horticulturae

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Description of Phenological Events of Persian Walnut (*Juglans regia* L.) according to the Extended BBCH Scale and Historical Scales

Julie Robin [1], Anthony Bernard [2], Lisa Albouy [3], Sibylle Papillon [3], Eloise Tranchand [4], Marie-Neige Hebrard [4], Jean-Baptiste Philibert [5], Marine Barbedette [5], Sadia Schafleitner [6], Bénédicte Wenden [2], Teresa Barreneche [2], Marine Delmas [6], Fabrice Lheureux [7] and Julien Toillon [1,*]

[1] Association Nationale des Producteurs de Noisettes (ANPN), 1500 Route de Monbahus, F-47290 Cancon, France; jrobin@anpn.eu
[2] Institut National de Recherche pour l'Agriculture, l'alimentation et l'environnement (INRAE), Université de Bordeaux, UMR BFP, 71 Avenue Edouard Bourlaux, F-33882 Villenave d'Ornon, France; anthony.bernard@inrae.fr (A.B.); benedicte.wenden@inrae.fr (B.W.); teresa.barreneche@inrae.fr (T.B.)
[3] SCA Unicoque, Noisettes et Noix de France, 1500 Route de Monbahus, F-47290 Cancon, France; lalbouy@layton.com (L.A.); spapillon@koki.com (S.P.)
[4] Station Expérimentale de Creysse, Perrical, F-46600 Creysse, France; e.tranchand@stationcreysse.fr (E.T.); mn.hebrard@stationcreysse.fr (M.-N.H.)
[5] Station Expérimentale Nucicole Rhône-Alpes (SENuRA), 385A Route de Saint Marcelin, F-38160 Chatte, France; jbphilibert@senura.com (J.-B.P.); mbarbedette@senura.com (M.B.)
[6] Institut National de Recherche pour l'Agriculture, l'alimentation et l'environnement (INRAE), Unité Expérimentale Arboricole, Domaine de la Tour de Rance, F-47320 Bourran, France; sadia.schafleitner@inrae.fr (S.S.); marine.delmas@inrae.fr (M.D.)
[7] Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL), Centre Opérationnel de Lanxade, 28 Route des Nébouts, F-24130 Prigonrieux, France; fabrice.lheureux@ctifl.fr
* Correspondence: jtoillon@anpn.eu

Citation: Robin, J.; Bernard, A.; Albouy, L.; Papillon, S.; Tranchand, E.; Hebrard, M.-N.; Philibert, J.-B.; Barbedette, M.; Schafleitner, S.; Wenden, B.; et al. Description of Phenological Events of Persian Walnut (*Juglans regia* L.) according to the Extended BBCH Scale and Historical Scales. *Horticulturae* **2024**, *10*, 402. https://doi.org/10.3390/horticulturae10040402

Academic Editor: Daniela Farinelli

Received: 28 March 2024
Revised: 10 April 2024
Accepted: 15 April 2024
Published: 16 April 2024

Abstract: Walnut trees are grown worldwide for their edible fruits, which have high nutritional value. To address climate change, researchers have studied walnut phenology to create cultivars adapted to warmer climates. The objective of this study is to propose a scale for phenological Persian walnut observations using the Biologische Bundesanstalt, Bundessortenamt, und CHemische Industrie (BBCH) codification and alignment with historical alphameric scales. Here, the principal growth stages (PGSs) of Persian walnut (*Juglans regia* L.) are described using stages from a previously available alphanumeric scale. This standardised phenological scale describes Persian walnut growth from the dormant vegetative state through reproductive budding and senescence. This phenological scale is expected to increase the efficiency of walnut phenological monitoring. Fifty-seven stages are used to describe the life cycle of Persian walnut in this BBCH scale. Of these 57 stages, 3 stages are dedicated to seed germination (PGS-0), 4 stages are dedicated to bud development (PGS-0), 7 stages are dedicated to leaf development (PGS-1), 4 stages are dedicated to stem elongation (PGS-3), 8 stages are dedicated to inflorescence emergence (PGS-5), 5 stages are dedicated to male flowering (PGS-6), 5 stages are dedicated to female flowering (PGS-6), 5 stages are dedicated to fruit development (PGS-7), 12 stages are dedicated to fruit ripening (PGS-8), and 4 stages are dedicated to leaf senescence (PGS-9).

Keywords: English walnut; phenology; phenophase; growth stages; climate change; global warming

1. Introduction

Persian walnut (*Juglans regia* L.), also known as English walnut, is a widespread tree of the *Juglandaceae* family. *Juglandaceae* comprises more than 50 species in 11 genera such as *Carya* (hickories, including pecans), *Pterocarya* (wingnuts), and *Juglans* (walnuts) [1–3]. The *Juglans* genus includes more than 20 species, all of which are diploid ($2n = 2x = 32$), such as *J. cinerea* (white walnut), *J. nigra* (black walnut), and *J. regia* (Persian walnut) [4–6].

