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A System of Defined Phenological Stages for Cold Tolerance and Development of Floricane Inflorescences of Primocane-Fruiting Blackberries

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Abstract

Phenological development charts for flower buds are commonly used for many fruit crops and phenology is linked to pest development and cold hardiness. However, no phenological development chart had been developed for blackberry (*Rubus* spp.). Observations were made of the floricanes of primocane-fruiting blackberry genotypes, including cultivars 'Prime-Jan'® and 'Prime-Jim'®, under high tunnels and ambient conditions in 2008 and 2009. Seven chronologically consistent stages were selected. The usefulness of these stages in predicting resistance to spring freezes and frosts was observed in a spring freeze (-5 °C) in 2008. Buds were selected and phenological stage and the presence or absence of necrosis were recorded. Phenological stage had a significant effect on bud mortality. Bud mortality increased greatly between the stage where the inflorescence was in a tight cluster and the stage where the pedicels had elongated.

Phenology is often used to map plant development for both ecological and crop management purposes (5). The primary horticultural purposes for making phenological observations are pest management and cold damage susceptibility. Freeze and frost susceptibility have been linked to stages of floral bud phenological development in multiple fruit crops including apple (*Malus × domestica* Borkh.) (6), peach (*Prunus persica* [L.] Batsch) (3), and sweet cherry (*Prunus avium* L.) (3). In a review of factors affecting tree fruit susceptibility to cold damage, Rodrigo (4) described four stages of increasing susceptibility to cold damage: dormancy, bud swell, between budbreak and the emergence of petal tips, and anthesis. Therefore, tracking phenological development is important in ascertaining the susceptibility of the crop to cold damage. However, clearly defined phenological stages have not been identified for blackberry as they have in apple, cherry, and peach (2). Although no phenological system was developed, Goulart (1) tracked phenological development 16 cultivars of red raspberries (*Rubus idaeus* L.) and found variation amongst cultivars, including differences of

up to 22 days of time between bloom and harvest of the fastest and slowest developing cultivars.

The objectives of this study were: 1) to determine a clear set of stages of blackberry (*Rubus* spp.) floricane inflorescence development on which observations of pest development and cold tolerance could be based and 2) to confirm the applicability of the results of phenological observations on cold temperature susceptibility.

Materials and Methods

Phenology observations. Two plots, one under a high tunnel and one in ambient conditions, were selected at the Arkansas Agricultural Research and Extension Center, Fayetteville, Ark. [lat. 36°5'4"N, long. 94°10'29"W; soil type Captina silt loam (Typic Fraguidults)]. There were three, 4 m rows in each plot, each with a different primocane-fruiting genotype: APF-46, 'Prime-Jim'®, or 'Prime-Jan'®. Beginning 12 March 2008, fifteen floricanes were randomly selected per row. The tenth node from the base of each cane was selected as this node usually was in the midsection of the cane. Buds closer to the growing tip were likely

to have already fruited the previous fall, and buds closer to the ground were more likely to remain vegetative. Selected buds were labeled at budbreak to avoid including buds which would remain dormant or die. Each bud was observed and photographed three times per week and more frequently after petals could first be seen. Each stage was recorded according to a set of pre-determined possible stages, later to be revised if necessary based on the photographic record.

At the beginning of this study we used some basic assumptions about blackberry inflorescence development to form a framework to test against our subsequent observations. In general blackberry inflorescences are mixed, having both leaves and flowers growing from a single stem. Prior to this study, we assumed that the blackberry inflorescence begins growing with leaf expansion and elongation of the main stem or axis of the inflorescence. The flower buds then form in the axils of the leaf petioles and at the apex of the inflorescence. The apical flower bud of the inflorescence, or "king bud," flowers first with anthesis continuing generally in a basipetal order. Based on the assumptions above, the stages we pre-determined were:

1. Budbreak: Visible growth of leaves from bud.
2. Leaf proliferation: Longer leaves overshadowing initial budbreak leaves, at least four leaves.
3. Elongation: Inflorescence axis elongation.
4. King bud: King bud visible.
5. Inflorescence opening: Individual flower buds other than king bud distinguishable.
6. Pedicels visible: Pedicels of individual flower buds elongated.
7. King bud popcorn: Petals of unopened king bud visible, but the flower has not opened.
8. Secondary popcorn: Petals of unopened buds other than king bud visible, but the flowers have not opened.
9. King bloom: King bud in bloom.
10. Secondary bloom: Buds other than king

bud bloom, not full bloom.

11. Full bloom: Anthesis of all flowers in inflorescence.
12. Petal fall: Loss of all petals from the king bud.

To cull out stages not common to all the genotypes and environments, data of each stage were first tabulated by frequency of observation. Stages with observation frequencies lower than 80% in any given combination of genotype and location were either combined with similar stages or eliminated from consideration. If the resultant combined stages did not result in greater than 80% observation frequency, they were eliminated. If two stages were frequent but were not sequentially different from each other (consistently in the same order across location and genotype combinations), the less easily identified stage was eliminated. The stages determined through the methods of 2008 were used to repeat the process in 2009.

