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LENTICEL AND WATER ROOT DEVELOPMENT OF SWAMP TUPELO UNDER VARIOUS FLOODING CONDITIONS

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ABSTRACT

Seedlings of swamp tupelo (*Nyssa sylvatica* var. *biflora* [Walt.] Sarg.) were grown in nonflooded soil or soil that was intermittently flooded, continuously flooded, or surface-saturated with moving or stagnant water. Lenticels on nonflooded seedlings were round, only slightly hypertrophied, and had few closing layers. Degree of hypertrophy and number of closing layers per lenticel increased with surface saturation and intermittent flooding, but closing layers were absent under continuous flooding. Flooded lenticels remained nearly round despite variations in hypertrophy. In all treatments, intercellular spaces were abundant in the complementary tissue and phellogen because of the spherical shape of these cells. Although the continuity of intercellular spaces was interrupted because the closing layers anastomosed, breaks within the closing layers prevented these spaces from being completely blocked. Water roots developed primarily under continuous flooding in moving water, some apparently originating beneath the phellogen of a lenticel and others within the phellogen or its derivatives.

Introduction

Aeration of interior tissues in the stems of woody plants is poorly understood. Lenticels are assumed to function in gas exchange because of the continuity of intercellular spaces in the lenticel tissues with those in the interior of the stem (ESAU 1965). However, the cambial zone forms a continuous sheath around the xylem and is apparently void of intercellular spaces. Because of the cambial sheath, gas exchange between the phloem and xylem is probably nil. Lenticels are interconnected apparently by the continuity of intercellular spaces in cortical and phloem tissues. This interconnection is verified in woody stems, in that air can be continuously pulled through submerged lenticels by applying a slight vacuum to the inundated portion of the stem if some lenticels are exposed to the atmosphere (THOMAS 1937). Also, SCHOLANDER, VAN DAM, and SCHOLANDER (1955) have demonstrated in black mangrove (*Avicennia nitida* Jacq.) that gas exchange occurs between the air and submerged main lateral roots through lenticels on exposed pneumatophores. The lateral roots of this species are very spongy and contain large amounts of intercellular space. Similarly, the swollen bases and roots of water tupelo (*Nyssa aquatica* L.), an ecological associate of swamp tupelo, are soft and parenchymatous; but, in contrast they contain practically no intercellular spaces in the xylem tissue (PENFOUND 1934).

Under flooding, numerous lenticels proliferate on the submerged stems of swamp tupelo seedlings, and new roots may develop concomitantly in the vicinity of the proliferated lenticels (HOOK 1968). Physiological studies indicate that the stem lenticels of swamp tupelo are functional in aerating the newly formed roots under flooded conditions (HOOK, BROWN, and KORMANIK, in press).

The purpose of this paper is to describe the anatomy of stem lenticels, phloem fiber differentiation, and the development of new roots (termed "water roots" in this paper) of swamp tupelo seedlings grown under various flooded and nonflooded conditions.

Methods

Seedlings were grown in the hydro-edaphytron (fig. 1) located on the Santee Experimental Forest, Berkeley County, South Carolina. This installation consists of six growth compartments, each of which is a 1.83-m cube. Each compartment has separate controls for regulating water flow and internal soil drainage. Depth, rate, and time of flooding, as well as vertical and horizontal movement of water across and within the soil, can be regulated. Tops of seedlings are exposed to the natural environment. The treatments imposed were:

1. Continuous surface saturation of the soil with stagnant water (SS-S).
2. Continuous surface saturation with moving water (SS-M).
3. Intermittent flooding with stagnant water: one week of flooding and two weeks of surface saturation. Cycle repeated throughout the growing season—stagnant soil water (Int-S).
4. Intermittent flooding with moving water: one week of flooding and two weeks of surface saturation. Cycle repeated throughout the growing season—moving soil water (Int-M).
5. Continuous flooding with stagnant water (Fld-S).
6. Continuous flooding with moving water (Fld-M).
7. Seedlings from the same seed sources were grown nearby in a moist but well-drained nursery bed (N-Fld), as a control.

Flooding depth was 20 cm in the Int and Fld treatments. (Between daily adjustments, the water level fluctuated about 2 cm.) One-year-old seedlings of swamp tupelo were planted in April 1964 and March 1965; the first planting was harvested in early November 1964 and the second in early November 1965.

