Mitotic Reactivation of the Terminal Bud and Cambium of White Ash

Abstract. *The first appearance of mitotic figures in reactivating buds of 1-year-old ash seedlings is in leaf primordia. The occurrence of mitotic figures then progresses sequentially to the procambial cells and finally to the cambium at the base of the bud. Mitotic reactivation of the cambium in progressively more proximal regions of the stem is slow, proceeding at a maximal rate of 6 centimeters per day.*

That bud and cambial reactivation are causally related and that the relationship is mediated by growth regulators (among them indoleacetic acid) is well supported (*1*). However, the pattern of mitotic reactivation in the two meristems has not been coordinated. Workers interested in bud break generally investigate this phenomenon without concern for cambial activity (*2*), whereas workers interested in cambial reactivation generally relate cambial changes to gross morphological changes in the bud (for example, bud swelling or expanding) (*3*). A description of the process of mitotic reactivation in the bud and its sequential progression into the cambium will broaden our insights into this problem and serve as a basis for future studies of sites of production of growth regulator and its path of movement in the bud and cambium.

One-year-old, potted white ash (*Fraxinus americana* L.) seedlings were utilized. They were chosen because they can be grown in a growth chamber and because the terminal bud is the only bud that elongates in the seedlings' second year of growth. This complete apical dominance is important because expanding axillary buds also can stimulate cambial reactivation (*4*), complicating the interpretation of the influence of terminal buds on cambial reactivation. However, this apical dominance does limit the extrapolation of the data to larger trees in which many buds expand simultaneously. The seedlings were between 19 and 26 cm in height and were chilled for 3 months (normal winter temperature in Syracuse) prior to use. Fifty-eight seedlings were placed in a growth chamber on 28 February 1966. A 16-hour day and 8-hour night with a temperature of 26°C during the photoperiod and 16°C in the night were utilized. Light intensity at plant level was about 30,000 lux. Seedlings were randomly collected at 2-day intervals over a period of 24 days. The date of bud break varied from seedling to seedling (6 to 16 days). As a result, a variety of developmental stages were collected at each date.

The terminal bud, a stem section 1 cm long immediately below the terminal bud, and a 1-cm-long stem section from the base of each seedling were collected. All materials were run through a tertiary butanol series, embedded in Tissuemat, and sectioned at 7 to 9 μ. Sections were stained with safranin and fast green.

The terminal bud of white ash is composed of a pair of scales (modified petioles) and four pairs of leaf primordia (Fig. 1). As a rule the scales absciss soon after the bud expands. All but the youngest pair of leaf primordia are composed of a petiole and from three to five leaflets.

The time at which mitotic figures first appear in the buds varies from individual to individual, and the description that follows is a composite deduced from shoot systems in various stages of bud break. Such a procedure is valid, since the degree of development or mitotic activity in the bud is always correlated with the cambial changes in the secondary body below the bud.

The first mitotic figures appear in the leaf primordia and not in the scales or in the cauline portion of the bud. We could not determine whether or not there was a distinctive pattern of mitotic reactivation within individual leaf primordia of differing ages (that is, from leaflets to the mid-

