Scanning Electron Microscopy of Floral Initiation in Sweet Cherry

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ABSTRACT. Flower initiation and development in ‘Bing’ sweet cherry (*Prunus avium* L.) was examined using scanning electron microscopy. There was a 1- to 2-week difference in the time of initiation of flower buds on summer pruned current season shoots (P) compared to buds borne on unpruned shoots (U) or spurs (S). By late July, this difference was obvious in morphological development. The P buds had already formed floral primordia, while the S and U buds showed little differentiation in the meristem until early August. In general, buds from unpruned shoots were similar developmentally to spur buds. By late August, primordial differentiation was similar in the buds from all the wood types; however, buds from pruned shoots were significantly larger (838 μm) than buds from spurs (535 μm) and unpruned shoots (663 μm). Early summer pruning may shift allocation of resources from terminal shoot elongation to reproductive meristem development at the base of current season shoots. The similarity in reproductive bud development between spurs and unpruned shoots, given the difference in active terminal growth, might suggest that developmental resources are inherently more limiting in reproductive buds on spurs.

Pruning has been used commonly as a management tool for controlling vegetative growth in fruit trees (Faust, 1989; Ferree et al., 1984; Forshey and Elfving, 1989; Mika, 1986). However, some studies indicate that summer pruning can also increase flowering on young shoots (Kesner et al., 1981; Miller, 1982; Webster and Shephard, 1984). Floral induction precedes floral initiation, the visible transition to a floral meristem. Floral induction is thought to be a biochemical signal that causes cells within a bud to begin programmatic transitions from a vegetative to a floral state (Faust, 1989; Westwood, 1993). In fruit trees this biochemical signal may occur when gibberellic acids (GAs) are below critical inhibitory levels and the balance of other hormones, including auxin, cytokinins, and ethylene, are favorable for floral initiation (Westwood, 1993).

Westwood suggested that floral initiation in sweet cherries occurs after fruit ripen. In apple trees flower buds generally initiate after harvest or when growth ceases (Forshey and Elfving, 1989). In sour cherries, Diaz et al. (1981) reported that the apex began doming at 28 d after anthesis, which signified the initial change from the vegetative to the reproductive stage. According to this evidence, floral initiation occurred well before fruit maturation. Bulatovic (1978) found that there was no direct correlation between sweet cherry flower bud initiation and the timing of fruit maturation in Yugoslavia. In early and late cultivars (‘Primavera’ and ‘Lambert’, respectively), floral bud initiation began at the same time (15 June) despite the differences in maturation dates.

When growth retardants, such as daminozide (SADH), were used to inhibit GA biosynthesis in ‘Bing’ cherry and pear trees,

flower buds tended to initiate (Ryugo, 1986). When GA was applied to apricot and cherry during fruit development, floral bud initiation was inhibited and return bloom decreased to almost none (Bradley and Crane, 1960). Applications of GA₃ to sweet cherry trees to improve fruit set have had adverse effects on return bloom, especially when applied repeatedly (Webster and Goldwin, 1983). There is a complex relationship between GA₃ and flowering of ‘Early Rivers’ sweet cherry. As GA₃ suppressed return bloom on old wood, it increased flowering on 1-year wood in proportion to the amount of GA₃ applied (Webster and Goldwin, 1983). GA interfered more with floral initiation than with differentiation in some species of *Prunus* (Bradley and Crane, 1960), so it would be important to know the time of sweet cherry floral initiation to avoid inadvertent inhibition during routine cultural practices, such as the preharvest use of GA₃ to improve fruit size and firmness. Summer pruning also can affect endogenous hormone balances and resource partitioning between meristematic tissues, which subsequently may affect flowering. The wound inflicted by pruning usually produces ethylene in tissues surrounding the wound (Klein and Faust, 1978). Ethylene at a high enough level may stimulate flower buds to initiate (Faust, 1989).

In general, the interval between the beginning and final stages of bud differentiation in sweet cherries ranges from 86 to 112 d, and in sour cherries from 56 to 100 d (Faust, 1989), depending on climate and cultivar. Batikov (1985) reported that differentiation in sweet cherries occurred in mid to late July in the Crimean foothills, and lasted for 22 to 52 d depending on the cultivar. Similar results were reported by Watanabe (1983) in Japan, with sweet cherries showing the first sign of floral initiation on 30 June (49 d after anthesis) and petal primordia forming by 10 Aug. The timing of floral induction, initiation and differentiation can be important for developing management strategies to enhance flowering location within the canopy and ultimately regulate fruit crop loads. Flowers are borne normally on second year shoots or on spurs from older wood (Webster and Shephard, 1984; Westwood,

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Table 1. Summary of the morphological changes of 'Bing' sweet cherry buds from current season shoots unpruned (U) and pruned (P) at 45 days after full bloom (DAFB), and buds from spurs (S).