At each harvest, stem sections (23-cm-long sections above the soil) were collected from two randomly selected seedlings in each treatment (except N-Fld) and stored in formalin-acetic acid-alcohol. These sections were later cut into approximately 2.5-cm segments and numbered 1 to 9 from the top.

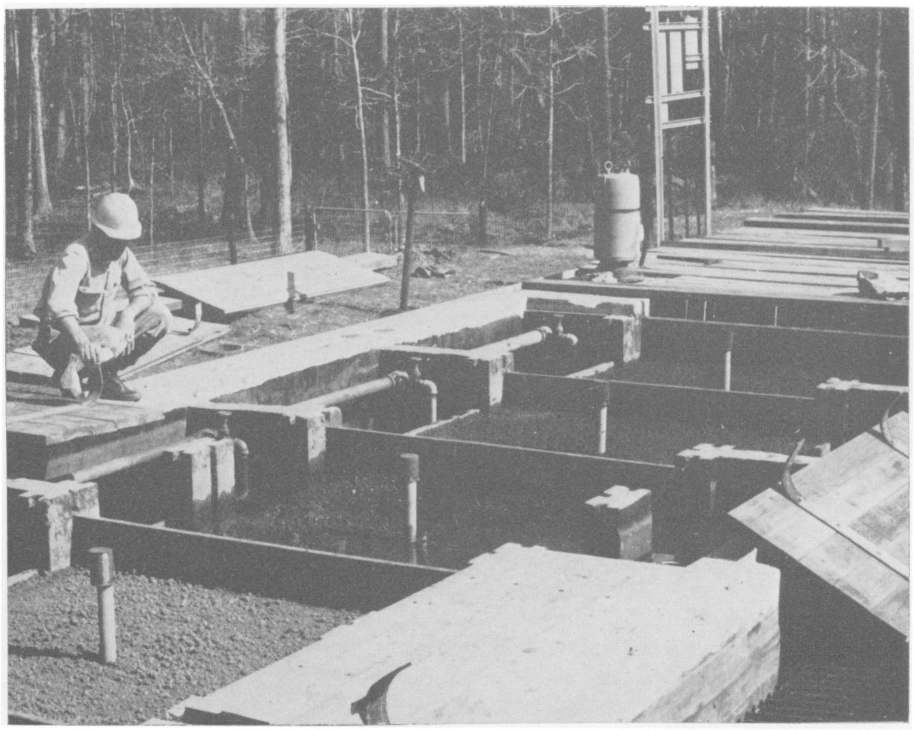


FIG. 1.—The hydro-edaphytron just prior to planting. Flood tanks abut each compartment on the right and left sides. Pipes in the center of the compartments are access tubes for neutron probes not used in this study.

The segments were then dehydrated in a graduated seven-step tertiary-butyl alcohol series, impregnated with tissuemat, and soaked in 1% detergent for 10 to 12 hrs. Segments 1 and 8 were serially cut into 12- to 15-μ thicknesses, stained in safranin-anilin blue (JOHANSEN 1940), and permanently mounted.