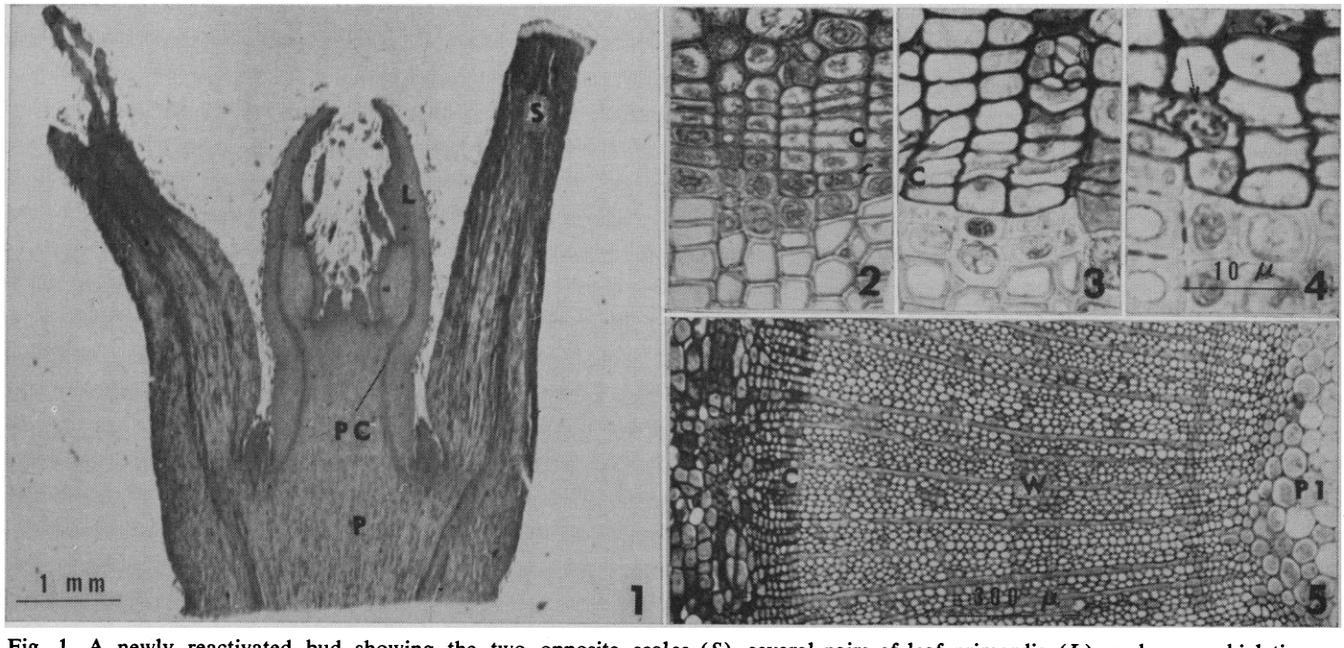

Fig. 1. A newly reactivated bud showing the two opposite scales (*S*), several pairs of leaf primordia (*L*), and procambial tissues (*PC*) embedded in the parenchyma (*P*). Fig. 2. Cross section of a stem showing the dormant cambium (*C*). Fig. 3. Cross section of cambium showing the thin tangential walls resulting from divisions in the fusiform initial of the cambium (*C*). Fig. 4. Cross section of a stem showing the cambium in process of reactivation and a prophase nucleus in a fusiform initial (arrow). Fig. 5. Cross section of 1-year-old stem showing the diffuse-porous nature of the wood (*W*) located between the pith (*PI*) and dormant cambium (*C*). (Figures 2–4 have the same magnification.)

rib or from older to younger leaves). Subsequently, mitotic activity in the leaves increases and when this occurs mitotic figures begin to appear in the cauline portions of the shoot. In the shoot, mitoses predominate in and near the procambium. In one bud 143 mitotic figures were visible in the seven most median sections. Of these, 40 percent were in the procambium and 36 percent in the five parenchyma cell layers that border the procambium. Another bud that was not as active had 43 mitotic figures of which 38 percent were in the procambium and 48 percent in the five parenchyma cell layers that border the procambium. This preponderance of mitotic figures in and immediately adjacent to the procambium, when considered in light of the relatively small proportion of the *area* of the bud occupied by the procambium (Fig. 1) and the limited *number* of procambial cells present in sectional view (approximately 13 percent of the total number of cells), suggests that the progression of mitotic reactivation is largely via the procambium (assuming equal mitotic time in all tissues). While this preponderance of mitoses in the procambium is no conclusive reason to expect that the growth regulators that trigger mitotic activity are translocated in the procambium, it certainly is suggestive of such a phenomenon.

Following the reactivation of the procambium and the immediately adjacent parenchymatous tissues, mitotic figures appear throughout the shoot. At the base of the bud where elongation is limited the new cell walls are largely longitudinal in orientation. In the more distal internodes, which do elongate, the new walls are predominantly transverse.

