Journal of Horticultural Science & Biotechnology (1998) **73** (5) 606–611

Vegetative and reproductive development of the Montana blue huckleberry (*Vaccinium globulare* Rydb.)

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SUMMARY
The Montana blue huckleberry (*Vaccinium globulare* Rydb.) is a popular and highly sought wild fruit in the western United States. Because it is becoming increasingly difficult to meet growing market demands from wild harvests, growers and state officials are funding projects to investigate cultivation of the fruit. This study investigates the vegetative and reproductive growth patterns of this species as a prelude to its cultivation. Such information is critical in properly scheduling fertilizing, pruning and other cultural practices. The species produces fixed sympodial, non-episodic vegetative extension growth over a four-week period each season, followed by apical abortion and the formation of vegetative or mixed buds. Floret differentiation begins shortly after cessation of shoot growth and all floral parts are differentiated by late summer of the year prior to anthesis.

The Montana blue huckleberry (*Vaccinium globulare* Rydb.) is widely prized in the western United States for its unique, intense flavour. It is one of 13 *Vaccinium* species native to the Pacific Northwest (Ballington *et al.*, 1985), and the one most commonly found in Montana (Stark and Baker, 1992). Though good economic data are not available on the importance of this fruit, the wild crop supports a vigorous local industry in western Montana and sells for up to $25 per gallon (Barney, 1995).

The intensity and method of harvest, using rakes and beaters, increases the potential for damage to the plants and destruction of its fragile ecosystem. Because of the potential for destruction of the native habitat through uncontrolled harvest and because of the increasing popularity and economic value of this fruit, some growers have begun to cultivate this species, and state and private groups have sponsored research to elucidate developmental patterns of the plant as the initial step toward its domestication.

Vegetative and reproductive development of some *Vaccinium* species, including the highbush blueberry (*V. corymbosum* L.) and the lowbush blueberry (*V. angustifolium* Ait.), have been documented (Bell, 1950; Gough *et al.*, 1978). However, little work has been published on growth and development of the Montana blue huckleberry. The purpose of this study was to outline the vegetative and reproductive growth sequences of this species in the plant's native northern Rocky Mountain habitat as a prelude to subsequent work leading to its domestication.

MATERIALS AND METHODS

Data were collected on mature plants growing in the Lee Metcalf Wilderness, part of the Greater Yellowstone ecosystem about 48 km southwest of Bozeman, Montana. The study site was in Gallatin County, Montana at 111° 14′ W, 45° 24′ N at an elevation of about 2,000 m. The site receives most of its precipitation during the autumn but soil moisture is not usually depleted at any time (Caprio and Nielsen, 1992). The last spring freeze usually occurs in mid to late June and the first autumn freeze occurs in late August, resulting in a growing season of approximately 80 days. Other pertinent meterological data are given in Table I.

All plants in this study were located within a quarter mile radius and probably represented multiple colonies.

Data on shoot length were collected over a three-year period by measuring all shoots on ten plants. Each shoot was measured from base to tip and weekly grand means and standard deviations plotted. Data for the 1996 season are shown as representative for other years.

Data on the relation of the length of shoots in the 1995 growing season to the number of buds on them forming shoots in the 1996 season were collected on 40 plants on 5 July 1996 after seasonal cessation of shoot growth. Shoot length was taken as one indication of vegetative vigour.

Distal penultimate buds were collected at weekly intervals from May to October over three seasons. Tissue was prepared for histological examination according to standard procedure (Johansen, 1940). Tissue was fixed in FAA, dehydrated through an ethanol and t-butanol series, and embedded in paraffin, then sectioned at 10 μm thickness, stained with safranin, counterstained with fast green, and mounted in Permount (Fisher Scientific CO., Fairlawn, New Jersey).

TABLE I
Meteorological data on the blue huckleberry collection site in the Greater Yellowstone ecosystem[z]

	Data
Mean annual precipitation	112 cm
Mean annual air temperature	1.7°C
Mean annual snowfall	312 cm
Number of days wih snowcover	200
Mean frost free season	80 d
Mean annual extreme low temperature	−34.7°C
Mean annual exteme high temperature	31.4°C
Mean daylength in mid June	15.6 h
Annual mean atmospheric relative humidity	50–60%

Nodes are numbered basipetally, with the distal node being number one. Only buds at node one are described anatomically.

