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THE MODE OF ORIGIN OF ROOT BUDS AND ROOT SPROUTS IN THE CLONAL TREE, *SASSAFRAS ALBIDUM*

By

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ABSTRACT

THE MODE OF ORIGIN OF ROOT BUDS AND ROOT SPROUTS IN THE CLONAL TREE, *SASSAFRAS ALBIDUM*

By

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The purpose of this study was to determine the mode of origin of root buds and root sprouts in *Sassafras albidum* (Nuttall) Nees. Root samples from 13 clones were sectioned and two types of buds were found, suppressed trace buds and exogenous buds. Suppressed trace buds form during the early growth of a root and they perennate by growing outwards with the vascular cambium. Exogenous buds from during the later growth of a root from proliferated pericycle cells. Instead of forming connections with the vascular cambium, exogenous buds cause the formation of nodules of wood, or sphaeroblasts. To determine if both buds were functional, 112 sprouts were sectioned at the root-stem junction. None of the sprouts were subtended by sphaeroblasts, but over 97% of the sprouts were subtended by traces, indicating that they had originated from suppressed trace buds. Although exogenous buds can form in great numbers, they appear to be dysfunctional.

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CHAPTER 1 - GENERAL INTRODUCTION

TAXONOMY

Sassafras albidum is a member of the Lauraceae family, which includes over 1000 species of aromatic trees and shrubs (Berry, 1923). Familiar species in the family include cinnamon (*Cinnamonum zeylanicum*), the camphor tree (*Cinnamonum camphora*), and true laurel (*Lauris nobilis*) (Dathe, 1984). Most Lauraceae species are tropical in distribution with the family being particularly well represented in northern South America (Berry, 1923). Only three genera are native to the eastern United States - *Sassafras, Persea,* and *Lindera* (Gleason and Cronquist, 1963).

Trees in the family are alternate-leaved and often dioecious (Gleason and Cronquist, 1963). The stamens of male flowers are arranged into four whorls of three, but the stamens of one or more whorls are typically reduced to staminodia, glands, or they are absent (Gleason and Cronquist, 1963). Functional anthers may be disporangiate or tetrasporangiate (Rohwer, 1994), and they open by means of uplifted valves or "anther flaps" (Gleason and Cronquist, 1963). The gynoecium consists of a simple ovary with a single suspended ovule (Gleason and Cronquist, 1963). Two perianth whorls are present in the flowers of each sex, but petals and sepals cannot be distinguished (Gleason and Cronquist, 1963). The fruit is a drupe, and the seeds lack an endosperm (Gleason and Cronquist, 1963).

GEOLOGIC HISTORY/RELATIVES:

The genus *Sassafras* formerly grew throughout North America, Greenland, and Europe, where it survived until the last continental glaciation (Dathe, 1984). The genus, which is at least 100 million years old, reached its peak of diversity during the upper Cretaceous Age (Berry,

1923; Dathe, 1984). Over 12 fossil species of *Sassafras* have been identified in rocks of the upper Cretaceous (Berry, 1923). Leaf fossils of *Sassafras* are especially common in shore deposits from the upper Cretaceous Sea that formerly covered the North American Plains (Berry, 1923). Today, there are three recognized species of *Sassafras* - *S. albidum, S. tzuma,* and *S. randaiense,* which are native to North America, China, and Taiwan, respectively (Dathe, 1984).

RANGE/HABITAT:

Sassafras albidum ranges from central Florida north to southern Maine, west through southern Ontario and lower Michigan to Missouri, and from Missouri south through eastern Kansas and Oklahoma to Eastern Texas (Peattie, 1964; Dathe, 1984; Gleason and Cronquist, 1991; Burns and Honkala, 1990). Throughout most of its range *S. albidum* grows at low elevations, but in the Appalachian Mountains it ascends to over 1000 meters (Fowells, 1965). The range of *S. albidum* is in a humid region with an average annual rainfall varying from 30 to 55 inches/year (Fowells, 1965).

Although *S. albidum* is a conspicuous invader of roadsides, old fields, and thickets, its also occurs in dry woods, mixed deciduous woods, and low wet woods (Voss, 1980; Gleason and Cronquist, 1991). *S. albidum* is considered to be a dominant or co-dominant tree in only two forest cover types, though, the Sassafras-Persimmon Type, a temporary cover which is characteristic of abandoned fields of the upper coastal plain north into the Ohio Valley, and the Bear Oak Type, which is restricted to sites with droughty, acidic soils, which have been disturbed in the recent past by heavy cutting , fire, or both. (Eyre, 1980)

At mid-elevations (620-840 m) in SW Virginia, *S. albidum* is distributed across stands with site indices$_{50}$ ranging from 11 to 21m (Martin et al., 1982a). The site index of a stand is a measure of the height of the dominant trees in the stand at an arbitrarily-chosen age, commonly 50 years (Burns and Honkala, 1990). Surveys by Martin et al. (1982a), have shown that in most

stands in SW Virginia, *S. albidum* is present as scattered individuals or small pure clumps, but in dry stands with site low indices it is a dominant understory tree. In several parts of Appalachia, *S. albidum* has increased in importance since the chestnut blight (Mackey and Sivec 1973). In Michigan, *S. albidum* is a common component of sand dune forests (Thompson, 1982) where it occurs in both the canopy and the understory. According to Peattie (1930), *S. albidum* is one of the most common woody plants in the Indiana Dunes National Lakeshore, but it grows as little more than a shrub in early-successional dune habitats.

Sassafras albidum prefers loamy, moist, well-drained acid soils (Dathe, 1984) and it has been observed to develop chlorosis in high pH soils (Dirr, 1975). In Ohio, *S. albidum* is absent from calcareous soils (Braun, 1961) and in Indiana it is thought to be an indicator of poor sites (Deam, 1931). *S. albidum* is most commonly found growing on soils of the orders Entisols, Alfisols, and Ultisols (Burns and Honkala, 1990) and its optimum pH is 6-7 (Spurway, 1941).

Sassafras albidum is common on droughty soils throughout its range. In the sandy barrens of central Pennsylvania, *S. albidum* saplings have been shown to maintain higher mid-day leaf water potentials, than co-occurring saplings of *Quercus velutina, Q. prinus*, and *Acer rubrum*, which are presumably less adapted for growth in xeric habitats (Kloeppel et al., 1994). Although *S. albidum* stems are intolerant of fire at all ages, it is quick to resprout following disturbances and it is therefore able to persist in habitats which are subject to frequent fires, such as prairies, glades, and barrens (Anderson, 1977). In the Pine Barrens of New Jersey, *S. albidum* occurs in association with *Q. ilicifolia* (bear oak), *Prunus serotina, Gaylussicia frondosa, Rhus copallina, Myrica carlinensis, Ilex glabra, Kalmia latifolia*, and *Smilax glauca* in the "dwarf oak understory" (Harshberger, 1970).

REPRODUCTIVE CHARACTERISTICS:

Sassafras albidum is subdioecious. Subdioecious species produce separate male and female individuals, but one or both of the sexes is often bisexual (Lloyd and Bawa, 1984). In *S. albidum* the males are polygamous and the females are unisexual. Polygamous trees produce both unisexual and bisexual flowers (Styles, 1972). Based upon the examination of three inflorescences per stem, Blount (1989) found that nearly 15% of the male stems *of S. albidum* occurring in the mid-elevational hardwood forests of Southwest Virginia had bisexual flowers, but he noted that pistils produced by the bisexual flowers of these stems were smaller than the pistils of female flowers. In Michigan, the pistils of bisexual flowers also tend to be small and often necrotic at their apices (Bosela, personal observation). Furthermore, it is not uncommon for these flowers to have extra tepals (7-8 instead of 6) and anomalous numbers of stamens (Bosela, personal observation).

The inflorescences produced by *S. albidum* are panicles which form in the scales of terminal buds (Voss, 1980). A single terminal bud may produce up to 8 panicles, each with as many as 10 spirally-arranged flowers. Because of the presence of these inflorescences, the terminal buds of *S. albidum* are swollen and ovoid in shape (Dathe, 1984). The inflorescences emerge early each spring before leaf expansion has occurred, (Voss, 1980) and each flower is subtended by an elongate, pubescent bract which may aid in bud opening (Bosela, personal observation).

The tepals and pedicels of *S. albidum* flowers are yellow-green in color (Gleason and Cronquist, 1991). A typical male flower has 6 persistent tepals, and 9 stamens, the inner three with pairs of glandular staminodia (Gleason and Cronquist, 1991). The anther of each stamen is introrse, tetrasporangiate, and dehiscent by means of "anther flaps". A typical female flower has 6 persistent tepals, 6 short staminodia, and a single superior pistil with one locule and a one ovule.

The fruits of *S. albidum* are dark, blue drupes with an ellipsoid shape (Gleason and Cronquist, 1991). The pedicel subtending each drupe turns a brilliant red and enlarges near its apex during fruit maturation. The apex of the pedicel and the persistent perianth together form a cuplike base beneath each drupe (Gleason and Cronquist, 1991). The seeds of *S. albidum*, and all members of the Lauraceae family, have a perisperm rather than an endosperm and the diploid chromosome number of the species is 48 (Cronquist and Gleason, 1991). Studies by Wendel (1977), have shown that most *S. albidum* seeds do not germinate until at least 1 to 2 years after their maturation. Stems of seed origin may bear fruit after 10 years (Fowells, 1965) and stems of sprout origin may bear fruit after even shorter periods of time (Blount, 1987).

VEGETATIVE CHARACTERISTICS - THE SHOOT SYSTEM:

The leaves of *S. albidum* are alternate in phyllotaxy and variable in shape (Peattie, 1964). They may be simple, two-lobed (mitten-shaped), or three-lobed. In young specimens, simple leaves predominate at the proximal and distal nodes of a shoot, but lobed leaves are typically found at the intermediate nodes (de Soyza et al, 1990). Lobed leaves tend to be larger than simple leaves and to have a higher chlorophyll content (de Soyza et al, 1990), but most of the leaves of older specimens are small, unlobed, and boat-shaped or keeled in appearance (Peattie, 1964).

Sassafras albidum leaves are glossy on their upper surfaces and chalky white below with smooth margins. Internally, the leaves contain both mucilage and oil "cells" as are characteristic of the Lauraceae family (Solereder, 1908). The function of the mucilage cells has not been investigated, but since mucilages are known to bind water tightly (Mauseth, 1988) they may be important for the maintaining hydrated leaf tissues in xeric habitats and/or during dry spells. The oil cells, which presumably function to deter herbivory, give *S. albidum* leaves a characteristic

lemon-like odor. The young stems of *S. albidum,* which are vivid green in color, (Peattie, 1964) also have a similar odor.

Sassafras albidum is fast-growing and under favorable conditions it produces sylleptic branches (Barnes and Wagner, 1981). Sylleptic branches are derived from axillary meristems which grow out immediately without overwintering as a bud (Fahn, 1987). Sylleptic branches lack terminal bud scale scars at their base and their first internodes are usually highly elongate. Sylleptic branches are readily produced by the terminal leaders of young, healthy stems of *S. albidum.* Since sylleptic branches form preferentially in the axils of those leaves which are produced near the distal end of each growth increment, they tend to be clustered so as to form pseudo-whorls. The first-order sylleptic branches produced by the terminal leader may produce higher-order sylleptic branches, but higher-order sylleptic branches tend to be produced singly or in pairs and to grow at a horizontal orientation such that tiers of long laterally-spreading branches are produced (Bosela, personal observation).

Although *S. albidum* grows as a small tree or shrub on poor sites and at the northern and southern limits of its range (Fowells, 1965), on better sites in the interior of its range, individual stems may grow to heights of 30 m or more (Gleason and Cronquist, 1991) and live for up to 1000 years (Collingwood, 19778). The largest *S. albidum* specimen on record is 165 cm in diameter and 33 meters tall (Dathe, 1984). Old stems of *S. albidum* can be recognized by their deeply-furrowed bark, which is red-colored in the furrows and gray on the ridges as a result of weathering (Dathe, 1984; Gleason and Cronquist, 1991).

The wood of *S. albidum* is ring-porous with aliform parenchyma (Core et al., 1979). It is light (32 lbs/ft^3), it shrinks less upon drying than the wood of most hardwoods (Peattie, 1964; Dathe, 1984), and resistant to decay, as is characteristic of the Lauraceae family. Despite its durability, *S. albidum* wood is brittle (Berry; 1923; Dathe, 1984) and older trees are susceptible to snapping in association with the toppling of neighboring trees or as a result of the

accumulation of heavy ice loads during periods of freezing rain. Surveys conducted following a severe glaze storm (i.e. freezing rain) in Western New York, showed that up to 60% of the *S. albidum* stems of several forested stands showed crown damage (Seischab et al., 1993) The thin, spindly shape of *S. albidum* stems and their susceptibility to *Nectria* cankers (Blount, 1987) also contributes to their susceptibility to breakage.

VEGETATIVE CHARACTERISTICS - THE ROOT SYSTEM:

The embryos of most members of the Lauraceae family have a coleorhiza which protects the radicle during its early growth (Mauseth, 1988). The radicles of *S. albidum* seedlings are long-lived and they develop as deep taproots which prohibit the transplanting of trees or saplings (Dathe, 1984). After one year the root system of a *S. albidum* seedling typically consists of a single downward growing root (the radicle) and many feeder roots, but by the time a seedling is two years old, though, its lateral roots are well-developed (Blount, 1987).

