Scientia Horticulturae 192: 141-147 (2015)

Flower development in sweet cherry framed in the BBCH scale

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ABSTRACT

In recent years a growing interest to widen the cherry (*Prunus avium* L.) production calendar results in cultivation out of the traditional cultivation areas. Since cherry has high chilling requirements, this often causes erratic cropping related to phenological alterations. However, appropriate phenological characterisation and comparison is hampered, due to the lack of a consensus phenological scale for this species. In this work we have characterised flower development in sweet cherry, framing it in the BBCH scale. For this purpose, the phenology of two cherry cultivars has been characterized over two consecutive years and adapted to the BBCH code, and flower development has been framed within the principal growth stages of this code. This provides a unified standardised approach for phenological comparative studies.

Keywords: BBCH scale, flower development, growth stage, phenology, *Prunus avium*, sweet cherry.

1. Introduction

Sweet cherry (*Prunus avium*) is well adapted to temperate regions with moderately cold winter temperatures (Iezzoni, 2008). But there is an increasing interest in expanding the range of ripening times to get profitable fruit offseason. This has prompted the extension of the traditional growing areas to warmer or cooler regions, and the breeding of new cultivars that wide open the ripening calendar (Kappel et al 2012). But this is often causing erratic cropping, and phenological alterations hampering the new market opportunities.

Phenological alterations and fruit set problems are also occurring in more traditional areas, which appear to be related to the effect of global warming on sexual plant reproduction (Hedhly et al., 2009; Hedhly, 2011). Indeed, cherry trees are particularly prone to these alterations since warm temperatures reduce fruit set (Hehdly et al., 2007), and shorten stigmatic receptivity (Hedhly et al., 2003), reducing the effective pollination period (Sanzol and Herrero, 2001). As it occurs for other temperate fruit trees, chilling is required in cherry for proper flowering (Perry, 1971; Vegis, 1964), and global warming is resulting in a decline of winter chilling temperatures, which cause alterations in flower development, and erratic cropping (Atkinson et al., 2013; Campoy et al., 2011; Hedhly et al., 2009; Luedeling, 2012). Finally, warm temperatures can compromise different phases of flower development, as early flower initiation during the previous summer (Thompson, 1996), or bud development close to flower opening, causing a lack of synchrony in the development of the different floral organs (Rodrigo and Herrero, 2002).

This new scenario has prompted a renewed interest in phenological characterisation, and in comparative cultivar adaptive studies. But this work is hampered by lack of a consensus phenological scale for sweet cherry. Following the classical work of Fleckinger (1948), phenological growth stages in sweet cherry were characterized using the external phenological stages of buds and flowers (Baggiolini, 1952; Westwood, 1993). In the last decades, a BBCH scale (*Biologische Bundesantalt, Bundessortenamt und Chemische Industrie*) has been put forward as a decimal coding system for both herbaceous and woody crops (Bleiholder et al., 1989; Lancashire et al., 1991; Hack et al., 1992), constituting a unified system for characterizing the entire developmental cycle of the plant for a wide range of crops, including the genus *Prunus* (Meier, 2001). In the last 10 years the application of the BBCH scale has been extended to fruit trees as persimmon (García-Carbonell et al., 2002), cherimoya (Cautín and Agustí, 2005), guava (Salazar et al., 2006), kiwi (Salinero et al., 2009), mango (Hernández Delgado et al., 2011), avocado (Alcaraz et al., 2013), cape gooseberry (Ramírez et al., 2013), peach (Mounzer et al., 2008), or apricot (Perez-Pastor et al., 2004).

While the BBCH scale has the advantages of standardising data and covering all plant cycle, it has the drawback that flower development, which is the plant development process most vulnerable to climate change effects (Hedhly et al., 2009; Hedhly, 2011; Luedeling, 2012) is not considered. To refer flower development to the external appearance of the tree, in this work a BBCH scale system is proposed for sweet cherry and flower development is framed within this code.

