Histological relationship of *Phytobia setosa* to *Acer saccharum*

ROBERT GREGORY
Northeastern Forest Experiment Station, Sugar Maple Laboratory, P.O. Box 968 Burlington, VT, U.S.A. 05402
AND
WILLIAM WALLNER
Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory, Hamden, CT, U.S.A. 06514

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The maple cambium miner, *Phytobia setosa* (Loew), attacks *Acer* spp., producing ray flecks which result in degrade in face veneer and furniture wood. Samples from infested sugar maple, *Acer saccharum* Marsh, trees demonstrated that while mines passed close to the vascular cambium the initial cells were not affected. Thus, although it is called a cambium miner it does not mine the cambium. Mines filled with parenchyma cells which proliferated from severed vascular rays. These cells, when mature, stored starch. In heavily infested trees the starch storage area in the xylem may thus be measurably increased. The zone of newly differentiating xylem provides the insect with the path of least resistance; variation in the condition of secondary xylem may account for the variability in host susceptibility.

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La mineuse du cambium de l'érable, *Phytobia setosa* (Loew), attaque des espèces d'*Acer*, produisant des mouchetures des rayons et amenant ainsi une dégradation du bois de placage et du bois d'ébénisterie. Des échantillons d'érables à sucre infestés (*Acer saccharum* Marsh) montrent que les mines passent près du cambium vasculaire, mais sans en affecter les cellules initiales. Par conséquent, malgré son nom, l'insecte n'est pas une mineuse du cambium. Les mines se remplissent de cellules parenchymateuses qui prolifèrent à partir des rayons vasculaires coupés. A maturité ces cellules accumulent de l'amidon. Chez les arbres fortement infestés, la région d'entreposage de l'amidon dans le xylème peut donc s'accroître de manière appréciable. La zone de xylème nouvellement différencié constitue le chemin de moindre résistance pour l'insecte; les variations dans le xylème secondaire expliquent peut-être la variabilité de la susceptibilité de l'hôte.

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Introduction

Larvae of *Phytobia* spp. reputedly mine via the cambium from young shoots to roots of many shrub and arborescent dicotyledons (Brown 1913) including at least one ring-porous species, *Fraxinus americana* L. (Skelly and Kearby 1969). This mining produces ray flecks, which cause as much as 40% degrade in face veneer and furniture wood (Hanson and Benjamin 1967) (Fig. 1) but are not known to affect the strength of wood (Panshen *et al.* 1964). According to Brown (1913), ray flecks were first observed by Hartig about 1840, while Kienitz in 1883 attributed the mining to dipterous insects in the cambium and soft tissue of the inner bark. Subsequent papers consistently refer to the basipetal larval migration as occurring in the cambium and neighboring regions (Panshen *et al.* 1964). The purpose of this study was to determine the location of *Phytobia setosa* (Loew) mines in relation to the vascular cambium in sugar maple, *Acer saccharum* Marsh, and to ascertain development of ray flecks relative to differentiating vascular elements.

Materials and Methods

Study material was taken at about breast height from young (ca. 25 years old) dominant and codominant sugar maple trees growing at the University of Vermont's Proctor Maple Research Farm, Underhill, Vermont. These trees were heavily infested with *P. setosa*. Cross sections from sugar maple stems taken at 30 cm aboveground exhibited an average of 2.02 mines/cm^2 of xylem transverse surface area.

Samples for studying the development of ray flecks were collected from each of three trees at weekly intervals from 12 March through 6 August 1975 and then at biweekly intervals through 1 October 1975. Most trees were sampled once; a few were sampled twice. The sample blocks, which were removed with razor blades and a wood chisel, measured ca. 1 × 3 cm on the tangential face and included bark, cambial zone, usually all of the 1974 xylem, and all of the 1975 xylem that had been produced. The sample blocks were placed in Navashin's fluid (Jensen 1962), evacuated, and then dehydrated and embedded in paraffin. Transverse, radial, and serial tangential sections were cut on a rotary microtome, usually at 10 μm thickness, stained by the periodic acid – Schiff's (PAS) reaction and counterstained with Heidenhain's iron hematoxylin. Duplicate transverse and radial sections were stained with safranin and fast green.

