Shoot morphogenesis associated with flowering in *Populus deltoides* (*Salicaceae*)[1]

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Temporal and spatial formation and differentiation of axillary buds in developing shoots of mature eastern cottonwood (*Populus deltoides*) were investigated. Shoots sequentially initiate early vegetative, floral, and late vegetative buds. Associated with these buds is the formation of three distinct leaf types. In May of the first growing season, the first type begins forming in terminal buds and overwinters as relatively developed foliar structures. These leaves bear early vegetative buds in their axils. The second type forms late in the first growing season in terminal buds. These leaves form floral buds in their axils the second growing season. The floral bud meristems initiate scale leaves in April and begin forming floral meristems in the axils of the bracts in May. The floral meristems subsequently form floral organs by the end of the second growing season. The floral buds overwinter with floral organs, and anthesis occurs in the third growing season. The third type of leaf forms and develops entirely outside the terminal buds in the second growing season. These leaves bear the late vegetative buds in their axils. On the basis of these and other supporting data, we hypothesize a 3-yr flowering cycle as opposed to the traditional 2-yr cycle in eastern cottonwood.

Key words: bud differentiation; eastern cottonwood; *Populus deltoides*; Salicaceae.

Eastern cottonwood (*Populus deltoides* Bartr. ex Marsh. var. *deltoides*) is a fast-growing forest tree species of commercial value native to the eastern United States (FAO, 1980; Burns and Honkala, 1990). Eastern cottonwood hybridizes with many other species in the genus, and there are numerous breeding programs for improvement of growth rate, wood properties, and disease and insect resistance (FAO, 1980; Land et al., 1996; Land and Singh, 1997). Genetic engineering is underway to develop reproductive sterility for transgene containment in hybrids with black cottonwood (*Populus trichocarpa* Torr. and Gray.) (Meilan, 1997; Strauss et al., 2001). Techniques to regulate flowering in eastern cottonwood are needed for (1) conventional breeding programs in order to shorten the generation interval for genetic improvement, (2) delaying floral induction in mature trees during the rotation period to increase wood biomass production by reallocating metabolites from reproductive to vegetative growth, and (3) environmental containment of transgenes via reproductive sterility of genetically engineered trees in order to minimize the unwanted spread of transgenes to interfertile wild relatives (Meilan, 1997; Meilan et al., 2001; Strauss et al., 2001). However, the fundamental mechanisms that regulate flowering in eastern cottonwood are unknown and must be determined before suitable techniques can be developed. One of the first steps in elucidating these mechanisms is to understand the

temporal and spatial formation and differentiation of axillary bud meristems in developing shoots of mature trees.

Shoots of eastern cottonwood trees at the juvenile stage consist of vegetative buds, leaves, and internodes; floral buds are added at maturity. Each shoot forms a terminal bud enclosed by bud scales that are formed by the enlargement of stipules to protect the foliage primordia of the following season's growth (Goffinet and Larson, 1981). However, shoots of mature eastern cottonwoods begin forming terminal buds early in the growing season, approximately 2 mo following spring bud flush in the southern U.S. Dormant terminal buds of relatively mature black cottonwood (6–9 yr old) exhibit leaf dimorphism with "early" and "late" leaves based on a difference in leaf ontogeny (Critchfield, 1960). Early leaves are relatively more developed than developmentally arrested late leaf primordia. When terminal buds open in the following growing season, early leaves protrude from terminal buds and expand rapidly. Late-leaf expansion then occurs in late spring. It is anticipated that molecular events in initiation of the following year's inflorescences occur either just prior to or during terminal bud opening in black cottonwood (Boes and Strauss, 1994).

Juvenile eastern cottonwoods do not show such clear-cut leaf dimorphism (Goffinet and Larson, 1981). It is not reported if mature eastern cottonwoods exhibit distinct leaf and bud types in relation to flowering during shoot development. The objectives of this work were (1) to define the shoot developmental pattern with bud/leaf types in relation to flowering, and (2) to determine the sequence of initiation and development of reproductive meristems in the axillary buds of eastern cottonwood.

MATERIALS AND METHODS

Two randomly selected genotypes of mature *Populus deltoides*, one male (ST-124, 32°55′ N, 91°05′ W, Mayersville, Mississippi, USA) and one female (ST-72, 32°37′ N, 91°00′ W, Issaquena, Mississippi, USA), were used for observations and sample collections. ST-124 was a cutting originating from
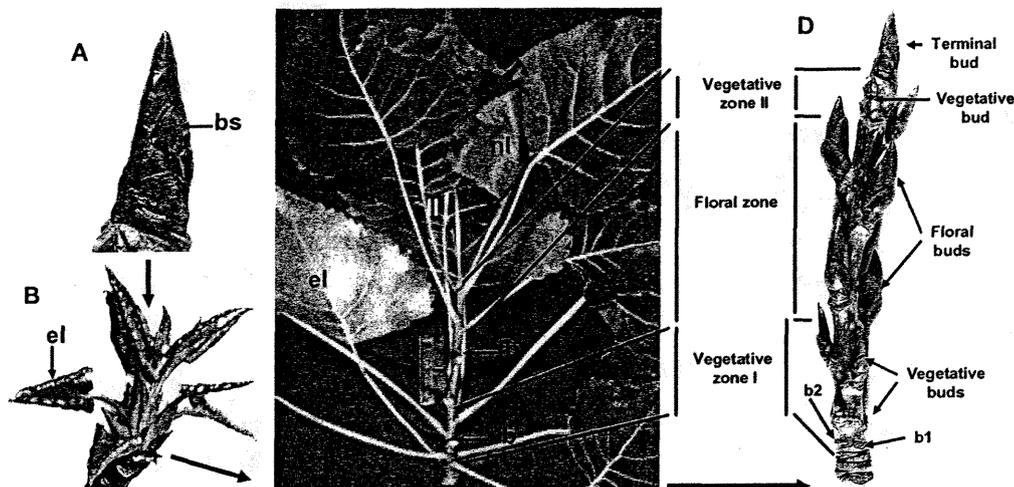
Fig. 1.   Pattern of mature eastern cottonwood shoot development over two growing seasons. (A) Each shoot formed a terminal bud covered by bud scales (bs) in the first growing season. (B) Terminal bud opened early in the second growing season, and early preformed leaves unfolded to form early leaves (el). (C) By the end of May, shoot growth and leaf expansion were almost complete. Floral (fb) and vegetative (vb) buds were visibly distinguishable. Early (el), late (ll), and neoformed (nl) leaves were identified by their development and locations. The razor blade is 4 cm in length. (D) Buds in (C) continued to grow until October. This shoot was collected in December. The zones were already established with respect to bud types. Underdeveloped axillary buds are represented by b1 and b2.

a male tree, and ST-72 was an open-pollinated progeny from a female tree. The cottonwoods were located in a plantation of Anderson-Tully Company about 32 km north of Vicksburg, Mississippi, USA. The experimental trees were 22 yr old in 1998. Terminal and axillary buds were collected in 1998 and 1999 from flower-bearing annual shoots in the upper crown (once in March, five times at 2-wk intervals from mid-April to mid-June, and once per month in July, August, September, and October). Fifty randomly chosen shoots from a main branch were collected on each date, and the bud at the ninth node (from the base of shoots) was excised. This node location was selected based on the personal observation that flower buds generally form around this node. Buds were fixed in half-strength Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde) with phosphate buffer (0.1 mol/L, pH 7.2) for 48 h at 4°C (Karnovsky, 1965). Buds were then transferred into a quarter-strength fixative for storage at 4°C.

