The Role of Spur Leaves, Bourse Leaves, and Fruit on Local Flower Formation in Apple: An Approach to Understanding Biennial Bearing

Mokhles A. Elsysis

Department of Horticulture and Landscape Architecture, Purdue University, 625 Agriculture Mall Drive, West Lafayette, IN 47907; and Department of Pomology, College of Agriculture, Assiut University, Assiut 71515, Egypt

Peter M. Hirst¹

Department of Horticulture and Landscape Architecture, Purdue University, 625 Agriculture Mall Drive, West Lafayette, IN 47907

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Abstract. Lack of consistent flower formation is the underlying cause of biennial bearing. Flower formation in apple (*Malus × domestica* Borkh.) has been associated with different factors, such as leaf area, shoot growth, bourse length, crop load, and seed number. However, it is unclear how these different factors interact to promote or inhibit flower formation. The effect of spur defoliation, fruit removal, and their interaction were evaluated on spur flower formation and bourse length in annual-bearing ‘Gala’ and the biennial-bearing ‘Honeycrisp’. Eight different combinations of spur defoliation and fruiting treatments were applied in three consecutive springs, 2013–15. Bourse shoot defoliation and fruiting treatments inhibited spur flower formation in both cultivars, but in different patterns from year to year. In addition, spur leaf defoliation did not affect flower formation in either cultivar. Furthermore, local defoliation and fruiting treatments did not affect bourse length. We propose that bourse leaves play a major role in both producing and transporting flower formation signals, but the effect depends on cultivar.

Consistent fruit production is highly dependent on flowering frequency. Flower formation is related to biennial bearing and continues to be a major challenge for the apple industry and many other fruit tree industries worldwide. Biennial (or alternate) bearing is the “fluctuation of cropping from year to year caused by irregular flowering” (Hirst, 2017). Despite considerable research, the underlying mechanisms of biennial bearing remain largely unknown (Bangerth, 2006, 2009; Hoad, 1978; Jonkers, 1979; Monselise and Goldschmidt, 1982; Singh, 1948; Xing et al., 2015). In apple trees, biennial bearing varies among cultivars and appears to be under both genetic and environmental control (Guitton et al., 2011; Hanke et al., 2007; Monselise and Goldschmidt, 1982). Factors that regulate flower formation in apple trees include leaf area (Davis, 2002; Sahulka, 1967), shoot growth (Luckwill, 1970; Sahulka,

1967), bourse length (Dennis and Neilsen, 1999), crop load (Embree et al., 2007; Meland, 2009), and seed number (Chan and Cain, 1967). It is unclear how these different factors interact to promote or inhibit flower formation in biennial cultivars or why these factors do not have the same effect in annual cultivars. For example, most annual cultivars, such as ‘Gala’ and ‘GoldRush’, tend to flower regularly despite characteristics usually associated with flowering inhibition such as high seed numbers. On the other hand, some biennial cultivars, such as ‘Honeycrisp’ and ‘Golden Delicious’, have moderate seed numbers yet often exhibit biennial bearing. Some researchers have suggested that leaves, fruit, and seeds activate or inhibit flower formation pathways by affecting the endogenous balance of plant growth regulators (Bangerth, 2009; Hanke et al., 2007). A recent study involving quantitative trait loci in apples suggested that biennial bearing is under control of plant growth regulators genes (Guitton et al., 2011).

The transition of apple buds from vegetative to a floral state requires sufficient leaf-area around the bud (Sahulka, 1967); however, the role of such leaves in flower formation pathways is not well understood (Grochowska, 1963). Leaves may affect flower formation in several ways. They provide carbohydrates and plant growth regulators, and work as the main receptors for environmental signals. Moreover, transpiring

leaves increase the flow of substances transported via the xylem stream. It is well understood that flower induction genes are active in the leaves (Hanke et al., 2007). It is commonly accepted that flower formation occurs in apple trees when shoot growth ceases (Luckwill, 1970). Removal of leaves can reduce flower bud formation (Childers et al., 1995; Fulford, 1960; Harley et al., 1942), and may be a practice to manipulate biennial bearing because partial defoliation in off-years prevents excessive flower formation in on-years (Davis, 1957; Fulford, 1960). In these studies, partial defoliation reduced but did not totally inhibit flower formation. Return bloom was suppressed with whole-tree and partial-tree defoliation in ‘Braeburn’, ‘Golden Delicious’, ‘Ramey York’, and ‘Fuji’ (Davis, 2002), but defoliation effects on flower formation varied among cultivars (Sahulka, 1967). There is a clear relationship between flower formation and defoliation time and severity because inhibition of flower formation declined as defoliation time was delayed (Davis, 1957; Liewelyn, 1968) and as defoliation severity was decreased (Harley et al., 1942; Tustin et al., 1997).