2. Materials and methods.

Three trees of two sweet cherry cultivars, ‘Bing’ and ‘Burlat’, were selected. Data were recorded from adult trees from an experimental orchard located at the CITA in Zaragoza (Spain) at 41º44´30” N, 0º47´00” and 220 m altitude. Zaragoza has an Arid Cold steppe climate, BSk (Köppen, 1900; Kottek et al., 2006). Long-term climate data for this region show annual average mean temperatures of 15ºC, average maximum temperatures of 31.5ºC in the hottest month (July) and an average minimum temperature of 2.4ºC in the coolest month (January) (Fig. 1).

Phenological observations were carried out weekly over two growing seasons (2011-2012; 2012-2013). Along this time, external phenological growth stages and flower development were sequentially characterised and photographed in the orchard with a digital camera DSC-R1 (Sony, Tokio, Japan). In order to characterise flower development, three flower buds of each cultivar were weekly collected during autumn and winter, and every two days from bud burst to full bloom. Buds were dissected under a stereoscopic microscope MZ-16 (Leica, Cambridge, UK), and photographed with a digital camera DC-300 (Leica, Cambridge, UK).

3. Results

Phenology covered the entire year cycle (Fig. 1), starting with vegetative bud dormancy (Stage 00) and ending with total leaf drop (Stage 97). This covered eight out of the ten principal growth stages of the BBCH scale (Table 1). Growth stages 2 (formation of side shoots) and 4 (development of harvestable vegetative plant parts) were not used, since they do not apply to sweet cherry growing.

3.1. Principal growth stage 0: Bud development

Sweet cherry vegetative bud entered in a dormant stage after been differentiated during the previous summer, and vegetative bud burst took place during the following spring, after flowering at early March.

00. Dormancy: leaf buds closed and covered by dark brown scales (Fig. 2 A).

01. Beginning of bud swelling (leaf buds); light brown scales visible, scales with light coloured edges (Fig. 2 B).

03. End of leaf bud swelling: scales separate, light green bud sections visible.

09. Green leaf tips visible: brown scales fallen, buds enclosed by light green scales.

3.2 Principal growth stage 1: Leaf development

During the first vegetative growth, most of the leaves emerged. This took place along April and was completed in approximately 30 days.

10. First leaves separating: green scales slightly open, leaves emerging (Fig. 2 C).

11. First leaves unfolded, axis of developing shoot visible.

19. First leaves fully expanded (Fig. 2 D).

3.3. Principal growth stage 3: Shoot development

First vegetative flush took place in spring (April-June) during the development (stage 7) and maturity of fruit (stage 8).

31. Beginning of shoot growth: axes of developing shoots visible (Fig. 2 E).
32. Shoots about 20% of final length.
33. Shoots about 30% of final length (Fig. 2 F).
35. Shoots about 50% of final length (Fig. 2 G).
39. Shoots about 90% of final length (Fig. 2 H).

3.4. Principal growth stage 5: Reproductive development or inflorescence emergence.

Flower initiation occurred during the previous season, once shoot growth was completed in midsummer (stage 91). During this period both flower and vegetative buds were differentiated (Fig. 3 A). Inside the flower bud it was possible to observe the sepal primordia (Fig. 3 B). Flower buds continued to develop (Fig. 3 C) until leaf fall (stage 93) when dormancy was established. Protected by external scales, there were three or four flowers inside each bud. Sepals were curved inward covering completely each flower (Fig. 3 D).

50. Dormancy: inflorescence buds closed and covered by dark brown scales (Fig. 3 E).

During dormancy, flower primordium stopped growing and the flower was enclosed within sepals (Fig. 3 F).

51. Inflorescence buds swelling: buds closed, light brown scales visible (Fig. 3 G).

At the end of dormancy, the flowers presented a spherical shape, with all the different whorls differentiated. Flowers were completely green, except petals, which were slightly translucent. Sepals and petals were very short, but sepals overpassed the petals. Stamens were conspicuous and, while filaments were very short, anthers had their characteristic shape. The pistil was located in the centre of the flower and its length was equivalent to flower height. Pistil parts were incipiently distinguished: the ovary, the style and the stigma, where stigmatic surface was initiating (Fig. 3 H).