By sprouting from its roots, *S. albidum* is able to from clones, or multi-stemmed trees, consisting initially of a stem of seed origin, known as a ortet, surrounded by stems of root origin, known as ramets (Stout, 1929; Barnes, 1966). Successful sprouts promote the continued growth of their parent roots as evidenced by the distal swelling of lateral roots just beyond the point of attachment of ramets (Duncan, 1935). In early successional and ruderal habitats, *S. albidum* almost always forms clones, but it is not known if clones are formed in late-successional habitats, such as woodlots.

The roots of *S. albidum*, which were once widely used to as a perfume and a flavor for medicines, candies, teas, and soft drinks including root beer, are fleshy and they produce a wide variety of fragrant isoprenoids (Kramer and Kozlowski, 1979; Clepper, 1989). Studies by Gant and Clebsch (1975) have shown that the types of isoprenoids which are present varies depending upon the size of the root. Small roots (< 1mm diameter) have high concentrations of alpha-

phellandrene, 2-pinene, and d-camphor, but larger roots (> 2mm diameter) have high concentrations of eugenol and safrole. The oil obtained from *S. albidum* roots is known medically as a demulcent (a substance capable of soothing abraded mucus membranes), an emollient (a substance which is soothing to the skin), a diaphoretic (a substance which increases perspiration), and a diuretic. (Clepper, 1989). Safrole, the main component of Sassafras oil has been shown to cause cancer in lab rats and it is now a federally regulated substance (Hagan et al., 1965)

ECOLOGY:

Sassafras albidum is a common invader of old fields and other types of early successional habitats. Although the fruits of *S. albidum* are dispersed by over 18 species of birds (Clepper, 1989), the research of Arnold (1966) suggests that *S. albidum* seedlings are more common in pine plantations than they are in eroded old fields. To explain these results, Bazzaz (1968) has hypothesized that the first *S. albidum* sprouts to appear in old fields following their abandonment may be derived from the roots of pre-existing fencerow trees which are broken by discing or plowing before the field's abandonment. According to Bazzaz (1969), the occasional occurrence of *S. albidum* sprouts in corn fields attests to the ability of broken root fragments to regenerate. After an initial shrub layer has formed, the number of birds visiting an old field (and the number of *S. albidum* seeds arriving at the field) would be expected to increase.

Studies of old field succession in Illinois, have shown that *S. albidum* and *Diospyros. virginiana.* are the dominant woody species in upland old fields for at least 40 years after their abandonment (Bazzaz, 1968). The success of *S. albidum* in old field habitats presumably depends upon its ability to form clones which can expand at rates of up to 112 cm per year (Duncan, 1935). Although clonal shrubs such as *Rhus* spp. and *Cornus* spp. are also common in abandoned fields, *S. albidum* is able to persist for much longer than either of these pioneers and

it has been hypothesized that *S. albidum* clones may be able to exclude competing vegetation by means of allelopathy. In support of this hypothesis, Gant and Clebsch (1975) have shown that leachates from the leaves, litter and soil samples of *S. albidum* clones are able to reduce the radicle growth of some tree seedlings

Sassafras albidum is typically thought of as shade-intolerant, but measurements by Bazzaz et al. (1972) suggest that it is moderately shade tolerant. For example, the dark respiration rates of *S. albidum* seedlings are equal to those which have been reported for *Quercus rubra* and lower than those which have been reported for *Liriodendron tulipifera* and *Populus tremuloides*, both of which are shade-intolerant. Similarly, the P_{50} values of *S. albidum* seedlings (600 ft-c) are comparable to those which have been reported for shade tolerant trees, such as *Fagus grandifolia* (500 ft-c), *Acer rubrum* (750 ft-c), and *Q. rubra* (750 ft-c). The P_{50} value is a measure of the light intensity at which photosynthetic rates are half of their maximum. The shade tolerance suggested by the data of Bazzaz et al. (1972) may account for the presence of *S. albidum* seedlings in pine plantations, but it is not great enough to allow for the indefinite height growth of stems beneath a closed canopy.

Pre-harvest surveys of four types of forest stands in SW Virginia (mixed oak, oak-pine, mixed hardwood, and mixed pine) showed that *S. albidum* was present in the canopy layer of only one of these stands (the mixed hardwood stand), but it was present in the shrub and herb layers of all of the stands (Martin et al., 1982). According to Blount (1987) *S. albidum* is able to persist for indefinite amounts of times at a site by means of a continual turnover of short-lived sprouts. In response to gap-formation or clearcutting, these sprouts are presumably released from suppression and they may assume canopy positions. In support of this hypothesis it was shown that three years after clearcutting in SW Virginia, *S. albidum* was the most common woody species in three of the four types of stands (Ross et al., 1982) and 7 years after clearcutting, it was the dominant shrub of mixed-oak sites and it was common in the forming tree layer of mixed

hardwood sites. Excavations conducted by Blount (1987) showed that over 90% of the *S. albidum* sprouts which appeared after clear-cutting on a xeric site were of sprout origin and 10% were of seed origin, but on a mesic site equal numbers were of seed and sprout origin. The rapid growth of *S. albidum* following clearcutting and its high nutrient use efficiency (Martin et al, 1982b) has lead to the suggestion that it may prevent nutrient losses following disturbances, much as has been proposed for pin cherry (*Prunus pennsylvanica*) seedlings in northern hardwood forests. (Marks and Borman, 1972)

CONCLUSIONS:

Sassafras albidum is a very distinctive tree. It is aromatic throughout, it produces unusual, heterophyllic leaves and green twigs, it is subdioecious, its flowers are specialized in so much as its pollen is released via the dehiscence of anther flaps, and it forms clones in many habitats. *S. albidum* is also unusual in that it occurs in North America, despite being a member of a tropical family of trees. Although *S. albidum* is perhaps best adapted for growth in early-successional habitats and xeric or highly disturbed habitats, it occurs in a wide diversity of habitats and is able to live for up to 1000 years. *S. albidum* was once an economically important tree species, but with the discovery of safrole's carcinogenic properties its use has become restricted. Ecologically, the importance of *S. albidum* is just beginning to be recognized. Its rapid regeneration may help to prevent the loss of nutrients from clearcut stands and its wide habitat tolerance and its ability to form clones may adapt it for use as a nurse tree in reforestation projects (Auten, 1945). Root sprouting appears to be an integral part of the ecological and evolutionary "strategy" of this species.

LITERATURE CITED

Anderson, R.C. 1977. Response of a southern Illinois grassland community to burning. Transactions Ill. Stat Acad. Sci. 69(4):399-414.

Auten, J.T. 1945. Relative influence of sassafras, black locust, and pines upon old-field soils. J. For. 43:441-446.

Barnes, B.V., 1966. The clonal growth habit of American aspens. Ecology 47(3):439-47.

Barnes, B.V. and W.H.Wagner. 1981. Michigan Trees. University of Michigan Press, Ann Arbor, MI.

Bazzaz, F.A. 1968. Succession on abandoned fields in the Shawnee Hills, Southern Illinois. Ecoogy. 49:924-36.

Bazzaz, F.A., V. Paape, and W.R. Boggess. 1972. Photosynthetic and respiratory rates of *Sassafras albidum*. For. Sci. 18:218-222.

Berry, E.W. 1923. Tree Ancestors. Williams and Wilkins Co., Baltimore, MD.

Blount, T.E. 1989. The biology of *Sassafras albidum* (Nutt.)Nees in regenerating upland hardwood stands in Southeast Virginia following clear felling. M.S. Thesis. Virginia Polytech Institute and State University.

Blount, T.E., J.E. Cook, D. Wm. Smith, and T.L. Sharik. 1987. Regeneration of Appalachian upland hardwood stands seven years after clearcutting. In. Proc. Fourth Bienn. South. Silv. Res. Conf. USDA For. Serv. Gen. Tech. Rep. SE-42. p. 113-19.

Braun, E.L. 1961. The Woody Plants of Ohio. Ohio State Univeristy Press. Columbus.

Burns, R.M. and B.H. Honkala (Eds.). 1990. Silvics of North America, Volume 2, Hardwoods. United States Department of Agriculture, Agriculture Handbook 654. 877 p.

Clepper, H. 1989. The singular sassafras. American Forests. 95(3/4):33-34, 57..

Collingwood, G.H. 1978. Knowing Your Trees. American Forestry Association. Washington, D.C.

Core, H.E., W.A Cote, and A.C. Day. 1979 Wood Structure and Identification. Syracuse University Press, Syracuse, NY.

Dathe, M. 1984. Sassafras in spite of itself. Horticulture. VXIII(9):19-24.

Deam, C.C. 1931. Trees of Indiana. Fort Wayne Printing Co, Fort Wayne, IN.

de Soyza, A.G., D.T. Kincaid, and C.R. Ramirez. Variability of leaf chlorophyll content in a population of *Sassafras*. Bull. Torrey Bot. Club. 117(2):167-72.

Dirr, M.A. 1990. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Popagation,and Uses. Stipes Publishing Company, Champaign, IL.

Duncan, W.H. 1935. Root systems of woody plants of old fields of Indiana. Ecology 16:554-67.

Eyre, R.H. (Ed.) 1980. Forest Cover Types of the United States and Canada. Society of American Foresters, Washington, D.C. 148 p.

Fowells, H.A. 1965. Silvics of Forest Trees of the United States. United States Deparment of Agriculture. Agriculture Handbook 271:762 pp.

Gant, R.E. and E.C. Clebsch. 1975. The allelopthic influences of *Sassafras albidum* in old-field succession in Tennessee. Ecology. 56:604-15.

Gleason, H.A. and A. Cronquist. 1963. Manual of the Vascular Plants of Northeastern United States and adjacent Canada. Willard Grant Press, Boston, MA.

Gleason, H.A. and A. Cronquist. 1991. Manual of the Vascular Plants of Northeastern United States and adjacent Canada. New York Botanical Garden, NY.

Hagan, E.C, P.M. Jenner, Wm. I. Jones, O. Garth Fitzhugh, E.L. Long, J.G. Brouwer, and W.K. Webb. 1965. Toxic properties of compounds related to Safrole. Toxicology and Applied Pharmacology. 7:18-24.

Harshberger, J.W. 1970. The Vegetation of the New Jersey Pine Barrens. Dover Publications, Inc. NY.

Keever, C. 1953. Present composition of some stands of the former oak-chestnut forests in the southern Blue Ridge Mountains. Ecology 34:44-54.

Kloeppel, B.D., M.E. Kubiske, and M.D. Abrams. 1994. Seasonal tissue water relations of four successional Pennsylvania barrens species in open and understory environmnets. Int. J. Plant. Sci. 155(1):73-79.

Liming, F.G. and J.P. Johnston. 1944. Reproduction in Oak-Hickory Forest Stands of the Missouri Ozarks. J. For. :42:175-180.

Lloyd, D.G. and C.J. Bawa. 1984. Modification of the gender of plants. In: Evolutionary Biology. M.K. Hecht, B.Wallace, and G.T. Prance (Eds). Vol. 17. Plenum Press, New York. p. 255-338.

Mackey, H.E., Jr. and N. Sivec. 1973. The present composition of a former oak-chestnut forest in the Allegheny Mountains of western Pennsylvania. Ecol. 54:915-18.

Marks, P.L. and F.H. Bormann. 1972. Regeneration following forest cutting: mechanisms for return to steady-state nutrient cycling. Sci. 176:914-15.

Martin, W.L., T.L. Sharik, and D. Wm. Smith. 1982a. Age-structure relationships of tree species in an Appalachian oak forest in southwest Virginia. Bull. Torr. Bot. Club 109(3):287-98.

Martin. W.L., T.L. Sharik, R.G. Oderwald, and D. Wm. Smith. 1982b. Phytomass and nutrient levels in the understory of an Appalachian oak forest in southest Virginia. Bull. Torr. Bot. Club 109(3):330-43.

Mauseth, J.D. 1988. Plant Anatomy. The Benjamin/Cummings Publishing Co. Reading, MA.

Peattie, D.C. 1977. A Natural History of Trees of Eastern and Central North American. Houghton Mifflin Co., Boston, MA.

Radford, A.E., H.E. Ahles, and C.R. Bell. 1983. Manual of the Vascular Flora of the Carolinas. Univ. of North Carolina Press, Chapel Hill, NC. 1183 p.

Rohwer, J.G. 1994. A note on the evolution of the stamens in the Laurales, with emphasis on the Lauraceae. Bot. Acta 107:110.

Ross, M.S., T.L. Sharik, and D. Wm. Smith. 1982. Species and site relationships among oak regeneration types after clearcutting in the Virginia Ridge and Valley. In: Proc. Second Bienn. South. Silv. Res. Conf. USDA For. Serv. Gen Tech. Rep. SE-24. Asheville, NC. p.151-56.

Ross, M.S., T.L. Sharik, and D. Wm.Smith. 1986. Oak regeneration after clear felling in the Ridge and Valley province of Southwest Virginia. For. Sci. 32:157:69.

Seischab, F.K., J.M. Bernard, and M.D. Eberle. 1993. Glaze storm damage to western New York forest communiites. Bull. Torrey. Bot. Club. 120(1):64-72.

Solereder, H. 1908. Systematic Anatomy of the Dicotyledons. The Clarendon Press, Oxford.

Spurway, C.H. 1941. Soil reaction (pH) preferences of plants. Michigan State College Agricultureal Experiment Station, Special Bulletin 306. East Lansing. 36 p.

Stout, A.B. 1929. The clone in plant life. J. New York Bot. Garden. 30:25-37.

Styles, B.T. 1972. The flower biology of the Meliaceae and its bearing on tree breeding. Silvae Genetica. 21(5):175-181.

Thompson, P.W. 1992. A unique old-growth Michigan hardwood stand with *Sassafras* as a major component. Michgan Bot. 31:31-34.