In 1948, Singh suggested that reducing carbohydrate supply by reducing leaf area inhibits flower formation. Although there have been positive correlations between carbohydrate content as an essential substrate for flower formation and flower intensity, results have been variable. It appears that sucrose could be the signal in flower induction (Xing et al., 2015).

Crop load affects flower formation, especially in biennial cultivars. Generally, higher crop loads result in lower return bloom (Meland, 2009). For 40 years, it has been widely accepted that it is the seeds within a fruit that are the major cause of biennial bearing in apple trees (Chan and Cain, 1967). In ‘Honeycrisp’, crop load reduction enhances flower formation (Embree et al., 2007). It is commonly accepted that high crop load inhibits vegetative growth (Singh, 1948), and light crop load promotes vegetative growth (Maggs, 1963). Therefore, there are negative relationships between both crop load and vegetative growth, and flower formation in apple trees.

An apple fruiting spur produces two different types of leaves, spur leaves (SL) and bourse leaves (BL). SL grow directly from the spur, and BL grow from subsequent vegetative growth on the spur called the bourse shoot (Fig. 1). If the flower formation signals are perceived in the terminal meristem of the bourse shoot, floral buds will be formed for the following year’s crop (Abbott, 1960).

It remains unclear whether the requirement for leaf area in close proximity to the potential flowering bud is similar in annual vs. biennial cultivars. Therefore, we investigated differences in the regulation of flower formation between two *Malus* cultivars, ‘Honeycrisp’, a biennial cultivar, and ‘Gala’, an annual cultivar. The effect of spur defoliation

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¹Corresponding author. E-mail: hirst@purdue.edu.

and fruiting treatments, and their interaction, on local flower formation were evaluated. We hypothesized that in response to these treatments, the cultivars would have different patterns of flower formation from year to year. Such information would help clarify the roles of SL and BL, and the presence of fruit, on flower promotion and inhibition signals within the spur.

Materials and Methods

Plant materials. This experiment was conducted on ‘Gala’ trees planted in 2001 and growing on B.9 rootstock, and mature ‘Honeycrisp’ trees planted in 2003 and growing on M.7 rootstock. Both cultivars were planted at a spacing of 3 m × 5 m at the Samuel G. Meigs Horticulture Facility of Purdue University in Lafayette, IN. The climatic conditions of the state of Indiana can be described as a hot-humid continental climate. Trees were trained to a central leader form and were managed according to standard commercial practices. Crop load was adjusted manually by hand thinning at petal fall to achieve a medium crop load of 120–140 fruit per tree, one fruit per spur. In 2012, the year preceding the experiment, a series of severe spring frosts resulted in total fruit loss, which offered a standard previous crop load for all trees.

Experimental procedure. The experiment was arranged as a randomized complete block design. Six uniform healthy trees of each cultivar were randomly chosen at bloom time, based on the number of flower clusters and tree size. This experiment was conducted over three consecutive seasons, spring 2013, 2014, and 2015, on different trees within the same plantings used each year.

One hundred flowering spurs were randomly tagged on each tree at bloom using durable, plastic tags identified by colors and numbers. Four manual defoliation treatments were applied at petal fall on each tree, and each treatment was randomly assigned according to tag color. Treatments were reapplied as necessary on the same spurs 2 weeks and 1 month later because after defoliation, new leaves emerged on some spurs as bourses continued to grow. Treatments were control, SL defoliated, BL defoliated, and both spur and bourse leaves (SL + BL) defoliated.

On each tree, each defoliation treatment was applied to 15 fruiting spurs thinned to one fruit per spur (king flower only) and 10 defruited spurs. Because not all flowers set fruit and some fruit abscission occurred through the growing season, a higher number of replications for fruiting spurs were selected compared with defruiting spurs. For fruiting treatments, only those spurs with fruit that persisted to the end of the growing season were included in analyses. Fruit were harvested and individually weighed during the commercial harvest period for each cultivar. During winter, while trees were dormant, tagged spurs were collected, bourse lengths measured using a ruler, and bourse

buds were dissected under a dissecting microscope (Olympus Corp., Center Valley, PA) to determine flower formation for each bourse shoot (Hirst and Ferree, 1995).

