FORMATION AND STRUCTURE OF LARVAL TUNNELS OF PHYTOBIA BETULAE IN BETULA PENDULA

by

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SUMMARY

Structural abnormalities in the wood of Betula pendula Roth caused by the larvae of Phytopia betulae Kangas have been examined by light microscopy. The larvae bore through the zone of differentiating xylem from the crown to the base of the tree. The wound callus formed by the tree in response to the damage becomes sclerified and appears as brown streaks running through the wood. In transverse section the tunnels are elliptical in shape up to 3 mm wide and correspond to ‘pith flecks’.

Key words: Betula pendula, Phytopia betulae, larval tunnels, pith flecks, wound callus.

INTRODUCTION

Phytopia betulae Kangas is one of a family of cambium miners (mainly larvae of the Agromyzidae) which attack genera including Populus, Salix, Acer, Alnus, Betula, Prunus and Sorbus, as well as some tropical hardwoods (Fink 1999). It feeds on species of Betula and also on Alnus, Carpinus and Corylus (Spencer 1990).

In early summer, the adult fly of P. betulae lays a single egg in the cambial zone at each position it visits in the crown of a tree. The eggs are laid in the younger shoots where the bark is soft. The eggs hatch in the cambial region and by the time the larvae are 2 weeks old, they may have already mined up to 2 metres of tunnels (Kangas 1935). It is thought that the tunnels made by P. betulae in Betula pendula are among the longest larval tunnels made in trees by any wood-boring insect larva (Ylioja et al. 1998). The tunnels so formed become filled with thick-walled parenchyma cells that store lipids (Ylioja et al. 1998). Swollen rays are often found adjacent to the tunnels. It was originally thought that the larvae fed directly on the cambium but studies on Acer saccharum infected by the larvae of Phytopia setosa have revealed that the mining takes place within the youngest xylem cells while the cambium remains intact (Gregory & Wallner 1979). This is probably because the newly differentiated xylem provides the insect with the path of least mechanical resistance compared to mature xylem, as these newly differentiated xylem cells are not yet fully lignified.

A study of xylem formed after wounding was carried out in Acer, Betula and Fagus by Rademacher et al. (1984). The wounded xylem was characterised by more ray and axial parenchyma, fewer and shorter fibres, fewer and smaller vessels and some axial tissue disorientation. Bhat (1980) also recorded an increase in width, height and frequency of rays occurring in the vicinity of pith flecks in Betula pendula and B. pu-
bescens, and found that larval injury triggers the initiation of aggregate rays (Bhat 1983). Similar cell changes have been observed in other species. In Pinus halepensis Mill., infected with Matsucoccus josephi Bodenheimer & Harpaz, Liphschitz and Mendel (1987) found the production of curved tracheids and lignified resin ducts, with lignified parenchyma cells replacing normal tracheids. There was also a large reduction in ring width below the attack site.

The work reported in this paper describes the anatomical changes in wood of Betula pendula that had been invaded by the larvae of Phytobia betulae.

MATERIALS AND METHODS

Wood samples were taken from eight 31–50-year-old trees of Betula pendula from sites in Hämeenlinna (lat. 61° 02', long. 24° 27') and Loppi (lat. 60° 38', long. 24° 27') (Finland), Kronoberg (lat. 56° 43', long. 14° 46'), Kristianstad (lat. 55° 55', long. 14° 01') and Älvsborg (lat. 58° 58', long. 12° 17') (Sweden). Samples were also taken from six 12-year-old trees grown in Kent, United Kingdom (lat. 51° 17', long. 0° 26'). Discs of 3 cm thick were removed from the stem at 2, 8 and 14 metres from ground level for each of the older trees and at 1, 3, 5 and 7 metres from ground level in the younger trees. Each disc was then polished with fine sandpaper so that the number of pith flecks could be observed. Radial samples were removed from each disc and blocks were prepared from the 2nd, 5th and every 10th ring out from the pith. Sections 20 μm thick were cut from the transverse, tangential and radial surfaces of these blocks and stained with safranin.

RESULTS AND DISCUSSION

Larval tunnels were found in all trees except those grown in Kent, UK. Phytobia betulae had previously been recorded in Finland, southern Sweden, Denmark and Germany but its presence in the UK has been questioned as no adults have been found, although a number of tunnels were found in trees sampled from the University of Reading, UK.

Formation of larval tunnels

In each tree the number of larval tunnels increased greatly from the crown region (disc 14) towards the base (disc 2) (Fig. 10). The occurrence of tunnels in disc 14 indicates that some larvae have mined in excess of 14 metres to reach the tree base. Kangas (1935) calculated that a larva could tunnel up to 1 metre per week whilst Ylioja et al. (1998) recorded tunnels up to 19 metres in length. However, the larval stage of P. betulae lasts for 1–2 months, so the larvae must mine at a much faster rate than Kangas suggested.

