Scientia Horticulturae 181 (2015) 76–80

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Identification of phenological growth stages of sugar apple (*Annona squamosa* L.) using the extended BBCH-scale

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ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form 26 October 2014
Accepted 27 October 2014
Available online 22 November 2014

Keywords:
BBCH-scale
Annonaceae
Developmental stages
Phenology

ABSTRACT

Codes and detailed crop-specific descriptions are presented for the growth stages of the sugar apple tree (*Annona squamosa*) in southern China, which contributes to the standardization of national and international testing systems for fruit growth. The codes used in this work are a three-digit numerical system that describes the growth stages of the sugar apple tree as based on the extended *Biologische Bundesantalt, Bundessortenamt, and Chemische Industrie* (BBCH)-scale. A total of eight principal growth stages are described: (0) bud, (1) leaf, and (3) shoot development, (5) inflorescence emergence, (6) flowering, (7) fruit development, (8) fruit maturity, and (9) senescence. The use of the BBCH phenological scale presented in this work for application to *A. squamosa* will assist in the development and implementation of agronomic management protocols and be valuable for future research into any of the plant's developmental stages.

1. Introduction

Sugar apple (*Annona squamosa* L.), which a commercially important fruit tree, is commonly grown in subtropical areas and tropical areas. The fruit of the tree is widely known and consumed due to its medicinal and nutritional properties, as well as its pleasant flavor. The health related components of the fruit include vitamins A, B, C, E, and K1, antioxidants, polyunsaturated fatty acids, and the presence of essential minerals (Liu et al., 2013).

A. squamosa belongs to the family Annonaceae, in which there are about 129 genera and more than 2000 species. Most of the commonly grown species belong to the genus *Annona*, including *A. muricata* L., *A. squamosa* L., *Annona cherimola* Mill., and *A. cherimola* × *A. squamosa* (Nakasone and Paull, 1998). Among these species, *A. squamosa* is a commercially important subtropical and tropical fruit tree that is well adapted to the edaphoclimatic conditions of southern China, and that is also grown commercially in Africa, South America, Australia, India, Mexico, the southern United States, the Philippines, and Thailand (Crane et al., 2005; Pinto et al., 2005).

A. squamosa is a semi-deciduous subtropical tree (new buds cannot sprout until the leaves have been shed) that progressively sheds its leaves in the spring. Most often, all the leaves will be shed, but on occasions some might persist into the followings season. Under natural, non-managed conditions, it has been known to reach 4–5 m in height, with its typical sympodial branching. Leaves are pale green, 6.4–10.2 cm long, hairy when young but smooth at maturity, thin, and arranged alternately. Due to the leaf bases grow over the buds, they must be abscised before the buds can develop. Flowers emerge during mid- to late spring, as the trees are flush in new vegetative growth. They appear from the current season's growth at the leaf axils either singly or in groups of two to four. The flowers are composed of three green fleshy petals, three small inconspicuous sepals, and numerous pistils on a common receptacle. Flowering lasts 3–6 months, or even more in some cases, with heavy peaks. The aggregate fruit is nearly heart-shaped, round, ovate, or conical, and 5–10 cm in length. The fruit is composed of loosely cohering segments, which project as rounded protuberances and are easily separated when the fruit is ripe. The fruit does not change skin color, but it loses brightness while growing. There are numerous, small, shiny, dark brown seeds embedded in the pulp. The plant has a long fruiting season as not all the fruits ripen at the same time, which means that fruit can be harvest from between 3 and 6 months in a season lasting from midsummer to possibly midwinter if there are no frosts (Crane et al., 2005).