Persian walnut is disseminated worldwide in temperate regions (Europe, North and South America, South Africa, Asia, Australia, and New Zealand) and was domesticated in central Asia (western Himalayas). It first spread to the west (northern Iran, the Caucasus, and eastern Türkiye) and then spread to the east (northern India and western China) [7,8]. Persian walnut was present in refugia in the Balkans and western Europe during the last glacial period [9,10], and human-mediated dispersal is thought to have occurred in the Early Bronze Age.

Persian walnut is a wind-pollinated, deciduous, monoecious, and dichogamous tree; its dichogamy limits self-fertilisation [2]. Usually, more than one hundred flowers form the male catkin, whereas two or three flowers form the female inflorescence.

The study of changes in the timing of seasonal events such as plant flowering is called phenology [11]. There is a scientific consensus that phenology is dependent on environmental conditions and impacted by climate change, as is the case for most deciduous tree species [12,13]. In Persian walnut, Charrier et al. [14] showed that the winter dormancy from September through January in the northern hemisphere is mainly under environmental control. Climate change leads to phenological shifts that disrupt the whole growth cycle of trees. During spring, a warming climate increases the fulfilment of heat requirements and promotes earlier flowering and leaf unfolding [15,16], making trees more susceptible to late frost. However, during winter, a warming climate counteracts phenological advancement because it decreases the fulfilment of chilling requirements [17,18]. Fu et al. [19] showed that higher temperatures also delay autumn leaf senescence, and the consequence of the phenological shift is the amplification of drought stress because of the extended growing season [20].

In Persian walnut, these phenological shifts have been observed in Slovenia [21] and Romania [22]; moreover, Luedeling [23] confirmed using partial least square regression that the phenology of Persian walnut in California is advanced by high temperatures in spring and delayed by high temperatures in winter. Therefore, the phenology of dormancy in Persian walnut is impacted by both spring and winter temperatures, which makes its response to climate change difficult to model [24–26]. For instance, fruit winter chill modelling approaches are numerous and may perform poorly, so the quantification of chilling requirements may be imprecise [23,27].

Differences in chilling and heat requirements are observable between the genotypes of a species: genotypes with low chilling requirements can flower earlier and may suffer frost damages, whereas genotypes with high chilling requirements flower later and may suffer insufficient chilling fulfilment, leading to abnormal growth and harvest loss [28–31]. In Persian walnut, strong variability in chilling and heat requirement traits is observed in orchards and within herbarium accessions. In 2019 in France, Bernard et al. [32] observed a 71-day gap in the bud break dates between the earliest and latest accessions in the INRAE (Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement) walnut germplasm collection. In California, the germplasm collection of the Walnut Improvement Program of the University of California-Davis showed a 44-day gap in the leafing dates in 2018 and 2019 [33]. This research has led to the identification of Single-Nucleotide Polymorphism–phenological trait associations [33]. It is crucial to create a precise and globally accepted scale for phenological observations of Persian walnut to ensure the efficient characterisation of genotypes whose data can be reused in models and statistical analyses and to better manage orchards.

Based on a decimal code used in plant breeding by Zadoks et al. [34], the Biologische Bundesanstalt, Bundessortenamt, und CHemische Industrie (BBCH) scale was first developed for cereal crops to help standardisation [35]. Later, Hack et al. [36] developed an extended BBCH scale for other species such as pome and stone fruits. BBCH scales are already available for other nut species, such as almond [37], cashew [38], chestnut [39], hazelnut [40,41], and pecan [42]. However, a BBCH scale for Persian walnut is still missing. For Persian walnut, a BBCH codification for the flowering, fruit development, and ripening stages was recently presented during the IX International Symposium on Walnut and Pecan organized by the International Society of Horticultural Science (ISHS) [43].

To describe the phenological events of Persian walnut, Germain et al. [44] released the first scale that provides a basis for the International Plant Genetic Resources Institute (IPGRI) descriptors of walnut phenology-related traits [45]. This scale has been used by breeders and genetic resource curators around the world to characterise Persian walnut genotypes; for instance, the IPGRI scale has been used in Iran and India to describe the phenological characteristics of promising genotypes [46–48]. The objective of this study is to propose an extended BBCH scale for Persian walnut, adapted from the scales of Germain et al. and the IPGRI [44,45], and we provide pictures of the main stages useful for genotype characterisation. We expect this work to contribute to meeting the findable, accessible, interoperable, and reusable (FAIR) data principles and to contribute to better genetic resource management and crop practices.

2. Materials and Methods

2.1. Study Area

The phenological phases of Persian walnut were monitored in a varietal collection orchard composed of 10 cultivars (Table 1) and located in Agmé (59 m above sea level, 44.495210 N, 0.357202 E) in southwestern France in the department of Lot-et-Garonne. The trees were 11 years old. The soil type was a clay loam (20.5% sand, 41.4% silt, and 38.1% clay). The soil had a pH of 8.3, an organic matter content of 1.8 $g \cdot kg^{-1}$, and a C/N ratio of 9.2.

