Flower Bud Initiation in Primocane-fruited Blackberry Germplasm

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Additional index words. *Rubus* sp., brambles, electron microscopy, light microscopy

Abstract. Scanning electron microscopy (SEM) and light microscopy (LM) were used to study the transition of meristems from vegetative to floral phase in erect primocane-fruited (PF) blackberries [*Rubus* (Tourn.) L. subgenus *Rubus*] developed at the Univ. of Arkansas. Dormant root cuttings of A-1836 and APF-13 blackberries were dug from the field and planted on 28 Dec. 1996 and 1 Mar. 1997 to produce plants for use in a greenhouse study. In a field study, terminal buds of field-grown A-1836, APF-13, NC194, and summer-fruited 'Arapaho' were sampled on 21 Mar. 1997 (before shoot emergence from soil), and then weekly from 14 to 28 May 1997. Flower bud primordia were first observed at five and six nodes of growth in greenhouse-grown A-1836 and APF-13 plants, respectively, 35 to 42 days after root cuttings were planted (DAP). Under field conditions, floral primordia were not observed until 21 May when A-1836 and APF-13 had at least 20 nodes of growth; NC194 did not differentiate floral structures until 10 July. The developmental patterns of the vegetative apical meristem in the PF selections, both field- and greenhouse-grown plants, were similar to those of 'Arapaho'. Opening of the terminal flower of the inflorescence occurred 32 to 35 days after floral initiation in APF-13, and 8 to 10 days later on A-1836. Field-grown NC194 bloomed in late August. The first fruits of greenhouse-grown APF-13 were harvested 120 DAP. These findings demonstrate that PF blackberries form flower buds after a short period of vegetative growth.

Primocane fruiting (PF), a trait that is present mainly in raspberry [*R. idaeus* L. (Daubeney, 1996; Keep, 1961; Ourecky, 1976)], has been introduced and intensified in erect blackberries at the Arkansas Agricultural Experiment Station. Blackberry germplasm of this type has already been released for breeding purposes (Ballington and Moore, 1995). The intensification of such a trait could result in several revolutionary possibilities in blackberry culture, including: 1) "off-season" (fall) production with enhanced market opportunities; 2) two crops per year could be harvested, fall and spring; 3) if only fall fruiting was practiced, all canes could be mowed to the ground in winter, avoiding most pruning needs, overwintering pests, and cold injury to overwintering canes; and 4) several crops per year might be harvested in tropical and subtropical climates (Moore, 1997).

To date, there are no reports on the process of flowering in PF blackberry. As to summer-cropping blackberries, floral initiation varies with cultivar and location; some cultivars initiate flower buds in autumn, others in midwinter, and still others in spring (MacDaniels, 1922; Robertson, 1957; Takeda and Wisniewski, 1989; Takeda et al., 1996; Waldo, 1933). For instance, two eastern thornless cultivars had different dates of floral initiation in West Virginia: October for 'Black Satin' and late March for 'Hull Thornless' (Takeda and Wisniewski, 1989). In another study, buds of 'Chester Thornless' remained undifferentiated until spring in Arkansas, Oregon, and West Virginia, while sepal development in buds of 'Cherokee' began in October in Arkansas and in November in Oregon (Takeda et al., 1996). In thorny blackberry, flower bud differentiation occurs basipetally within canes and inflorescences, with formation of the terminal flower in first place (Daubeney, 1996); in eastern thornless cultivars, however, once the terminal flower forms, subsequent differentiation occurs at the base of the inflorescence and proceeds acropetally (Takeda, 1987; Takeda and Wisniewski, 1989).

In summer-cropping red raspberries, the time of flower bud initiation is believed to be triggered mainly by shortening daylengths and falling temperatures (Williams, 1959, 1960), with the age and size of canes, expressed by the number of nodes, also playing a role (Williams and Hudson, 1956). In PF red raspberries, on the other hand, floral initiation can occur independently of these factors (Lockshin

and Elfving, 1981; Takeda, 1993; Vasilakakis et al., 1979a, 1980), although air temperature, daylength, and solar radiation are associated with early flowering (Privé et al., 1993).

Knowledge of the time of onset of flower bud initiation in primocane-fruited blackberries may prove useful for the implementation of strategies aimed at manipulating fruit production. The objective of this research was to determine the time of floral initiation and development under field and greenhouse conditions in erect PF blackberry selections.