Phenology is a useful tool to better understand plant life cycles, and it has been studied in numerous herbaceous plants, such as rice, maize, sunflower, beans, and peas among others (Meier, 1997). Phenologically similar growth stages in plants can be described

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http://dx.doi.org/10.1016/j.scienta.2014.10.046
0304-4238/

using the extended *Biologische Bundesantalt*, *Bundessortenamt*, *and Chemische Industrie* (BBCH)-scale with its uniform coding and description system (Lancashire et al., 1991; Hack et al., 1992; Meier, 1997). This scale generally consists of 10 principal stages (0–9), which are further divided into 10 secondary (0–9) growth stages. For certain crops, this has been further extended to become a three-digit “extended BBCH-scale” (Finn et al., 2007; Hernández Delgado et al., 2011). So far, the BBCH-scale has been widely used with many crop species, such as cereals, colza, bean (Lancashire et al., 1991), beet (Meier et al., 1994), and potato (Hack et al., 1993). Fruit bearing plants, such as grapevine (Lorenz et al., 1994), loquat (Martinez-Calvo et al., 1999), guava (Salazar et al., 2006), kiwifruit (Salinero et al., 2009), mango (Hernández Delgado et al., 2011; Rajan et al., 2011), avocado (Alcaraz et al., 2013), cape gooseberry (Ramírez et al., 2013), and lychee (Wei et al., 2013) have been described in detail according to the BBCH-scale. Phenology has also been studied using landmark stages of vegetative growth and development (Ramírez et al., 2014).

Cautín and Agustí (2005) proposed a general two-digit BBCH-scale for *A. cherimola*, describing 7 princial stages. Although *A. squamosa* and *A. cherimola* all belong to the genus *Annona*, they are two different species. Moreover, they have much difference in most parts of life cycles. The codes describe growth stages in previous research focused on the phenological growth stages of the cherimoya were using a two-digit numerical system. This system based on the general BBCH-scale and identified the different stages of development with two digits; the first uses 10 principal growth stages (0–9) divided into 10 secondary (0–9) growth stages.

However, a three-digit numerical system based on the extended BBCH-scale has not yet been employed to specifically describe the development of *A. squamosa*. Therefore, the objective of this study was to describe the phenological stages of *A. squamosa* using the extended BBCH-scale, and the codes used in our work are a three-digit numerical system. This work will contribute to the standardization of phenology studies in this fruit and the quantitative analysis of sugar tree growth cycles.

2. Materials and methods

2.1. Plant material

Phenological data were collected from adult trees (10–12 years old) of *A. squamosa* cv. ‘Bendi’, which were planted in a 4 m × 4 m arrangement with drip irrigation and fertilizer applications as required. Trees were located at the Ling Nan Normal University (LNNU) field experimental station in Zhanjiang City (Guangdong Province, China) at 21°7′36″N latitude, 110°14′24″E longitude, and an altitude of 21.34 m above sea level. The area has a tropical oceanic monsoon climate with daily average, minimum, and maximum temperatures of 22.8 °C, 15.7 °C, and 28.8 °C, respectively, as well as a total yearly rainfall of 1100–1800 mm (Xiao et al., 2008).

Five healthy trees were randomly selected, and three branches from each tree were studied. A total of 80 buds located in 15 different branches were marked and measured. In addition, the predominant developmental stage was determined by monitoring all the trees in the orchard. Measurements and observations of vegetative and reproductive development were carried out two to three times per week from March to the following February. The developmental stage and morphological characteristics of each developing organ were recorded during two growing seasons (2012–2014). Photographs were taken of ideal examples of each organ, and these are used to illustrate the various phenological growth stages.

2.2. BBCH scale characteristics for A. squamosa

The proposed BBCH phenological scale for *A. squamosa* is represented by eight principal growth stages out of the ten possible stages. These principal stages begin with bud development (stage 0) and end with senescence (stage 9). Three principal growth stages are assigned to vegetative growth, which describes bud development (stage 0), leaf development on tree branches (stage 1), and shoot growth elongation on the branches (stage 3). Then two stages describe the flowering, which is divided into inflorescence emergence (stage 5) and flowering (stage 6). The other two stages are fruit development (stage 7) and fruit maturity (stage 8). Senescence (stage 9) completed the description. Development of rosette leaves (stage 2) and vegetative harvestable parts (stage 4) are not considered because they do not apply in *A. squamosa*.