Table 1. List of cultivars planted in the varietal collection orchard.

Cultivar Name	Reference
Franquette	[2]
Ferbel	[3]
Lara	[2]
Livermore	[49]
Forde	[50]
Gillet	[51]
Durham	[52]
Tulare	[53]
Howard	[54]
Chandler	[54]

2.2. Monitoring of Phenophases and Fruit Growth Measurements

The phenological stages of flowering and leaf development were monitored weekly from April to September on 16 trees per cultivar. These observations were repeated in 2019, 2020, 2021, 2022, and 2023.

Fruit development was monitored weekly from early May to late August 2022 in 40 fruits on trees of the 'Lara' cv. The fruits were randomly chosen in the orchard. On each fruit, the length (from the basal scar to the apical scar) and the width were measured with an electronic calliper (MarCal 16 EWRi digital calliper IP66, Mahr, Göttingen, Germany). Then, the fruits were weighed with a precision balance (Mettler Toledo model MS-S/MS-L, Mettler Toledo SAS, Viroflay, France). Means (n = 40) are presented with their standard deviations. For lignification, the method described by Paradinas et al. [40] was used.

2.3. BBCH Scale Characteristics

The development of Persian walnut was described using main growth stages numbered from 0 to 9 according to the extended BBCH reference scale [36]. The numerical order of the main growth stages was respected in the first digit of this scale. The second digit of this scale described the plant or organ stage in further detail. To avoid errors during phenology monitoring, male flowering was described using a two-digit code, while female flowering was described using a three-digit code. As for hazelnuts [40], once the walnut husk and the shell were fully developed, a third digit was added to describe kernel development. For fruit development, a third digit was added to the two-digit scale of fruit ripening to detail the percentage of falling fruits at maturity.

3. Results

All stages presented in this section are described in Table 2 and illustrated in Figure 1.

