**. Frequency of number of eggs per roll.

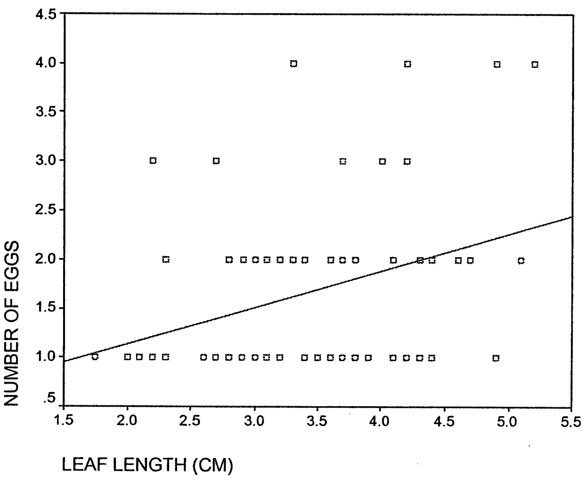


**Figure 3.29.** The number of eggs in a roll is positively correlated with the length of leaf roll. (Captive + wild).

## 3.6 Adult mobility

The recapture number was low (13). Males moved an average of 16.56m in a 24 hour period (N=7), females moved an average of 3.56 m (N = 6) (Figure 3.28). The data has a normal distribution and equal variances so a t-test was carried out. The difference in mean distance moved for males and females was not significant ($t_{11}$ = 1.340, P = 0.207) (Figure 3.30). The minimum distance moved by females was 0m, as the female was still on the release bush. For males the minimum distance moved was 1.43 m. The maximum distance moved was 9.78 m for females and 68.90 m for males.

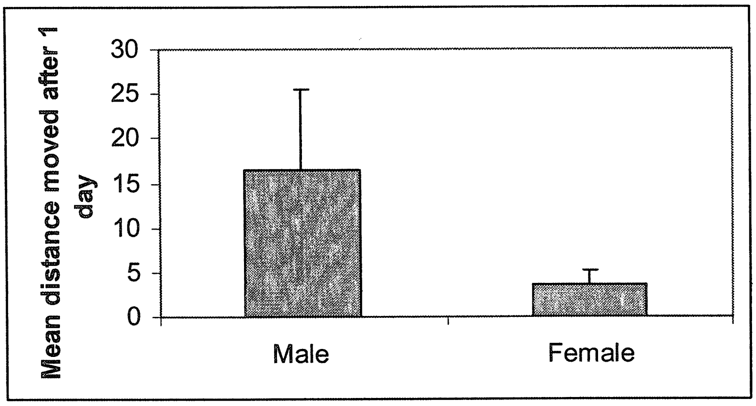


**Figure 3.30.**The difference in mean distance travelled by males and females in a 48 hour period is not significant ($t_{11} = 1.340$, $P = 0.207$).
Error bars represent + standard error.

## 3.7 The process of leaf rolling.

The main features of leaf rolling are described below, including an apparent phase of pre-rolling leaf assessment.

On a number of occasions, females were observed landing on a leaf of an aspen tree. They walk around the circumference of the upper surface of the leaf, following an extended pause at the point where the leaf stalk meets the leaf blade. The female may also walk down the midrib still on the upper leaf surface and veer off to one side until the edge of the leaf is reached. If a leaf was not suitable for rolling (e.g. in one case the leaf was badly torn, or the leaf is very small) females were seen to either move down the branch to another leaf, fall off the leaf landing on a lower leaf or fly to a different aspen. Abandonment of a leaf following this apparent assessment phase was observed to occur both before and after the initial incision of the leaf stalk. Nibbling of the leaf surface also often occurs during this phase.

When the decision to roll has been made, females were observed to remain at the point where the leaf stalk meets the leaf blade for an extended time, apparently feeding. (This early feeding damage at this specific site may make the leaf wilt). Females then passed to the leaf underside and back to the upper surface and circumference walking commenced for a variable length of time. This was interspersed with walks up or down the midrib. The incision point (IP) was then made on the leaf stalk about 0.5mm from the leaf blade. The rostrum is inserted into the leaf stalk and the female engages in a 'rocking' motion, levering up and down with her rostrum. This process takes about 1minute.