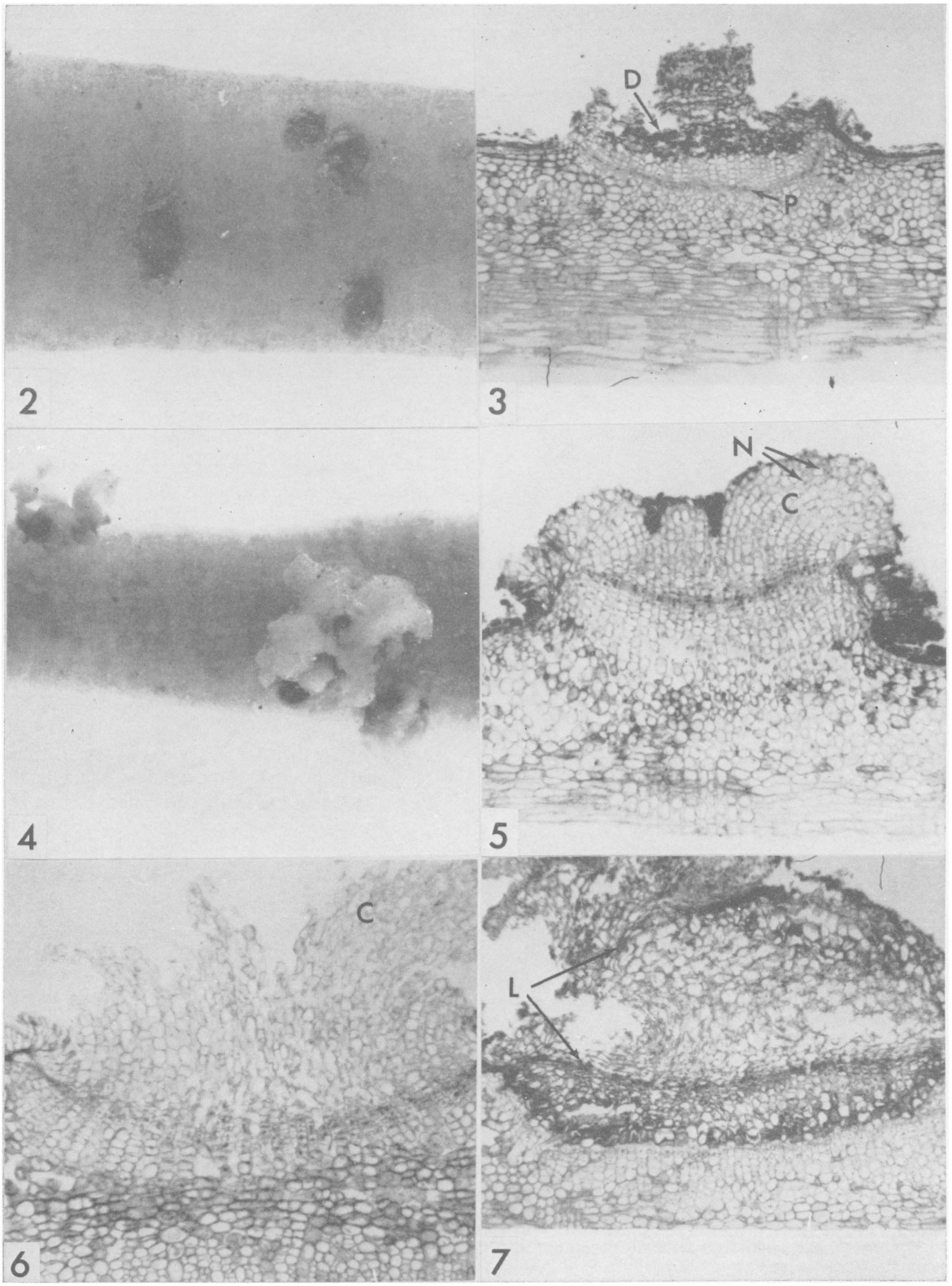
In addition, slivers of bark and xylem were cut from two seedlings in each treatment (including N-Fld) on May 21 and 28, June 11, and July 9, 1965. The slivers from the Fld-M, Fld-S, Int-M, and Int-S treatments were cut at 2–7 cm and at 20–23 cm above the soil, and those from the SS-M, SS-S, and N-Fld treatments were cut at 2–7 cm above the soil. These slivers were stored immediately in FAA.

During the summer of 1967, additional samples were collected from seedlings subjected to the Fld-M treatment. These fresh stem segments were sectioned on a freezing microtome and stained as previously described to verify further the origin of water roots.

Results

LENTICEL ANATOMY.—In the N-Fld treatment, lenticels of swamp tupelo seedlings were round and protruded only slightly above the periderm (fig. 2). Such lenticels had well-defined phellogens and complementary tissues beneath the phellogens. Degenerated cells (closing cells) were located only a few cells exterior to the phellogen (fig. 3). Generally, the phellogen was orientated in a concave manner to the cambium, and all cells distal to the phellogen were devoid of nuclei. Degenerated cells rapidly absorbed safranin, indicating they were probably suberized.

In the Fld-M and Fld-S treatments, lenticels below the water surface protruded well above the periderm. Although these proliferated masses of cells projected back toward the bark in flanges, they retained the round shape characteristic of swamp tupelo lenticels (fig. 4). Well-defined complementary cells were present; these were interior and exterior to the phellogen (fig. 5). Closing layers were absent. Large, well-defined nuclei were observed 15 to 20 cell layers exterior to the phellogen. On some of the