The principle growth stages were divided into secondary stages corresponding to shorter developmental intervals that were linked to specific time points. The first digit of the code in the BBCH scale identifies the principal growth stage, which ranges from 0 to 9, whereas the second digit, which ranges from 1 to *n*, specifies the mesostages that occur between the principal and secondary growth stages. The third digit is a numerical value between 0 and 9 that reflects the percentage growth of the buds, leaves, shoots, flower buds, panicle, fruit development, fruit maturity, and senescence (Meier, 1997; Finn et al., 2007; Hernández Delgado et al., 2011).

3. Results

Description of phenological stages of *A. squamosa* (BBCH scale).

3.1. Principal growth stage 0: Bud development

3.1.1. First vegetative flush (mesostage 1)

010 Dormancy: buds are completely closed and covered by green-brown scales. A small ostiole (<2 mm in diameter) is visible (Fig. 1).

011 Beginning of bud swelling: buds start to swell and buds visibly swollen (Fig. 1).

013 End of leaf bud swelling: bud scales completely separated, lighter green sections of inner bud scales visible (Fig. 1).

017 Beginning of bud burst: green leaf tips start to become visible.

019 Bud burst: green shoot tips about 5 mm above bud scales are clearly visible (Fig. 1).

3.1.2. Second vegetative flush (mesostage 2)

020 Dormancy: foliar buds are completely closed and covered by brownish scales with no sign of growth.

021 Beginning of bud swelling: buds start to swell and buds visibly swollen.

023 End of leaf bud swelling: bud scales completely separated, lighter green sections of inner bud scales visible.

027 Beginning of bud burst: green leaf tips start to become visible.

029 Bud burst: green shoot tips about 5 mm above bud scales are clearly visible.

3.2. Principal growth stage 1: Leaf development

3.2.1. First vegetative flush (mesostage 1)

110 First leaves separate: green scales open slightly, and leaves emerging (Fig. 1).

111 First leaves unfolded: first leaves visible and spread away from the shoot (Fig. 1).

115 More leaves unfolded: petioles become visible (Fig. 1).

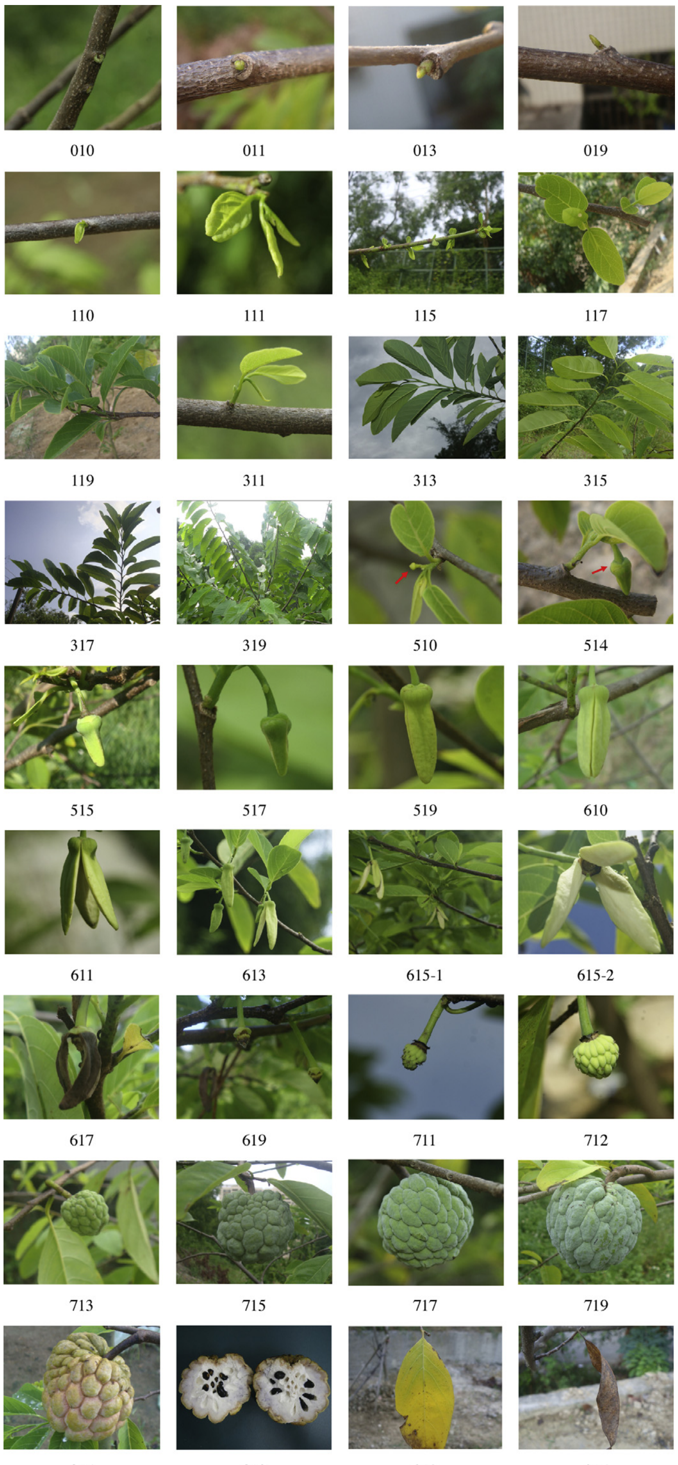

Fig. 1. Illustrations of the main phenological growth stages of sugar apple according to the extended BBCH-scale in southern China.

117 First leaves fully expand (Fig. 1).
119 All leaves unfolded and fully expand (Fig. 1).

3.2.2. *Second vegetative flush (mesostage 2)*