RESULTS AND DISCUSSION

Vegetative growth

The dormant bud of the blue huckleberry is a small, pointed structure approximately 5 mm long and 3 mm wide, measurements approximating those of similar buds in the highbush blueberry (Gough and Shutak, 1978). The interior of the bud is enclosed by two exterior budscales. The dome-shaped vegetative apex, typically about 62 μm in its widest diameter, the point of insertion of the youngest leaf primordium, extends about 80 μm from the distal surface to the distal portion of the rib meristem (Figure 1). These apical diameters are within the range given for most plant species (Steeves and Sussex, 1972). The apex is biseriate and capped with a double-layered tunica about 16 μm thick. This is similar to, but smaller than, that described for the highbush blueberry (Gough *et al.*, 1978) and contrasts with the single-layered tunica of the lowbush blueberry (Bell, 1950). Although tunica layering varies relative to apical age (Chakravati, 1953) and stage of leaf inception (Gifford, 1954), other ericaceous species such as *Rhododendron* (Foster, 1937) and cranberry (*Vaccinium macrocarpon* Ait.) (Dermen, 1947) generally have two layers.

The shoot axis is embedded in either a vegetative bud or in a mixed bud, both of which produce "fixed growth" in that the apex has initiated all leaves on the shoot axis prior to spring budbreak (Kozlowski and Ward, 1961). Lamina of these leaves, approximately five in number but ranging from three to eight on individual shoots, are rolled about the shoot axis within the bud. Seasonal shoot extension growth involves mostly internode elongation. In averaging five leaves formed in the bud before budbreak, the shoots of this plant strongly resemble those of the first flush of growth in the highbush blueberry, though the final number of leaves on shoots of the latter species is considerably greater due to the presence of multiple growth flushes (Gough and Shutak, 1978).

Mitotic figures are visible in late April about six weeks before anthesis and before there is any visible extension of the shoot axis, though budscales had begun to split to reveal green leaf tissue beneath. At this time there were still 8 cm of snowpack beneath the plants and the soil surface was frozen to a depth of approximately 5 cm.

Shoot growth begins from the vegetative apex in mid May as soon as the soil has thawed and about three weeks before anthesis. Similar buds examined at the same time on plants beneath which the soil was still frozen showed no swelling, so budswell may in part depend upon the availability of soil moisture. Buds on thicker shoots approximately 2 mm in diameter begin to swell before those on thinner, less vigorous shoots.

If a floret is present, the shoot axis initially lies lateral and subordinate to the floret base, then assumes a central position by displacement of the floret to a lateral basal position (Figure 2). The shoot continues to elongate while remaining enveloped in leaves until late May, at which time the leaves unfold away from the shoot axis, exposing it and the floret, if one is present. About a month elapses between the time of the first noticeable budswell and the time the buds open about two weeks prior to anthesis. Highbush blueberry also opens its inflorescence buds about two weeks before anthesis (Gough and Shutak, 1978).

Shoots continue to elongate rapidly, following a sigmoid growth pattern, until growth ceases with apical abortion in mid to late June, about two weeks after anthesis (Figure 3). There is no significant difference