FIGS. 2–7.—Fig. 2, Tangential view of lenticel development on nonflooded seedling grown in the nursery bed; ×5. Fig. 3, Radial section of nonflooded lenticel from the nursery bed; note degenerated complementary cells (*D*) exterior to the phellogen (*P*); ×225. Fig. 4, Tangential view of continuously flooded lenticel (Fld-M); note proliferation, flanges, and round shape; ×5. Fig. 5, Radial section of continuously flooded lenticel (Fld-M); note active complementary cells (*C*) with nuclei (*N*) exterior to the phellogen, elevated phellogen, and absence of closing layers; ×180. Fig. 6, Cross-sectional view of continuously flooded lenticel (Fld-S); note active complementary cells (*C*) exterior to phellogen and a recessed phellogen lying beneath the previous plane of the periderm; ×180. Fig. 7, Radial view of lenticel from the SS-S treatment; note proliferation with degenerated complementary cells and several closing layers (*L*); ×180.

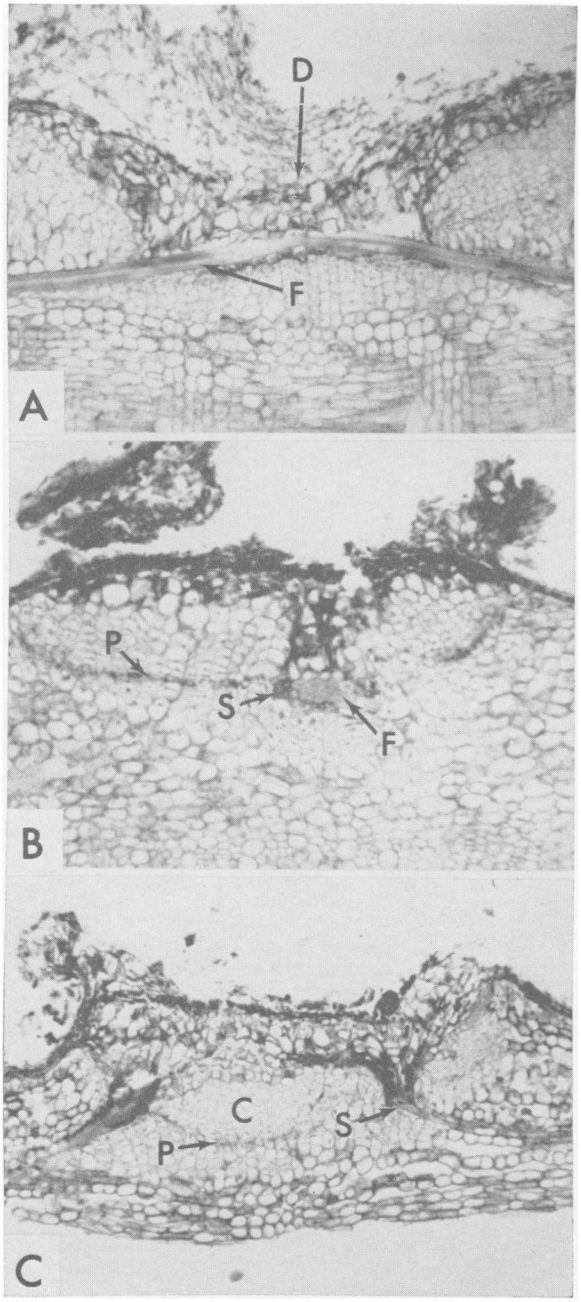


FIG. 8.—Cortical fiber and sclereid development in lenticels subjected to intermittent flooding. *A*, Radial view of longitudinal cortical fiber (*F*) in lenticel with degenerated complementary cells (*D*) above the fiber; ×185. *B*, Cross-sectional view of fibers (*F*) and associated sclereids (*S*) in lenticel with newly differentiated phellogen (*P*) beneath the fiber bundles; ×185. *C*, Radial view of lenticel with complementary cells (*C*) and phellogen (*P*) interrupting the sclereid bundle (*S*); ×185.

lenticels the phellogens were exterior to the surface of the periderm (*see* fig. 5), but on others the phellogens were typically receded beneath the periderm (fig. 6).

Lenticels on the stems of seedlings grown in the SS-S and SS-M treatments (fig. 7) showed more hypertrophy than did lenticels on the seedlings in the N-Fld treatment (fig. 3). There were several well-defined closing layers protruding outward on lenticels in the SS treatments, but the closing layers were compacted or were singular on lenticels in the N-Fld treatment. Either the closing layers sloughed off more readily in the N-Fld treatment than in the SS treatment or the complementary cells were more active (repeatedly rupturing the phellogen) in the lenticels in the SS treatments than in the N-Fld treatment.