53. Bud burst: scales separated, light green bud sections visible (Fig. 3 I).

Sepals enclosed the whole flower. Petals turned into a pale white, but the most striking change was in the colour of the anthers, which turned into a bright yellow. Anthers continued to occupy most of the space inside the flower. The pistil had significantly elongated (Fig. 3 J).

54. Inflorescence enclosed by light green scales (Fig. 4 A).

The anthers filament was still short. But the style grew and surpassed the anthers, being the stigma at the same height than petals and sepals (Fig. 4 B).

55. Single flower buds visible (still closed) borne on short stalks, green scales slightly open (Fig. 4 C).

The green sepals appeared with red spots, especially at the apex, and continued enclosing the whole flower. The hypanthium, a cup-shape tube structure in which basal portions of the calyx, the corolla, and the stamens are inserted, developed as a cavity around the ovary. Anther filaments began to elongate. Pistil continued growing and reached the upper part of the flower and even it could surpass it, in some cases. The stigmatic surface was apparent, and the stigma edges started to curve down (Fig. 4 D).

56. Flower pedicel elongating; sepals closed; single flowers separating (Fig. 4 E).

The flower had acquired an elongated shape with a narrowing in the middle of the flower, which corresponded to the hypanthium. The white petals began to protrude above the sepals showing a white tip (Fig. 4 E). Inside the flower, anthers were grouped in the upper half of the flower staggered at different heights, since filaments were significantly elongated. The style continued growing over the anthers. The swelled ovary was completely surrounded by the hypanthium cavity (Fig. 4 F).

57. Sepals open: petal tips fully visible; flowers with white petals (still closed) (Fig. 4 G).

The sepals began to open and separate, forming a 120º angle with the hypanthium. The petals completely enclosed the flower. The anther filaments were significantly elongated reaching its final length. The style also reached their final length and the ovary was laterally placed. The stigma and the anthers were at the same height (Fig. 4 H).

59. Balloon stage: Sepals completely opened, petals completely extended and rounded but still closed (Fig. 4 I).

The sepals were completely open, forming a 90º angle with the hypanthium. The petals were completely extended, closing with a balloon shape (Fig. 4 J).

3.5. Principal growth stage 6: Flowering

Full bloom for both cultivars occurred between the end of March and the beginning of April, about 4 - 6 weeks after bud burst.

60. First flowers open (Fig. 5 A).

61. Beginning of flowering: about 10% of flowers open.

62. About 20% of flowers open (Fig. 5 B).

63. About 30% of flowers open.

64. About 40% of flowers open.

65. Full flowering: at least 50% of flowers open, first petals falling (Fig. 5 C).

67. Flower fading: majority of petals fallen (Fig. 5 D).

69. End of flowering: all petals fallen.

3.6. Principal growth stage 7: Fruit development

Fruit development lasted a month and a half for ‘Burlat’ and two months for ‘Bing’. Sweet cherry fruit exhibits a double sigmoidal seasonal growth pattern because of a period of slow growth during pit hardening (Westwood, 1993). Flower/fruit drop occurs 2-4 weeks after pollination, and fruit set gets established 3-4 weeks after pollination (Hedhly et al., 2007).

71. Ovary growing; flower/fruitlet drop (Fig. 5 E).

72. Green ovary surrounded by drying sepals that begin to fall.

75. Fruit about half final size.

76. Fruit about 60% of final size.

77. Fruit about 70% of final size (Fig. 5 F).

78. Fruit about 80% of final size.

79. Fruit about 90% of final size.

3.7. Principal growth stage 8: Ripening or maturity

'Burlat', an early maturing cultivar, could be harvested at mid May, while 'Bing' ripened during the first week of June. Sweet cherry produces non-climacteric so fruits so they are harvested at maturity (Hartmann, 1989).

81. Beginning of fruit colouring (Fig. 5 G).

85. Colouring advanced (Fig. 5 H).

89. Fruit ripe for harvesting (Fig. 5 I).

3.8. Principal growth stage 9: Senescence, beginning of dormancy

Leaf fall started at the beginning of October and lasted approximately a month. During this period dormancy got established (Westwood, 1993).

91. Shoot growth completed; foliage still fully green (Fig. 5 J).

92. Leaves begin to fade colour.

93. Beginning of leaf fall.

95. 50% of leaves discoloured or fallen (Fig. 5 K).