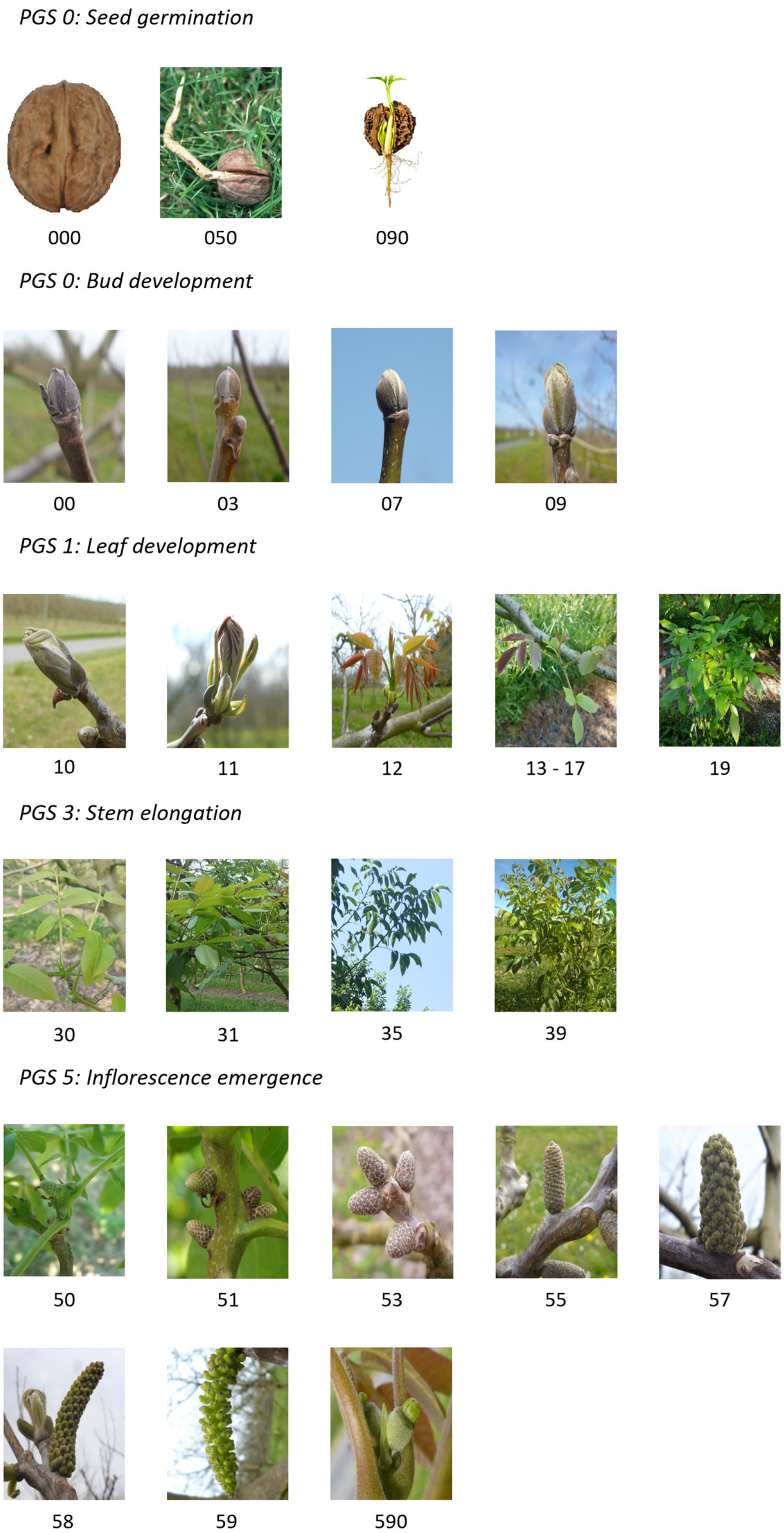

Figure 1. *Cont.*

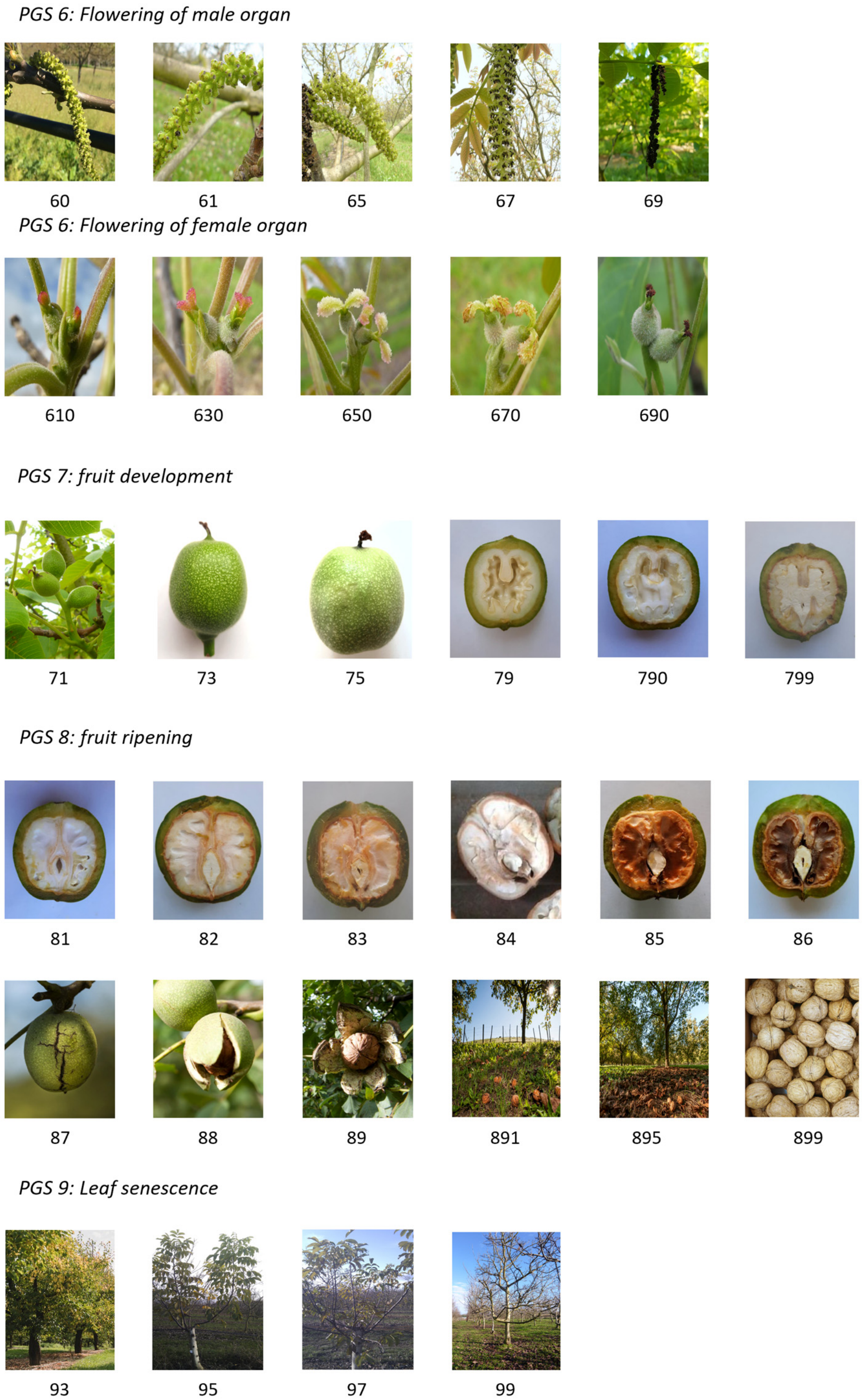

Figure 1. The phenological growth stages of Persian walnut according to the BBCH codification.