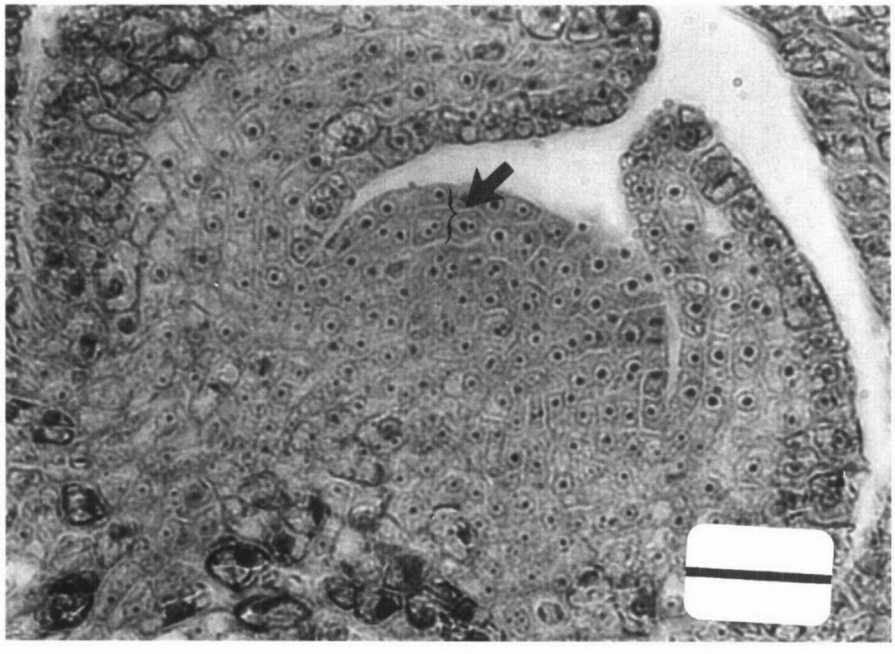

FIG. 1
Longitudinal section of a vegetative apex of Montana blue huckleberry, 24 May 1996. Note the biseriate tunica. Bar = 60 μm.

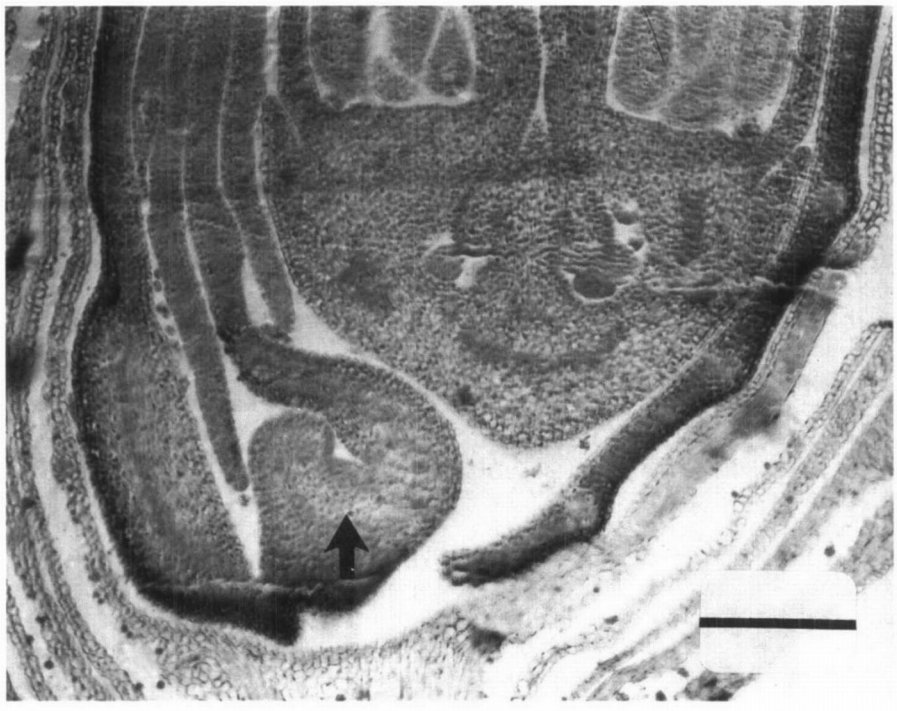

FIG. 2
The floret emerges from the proximal node of the shoot axis but occupies a central position in the mixed bud. Both pedicellate and vegetative axes appear discontinuous because they move out of the plane of sectioning. 26 April 1996. Note vegetative apex (arrow). Bar = 240 μm.

between the lengths of distal and penultimate shoots. By this time the shoots have begun to harden and axillary buds for the following year are visible. The shoot epidermis assumes a reddish cast shortly after axis elongation ceases. Seasonal shoot growth in the blue huckleberry resembles that of many temperate woody plants by being completed within about a four-week period in late spring by internodal elongation alone (Kozlowski, 1971). The single flush also requires about the same time to extend as the first flush of the highbush blueberry, and occurs in a similar four-week period centred on anthesis (Gough and Shutak, 1978).