The buds were prepared for scanning electron microscopy (SEM) by removing bud scales, leaf primordia, and other extraneous parts in a 0.2 mol/L phosphate buffer (pH 7.2) under a dissecting microscope to expose the bud meristems. The peeled buds were dehydrated through a graded ethanol series and stored in 100% ethanol. They were critical point dried in a Polaron E3000 Critical Point Dryer (Quorum Technologies, Newhaven, UK) using liquid CO_2. The tissues were then mounted on stubs and coated with gold-palladium using a Polaron E5100 sputter coater (Quorum Technologies). Samples were subsequently examined with a S360 SEM (Leo Electron Microscopy, Thornwood, New York, USA) at 15 kV. Representative tissues of each date were photographed using Polaroid Type 55 4 × 5 black and white film (Polaroid, Cambridge, Massachusetts, USA). The tissues for histological work were dehydrated in an ethanol series and cleared in tertiary butyl alcohol. Tissues were then embedded in Paraplast (Fisher, Burr Ridge, Illinois, USA), sectioned at 8 μm, mounted, and stained with safranin-fast green (Jensen, 1962). The sections were examined on a Leica TCS NT confocal microscope system (Leica Microsystems, Heerbrugg, Switzerland).

RESULTS

Each eastern cottonwood shoot formed a terminal bud covered with several layers of bud scales during the first growing season (Fig. 1A). Terminal buds flushed in the second half of March of the following growing season, and the preformed leaves rapidly unfolded (Fig. 1B). Shoot expansion was complete by late May and floral and vegetative buds were visibly

distinguishable (Fig. 1C). Morphological investigation of annual shoots at the end of the second growing season revealed a common pattern. The first set of axillary buds at the base of the shoot was vegetative (Vegetative Zone I), the second set of axillary buds was reproductive (Floral Zone), and the distal set of buds was vegetative (Vegetative Zone II) (Fig. 1D).

Removal of the bud scales of a fully developed terminal bud at the end of the first growing season and its examination by SEM showed that it contained preformed leaves and stipules (Fig. 2A). The preformed leaves were 8–10 mm in length (lamina length + petiole length). Both adaxial and abaxial surfaces of the leaves were covered with dense trichomes and sticky resin (Fig. 2A1). The number of preformed leaves 8–9 mm in size was approximately eight. The preformed leaves were further removed to display the subtended structures in their axils. A stipule formed at each side of each preformed leaf (Fig. 2B–C). There were preformed buds in the axil of preformed leaves 3–8 (Figs. 2B, 2B1, 2B2, 2C, 2C1, and 2C2). The first and second preformed leaves did not have definitive preformed buds in their axils, although there were protuberances (Fig. 3A–B). The protuberance was larger in the second preformed leaf axil than in the first. These protuberances did not develop into fully grown buds in the following growing season (see b1 and b2 in Fig. 1D). Some of the preformed buds had organized structures (Fig. 4A). For example, the third preformed bud in the axil of the third preformed leaf had a well-defined, two-layer tunica (Fig. 4A). No leaf primordium or bud scale formation was observed at this developmental stage. All preformed buds were covered with petioles of the surrounding early preformed leaves.

Close examination of the distal end of the terminal bud with SEM revealed some leaf-like structures (Fig. 2B–C). This region was sectioned and examined with a confocal laser microscope. There were 3–5 preformed leaf primordia (all smaller than 1 mm in length) close to the shoot apical meristem (Fig. 5). Trichome formation was not observed on these leaf primordia, nor were preformed buds in the axils.

These observations indicated that two distinct developmen-

Fig. 2. Content of a terminal bud of a mature eastern cottonwood shoot in December. (A) Terminal bud with the bud scales removed. Early preformed leaves (epl) and several stipules (s) are shown. (A1) Abaxial surface of the fourth early preformed leaf (4epl) showing dense trichomes and sticky resin that covered the surface. (B) Early preformed leaves removed to display the preformed buds. First (1epl) and second (2epl) early preformed leaves without definitive preformed buds in their axils. The fourth (4epl) (B1) and seventh (7epl) (B2) early preformed leaves had preformed buds (b4 and b7, respectively). (C) Reverse side of the embryonic shoot. Locations of the third (3epl), fifth (5epl), sixth (6epl), and eighth (8epl) early preformed leaves are shown. The 3epl (C2), 5epl (C1), and 6epl (C) had preformed buds (b3, b5, and b6, respectively) in their axils. lls = leaf-like structure. Bars: (A), 2 mm; (A1), 250 μm; (B), 2 mm; (B1), 225 μm; (B2), 100 μm; (C), 1 mm; (C1), 100 μm; (C2), 250 μm.
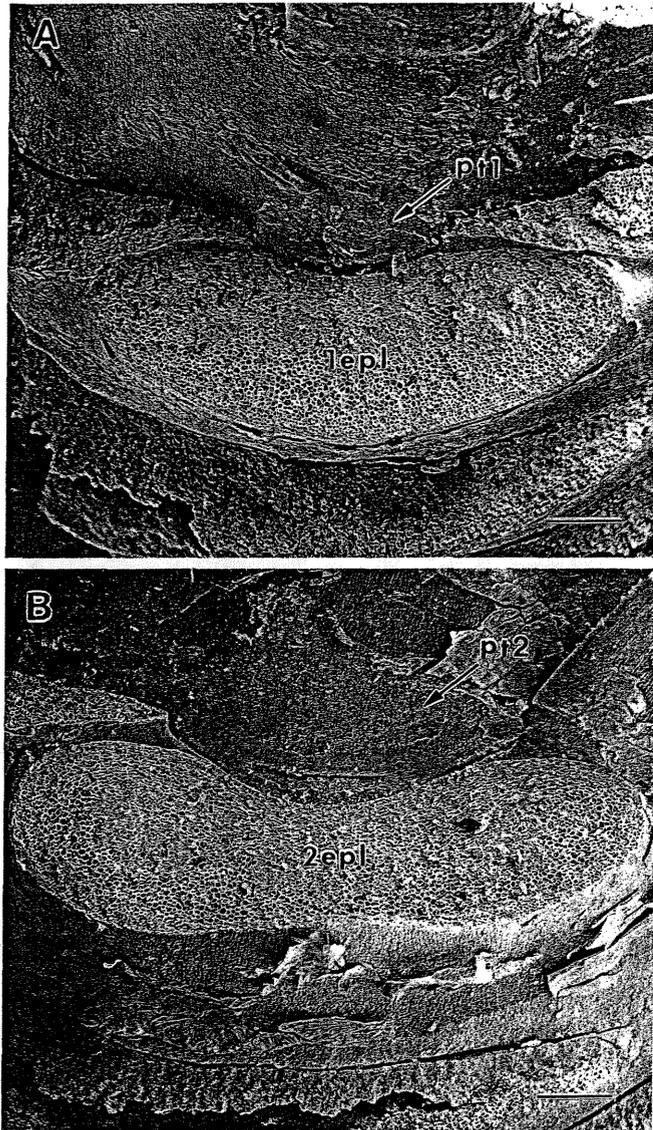
Fig. 3. Protuberances (pt) shown in the axils of the first (A) and second (B) early preformed leaf indicate the locations of underdeveloped axillary buds (b1 and b2) in Fig. 1D. Bars: 250 μm.