97. All leaves fallen (Fig. 5 L).

4. Discussion

The adaptation of the BBCH scale to sweet cherry has the advantage that it gives a wide overview of all plant development stages, including vegetative development and fruit ripening. But has the disadvantage that flower bud development and flowering are covered at the whole tree scale. As an alternative, specific scales, as Baggiolini (1952) or Westwood (1993), focused only in flower bud development and flowering, because those are the most delicate phases to determine harvest. To overcome this gap these phenological scales have been framed within the BBCH scale (Table 2). Still the longest process along the year is flower development, which starts at the end on the previous summer and lasts up to flowering in the spring. In this work the detailed description of flower developmental stages framed within this scale contributes to the standardization of phenological studies and connects flower development with external phenology. The adaptation of the BBCH code to sweet cherry is useful apart from agronomic treatments (Leather, 2010) for climate change studies, and to evaluate the adaptation of particular cultivars to different conditions.

So far, flower development in sweet cherry was fragmented, early stages from flower induction until dormancy were characterised (Guimond et al., 1998). Once flowers open, information is also available on stigmatic receptivity (Hedhly et al., 2003), pollen tube kinetics and dynamics (Hedhly et al., 2004), and the progamic phase and fruit set (Hedhly et al., 2007). However, from dormancy to bloom, only the characterization of the external appearance of the flower bud was so far available (Baggiolini, 1952; Westwood, 1993). Results herein fill in this gap, characterizing flower development also in this period. There are equivalent descriptions of flower development for other model species as the annuals Arabidopsis (Smyth et al., 1990), tobacco (Koltunow et al., 1990) and tomato (Brukhin et al., 2003), and *Populus* as a woody plant model (Bradshaw et al., 2000; Brunner and Nilsson, 2004). These descriptions offer morphological landmarks to understand the genetic control of flower development (Scott et al., 2004). The reference points provided in this work for sweet cherry establish the first step for further transfer floral genetic studies to this crop.

Detailed characterisation of flower developmental stages framed in the BBCH code allows connecting studies on flower biology with field observations, and provides a consensus unified approach contributing to the standardisation of phenology studies.

Acknowledgements

This work was supported by Ministerio de Economía y Competitividad (MINECO) - European Regional Development Fund, European Union (Project grants: AGL2009-12621-C02-00, AGL2012-40239-C02, INIA RF2011-00029-C03 and INIA RFP2012-00017-C03) and Gobierno de Aragón (Grupo Consolidado A-43). E. Fadón was supported by a FPI fellowship of MINECO [BES- 2010-037992].

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Figure legends

Figure 1. Flower and fruit development framed in the principal growth stages of the BBCH scale in Zaragoza. Time elapsed in each stage (horizontal bars), weekly mean temperature (2012-2013) (continuous line) and photoperiod (dashed line).

Figure 2. Vegetative development. Principal growth stages 0: Bud development, 1: Leaf development, and 3: Shoot development of sweet cherry according to the extended BBCH scale.

Figure 3. Stages of flower development framed in principal growth stages 9: Senescence, beginning of dormancy, and 5: Reproductive development of sweet cherry according to the extended BBCH scale. Scale bar = 0.2 mm.

Figure 4. Stages of flower development framed in principal growth stage 5: Flower bud development of sweet cherry according to the extended BBCH scale. (B, D, F) Scale bar = 1 mm; (H, I) Scale bar = 2 mm.