Lenticels from the cyclic water treatments (Int-S and Int-M) and lenticels along the mean flood line in other treatments often contained groups of sclereids associated with the longitudinal cortical fibers (fig. 8*A*). In cross-section, active ground parenchyma cells (large nuclei) surrounded the fibers and sclereids, particularly those beneath the bundle (fig. 8*B*). As successively new phellogens differentiated centripetally, groups of cortical fibers were occasionally cut off exteriorly, and new sclereids differentiated in association with the fibers from phellogen derivatives (fig. 8*C*).

Tangential sections of lenticels from all treatments showed abundant intercellular spaces in the phellogen; these spaces resulted primarily from the spherical shape of phellogen cells (fig. 9*A*). However, the cells in the closing layers collapsed and anastomosed upon senescence, apparently interrupting or impeding the continuity of intercellular spaces (fig. 9*B*).

Cortical fibers were a consistent feature of all stem sections on the young swamp tupelo seedlings (fig. 10). In contrast, secondary phloem fibers were absent in stem sections that were not flooded, but were prevalent just exterior to the cambium in stem sections that were flooded or came from near the flood line (fig. 10*A*). In the seasonal observations, secondary phloem fibers were found as early as May 21 in the Int-M treatment, but not until July 9 in the Int-S, Fld-S, and Fld-M treatments. Protoplasmic bodies were present in many phloem fibers in May and June, but fibers were fully differentiated by July in the Int-M treatment.

WATER ROOT DEVELOPMENT.—Development of water roots was restricted almost exclusively to the Fld-M treatment, but a few water roots were found in the Fld-S treatment. Water roots were adventitious in origin because their vascular connections

terminated within the annual ring formed during treatment (fig. 11*A*). The loci of root initiation indicated that water roots arise in several different ways. Most water roots had lenticel remnants around their bases; these roots probably originated beneath the phellogen of the lenticel and erupted through the lenticel, destroying most of the phellogen in the process (fig. 11*B*). Some water roots were found in lenticels with the phellogen intact, except directly in the center of the root, indicating that these roots originated in the phellogen or its derivatives (fig. 11*C*). One water root was found without lenticel remnants around its base; this root probably originated deep within a phloem ray or from the vascular cambium independent of a lenticel (fig. 11*D*).

Discussion

WETMORE (1926) and ESAU (1965) separate transverse from longitudinal lenticels by the orientation of the fissure. Furthermore, WETMORE found that the phellogens of longitudinally orientated lenticels were either less organized or more frequently interrupted than those of transverse lenticels. He concluded the former were better adapted for gas