Table 2. Description of BBCH stages applied to Persian walnut.

BBCH Codification	Scale from Germain et al. [2]	IPGRI Scale [45]	Description
			Principal growth stage 0: seed germination
000			Dry seed
050			The radicle emerges from the seed.
090			Rootlet elongation, appearance of absorbent hairs and secondary root development, and soil emergence of the beetle

Table 2. *Cont.*

BBCH Codification	Scale from Germain et al. [2]	IPGRI Scale [45]	Description
		Principal growth stage 0: bud development	
00	Af		Dormant buds: scale-covered buds
03	Af2		Fall of the hard scales of the first order. Bud still enveloped by other poorly differentiated semi-membranous scales
07	Bf		The bud swells: The outer envelopes loosen, and the ends of underlying bracts covered with a whitish down appear. This is the so-called "woolly" or "white bud" stage.
09	Cf		The bud elongates, and the extremities of the terminal leaves of the outermost leaves can be distinguished. This is the bud burst.
		Principal growth stage 1: leaf development	
10	Cf2		The scales and bracts move apart, and the first leaves begin to separate.
11	Df		The bud is open, the first leaves separate, and their leaflets are well individualised.
12	Df2		The first leaves are completely unfolded. They are erect first, then take on a more or less oblique habit, revealing the female flowers at their centres.
13			First leaf fully developed, loss of red foliage colour
15			More than two leaves are fully developed with green foliage.
17			All leaves are fully expanded. They are growing and turn dark green.
19			All leaves are mature and have their final lengths.
		Principal growth stage 3: stem elongation	
30			Starting stem elongation
31			<10% of final stem length
33			>10% and <50% of final stem length
35			>50% and <90% of final stem length
39			>90% of final stem length
		Principal growth stage 5: inflorescence emergence	
50	Amr		In early summer, the differentiated male catkin, globose in shape, has a pinkish hue, while the buds remain green.
51	Amv		During the summer, the catkin grows slightly, becomes conical, reaches about 0.5 cm in length, and takes on a green colour.
53	Amg		At the beginning of October, the catkin stops growing. It measures 0.5–0.8 cm and takes on a grey colour that it will keep all winter.
55	Bm		About 3 weeks before the bud break, growth resumes. The catkin swells and lengthens to reach 1.3–2 cm in length.
57	Cm		The catkin, stiff and oblique, reaches the size of a pencil and measures 3–4 cm. Its colour gradually changes from green-brown to light green. The flower clusters are distinct.
58	Dm		The catkin loses its rigidity and becomes semi-drooping. The glomeruli separate.
59	Dm2		The glomeruli spread out and begin to open. The catkin hangs.
590	Ef		Appearance of female flowers
		Principal growth stage 6: flowering	
60	Em	First male bloom date	Complete opening of the glomeruli and separation of the anthers, which begin to turn yellow
61	Fm	Peak male bloom date	Beginning of anther dehiscence from base of catkin
65	Fm2	Peak male bloom date	Complete anther dehiscence and full pollen emission
67	Gm	Last male bloom date	The anthers are emptied of their pollen and turn black.
69	Hm		The catkin falls to the ground and dries up.
610	Ff	First female bloom date	Appearance of stigmata
630	Ff1	Peak female bloom date	The orange-yellow stigmata are divergent. Their receptivity is optimal. This is full female flowering.
650	FF2	Peak female bloom date	The stigmata take on a pale green-yellow colour and are completely recurved.
670	FF3	Last female bloom date	The stigmata begin to become necrotic and are streaked with fine brown threads.
690	Gf		Drying and blackening of the stigmata

Table 2. *Cont.*

BBCH Codification	Scale from Germain et al. [2]	IPGRI Scale [45]	Description
Principal growth stage 7: fruit development			
71			Beginning of fruit husk growth
75			Fruit husk is 50% of its final size.
79			Husk is 100% of its final size and lignification begins, beginning resistance to knives.
790			The shell is 100% lignified, and kernel filling begins.
799			The shell is 100% lignified, and kernel filling is completed.
Principal growth stage 8: fruit ripening			
81			White septum in kernel
82			Beginning of browning with some brown pitting on the septum
83			Internal septum is brown on 1/3 of its surface.
84		Packing-tissue-brown date	Internal septum is brown on 3/3 of its surface.
85			Septum is brown all over but damp and matte.
86			Shiny dry brown septum
87			Cracking of the husk
88			Opening of the husk: the nut remains trapped in the husk.
89			The husk opens enough for the nut to freely fall to the ground.
891		Harvest date	In total, <10% of the nuts have fallen to the ground.
895		Harvest date	In total, >50% of the nuts have fallen to the ground.
899		Harvest date	All nuts have fallen to the ground.
Principal growth stage 9: leaf senescence			
93			Beginning of leaf fall and increase in leaf discolouration
95			In total, 50% of the leaves have fallen.
97			In total, 70% of the leaves have fallen.
99		Defoliation date	All leaves have fallen.

