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Abstract

Chestnuts (*Castanea spp*.) are ecologically and economically important forest and fruit trees. They are cultivated for their nutritious nuts. To select varieties to be cultivated in chestnut orchards, their phenology needs to be considered. In this work, we adapt the international BBCH system to chestnuts by building on an existing phenological scale used in some European countries for these species. The proposed BBCH scale uses eight of the ten principal growth stages for fruit trees and secondary growth stages that are specific to chestnut trees. We tested it by monitoring chestnut trees phenology during three growing seasons, illustrating its suitability for high-throughput phenotyping studies. Overall, the approach used, despite its inherent limitations, is straightforward, accessible and flexible, allowing particularly precise description of the complex flowering phenology of these trees.

Keywords

BBCH, Flowering phenology, Pollination, Monoecious, Male-sterile, Fagaceae, Castanea

Introduction

Plant phenology is the timing of plant life seasonal events, such as bud burst, flowering, fruiting, and leaf abscission. It plays a fundamental role in the functioning of both natural ecosystems and agrosystems (Stucky et al., 2018). Because plant phenology is influenced by climatic variables and affects plant growth and reproduction, it is key to studies of the consequences of climate change. In agriculture, plant phenology is also particularly useful for the planning of cultivation operations such as planting, fertilizing, irrigating or harvesting (Chmielewski, 2003) and for breeding programs (Meier et al., 2009). Therefore, phenological data are currently collected around the world at an accelerated pace. However, phenological descriptions are often not standardized, making it difficult to make sense of newly collected data in large-scale multispecies comparisons (Stucky et al., 2018). Hence, it is crucial to develop universal phenological scales for all major cultivated species.

The *Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie* (BBCH) scale developed for monitoring phenological growth stages is the result of a teamwork conducted by the German Federal Biological Research Centre for Agriculture and Forestry, the German Federal Office of Plant Varieties, the German Agrochemical Association and the Institute for Vegetables and Ornamentals (Bleiholder et al., 1989). This work generated much interest, and Hack et al. (1992) subsequently published the principles of the extended BBCH, a universal scale that works with mono- and dicotyledons. Since 1992, new BBCH extended scales for specific crops have regularly been published, including some for fruit trees: pome fruits and stone fruits (Meier et al., 1994), *Citrus* (Agusti et al., 1995), apricots (Pérez Pastor et al., 2004), mango (Hernández Delgado et al., 2011), or sweet cherry (Fadón et al., 2015). Despite some limitations (Stucky et al., 2018), this method has many advantages for practical applications and has become a standard in agronomy.

There are seven consistently recognized chestnut tree species growing in subtropical, Mediterranean and temperate forests from the Northern hemisphere (Pereira-Lorenzo et al., 2012). Some are of special economic importance. In particular, the Chinese chestnut (*C. mollissima*), Japanese chestnut (*C. crenata*) and European chestnut (*C. sativa*) are widely cultivated for their fruits (Pereira-Lorenzo et al., 2012). Chestnut production is mostly concentrated in Asia and Europe (Food and Agriculture of the United Nations, 2020). China is by far the main producer of chestnuts worldwide, with over 1.9 million tons of fruits harvested annually. Fruit production in China has tripled in the last two decades. In Europe, chestnut production, which had steadily decreased since the 19th century, has slowly started to recover since the 2010s, currently reaching about 0.15 million tons per year, thanks to a renewed interest of consumers for chestnut products.

Chestnut trees are self-incompatible (Stout, 1926; Xiong et al., 2019). Hence, orchards must include enough varieties for successful cross-fertilization. When female flowers of a variety are receptive, male flowers of nearby trees from different compatible varieties must release pollen at the same time for successful pollination, fertilization and fruit production (Solignat and Chapa, 1975). Because there is a wide variation in flowering phenology across varieties, a knowledge of that variation is essential for the design of productive orchards. Chestnut trees are monoecious and have a complex flowering phenology with two separate peaks of pollen emission (Stout, 1928; Hasegawa et al., 2017). To monitor chestnut phenology, it is therefore critical to develop an effective method taking into account these biological features.