Voss, E.G. 1985. Michigan Flora, Part II Dicots. Cranbrook Institute of Science, MI.

Wells, J.R. and P.W. Thompson, 1982. Plant communities of the sand dune regions of Berrien County, Michigan. Michigan Bot. 21:3-38.

Wendel, G.W. 1977. Longevity of black cherry, wild grape, and sassafras seed inthe forest floor. USDA For. Serv. Res. Pap. NE-375. 6 p.

CHAPTER 2 - THE MODE OF ORIGIN OF ROOT BUDS AND ROOT SPROUTS IN *SASSAFRAS ALBIDUM*

ABSTRACT

The objectives of this study were to determine (1) the mode of origin of root buds in *S. albidum*, (2) the mode of origin of successful root sprouts, and (3) whether different types of root buds are produced in early versus late successional habitats or disturbed versus undisturbed clones. Root samples were collected from 13 clones which differed in their successional status and disturbance histories. Each sample was examined for swellings, which may have represented the positions of buds, and these swellings were sectioned. Two types of buds were found, which are referred to here as suppressed trace buds and exogenous buds. Suppressed trace buds form during the early secondary growth of a root and they perennate by growing outwards in concert with the vascular cambium such that bud traces are produced in the secondary xylem. Exogenous buds of *S. albidum* form during the subsequent growth of a root from a band of proliferated pericycle cells between the secondary phloem and the periderm. Instead of forming connections with the vascular cambium of a root, the exogenous buds of *S. albidum* cause the formation of adventive, spherical vascular cambia which give rise to nodules of wood, or sphaeroblasts. Since exogenous buds were most common on the roots of disturbed clones and old clones in late-successional habitats, they are presumably formed in response to injuries or senescence. To determine if both types of buds were functional, 112 sprouts, including 35 sprouts which had formed from incubated root samples, were serially sectioned at the root-stem junction to determine their mode of origin. Although many of the incubated root samples had contained exogenous buds, none of the resultant sprouts were subtended by sphaeroblasts as would be expected if they had developed from exogenous buds. Of the 112 sprouts sectioned, 109 were subtended by traces, indicating that they had originated from suppressed trace buds,

and three were subtended by wide rays, which suggested that they had formed from reparative buds of ray parenchyma origin. Although exogenous buds may form in great numbers they presumably unable to sprout. *De novo* buds of ray parenchyma origin can sprout, but they were uncommon. Over 97% of the root sprouts were derived from suppressed trace buds, which were present on the root samples of all 13 of the clones examined.

INTRODUCTION

Many trees are able to sprout from their roots (Busgen, 1929; Zimmerman and Brown, 1971; Kramer and Kozlowski, 1979). Although some trees produce root sprouts in response to disturbances alone, others do so apparently as a normal part of their growth and development (Busgen, 1929). Under favorable conditions, trees of the second type, such as *Liquidambar styraciflua, Nyssa sylvatica, Robinia pseudoacacia*, and *Sassafras albidum* form multi-stemmed individuals, or clones, consisting of a stem of seedling origin, known as an ortet, surrounded by one or more stems of root origin, known as ramets (Stout, 1929; Barnes, 1966). Trees that require disturbances to induce their root sprouting may also form clones readily if slight disturbances are able to cause sprouting as in *Populus tremuloides* (Barnes, 1966; Schier, 1971). Trees that require severe disturbances as a prerequisite to root sprouting do not normally form clones, but they may do so if they are cut or if their roots are injured frequently as a result of natural disturbances, such as freeze-thaw cycles, as has been postulated for *Fagus grandifolia* in the northern portions of its range (Forcier, 1973; Jones and Raynal, 1986, 1988).

Previous studies have shown that root sprouts may originate from two types of buds, which are referred to here as reparative buds or suppressed trace buds (Kormanik and Brown, 1967; Schier, 1971; Zimmerman and Brown, Jones and Raynal, 1986). In Table 1, these buds are compared and their synonyms are listed. Since suppressed trace buds form during the early growth of uninjured roots, they are analogous to the preventitious buds of shoots (Hartig, 1878,

Table 1: Types of buds which have been shown to give rise to root sprouts in trees.

	Suppressed trace buds[6,15]	**Reparative buds**[1,2,3,4]
Synonyms	Additional buds[1, 2,3,4], endogenous buds[5], pre-existing buds	*De novo* buds, exogenous buds[5].
Origin	The pericycle, early secondary growth[12,15]	In the bark, from preformed primordia[7,8] or induced primordia[9,10, 13]
Traits	Bud traces to the center of a root	Bud traces absent or not to the center of a root
Examples	*Liquidambar styraciflua*[6,15], *Sassafras albidum*[11], *Alnus incana*[12], *Populus balsamifera*[9], *P. nigra*[9], *P. angustifolia*[9]	*Fagus grandifolia*[10], *Populus tremuloides*[7] *Sassafras albidum*[11], *Pyrus malus*[8, 13] *Ailanthus*[14], *Maclura aurantiaca*[14], *Populus deltoides*[9], *P. balsamifera*[9], *P. angustifolia*[9]

1 Polowick and Raju (1982); 2, Raju et al. (1966); 3, Holm (1925); 4, Wittrock, (1884); 5: Zimmerman and Brown (1971); 6, Kormanik and Brown (1967a); 7, Schier (1971); 8, Siegler and Bowman (1939); 9, Schier and Campbell, (1976); 10, Jones and Raynal (1986); 11, present study; 12, Paukkonen, et al. (1992); 13, Robinson and Schwabe (1977); 14, Trecul (1847); and 15, Kormanik and Brown (1967b)

Swingle and Preistley, 1939; Aaron, 1946; Stone and Stone, 1955, Fink, 1983). Reparative root buds, which require disturbances for their formation, may arise during either the early growth or later growth of a root. Two classes of reparative buds can be recognized based upon whether they originate from preformed or induced primordia. Primordia are organs, cells, or organized series of cells in their earliest stage of differentiation (Esau, 1965). Preformed primordia are initiated during the normal growth of stems or roots, but they lack apical meristems or leaf primordia, and they remain dormant and undifferentiated for variable amounts of time (Van der Lek, 1924; Carlson, 1950; Hassig, 1972; Fink, 1982: Lovell and White, 1986). Induced primordia form *de novo* in response to a treatment from either pre-existing tissues or wound callus tissues (Van der Lek, 1924; Carlson, 1950; Hassig, 1972; Fink, 1982: Lovell and White, 1986).

The mode of origin of reparative root buds has been studied by injuring roots in the field and collecting them for observation at later dates (Jones and Raynal, 1988) or by incubating root cuttings in vermiculite and/or other media (Trecul, 1847; Preistley and Swingle, 1929; Murray, 1957; Bonnett and Torrey, 1956; Wilkinson, 1969; Campbell and Schier, 1976; Wehtje et al., 1987). The root cuttings of several herbaceous species and two varieties of *Pyrus malus* (Lord Lambourne and Lord Derby) form reparative buds from induced primordia which appear in the bark upon incubation (Charlton, 1956; Goforth and Torrey, 1977; Robinson and Schwabe, 1977; Rao and Mohen Ram, 1981; Schirman and Zamora, 1987). In contrast, the reparative buds of *F. grandifolia* originate from induced primordia of callus origin. (Jones and Raynal, 1986). Reparative buds of callus origin have also been reported to form at the ends of root cuttings in *Ailanthus, Maclura aurantiaca, Populus balsamifera, P. deltoides,* and *P. angustifolia* (Trecul, 1847; Schier and Campbell, 1976).

The reparative root buds of *Populus tremuloides* and two varieties of *Pyrus malus* (Delicious apple and Florence crab) arise from pre-formed, rather than induced shoot primordia

(Siegler and Bowman, 1939; Schier, 1971). Although preformed root primordia have been found on the stems of many trees, including several species of *Salix* and *Populus* (Carlson, 1938; Carlson, 1950; Fink, 1982; Fjell, 1985; Lovell and White, 1986), *P. tremuloides* and *Pyrus malus* are the only trees that have been reported to produce pre-formed shoot primordia on their roots. Preformed root primordia appear as small groups of meristematic cells which interrupt the continuity of the surrounding tissues of a stem (Carlson, 1950; Lovell and White, 1986). Preformed primordia may remain dormant or little changed for years and they may be aborted without further developing (Fink, 1982; Lovell and White, 1986). In each case where the ontogeny of preformed primordia has been examined, they have been found to arise from secondary tissues, particularly the parenchyma cells of branch gaps, leaf gaps, and wide rays (Swingle, 1927; Carlson, 1950; Fink, 1982; Fjell, 1985).

The preformed shoot primordia of *P. tremuloides* form in the phellogen and they are visible externally as small ellipsoidal swellings with a transverse orientation after the phellem of a root has been removed (Schier, 1971). Anatomically, they appear as dense, spherical groups of undifferentiated cells (Brown, 1935; Schier, 1971). Since the roots of disturbed *P. tremuloides* clones tend to have greater numbers of shoot primordia than the roots of undisturbed clones, Schier and Campbell (1976) have postulated that shoot primordia form continuously and that their formation is facilitated by disturbances. Disturbances (presumably of a larger magnitude) are also thought to cause their differentiation, a process that culminates with the formation of an apical meristem surrounded by many leaf primordia, each with an associated procambial strand which extends towards the vascular cambium (Brown, 1935; Kormanik and Brown, 1967; Schier, 1971). After these strands have become continuous with the subtending cambium, the cells to their interior differentiate as cylindrical "vascular peg" which becomes embedded in the later growth rings of the root. (Brown, 1935). Although *P. tremuloides* roots may contain pre-existing buds with vascular pegs, incubation experiments have shown that they are less likely to

sprout than buds which form *de novo* from undifferentiated, preformed shoot primordia (Sandburg, 1951; Schier, 1971).

The root sprouts of *L. styraciflua, P. balsamifera, P. nigra,* and *Alnus incana* develop from suppressed trace buds (Kormanik and Brown, 1967; Schier and Campbell, 1976; Paukkonen et al., 1992). In each case, the responsible buds have been interpreted to form from pericycle cells slightly before or concurrent with the onset of secondary growth, since the bud traces that subtend them are contiguous with the primary xylem of a root. Bud traces are wedge-shaped groups of parenchyma cells in the secondary xylem that are produced as buds grow outwards in concert with the vascular cambium of a root or stem. Reparative buds may also produce traces if they don't sprout immediately, but instead grow outwards with the vascular cambium of a root. However, reparative bud traces do not extend to the primary xylem of a root (Stone and Stone, 1943; Kormanik and Brown, 1964, 1967).

Since the shoot primordia can form continuously on the roots of *P. tremuloides*, the sprouting potential of a single root is tremendous. In fact, 10 cm long root cuttings of *P. tremuloides* have been reported to produce as many as 200 shoot apices upon incubation (Schier, 1971). To sprout as prolifically, the roots of trees that produce suppressed buds might be required to also produce reparative buds. In an attempt to induce the formation of reparative buds on *L. styraciflua* roots, Kormanik and Brown (1967) exposed the uppermost lateral roots of 12 trees to the air. However, all of the sprouts which developed in response to this treatment were subtended by traces. Since some of these traces ended prematurely in the secondary xylem at points of previous injury or root abortion, the authors concluded that *L. styraciflua* roots can form reparative buds, but that they required more of a stimulus than exposure alone to do so. Similarly, the roots of *P. deltoides, P. angustifolia*, and *P. balsamifera* are able to sprout from the callus tissues which form at their cut ends upon incubation (Schier and Campbell, 1976).

In the present study, the mode of origin of root buds in *S. albidum* was examined for the first time. The objectives were (1) to determine if different types of buds (i.e. reparative vs. suppressed trace buds) are produced in early vs. later successional habitats or in disturbed vs. undisturbed clones and (2) to determine if each bud type was capable of sprouting to form successful ramets. To ensure that no types of buds were overlooked root samples were collected from 13 separate clones varying in age, size, successional status, and disturbance history and examined them for swellings which may have represented the positions of buds. All of the buds found on the samples, which were of two distinct types, were examined for clues relating to their mode of origin and/or interconnections with their parent roots. To determine if each bud type was functional several root samples were incubated in moistened plastic bags and the root-stem junctions of the 35 sprouts which developed from these samples were sectioned for clues about their mode of origin. In addition, 77 pre-existing sprouts were collected from several of the clones and sectioned them in a like manner for clues about their mode of origin.

MATERIALS AND METHODS

Plant Material:

Figure 1 shows the locations of the 13 sampled genets of *Sassafras albidum* (Nuttall) Nees within the state of Michigan, USA and Table 2 provides more detailed information about the location of each of these genets. The term "genet" is used here to refer to a single genetic individual irrespective of whether or not clone formation has occurred (Stout, 1929; Barnes, 1966). The 13 genets sampled included 12 clones (Figure 2) and a single tree that had not yet produced any root sprouts at least 120 cm tall. The clones were identified based upon the close spacing and similar sex expression of their ramets (since *S. albidum* is subdioecious), as well as

Figure 1. Genet Locations in Michigan, USA.

Table 2. Locations of the genets studied.