3.1. Principal Growth Stage 0: Seed Germination

Principal growth stage 0 for seed germination is subdivided into three developmental stages and presented with three-digit codes to avoid ambiguity between seed germination and bud development, which is expressed with a two-digit code.

Stage 000, which corresponds to stage 89 of fruit ripening in this scale, represents the dry seed. When the radicle emerges from the seed, stage 050 is reached. Finally, stage 090 represents the beginning of root growth, when the rootlet elongates, absorbent hairs appear, secondary root development begins, and the shoot emerges from the seed and pierces the soil surface.

3.2. Principal Growth Stage 0: Bud Development

Principal growth stage 0 for bud development is subdivided into four developmental stages. Bud development stage 00, which was ‘Af’ in the Germain scale, is reached when buds are dormant and covered by scales. When the hard scales of the first order fall and buds are still enveloped by poorly differentiated semi-membranous scales, stage 03 is reached, which was historically described as stage Af2. Once the bud swells (stage Bf), the outer envelopes loosen and the ends of underlying bracts covered with whitish down appear. This is the so-called ‘woolly’ or ‘white bud’ stage described in this scale as stage 07. The final stage, initially described as stage Cf, represents bud elongation, when the extremity of the outermost leaves can be distinguished, also known as bud burst. Here, bud burst is described as stage 09.

3.3. Principal Growth Stage 1: Leaf Development

Principal growth stage 1 for leaf development is subdivided into seven developmental stages. Historically, three stages were used to describe leaf development. Stage Cf2, which represents the separation of the scales and bracts and the beginning of the separation of the first leaf, is described here as stage 10. When the bud is open, the first leaves are separated, and their leaflets are well individualised, stage 11 is reached, which was initially described as stage Df. Finally, stage 12 represents the point at which the first leaves are completely unfolded and erect and have taken on an oblique habit, revealing the female flower at their centre. Four new stages were added to the historical description. These are stage 13, which represents the point at which the first leaf is fully developed with a loss of red colour; stage 14, representing the point at which more than two leaves are fully developed with a green colour; stage 17, corresponding to the point at which all leaves are fully expanded and have a dark green colour; and stage 19, which represents the complete development of the leaves on the tree.

3.4. Principal Growth Stage 3: Stem Elongation

Principal growth stage 3 for stem elongation is subdivided into four developmental stages. The beginning of stem elongation is described as stage 30. This stage is reached when the stem has elongated to less than 10% of its final length. Stage 31 is reached when the stem has elongated to between 10% and 50% of its final length. When the stem has elongated to between 50% and 90% of its final length, stage 35 is reached. Finally, when the stem has elongated to more than 90% of its final length, stage 39 is reached.

3.5. Principal Growth Stage 5: Inflorescence Emergence

Principal growth stage 5 for inflorescence emergence is subdivided into seven developmental stages for male inflorescence emergence and one stage for female inflorescence emergence. These stages are an adaptation of the Germain et al. and IPGRI scale into BBCH codes. The first stage (stage 50), initially described as stage Amr, occurs in early summer. The differentiated male catkin, globose in shape, has a pinkish hue, while the buds remain green. Then, during summer, the catkin grows slightly, becomes conical, reaches about 0.5 cm in length, takes on a green colour, and reaches stage 51 (Amv). Stage 53 occurs at the beginning of October, when the catkin stops growing, measures 0.5–0.8 cm in length, and takes on a grey colour that will be kept during winter. About 3 weeks before bud break, growth resumes and the catkin swells, reaches 1.3–2 cm in length, and reaches stage 55 (Bm). When the catkin remains stiff and oblique, reaches the length of a pencil, and measures 3–4 cm, stage 57 is reached (Cm). Its colour gradually changes from green-brown to light green, and the flower clusters become distinct. Stage 58 (Dm) is reached when the catkin loses its rigidity and becomes semi-drooping and the glomeruli separate. When the glomeruli spread out and begin to open, the catkin hangs and reaches stage 59 (Dm2).

The appearance of the first female flowers is described here as stage 590. Generally, this stage occurs just after stage 12, when the first leaf is completely unfolded. This stage was historically named Ef.

3.6. Principal Growth Stage 6: Flowering

As Persian walnut develops male and female reproductive organs on separate flowers, two codifications of the developmental stages were developed here, a two-digit code for male flowering and a three-digit code for female flowering.

3.6.1. Male Flowering

Principal growth stage 6 for male flowering is subdivided into five developmental stages. The first male bloom, historically named Em, is described here as stage 60 and represents the complete opening of the glomeruli and the separation of the anthers, which begin to turn yellow. When male blooming peaks, the dehiscence of the anthers begins from the base of the catkin. This is stage 61 (Fm). Stage 65 represents full pollen emission and complete anther dehiscence, which was initially described as Fm2. When anthers that are emptied of their pollen turn black, stage 67 is reached. Stage 67 is also known as the late male bloom date or Gm. Finally, when the catkin falls to the ground and dries up, stage 69 is reached (Hm).