All occurrences thus far described take place at the time the bud is just beginning to expand noticeably. Cambial reactivation also occurs in the upper stem sections at this time. This is in agreement with Lodewick's (*3*) generalization that in species with ring-porous wood (white ash is ring-porous) cambial reactivation occurs at about the time buds open. In diffuse-porous species leaf expansion generally precedes cambial reactivation (*3*).

The dormant cambial zone in white ash is generally three to four cells wide. The fusiform initials are tabular in cross section, undifferentiated, and cannot be distinguished from one another (Fig. 2). Generally these cambial cells abut on the mature secondary xylem of the first annual ring, but occasionally they are separated from the latter by a partially differentiated xylem mother cell. Regardless of which condition applies, the first sign of cambial reactivation is the enlargement of the xylem mother cell or the centripetal cell of the cambial zone (Fig. 3). Subsequently, the first mitotic figures (Fig. 4, arrow) or thin, new tangential walls (Fig. 3), indicative of a mitotic division, appear in cambial cells which apparently do not expand prior to division. The initial divisions generally occur in the cambial cells adjacent to the xylem mother cells (Figs. 3 and 4) and spread from these loci to the other cells in the cambial zone. As the cambium continues to function, earlywood vessels begin to differentiate and generally they develop directly from the xylem mother cells immediately adjacent to mature secondary xylem.

Ray initials in the cambial zone enlarge and divide commensurate with the reactivation of the fusiform initials. However, the first mitotic figure in a ray is generally found in the initial immediately adjacent to the mature secondary xylem.

In many cases the reactivation of the cambium at the level immediately below the bud does not occur simultaneously over its circumference. Frequently portions of the cambium below the bud scales remain dormant for longer periods of time than the remainder of the cambium. This occurrence may bear a direct relationship to the fact that mitoses in the scales are few in number, and they rarely develop into functional leaves. The scales, therefore, probably do not produce the growth regulators necessary for cambial reactivation. As the vascular system in the stem below the terminal bud is often oval in cross section, with the wide extremities of the oval beneath the scales, this delayed reactivation provides the opportunity for the cambium to "round out."

The subsequent reactivation of cambial initials in basal portions of the stem is similar in occurrence to that in the upper stem section. However, the stimulus to divide proceeds down the stem relatively slowly. The cambium in the basal stem section does not become active until the buds that terminate the system are about 2 cm in length and are expanding three pairs of leaves, the largest of which is 2 to 3 cm long. The time required for a bud to proceed from a state such as that in Fig. 1, in which the cambium at the base of the bud has just reactivated to one that is about 2 cm long, is 2 to 3 days. As the seedlings were only 19 to 26 cm long, this indicates a rate of spread of cambial reactivation of at most 6 cm per day. This is in contrast to earlier observations on trees with ring-porous wood (white ash is a ring-porous species) in which the rate of the basipetal movement of cambial divisions was so fast that a time lapse could not be detected (*1*). However, the wood present in the 1-year-old ash seedlings is diffuse-porous (Fig. 5) and the seedlings do not begin to produce ring-porous wood until their second year of growth.

Wareing (*4*) speculated that the rapid spread of cambial activity in ring-porous species is due to the presence of a hormone precursor in the cambial or cortical tissue before bud break. More recently Digby and Wareing (*5*) have demonstrated a growth-promoting substance in the cambium of ring-porous (*F. excelsior*) but not diffuse-porous species. This substance has an R_F which corresponds closely to the R_F of tryptophan. Digby and Wareing suggest that the rapid conversion of tryptophan to indoleacetic acid is responsible for the rapid spread of cambial activity in ring-porous species. The absence of rapid cambial reactivation and ring-porous wood in the 1-year-old seedlings and the presence of ring-porous wood in older seedlings make *F. americana* an ideal test object for further exploration of the interrelationship of wood porosity, the presence of tryptophan, and the spread of cambial reactivation.

HERBERT B. TEPPER
CHARLES A. HOLLIS
Department of Forest Botany and Pathology, State University, College of Forestry, Syracuse, New York

References and Notes

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