Solignat and Chapa (1975) have proposed the first phenological scale for chestnuts growth stages. This system has been widely used by chestnut breeders and germplasm curators to screen and characterize chestnut cultivars phenology and for establishing plant variety rights in Europe using harmonized descriptions of new varieties fulfilling criteria of distinctness, uniformity and stability, as defined in Kiewiet (2005). Badeau et al. (2017) then proposed a very simplified BBCH scale for chestnut to be used in a citizen science program, in which they monitored only male flowers. Here we propose a complete phenology scoring system combining Solignat and Chapa (1975) stages and the international BBCH system to facilitate comparisons across studies thanks to a uniform coding system of phenologically equivalent growth stages in plants (Hack et al., 1992).

Materials and methods

Development of a BBCH scale

The BBCH scale is a decimal code (from 00 to 99) divided into principal and secondary growth stages. An arithmetically greater code always indicates a plant at a later growth stage (Meier et al., 2009). The first digit corresponds to the main growth stage common to all plants. It allows comparisons between different crops, including mono- and dicotyledons. These stages begin from stage 0: “Germination / sprouting / bud development” and end with stage 9: “Senescence, beginning of dormancy" (Hack et al., 1992). The second digit corresponds to secondary growth stages, i.e. short developmental stages characteristics of the studied plant species. These secondary growth stages, also coded from 0 to 9, can represent percentages or average developmental stages: for example, stage 5 could represent a plant with 50% of flowers open or a plant with a relatively high proportion of unfolded leaves (Meier, 2001). If two or more growth scales are used to describe separate phenological events proceeding in parallel, such as the phenology of male and female flowers in monoecious species, they are separated by a slash.

To develop a BBCH phenological scale for chestnut trees, we relied on the principal growth stages used for pome fruits and stone fruits by Meier et al. (1994). To further describe chestnut flower development, we identified secondary growth stages and assigned them specific scores. For this purpose, we selected as much as possible phenological scores matching with those proposed by Solignat and Chapa (1975).

Chestnut flowering

Chestnut trees (genus *Castanea*) have a remarkably complex reproductive system. At the flower level, they are monoecious. Their small female and male flowers are borne on inflorescences called catkins. At the inflorescence level, however, chestnut trees are andromonoecious: they have two types of catkins, unisexual male catkins and bisexual catkins. Bisexual catkins are composed of a few female flowers generally grouped by three at the basis of the catkin. After pollination, they develop into an infrutescence composed of a spiny burr enclosing up to three fruits, one per flower. The distal part of bisexual catkins harbors numerous male flowers grouped into small glomerules spirally organized on the catkin. Finally, at the tree level, chestnut trees are either bisexual or unisexual female. In female trees, also called male-sterile trees, male catkins are still present, but their flowers have aborted anthers that produce little or no pollen. Interestingly, these male flowers still produce nectar and attract insects (Pereira-Lorenzo et al., 2017 ; Larue et al., in press).

The phenology of chestnuts is particularly complex. Male flowers of unisexual catkins bloom much earlier than male flowers of bisexual catkins, whereas female flowers have a long receptivity period. This rare flowering system (Renner, 2014), first described by Meehan (1879), is called duodichogamy (Stout, 1928). The two peaks of pollen emission do not overlap (Hasegawa et al., 2017) and are very unequal, the first one being an order of magnitude greater than the second one in terms of number of flowers produced and amount of pollen released (Larue et al., in press).

Study site and monitoring

The studied trees belong to the INRAE chestnut genetic resources collection (Figure S1). They grow in two nearby orchards located near Bordeaux in southwestern France (44.788319 N, -0.577062 E). The collection includes 117 *C. sativa*, 22 *C. crenata*, 20 *C. mollissima* and 81 interspecific hybrids, including 56 *C. sativa x C. crenata* hybrids, some of which belong to popular varieties widely cultivated in the region. All the 240 trees are grafted on two well-known rootstocks: ‘Marsol’ (CA07) or ‘Maraval’ (CA74). The first orchard was planted in 1970 and comprises 29 widely spaced trees on 2.3 ha. The second orchard was planted in 1990 and includes 211 trees on 3.5 ha.

In late spring of 2018, we monitored flowering phenology of all trees twice a week. In 2019 and 2020, we monitored a subset of these trees. We photographed phenological growth stages in the field with APS-C camera (Fujifilm X-T3 and Nikon D500) equipped with a macro lens objective (Fujinon XF 80 mm f/2.8 R LM OIS W Macro and AF-S VR Micro-Nikkor 105mm f/2.8G).