120 First leaves separate: green scales open slightly, and leaves emerging.
121 First leaves unfolded: first leaves visible and spread away from the shoot.
125 More leaves unfolded: petioles become visible.
127 First leaves fully expand.
129 All leaves unfolded and fully expand.

3.3. *Principal growth stage 3: Shoot development*

3.3.1. *First vegetative flush (mesostage 1)*

311 Beginning of shoot growth: axes of developing shoots become visible and are approximately 10% of final length (Fig. 1).
313 Shoots are about 30% of final length (Fig. 1).
315 Shoots are about 50% of final length (Fig. 1).
317 Shoots are about 70% of final length (Fig. 1).
319 Shoots are about 90% of final length (Fig. 1).

3.3.2. *Second vegetative flush (mesostage 2)*

321 Beginning of shoot growth: axes of developing shoots become visible and are approximately 10% of final length.
323 Shoots are about 30% of final length.
325 Shoots are about 50% of final length.
327 Shoots are about 70% of final length.
329 Shoots are about 90% of final length.

3.4. *Principal growth stage 5: Inflorescence emergence*

3.4.1. *Principal flowering (mesostage 1)*

510 Inflorescence bud swelling: buds closed and covered with faint yellow scales, with no peduncle (Fig. 1).
511 Beginning of bud swelling: scales begin to separate.
513 Bud burst: first floral primordia just visible.
514 Petals begin to elongate (Fig. 1).
515 Petals continue to elongate (Fig. 1).
517 Flowers still closed, but petals elongating (Fig. 1).
519 Most flowers closed, with petals fully elongated forming a long corolla (Fig. 1).

3.4.2. *Secondary flowering (mesostage 2)*

520 Inflorescence bud swelling: buds closed and covered with faint yellow scales, with no peduncle.
521 Beginning of bud swelling: scales begin to separate.
523 Bud burst: first floral primordia just visible.
524 Petals begin to elongate.
525 Petals continue to elongate.
527 Flowers still closed, but petals elongating.
529 Most flowers closed, with petals fully elongated forming a long corolla.

3.5. *Principal growth stage 6: Flowering*

3.5.1. *Principal flowering (mesostage 1)*

610 First flowers open: petals begin to separate (Fig. 1).
611 Flowers partially open (Fig. 1).
613 Early flowering: about 30% of flowers open (Fig. 1).
615 Full flowering: more than 50% of flowers open completely (Fig. 1).
617 Flower fading: majority of petals fall off or dry out (Fig. 1).
619 End of flowering: all petals have fallen off or dried out (Fig. 1).