Circumference walking then recommences, interspersed with walking up or down the mid-rib, moving from the upper to the lower leaf surface and back again. The duration of this stage was variable. Occasional nibbling of the leaf surface may occur. The female uses her rostrum to 'tenderise' the leaf surface at the periphery of the leaf as she walks along the circumference, paying particular attention to the side of the leaf which is to be rolled first. This may make rolling easier, in a similar way that scoring paper facilitates folding. This 'tenderising' of the leaf also occurs along prominent secondary veins and damage caused in this way is only slightly visible as a faint 'track-way' left on the leaf surface. Once this 'stamping walk' has started it seems that rolling *per se* has commenced, as this walking is more deliberate and slow than that during the assessment phase. The incision point may be returned to at any point and further incision may occur. Circumference and vein walking continues with 'tenderising'. The female frequently moves from the upper surface to the under surface of the leaf. The incision point may be used as a reference point as females were frequently observed to walk up the stem on reaching it during a circumference walk, reach the IP and turn around at this point.

Small patches of feeding damage may be created on the leaf, mainly on the side of the leaf where rolling is initiated. At some point following tenderising of the leaf, females commence rolling. This is performed during a circumference walk, females position themselves so that they straddle the leaf edge, with the left side is on one surface of the leaf whilst the other side of the body is on the opposite leaf surface. They then pull the leaf edge around with their foremost pair of legs, stamp it down with the rostrum and hold the folded part down with their hind pair of legs. Tenderising may then recommence, females seem to ignore leaf veins and use the rolled section as a reference, tenderising the leaf surface parallel to the section that is rolled as they walk vertically up or down. This is repeated. As the rolled section becomes larger, the female starts to walk up or down it, stamping at it with her rostrum. When she reaches the bottom part of the rolled section, the female may tuck the bottom part up into the rolled section by pushing firmly with the rostrum. The top and the bottom of the rolled section seem to indicate to the female that she should turn around.

At this stage of rolling females do not appear to be using anal secretions, the stamping action of the rostrum fixes the roll in place. Females were then observed to open up the rolled section and get inside it, staying still for around 3minutes. As oviposition was not directly observed, it seems likely that it occurs 'under cover' in this way, this may protect the egg, which may be particularly vulnerable shortly after oviposition to desiccation. Females were visible from above when inside the roll. They visibly squeezed the rolled section together with their legs as they moved up or down. Upon emerging, the females tuck the bottom section of the leaf up into the roll and then squeeze the roll together as they move along the rolled section. This may be repeated 3 or 4 times after further periods of rolling. When females are rolling, they keep their legs on one side of the body on the unrolled leaf surface. When this 'runs out' at the bottom of the rolled section, they turn around. Upon completion of the roll, the females use anal secretions, they visibly dab at the seal about 2 or 3 times with their anus. This is supported by the fact that only the edge of the roll needs separating with a retractable pencil when rolls are being opened, the rest of the roll opens easily. On 4 occasions females were observed to then return to the IP and incise it further until the roll fell to the ground. In most cases however the roll is left suspended upon the tree. The whole process of rolling in the field took between 1 hour and 50 minutes to 2 hours 30 minutes.

Males were frequently observed 'helping' females to roll, this has also been observed in the UK (Binding, A.; Mellings, J. *Pers com.*). Both male and female were seen rolling the same leaf simultaneously, one above the other on a number of occasions. This helping behaviour may be a means by which the male can guard the female from other males following copulation (Smith, 1984). Female *B. populi* are capable of storing sperm for at least 2 days; a female that was isolated in captivity began to roll leaves 2 days after being removed from the wild and laid eggs that were viable (they hatched into larvae). This makes sperm competition likely; the last male to mate with a female prior to oviposition is likely to father the majority of offspring (Parker, 1970). Indeed battles between males over females were very common. Males push each other in a head-on position and jostle until one is displaced from the leaf. It is not clear if the spines possessed by males are important in these battles, but they may be important features used in assessment. The victor usually immediately copulates with the female. Helping behaviour is interspersed with bouts of copulation, a common feature in post-copulatory mate guarding.