3.6.2. Female Flowering

Principal growth stage 6 for female flowering is subdivided into five developmental stages. The appearance of stigmata, initially described as stage Ff or 'first female bloom date,' is described here as stage 610. When orange-yellow stigmata are divergent, their receptivity is optimal. This stage is known as full female flowering. Full female flowering was previously described as stage Ff1 and is described here as stage 630. The next stage, described here as stage 650, occurs when stigmata take on a pale green-yellow colour and are completely recurved (Ff2). When the stigmata begin to become necrotic, they are streaked with fine brown threads and stage 670 is reached (Ff3). This stage is known as the last female bloom date. The last stage of female flowering, stage 690, occurs when the stigmata dry and blacken (Gf).

3.7. Principal Growth Stage 7: Fruit Development

Principal growth stage 7 for fruit development is subdivided into five developmental stages. The beginning of fruit husk growth is described here as stage 71. When the fruit husk has reached 50% of its final size, stage 75 is reached. Complete growth, that is, when the fruit husk has reached 100% of its final size and lignification begins, manifested by low resistance to a knife when the nut is cut, is described here as stage 79. When 100% of the shell is lignified and the kernel begins to fill, stage 790 is reached. When kernel filling is complete, stage 799 is reached. Persian walnut fruit development is illustrated in Figure 2. Fruit size (length and width) and weight change over time follow similar curves. These growth curves can be divided into two phases: a first phase that represents rapid fruit husk and shell growth between BBCH stages 71 and 79 and a second phase with slower fruit growth, during which the kernel grows, between BBCH stages 790 and 799.

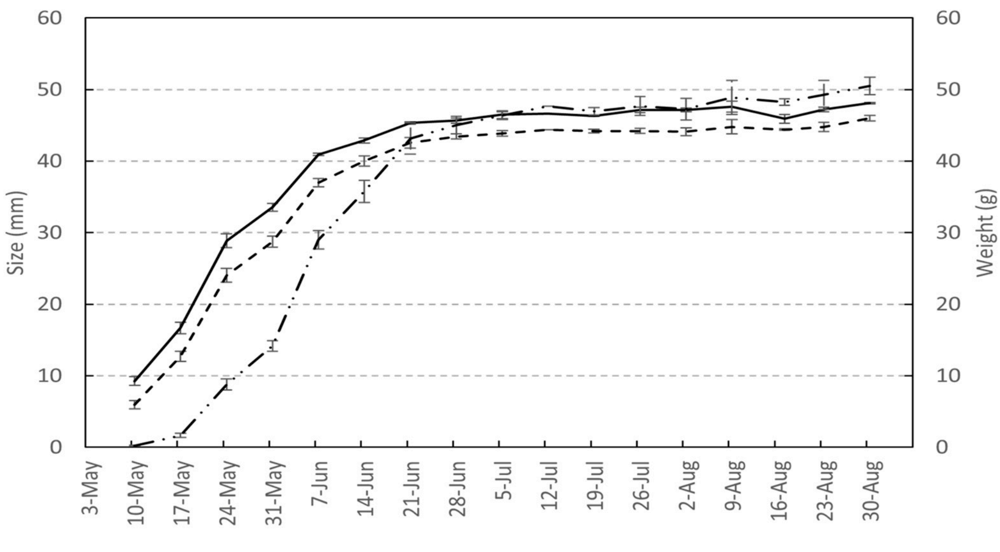

Figure 2. Growth dynamics of nut length (black line), width (dash line), and weight (dash–point line) of 'Lara' cultivar associated with their standard deviations of means.

3.8. Principal Growth Stage 8: Fruit Ripening

Principal growth stage 8 for fruit ripening is subdivided into 12 developmental stages. The first stage, described here as stage 81, is reached when the white septum is observable. When the septum begins to brown and develops some brown pitting, stage 82 is reached. Stage 83 occurs when one-third of the internal septum is brown. When the entire surface of the internal septum is brown, stage 84 is reached. This stage is known as the septum browning date. Stage 85 describes the point at which the septum is completely brown but still damp and matte. When the septum is shiny, dry, and brown, stage 86 is reached. Then, when the husk cracks, stage 87 is reached. The next stage, stage 88, is reached when the husk begins to open but the nuts remain trapped in the husk. When the nuts fall freely to the ground, stage 89 occurs. This stage can also be described as stage 000 if the BBCH code for seed germination is used.

The harvest date is subdivided into three stages: stage 891, which represents the point at which fewer than 10% of the nuts have fallen to the ground; stage 895, the point at which more than 50% of the nuts have fallen to the ground; and stage 899, the point at which 100% of the nuts have fallen to the ground.