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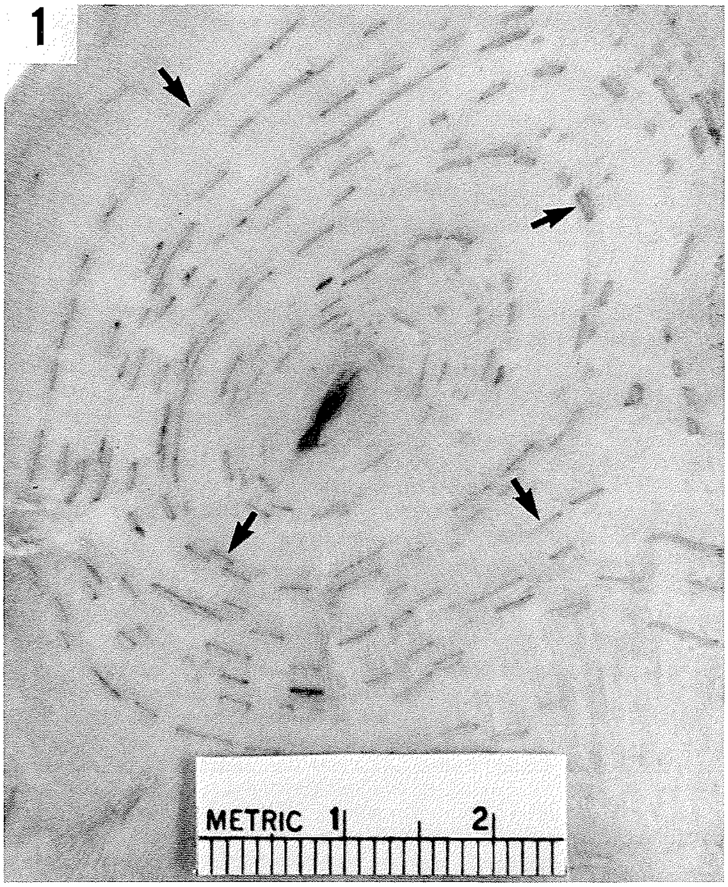


FIG. 1. Transverse face of a section taken near the base of a sugar maple stem illustrates the abundance (6.8/cm^2) and distribution of ray flecks (arrows) commonly found in the xylem of this species.

For the reader to interpret the following results it is essential that we describe precisely what we regard as the cambium. We consider the 'vascular cambium' or 'cambium' to be a self-perpetuating, single layer of initials within a region of actively dividing xylem and phloem mother cells. This is the "uniserate concept" as most recently described by Schmid (1976). The 'cambial zone' is the entire meristematic region including the vascular cambium (fusiform and ray cell initials) and its most recent derivatives, the xylem and phloem mother cells. In stems of many tree species the cambial zone may contain 10 or more cells per radial file during the growing season, most being xylem mother cells.

Results

Thirty-three of the 78 samples taken in 1975 had one or more ray flecks in the 1974 or 1975 xylem. The first wounds caused by *P. setosa* in 1975 were observed on the xylem side of the cambial zone in the breast height samples taken 25 June, about 40 days after the first of the current year's xylem elements began to differentiate.

The proximity of mines to the cambial zone (Fig. 2) indicated that the larvae passed very close to the initial cells of the cambium. However, subsequently produced xylem elements radial to the mined area were always normal in structure and number (Fig. 3). Furthermore, there was no evidence of abnormality in the phloem tissue radial to the mines (Fig. 3). The only abnormal elements other than the cells that filled the mines were those immediately surrounding the cavity. There, abnormal axial elements could usually be seen in radial sections in mature xylem, especially on the cambium side of the ray flecks (Figs. 3, 5). Therefore, effects of mining did not extend to elements of the cambium, although they may have extended to xylem mother cells as well as to the normally non-meristematic, differentiating xylem elements.

In transection, the mines which subsequently contained the ray flecks were always elongated tangentially as reported by Brown (1913) and were filled with parenchyma cells which proliferated from severed vascular rays (Figs. 4–6). In mature secondary xylem the mines appear in transection as small tangentially elongate spots or flecks (Figs. 1, 4).