Sample date	DAFB ^z	GDD ^y	Description of morphological development
9 July	91	423	P: Smooth, rounded apex. No obvious signs of floral initiation. S, U: Smooth, rounded apex.
23 July	105	587	P: Floral primordia form. S, U: Meristem still rounded, but may show slight changes.
6 Aug.	119	759	P: Apex of floral primordia flattens. Sepal primordia developing (in a pentagonal whorl). S: Floral primordia formed, apex of primordia still rounded but starting to flatten. U: Floral primordia formed, apex of primordia flattening. Sepal primordia starting to form.
27 Aug.	140	1003	P, S, U: Sepals, petals, carpel and stamen differentiating.
24 Sept.	168	1168	P, S, U: Sepals, petals, carpel, and stamen differentiating. Flower continues to develop.

^zFull bloom was 9 Apr. and harvest was 79 DAFB in Prosser, Wash.

^yAccumulated degree days calculated from full bloom using base temperature of 10 °C.

1993). It is desirable to increase the proportion of fruit on 1- and 2-year shoots, relative to spurs, because these fruit usually are of higher quality (Faust, 1989; Roper and Loescher, 1987). Young shoots are usually exposed to more light than the fruiting spurs on older wood, therefore providing more photoassimilates for fruit growth and higher total soluble solids (Ryugo, 1986). Early summer pruning of current season growth in sweet cherries can increase the number of floral meristems that develop at the basal nodes of such growth (Guimond et al., 1998).

This study documents the morphological changes of 'Bing' sweet cherry meristems during the transition from a vegetative to a reproductive bud, specifically examining initiation on three types of wood: second year spurs, unpruned current season shoots, and summer-pruned current season shoots.

Materials and Methods

This study used a block of 8-year 'Bing' trees on mazzard (*P. avium*) seedling rootstock at Washington State University's Roza Experimental Farm near Prosser, Washington. Samples were collected randomly from three types of wood: spur buds on 1- to 2-year wood, basal buds on current season shoots summer pruned (Guimond et al., 1998) to 20 cm at 45 d after full bloom (DAFB; full bloom in 1996 was 9 Apr.), and basal buds on current season shoots that were unpruned. Twenty buds were sampled from each wood type for each sample date. Sampling dates ranged from 22 May to 24 Sept. 1996. The samples were fixed and stored in a solution of FAA (10% Formalin, 50% ethanol, 5% glacial acetic acid) for 24 h or longer.

Ten buds from each sample were dissected using a Bausch and Lomb dissecting microscope before processing for scanning electron microscopy (SEM). Buds were rinsed twice, ≈10 min each, in 50% ethanol (EtOH) to remove the FAA from the plant tissue and were kept in 50% EtOH during dissection to prevent desiccation. The outer bud scales were removed to expose the meristem(s) at the base of the bud. After the outer bud scales were removed, the samples were dehydrated in an EtOH series (50%, 70%, and 95% for 10 min each, then twice in 100% for 10 min each). The samples were dried with a critical-point dryer (Samdri-PVT-3B; Tousimis Research Corp., Rockville, Md.) and then mounted on stainless-steel stubs with carbon tape before being gold coated with a sputter coater (Hummer IV; Technics). The earlier samples (9 July to 6 Aug.) were coated for 7 min, while the later samples (27 Aug. and 24 Sept.) were coated for 4.5 min on three sides tilted at a 45-degree angle. Samples were examined with a scanning electron micro-

scope (Hitachi S-570) at 15 kV. The diameters of individual meristems within the buds were measured from the photographic images produced with a Polaroid camera mounted on the microscope. The bud measurement data were analyzed using Duncan's multiple range tests (SAS, Cary, N.C.) to determine significant differences in meristem size between bud types and sample dates. Accumulated growing degree days (GDD), using a base temperature of 10 °C (Eisensmith et al., 1980), were calculated for each of the timings as a measure of climatic differences between the different dates (Table 1).

at a relatively similar stage of development. This also was seen in a study by Kappel et al. (1990), in which basal buds collected from 1-year-old shoots on 12-year-old 'Bing' trees lagged in development compared to those from current season growth or buds from other types of wood; by the end of the season, buds from all wood types were developmentally similar.