Statistical analysis. Data analyses were conducted using flower formation and bourse length as response factors. Many spurs, especially on ‘Honeycrisp’, formed more than one bourse shoot. For flower formation, comparisons were made among spurs so that if any bourse on a spur formed a flower, it was considered to be a flowering spur. However, for bourse length, all bourses on each spur were summed resulting in total bourse length per spur. For each cultivar, treatments were compared within each year separately. Data are presented as proportion flower formation for each cultivar in each year (Tables 1 and 2). Comparisons were conducted using Tukey’s Studentized range test, package multcomp 1.2–12 (Torsten et al., 2008), procedure GLM, R 3.2.2 (14 Aug. 2015), “Fire Safety” within the R statistical package (R Foundation, Vienna, Austria).

Results

Similar trends in flowering of ‘Honeycrisp’ were observed in 2013 and 2015, where defoliating SL did not inhibit flower formation compared with controls, but defoliating BL only and BL + SL significantly inhibited flower formation compared with controls and SL defoliated (Table 1). Conversely, very low flowering of all ‘Honeycrisp’ trees across all treatments was observed in Spring 2014, and no significant differences were apparent. In years from 2013 to 2015, flowering in ‘Gala’ showed similar trends as seen in ‘Honeycrisp’, where defoliation of BL and BL + SL resulted in lower flowering, although the differences were only statistically significant in 2015 (Table 2).

Flower formation on fruiting spurs was significantly inhibited compared with defruited spurs for both cultivars in 2015 (Tables 1 and 2). Similar trends were evident for both cultivars in other years, but were not statistically significant, although this was almost the case for ‘Honeycrisp’ in 2013 ($P = 0.057$) (Tables 1 and 2). Defoliation and fruiting effects were independent with no significant interactions apparent for either cultivar in any of the years of the experiment (Tables 1 and 2).

In ‘Honeycrisp’, in 2014, defoliating SL resulted in significantly longer bourses compared with control and defoliating SL + BL (Table 3), but in 2013 and 2015, no differences in bourse length were observed. With ‘Gala’, in 2015 there was significant inhibition of bourse length when defoliating BL and SL + BL compared with control (Table 4), but no differences in bourse length were observed in 2013 and 2014. In all years in both cultivars, there were no significant differences in bourse length between fruiting and nonfruiting treatments (data not presented).

Fig. 1. Apple fruiting spur showing spur leaves emerging directly from the spur and bourse leaves emerging from the bourse shoot.

Table 1. Effect of defoliation treatments, fruiting treatments and their interaction on flower formation in ‘Honeycrisp’ apple during 2013, 2014, and 2015.

Defoliation treatments	Flowering (%)		
	2013	2014	2015
Control	55 a	4 a	58 a
Spur	68 a	1 a	51 a
Bourse	19 b	0.0 a	23 b
Spur + bourse	17 b	0.0 a	24 b

Fruiting treatments

Fruit	36 a	1 a	28 a
No fruit	52 a	2 a	52 b
Defoliation × fruiting	NS	NS	NS

Values followed by different letters are significantly different ($P < 0.05$).

Table 2. Effect of defoliation treatments, fruiting treatments and their interaction on flower formation in ‘Gala’ apple during 2013, 2014, and 2015.

Defoliation treatments	Flowering (%)		
	2013	2014	2015
Control	100 a	76 a	62 a
Spur	100 a	86 a	72 a
Bourse	79 a	62 a	29 b
Spur + bourse	71 a	68 a	25 b

Fruiting treatments

Fruit	87 a	74 a	43 a
No fruit	93 a	77 a	56 b
Defoliation × fruiting	NS	NS	NS

Values followed by different letters are significantly different ($P < 0.05$).

Discussion

Crop load (Embree et al., 2007) and seeds within the fruit (Chan and Cain, 1967) have been suggested to be the source of flower formation inhibiting signals, and leaves have been considered to be the main source of flower formation promoting signals (Hanke et al., 2007). Nevertheless, the contribution of the whole tree to stimulate or inhibit local flower formation within the spurs of biennial and annual cultivars was rarely discussed. Our data suggested that both local defoliation and local fruiting are flower formation

inhibitors. However, trees of ‘Gala’, an annual cultivar, and trees of ‘Honeycrisp’, a biennial cultivar, manipulate the local inhibition effects of fruiting and defoliation within their spurs in different ways. ‘Gala’ trees overcame the local inhibition effects in 2 years 2013 and 2014, but ‘Honeycrisp’ trees were not able to resist the local inhibition effects in any year (Tables 1 and 2). On the other hand, local fruiting and local

defoliation affected flower formation, but did not affect bourse length; hence, it appears that bourse length was predetermined before bourses emerged, but flower formation was not predetermined before bourse emergence (Tables 3 and 4).