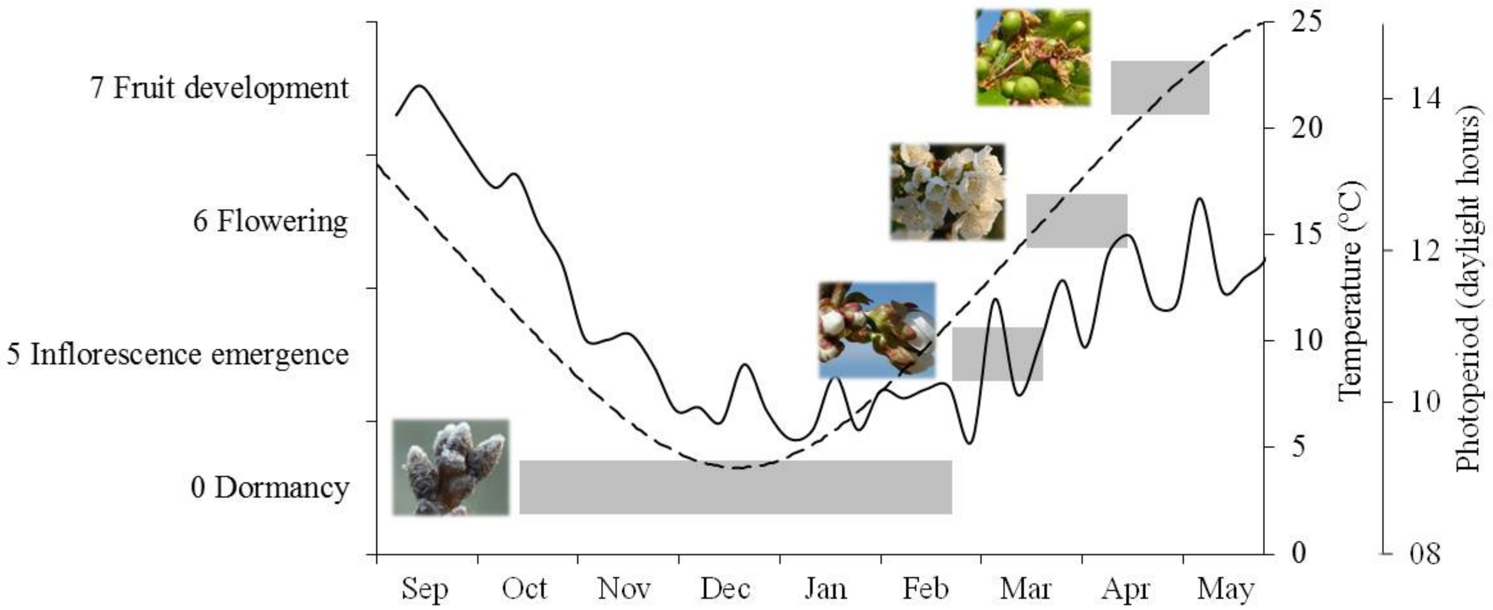
Figure 5. BBCH principal growth stages 6: Flowering, 7: Fruit development, 8: Ripening or maturity and 9: Senescence, beginning of dormancy of sweet cherry according to the extended BBCH scale.

Table 1. Phenological growth stages of sweet cherry according to the BBCH scale.