Results

The proposed BBCH phenological scale for chestnut trees includes eight principal growth stages (Table 1). We used three different scores to describe accurately the whole complexity of flowering phenology in this andro-monoecious species. The first score is for male flowers from unisexual catkins. The second score is for female flowers from bisexual catkins. The third score is for male flowers from bisexual catkins. For example, at chestnut tree scored 65/65/59 (Figure 1) corresponds to unisexual catkins with at least 50% of opened male flowers and to bisexual catkins with at least 50% of receptive female flowers and with all male flowers still unopened. Note that the scores used for the two types of male flowers are the same. We illustrate these flower developmental stages in Figure 2.

Figure 1: [Color**:** online only] This flowering shoot is composed of eight unisexual male catkins (black symbols) and two bisexual catkins (white symbols) at the tip. The phenological score that we attributed to this tree on that particular day using the BBCH scale was 65/65/59. On that branch, seven unisexual catkins are at full bloom, whereas the most distal unisexual catkin is just starting to flower (overall BBCH score for unisexual catkins: 65). Each bisexual catkin has a single female inflorescence formed by three female flowers at full receptivity (BBCH = 65) and a short male catkin. Flowers of the male part of the bisexual catkins are still not open, but catkins are already well elongated (BBCH = 59).

Figure 2: [Color**:** online only] Illustration of flowering stages. Left pictures: unisexual male catkins. a) 59: Male catkins just before flowering. Catkins have almost their final length and have turned yellow but their flowers grouped in well-differentiated glomerules are still closed. b) 65: Male catkins from male-sterile trees at full flowering. Flowers are open but stamens are not visible. c) 65: Male catkins from male-fertile trees at full flowering. Stamens are visible. d) 67: Male catkins are fading and turning brown. Central pictures: bisexual catkins. e) 59: Male part of bisexual catkins just before flowering. f) 65: Male part of bisexual catkins from male-sterile trees at full bloom. Flowers are open, aborted stamens have short filaments and do not protrude from the flowers. g) 65: Male part of bisexual catkins from male-fertile trees at full bloom. Flowers are open and stamens have long filaments. h) 67: Male part of a bisexual catkin that has turned brown. Right pictures: female inflorescence. i) 61: Only the stigmas of the central flower are visible. j) 63: Stigmas of central flower are well developed and stigmas of lateral flowers are visible. k) 65: full receptivity. Stigmas of the three flowers are well developed. l) 67: tips of stigmas from female flowers have turned brown.

To assess the phenology of the male flowers from male-sterile trees, we relied on the proportion of open male flowers instead of estimating the proportion of flowers with conspicuous stamens emerging from the catkins (Table 1, Stage 5). To evaluate the phenology of female flowers, we propose two options. One option is to rely on the appearance of diagnostic stages, following Solignat and Chapa (1975) (Table 1, Stage 6, Female flowers option 1). Alternatively, a semi-quantitative scale can be used to describe tree receptivity, similar to that used for male flowering (Table 1, Stage 6; Female flowers option 2).

BBCH Code	**Description**	**Conversion**
Stage 0: Sprouting/Bud development		
0	Dormant buds	A - Af
07	Beginning of bud break	B
09	Green leaf tips visible: first green leaf tips just visible	C
Stage 1: Leaf development		
11	First leaves unfolded	D
15	More leaves unfolded, not yet at full size	D
19	All leaves unfolded and fully expanded	Dl
Stage 3: Shoot development		
31	Beginning of shoot growth	
35	Shoots about 50% of final length	
39	Shoots about 90% of final length	
Stage 5: Catkins growth (unisexual catkins / Female inflorescences / bisexual catkins)		
Male catkins (unisexual or bisexual)		
50	Appearance of male catkins	Dm-Da
55	Glomerules are visible, male catkins grow	
59	Glomerules well differentiated, male catkins about 90% of final length	Em
Female inflorescences		
50	Appearance of buds of female inflorescences	Df
55	Buds of female inflorescences are visible, bisexual catkins grow	
59	Female inflorescences well differentiated, bisexual catkins about 90% of final length	Ef
Stage 6: Flowering (Male flowers of unisexual catkins / Female flowers / Male flowers of bisexual catkins)		