tal stages of preformed leaves exist in the terminal buds of mature cottonwood trees. The first group, those with axillary buds and trichomes, are "early preformed leaves," and the second group, those 1 mm or less in length with no trichomes and no axillary buds, are "late preformed leaves." The early preformed leaves began forming in May along with the cessation of shoot expansion and continued development through summer and early fall. In contrast, late preformed leaves formed in late summer and early fall; the winter interrupted their development.

In the second growing season, the terminal buds opened in the second half of March, and the tips of the early preformed leaves protruded from the bud. Afterwards, the blades emerged and unfolded to form "early leaves" (Fig. 1B). The early leaves all expanded rapidly to reach almost their full size in 1 mo (by the third week of April). The late preformed leaves began expanding to form "late leaves" in the middle of April

(3 wk after the terminal bud flush). The expansion was almost complete by the end of May (Fig. 1C). There were also "neo-formed leaves" that began forming very close to the shoot apical meristem in the second half of April along with the beginning of the late-preformed leaf expansion (Fig. 1C). These neoformed leaves completed leaf expansion by the middle of June. The neoformed leaves formed entirely within the second growing season. Thus, they were not present in the terminal bud during the first growing season.

All preformed buds in the terminal bud during the first growing season formed vegetative buds in the axils of early preformed leaves. The preformed buds grew to various sizes by the end of the second growing season in Vegetative Zone I (Fig. 1D). Although the preformed leaves close to the base of the shoot form early during the first growing season and expand first during the second growing season, the most basal vegetative buds in Vegetative Zone I were smaller in size than the upper ones. We currently do not know what regulates their size.

The floral buds in the Floral Zone began forming in the axils of late leaves during the second growing season, because bud formation in the axils of these leaves was not observed during the first growing season. Examination of the bud meristem by SEM at node nine in April, May, and June indicated morphological changes that led to floral meristem development and organ formation. The meristem formed bud-scale primordia in both male and female buds during April (Figs. 6A, 6B, 7A, and 7B). Formation of the dome-shaped apical meristem during April indicated high mitotic activity. Bract formation was first detected in the inflorescence meristem in both sexes in the first half of May (Figs. 6C and 7C). No additional bud-scale primordia were formed after bract formation. Numerous bracts in a helical orientation were formed centripetally (from outside to the center) by late May (Figs. 6D and 7D). However, there were more bracts formed on the male inflorescence meristem than on the female. Floral meristems developed in the axils of the bracts slightly after bract primordia formation. The bracts elongated and formed leaf-like structures by the middle of June (Figs. 6E and 7E). Floral meristems at this time were enlarged and easily visible when the bracts were removed (Figs. 6E and 7E).

Male and female axillary floral buds formed a cup-like, reduced perianth in the axils of fully elongated bracts by winter in the second growing season (Fig. 8A and C). The stigmas and tetrasporangiate anthers were surrounded by the reduced perianth (Fig. 8A–C). These lateral floral buds formed mature male and female catkins during anthesis in March of the third growing season.

DISCUSSION

Shoots of mature eastern cottonwood trees possess a well-defined pattern of structural development that includes three distinct leaf types and specific locations of vegetative and floral buds. The first set of leaves bears vegetative buds in their axils. These leaves have been designated "early preformed leaves" (Critchfield, 1960) and comprise all the leaves in Vegetative Zone I. The next set of leaves has been designated "late preformed leaves" (Critchfield, 1960). They exist in the first growing season's overwintering terminal bud as relatively undeveloped leaf primordia. These leaves subsequently bear flower buds in their axils and comprise the Floral Zone during the second growing season. Leaves at the distal end of the
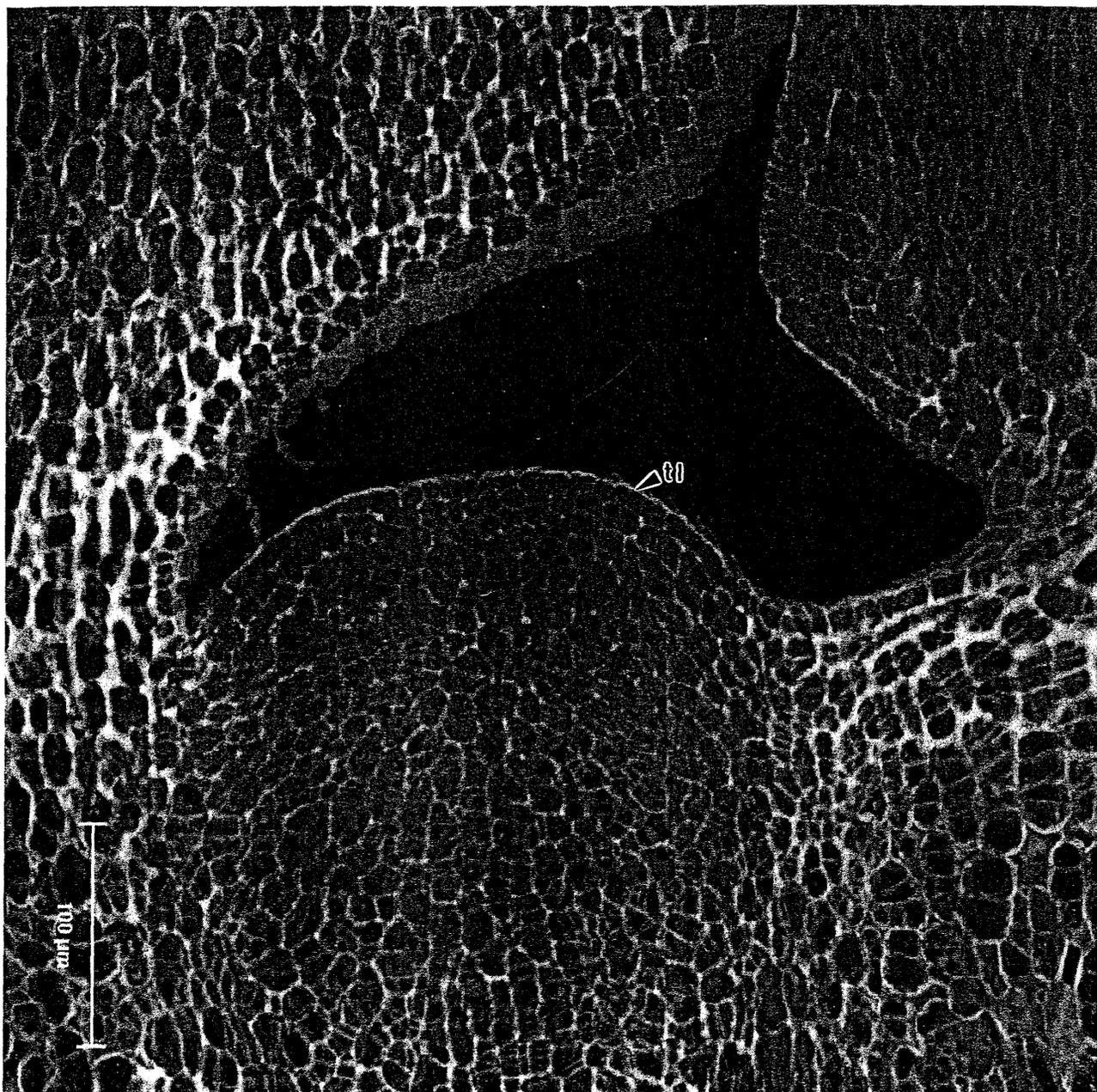
Fig. 4.   A thin section of the third preformed bud in the axil of the third early preformed leaf in a dormant terminal bud in December in the first year. The thin section shows a two-layer tunica formation (tl).