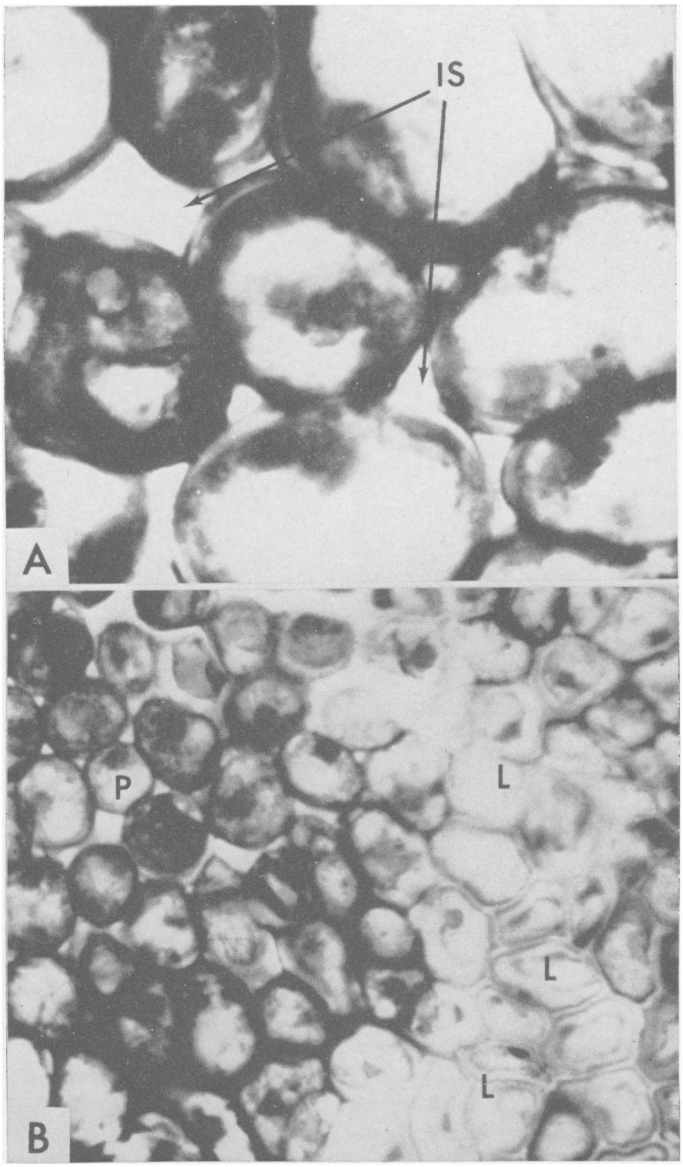


FIG. 9.—Tangential sections of lenticels. *A*, Continually flooded lenticel; note intercellular space (*IS*) in the phellogen; ×1,750. *B*, Nonflooded lenticel; note closing layer (*L*) and phellogen (*P*); ×925.

exchange. The nearly circular shape of the swamp tupelo lenticels does not fit into either the transverse or longitudinal classification, but they do have ample intercellular spaces, at least under continuously flooded conditions. The intact phellogen has intercellular space because of the spherical shape of the cells, but lenticels on nonflooded stems (SS and N-Fld treatments) may be less pervious to gas exchange because the cells in the closing layers collapse and anastomose.

The absence of closing layers on flooded lenticels was reported by DEVAUX (1900). Lenticels on continually flooded stems of swamp tupelo exhibited this characteristic. However, little evidence was obtained to support TEMPLETON'S (1926) observations that lenticels in poorly aerated water were more hypertrophied than those in periodically aerated tap water. On swamp tupelo, the degree of hypertrophy is similar in plants both in the poorly aerated and in the well-aerated treatments (*see* figs. 3, 4). However, there are more closing layers in lenticels on stems grown in surface-saturated soils than in those grown in a well-drained nursery bed. This difference in lenticel activity may have arisen because moisture content of stems and air is higher above surface-saturated soil than above well-drained soil. DEVAUX reported that air saturated with water vapor resulted in similar lenticel development as did submersion in water.

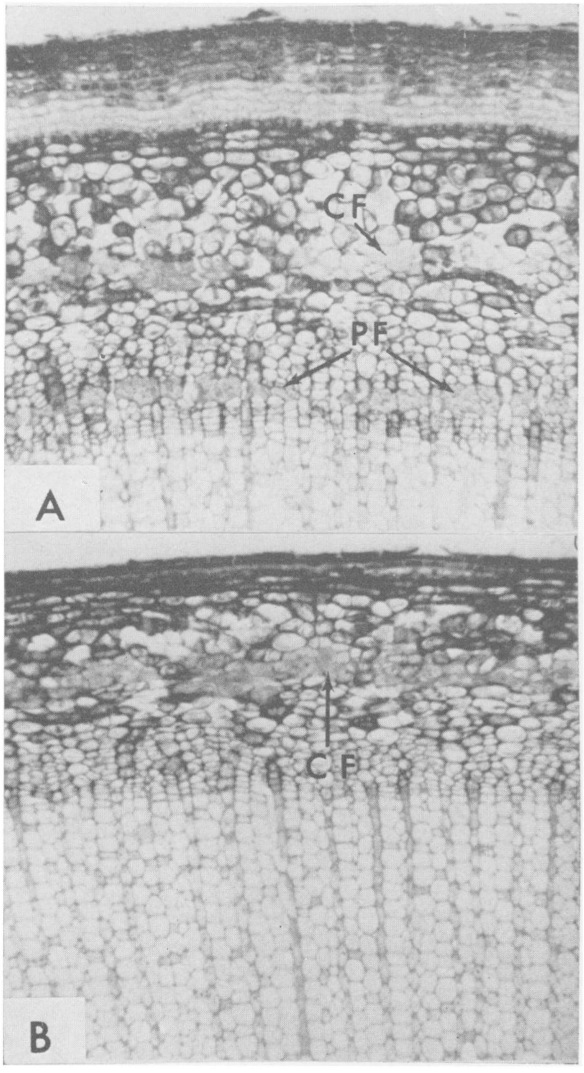


FIG. 10.—Cortical and secondary phloem fibers in flooded and nonflooded stem segments from the Int-M treatment. *A*, Cross section of intermittently flooded stem segment with phloem fibers (*PF*) differentiating near cambium and cortical fibers (*CF*) in cortex; ×205. (This segment was below the flood line.) *B*, Cross section of nonflooded stem segment with cortical fibers only; ×215. (This segment was above the flood line.)