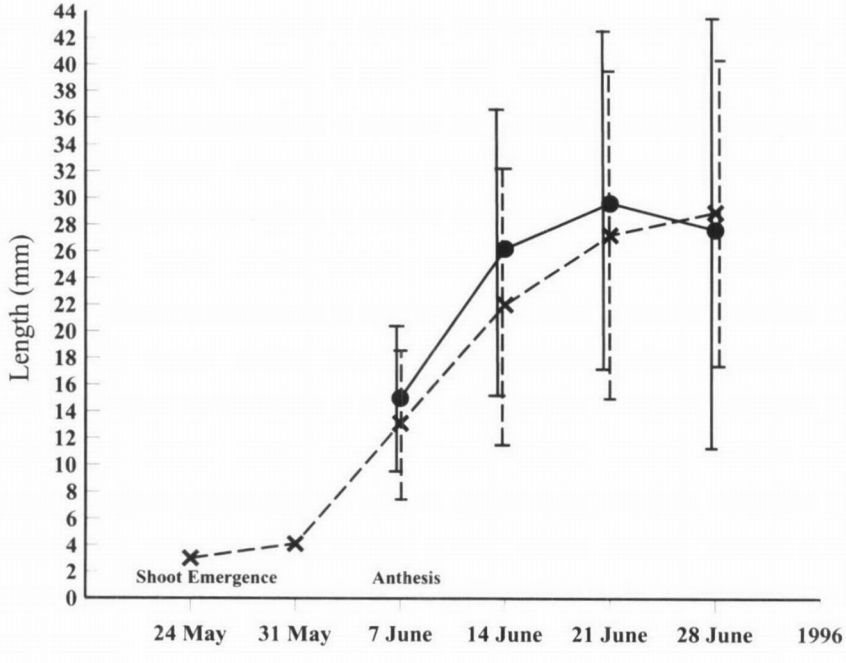

FIG. 3
Seasonal vegetative extension growth in the Montana blue huckleberry is fixed and continues for about a month centred on anthesis. The grand period of growth occurs for about a week on either side of anthesis. Data plots represent means and standard deviations. The 1996 shoot growth shown for circles: penultimate shoots, and for crosses: distal shoots, is representative of that in other years.

TABLE II
The length of shoots in 1996 formed in various positions along the stem axis. The data were collected on 40 plants selected at random (163 shoots)

Shoot position	Length (mm)	Percent of all cases
Shoot at node one only	30.8 ± 13.9[z]	18
Shoots at nodes one and two:		55
Distal shoot	46.8 ± 15.9	
Penultimate shoot	31.9 ± 11.5	
Shoots at distal three nodes:		27
Distal shoot	55.6 ± 25.3	
Penultimate shoot	51.2 ± 14.9	
Antepenultimate shoot	38.8 ± 20.3	

[z] Mean and standard deviation.

Growth of individual shoots is sympodial and non-episodic. Following apical abortion, the penultimate bud develops into a vegetative bud or differentiates into a mixed bud. Three to eight additional proximal buds develop basipetally along the shoot axis. In *V. corymbosum* production of more than a single flush is dependent upon cultivar, ripening season, and vigour (Gough and Shutak, 1978). It may be possible for blue huckleberry plants to undergo intraseasonal episodic growth by improving conditions for growth through pruning, irrigating, and fertilizing. With this, the distal bud of each flush may differentiate into a mixed bud, thus increasing potential yields twofold or threefold.

Length of shoots at different nodes is highly variable among the wild population and is dependent in part upon the number of buds along the previous year's stem axis that have broken into shoots (Table II). This appears to be a function of vigour. Further, damage to the distal portion of the plant by browsing animals or winter injury tends to stimulate vigourous shoot growth. While some shoots longer than 100 mm were observed, most grew to about 30 mm. Thin, weak shoots from 1995, comprising about 18% of all shoots and generally occurring on less robust plants formed new shoots only from their distal buds. These had a length of about 30 mm (Table II). This is precisely the length of weak shoots on the highbush blueberry (Gough and Shutak, 1978). More robust plants had stronger growth from the previous year, and shoots formed at nodes one, two, and sometimes three. About half of the 1995 shoots formed 1996 shoots at two nodes and 27% at three nodes. Few formed shoots from more than three nodes. When multiple shoots develop along a stem axis, the lowest shoot attains a length similar to the length of a single shoot produced from the distal bud alone. Shoot length appeared to be independent of the presence of fruit (Table III).