Genet	County	Township	Range	Section	Site
1	Clinton	5N	1W	23 (NW 1/4)	E. margin of Upton Rd, ≈ 600 ft. before Clark Rd.
2	Clinton	5N	1W	14 (S 1/2)	Woodlot north of Clark Rd.
3	Clinton	5N	1W	24 (N 1/2)	E. Margin of Peacock Rd.
4	Kalamazoo	1S	9W	5	Louden Field, Kellogg Biological Station
5	Kalamazoo	1S	9W	5	Louden Field, Kellogg Biological Station
6	Allegan	3N	16W	3 or 4	S. margin of Route A2, opposite Saugatuck Camp.
7	Ingham	3N	1W	6 (SW 1/4)	N. margin of Sandhill Rd. (Lott Woodlot)
8	Allegan	4N	16W	35 (SW 1/4)	Margin of Exit Ramp 41 off I-196 South
9	Van Burean	1S	17W	32	V. Burean State Park, "Erosion control area"
10	Clinton	5N	1W	23 (NW 1/4, N1/2)	S. margin of Clark Rd, ≈ 300 ft. before Peacock Rd.
11	Jackson	4S	1W	4 (SE 1/4)	NW corner of S.Jackson and Hatch Rds.
12	Allegan	3N	16W	8 (W 1/2)	Church Retreat Camp; off Center Rd.
13	Jackson	4S	1W	4 (SE 1/4)	NW corner of S. Jackson Rd. and Hatch Rd.

by the partial excavation of their interconnecting roots. Most of the clones studied occurred in isolation, but in one case a male and female clone (Genets 2 and 3) were overlapped such that, without excavation, those ramets which had not yet reached reproductive age could not be positively identified as belonging to either of these genets

The genets studied were selected to represent a wide range of habitats (Table 3). Eight of these genets were found in early successional or ruderal habitats (i.e. old fields and road edges) and four were found in later successional habitats (i.e. woodlots). Most of the genets were undisturbed, but some of them experienced recent disturbances as indicated in Table 3. During the Fall of 1992, for example, a large portion of Genet 8 was clear-cut for highway maintenance. Similarly, in the Fall of 1993, Genet 13 was cut in association with the creation of a utility right-of-way. Although Genet 12 was not disturbed during the study, the uniform size of its stems and its position along the margins of a road-cut through a sand dune forest suggested that it had either been partially cut a few years previously or it had been released from suppression in association with the cutting of several taller trees.

In Table 3, these genets are arranged based upon their overstory conditions and whether or not they were surrounded by taller trees around their margin. Genet 1 was open-grown and its margins were open, Genets 2-3 were boxed in by taller trees, but most of their ramets were not overtopped, Genets 4-7 were open-grown and they had open margins, but their ramets were interspersed with some occasional taller trees, Genet 8 was open-grown but its ramets were interspersed with taller trees and it was surrounded by taller trees for over half of its margin, Genets 9-12 were partly to mostly overtopped, and Genet 13 was completely overtopped. Since Genets 10-12 were located in woodlots, their margins were not distinct, and they may have represented expanding clones with widely-dispersed ramets or older, fragmenting clones.

All of the ramets (> 120 cm tall) of the undisturbed genets were numbered, measured for dbh and classified as living or dead, with the exception of Genet 9. Since Genet 9 covered a

Table 3: Descriptions of the sampled genets. The genets are listed based upon their overstory conditions, genets with lower numbers being more open grown. The margins column refer sto whether or not taller trees "boxed in " the clone. The margins of most of the genets that were found in woodlots (Genets 10-13) could not be precisely delimited.

Genet	Habitat	Overstory Conditions	Margins	Number of Stems	DBH of largest (cm)	Disturbance History
1	road edge	open-grown	open	12	12	undisturbed
2	dry hilltop	open-grown	boxed-in	11	17	undisturbed
3	road edge	open-grown	boxed-in	295	28.7	undisturbed
4	old field	interspersed with taller trees	open	≈60	21.1	undisturbed
5	old field	interspersed with taller trees	open	≈60	17	undisturbed
6	old field	interspersed with taller trees	open	150	11	undisturbed
7	old field	interspersed with taller trees	open	170	23	undisturbed
8	highway edge	partially overtopped	partially open	>300	n/a	cut in the Fall of 1992
9	forested sand dune	partially overtopped	partially open	>500	21.1	undisturbed
10	woodlot margin	partially overtopped	indefinite	≈20	24	undisturbed
11	oak woodlot	mostly overtopped	indefinite	260	35	undisturbed
12	road cut	mostly overtopped	indefinite	n/a	n/a	cut 5-10 years earlier
13	oak woodlot	completely overtopped	indefinite	1	n/a	cut in the Fall of 1993

much larger area than any of the other genets, its ramets were surveyed in a single 10 x 60 m plot. Although Genet 11 contained fewer ramets than Genets 7-9 (Table 3), it was the only clone with ramets of 30 cm dbh or greater. The presumed progenitor stem of Genet 11 was 35 cm in dbh and it was determined to be 125-130 years old based upon the ring count of a core obtained from its base. Since Genet 11 occurred in an oak-hickory woodlot many of its ramets were over-topped by taller trees. In fact, nearly 1/3 of the ramets of Genet 11 were dead and equally as many appeared to be senescing, but several sprouts of presumed root origin which could be identified by the vivid green color of their stems, were intermixed with the larger stems of this clone.

The ramets of the disturbed clones (Genets 8 and 12) were not surveyed in a like manner. Although the total number of ramets of each of these genets was estimated, these numbers are not directly comparable with the counts for the undisturbed genets since the ramets of these genets had not yet self-thinned. Nonetheless, the large area covered by the Genet 8 suggested that it was one of the oldest genets studied. Since Genet 12 was situated at the margin of a woodlot and a road cut its boundaries could not be precisely delimited.

Microscopy of Root Buds:

During the 1993 and 1994 growing seasons portions of the root system of each genet were excavated by hand and samples up to two cm in diameter and one meter in length were collected. Although some of the samples still contained healthy cortical tissues, most of the samples were older and they lacked a cortex. Most of the samples were cut into 5 or 10 cm long sections and vacuum-infiltrated on site with formaldehyde-acetic acid-alcohol (FAA), but some were collected in a living condition and stored in plastic bags with wet paper towels to prevent their desiccation. In the lab, all of the samples were examined for swellings (up to 2 mm tall)

which may have indicated the positions of buds. Two distinct types of swellings were found on the root samples examined, low ellipsoidal swellings (Figure 4), and more pronounced, hemispherical swellings (Figure 5).

All of the swellings found on the fixed samples and most of the swellings found on the non-fixed samples were sectioned for anatomical study, but some of the non-fixed samples were left in plastic bags to induce their sprouting. In total, 150 root samples including 200 swellings were serially sectioned, 90% of them transversely and 10% of them longitudinally. Most of these samples were sectioned with a sliding microtome at 40 um. However, all of the young samples with cortical tissues, plus several older samples that were judged to be too small for sectioning with a sliding microtome, were embedded in paraffin and sectioned with a rotary microtome at 15 to 25 um. (Johansen, 1940).

The serial sections from the paraffin-embedded samples were stained with safranin, counter-stained with fast green, and then mounted in Permount. The sections cut with the sliding microtome were mounted in glycerol without being stained. All of the sections were examined with a Zeiss compound microscope. Some of the sections were also examined with a Zeiss Laser Scanning Confocal Microscope (LSM) in epifluorescent mode, with a 488/512 argon/helium laser and a red barrier filter (LP 600) to better distinguish between the lignified and non-lignified cells of the specimens.

All of the buds observed were surveyed for tracheary elements and/or procambial strands, and, in median (or near-median) section, their longitudinally-sectioned leaf primordia were counted as a measure of their size. To test for correlations between root size and bud size, the wood radius of each sample was measured. Subsequent measurements varied depending upon the type of bud present. Representative sections were photographed with a Zeiss photomicroscope III, a Nikon Labophot, a Nikon dissecting scope equipped with a camera tube,

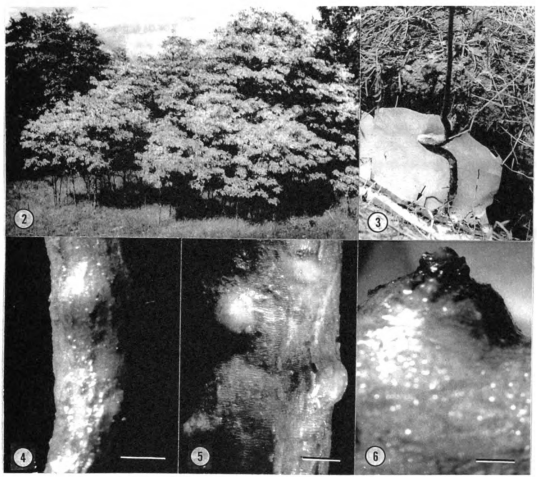
Figure 2. A young *S. albidum* clone. This clone was found growing along the margins of highway in SW Michigan. Notice the decrease in ramet size from the center of this clone towards its periphery. Strangely, no young root sprouts are visible along the margins of this clone.

Figure 3. A root sprout. Notice that the original root sprout died-back to ground level, but it has since coppiced. The arrow indicates the parent root of the sprout.

Figure 4. Suppressed trace bud as seen on the surface of a root. Low, ellipsoidal swellings with a transverse orientation indicated the positions of suppressed trace buds. Bar = 2 mm

Figure 5. Exogenous bands as seen on the surface of a root. Discrete hemispherical swellings indicated the positions of exogenous buds. The root sample shown was collected from Genet 11, which contained large numbers of exogenous buds. Bar = 2 mm.

Figure 6. A conical protuberance, or presumed short shoot. Several conical protuberances similar to this one were seen on the roots of the genets studied. All of these protuberances were much taller and larger in area than typical root bud swellings, and many of them had emergent buds at their apices which suggested that they may have represented short shoots. Bar = 2 mm.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and the LSM. The fluorescent images obtained with the LSM were enhanced by frame averaging before being photographed.

Origin of Sprouts:

The parent roots of 35 sprouts which had developed from the incubated root samples were sectioned transversely with a sliding microtome and serial sections from the root-stem junction were mounted in glycerol and examined for clues about the mode of origin of the sprouts. Seventy-seven field-collected root sprouts (Figure 3), including 30 which had regrown from the clear-cut regions of Genet 8, were also sectioned and examined for evidence relating to their mode of origin. Most of the sprouts that were sectioned occurred alone on a root, but some of them occurred in pairs at the same position (Figure 40). Fifteen conical protuberances that were found on the parent roots as the 77 field-collected sprouts were also sectioned (Figure 6). Most of the protuberances were at least 4 mm tall, or 2-3 times as tall as a typical root bud swelling and much larger in area and volume. Most of the conical protuberances had buds at their apices, which suggested that they may have been short shoots (Figure 6).

RESULTS

Of the 200 swellings sectioned, 184 represented buds. Of these, 117 were suppressed trace buds and the remainder (n=67) were exogenous buds. The 16 swellings that lacked buds represented local proliferations of the phelloderm or pericycle that were typically subtended by buried root traces or wide primary rays. Pairs of wide rays that originated at a single protoxylem pole were common below these swellings (Figure 8). The outermost xylem parenchyma cells of some of the wide rays were elevated above the general level of the vascular cambium as shown in Figure 8, but the outermost xylem parenchyma of others were sunken below the level of the

cambium. Developing root apices were seen in the phloem regions of several of the wide rays (Figure 9). Groups of amber-colored meristematic cells, which may have represented a second type of exogenous bud were also seen in the phloem regions of some of the wide rays, but these cells could not be positively identified as either shoot primordia or developing buds.

Suppressed Trace Buds:

Suppressed trace buds were found on the roots of all 13 genets. Suppressed trace buds were visible externally as low, ellipsoidal swellings (Figure 4). Although pairs of suppressed trace buds were sometimes seen in the same sectional view, solitary suppressed trace buds were much more common. In root transverse section, all of the suppressed trace buds appeared to "sit" on the vascular cambia and they were subtended by wedge-shaped traces in the secondary xylem. Figure 10 shows a typical trace that is labeled to indicate its inner tip, its outer face, and its margins. The margins of several of the traces were bordered by "wings" of ray parenchyma cells which made them difficult to delimit (Figures 11, 13), but the inner tips of most of the traces were easier to distinguish.

The inner tips of all of the traces were located within 500 um from the primary xylem tissues marking the center of a root, but they ended at different positions. Thirty-two percent of the traces were contiguous with protoxylem poles, 56% with early-formed secondary vessels (Figure 11), and 12% with neither vessels nor poles. Most of the vessels that were contiguous with a trace bordered a protoxylem pole, but several of them were located midway between neighboring protoxylem poles. Although most of the traces that were contiguous with neither a pole nor a vessel ended among groups of axial parenchyma cells, several of them were contiguous with rays that had originated at a protoxylem pole or an early vessel.

Figure 7. The anatomy of a young root in cross section. The secondary phloem and the periderm are separated by a band of proliferated pericycle cells (P) which widens during the secondary growth of a root. Bar = 500 um.

Figure 8. A pair of wide primary rays. The rays shown originated at the same protoxylem pole and they are elevated above the general level of the vascular cambium. Bar = 1 mm.

Figure 9. A forming root apex in the phloem region of a wide ray. Bar = 500 um.

Figure 10. A suppressed trace bud as seen in root cross-section. The upper arrow indicates the outer face of the trace, the middle arrows indicate the margin of the trace, and the lower arrow indicates the inner tip of the trace. The margins of this trace are defined by strands of elongate parenchyma cells as shown in Figure 12. Bar = 800 um.