shoot may be designated "neoformed leaves," because they are initiated and expand entirely within the second growing season (do not exist as primordia in the first season's terminal bud). These leaves comprise Vegetative Zone II and bear vegetative buds in their axils. It is likely that preformed leaves in terminal buds represent a continuum of primordial development. Moreover, it is possible that axillary buds associated with late leaves may exist at some extreme rudimentary stage in the first season's terminal bud. Nonetheless, we conclude that the absence of intermediate leaf sizes, presence or absence of trichomes, and visibly recognizable axillary buds represent two distinct developmental stages among preformed leaves in the terminal buds of mature eastern cottonwood.

The lateral vegetative and flower buds depicted in Fig. 1 were thought to form during spring leaf expansion, opening a year later to produce lateral branches and flowers. Thus, flowering was said to occur over two growing seasons with flower bud formation in the first growing season and anthesis in the following growing season (Pauley, 1950; Nagaraj, 1952; Farmer, 1976; Boes and Strauss, 1994). However, we found primordial lateral buds in the axils of the early preformed leaves in terminal buds well in advance of spring leaf expansion. Therefore, year one can be considered the year when these primordial buds are formed. Year two is the year when they remain dormant in the axils of the fully expanded early leaves and when floral meristems form in the late leaf axils
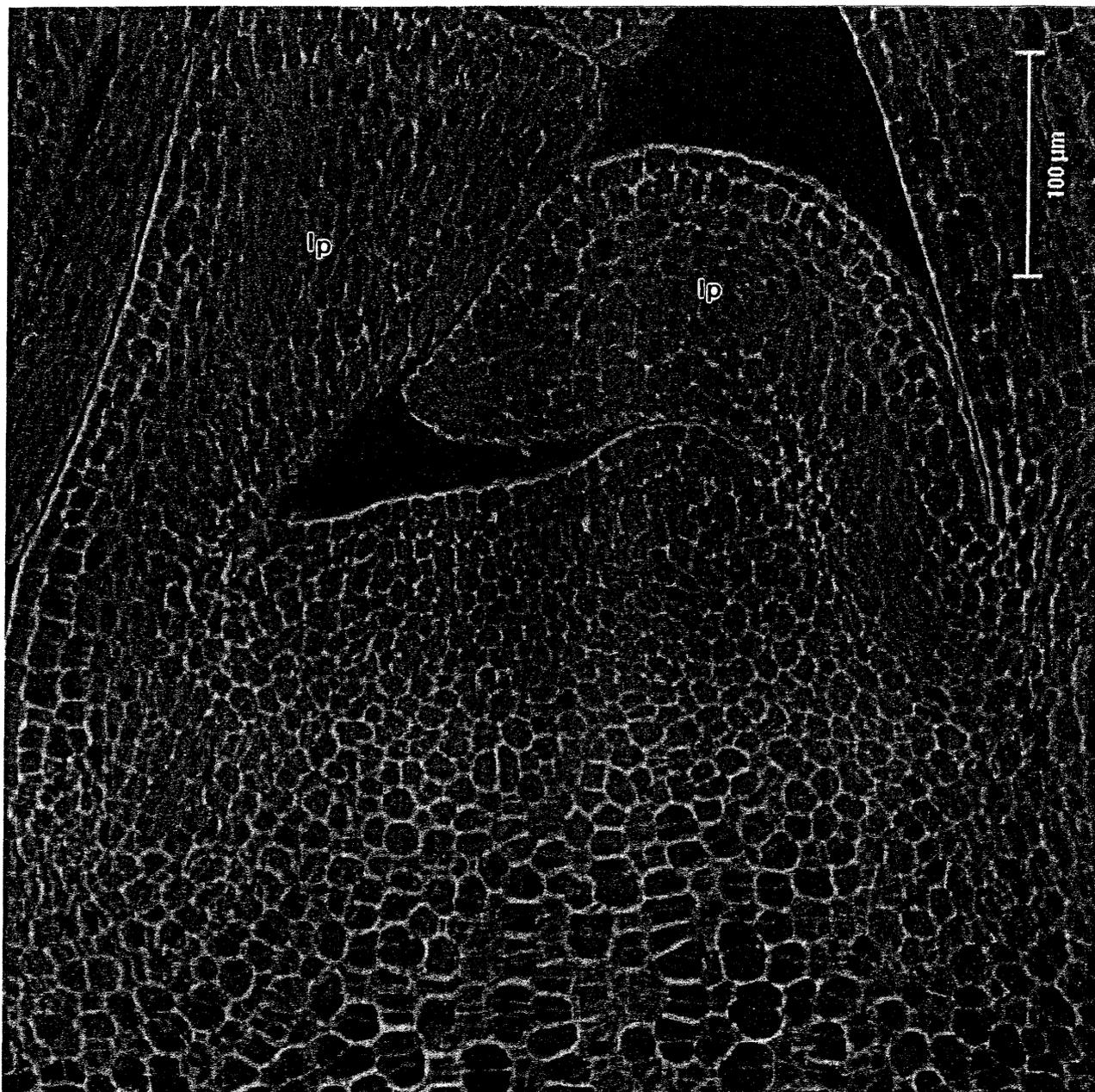
Fig. 5. Late preformed leaf primordia (lp) formed around the shoot apical meristem in a dormant terminal bud in December at the end of the first growing season. No axillary buds are apparent at this time.

and reproductive organs develop. Year three is the year of lateral branch growth and flower anthesis.