On average, about 60% of buds formed in one year form no shoots in the following year (Table IV). These lower buds remain dormant and break into shoots only when upper portions of the main axis are destroyed. These observations are consistent with those reported by

TABLE III
Length of all distal shoots in 1996 relative to the presence of fruit

Fruit	Shoot length (mm)
Present	25.8 ± 12.0[z]
Absent	31.5 ± 12.3

[z] Mean and standard deviation.

TABLE IV
Total number of nodes on 1995 shoots and the number of axillary buds that formed shoots in 1996

	Number of ndoes
Axillary buds on 1995 shoots	4.8 ± 1.3[z]
Number of shoots in 1996	2.4 ± 0.9
Total number of nodes on shoots in 1996	5.4 ± 1.2

[z] Mean and standard deviation.

Ward (1964) for northern red oak (*Quercus rubra* L) and by MacDaniels (1953) for apple (*Malus pumila* L. (Mill.)).

By completing most of its vegetative growth in about a month during late spring and early summer, the Montana blue huckleberry is similar to most temperate-zone perennials (Street and Opik, 1970). Its vegetative growth habit resembles that of other genera, including *Betula*, *Carpinus*, *Rhamnus*, and other *Vaccinium* species, by being sympodial and ceasing with formation of pseudoterminal buds (Kozlowski, 1971; Millington, 1963; Romberger, 1963).

Reproductive growth

Prior to the cessation of shoot growth in late June, two vegetative apices are visible near the distal end of the vegetative axis within the penultimate bud (Figure 4).

The first indication of differentiation occurred in early July, a few days after cessation of shoot growth (Figure 5). All floral parts had begun to differentiate by mid August (Figure 6) and differentiation had stopped for the season by mid October (Figure 7).

The ovary was about 860 μm at its greatest diameter (intersepallary distance) at the beginning of the winter dormancy period. Ovule primordia were apparent and about 45 μm in diameter. An intralocular distance of about 40 μm separated the ovule lobes from the interior of the ovary wall. Ovules and placental tissue were situated in kidney- shaped locules about 320 μm in length (distal to proximal) by 190 μm in width (interior to exterior). Microspore mother cell formation had begun with some separation of sporogenous tissue. The ovarian wall surrounding the ovules was approximately 124 μm thick.

The petals, about 45 μm thick at their bases, were well-formed and completely enclosed the style and stamens. Typical petaloid epidermal cells were apparent distal to the approximate point of anther attachment to the filament. The style was about 220 μm in diameter at the base and about 600 μm long, with stylar canals apparent.

By this time buds had entered their resting stage and further autumnal development was not apparent, nor was further increase in gross size of the flower buds evident until spring.

Meiotic activity was apparent in anthers and ovules by late April. Bud swell had barely begun at that time, with light green cataphylls showing beneath slightly opened scales. The flower buds appeared anatomically similar to those sampled the previous October. At this time soil temperature was 0°C and about 2–3 cm of snow remained beneath the plants.

Bud activity resumed in early May. By mid May, pollen grains appeared angular and stained faintly with safranin. The microsporogenous tissue in the anthers