Materials and Methods

Field-grown root cuttings (5–7.5 cm long) of A-1836 and APF-13 PF blackberries from the Arkansas Agricultural Experiment Station, Fayetteville, were harvested and planted in metal trays containing Sunshine LC1 mix (Sungro Horticulture, Bellevue, Wash.) on 28 Dec. 1996 or 1 Mar. 1997 under greenhouse conditions. The soil mix was fortified with Osmocote 14N–4.2P–11.6K (Scotts-Sierra Horticultural Products, Marysville, Ohio) at 100 g per 0.06 m³ of compressed potting soil. The greenhouse was maintained under natural daylight and 29 °C day/21 °C night temperatures. Ten terminal buds of each selection were randomly sampled when plants from the first planting date reached five and 10 nodes of growth (11 and 28 Feb. 1997, respectively). From the second planting, five terminal buds of each selection were sampled weekly from 21 Mar. (just before shoot emergence from soil) to 15 Apr. 1997 at two, four, six, eight, and 10 nodes of plant growth. In a field study, five terminal buds each of A-1836, APF-13, NC194, and 'Arapaho' [a summer-cropping cultivar (Moore and Clark, 1993)], were sampled on 21 Mar. (before emergence from soil), and again from 14 to 28 May 1997 when the plants reached five, 10, 15, 20, and 25 nodes of growth (± 1 node at each stage).

The plant material was fixed in a modified Karnovsky's fixative (Karnovsky, 1965) containing 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.2 and postfixed in 1% osmium tetroxide in the same buffer. The specimens were en bloc stained overnight in 0.5% uranyl acetate at ≈ 4 °C and then dehydrated in a graded ethanol series. For SEM, buds were critical-point-dried in hexamethyldisilazane, sputter-coated with gold, and observed on a 30-kV ISI-60 scanner (International Scientific Instruments, Mountain View, Calif.). For LM, dehydrated buds were embedded in Spurr's medium (Spurr, 1969). One-micrometer sections were microtomed, flattened on glass slides coated with Haupt's adhesive, and stained with 1% toluidine blue (Harris, 1972) and 1% Azure II in 1% borax. At each sampling date, the developmental stage of each bud was recorded on a 1-to-10 scale as described by Takeda and Wisniewski (1989), with minor adaptations (Table 1).

All experiments were conducted using completely randomized designs. At each sampling, each bud was taken from a different plant, each plant representing a replication.

Received for publication 16 Mar. 1998. Accepted for publication 22 July 1998. Published with the approval of the Director, Arkansas Agricultural Experiment Station, as manuscript #98010. We thank Dr. R.W. McNew for assistance with statistical analysis. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 1. Developmental stages of buds of primocane-fruited blackberry selections. (Adapted from Takeda and Wisniewski, 1989).

Stage	Description
1	Buds in vegetative phase with leaf primordia encircling the flat apical meristem.
2	A ₁ inflorescence apex beginning to develop with a few leaf, phyllome, and bract primordia evident.
3	Terminal flower of A ₁ axis is differentiated. The apex is enlarged and sepal primordia is evident.
4	Terminal flower of A ₁ apex is developed. Sepal primordia enlarge and become three-lobed. Petal primordia start differentiating.
5	The central receptacle in the terminal flower of the A ₁ axis is enlarged. Sepals fuse and enclose central receptacle.
6	Receptacle of terminal flower is elongate dome and petal primordia are enlarged. Sepals and petals are in alternate arrangements.
7	Stamen primordia are differentiated on floral cup.
8	Gynoecial structures are noticeable at the base of the receptacle.
9	Gynoeceia cover the central receptacle. Petals expand and enclose stamen and receptacle.
10	Anthers and filaments are developed. Style and stigma are evident.

The values for the developmental stage of sampled buds were analyzed by the nonparametric Jonckheere-Terpstra (JT) test using the frequency procedure of SAS (SAS Institute, Cary, N.C.).

Results

The change from the vegetative to the reproductive phase was evident in at least four out of 10 five-node plants of A-1836 blackberry originating from the root cuttings collected on 28 Dec. 1996, while the meristems of similar APF-13 plants remained vegetative (data not shown). In 10-node plants (28 Feb. 1997), however, floral differentiation was already in progress in all sampled buds of both blackberry selections, with inception of gynoecial structures at the base of the receptacle in at least 10% of A-1836 buds. Although flower buds were initiated slightly earlier in A-1836 than in APF-13 ($P=0.08$), anthesis of the terminal flower at the A₁ inflorescence axis (Takeda, 1987) occurred more rapidly in APF-13, since this selection started to bloom ≈ 10 d earlier than did A-1836. On average, plants of both PF blackberry selections reached five and 10 nodes of growth 45 and 62 d after the root cuttings had been planted, respectively.