On one occasion, 2 males and 4 females were all observed rolling a very large leaf. Instances of multi-adult rolling have been observed in the UK (Mellings, J. *Pers com.*). This phenomenon raises interesting questions concerning co-operation in this species.

# 4. Discussion

## 4.1 Habitat preferences.

Aspen height is positively correlated with the number of leaves on a tree, tree radius and with the trunk circumference, all of these measurements can therefore indicate the age of a tree.

At all sites, the number of rolls and roll scars is positively correlated with the number of adults on a tree. This may be because the main activity adults seemed to be engaged in was rolling leaves. Feeding seems to occur in close proximity to leaf rolls. There were more rolls on trees that have no bare soil at their base, this could be beneficial to larvae they may be shielded from hot sunlight and protected against desiccation, which proved to be an important larval mortality factor.

In sites 1 and 2 aspen height is positively correlated with the number of rolls, (and at site 1 with the number of adults) up to a certain aspen height, above which no rolls were found. When data for sites 1, 2 and 3 were combined and height plotted against number of rolls plus roll scars, there was no significant correlation, however it seems that there are more rolls at intermediate heights of about 2m. The transect showed that in fact, rolls are generally only found on trees under 2.80m tall. The absence of *B. populi* rolls on the young aspen trees that were amongst taller trees may be due to the high level of shading by surrounding trees. *B. populi* may therefore avoid shade when deciding where to create leaf rolls. It was noted that the shaded young aspens had very little new growth. It may be the case that the growth period of young aspen is reduced when the tree is fully shaded. Relevant data on aspen growth periods at different ages and under various environmental conditions is required, however such information is not available.

The anecdotal reports that state that *B. populi* prefers young aspen on which to create rolls seem to be validated by this data.

**Why does *B. populi* prefer young aspen?**

The results of the transect study suggest that height *per se* is not the crucial factor in determining presence or absence of *B. populi* rolls. Rather it is likely that the nature of these young aspen that is important. During this study it was found that most *B. populi* rolls are situated at or very near to the tips of branches or the very top of the tree. Foliage at these points tends to be tender new growth, which is often a deep red in colour. The shape and size of leaves at the growing points tends to be very different to that of more mature growth; often they are longer, narrower and larger than the typical 'rounded' aspen leaf (Figure 4.1). Young aspen trees continue to produce more of this new growth at the branch tips for a much longer period than do mature aspens. This may be due to young trees having a longer growth period than mature ones.