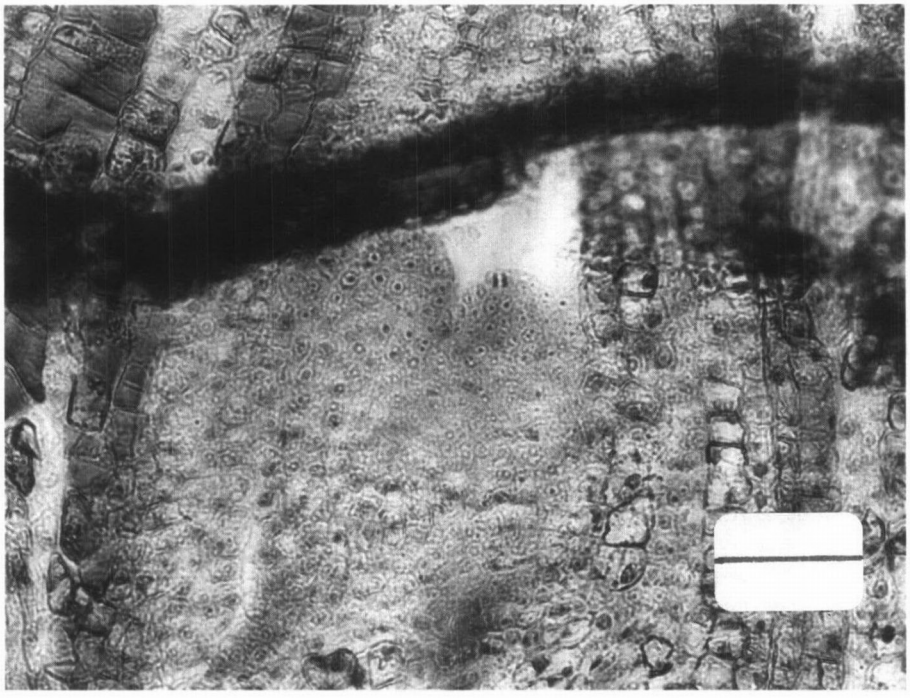

FIG. 4
Two vegetative apices appear in the distal bud at cessation of shoot extension growth in late June. One will differentiate into a floret within a few weeks. Bar = 60 μm.

had separated and tapetal disintegration had begun. The ovarian locules had increased in size and the ovules had begun their final phase of development.

The budscales abscinded and the leaves enfolding the vegetative axis and floret reflexed in late May, exposing the unopened floret. The first indication of the future testa became plainly visible, with the outer ovular integuments highly vacuolated. An integumentary tapetum surrounded the embryo sac, and the ovules nearly filled their locules. Pollen was nearly completely formed and loosely packed in the antherine locules.

Anthesis occurred during the first week of June in each study year. At anthesis the stigma protruded about 570 μm above the lip of the corolla and about 2.3 mm above the distal edge of the anthers. Total stylar length at anthesis was about 4.75 mm. Total time for floret development is about four months (mid July to mid October plus May).

Contribution no. J-5093 of the Montana Agricultural Experiment Station. This work was supported in part by a grant from the Montana Native Plant Society.

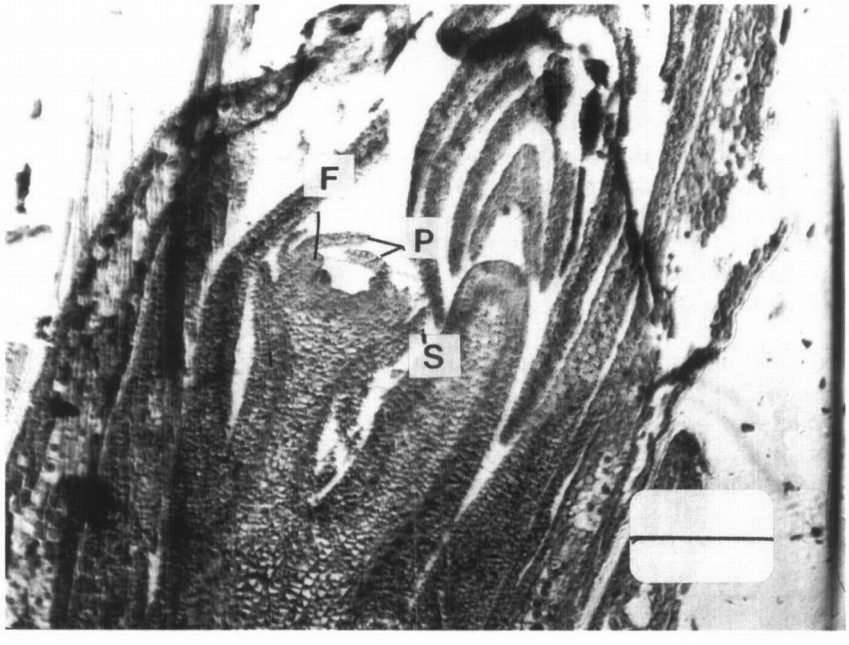

FIG. 5
Centripetal differentiation of floral primordia in the distal bud in early July in the apex on the left. Right apex remains vegetative. S = Sepal; P = Petal; F = Filament. Bar = 240 μm.