3.5.2. *Secondary flowering (mesostage 2)*

620 First flowers open: petals begin to separate.
621 Flowers partially open.
623 Early flowering: about 30% of flowers open.
625 Full flowering: more than 50% of flowers open completely.
627 Flower fading: majority of petals fall off or dry out.
629 End of flowering: all petals have fallen off or dried out.

3.6. Principal growth stage 7: Fruit development

3.6.1. Main season fruit development (mesostage 1)

711 Fruit set: initial ovary growth (Fig. 1).

712 Fruit is about 10% of final size, already showing characteristic heart-shape, round, ovate, or conical, green pericarp of cultivar (Fig. 1).

713 Fruit is about 30% of final size (Fig. 1).

715 Fruit is about 50% of final size (Fig. 1).

717 Fruit is about 70% of final size (Fig. 1).

719 Fruit is about 90% of final size (Fig. 1).

3.6.2. Second season fruit development (mesostage 2)

721 Fruit set: initial ovary growth.

722 Fruit is about 10% of final size, already showing characteristic heart-shape, round, ovate, or conical, green pericarp of cultivar.

723 Fruit is about 30% of final size.

725 Fruit is about 50% of final size.

727 Fruit is about 70% of final size.

729 Fruit is about 90% of final size.

3.7. Principal growth stage 8: Maturity of fruit

3.7.1. Main season fruit development (mesostage 1)

811 Seeds reach their full size, harden and change color from white to brown, progressing through tan to dark brown.

815 Fruit ripe for consumption: fruit is at standard cultivar size, seed color becomes black, fruit appears plump and yellow green (Fig. 1).

819 Fruit fully ripe for consumption: fruit has typical taste and firmness (Fig. 1).

3.7.2. Second season fruit development (mesostage 2)

821 Seeds reach their full size, harden and change color from white to brown, progressing through tan to dark brown.

825 Fruit ripe for consumption: fruit is at standard cultivar size, seed color becomes black, fruit appears plump and yellow green.

829 Fruit fully ripe for consumption: fruit has typical taste and firmness.

3.8. Principal growth stage 9: Senescence

3.8.1. Principal vegetative flush (mesostage 1)

911: Shoot growth complete.

912: Start of senescence in old leaves; leaves start to fall (Fig. 1).

915: Leaves falling (Fig. 1).

917: Most leaves fallen.

3.8.2. Second vegetative flush (mesostage 2)

921: Shoot growth complete.

922: Start of senescence in old leaves; leaves start to fall.

925: Leaves falling.

927: Most leaves fallen.

4. Discussion

The phenology of a fruit tree is studied so that the periodicity of its life-cycle can be determined and used in agronomical management. The extended BBCH-scale can provide a more detailed and accurate description of the main phenological events than any of the other coding systems for *A. squamosa*. When using the extended BBCH-scale, *A. squamosa*'s life-cycle can be divided into eight main growth stages: bud, leaf, and shoot development, inflorescence emergence, flowering, fruit development, fruit maturity, and senescence.

Vegetative growth: in southern China, vegetative growth normally includes three processes of bud (010–019), leaf (110–119), and shoot (311–319) development, in a typical growth cycle of *A. squamosa*. In the spring, it is from the axillary buds that the new vegetative growth generally occurs. It takes 8–10 weeks form bud to shoot. The elongation of the vegetative shoots is continuous from the spring until the follow winter. And the vegetative stage lasts 24–28 weeks. Axillary bud break occurs in two ways: following leaf abscission; and during early shoot development, amongst the first few nodes, with the subtending leaves still attached. For the rest of the season, however, branch development is strictly linear. By the end of the season, branches can have extended 3 m or more away from the nearest branch junction (Olesen and Muldoon, 2009).