Histological examinations of terminal buds from plants of A-1836 and APF-13 sampled just before emergence from the soil revealed the typical features of a vegetative meristem as described by Bernier et al. (1981): a central zone, composed of the corpus and the centrally located cells of a three-layered tunica; a peripheral zone, from which leaf primordia originated; and the pith-rib meristem (Fig. 1A). Observations by SEM of those buds revealed whorls of leaf primordia tightly encircling the somewhat flat apical meristem (Fig. 1B). These features were similar in greenhouse- and field-grown PF blackberry plants, and field-grown 'Arapaho' plants. When shoots reached four nodes, the buds remained vegetative in the two PF blackberry selections, but slight "doming" was evident on the apical meristem of the terminal flower (Fig. 1C). At six nodes, there was a marked "doming" and broadening of the apical meristem, with bracts being formed and whorls of leaves loosely surrounding the meristem (Fig. 1D). Thus, floral initiation was

started. Differentiation of floral structures then followed and continued uninterrupted until completion (Fig. 1E-H). Although floral differentiation seemed to be slightly more advanced in A-1836 than in APF-13, bud developmental stages were statistically similar ($P \geq 0.10$) in both PF blackberry selections within the same number of nodes (Fig. 2). First bloom on terminal buds of the inflorescence occurred 32 to 35 d after floral primordia initiation in APF-13, and ≈ 10 d later on A-1836, with harvest of the first APF-13 fruits 120 d after the root cuttings were planted in the greenhouse.

In the field study, floral primordia were not observed until the plants of A-1836 and APF-13 reached at least 20 nodes of growth (21 May). Plants of NC194, however, were still in their vegetative phase at 25 nodes, similar to those of summer-cropping 'Arapaho' (Fig. 3). The first evidence of floral initiation in NC194 occurred on 10 July in plants ranging from 35 to 40 nodes of growth. First bloom on the terminal flowers of the inflorescence occurred on 22 June and 30 June in APF-13 and A-1836, respectively. In NC194, only a few primocanes showed open flowers at their tips by late August; the remaining primocanes continued to be vegetative until the end of the season, similar to 'Arapaho.'

Discussion

Buds of fall-fruited and summer-fruited types of blackberry were similar in their vegetative stage. After a transitional stage, a whorl of sepal primordia arose at the outer edge of the terminal apex of the A₁ inflorescence axis and began to elongate (Fig. 1E). Soon after the sepals differentiated and became three-lobed, a whorl of petal primordia in alternate arrangement with the sepals developed (Fig. 1F). Sepals began to enlarge and enclosed the terminal apex (Fig. 1G), which by then had become the central receptacle (Fig. 1H). By the time the terminal apex of the A₁ inflorescence axis was enclosed by the sepals, some degree of differentiation in the stamen and gynoecial structures was evident, and subtending buds began differentiating acropetally (Fig. 1G). This sequence of floral development is similar to that reported for summer-

fruiting cultivars (Takeda and Wisniewski, 1989), but occurs much sooner in fall-fruited genotypes.

Under greenhouse conditions, the time required from the date of planting of the root cuttings to the time of floral initiation ranged from 32 d in the second planting (1 Mar.) to 45 d in the first planting (28 Dec.); however, the number of nodes at which the floral initiation process took place was about the same in both planting dates. This difference of 2 weeks might be attributable to more chilling unit (CU) accumulation in plants from the second planting date. In 'Heritage' red raspberry, Takeda (1993) showed that the time to flower was negatively correlated with CU accumulation prior to cane emergence. In the present study, the accumulation of CU may explain why young plants initiated flower primordia at an early stage of growth (as early as five nodes).

Dormant buds of 'Arapaho', A-1836, and APF-13 sampled on 28 Feb. 1997 from overwintering plants in the field were at an advanced stage of differentiation (data not shown). The process of floral differentiation, however, was still incomplete, since anther and gynoecial structures in terminal flower buds on primary inflorescence axes were not totally developed. Presumably, the process of floral differentiation started earlier in the fall, but probably was arrested by low temperatures during winter.