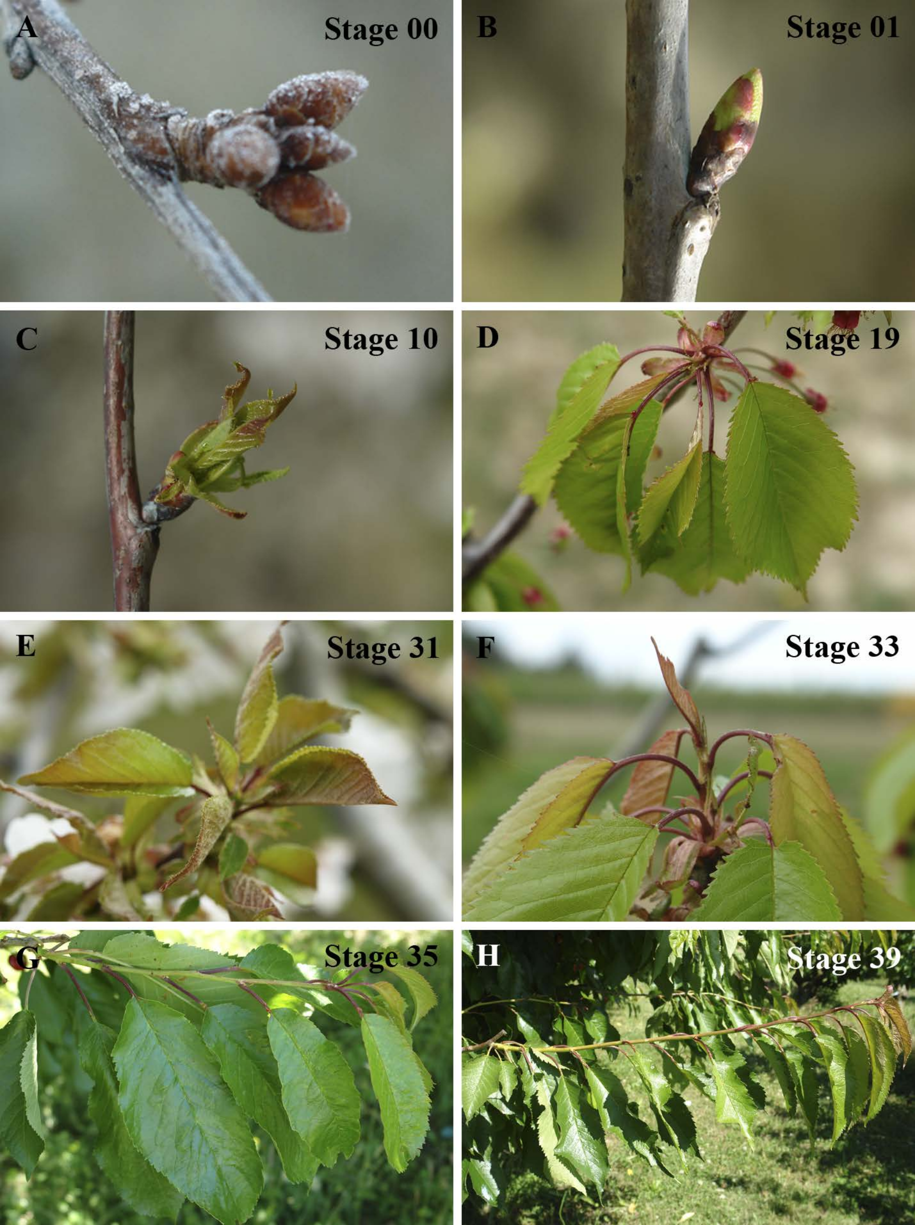
BBCH code	**Description**
Principal growth stage 0: Bud development	
00	Dormancy
01	Beginning bud swelling
03	End of leaf bud swelling
09	Green leaf tips visible
Principal growth stage 1: Leaf development	
10	First leaves separating
11	First leaves unfolded
19	First leaves fully expanded.
Principal growth stage 3: Shoot development	
31	Beginning of shoot growth
32	20% of final shoots length
33	30% of final shoots length
3…	Stages continuous till …
39	90% of final shoots length.
Principal growth stage 5: Reproductive development or inflorescence emergence.	
50	Dormancy, inflorescence bud closed
51	Inflorescence buds swelling
53	Bud burst
54	Inflorescence enclosed by light green scales
55	Single flower buds visible
56	Flower pedicel elongating
57	Sepals open
59	Balloon
Principal growth stage 6: Flowering	
60	First flowers open
61	Beginning of flowering
62	20% of flowers open
63	30% of flowers open
64	40% of flowers open
65	Full flowering
67	Flower fading
69	End of flowering
Principal growth stage 7: Fruit development	
71	Ovary growing
72	Sepals beginning to fall
73	Second fruit fall
75	50% of final fruit size
76	60% of final fruit size
77	70% of final fruit size
78	80% of final fruit size
79	90% of final fruit size
Principal growth stage 8: Ripening or maturity	
81	Beginning of fruit colouring
85	Colouring advanced
87	Fruit ripe for picking
Principal growth stage 9: Senescence, beginning of dormancy	
91	
92	Leaves begin to discolour
93	Beginning of leaf fall
95	50% of leaves fallen
97	All leaves fallen