Application of phenological stages to cold temperature survival. A freeze occurred on 15 April 2008 in which ambient temperatures reached -5 °C. Thirty buds were randomly selected from each of the three primocane-fruited genotypes. Data were collected under high tunnels, but because temperatures did not drop below 0 °C, the data were not presented because there was no necrosis. Buds were classified according to the phenological stages based on the results of the observations described above. Buds were sectioned and the carpelate material was inspected for necrosis as in Proebsting and Mills (3). Buds were classified as dead or alive. Frequencies were analyzed in the GENMOD procedure in SAS (SAS version 9.1; SAS Institute, Cary, NC).

Results

Phenological observations. Of the 12 proposed stages, five were not observed frequently enough to be considered defined stages (Table 1). Leaf proliferation was frequent in 'Prime-Jim'[®] and 'Prime-Jan'[®], but

Table 1. Proportion of flower buds observed at each phenological development stage for inflorescences of floricanes of primocane-fruited blackberry genotypes APF-46, ‘Prime-Jan’®, and ‘Prime-Jim’® under ambient and high tunnel locations. Shaded cells had less than 80% observation frequency.

Stage	Ambient			High tunnel		
	APF-	Prime-	Prime-	Prime-		
	46	Jan	Jim	APF-46	Jan	Jim
1. Budbreak	1.00	1.00	1.00	1.00	1.00	1.00
2. Leaf proliferation	0.73	1.00	0.87	0.50	1.00	1.00
3. Elongation	0.20	0.50	0.33	0.00	0.77	0.13
4. King bud visible	1.00	1.00	0.92	1.00	0.83	1.00
5. Flower bud proliferation	0.91	1.00	1.00	1.00	0.80	0.92
6. Flower bud pedicels visible	1.00	1.00	1.00	1.00	1.00	0.92
7. King bud popcorn	0.67	1.00	0.67	0.29	0.75	0.89
8. Secondary popcorn	0.83	1.00	0.83	0.83	1.00	1.00
9. King bud bloom	0.60	1.00	0.63	0.67	0.75	0.91
10. Secondary bloom	0.50	1.00	1.00	1.00	0.50	0.88
11. Full bloom	1.00	1.00	- ^z	1.00	1.00	1.00
12. Petal fall	1.00	1.00	1.00	1.00	1.00	0.91

^z Although it was apparent that ‘Prime-Jim’® underwent this stage, the frequency of actual observation was low due to the brief duration of the stage, so the data were omitted.

not in APF-46, because many buds of this genotype did not unfold more than the two initial leaves before the flower buds became visible. Elongation was both infrequent and genotype dependent. These two stages were eliminated because they were not associable with any other stage. Because of the infrequencies of observation, the king bloom and secondary bloom stages were combined (Table 2). Although the secondary popcorn stage was observed frequently enough to stand alone, combination with king bud popcorn, which could not stand alone, yielded a higher fre-

quency. This combination was also simpler because observing the first appearance of popcorn in the inflorescence avoids the ambiguities resulting from loss of the king bud.

When the resulting seven stages were compared by date, the full bloom and petal drop stages were not sequentially different (Table 3). In some treatment combinations average date of petal fall occurred before full bloom and in others it occurred after full bloom. Thus, the stage of full bloom was eliminated. Hence, between the first popcorn stage and the petal fall stage, the finally selected observations

Table 2. Proportion of flower buds observed at finally selected stages of phenological development of floricanes in-florescences of floricanes of primocane-fruiting blackberry genotypes APF-46, 'Prime-Jim'®, and 'Prime-Jan'® under high tunnel and ambient conditions in 2008 and 2009.

Stage	Ambient						High tunnel		
	APF-46		Prime-Jan		Prime-Jim		Prime-		Prime-
	2008	2009	2008	2009	2008	2009	APF-46	Jan	Jim
1. Budbreak	1	1	1	1	1	1	1	1	1
2. King bud visible	1	1	1	0.91	0.92	0.75	1	0.83	1
3. Flower bud cluster	0.91	1	1	1	1	1	1	0.8	0.92
4. Pedicels visible	1	1	1	1	1	0.92	1	1	0.92
5. Popcorn	1	1	1	0.86	1	0.92	0.89	1	1
6. First bloom	1	0.8	1	0.86	1	1	1	1	0.91
7. Petal fall	1	1	1	1	1	1	1	1	1

focused only on the king bud (or the first bud if the king bud was absent) rather than on the entire inflorescence (Table 2).

In February 2009, high tunnel temperatures exceeded 45 °C, killing the flower buds; therefore only data from ambient plots are presented. Results from 2009 were similar to 2008 in terms of frequency and order of observation (Table 2), although the dates of occurrence differed by nearly 3 weeks (Table 3). The resulting selected stages were (Fig. 1):

1. Budbreak: Visible growth from bud after dormancy.
2. King bud: The king bud is first visible.
3. Flower bud cluster: Multiple flower buds are visible, but they remain together in a tight cluster.
4. Pedicels of flower buds visible: Pedicels begin to elongate, opening up the cluster.
5. Popcorn: The first observation of petals on any bud in the inflorescence, but the flower has not opened.
6. Bloom: The first observation of full anthesis of any bud in the inflorescence.