The primordial buds at the end of the first growing season have well-defined, two-layer tunica, but there are no leaf primordia or bud scales associated with them. Bud primordia are associated with the early preformed leaves. There are approximately eight early preformed leaves located at the base of the embryonic shoot, and 3–5 late preformed leaves at the apex. Early preformed leaves are initiated early in the development of the terminal bud (mid-May of the first growing season) and have a long embryonic developmental period interrupted by a cold period (vernalization) prior to expansion in the spring of the second growing season. Late preformed leaf primordia de-

velop late during terminal bud development and stay in a primordial stage during vernalization. There are no discernable primordial buds associated with late preformed leaf primordia, which may indicate that the development of flower buds in the axils of late preformed leaves is initiated during leaf expansion of the second growing season.

After their long embryonic developmental and vernalization periods during the first growing season, the early preformed leaves are the first structures to expand in the spring of the second growing season. These leaves reach their full size by the third week of April. The preformed buds in the axils of the early preformed leaves do not develop into flower buds. The buds of the Floral Zone appear to form in the axils of the
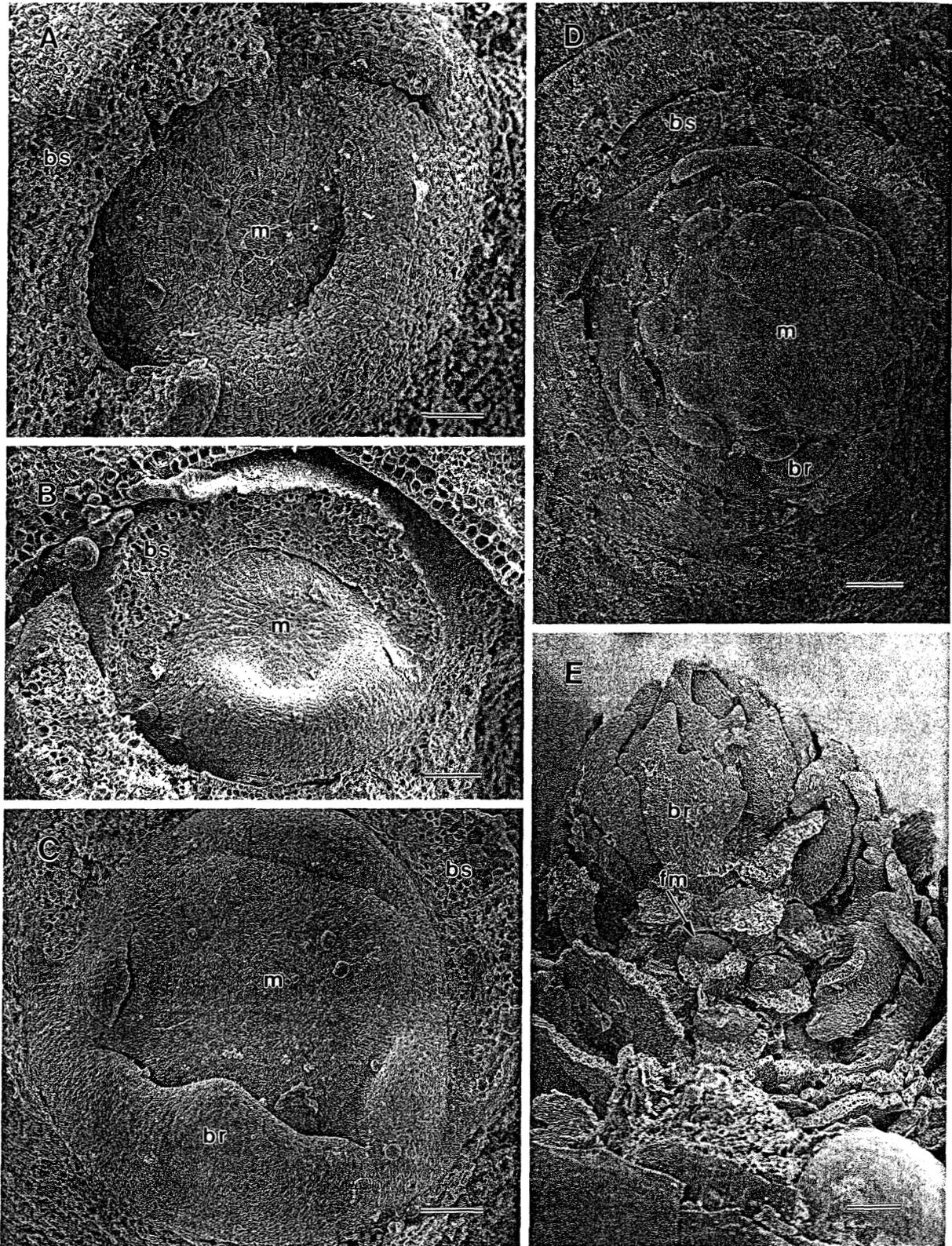
Fig. 6.   Sequence of morphological events at the meristem of the male axillary bud at node nine (from the base of the current year's shoot) in the axil of the late leaf in April, May, and June of the second growing season. The meristem (m) formed bud scales (bs) (A) on 11 April