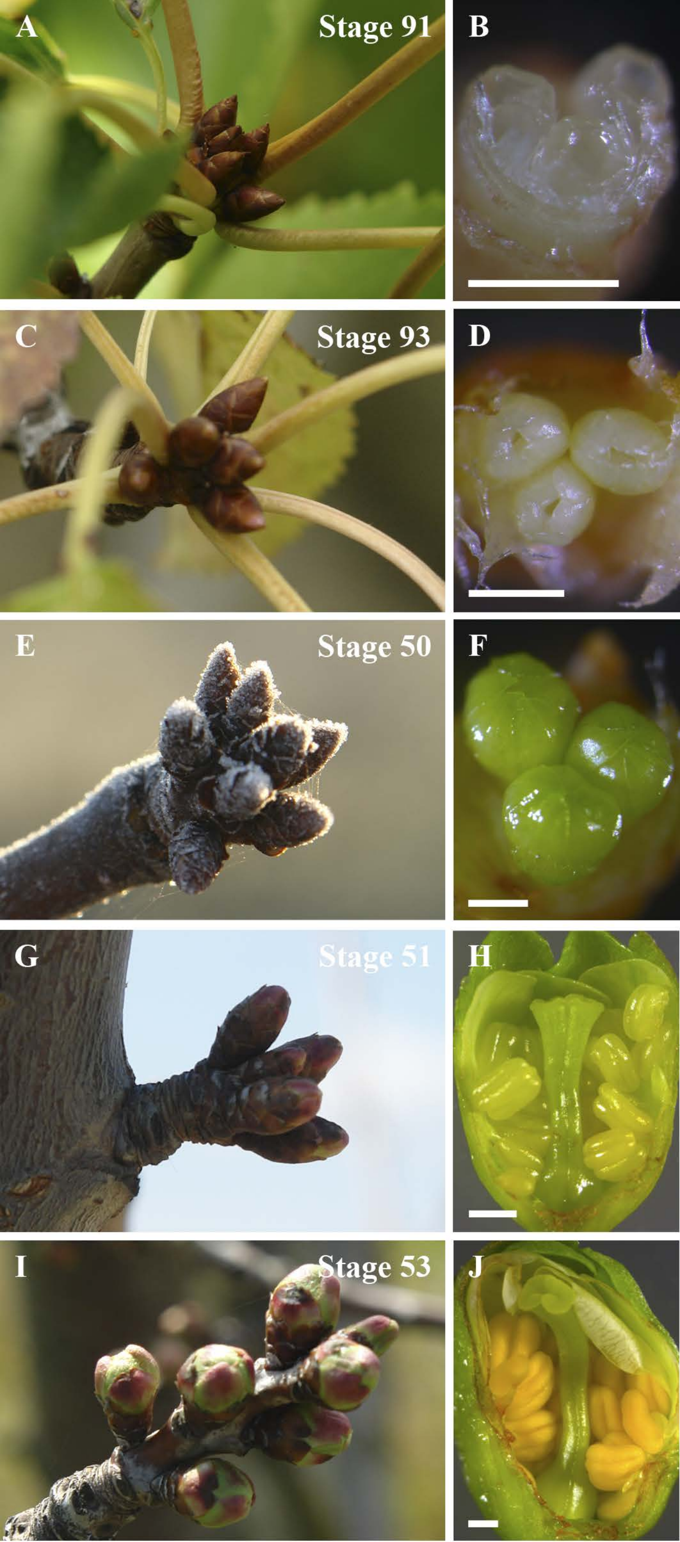
Table 2. Comparison among flower bud phenological growth stages of sweet cherry described according to the BBCH scale, Baggiolini (Baggiolini, 1952) and Westwood (Westwood, 1993).