In raspberry, flower induction in primocane-fruited cultivars occurs independently of photoperiod and temperature (Takeda, 1993; Vasilakakis et al., 1979b, 1980; Williams, 1960). This might also be true for the PF blackberries used in this research. These plants formed flower buds and bloomed while the temperature remained relatively constant (29 °C day/21 °C night), and the natural daylength was gradually increasing rather than decreasing throughout the period of study (early Feb. to 15 Apr.). However, a discrepancy existed between greenhouse- and field-grown plants as to cane length at which the onset of flower bud initiation occurred (five nodes vs. 20 nodes, respectively). The temperatures prevailing in the field were milder (20.8 and 9.7 °C average maximum and minimum, respectively, from 1 Apr. to 28 May) than those in the greenhouse. Lockshin and Elfving (1981) reported that higher temperatures (29 °C day/24 °C night) induced flowering of 'Heritage' red raspberry 2 weeks earlier than did lower temperatures (25.5 °C day/20 °C night); however, canes in both temperature regimes flowered at 24 to 25 nodes of growth. In the same raspberry cultivar, exposure to 25 °C quickly stopped cane elongation and resulted in flowering and fruiting, while canes exposed to 16 °C were extremely long (Ourecky, 1976). Another factor that might be closely related with the discrepancies mentioned above is the environment of the root system. Factors that are associated with the slowing or suppression of primocane growth usually hasten the development of floral primordia in summer-fruited raspberries (Crandall and Chamberlain, 1972). This might also be applicable to PF blackber-

Fig. 1. Flower bud initiation and differentiation in erect primocane-fruited blackberry. (A) Longitudinal section of the terminal bud sampled just before emergence from the soil. All features correspond to the vegetative stage. c = Corpus, t = tunica, l = leaf primordium, p = pith-rib meristem, i = primordial internode, v = vascular trace-procambium. (B) Terminal bud as in (A) showing leaf primordia developing and encircling the apical meristem (m). (C) Terminal bud at four nodes of growth. (D) Terminal bud at five nodes of growth. The apical meristem has broadened and become the primary inflorescence axis (A_1). b = Bract, L = leaf. (E) Development of sepal primordia (s) in terminal flower of the A_1 axis. (F) Primary inflorescence axis showing inception of petal primordia (arrows) in alternate arrangement with the sepals. a = Axillary buds. (G) Primary axis showing differentiation of axillary buds. Sepals have fused and enclosed the central receptacle of the terminal flower. (H) Terminal flower ("floral cup") showing advanced carpel development in central receptacle (r). Sepals were removed to facilitate view of petals (p), gynoecial (g), and stamen structures (at arrows). Solid bar in all micrographs = 0.1 mm.

Fig. 1 continued on next page

Fig. 1. Continued.

ries since root system in the field was not restricted, in contrast with plants in the greenhouse, which grew on metal trays with a layer of soil no deeper than 6 cm.

With an understanding of floral initiation and differentiation, cultural practices may be used to promote or suppress flowering in PF blackberry. For example, the use of plant growth regulators (Braun and Garth, 1984, 1986; Crandall and Garth, 1981; Goulart,

1989), a combination of N fertilizer and high temperature (Lockshin and Elfving, 1981), row covers (Pritts et al., 1992), and cold temperature pretreatments (Takeda, 1993; Vasilakakis et al., 1980) performed on fall-fruited red raspberry could be adopted to

Fig. 2. Stages of bud development (see Table 1 for description of rating) in terminal buds of primocane-fruited blackberries grown under greenhouse conditions. Buds were sampled from 21 Mar. to 15 Apr. 1997 from plants originated from root cuttings collected from the field on 1 Mar. 1997. Each value represents the mean for five buds. Differences among selections at each stage of growth are nonsignificant (Jonckheere-Terpstra test).

Fig. 3. Stages of bud development (see Table 1 for description of rating) in terminal buds of three primocane-fruited blackberries (A-1836, APF-13, and NC194) and summer-fruited 'Arapaho' grown under field conditions. Buds were sampled from 14 to 28 May 1997. Each value represents the mean for five buds. Once floral initiation occurred, genotypes were significantly different ($P = 0.02$ and ≤ 0.01 at 20 and 25 nodes of growth, respectively; Jonckheere-Terpstra test).

manipulate flowering to yield fruit at dates when the market opportunities are more attractive. Since plants used in this study were capable of producing harvestable fruit as early as 120 d after root cuttings were planted, greenhouse production during the winter months, as well as multiple cropping in tropical and subtropical climates, may be possible.

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