and (B) on 28 April. (C) Bract (br) formation began in the first half of May (9 May). (D) Centripetal bract formation continued on 23 May, and floral meristems were subtended by the bracts (not seen on the surface). (E) Highly developed floral meristems (fm) in the axils of bracts after some of the bracts were removed. Bars: (A), 25 μm; (B) and (C), 50 μm; (D) and (E), 100 μm.
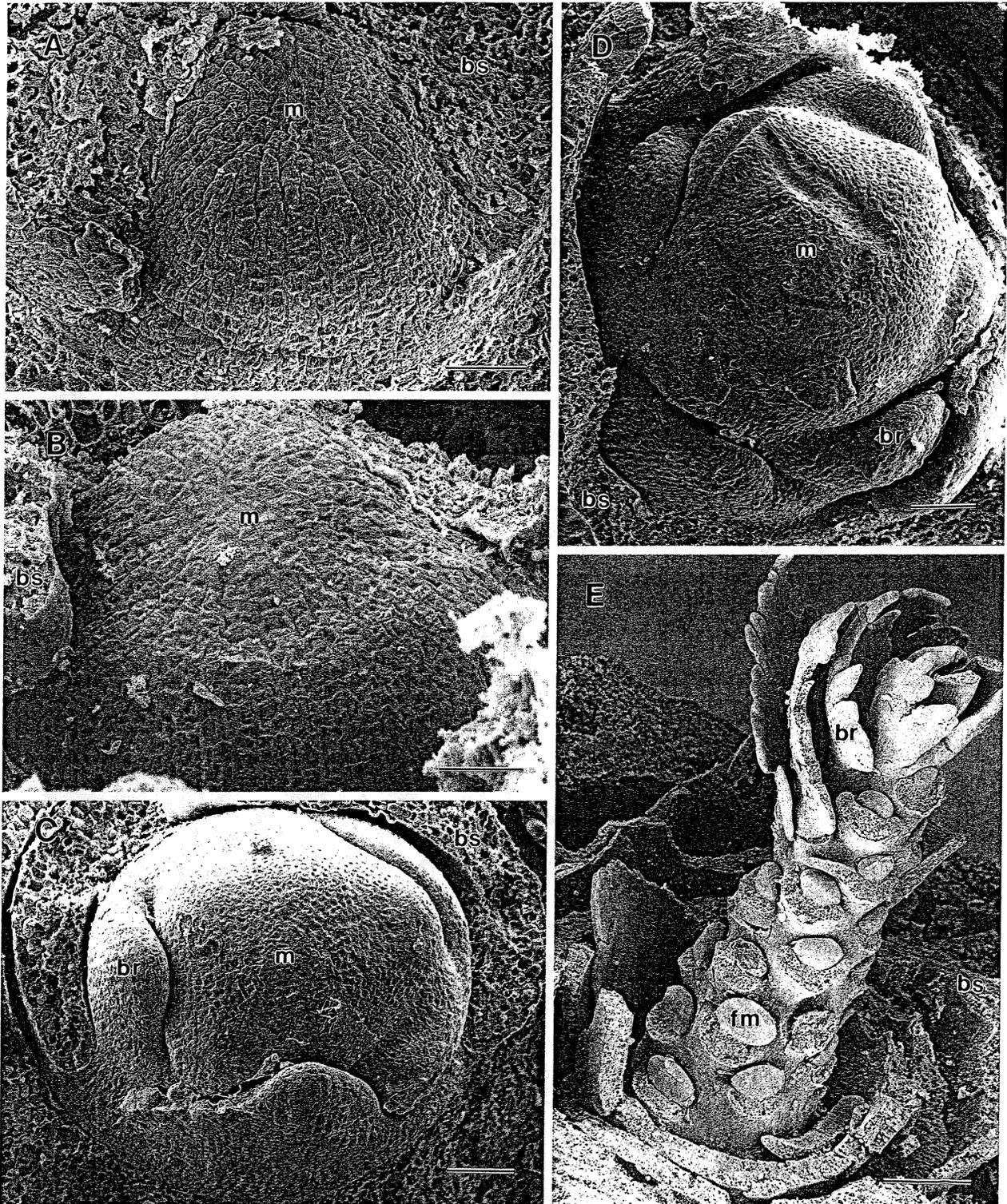
Fig. 7.   Sequence of morphological events at the meristem of the female axillary bud at node nine (from the base of the shoot) in the axil of the late leaf in April, May, and June of the second growing season. The meristem (m) form bud scales (bs) (A) on 11 April and (B) on 28 April. (C) Bract (br) formation began in the first half of May (9 May). (D) Centripetal bract formation continued on 23 May. (E) Highly developed floral meristems (fm) in the axils of bracts after some of the bracts were removed. Bars: (A) and (B), 25 μm; (C) and (D), 50 μm; (E), 250 μm.
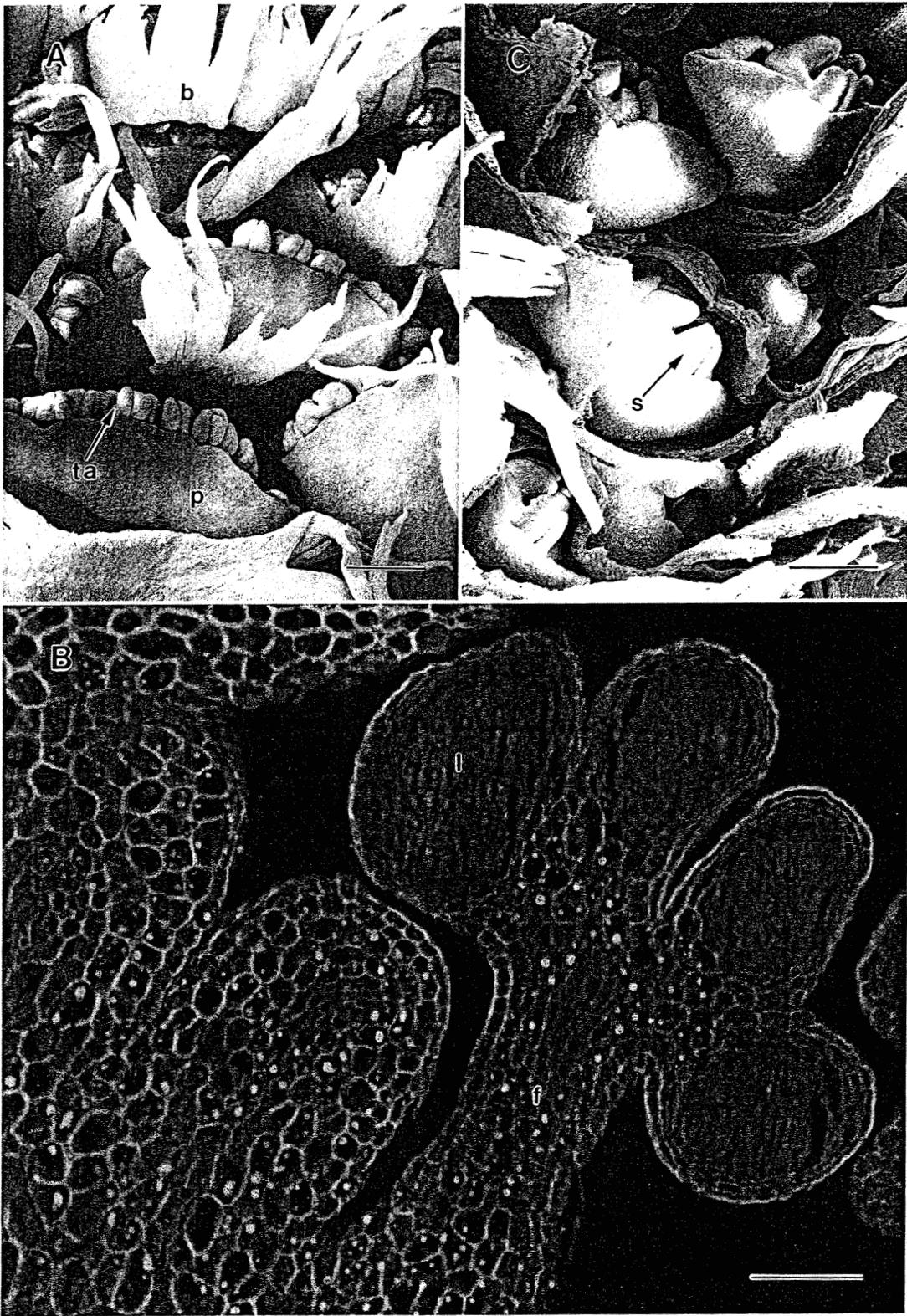
Fig. 8. Late morphological events in the axillary male and female floral buds at the end of the second growing season (December). (A) Tetrasporangiate anthers (ta) surrounded by a cup-like, reduced perianth (p) in the axils of mature bracts (b) in a male flower bud. (B) Longitudinal section of a tetrasporangiate anther with a filament (f). Loosely arranged pollen grains are seen in locules (l). (C) Stigmas (s) surrounded by a cup-like, reduced perianth in a female flower bud. Bars: (A) and (B), 250 μm; (C), 50 μm.
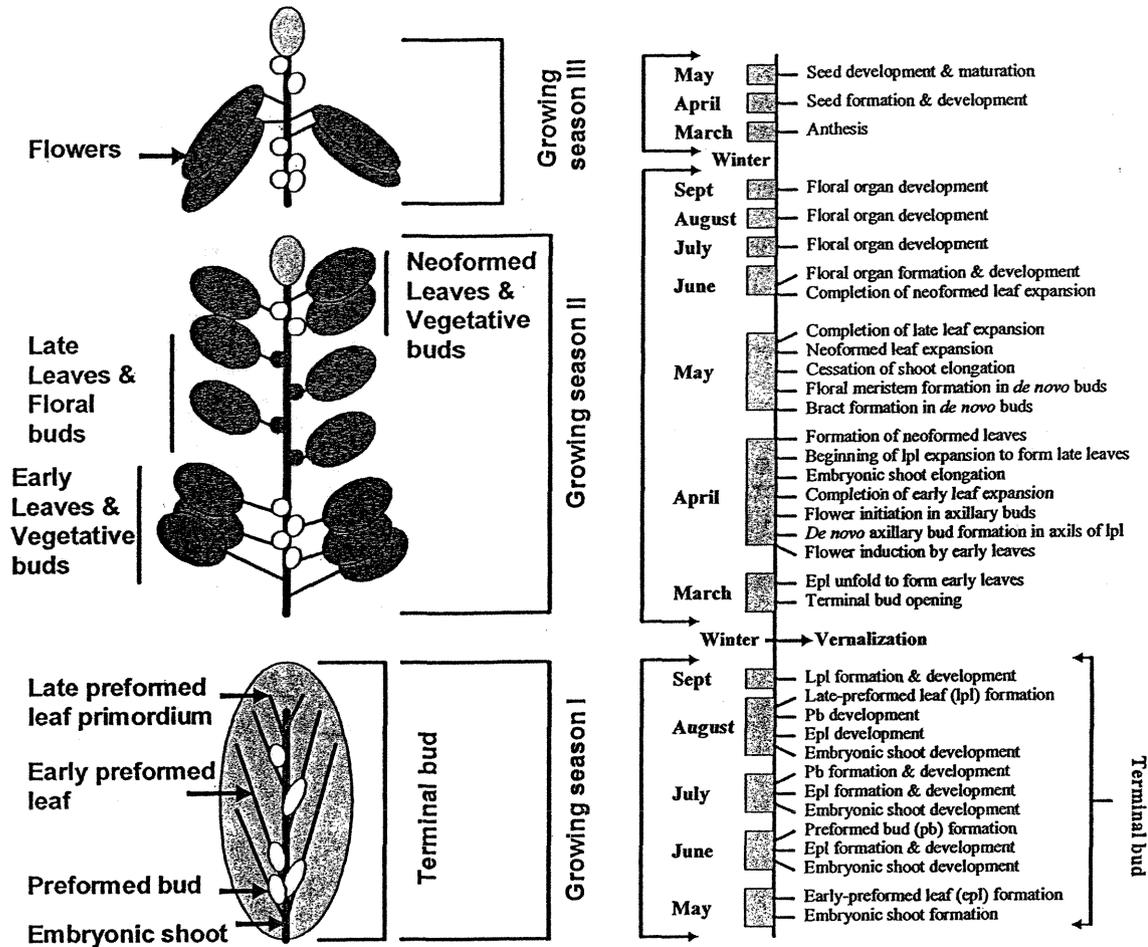
Fig. 9. Diagram of the hypothesized three-growing-season's shoot morphogenetic events leading to flower induction, initiation, and formation in mature eastern cottonwood shoots.