The greater number of tunnels in disc 8 compared with disc 14 in each tree suggests more larvae entered the tree between 8 and 14 metres above ground. These results confirm those of Ylioja et al. (1998) who found tunnels starting at an average height of 11 (± 3) metres. The tunnels were most abundant in disc 2 suggesting either that some larvae may have begun mining below 8 metres or that the larvae may mine...
Fig. 1–6. Examples of larval damage in silver birch. – 1: Cross section of a disc with numerous larval tunnels dispersed throughout the latewood. – 2: Transverse section through the edge of a larval tunnel composed of darkly stained sclerified cells with thick, pitted walls. – 3: Transverse section of an elliptical larval tunnel that has caused an irregularity in the growth ring boundary. – 4: Transverse section of rays that can be traced through a larval tunnel. – 5: Radial longitudinal section of part of tunnel shown in Fig. 3. – 6: Tangential section of part of the larval tunnel shown in Fig. 3. — Scale bar for 1 = 10 mm, for 2 = 50 μm, for 3–6 = 150 μm.
up and down if they reached the base before they are ready to pupate. This creates multiple tunnels at the base of the tree (Gregory & Wallner 1979; Ylioja et al. 1998).

According to Spencer (1973) trees growing on damp ground appeared to be favoured by P. betulae over those growing in a dry, sandy locality. Ylioja et al. (2000) reported that slow-growing clones were the most resistant to Phytobia. Each of the trees in this study was growing on fine, sandy soils with similar levels of soil mois-

Fig. 7–9. A comparison of frost and larval damage in silver birch. – 7: Cross section of a disc containing a frost ring (indicated by arrow). – 8: Transverse section of a larval tunnel in earlywood with rays protruding from the callus. – 9: Transverse section of frost-damaged wood comprising 4 distinct bands: A: ‘frost ring’, B: band of vessels, C: latewood-type region, D: continuation of normal earlywood growth. — Scale bar for 8 = 10 mm, for 7 = 150 μm, for 9 = 200 μm.
ture, and all appeared to be growing at similar rates. Therefore different levels of larval infestation may depend on a combination of factors which might also include the availability of other suitable host trees and the abundance of the _P. betulae_ in the area.

**Structure of larval tunnels**

In transverse section, larval tunnels were visible as dark-brown spots 1–3 mm wide, usually situated in the latewood part of the growth rings (Fig. 1). The tunnels were variable in shape in transverse section, the most common form being an ellipse, 1–3 mm wide (Fig. 3). The presence of large tunnels such as these caused the growth ring boundary to deviate from a circular outline.

The path followed by the larva was marked by a proliferation of callus cells filling the tunnel. Many of the cells had sclerified walls with simple pitting (Fig. 2). The path of the rays could be followed in some cases (Fig. 4) although there was not always correspondence between rays entering and leaving (Fig. 3). The cells were filled with dark stained contents with the contents of the sclerified cells staining most strongly.

In RLS (Fig. 5) the cessation and recommencement of normal xylem production can be seen clearly. The isodiametric nature of the callus cells and their origin from ray parenchyma can also be distinguished in places.

In TLS (Fig. 6) the callus is interspersed with axially elongated cells. The whole structure has the appearance of an aggregate ray. However, combining the image with the TS in Figure 2 suggests that there is merely a passing resemblance brought about by a mixture of callus cells and the cells derived from fusiform cells of the vascular cambium which have survived the larval attack.

Smaller tunnels were generally circular with swollen rays protruding from the central area, giving them a star-like appearance (Fig. 8). An array of such tunnels could...
be confused with damage caused by frost (Fig. 7 & 9), as both forms of damage cause swelling of rays and general cell disruption. Frost and larval damage is most difficult to distinguish in the upper parts of a tree where the larval tunnels are relatively small in diameter and occur in earlywood where frost rings are most likely to also occur. Larval damage is easier to distinguish from frost damage nearer the base of the stem as the tunnels are much wider, more elliptical, stained dark brown and found in the latewood part of the growth ring. In frost rings, the loss of turgor in the damaged area allows the rays to swell resulting in the deformation of the unlignified xylem cells. Radial files of xylem can be traced through the frost-damaged area, unlike the situation in the case of larval damage.

The pockets formed by removal of tissue by the feeding larvae are smaller versions of the cavities formed in stems by splitting during growth (Harris et al. 1975). Here, the breaking of rays and the creation of a space resulted in the formation of callus nodules. A similar mechanism is probably operating in the case of pith flecks.

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REFERENCES