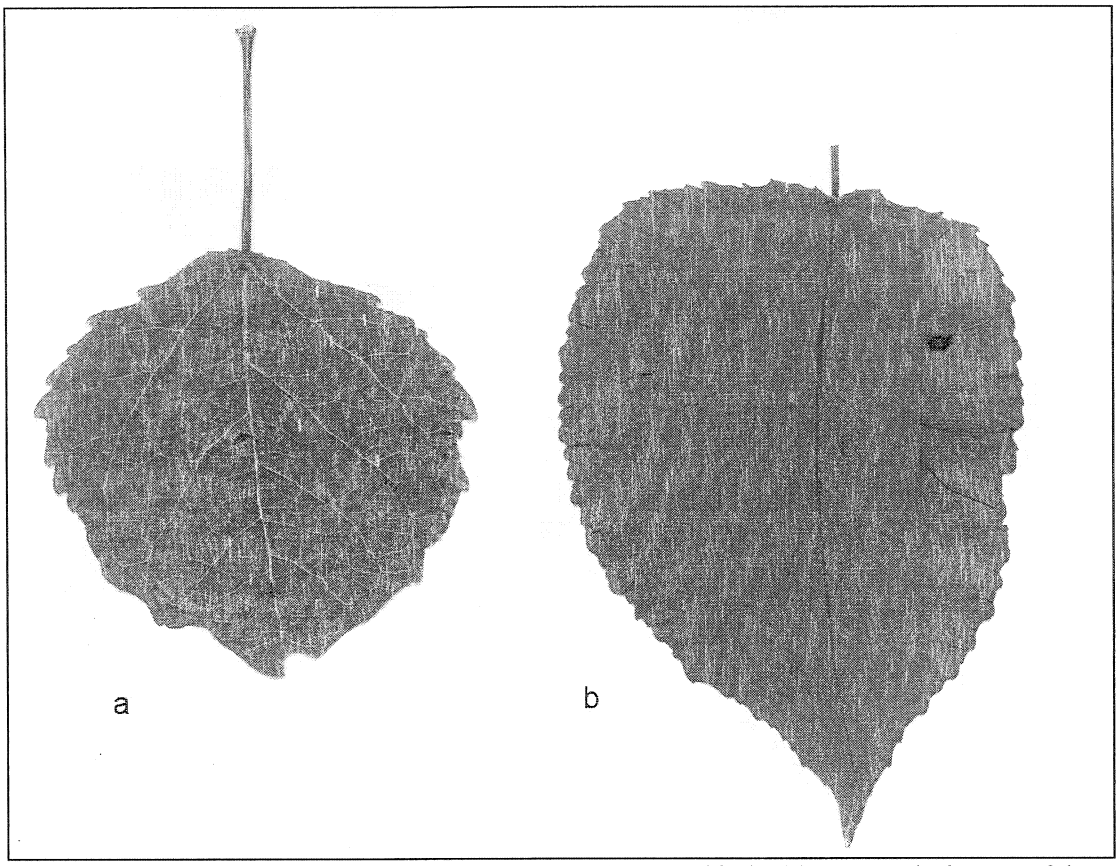


**Figure 4.1.** These leaves are from the same aspen tree; a) is an 'old' leaf from near the bottom of the tree. b) is a new leaf from the tip of a branch. The difference in size and shape of the two leaf types is obvious. (Actual size).

By rolling the leaves at the branch tips *B. populi* activity may actually stimulate the production of further new growth which can then be utilised.

The young leaves, more readily available on younger aspen, may be easier to roll due to their longer, narrower shape. Alternatively, due to their age they may contain fewer defensive chemicals such as phenolic compounds. This may favour larval (and adult) feeding. They may also wilt faster allowing females to create rolls at a faster rate. It seems feasible that all of the above may be true.

It is interesting to note that rolls were found on a 29.2m tall tree. Three of the mature aspen that fell in the storm did not have any new growth at the branch tips. These trees did not have any rolls on them. The tree that supported rolls, however, did have a small amount of new growth at the tips and it is at these locations where the rolls were found.

## 4.2 Roll position

Rolls tended to be located at or near to the tips of branches or at the very top of the tree. Although there were small but significant differences in temperature at different points on the trees, it seems unlikely that this explains the positioning of leaf rolls. As rolls were mainly found at both branch tips and at the very tops of trees, it seems unlikely that the differences in temperature are the important factor. It is more likely that at these locations the foliage is younger, as explained above.

## 4.3 Life history.

Temperature is likely to affect the speed of development, it was not possible to maintain rolls at constant temperatures in Latvia. Laying to hatching took on average 3.7 days, after hatching larvae remain inside the leaf rolls for 15.57 days on average, larvae then burrow into the substrate and pupate in pupal chambers. Adults emerge above ground but it is not clear where they over-winter. *B. betulae* over-winter as adults in the pupal chambers (Bily, 1990). *B. populi* larvae are able to exit rolls early if conditions are unsuitable, and enter the substrate.