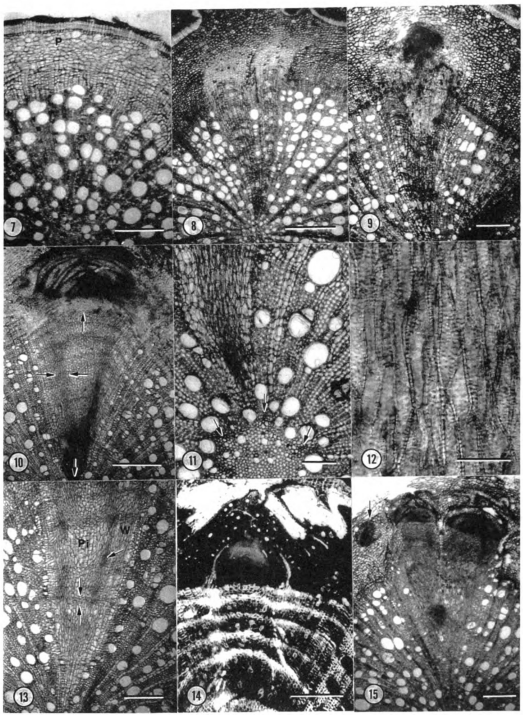
Figure 11. The inner tip of a root bud trace. The trace shown is contiguous with an early-formed secondary vessel. Notice the "wings" of ray parenchyma cells which border the elongate parenchyma cells of the trace. The arrows indicate protoxylem poles. Bar = 200 um.

Figure 12. The elongate parenchyma cells of a trace as seen in root transverse section. Notice that several of the cells shown are fusiform-shaped. During the winter these parenchyma cells store large quantities of starch as do all of the wood parenchyma of *S. albidum* roots. Bar = 50 um.

Figure 13. A well-differentiated trace. This trace consist of a central pith-like region (Pi) of relatively larger parenchyma cells which are surrounded by strands of elongate parenchyma cells (upper arrow). Notice the bands of small, relatively thick-walled pith cells (lower arrows) which appear to be continuous with the growth rings of the conducting wood of the root. The trace is bordered by wings of ray parenchyma cells. Because the trace was cut at an oblique angle its inner tip is seen in non-median section. Bar = 500 um.

Figure 14. A trace and surrounding wood tissues as seen with epifluorescent illumination. A argon-neon laser (488 and 512 nm) was used in combination with a red barrier filter (LP 600) to produce this image. The bands of small, thick-walled pith cells in the trace are continuous with the growth rings of the root. Tracheary strands are visible in two of the inner leaf primordia of the bud (arrow). Buried leaf traces can be seen in the secondary xylem along the margins of the trace. Bar = 500 um.

Figure 15. A branched trace bud. Because the trace was cut at an oblique angle its tip is not visible. The arrow indicates a forming exogenous bud in the bark. Bar = 500 um.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Early in this study, several of the traces examined appeared similar to wide rays. To ensure that the "traces" did not represent modified rays, they were compared with 50 wide rays which had been encountered during the sectioning of the root samples. The width of the outer face of each trace and/or wide ray was measured and compared with the wood radius of its parent root to determine if the traces were as wide or wider than rays. Although most of the wide rays were less than 20% as wide as the radii of their parent roots, most of the traces were 40% to 60% as wide as the radii of their parent roots and some were as much as 80% to 100% as wide.

The traces also showed a more complex cellular organization than the wide rays. In median longitudinal section all of the traces contained large numbers of radially-elongate, often fusiform-shaped, parenchyma cells (Figure 12) instead of files of rectangular or square-shaped parenchyma cells as are characteristic of rays. These elongate parenchyma cells formed a solid mass near the inner tip of each trace (Figure 11), but towards the outer face, they were less conspicuous and they frequently appeared to be organized as a cylinder of strands surrounding a region of larger parenchyma cells (Figures 10, 13). Because they are located in the center of a trace and are typically isodiametric in shape (Figure 18), the larger parenchyma cells are referred to subsequently as pith cells.

Pith cells could be identified in the traces of 72% of the suppressed trace buds. Based upon their size and wall characteristics, two types of pith cells could typically be distinguished: large, thin-walled pith cells, and smaller, thicker-walled pith cells that were organized as discrete bands which appeared to be aligned with the latewood cells of the growth rings of the conducting regions of their parent roots (Figure 13). Since the latewood cells of growth rings tend to be more highly lignified (per volume of wood tissue) and, consequently, more autofluorescent than the earlywood cells of growth rings (Esau, 1965), several traces were examined with the Zeiss LSM in epifluorescent mode to clarify the nature of the relationship between the circumferential bands of pith cells in a trace and the growth rings of their parent roots. The resultant images

clearly showed that the bands of pith cells and the latewood cells of the growth rings of a root were continuous (Figures 14, 22)

The circumferential bands of many of the traces were bordered towards the exterior (as seen in root transverse section) by putative procambial strands or strands of mature tracheary elements with either annual or helical wall thickenings (Figure 14). In each case, these strands turned "upwards" after entering a trace and extended radially, or towards the outer face of a trace. On several occasions, strands which originated near the outer growth rings of a root were seen to enter the leaf primordia of a bud (Figures 19, 22). In trace cross section, that is, root longitudinal section, these strands appeared to form cylinders which presumably reflect the arrangement of a bud's leaf primordia. Some traces contained isolated tracheary elements instead of strands of tracheary elements. In total, 79% of the traces contained either isolated tracheary elements, strands of tracheary elements, or both.

Eighty-five percent of the suppressed trace buds contained mature tracheary elements which were associated with one or more of their leaf primordia. Many of the buds also contained putative procambial strands in various stages of development. The more well-developed procambial strands were multiseriate and they could be seen to connect leaf primordia and the neighboring regions of a root's vascular cambium as seen in root transverse section (Figure 18). Most of the multiseriate procambial strands were bordered by strands of mature tracheary elements on their inner margins which appeared to interconnect them with the conducting wood of their parent root. Some suppressed trace buds contained many multiseriate procambial strands; each strand within a separate leaf primordium.

Although most of the suppressed trace buds contained less than 12 longitudinally-oriented leaf primordia as seen in root transverse section, some of the larger buds contained up to 20 such primordia. The youngest leaf primordia bordered the apices of the buds, but the older leaf primordia were displaced both laterally and centrifugally (Figures 10, 14, 23). Wood radius,

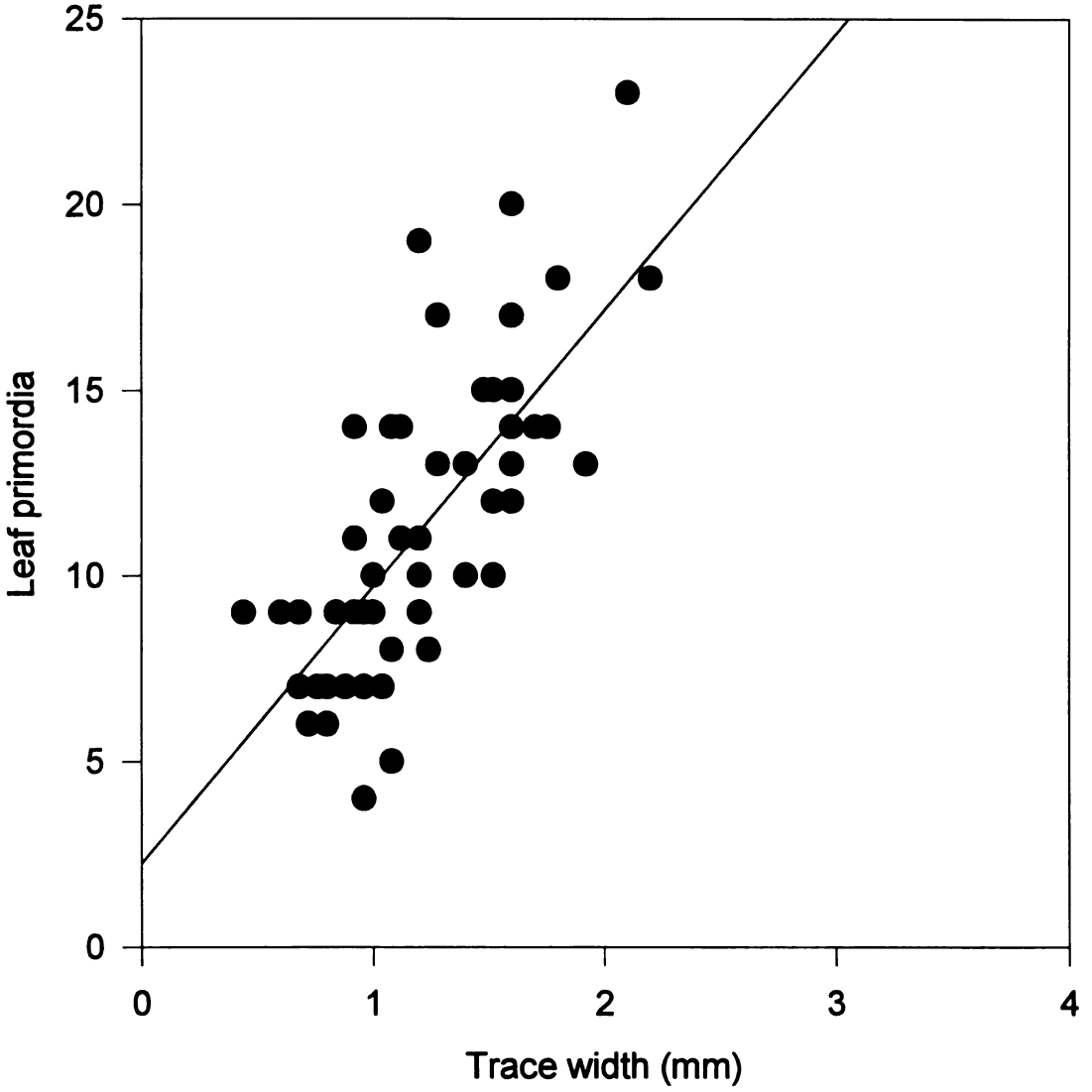


Figure 16. Leaf primordia vs. trace width. Trace width was measured at the level of the vascular cambium, or the outer face of each trace. Leaf primordia were counted in bud median section. Only longitudinally-sectioned leaf primordia were counted. n=57, r=.73, p <.001

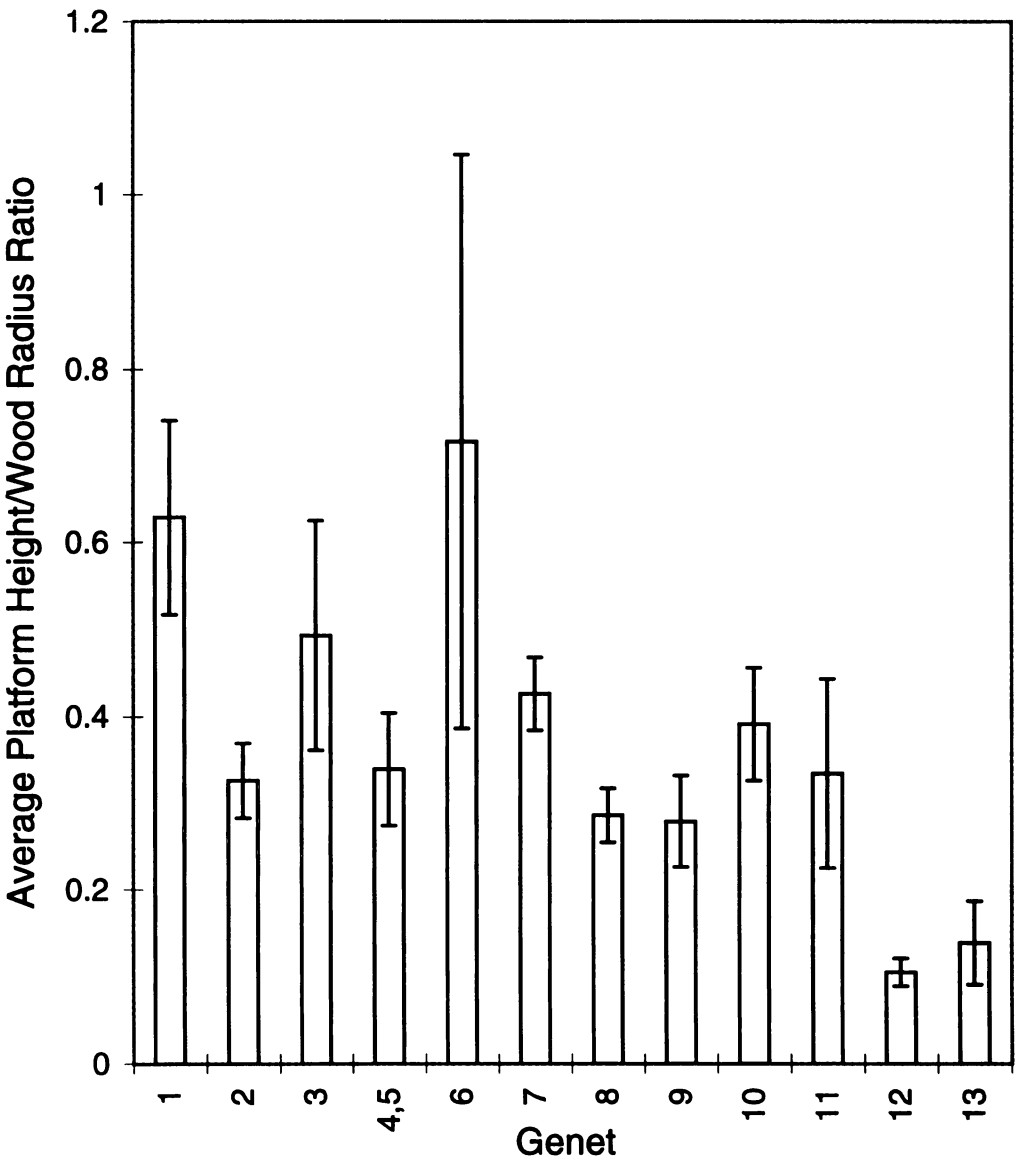


Figure 17. Ratio of average platform height/wood radius vs. genet number. The bars shown represent the standard error of each average. The genets with higher numbers were overtopped to a greater degrees than genets with lower numbers. Genets 2, 4, 5, 7, 9, and 12 were growing in soils that appeared to be susceptible to drying as a result of either the local topography or their composition.