The size of the different buds varied by wood type and by stage of development. Early in the summer, there was little significant difference in bud size between the samples (Table 2). By 27 Aug., although all samples were at similar visual stages of differentiation, meristems from pruned shoots were significantly ($P = 0.05$) larger (838 μm) than those from spurs (535 μm) or unpruned shoots (663 μm). This could be of interest if the primordial size of the flower is relative to the potential size of the fruit produced from it, suggesting that fruit would be larger at the base of the pruned shoots and smallest on spurs.

It is desirable to have more fruit on 1-year-old shoots than on spurs because these fruit are usually of higher quality (Faust, 1989). Early summer pruning may shift allocation of resources from terminal shoot elongation to reproductive meristem development at the base of current season shoots. Young shoots usually are exposed to more light than the fruiting spurs on older wood. Spurs tend to be shaded and the older the spurs, the lower the fruit quality. The fruit on young shoots are higher quality due to a greater proportion of source leaves that receive more light for photosynthesis, therefore resulting in fruit with higher total soluble solids (Ryugo, 1986). The similarity in reproductive bud development between spurs, which do not have active terminal growth, and unpruned shoots that have a terminal vegetative meristem compet-

Fig. 2. SEM micrographs of 'Bing' sweet cherry buds from spurs, showing primordial changes from initiation to differentiation. (A) Rounded meristem (m), bract (b) primordia forming; 9 July; bar = 25 μm . (B) Rounded meristem (m) with bract (b) primordia; 23 July; bar = 40 μm . (C) Flower (f) primordia; 6 Aug.; bar = 45 μm . (D) Sepals (s) and petals (p) differentiate; 27 Aug.; bar = 100 μm . (E) Flower enlarges and continues to differentiate; 24 Sept.; bar = 200 μm .

Results and Discussion

The use of SEM revealed the time frame for initiation of visual changes from a vegetative to a reproductive state in sweet cherry buds. The first sign of floral initiation occurs when the meristematic apex of the bud becomes dome-like in shape, on which floral organs are destined to differentiate laterally. The buds that were collected from pruned shoots began such changes in the meristem between 9 and 23 July (Fig. 1 A and B), which was 91 to 105 DAFB. Harvest was on 27 June (79 DAFB). For comparison, a vegetative bud at this time was relatively smooth, perhaps slightly rounded, and surrounded by many leaf and bract primordia. Buds from spurs did not appear to initiate until just before 6 Aug. (Fig. 2C), ≈ 119 DAFB. Buds from unpruned shoots were similar developmentally to spur buds, without any meristematic indications of floral initiation until 6 Aug. (Fig. 3C). This evidence indicates that 'Bing' sweet cherry flower buds are not initiated in the Pacific Northwest until after harvest, with at least 12 d between fruit ripening and the earliest signs of floral bud initiation.

The buds from pruned shoots formed a pentagonal whorl of sepal primordia (Fig. 1C) by 6 Aug. (Table 1). Sepal primordia could not be observed on any of the buds from unpruned shoots or spurs by this time, but were readily apparent by 27 Aug. (Table 1). Thus, by 140 DAFB the differences in meristematic differentiation became less obvious between the samples: sepal and petal differentiation was readily apparent (Figs. 1D and 2D). Differentiation of the stamen and carpel could be observed at this time in longitudinal sections of individual flowers (not shown). Even though buds from unpruned shoot and spur samples before 27 Aug. were less advanced developmentally compared to those from pruned shoots, toward the end of the growing season (24 Sept.) all floral buds were

Fig. 3. SEM micrographs of 'Bing' sweet cherry buds from unpruned current season shoots, showing primordial changes from initiation to differentiation. (A) Rounded meristem (m), bract (b) primordia forming; 9 July; bar = 23 μm . (B) Rounded meristem with bract (b) primordia; 23 July; bar = 40 μm . (C) Flower (f) and sepal (s) primordia; 6 Aug.; bar = 45 μm . (D) Sepals (s) and petals (p) differentiate; 27 Aug.; bar = 100 μm . (E) Flower enlarges and continues to differentiate; 24 Sept.; bar = 200 μm .

Table 2. Changes in size of the bud apices during different stages of floral development in 'Bing' sweet cherry at Prosser, Washington (1996).

Date	Meristem diam (µm)		
	Spur buds	Unpruned shoot buds	Pruned shoot buds
9 July	193 i ²	224 i	195 i
23 July	198 i	229 hi	313 hi
6 Aug.	347 g	355 g	409 g
27 Aug.	532 f	663 e	838 d
24 Sept.	1100 b	985 c	1200 a

²Mean separation by Duncan's multiple range test, $P = 0.05$.

ing for resources, suggests that developmental resources may be limiting in spur buds as well.