Now that it is known that *B. populi* pupate in the soil the nature of the substrate becomes an important factor. Future research should attempt to investigate soil conditions and their effects on larval survival, e.g. soil acidity, the level of compaction, litter content and other factors. In the case of larvae exiting rolls at early stages what do the larvae feed on when in the soil? The precise whereabouts of adult over-wintering sites remains the most interesting question yet to be answered.

## 4.4 Mortality and predation.

Desiccation was the most significant mortality factor for larvae inside rolls. It should be noted that the rolls used in this part of the study contained various numbers of eggs. Egg /larvae numbers per roll may influence survivorship and may therefore have been a confounding factor. It would be interesting to repeat this study but use rolls containing the same number of eggs and comparing the results.

The pattern of damage seen on leaf rolls indicates that larvae inside leaf rolls on the ground may be at risk from predation, possibly by shrews. The extent of this predation indicates that this may be a significant mortality factor to larval *B. populi*. Future research should attempt to elucidate precisely which species predate upon larvae inside leaf rolls possibly by using Longman traps containing leaf rolls. In localities where *B. populi* struggles to persist in the UK, or in the future if the species is reintroduced to former sites, a substantial reduction in losses may be brought about by placing leaf rolls in areas from which small mammals are excluded.

Ants may indirectly provide protection for larvae whilst leaf rolls are suspended on trees, as they protect aphids.

Leaves are rolled more often so that the upper surface forms the roll exterior. This may benefit the developing larvae, as the upper leaf surface may be more reflective of light and heat, so preventing excessive desiccation.

## 4.5 Leaf length and number of eggs

The results of this study show that rolls can contain between 1 and 4 eggs, and that the number of eggs is associated with leaf length. This suggests that female *B. populi* are able in some way to assess leaf size and lay an appropriate number of eggs. This ensures that larvae have sufficient food. The correlation between leaf length and egg number was stronger in captive situations. This may have arisen as a result of the supply of suitable leaves being limited in captivity; females may be able to perceive leaf availability and ensure that the maximum number of eggs possible for that sized leaf are laid.

## 4.6 Adult mobility

This part of the study indicates that males moved greater distances than females. This difference was not significant, however the sample size was extremely small. Further work is required in this area. It seems likely that males may move greater distances in search of females. The distances moved by the marked adults may be exceptional for a 48 hour period due to stress caused by capture and marking. However circumstances precluded a further attempt at mark and recapture work.

## 4.7 The process of leaf rolling

Despite styles of leaf rolling varying greatly within the family Attelabidae, there are broad similarities in this behaviour between species nonetheless. *Chonostropheus chujoi* makes cradles from the apical part of leaves and cuts across the leaf prior to rolling so that the bottom section can be rolled. This species performs stereotypical pre-rolling assessment of the leaf with circumference walking a prominent feature (Sakurai, 1988). During this circumference walking, the females often stop to nibble at the leaf tissue as observed in *B. populi*.

Walking allows females to gather information on leaf size and shape, nibbling allows females to determine leaf suitability in chemical and physical terms, allowing information on leaf age and plant species to be gathered. Many other herbivorous insects assess leaf suitability by nibbling at leaf tissue (Sakurai, 1988).

Sakurai (1988) found that when given leaves of fixed sizes, with no choice of leaf size, more eggs were laid in rolls made from larger leaves. Female *C. chujoi* therefore have an ability to control the number of eggs laid according to leaf size or the size of the apical part of the leaf which forms the roll. As we have seen this is also the case for *B. populi* females; more eggs are laid in longer leaf rolls. In *C. chujoi*, providing females with model leaves showed that circumference is not the determinant of egg number but a measure of leaf area is used for this purpose. If circumference alone was used, narrow leaves would have too many eggs laid in them and larvae would starve. It seems likely that this is the case in *B. populi*, but more work is required in this area. Studies have indicated that *Deporaus betulae* and *C. chujoi* use walking of the leaf surface as a measuring technique (Sakurai, 1990). The process of walking may allow the leaf to be measured through visual or kinetic means (muscle fatigue or the number of steps taken) (Sakurai, 1988).