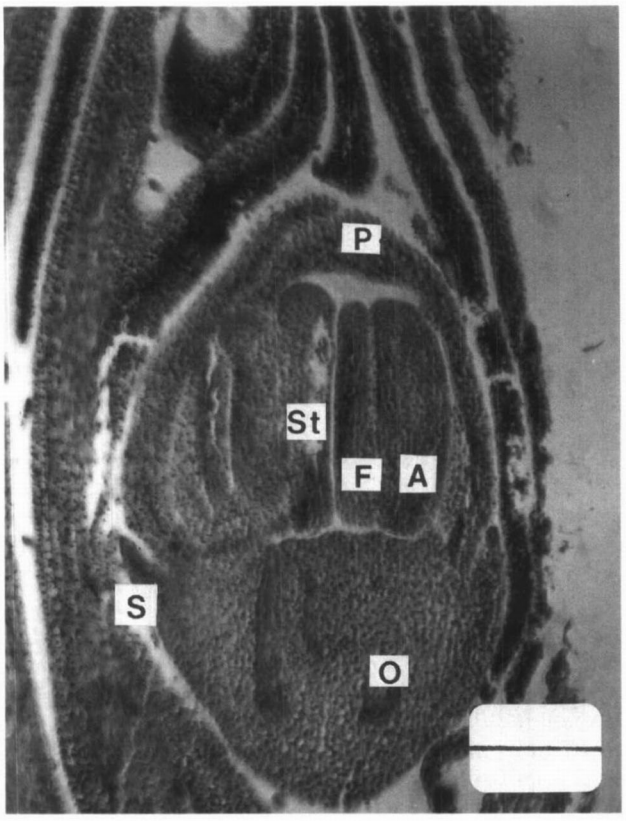

FIG. 6
All floral parts have begun to differentiate in the distal bud by mid August. S = Sepal; P = Petal; F = Filament; A = Anther; St = Style; O = Ovule. Bar = 240 μm.

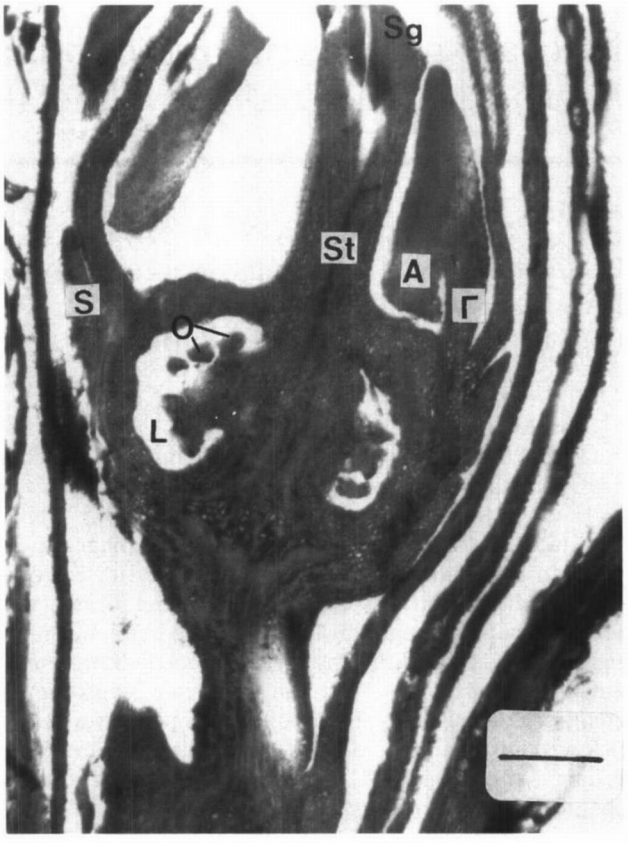

FIG. 7
All floral parts are visible in the distal bud as the plant enters its winter resting stage in mid October. S = Sepal; P = Petal; F = Filament; A = Anther; St = Style; Sg = Stigma; Pl = Placenta; O = Ovule; L = Ovarian locule. Bar = 240 μm.

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(Accepted 26 February 1998)