The differentiation of sclereid bundles associated with lenticels could be related to differences in gas exchange and spatial relationships at sites of proliferation. During flooding, complementary cells may be produced in abundance and give rise to more intercellular space, whereas in the nonflooded phase, sclereids may be stimulated to differentiate and fill the air space. FOSTER (1947) found that sclereids grew into the intercellular space in the leaves of *Mouriria*, and BLOCH (1946) reported the intrusion of sclereids into the intercellular space in air roots of *Monstera*. If sclereids fill intercellular spaces in lenticels of nonflooded swamp tupelo, it suggests that flooding increases the amount of intercellular space in the vicinity of the lenticel, thereby facilitating gas exchange.

During the flooded phase, complementary cells remain active above the phellogen and no closing layer is produced. In the presence of moving water, a higher oxygen potential could exist at the lenticel surface than in the phloem and thus create an inward diffusion of oxygen. At the beginning of the nonflooded phase, the lenticel would be hypertrophied and should be highly pervious to gas exchange. Hence, it seems plausible that, as a water table recedes, there is an influx of oxygen into the cortex and phloem through the lenticel of swamp tupelo. Because lenticels collapse in the absence of water (HOOK 1968) and closing layers typically develop on all but continuously flooded lenticels, it is assumed that there is a reduction in gas exchanged via lenticels shortly after a water table recedes.

There appear to be sufficient breaks or spaces within the closing layers to permit limited gas exchange. HOOK et al. (in press) found that oxygen was transported down the stem of nonflooded and flooded swamp tupelo seedlings, but that oxygen transport was effectively blocked if all stem lenticels were covered by a paraffin-lanolin mixture.