## 4.8 Implications for conservation in the UK

The major factor preventing *B. populi* from colonising suitable habitat in Latvia appears to be the isolation of the habitat in an unfavourable matrix. In Latvia this tended to be large pine forest, in the UK this is likely to be the intensive agricultural landscape. One potential method of linking suitable habitat patches in the UK would be to utilise field margins. The transect study site at Slitere National Park supported a large population of *B. populi* and was located in a field margin. There is potentially scope under agri-environment schemes such as the Countryside Stewardship Scheme to plant aspen in field margins in areas adjacent to existing *B. populi* habitat. This aspen should be cut on rotation roughly every four years in order to maintain aspens at a suitable height. Such a scheme may enable the species to disperse to new habitat.

It may be possible to stimulate new growth on aspens that would otherwise be unsuitable for *B. populi* by pruning branches.

Larvae remain inside rolls that fall to the ground and are subject to predation. For these reasons, it may be necessary to cordon off patches of aspen occupied by *B. populi* in order to exclude potential mammalian predators and to prevent unintentional trampling by humans. Interpretation boards at UK *B. populi* sites are essential in order to increase public awareness and appreciation of the species.

# 5. Acknowledgements

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# 6. References

BILY, S., 1990. *A colour guide to beetles*. London: Hamlyn.

DYTHAM, C., 1999. *Choosing and using statistics: a biologists guide*. Oxford: Blackwell Sciences Ltd.

FOWLER, W.W., 1891. *The Coleoptera of the British Isles*, vol 5. London: Reeve and Co.

GRUPPE, A., FUßEDER, M., SCHOPF, R., 1999. Short rotation plantations of aspen and balsam poplar on former arable land in Germany: defoliating insects and leaf constituents. *Forest ecology and management*, **121**: 113-122.

HARDE, K.W. & SEVERA, F., 1998. A Field Guide in Colour to Beetles. Leicester: Blitz Editions.

HYMEN, P.S. & PERSONS, M.S., 1992. *A review of the scarce and threatened Coleoptera of Great Britain Part 1.* Peterborough

Minutes of meeting on phytophagous beetle conservation. London: Royal Entomological Society.

JNCC, 2001. *Byctiscus populi* Action Plan.

LATVIAN MINISTRY OF ENVIRONMENTAL PROTECTION AND REGIONAL DEVELOPMENT, 1998. *National report on biological diversity of Latvia.*

LATVIAN MINISTRY OF ENVIRONMENTAL PROTECTION AND REGIONAL DEVELOPMENT, 2000. National Programme on Biological Diversity. Strategy Section.

LATVIAN MINISTRY OF AGRICULTURE, 1995. Latvia: Country Report to the FAO International Technical Conference on Plant Genetic Resources.

SAKURAI, K., 1988. Leaf size recognition and evaluation of some Attelabid weevils (1) *Chonostropheus chujoi*. *Behaviour,* **106**:279-300

SAKURAI, K., 1988. Leaf size recognition and evaluation of some Attelabid weevils (2) *Apoderus balteatus*. *Behaviour,* **106**: 301-317