Figure 18. A leaf primordium with a multiseriate procambial strand. The procambial strand shown (arrow) appears to be continuous with cambial initials bordering to the apex of bud. The xylem derivatives of these initials will probably differentiate as strands of circumferentially-oriented tracheary elements interconnecting like strands in the leaf primordium with the sapwood of the conducting regions of the parent root. Bar = 200 um.

Figure 19. A suppressed trace bud and associated vasculature. The strands of circumferentially-oriented tracheary elements shown (arrow) connect an inner leaf primordium and the conducting wood of the root. Bar = 300 um.

Figure 20. Early-differentiating, circumferentially-oriented strands of tracheary elements (arrow) lateral to the apex of a suppressed trace bud. A young leaf primordium bordering the apex of the bud (A) has apparently caused the differentiation of these tracheary elements. The neighboring xylem derivatives have not yet matured. Bar = 300 um

Figure 21. A suppressed trace bud and associated trace platform. Trace platforms (TP) are swellings of secondary xylem which elevate a bud above the general level of the vascular cambium (dashed line). Bar = 200 um.

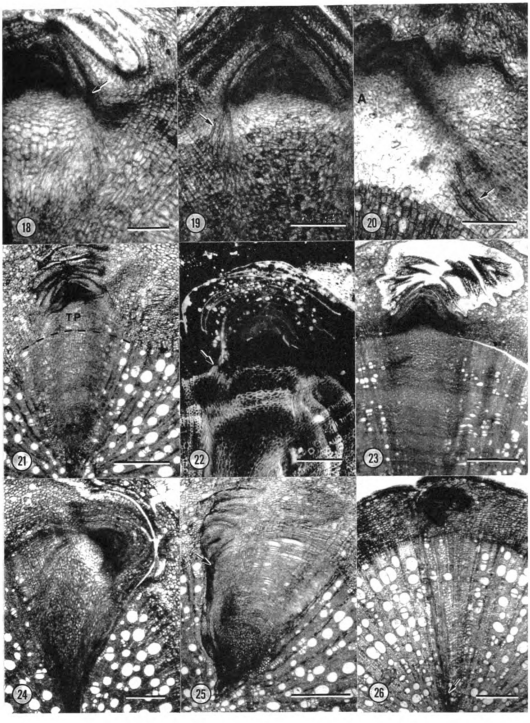
Figure 22. A suppressed trace bud as seen with epifluorescent illumination. A argon/neon laser (488/512 nm) was used in association with a red barrier filter (LP 600) to produce this image. Notice the tracheary elements which appear to be continuous between an inner leaf primordia and the adjacent conducting wood of the root (arrow). The bright, spherical structures in the leaf primordia may represent mucilage cells or oil cells, both of which are characteristic of the leaves of *S. albidum*. Bar = 500 um.

Figure 23. A large suppressed trace bud with necrotic outer leaf primordia. The necrotic leaf primordia are surrounded by a wound periderm but the apex and innermost leaf primordia of the bud are healthy. Bar = 1 mm.

Figure 24. A suppressed trace bud which has started to slip onto the margins of its trace. Several of the leaf primordia of this bud are buried in the secondary xylem along the margins of its trace. Bar = 500 um.

Figure 25. A suppressed trace bud which has completely slipped onto the margins of its trace. The arrow indicates the position of the bud's apex. Bar = 1 mm.

Figure 26. A displaced bud as seen in root transverse section. The arrow indicates a relict trace in the secondary xylem. Bar = 800 um.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trace width, and trace area (1/2 trace width multiplied by wood radius) were all found to be positively correlated with the number of leaf primordia counted ($r = 0.51$, 0.73, and 0.69, respectively; $n = 57$, $p < 0.001$). The scatter graph in Figure 16 shows the relationship between trace width and leaf primordia number.

Twenty-four percent of the traces were branched (Figure 15). Branched traces were found on the roots of all 13 genets. In fact, over 50% of the suppressed trace buds found of the roots of Genet 1 (one of the youngest and healthiest genets) were branched. Most of the branched traces observed had branched only once, but some of them had branched multiple times such that they were associated with clusters of up to six interconnected buds. Although the branching points of these traces were difficult to pinpoint, they appeared to be aligned with the growth rings of the conducting regions of their parent roots. The interconnected buds of 53% of the branched traces had the same number of leaf primordia (plus or minus one leaf primordium), but the interconnected buds of the remainder differed in leaf primordia number by two or more, and the interconnected buds of some of the traces differed by as many as five leaf primordia. Most of the traces that were branched close to the inner tip of the trace supported buds with nearly equal numbers of leaf primordia, but most of the traces that were branched close to the outer face of the trace supported buds with large differences in leaf primordia number, suggesting the lateral branching of the main trace bud.

Almost all of the suppressed trace buds were elevated on platforms or swellings of secondary xylem, which are referred to here as trace platforms (Figure 21). Although most of the trace platforms examined were less than 35% as tall as the radius of their parent root, some of them were taller than the radius of their parent root and genet-specific differences in platform height were also noted. For example, the platforms subtending the trace buds of Genets 1, 3, and 6 were greater than 45% as tall as the radius of their parent roots on average, but the platforms subtending the trace buds of Genets 12 and 13 were less than 20 % as tall as the radius of their

parent roots on average (Figure 17). For the data set as a whole, the ratio of platform height to root radius was inversely proportional to root radius ($r = 0.34$, $n = 95$; $p < 0.001$) and many of the suppressed trace buds seen on the larger root samples were sunken in local depressions at the apex of larger platforms.

Although most of the suppressed trace buds faced directly outwards, 20% appeared to be "slipping" onto the margins of their traces such that they faced laterally or outwards at an oblique angle (Figures 24). In some cases, single leaf primordia were seen along the margins of a trace, but at other times groups of leaf primordia or entire buds were seen (Figure 25). The slipped tissues were always amber-colored and their nuclei were enlarged with large nucleoli. Three "displaced" buds were also seen (Figure 26). These buds, which were discolored and indistinct in appearance, were located in the secondary phloem of their parent roots. As their name implies, the displaced buds were separated from the subtending vascular cambia of their parent roots and they appeared to mirror aborted traces in the secondary xylem (Figure 26) Some of the buds examined were neither slipped nor displaced, but their outermost leaf primordia were necrotic and surrounded by a periderm (Figures 14, 23).

Exogenous Buds:

Exogenous buds were found on the roots of seven of the 13 genets, but they were common on the roots of only three of the genets - Genets 6, 8, and 11. Genet 11, in particular, contained large numbers of exogenous buds. In fact, a single 90 cm long root sample from this genet was found to contain over 30 exogenous buds. Exogenous buds were visible as discrete, hemispherical swellings on the surface of a root. In sectional view, the exogenous buds were located among proliferated pericycle cells between the periderm and the secondary phloem of a

root (Figures 5, 29). None of the exogenous buds examined were associated with wide rays, traces, injuries, or any other obvious internal features which may have induced their formation.

The apices of the exogenous buds were irregularly oriented. The apices of most of the exogenous buds faced outwards (Figure 29) or laterally (Figure 28), but the apices of nearly 15% of the buds faced inwards, or towards the center of their parent roots (Figure 30). All of the larger exogenous buds were subtended by spherical to oval-shaped nodules of wood (Figures 27, 28, 29, 33, and 34). In dissected nodules and in non-median sectional view, the surfaces of the nodules were seen to contain discrete sectors of xylem the cells of which were arranged so as to form fishbone or V-shaped patterns (Figure 27). Because each of these sectors was slightly convex in profile, the surfaces of the dissected nodules were uneven, rather than smooth.

In median view, each nodule consisted of a central region of pith-like parenchyma cells, surrounded by a ring of secondary xylem, a spherical vascular cambium (Figures 31, 32), and presumably a thin ring of phloem. The xylem tissues of each of the larger nodules contained multiple cell types including vessels, ray parenchyma, and axial parenchyma. The vessels and axial parenchyma of the nodule were variously oriented in median section. In some sectors they were circumferentially oriented, but in others they were transversely-oriented and the resembled the secondary xylem tissues of a root (Figures 29, 33). Although the vascular cambia of the larger nodules contained both fusiform and ray initials, the initials below each exogenous bud were modified (or absent) such that bud traces (Figure 33) were produced. These bud traces of most of the nodules differed little from wide rays and they appeared to exhibit delayed lignification as shown by fluorescent microscopy (Figure 34).

The youngest exogenous buds seen were not subtended by nodules, but rather by spherical groups of relatively large parenchyma cells which may be surrounded by recently initiated adventitious cambia and their first derivatives (Figures 30, 31). Although the vascular cambia of the forming nodules appeared to consist of isodiametric initials alone, the first xylem

Figure 27. The secondary xylem of a wood nodule, or sphaeroblast, in non-median section. Notice the two sectors (A and B) which are implied by the V-shaped arrangement of the xylem cells of the nodule. Sectors such as those shown are typically convex in profile such that the surface of a nodule is uneven. Bar = 250 um

Figure 28. A sphaeroblast as seen in median section. Notice the pith-like parenchyma cells at the center of the sphaeroblast and the lateral orientation of the exogenous bud. Bar = 800 um.

Figure 29. A sphaeroblast in near median-section and surrounding tissues. Notice its location in the proliferated pericycle (P) the hemispherical swelling the sphaeroblast has caused in the bark. The vessels of the upper half of the sphaeroblast are seen in cross section, but the vessels of the lower half are circumferentially oriented. Bar = 800 um.

Figure 30. A young exogenous bud and forming sphaeroblast as seen with epifluorescent illumination. An argon-neon laser (488/512 nm) was used in association with a red barrier filter (LP 600) to produce this image. The first xylem derivatives produced by the sphaeroblast have differentiated as circumferentially-oriented tracheary elements (arrow) opposite the apex of the bud, which is facing towards the center of the root. Bar = 300 um.

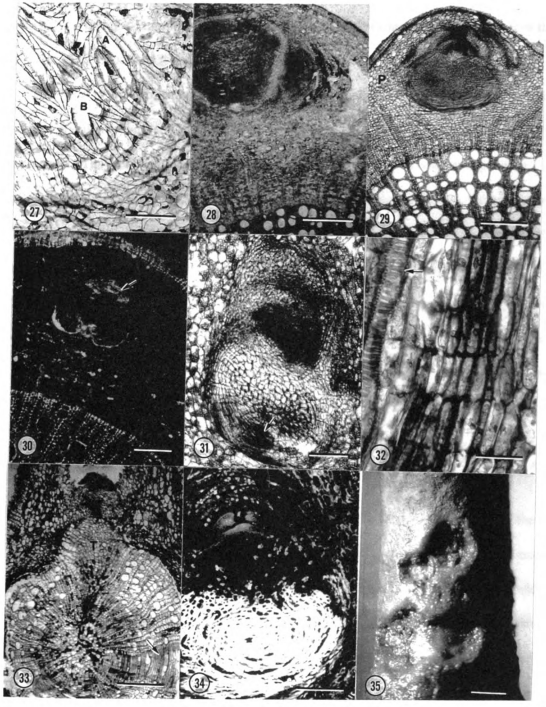
Figure 31. A young exogenous bud and associated spherical vascular cambium. This bud presumably formed at one pole of an induced primordium and then caused the cell around the periphery of the remainder of the primordium to become organized as a spherical cambium. The first xylem derivatives produced by the cambium have differentiated a circumferentially-oriented tracheary elements opposite the bud (arrow). Bar = 200 um.

Figure 32. The anomalous vascular cambia of a sphaeroblast as seen in sectional view. The xylem derivatives of the cambium have differentiated as circumferentially-oriented vessels with sclariform pitting (arrow). Bar = 50 um.

Figure 33. A large sphaeroblast as seen in median section. The associated exogenous bud is subtended by a trace (T) which extends to the center of the nodule. Notice the parenchyma cells at the center of the sphaeroblast and that the xylem cells of one sector of the sphaeroblast are circumferentially oriented (arrow). Bar = 500 um.

Figure 34. A sphaeroblast as seen in median section with epifluorescent illumination. An argon-neon laser (488/512 nm)was used in association with a red barrier filter (LP 600) to produce this image. The portions of the sphaeroblast beneath its the exogenous bud are weakly autofluorescent, and presumably unlignified or little lignified. Bar = 500 um.

Figure 35. A root sample with several crater-like depressions on its surface which may represent the positions of former sphaeroblasts. The root shown was collected from Genet 11 which contained large numbers of sphaeroblasts Bar = 2 mm.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derivatives to mature were frequently elongate in shape and circumferentially oriented (Figures 30, 31). Recently-formed exogenous buds were found on roots from 2 to 4 mm in diameter. Large and small exogenous buds were found side by side in some of the sectioned swellings. Most of the nodules examined were associated with a single exogenous buds, but five of the nodules were associated with pairs of buds.

Most of the exogenous buds had five to eight longitudinally-oriented leaf primordia as seen in nodule median section (Figures 28, 29), but some had as many as twelve such leaf primordia. The leaf primordia of many of the exogenous buds often contained putative procambial strands, but only 20% of these buds had mature vascular tissues. Nodule volume and leaf primordia number were positively correlated ($r = 0.66$, $n = 28$, $p < 0.001$). Root wood radius and leaf primordia number were also positively correlated but with lower r-values ($r = 0.33$, $n = 28$, $p < 0.1$). The volume of the largest nodule examined was 75 mm^3 with 12 longitudinally-oriented leaf primordia. Many of the root samples from Genet 11, particularly those of older roots contained both exogenous buds and crater-like depressions which may have represented the positions of former nodules (Figure 35).