late leaves during spring leaf expansion of the second growing season. It is unknown whether the floral induction signal is available either prior to or during formation of the buds subtended by the late leaves. Equally important is by which leaves the floral signal is perceived and how the floral signal is translocated to the developing axillary buds. It is possible that the floral signal is perceived by developmentally advanced and vernalized early preformed leaves and translocated to the developing buds in the late-leaf axils through direct vascular connections. Not all of the leaves along the axis of a shoot are interconnected by vascular tissues in eastern cottonwood. Rather, a specific, repeating pattern of primary vascular tissues exists, such that a leaf at the base of the shoot is directly connected to nodes three and five positions above it (Larson and Pizzolato, 1977; Pizzolato and Larson, 1977; Dickson, 1986). This pattern of vascular connections is important in stress signal transduction among nodes, because a stress response from any particular leaf can affect the development of tissues with direct vascular connections to it (Dickson and Isebrands, 1991). The primary vascular connections are formed in the primordial stem tissues of the overwintering terminal bud as a continuation of acropetal elongation of the shoot, and the connections may play a role in determining the positioning of primordial leaves in the overwintering terminal bud (Larson, 1975).

The axillary bud meristem at node nine forms bud scales in both sexes by the end of April in the second growing season. The first morphological evidence of the transition from vegetative to reproductive growth in the axillary meristem comes with bract primordia formation in early May. Boes and Strauss (1994) observed similar differentiation events in black cottonwood in the northwestern U.S. The inflorescence meristem initiates more bract primordia centripetally in a helical orientation by the end of May. However, this process begins slightly later in the female inflorescence meristem. By mid-June, the floral meristems are well developed in the axils of the maturing bracts of the inflorescence meristems in both sexes. The first floral meristems in both sexes are likely initiated in the axils of the older bracts in the middle of May as seen in Figs. 7 and 8. Reproductive buds form inflorescences (catkins) in both sexes of cottonwood by the end of the second growing season. Anthesis occurs in March of the third growing season.