7. Petal drop: No petals remain on the flower.

Cold damage application. Because there were genotypic differences in the average developmental stage, genotype and stage were confounded in the analysis, making it impossible to test for interactions. There was a significant effect of genotype ($X^2=14.28$ $P=0.0008$) and phenological stage ($X^2=66.95$ $P<0.0001$) on bud mortality. APF-46 was decimated, with 98% bud kill. 'Prime-Jim'® had 70% kill (Table 4). 'Prime-Jan'® had the lowest mortality rate, with 42%, though it was not significantly different from that of Prime-Jim'®.

Bud development stage also impacted sensitivity to low temperatures (Table 4). There were only three stages present at the time of the freeze: tight flower bud cluster, pedicels visible, and popcorn. More advanced stages were more sensitive. The greatest differences were between tight flower bud cluster (Stage 3) and elongated pedicels (Stage 4).



Figure 1. Phenological stages of floricanes of primocane-fruiting blackberry from dormancy top petal fall.

Discussion

The bud phenology stages we selected based on our observations were consistently observed across three genotypes, two types of locations (high tunnels and ambient conditions), and two years. The stages were chronologically consistent, occurring in the same order across genotype, location, and year. Varying temperature conditions between years and location resulted in very different dates of each phenological progression. Although the observations were based on floricanes, the genotypes were primocane-

fruiting types. Because primocane-fruiting blackberries are relatively new, standard practices are not yet developed. Thus it is not known whether commercial growers will prefer to mow canes down in winter or to harvest floricanes. In cases where floricanes are not mown, phenological charts could be of great use, though they are less important where only the fall crop is harvested.

Some possibility exists of differences in phenological development between primocane-fruiting and floricanes-fruiting types. Further observation is suggested to

Table 3. Mean calendar dates of finally selected phenological states of three genotypes of primocane-fruited blackberry under high tunnels and ambient conditions in 2008 and 2009.

Stage	Field						High tunnel		
	Prime-Jim		APF-46		Prime Jan		Prime-Jim	APF-46	Prime-Jan
	2008	2009	2008	2009	2008	2009	2008	2008	2008
Budbreak	14 Mar	5 Apr	13 Mar	6 Apr	15 Mar	6 Apr	7 Mar	7 Mar	7 Mar
Kingbud									
visible	29 Mar	1 May	31 Mar	17 Apr	9 Apr	23 Apr	22 Mar	15 Mar	1 Apr
Flower bud									
cluster visible	5 Apr	2 May	5 Apr	29 Apr	19 Apr	3 May	28 Mar	21 Mar	4 Apr
Pedicels of									
flower buds									
visible	11 Apr	9 May	10 Apr	1 May	20 Apr	9 May	5 Apr	26 Mar	7 Apr
Popcorn	23 Apr	16 May	20 Apr	5 May	30 Apr	14 May	13 Apr	13 Apr	17 Apr
Bloom	27 Apr	20 May	25 Apr	13 May	2 May	21 May	17 Apr	16 Apr	18 Apr
Petal fall	4 May	24 May	29 Apr	16 May	3 May	24 May	22 Apr	23 Apr	20 Apr

confirm these stages or adjust the chart to include differences for florican-fruited types. Based on experience and observations, we expect florican-fruited types generally will have a similar progression but will include the inflorescence axis elongation phase, which was eliminated from the primocane-fruited chart.

The observations on bud mortality from cold spring temperatures are consistent with results of studies in fruit trees (3), in that susceptibility to damage increased in buds that were more advanced in development. Susceptibility increased between the tight bud and elongated pedicel stages, indicating that this may be an important stage in predicting possible damage from spring frosts and freezes.

As in raspberry (1), there was significant variation among genotypes in timing of

phenological development. APF-46 bloomed on average between 1 and 8 days earlier than 'Prime-Jim'[®] and between 8 and 12 days before 'Prime-Jan'[®] within any year or location. These differences in bloom dates paralleled the much greater mortality of APF-46 than the other two genotypes in the spring freeze. APF-46 generally reached every phenological stage earlier than 'Prime-Jim'[®] and 'Prime-Jan'[®]. Additional controlled studies to determine critical low temperatures at the various stages for both primocane- and florican-fruited types would be beneficial.

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Table 4. Frequency of bud mortality of floricanes of primocane-fruited blackberry genotypes APF-46, 'Prime-Jim'®, and 'Prime-Jan'® after a spring freeze in 2008 during which temperatures reached -5°C.

Genotype	Bud mortality (%)
APF-46 ^z	98 a ^z
Prime-Jim	70 b
Prime-Jan	42 b
Stage	
3. Tight flower bud cluster	25 a
4. Pedicels visible	96 b
5. Popcorn	100 -

^z Frequencies followed by different letters are significantly different within genotype or stage at P<0.05. Mean separation was performed using a X² analysis. No letter is assigned to stage 5 because 100% mortality yields no estimate of variance.

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