A thick, wall-like substance that stained heavily by the PAS reaction was present around the periphery of newly formed mines (Figs. 2–7) and often extended between parenchyma cells within

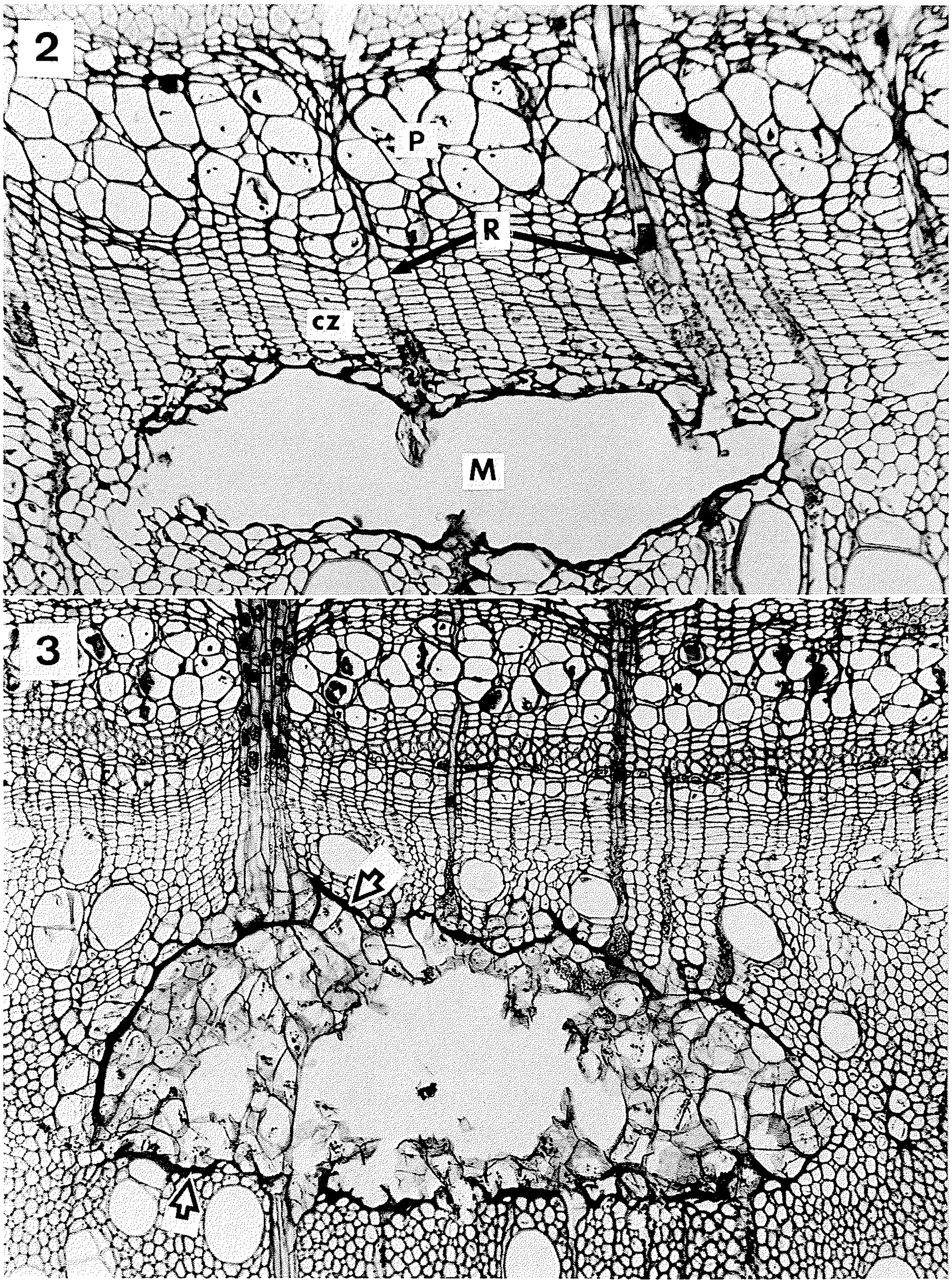
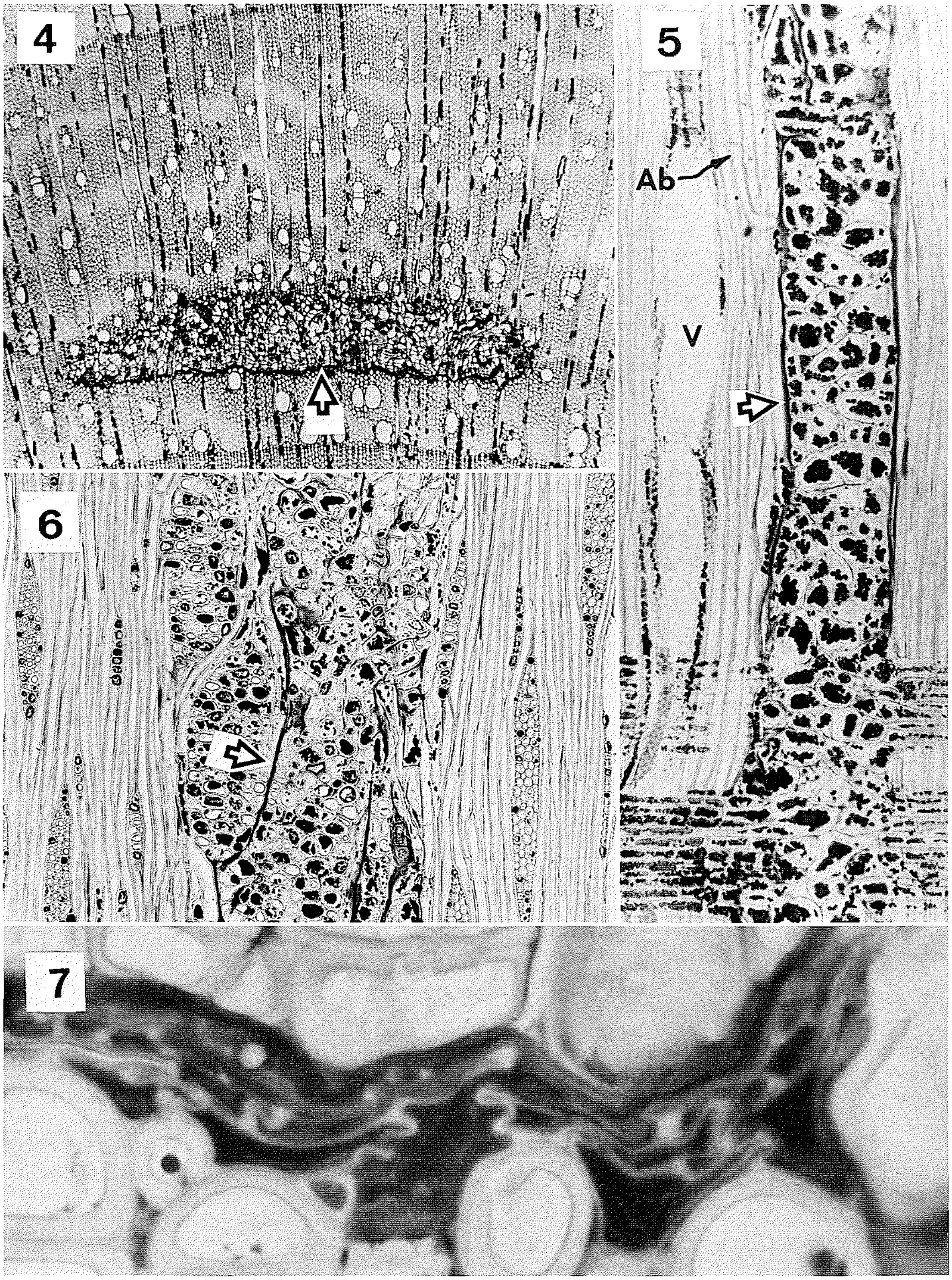