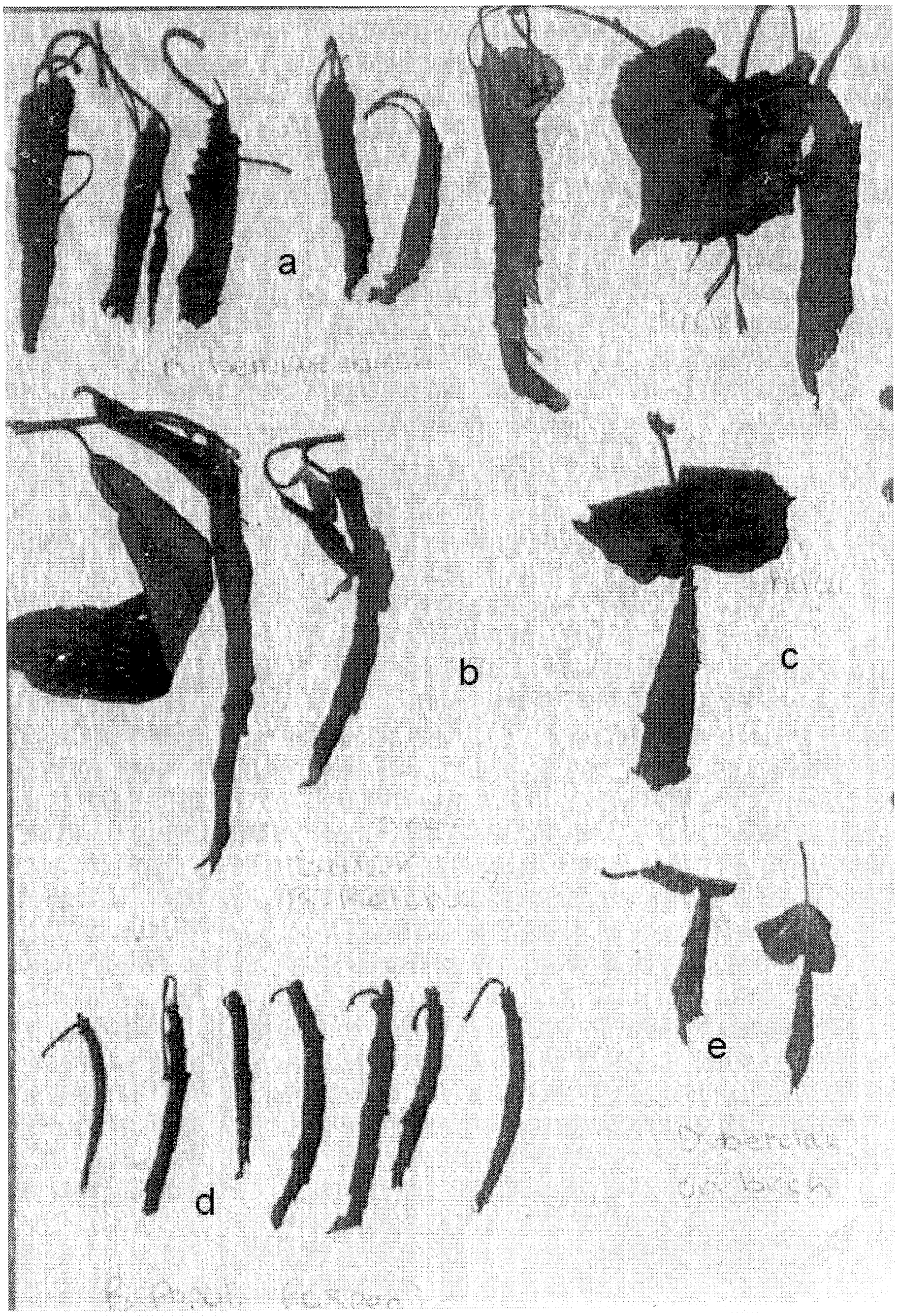
SAKURAI, K., 1990. Leaf size and recognition by some Attelabid weevils (3) *Deporaus* sp. *Behaviour,* **115**: 348-369.

PARKER, G.A., 1970. Sperm competition and its evolutionary consequences in the insects. *Biological Review*, **45**: 525-567.

SMITH R.L., 1984. Sperm competition and the evolution of animal mating systems. New York: Academic Press.

SOUTHWOOD, T.R.E., 1978. *Ecological Methods with particular reference to the study of insect populations.* Second edition. London: Chapman and Hall.

# Appendix 1. Leaf rolls of various attelabid species




a) *B. betulae* rolls on birch (left 5), and on hazel (right 2) Adult females were observed rolling these rolls.
b) Possibly *B. betulae* rolls on *Salix*.
c) *Apoderus coryli* roll on hazel.
d) *B. populi* rolls on *Populus tremula*.
e) *Deporaus betulae* rolls on birch.