Sprouts:

One of the 77 field-collected sprouts was subtended by a ray (Figure 38), but the rest were subtended by traces (Figure 36) including five that were subtended by branched traces (Figures 39). Likewise, two of the 35 incubation-induced sprouts were subtended by wide rays and the rest were subtended by traces including nine that were subtended by branched traces. None of the sprouts were subtended by nodules of wood although many of the incubated root samples had contained exogenous buds as evidenced by their subsequent dissection. Short regions of highly elongate, transitional parenchyma separated the traces (or wide rays) and the

Figure 36. The junction of a sprout and its parent root as seen in root transverse section. Notice the trace of the bud which sprouted and the highly elongate, transitional parenchyma cells (arrow) which separate it from the pith cells at the base of the sprout. Bar = 1 mm.

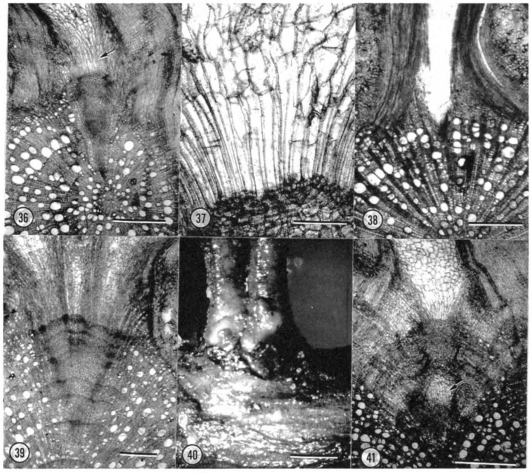
Figure 37. Elongate, transitional parenchyma cells produced during sprouting. Here, the transitional parenchyma cells give rise to the pith cells very quickly, but in most of the sprouts examined, the region of transitional parenchyma was more extensive. Bar = 200 um.

Figure 38. The junction of a field-collected sprout of ray origin and its parent root. The sprout shown is subtended by a xylem ray which suggests that it originated from a ray bud. Bar = 800 um

Figure 39. The junction of a pair of incubation-induced sprouts. These sprouts developed from a branched trace. Bar = 1.5 mm.

Figure 40. A pair of incubation induced sprouts as seen with a dissecting microscope. Bar = 4 mm.

Figure 41. The trace of a sprout which had presumably grown for several years as a short shoot. Notice the inclusion of transitional parenchyma cells (arrow) in the trace. Bar = 1 mm.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pith tissues of all of the sprouts examined (Figures 36, 37). Most of the branched traces had produced pairs of sprouts as shown in Figure 40.

All of the conical protuberances (Figure 6) were subtended by traces, but most of these traces contained one or more inclusions of apparent transitional parenchyma (Figure 41). Those traces which did not contain inclusions of transitional tissue were bordered laterally by broad sectors of wood which consisted of a mixture of transversely and circumferentially-oriented tracheary elements as seen in cross section. Most of the traces terminated in normal-appearing suppressed trace buds, but many of them terminated in necrotic stubs which suggested that the original bud had sprouted but failed.

DISCUSSION

Suppressed Trace Buds:

Ninety-seven percent of the sprouts sectioned were derived from suppressed trace buds. Since suppressed trace buds are formed early in development and were found on the roots of all 13 genets, they appear to be an obligate feature of root development and thus are analogous to the preventitious buds of shoots. The suppressed trace buds of stems and roots differ in origin (endogenous in roots versus exogenous in stems) but they perennate in a similar manner, that is, they grow outwards in concert with the vascular cambium. The sprouting of suppressed trace buds is presumably facilitated by the starch reserves which are stored in the parenchyma cells of their traces and their positions at the level of the vascular cambium.

Since none of the sectioned root swellings showed suppressed trace buds in an early stage of development, their initial ontogeny is uncertain. Although some of the traces examined ended among groups of axial parenchyma cells, most of them were contiguous with a protoxylem pole or an early-formed secondary vessel. Since buds with traces that end at protoxylem poles

occupy the same positions as lateral roots, they have been interpreted by previous authors to be of pericylic origin (Brown and Kormanik, 1967). Although 30% of the traces examined were contiguous with protoxylem poles, but a greater number (50%) were contiguous with early-formed secondary xylem vessels. Since the inner tips of all of the traces were located within 500 um of the primary xylem, the trace buds may have been derived from pericycle cells which had been induced to become organized as buds by substances that are produced in association with the differentiation of tracheary elements. Later, in secondary growth, the pericycle becomes widely separated from the vascular cambium and apparently no new trace buds are formed.

As they perennate, suppressed trace buds continually produce new leaf primordia, as evidenced by the positive correlation between trace width and leaf primordia number. As suppressed trace buds grow in size (and in leaf primordia number), they modify the physical structure of their parent roots. Since cambial cells beneath the apex of a suppressed trace bud (and their neighbors) are more active than typical cambial cells, platforms of secondary xylem are produced over time which elevate suppressed trace buds above the general level of a root's vascular cambium. The traces produced by suppressed trace buds also widen continuously, as evidenced by their wedge-shaped appearance in root transverse view. In fact, the traces of several of the older roots were so large at the level of their outer faces that they caused the significant distortion or bending of many conducting vessels in the secondary xylem.

Suppressed trace buds also impact the differentiation of surrounding tissues in a root. For example, the xylem derivatives that are produced by cambial initials bordering the apex of a suppressed trace bud may be caused to differentiate as circumferentially-oriented strands of axial parenchyma cells or tracheary elements that are continuous with like strands in leaf primordia near the apex of a trace bud. Since the differentiation of strands of circumferentially-oriented tracheary elements is promoted by stimuli produced by leaf primordia, their differentiation precedes that of neighboring cambial derivatives which are presumably dependent upon more

distant stimuli which arrive later via the proximal tissues of a root, Thus, among the parenchyma cells bordering bud traces, it is much more common for the first formed cambial derivatives in a year to differentiate as circumferential elements and for the subsequently-formed derivatives to differentiate as ray parenchyma cells.

In herbaceous plants, buds which form near the vascular cambium of a root become vascularized by means of the acropetal differentiation of procambial strands (Kondrat'eva Mel'vil, 1957). Since the suppressed trace buds of *S. albidum* roots perennate at the level of the vascular cambium, their leaf primordia presumably become vascularized in a similar manner. Although the procambial strands and/or tracheary strands of the innermost leaf primordia of many of the buds examined were continuous with the xylem cells bordering the apices of these buds, the procambial strands and/or tracheary strands of the outermost leaf primordia were never similarly connected. These observations suggest that as the leaf primordia of a suppressed trace bud are displaced both laterally and peripherally they are no longer able to influence the differentiation of the xylem derivatives produced by initials bordering the apex of the bud. Over time, the connections with older leaf primordia are apparently broken and the strands of circumferential elements become buried in the secondary xylem.

Although suppressed trace buds form during the early growth of a root alone, the number of suppressed trace buds on a length of root is able to increase by means of the proliferation, or branching, of pre-existing buds. In fact, nearly 30% of the suppressed trace buds examined were subtended by branched traces. Since the branch points of these traces were aligned with the growth rings of their parent roots, branching appears to occur preferentially at the start of a growing season or, less likely, at the end of a growing season. The branching of suppressed trace buds has also been reported in the roots of *Alnus incana* (Paukkonen et al., 1993) and in the stems of several trees (Hahne, 1926; Busgen and Munch, 1929; Chandler, 1947; Gifford, 1959; Kormanik and Brown, 1964; Kramer and Kozlowski, 1979). Previous investigators have

concluded that the branching of suppressed trace buds results from the outgrowth of buds which form in the axils of their leaf primordia (Hahne, 1926; Chandler, 1947; Kormanik and Brown, 1967).

The conical protuberances sectioned were taller and larger than typical root bud swellings and they typically contained inclusions of elongate, transitional parenchyma which suggest that they are dormant short shoots. The discovery of short shoots on the roots of *S. albidum* suggests that suppressed trace buds may begin to grow outwards, but stop short of sprouting for unknown reasons. Short shoots have also been found of the roots of *P. tremuloides* and *L. styraciflua* (Kormanik and Brown, 1967b; Schier, 1971), but the short shoots of *S. albidum* differ from those previously described, since in *S. albidum* they remain almost completely hidden beneath the periderm (Figure 6). The traces which subtended several of the field-collected root sprouts contained inclusions of transitional parenchyma which suggested that they had formed as a result of the conversion of short shoots to long shoots.

Healthy suppressed trace buds were found on roots up to 16 years old, but trace buds of this age appear to be uncommon. Although no roots greater than 1.5 cm in xylem diameter were sectioned, many larger roots were examined in the field and most of them contained few or no ellipsoidal swellings that would have indicated the positions of trace buds. Furthermore, none of the sprouts collected from the clearcut regions of the disturbed clones had developed from roots greater 2 cm in wood diameter although roots of this size were present. These results corroborate with those of an earlier study which found that most of the *S. albidum* sprouts which appeared after clearcutting in Virginia had developed from roots which were less than 4 mm in diameter (Blount, 1987).

Two types of dysfunctional suppressed trace buds were found on the root samples - slipping buds and displaced buds. The slipping of a suppressed trace bud presumably results from the death of some of the cambial cells subtending it such that one part of the bud continues

to grow outwards but the other part remains in place and is buried alive in the secondary xylem. In comparison, displaced buds are suppressed trace buds which have become separated from the vascular cambium of a root. Their discoloration implies that their displacement is caused by their own senescence. After a bud has become displaced the cambial cells which had subtended it resume their normal activity and its former trace becomes buried in the secondary xylem.

Although suppressed trace buds were found on the roots of all 13 genets, the buds of the genets appeared to differ in vigor as measured by the height of the trace platforms subtending them. In general, the height of the platforms examined was lower in the overtopped clones than the open-grown clones. Genets 12 and 13, which were both partially or completely overtopped had highly reduced trace platforms compared to the other genets. These differences suggest that although suppressed trace buds are produced in all habitats, they perennate with greater vigor in some habitats rather than others. Suppressed trace buds may also form in greater numbers in some habitats rather than others, but too few roots samples were collected to detect such differences.

Exogenous Buds:

The exogenous buds of *S. albidum*, which were found on only 7 of the 13 genets, are apparently reparative in nature. The genet which contained the greatest number of exogenous buds (Genet 11) was naturally senescent and the genet which contained the second greatest number of exogenous buds (genet 8) had been partially clear-cut before samples were collected. Although some of the genets that had exogenous buds were young and healthy-appearing, the exogenous buds of these genets were presumably located on injured roots. However, none of the exogenous buds on any of the genets were associated with wound tissues or point injuries. Although exogenous buds can form in large numbers, they appear to be dysfunctional since none

of the sectioned sprouts were subtended by sphaeroblasts as would be expected if they had been derived from exogenous buds.

The exogenous buds of *S. albidum* develop from residual pericycle cells which are located between the periderm and secondary phloem of a root. During the secondary growth of a root, these cells divide diffusely so as to form a band of loose-appearing parenchyma cells which has been referred to by previous authors as either a starch-storing parenchyma or a secondary cortex. (Goforth and Torrey, 1977; Baird et al., 1992). The root buds of *Euphorbia esula* and *Chondrilla juncea* also form from proliferated pericycle cells, but in both species these buds cause the basipetal differentiation of procambial strands which join with the vascular cambia of their parent roots (Bakshi and Coupland, 1959; Schirman and Zamora, 1978). In contrast, the exogenous buds of *S. albidum* cause the formation of adventitious spherical vascular cambia and nodules, or sphaeroblasts, which allow for the ready detection of exogenous buds on the surface of a root.

Sphaeroblasts are independent nodules of wood in the bark which have been reported to form in the stems of many trees (Baldini and Mosse, 1956). Sphaeroblast-like structures have also been observed in the roots of several plant species other than *S. albidum* but they have not been identified as such. Murray (1957), for example, described the formation of whorls of vascular tissue from swellings of phellogen on the roots of non-creeping *Medicago* varieties, but he did not investigate these whorls closely enough to determine if they were surrounded by vascular cambia. Similarly, in his paper on the formation of root buds in *Populus tremuloides*, Schier included a photograph of a whorl of xylem cells in the bark of a root which appeared to represent a sphaeroblast but it was interpreted to be a normal stage in the differentiation of a shoot primordium. Sphaeroblast-like structures have also been observed in plant cell cultures. In auxin-starved suspension cultures of *Fraxinus*, nodules of wood which are surrounded by radial files of cambial-like have been reported to develop (Preece, 1981).

Since none of the swellings sectioned were subtended by groups of meristematic cells in the proliferated pericycle, the exogenous buds of *S. albidum* are presumably derived from induced primordia rather than preformed, or pre-existing, primordia. The sphaeroblasts of *Pyrus malus* rootstocks are also derived from induced primordia, but these primordia differentiate as either sphaeroblasts or buds, but not both (Baldini and Mosse, 1956). In *S. albidum*, the cells at one end of each primordium become organized as an shoot apex which apparently causes the remainder of the cells around the periphery of the primordium to become organized as an adventive spherical cambium. Roots with forming sphaeroblasts ranged in size from 2 to 4 cm in wood diameter. The first xylem derivatives of a sphaeroblast to mature are located opposite the exogenous bud. Once sphaeroblasts have formed, they appear to grow continuously as evidenced by the positive correlation between leaf primordia number and nodule volume.