Effect of leaf and fruit on flower formation. Different apple cultivars show different patterns of flower formation in response to defoliation (Sahulka, 1967), and fruiting inhibits flower formation in biennial cultivars (Meland, 2009). Hence, fruit and leaves play the main role in regulating biennial bearing and flower formation. BL are essential for flower formation, and defoliating BL led to reduced flower formation in both cultivars. However, SL do not contribute to flower formation, because in both cultivars in all years defoliating SL did not reduce flower formation compared with control (Tables 1 and 2). Furthermore, in an associated experiment we found that the proportion of SL to BL within each spur in both cultivars was around 80% BL to 20% SL (data not presented). Therefore, most of the leaf area on a spur is attributable to BL and not SL. From these data, it is unclear whether BL and SL play different roles or whether defoliation of SL did not remove sufficient total spur leaf area to have an effect on flowering.

In this experiment, local BL defoliation inhibited local flower formation (Tables 1 and 2). This may have been a result of: 1) reducing transpiration and therefore xylem flow from the roots to the spurs, 2) inhibiting both production, transportation of flower formation signals to the meristematic buds, or both, and/or 3) reducing carbohydrate supply by the leaves to the meristematic buds. In addition, it appears that local fruiting reduced flower formation, which may be a result of: 1) local interruption by fruiting on the flower formation signals and/or 2) local consumption of carbohydrates or other local resources.

High sucrose levels in the meristematic buds is essential for apple flower formation, and promotes *FT* gene expression (Xing et al., 2015). Both fruiting and defoliation would be expected to result in reduced total sugar content in the spur, so this could explain the mechanism of these effects.

Effect of whole tree on treated spurs flower formation. Effects of local, within spur inhibitions such as fruiting and defoliation may be related to the availability of flower promotion signals within the whole tree. The balance of whole tree promotive effects and local inhibition effects may help explain the response of different cultivars to spur defoliation treatments. We suggest that in ‘Gala’, these local inhibition effects could be overridden by the whole tree promotive effect, resulting in flower production (Fig. 2). By contrast, it would seem that in ‘Honeycrisp’ these local inhibition effects were not overridden by whole tree effects, and flowering inhibition occurred on treated spurs. So, if indeed the balance between whole tree promotive signals and spur inhibition is important, the influence of cultivar remains unclear. It could be that ‘Gala’ produces more whole-tree promotive signal or that spur inhibition is stronger in ‘Honeycrisp’. Such local inhibition effects appeared to have less effect on ‘Gala’ which may contribute to its annual-bearing habit and had a greater impact on ‘Honeycrisp’ which may contribute to its biennial bearing habit. Hence, the regulation of flower formation signals may not only be localized within the spur, but also exist throughout the tree.

In conclusion, in this experiment, it was confirmed that BL play the main role in regulating flower formation, with SL having little effect. In addition, both bourse leaf defoliation and fruiting were shown to cause local inhibition of flowering, but their inhibition pattern varied between the two cultivars. Such differences between cultivars and may be related to the availability and metabolism of carbohydrates and/or flower promotion signals in the tree. Further understanding of the expression patterns of apple flowering genes within spurs of annual and biennial cultivars may contribute to increased understanding of the complex networks involved in apple flower formation and biennial bearing.

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Table 3. Effect of defoliation treatments, fruiting treatments and their interaction on bourse length in ‘Honeycrisp’ apple.

Defoliation treatments	Bourse length (cm)		
	2013	2014	2015
Control	18.48 a	4.57 a	5.43 a
Spur	15.55 a	6.34 b	4.6 a
Bourse	10.79 a	4.58 ab	4.24 a
Spur + bourse	11.37 a	4.2 a	4.9 a
Fruiting treatments			
Fruit	15.8 a	4.61 a	4.63 a
No fruit	14.22 a	5.08 a	5.05 a
Defoliation × fruiting	NS	NS	NS

Values followed by different letters are significantly different ($P < 0.05$).

Table 4. Effect of defoliation treatments, fruiting treatments and their interaction on bourse length in ‘Gala’ apple.

Defoliation treatments	Bourse length (cm)		
	2013	2014	2015
Control	6.83 a	3.01 a	5.2 a
Spur	8.32 a	3.37 a	3.9 ab
Bourse	5.23 a	3.28 a	3.3 b
Spur + bourse	6.20 a	3.26 a	3.3 b
Fruiting treatments			
Fruit	5.38 a	3.29 a	4.24 a
No fruit	7.55 a	2.98 a	3.94 a
Defoliation × fruiting	NS	NS	NS

Values followed by different letters are significantly different ($P < 0.05$).

Fig. 2. Proposed model of flowering signals regulation. No flower formation refers to nonavailability of flower promotion signals. Local inhibition effects refer to flowering promotion signal availability, but local inhibitions will inhibit flower formation. No local inhibition effects refers to flowering promotion signal availability, and local inhibitions will not inhibit flower formation.

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