FIG. 2. Transection of a recently made mine (M) in a 2 July sample. The cavity is within differentiating xylem slightly beyond the cambial zone (CZ). Note the severed xylem rays (R) and the normal appearance of the cambial zone and recently produced phloem (P) radial to the cavity. × 229. FIG. 3. Transection of a recently made, partially filled mine in a 9 July sample. Thin-walled parenchyma from xylem rays have proliferated within the mine, which is sharply outlined by a thick layer of darkly stained material (arrows). Note the normal appearance of the cambial zone, the recently produced phloem, and the xylem produced after wounding. × 148.



FIGS. 4–6. Ray flecks containing thick-walled parenchyma cells in mature sugar maple xylem as seen in transverse (Fig. 4, × 36), radial (Fig. 5, × 145), and tangential (Fig. 6, × 118) sections. Note the darkly stained, thick-layered material (arrows), presumably debris from dead cells, in the boundaries and in the flecks. The heavily stained material within the ray fleck cells of Figs. 5 and 6 is starch. V, vessel members; Ab, abnormal axial elements. FIG. 7. Transverse view of convoluted and compressed cell wall debris at the boundary of a ray fleck. × 1120.

the cavity. This substance may be what Brown (1913) referred to as "a compact yellowish-brown substance, the remains of dead cells and excrement of larvae," which, he noted, occasionally separated cells of ray flecks.

Proliferating ray parenchyma cells with thin walls and numerous primary pit fields soon filled newly formed mines (Fig. 3). Some mines were completely filled by mid-July. By then, the parenchyma cell walls were much thickened and profusely pitted, and starch grains were beginning to appear in the cell lumens in advance of starch accumulation elsewhere in the new (1975) xylem. In early August, starch was abundant in the wound parenchyma and in most other living elements of the new xylem. Near the end of the growing season the wound parenchyma cells were filled with starch. We observed ray fleck parenchyma in xylem more than 10 years old (which we presumed to be alive since it contained starch grains).

Discussion

The ray fleck parenchyma is obviously a part of the interconnected three-dimensional network of living axial and ray elements in sugar maple xylem, all of which probably have similar longevity. Since the mines created by the larvae serve as reservoirs for starch, they increase storage capacity and ostensibly benefit the tree. Our observations of the cambial zone and differentiating secondary vascular tissues in sugar maple indicate that the larvae do not pass through or noticeably affect cells of the vascular cambium. Obviously, ingress and egress by larvae create wounds which could serve as portals of entry for pathogens.

We believe that *P. setosa* larvae mine in the zone of newly differentiating xylem because this is the path of least mechanical resistance within a nutritionally favorable environment. As the oldest xylem mother cells begin to differentiate into xylem elements, they undergo considerable transverse enlargement, especially those axial elements destined to become vessel members. The walls of these newly differentiating cells are unusually thin, thinner than in any other part of the secondary vascular system including the cambial zone. This narrow area, just beyond the cambial zone, would be the path of least resistance for larval mining and may indeed explain the insects' presence there. It is also a metabolic sink, presumably rich in carbohydrates. The larvae could wander tangentially much more easily than radially (which would also explain the tangentially elongate shape of the ray flecks as seen in transection).

The 'turnaround' habit of the larvae near the base of the bole has been reported as being common. Greene (1914) stated that, "if the larva reaches the base of the tree before the time to pupate, it will turn and mine up the cambium for some distance." It would be of practical value to determine if the incidence of turnaround is greater in young trees with live branches close to the ground or if differences in nutrient concentration or root versus stem maturation were the determining factor. Hanson and Benjamin (1967) reported that the point of entrance of *P. setosa* in sugar maple and red maple was in 1-year-old twigs and that the larvae exited from primary roots. Thus, total migration distance from twigs to roots would vary considerably depending on the distance of the live crown from the ground. This distance, in older, taller, forest-grown trees, might simply be too great for the insect to mine during its larval stages, so the larvae may not reach the roots to exit and pupate. Hence, young, open-grown trees might be more favorable hosts. Conversely, by pruning up the crown, the distance of migration from twigs to roots could be increased and larval survival reduced.

Brown (1913) reported that *Phytobia* sp. have favorite hosts and that some trees in some localities are infested much more than others. Certain characteristics of differentiating secondary xylem may possibly limit the survival of larvae so that some trees may be more favorable hosts than others. Vertical and tangential movement of the larvae, and hence their ease of access to new food sources, would be least restricted when the cambium is highly active and when the zone of differentiating xylem is relatively wide, that is, when radial growth rate is high. Conversely, larval movement might be critically restricted when growth rate is low and when the seasonal period of xylem differentiation is short. Thus, factors which might influence the period of xylem differentiation (such as defoliation by other insects or meteorological conditions) could jeopardize successful larval development.

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