It appears that both the developmental state of leaves and the positions of axillary buds on a stem are important factors in flower initiation. Based on the observations reported in this paper, we hypothesize that the flowering sequence in eastern cottonwood spans a 3-yr period (Fig. 9), rather than a traditional 2-yr period as previously reported. The flowering process may begin as signal perception in early preformed leaves of the first growing season, prior to flower bud formation dur-

ing the second growing season, and anthesis during the third growing season. Negative regulators of flowering-time genes in early preformed leaves may be dominantly expressed to suppress flower induction during the first growing season, because the bud primordia associated with early preformed leaves in Vegetative Zone I do not differentiate into a floral state. The prolonged developmental and vernalization sequence of early preformed leaves during the first growing season is likely required to suppress as yet unidentified negative regulators of flowering-time genes. Negative regulators of flowering-time genes *FRIGIDA, FLOWERING LOCUS C,* and *EMBRYONIC FLOWER* in *Arabidopsis* play major roles in preventing plants from flower induction (Clarke and Dean, 1994; Sheldon et al., 1999; Aubert et al., 2001; Yoshida et al., 2001). Identification of such negative regulators in eastern cottonwood would allow the testing of the significance of preformed leaf development and vernalization on regulation of flowering-time genes in developing shoots during the first and second growing seasons. It is also important to characterize if a control mechanism is imposed by early preformed leaves in Vegetative Zone I on the initiation of floral buds in the Floral Zone.

LITERATURE CITED

AUBERT, D., L. CHEN, Y. H. MOON, D. MARTIN, L. A. CASTLE, C. H. YANG, AND Z. R. SUNG. 2001. EMF1, a novel protein involved in the control of shoot architecture and flowering in *Arabidopsis*. *Plant Cell* 13: 1865–1875.

BOES, T. K., AND S. H. STRAUSS. 1994. Floral phenology and morphology of black cottonwood, *Populus trichocarpa* (Salicaceae). *American Journal of Botany* 81: 562–567.

BURNS, R. M., AND B. H. HONKALA. 1990. Silvics of North America. 2. Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, D.C., USA.

CLARKE, J. H., AND C. DEAN. 1994. Mapping *FRI,* a locus controlling flowering time and vernalization response in *Arabidopsis thaliana. Molecular and General Genetics* 242: 81–89.

CRITCHFIELD, W. B. 1960. Leaf dimorphism in *Populus trichocarpa. American Journal of Botany* 47: 699–711.

DICKSON, R. E. 1986. Carbon fixation and distribution in young *Populus* trees. *In* T. Fujimori and D. Whitehead [eds.], Proceedings: crown and canopy structure in relation to productivity, 409–426. Forestry and Forest Products Research Institute, Ibaraki, Japan.

DICKSON, R. E., AND J. G. ISEBRANDS. 1991. Leaves as regulators of stress response. *In* H. A. Mooney, W. E. Winner, E. J. Pell, and E. Chu [eds.], Response of plants to multiple stresses, 3–34. Academic Press, San Diego, California, USA.

FAO (FOOD AND AGRICULTURAL ORGANIZATION OF THE UNITED NATIONS). 1980. Poplars and willows in wood production and land use. FAO (Food and Agriculture Organizations of the United Nations) Forestry Series no. 10, Rome, Italy.

FARMER, R. E., JR. 1976. Sexual reproduction of eastern cottonwood. *In* B. A. Thielges and S. B. Land, Jr. [eds.], Proceedings: symposium on eastern cottonwood and related species, 89–96. September 28–October 2, 1976, Greenville, Mississippi. Louisiana State University, Baton Rouge, Louisiana, USA.

GOFFINET, M. C., AND P. R. LARSON. 1981. Structural changes in *Populus deltoides* terminal buds and in the vascular transition zone of the stems during dormancy induction. *American Journal of Botany* 68: 118–129.

JENSEN, W. A. 1962. Botanical histochemistry. W. H. Freeman, San Francisco, California, USA.

KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology* 27: 137A.

LAND, S. B., JR., A. W. EZELL, S. H. SCHOENHOLTZ, G. A. TUSKAN, T. J. TSCHAPLIN, M. STINE, H. D. BRADSHAW, R. C. KELLISON, AND J. PORTWOOD. 1996. Intensive culture of cottonwood and hybrid poplars. *In* M. C. Carter [ed.], Growing trees in a greener world: industrial forestry in the 21st century, 167–189. 35th Louisiana State University Forestry Symposium. School of Forestry, Wildlife & Fisheries, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, USA.

LAND, S. B., JR., AND B. SINGH. 1997. *Populus* tree improvement in northern India. *In* T. White, H. Dudley, and G. Powell [eds.], Proceedings of the 24th Biennial Southern Forest Tree Improvement Conference, 1997 June 9–12; Orlando, Florida, 224–233. Sponsored publication no. 46; Southern Forest Tree Improvement Committee. Available from National Technical Information Service, Springfield, Virginia, USA.

LARSON, P. R. 1975. Development and organization of the primary vascular system in *Populus deltoides* according to phyllotaxy. *American Journal of Botany* 62: 1084–1099.

LARSON, P. R., AND T. D. PIZZOLATO. 1977. Axillary bud development in *Populus deltoides*. I. Origin and early ontogeny. *American Journal of Botany* 64: 835–848.

MEILAN, R. 1997. Floral induction in woody angiosperms. *New Forests* 14: 179–202.

MEILAN, R., A. BRUNNER, J. SKINNER, AND S. STRAUSS. 2001. Modification of flowering in transgenic trees. *In* A. Komamine and N. Morohoshi [eds.], Molecular breeding of woody plants, progress in biotechnology series, 247–256. Elsevier Science BV, Amsterdam, Netherlands.

NAGARAJ, M. 1952. Floral morphology of *Populus deltoides* and *Populus tremuloides. Botanical Gazette* 114: 222–243.

PAULEY, S. S. 1950. Flowering habits in *Populus. Genetics* 35: 684.

PIZZOLATO, T. D., AND P. R. LARSON. 1977. Axillary bud development in *Populus deltoides*. II. Late ontogeny and vascularization. *American Journal of Botany* 64: 849–860.

SHELDON, C. C., J. E. BURN, P. P. PEREZ, J. METZGER, J. A. EDWARDS, W. J. PEACOCK, AND E. S. DENNIS. 1999. The *FLF* MADS box gene. A repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *Plant Cell* 11: 445–458.

STRAUSS, S. H., P. COVENTRY, M. M. CAMPBELL, S. N. PRYOR, AND J. BURLEY. 2001. Certification of genetically modified forest plantations. *International Forestry Review* 3: 87–104.

YOSHIDA, N., Y. YANAI, L. CHEN, Y. KATO, J. HIRATSUKA, T. MIWA, Z. R. SUNG, AND S. TAKAHASHI. 2001. EMBRYONIC FLOWER2, a novel polycomb group protein homolog, mediates shoot development and flowering in *Arabidopsis. Plant Cell* 13: 2471–2481.