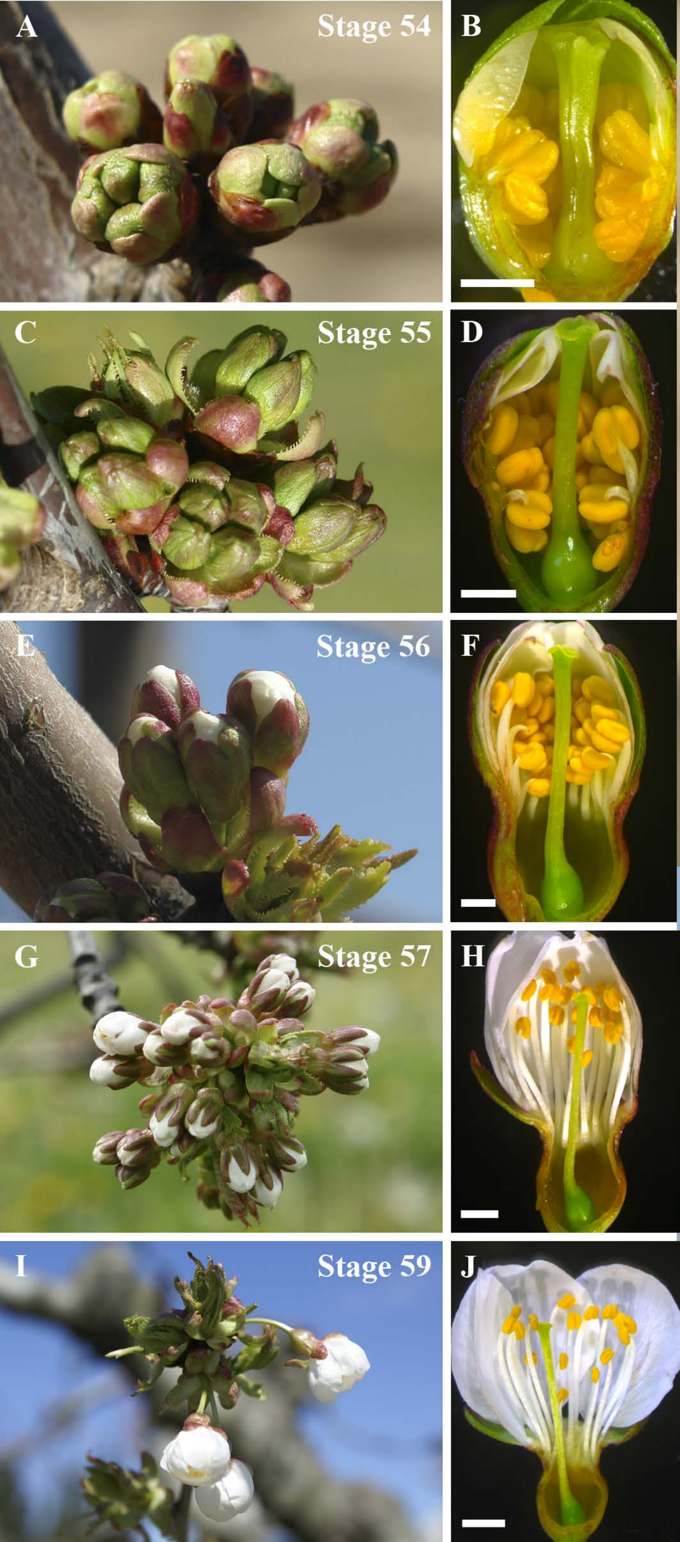
BBCH	**BAGGIOLINI**	**WESTWOOD**
50	A. Winter bud. Dormancy	0. Dormancy
51		1. First swell
53	B. Flower bud swelling.	2. Side white
54		3. Green tip
55	C1. Flower buds appearent	4. Tight cluster
56		5. Open cluster
57	D. Flower bud open.	6. First white
59	E. Stamina are appearent.	7. First bloom
65	F. Full bloom	8. Full bloom
67	G. Petals are falling.	9. Post bloom
71	H. Settling	
72	I. Calyx is falling.	
75 - 79	J. Young fruit.	



7 Fruit development
6 Flowering
5 Inflorescence emergence
0 Dormancy
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
25
20
15
10
5
0
Temperature (°C)
14
12
10
08
Photoperiod (daylight hours)


A
Stage 00
B
Stage 01
C
Stage 10
D
Stage 19
E
Stage 31
F
Stage 33
G
Stage 35
H
Stage 39


A
Stage 91
B
C
Stage 93
D
E
Stage 50
F
G
Stage 51
H
I
Stage 53
J


A
Stage 54
B
C
Stage 55
D
E
Stage 56
F
G
Stage 57
H
I
Stage 59
J


A
Stage 60
B
Stage 62
C
Stage 65
D
Stage 67
E
Stage 71
F
Stage 77
G
Stage 81
H
Stage 85
I
Stage 89
J
Stage 91
K
Stage 95
L
Stage 97