Flowering development: flowering in *Annona* was closely associated with vegetative flushing, with most flowers being produced on the basal nodes of newly emerging vegetative laterals (George and Nissen, 1987). Many of the researchers that study *A. squamosa* are focused on the flower induction and development. After flower initiation, the development of the flower (510–519) continues uninterrupted and leads to anthesis, which lasts about 3–4 weeks for *A. squamosa* cv. 'Bendi'. The female flowers of *A. squamosa* only have a sight opening of the petals and the surfaces of the stigmatic glisten (610–611). The male flowers were characterized by flower petals being wide open (613–615), which may easily fall when touched. Flowers that open under conditions of high humidity and warm temperatures are more likely to set fruit than the flowers that opening during low humidity and/or cool temperatures (Crane et al., 2005).

Fruit development*:* *A. squamosa* fruit maturation (811–819) occurs between July and February the following year. Previously it has been shown that the fruit of *A. sqamosa*, when artificially pollination had been performed, had good characteristics for consumption and a six day shelf life when harvested 104 days after pollination, but if harvested 108 days after pollination the fruit only had a three day shelf life (Pereira et al., 2010). The fruit is considered to be mature and at its harvesting point (815) when the skin changes color and when the segments spread far apart, exposing a creamy yellow skin (Salunkhe and Desai, 1984). Skin color can be used as a maturity index in sugar apples (Pereek et al., 2011).

An accurate understanding of these stages is very important for the correct timing of general orchard management. The management of *A. squamosa* orchards involves many horticultural practices (such as pruning, defoliation, fertirigation, pollination techniques, flush control, application of plant growth regulators, pesticide applications, and postharvest control), all of which require a good knowledge of the phenology to enable them to be performed at the optimal times.

In *Annona* species, pruning and defoliation are frequently used to give a second wave of flowering, which is used to improve the fruit set on the trees (Olesen and Muldoon, 2009, 2012). Therefore, considering the BBCH-scale presented, pruning and defoliation should be performed before the time of dormancy (stage 010).

Fertirrigation is commonly used in *A. squamosa* orchards, and it provides N, P, K, Ca, and Mg during the spring–summer period (Crane et al., 2005). In southern China, the availability of P and Ca is low due to the soils being acidic. The application of nutrients through fertirrigation starts approximately at stage 510, when buds are still closed, and further foliar applications of P, Ca, and K are usually done before anthesis (from stages 513–519) and after fruit set (from stages 711–713).

It is crucial for the production of sugar apples to be able to determine the developmental stage during the development of the floral bud and flower: this stage is when the pollination success can be enhanced and when the flowers and fruit need to be thinned to ensure optimal production. In general, pollen is collected in the late afternoon, early evening, and early morning from the stamens of flowers in the male stage. The collected flowers are placed on paper where the anthers (male flower parts) are allowed to dehisce (release pollen). The next morning, the pollen is mixed with talcum powder, to improve handling, and transferred to the flowers in the female stage of development (Crane et al., 2005; Orwa et al., 2009). Considering the BBCH-scale presented, pollination should be performed between stages 613 and 615.

A. squamosa can be affected by a number of pests and diseases (Orwa et al., 2009). Symptoms of insect damage include small, black holes on the fruit surface and brown to black decay of the fruit. Most damage is commonly observed between stage 811 and stage 819. The fruit can be protected from pests and diseases by bagging them in small paper lunch bags.

Although the extended BBCH-scale for *A. squamosa* can be used widely, yet there are some limitations of the BBCH-scale. At initial panicle or shoot development stage, the percent growth of vegetative shoots (311 to 315), inflorescence emergence (510 to 513) or fruit development stage (711 to 715) cannot be assigned/predicted precisely, thus some of the modifications are suggested to make it more useful for the users.

In conclusion, the use of the BBCH-scale presented here could help growers to enhance their yield and improve fruit quality despite of its limitations. In addition, it could also help minimize the use of pesticides and fertilizers, which would offer further economic and ecological benefits.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant no. 31201586); Science and Technology Program of Guangdong, China (grant no. 2013B020304008); Foundation for Distinguished Young Talents in Higher Education of Guangdong, China (grant no. LYM11087); National Sparking Plan Project of China (grant no. 2013GA780081, 2013GA780093); and the (Key) Project of Department of Education of Guangdong Province (grant no. 2013KJCX0124).

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