Sweet cherry flower initiation may differ by cultivar and climates, and this study indicates it can be influenced by pruning as well. For 'Bing' in central Washington, initiation occurs in mid-to late July. In Japan, sweet cherry flower initiation is generally thought to occur ≈30 June (Watanabe, 1983); in British Columbia, Canada, late June to early July (Kappel et al., 1990). Knowing this, orchard management and cultural practices at this critical time can be optimized to favor floral initiation. For example, different timings of summer pruning or postharvest fertilization may affect hormonal balances or sink activities within the tree and alter initiation. Further study of primordial development during initiation may prove useful in determining how other cultural and/or climatic factors, and their timing during the growing season, affect sweet cherry cropping and potentially abnormal (e.g., double or spur) fruit formation.

Literature Cited

- Batikov, S.G. 1985. Differentiation of sweet cherry flower buds in the Crimean foothills (in Russian). Trudy po Prikladnoi Botanike, Genetike i Selektzii. 78:43–49 (Hort. Abstr. 56:4961; 1986).
- Bradley, M.V. and J.C. Crane. 1960. Gibberellin-induced inhibition of bud development in some species of *Prunus*. Science 131:825–826.
- Bulatovic, M. 1978. Studies on the relationship between the start of flower bud differentiation and fruit ripening date in cherries at Caak. Preliminary communication. Archiv za Poljoprivredne Nauke 31:159–164 (Hort. Abstr. 49:4825; 1979).
- Diaz, D.H., H.P. Rasmussen, and F.G. Dennis, Jr. 1981. Scanning electron microscope examination of flower bud differentiation in sour cherry. J. Amer. Soc. Hort. Sci. 106:513–515.
- Eisensmith, S.P., A.L. Jones, and J.A. Flore. 1980. Predicting leaf emergence of 'Montmorency' sour cherry from degree-day accumulations. J. Amer. Soc. Hort. Sci. 105:75–78.
- Faust, M. 1989. Physiology of temperate zone fruit trees. Wiley, New York.
- Ferree, D.C., S.C. Myers, C.R. Rom, and B.H. Taylor. 1984. Physiological aspects of summer pruning. Acta Hort. 146:243–252.
- Forshey, C.G. and D.C. Elfving. 1989. The relationship between vegetative growth and fruiting in apple trees. Hort. Rev. 11:229–287.
- Guimond, C.M., G.A. Lang, and P.K. Andrews. 1998. Summer pruning affects flower initiation and shoot regrowth in sweet cherry (*Prunus avium* L.). HortScience (In press.)
- Kappel, F., M. Bouthillier, and L. Veto. 1990. A study of flower bud differentiation in sweet cherry by scanning electron microscope. HortScience 25:1106. (Abstr.)
- Kesner, C.D., C.M. Hansen, and S.B. Fouch. 1981. Tree training and mechanical pruning of tart cherry in high density plantings. Compact Fruit Tree 14:135–139.
- Klein, J. and M. Faust. 1978. Internal ethylene content in buds and woody tissues of apple trees. HortScience 13:164–166.
- Mika, A. 1986. Physiological responses of fruit trees to pruning. Hort. Rev. 8:337–378.
- Miller, S.S. 1982. Regrowth, flowering, and fruit quality of 'Delicious' apple trees as influenced by summer pruning. J. Amer. Soc. Hort. Sci. 107:975–978.
- Roper, T.R. and W.H. Loescher. 1987. Relationships between leaf area per fruit and fruit quality in 'Bing' sweet cherry. HortScience 22:1273–1276.
- Ryugo, K. 1986. Promotion and inhibition of flower initiation and fruit set by plant manipulation and hormones, a review. Acta Hort. 179:301–307.
- Watanabe, S. 1983. Scanning electron microscope observations of flower bud differentiation in sweet cherry (*Prunus avium*). J. Yamagata Agr. For. Soc. 0:15–18 (Biol. Abstr. 77:46965; 1984).
- Webster, A.D. and G.K. Goldwin. 1983. The effects of setting mixtures on cropping, flowering and vegetative growth of sweet cherries. Acta Hort. 149:13–23.
- Webster, A.D. and U.M. Shepherd. 1984. The effects of summer shoot tipping and rootstock on the growth, floral bud production, yield and fruit quality of young sweet cherries. J. Hort. Sci. 59:175–182.
- Westwood, M.N. 1993. Temperate-Zone Pomology- Physiology and Culture. 3rd ed. Timber Press. Portland, Ore.