Although exogenous buds can form in great numbers, many of the larger roots we examined contained circular depressions, or "craters", on their surface which may have indicated the positions of former exogenous buds which had aborted or sprouted without success. Roots larger than approximately 4 cm in diameter did not have any swellings which would have indicated the position of exogenous buds, but they often had craters on their surface. These results suggest that although exogenous buds can form continuously during a window of time, they cannot form for the entire life of a root and as they age they tend to be lost from a root. Although the nodules which are produced by *Fraxinus* suspension cultures can withstand up to 2 weeks of desiccation and still regenerate as callus, and the sphaeroblasts of *Laburnum* can sprout upon being planted in peat (Baldini and Mosses, 1956), experiments to determine whether the sphaeroblasts of *S. albidum* roots are able to sprout upon being removed from the bark were not performed.

Root Bud Formation and Root Sprouting as a Function of Habitat:

Although *S. albidum* is best known as a short-lived pioneer tree of old fields and fence rows, it also occurs in dry woods and its stems can live for up to 1000 years and grow to heights of over 90 feet (Thompson, 1982; Collingwood, 1987). Even if tree-sized stems of *S. albidum* are absent from a stand, the roots of pre-existing clones may be able to persist for indefinite amounts of time by producing a succession of ephemeral sprouts (Liming and Johnston, 1944; Martin et al., 1982b, Blount, 1989). Similarly, in habitats which are subjected to frequent disturbances, such as prairies and pine barrens, *S. albidum* is able to persist by means of a turnover of sprouts (Harshberger, 1970, Anderson, 1977). Although *S. albidum* roots can produce reparative buds, the results of our research suggest that most of the sprouts and/or ramets of *S. albidum* clones are derived from suppressed trace buds, irrespective of the successional status or disturbance history of the clone in question.

After the formation of suppressed trace buds has ceased on a length of root, reparative buds may form. Nearly 1/3 of the sectioned swellings were subtended by exogenous buds of pericylic origin and their associated sphaeroblasts, but these buds are apparently unable to sprout since none of the root sprouts that we sectioned were subtended by buried sphaeroblasts. The sphaeroblasts of *S. albidum* may function as dispersive propagules, which sprout upon being shed from the bark of a root or dispersed by biotic factors, but these possibilities seems unlikely.

Reparative buds can also form from the phloem parenchyma cells of rays, but such buds are rare. Only three of the sprouts which we sectioned were subtended by rays instead of traces. Shoot buds derived from wide rays have been found in the stems of *Tilia platyphyllos*, *Fraxinus excelsior*, and *Couroupita guianensis* (Fink, 1983) and they can be induced to form in the root cuttings of several varieties of *P. malus* (Siegler and Bowman, 1939). Since buds of ray origin are located near to the vascular cambium of a root, they can presumably form tracheary

connections with the vascular cambia of their parent roots as readily as suppressed trace buds but the rays which subtend them presumably have fewer stored reserves than the traces which subtend suppressed trace buds.

Since sphaeroblast buds are apparently unable to sprout and buds derived from the parenchyma cells of rays are uncommon, the ability of *S. albidum* clones to persist in later-successional habitats and to regrow following disturbance, is presumably dependent upon their ability to form additional suppressed trace buds near the apices of young roots, or to coppice from the base of failed sprouts. In fact, in late successional habitats coppice sprouts may be more numerous than new root sprouts (Blount, 1987, Bosela, personal observation). These results suggest that although root buds facilitate the spread of expanding clones into open areas, they contribute little to the maintenance of old clones in an understory environment.

LITERATURE CITED

Aaron, I. 1946. Dormant and adventitious buds. Science 104:329.

Anderson, R.C. 1977. Response of a southern Illinois grassland community to burning. Transactions Ill. State Acad. Sci. 69(4):399-414.

Baird, J.H., R.R. Dute, and R. Dickens. 1992. Ontogeny, anatomy, and reproductive biology of vegetative reproductive organs of *Diodia virginiana* L. (Rubiaceae). Int. J. Plant Sci. 153:320-28.

Bakshi, T.S. and R.T. Coupland. 1959. An anatomical study of the subterranean organs of *Euphorbia esula* in relation to its control. Can. J. Bot. 37:613-619

Baldini, E and B. Mosse. 1956. Observations on the origin and development of sphaeroblasts in the apple. J. Hort. Sci. 31:156-62.

Barnes, B.V. 1966. The clonal growth habit of American aspens. Ecology 47(3):439-47.

Blount, T.E. 1989. The biology of *Sassafras albidum* (Nutt.) Nees in regenerating upland hardwood stands in southeastern Virginia following clear felling. M.S. Thesis. Virginia Polytechnical Inst. and State Univ.

Bonnett, H.T. and J.G. Torrey. 1966. Comparative anatomy of endogenous bud and lateral root formation in *Convolvulus arvensis* roots culture in vitro. Amer. J. Bot. 53(5):496-507.

Brown, A.B. 1935. Cambial activity, root habit, and sucker development in two species of poplar. New Phytol. 34:163-79.

Burns, R.M. and B.H. Honkala. 1990. Silvics of North America, Volume 2 (Hardwoods), USDA Forest Service, Agricultural Handbook, Agriculture Hanbdbook 654.

Busgen, M. 1929. The Structure and Life of Forest Trees. John Wiley & Sons, New York.

Carlson, M.C. 1938. The formation of nodal adventitious roots in *Salix cordata*. Am. J. Bot. 25:721-25

Carlson, M.C. 1950. Nodal adventitous roots in willow stems of different ages. Am. J. Bot. 37: 555-61.

Chandler, W.H. 1925. Fruit Growing. Houghton Mifflin Co., Boston.

Chandler, W.H. 1947. Deciduous Orchards. Lea & Febiger, Philadelphia.

Charlton, W.A. 1965. Bud initiation in excised roots of *Linaria vulgaris*. Nature: 207:781-82.

Clepper, H. 1989. The singular Sassafras. American Forests. 95(3/4):35-35, 57.

Collingwood, G.H. 1978. Knowing Your Trees. American Forestry Association. Washington, D.C.

Esau, K. 1965. Plant Anatomy (2nd edition). John Wiley & Sons, Inc., New York. 550 pp.

Fink, S. 1982. Adventitious root primordia - the cause of abnormally broad xylem rays in hard- and softwoods. IAWA Bulletin. 3(1):31-39.

Fink, S. 1983. The occurrence of adventitious and preventitious buds within the bark of some temperate and tropical trees. Amer. J. Bot. 70:532-42.

Fjell, I. 1985. Preformation of root primordia in shoots and root morphogenesis in *Salix viminalis* Nord. J. Bot. 5:357-76.

Forcier, L.K., 1973. Seedling pattern and population dynamics, and the reproductive strategies of sugar maple, beech, and yellow birch at Hubbard Brook. Ph.D. Thesis, Yale University, New Haven, CT, 162 pp.

Gofoth, P.L. and J.G. Torrey. 1977. The development of isolated roots of *Comptonia peregrina* (Myricaceae) in culture. Amer. J. Bot. 64(4):476-82.

Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill Book Company, New York. 523 pp.

Jones, R. H. and D.J. Raynal. 1986. Spatial distribution and development of root sprouts in *Fagus grandifolia* (Fagaceae). Am. J. Bot. 73(2):1723-31.

Jones, R.A. and D.J. Raynal. 1988. Root Sprouting in American Beech (*Fagus grandifolia*): Effects of root injury, root exposure, and season. For. Ecol. Mgmt. 25:79-90.

Hahne, B. 1926. The origin of secondary dormant buds in deciduous fruit trees. Univ. Calif. Pub. Bot. 13(5): 125-27.

Harshberger, J.W.. 1970. The vegetation of the New Jersey Pine Barrens. Dover Publications, Inc., New York.

Hartig, T. 1878. Anatomie und Physiologie der Holzpflanzen. Springer-Verlag, Berlin.

Hassig, B.E. 1974. Origins of adventitious roots. New Zeal. J. For. Sci. 4: 299-310.

Holm, T. 1925. On the development of buds upon roots and leaves. Ann. Botany London, 39: 867-81.

Kondrat'eva-Mel'vil', E.A., 1957. "Obrazovanie kornevykh otpryskov u nekotorykh travyanistykh dvudol'nykh. [Formation of shoots on roots in some herbaceous dicotyledons]" Lenigrad Univ. Vest. Ser. Biol. Bull., 12:23-37.

Kormanik, P.P. and C.L. Brown. 1964. Origin of secondary dormant buds in sweetgum. U.S. Forest Service Research Note SE - 36.

Kormanik, P.P. and C.L. Brown. 1967a. Suppressed buds on lateral roots of *Liquidambar styraciflua*. Bot. Gaz. 128:208-211.

Kormanik, P.P. and C.L. Brown. 1967b. Root buds and the development of root suckers in Sweetgum. For. Sci. 13:338-345.

Kormanik, P.P. and C.L. Brown. 1967c. Adventitious buds on mature trees. Forest Service Research Note SE-82.

Kramer, P.J. and Kozlowski, T.T. 1979. Physiology of Woody Plants. Academic Press, New York.

Lek, H.A.A. van der. 1924. Over de wortelvorming van houtige stekken. Meded Landbouwhogeschool Wageningen 28:1-230.

Liming, F.G. and J.P. Johnston. 1944. Reproduction in Oak-Hickory forest stands of the Missouri Ozarks. J. For. 42:175-80.

Lovell, P.H. and J.White. 1986. Anatomical changes during adventitious root formation. In M.B. Jackson (ed), New Root Formation in Plants and Cuttings, Marinus Nighoff Publishers, Boston. pp.111-41.

Martin, W.L., T.L. Sharik, R.G. Oderwald, and D. Wm. Smith. 1982. Phytomass and nutrient levels in the understory of an Appalachian oak forest in southeast Virginia. Bull. Torr. Bot. Club. 109(3):330-43.

Murray, B.E. 1957. The ontogeny of adventitious stems of roots of creeping-rooted alfalfa. Can. J. Bot. 35:463-75.

Paukkonen, K., A. Kauppi, and A. Ferm. 1992. Root and stump buds as structural faculties for reinvigoration in *Alnus incana*. Flora 187:353-67.

Peterson, R.L. 1975. The initiation and development of root buds. In J.G. Torrey and D.T. Clarkson (eds.), The Development and Function of Roots, pp. 125-161. Academic Press. London, New York.

Polowick, P.L. and M.V.S. Raju. 1982. The origin and development of root buds in *Asclepias syriaca*. Can. J. Bot. 60:2119-125.

Preece, J.E. and S. Bates. 1991. *In vitro* studies with wite ash (*Fraxinus americana*) nodules. In M.R. Ahuja (ed.), Woody Plant Biotechnology. Plenum Press, New York.

Priestley, J.H. and C.F. Swingle. 1929. Vegetative propagation of plants from the standpoint of plant anatomy. U.S. Dept. Agr. Tech. Bull. 151.

Raju, M.V.S., R.T. Coupland, and T.A. Steeves. 1966. On the occurrence of root buds on perennial plants in Saskatchewan. Can. J. Bot. 44:33-37.

Rao, S. and H.Y. Mohan Ram. 1981. Regeneration of whole plants from cultured root tips of *Limnophila indica*. Can. J. Bot. 59:969-73.

Robinson, J.C. and W.W. Schwabe. 1977. Studies on the regeneration of apple cultivars from root cuttings I. Propagation aspects. J. Hort. Sci. 52:205-220.

Sandburg, D. 1951. The regeneration of quaking aspen by root suckering. M.F. Thesis, Univ. Minn.

Schier, G.A. 1973. Origin and Development of Aspen Root Suckers. Can. J. For. Res. 3:45-53.

Schier, G.A. and R.B. Campbell. 1976. Differences among *Populus* species in the ability to form adventitious shoots and roots. Can. J. For. Res. 6:253-61.

Schier, G.A., J.R. Jones, and R.P. Winokur. 1979. Vegetative regeneration. In N.V. DeByle and R.P. Winokur (Eds) Aspen: Ecology and Management in the Western United States. USDA Forest Serv. Gen Tech.Report RM-119. pp.29-33.

Schirman, R. and B..A. Zamora. 1978. Bud development in excised roots of rush skeletonweed (*Chondrilla juncea*). Weed Science 26:582-84

Siegler, E.A. and J.J. Bowman. 1939. Anatomical studies of root and shoot primordia in 1-year apple roots. J. Agr. Research 58:795-804.

Stone, E.L., Jr. and M.H. Stone. 1943. "Dormant" versus "adventitious" buds. Science 98:62.

Stout, A.B. 1929. The clone in plant life. J. New York Bot. Garden 30:25-37.

Swingle, C.F. 1927. Burrknot formations in relation to the vascular system of the apple stem. J. Agr. Res. 34(6):533-44.

Trecul, A. 1847. Recherches sur l'origine des bourgeons adventifs. Ann. D. Sci. Nat., Bot., ser. iii, vol. viiii, Paris, p. 268.

Wehtje, G., J.W. Wilcut, T. Vint Hicks, and G.R. Sims. 1987. Reproductive biology and control of *Solanum dimidiatum* and *Solanum carolinense*. Weed Sci. 35:356-59.

Wilkinson, R.E. 1966. Adventitious shoots on saltcedar roots. Bot. Gaz. 127(2-3):103-104.

Wittrock, V. B. 1884. Om rotskott hos ortartade vaxter, med sarskild hansyn till deras olika biologiska betydelse. Botan. Notiser, 21-37.

Zimmerman, M.H. and C.L. Brown. 1971. Trees: Structure and Function. Springer-Verlag, Berlin. 336 pp.