Male flowers (unisexual or bisexual catkins)		
60	First male flowers open	Fm-Fa
61	Beginning of the flowering: 10-20% of male flowers open	
62	20-30% of male flowers open	
63	30-40% of male flowers open	
64	40-50% of male flowers open	
65	Full flowering: at least 50% of male flowers open	Fm2-Fa2
67	Catkins fading: at least 50% of brown male catkins	Gm-Ga
69	End of flowering: at least 50% of male catkins have fallen	Hm
Female flowers (Option 1): Phenotypic stages		
60	Female flowers visible	
61	Stigmas of the central flower of the inflorescence visible	Ff
63	Stigmas of the central flower elongated, stigmas of lateral flowers visible	
65	Full receptivity: stigmas of three female flowers are well developed	Ff2
67	At least 50% of female flowers have brown stigmas	
69	End of flowering: all female flowers have brown stigmas	
Female flowers (Option 2): Receptivity		
61	Beginning of the flowering: 10-20% of female flowers are receptive	
62	20-30% of female flowers are receptive	
63	30-40% of female flowers are receptive	
64	40-50% of female flowers are receptive	
65	Full flowering: at least 50% of female flowers are receptive	
67	At least 50% of female flowers have brown stigmas	
69	End of flowering: all female flowers have brown stigmas	
Stage 7: Burr development		
72	Involucre is 3x larger than when the female inflorescence was receptive	I
75	Burrs about 50% of final volume	J
79	Burrs about 90% of final volume	J
Stage 8: Fruit maturity		
81	Burrs turn brown	K
83	First burrs open	Lo
85	At least 50% of burrs open	Mo
87	At least 50% of chestnuts/burrs fallen	N - O
89	All chestnuts/burrs fallen	N - O
Stage 9: Leaf senescence		
90	Leaves begin to discolor or start to fall	Dj
91	About 10% of leaves discolored or fallen	Dz

95	About 50% of leaves discolored of fallen	Dz
97	All leaves fallen	Dz

Table 1: Phenological growth stages of chestnuts according to the BBCH scale and conversion from Chapa and Solignat (1975) scale.

We successfully applied this new BBCH scale to the trees from INRAE chestnut genetic resources collection during three years. To briefly illustrate the type of results obtained, mean date, minimum date and maximum date for onset of full flowering of the two types of male catkins are provided in Table 2 for measures performed in 2018 on all trees, distinguishing the three pure species and one class of hybrids. On average, male flowers from bisexual catkins reach full bloom about 10 days after male flowers from unisexual catkins. Date of flowering of male catkins vary slightly among species but greatly within each species. Overall, for unisexual catkins, 25 days separate the earliest and the latest chestnut trees, whereas for bisexual catkins, 20 days separate the earliest and the latest trees.

Type	**N**	**Unisexual male catkins (BBCH = 65)**	**Male flowers of bisexual catkins (BBCH = 65)**
C. sativa	117	163.8 (155.5-177)	173.7 (165.5-182)
C. sativa x C. crenata	56	161.3 (155.5-170)	172 (162-180)
C. crenata	22	160.2 (152-165.5)	169.9 (162-178.5)
C. mollissima	20	159.8 (155.5-170)	173.2 (170-177)

Table 2: Date in Julian days of onset of full flowering for male catkins in 2018 in the INRAE chestnut collection, according to chestnut species.

To illustrate the dynamic of chestnut flowering, we provide an example of the flowering phenology of two trees from two different varieties in year 2018, illustrating the two peaks of male flowering in this duodichogamous species (Figure 3).