3.9. Principal Growth Stage 9: Leaf Senescence

Principal growth stage 9 for leaf senescence is subdivided into four developmental stages. Stage 93 occurs when leaves begin to fall and the discolouration of leaves increases. When more than 50% of leaves have fallen, stage 95 is reached. Stage 97 is reached when 70% of leaves have fallen. Finally, when all leaves have fallen, the defoliation date is reached, which is described here as stage 99.

4. Discussion

The Persian walnut BBCH scale that is outlined in this paper uses 9 principal growth stages and 57 detailed stages to describe the developmental phenophases of Persian walnut. This level of detail is adequate to describe the Persian walnut growth stages. Moreover, this level of detail is in agreement with other BBCH scales of nut tree fruits that have been developed, such as the scales for hazelnut, with 53 stages [41]; chestnut, with 48 stages [39]; almond, with 39 stages [37]; and pecan, with 51 stages [42]. The BBCH scale outlined in Table 2 allows walnut phenological monitoring data that have been recorded in one of the three historically used scales to be translated into BBCH codes.

4.1. Flowering Phenophases

To develop a strictly numerical scale, male flowering is presented here using a two-digit code and female flowering is presented using a three-digit code using the same method that has previously been used for hazelnut [40,41]. This method has the advantage of preventing confusion between male and female flowering because Persian walnut can be protogynous, protandrous, or homogamous depending on the cultivar.

4.2. Fruit Development

The development of the fruit, that is, the nut, is described using an outline view. Fruit development is divided into two steps. The first step, concerning husk development, is described by stages 71 to 79 in this scale, and the second step, concerning shell development and lignification, is described by stages 790 to 799. Kernel development and ripening are described using stages 81 to 89. Nut fall is detailed using three-digit codes from the beginning of nut fall (stage 891) to the point at which all nuts have fallen to the ground (stage 899). Nut growth shows a sigmoidal curve, with a rapid growth phase from May to mid-June, a decrease in growth from mid-June to late June, and finally a halt in growth at the beginning of July. Nut weight shows a slight increase of 5 g during fruit ripening from the gelatinous phase of the kernel (stage 79) to the time when the nut is dry and rich in lipids and proteins (stage 899) [55].

4.3. Orchard and Research Implications of BBCH Codification

The BBCH scale that is outlined here will allow the standardisation of phenological stage descriptions between research teams and advisors. The use of numerical stages will simplify the use of phenological stages in models using phenological characteristics. In commercial orchards, the application of phytosanitary products is generally recommended for a specific BBCH stage that was not described for Persian walnut before 2023. Indeed, the first communication presenting a BBCH scale for Persian walnut was presented by Papillon et al. during the XI International Symposium on Walnut and Pecan in June 2023 hosted by the ISHS [43]. The detailed description of phenological events can help researchers and advisors in their comparison of cultivars or other data such as crop evapotranspiration collected in different years or locations. Indeed, as the BBCH codification is unique, the standardisation of stages helps to compare plant processes at the same physiological stages. For example, crop evapotranspiration differs from the early season to the full or late leafy season due to leaf physiology and climate.

5. Conclusions

The BBCH scale described here is the first to detail Persian walnut phenophases in 57 stages from seed germination to leaf fall. The two-digit and three-digit codes used to present male and female flowering provide the advantage of avoiding ambiguity during phenology monitoring. Finally, the use of strictly numerical codes provides an advantage when data are stored in computer files, including tables. The proposed phenological scale is expected to increase the efficiency of both worldwide germplasm characterisation and breeding and to provide help for future comprehensive studies of the adaptation of Persian walnut to climate change.

Author Contributions: Conceptualization, J.T.; methodology, J.T., J.R. and S.P.; validation, J.R., A.B., L.A., S.P., E.T., M.-N.H., J.-B.P., M.B., S.S., B.W., T.B., M.D., F.L. and J.T.; formal analysis, J.T. and L.A.; investigation, J.T., J.R., S.P. and L.A.; resources, J.T.; data curation, J.T. and L.A.; writing—original draft preparation, J.R., A.B. and J.T.; writing—review and editing, A.B. and J.T.; supervision, J.T.; project administration, J.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article. Additional data can be obtained by contacting the corresponding author of the article. The data are not publicly available due to the financial support of the members of the French National Association of Hazelnut Growers (ANPN) and the cooperative Unicoque.

Acknowledgments: The authors thank the three anonymous reviewers for their comments on a previous version of this manuscript. The authors thank the technical staffs of the Association Nationale des Producteurs de Noisettes (ANPN), Station Expérimentale de Creysse, Station Expérimentale Nucicole Rhône-Alpe (SENuRA), Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL), INRAE, and the cooperative Unicoque Noisette et Noix de France. The authors are specifically grateful to Charlène Thiriet for providing some of the pictures used to illustrate the scale.

Conflicts of Interest: The authors declare no conflicts of interest.

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