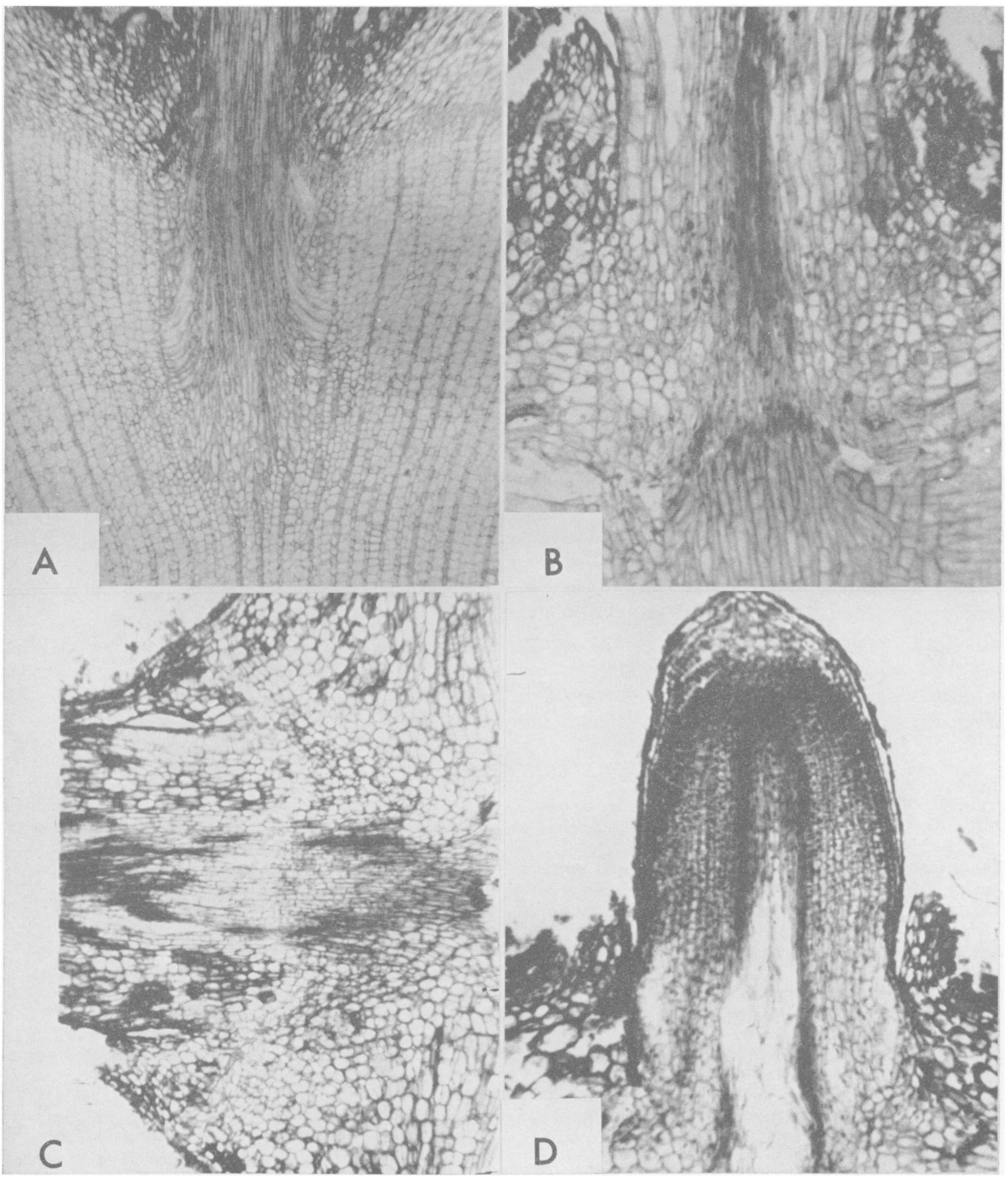


FIG. 11.—Adventitious nature of water roots and differences in loci of origin. *A*, Cross section of a stem with vascular connections of a water root terminating within the outer growth ring; ×110. *B*, Radial view of water root (Fld-M); note lenticel remnants around the base of the root; ×185. *C*, Radial view of water root; note the phellogen flanking the periphery of the root; ×140. *D*, Radial view of water root immediately after erupting through the periderm; lenticel remnants are absent around the base of the root; ×195.

Differentiation of phloem fibers around the periphery of the cambium appears to be related to flooding of the stem. In the absence of flooding, only cortical fibers are present; but, in sections subjected to flooding or sections near the flood level, phloem fibers are abundant adjacent to the cambium. Early differentiation of phloem fibers under intermittent flooding with moving water suggests that alternation of flooding and aeration of phloem may be a factor in early differentiation of phloem fibers and increased sclereid activity. Furthermore, the development of phloem fibers is directly correlated with the production of a soft and parenchymatous xylem. PENFOUND (1934) found that, in water tupelo, the number of parenchyma cells is greater and cell walls are thinner in the swollen base than in the trunk above the swollen base. He also found that water content is higher in the swollen base than in the trunk. In the present study, observations indicate that the early initiation of phloem fibers near the cambium of flooded seedlings strengthens the phloem at the same time that the xylem is weakened by more parenchyma cells and thinner cell walls.

The sclerenchyma cells in the mature bark of black tupelo (*Nyssa sylvatica* var. *sylvatica*) differ distinctly from those in water tupelo. Water tupelo has almost continuous bands of phloem fibers around the stem, but sclereids are either poorly developed or absent. In contrast, black tupelo has fiber bands and sclereid groups; sclereids predominate, especially in the outer bark (CHANG 1954).

Water roots arise adventitiously in the stems of swamp tupelo, primarily under the stimulation of continuous flooding with moving water. No preformed initials were found in the stem sections observed; hence, differentiation and elongation of root primordia must rapidly follow the inception of root initials. GIROUARD (1967) found that adventitious roots in stem cuttings of *Hedera helix* arose from parenchyma cells in the phloem rays. HARTMAN and KESTER (1959), SACHS, LORATI, and DEBIE (1964), and SATOO (1955) have reported similar origin of adventitious roots in several woody species. Adventitious roots may also arise from vascular cambium (SINNOTT 1960). In swamp tupelo, the adventitious water roots that erupt through lenticels and those that erupt through the periderm in the absence of lenticels could originate from the phloem ray cells or vascular cambium. However, water roots that develop through a lenticel and destroy only a small segment of the phellogen could originate from the phellogen or its immediate derivatives.

The formation of water roots appears to be beneficial because they occur in the upper flood water, where the oxygen content is higher, and toxic compounds such as CO_2 are lower, than in the soil. Water roots also increase the absorption area for water and nutrients, and eventually function in support of the tree.

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