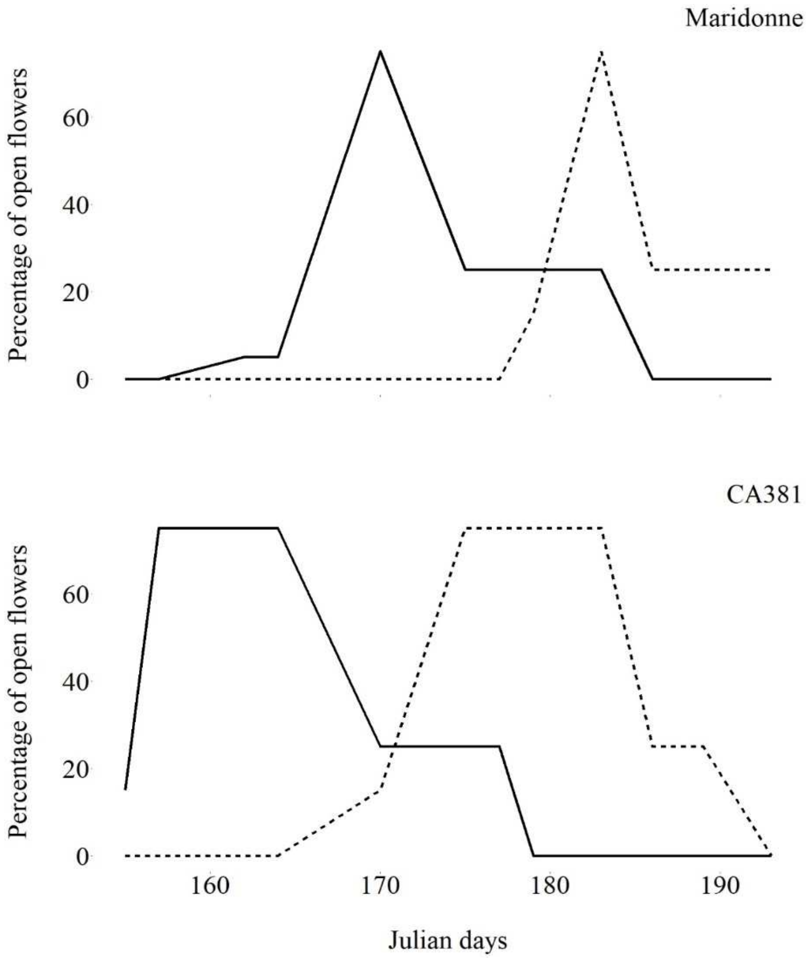

Figure 3: Male flowering phenology of two trees of the 'Maridonne' and CA381 varieties in year 2018. The x-axis is expressed in Julian days and the y-axis in percentage of open male flowers. Phenology of male flowers from unisexual catkins is represented by a continuous line and phenology of male flowers from bisexual catkins is represented by a dotted line. CA381 flowers two weeks earlier than Maridonne and its pollen emission lasts longer. Percentage of open flowers can be estimated from the BBCH scale, with the scores 60, 61, 62, 63, 64, 65 and 67 representing respectively roughly 5%, 15%, 25%, 35%, 45%, 75% and 25% of open flowers.

Discussion

In principle, to model accurately the phenology of a studied species, one should rely on a complete ontology of phenological traits to be measured exhaustively at the whole plant level (Stucky et al., 2018). However, this can quickly become time consuming. Diagnosing phenological stages using preestablished scores, as performed with the BBCH scale, is much faster, making it possible to monitor many more trees. We chose that latter strategy to compare varieties in orchards.

We successfully tested this new BBCH scale on trees from three chestnut species and their hybrids. Given the great homogeneity of the genus, it is likely that other chestnut species, including the American chestnut (*C. dentata*), can be scored with the proposed system. Furthermore, the proposed chestnut BBCH scale is suitable for both male-fertile and male-sterile trees. On male flowers from male-fertile trees, the stamens are conspicuous and easily scored. For male-sterile trees, the aborted stamens do not emerge out of the flower so a closer look on the opening of male flowers themselves is required.

The scoring of the phenology of female flowers can be adapted to meet different objectives. A simple approach is to monitor what happens at the scale of inflorescences, allowing comparison with Solignat and Chapa (1975) phenological stages. Instead, if the objective is to study the temporal compatibility between pollen emission and female flower receptivity, we recommend evaluating the percentage of receptive female flowers using class intervals. To investigate in even more details the mating system of the species, a more rigorous but more labor-intensive approach is to monitor and track individually a sample of flowers on each tree, as performed by Hasegawa et al. (2017).

To the best of our knowledge, this study is the first to use phenological stages based on the BBCH scale in chestnut. Despite some limitations inherent in the approach used, this scale allows a rapid semi-quantitative assessment of the growth stages of the three types of flowers found in this tree, making it possible to gather precious phenological knowledge on all chestnut species worldwide.

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Photo credits

Photography of Figure 1 and of the two insets of Figure 2 are by RJP, all others are by CL.

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