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Phenological characterization and temperature requirements of *Annona squamosa* L. in the Brazilian semiarid region

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ABSTRACT

The phenological growth stages of various species of monocotyledonous and dicotyledonous plants can be uniformly coded using the Biologische Bundesanstalt, Bundessortenamt, und Chemische Industrie (BBCH) scale. The aim of the present study was to determine the duration of different phenological stages and the temperature requirements of the sugar-apple, *Annona squamosa*, during two crop cycles in the semiarid region of Brazil. Phenological stages were divided into eight of ten possible principal stages: (0) bud development, (1) leaf development, (3) shoot/branch development, (5) inflorescence emergence, (6) flowering, (7) fruit development, (8) fruit maturity and (9) senescence and the beginning of dormancy. The phenological cycle of the sugar-apple from having closed leaf buds to the fruit ripening stage lasted 149 and 164 days with temperature requirements of 1684.5 and 1786.7 degree days (DD) for the first and second crop cycles, respectively. The results provided important information that will inform the correct timing for crop management practices.

Key words: BBCH, sugar-apple, degree days, phenology, pruning timing.

INTRODUCTION

Genus *Annona* includes more than 50 species and interspecific hybrids, most of which originate from tropical and subtropical regions of the Americas. *Annona squamosa* L. (sugar-apple) is the best known of the *Annonaceae* species grown in Brazil, and the Brazilian semiarid region is responsible for more than 90% of the sugar-apple production in the country.

The sugar-apple is a short (4 to 6 m high) and highly branched tree (Kiill and Costa 2003) with deciduous leaves, approximately 5 to 15 cm long and 2 to 6 cm wide, with a bright green upper surface and bluish-green lower surface (Manica 1994). Flowers form on new branches, from which they hang singly or in groups of two to four (Araújo-Filho et al. 1998). Flowers are hermaphroditic but exhibit

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dichogamous behavior, i.e., protogynous, with the female reproductive organ (gynoecium) reaching maturity before the male organ (androecium), so self-fertilization is difficult (Pinto and Silva 1994). The fruits are syncarpous, rounded, ovoid, spherical or cordiform, 5 to 13 cm in diameter and formed by very prominent carpels in most cultivars and selections. The skin of the fruit is usually green but can also be yellow or purple (Manica 2003).

Phenology is the study of periodical plant development events, how they are affected by environmental conditions, and their correlation with plant morphology. Studying the developmental stages of a given plant species elucidates the relationship between its morphological and physiological characteristics and environmental factors, especially climatic factors.

The accurate characterization of plant phenological stages has always been a challenge to the scientific community. In the 1970s, Zadoks et al. (1974) published the first decimal code to standardize the description of the homologous developmental stages of different crops, and Bleiholder et al. (1989) and Hack et al. (1992) later developed the Biologische Bundesanstalt, Bundessortenamt, und Chemische Industrie (BBCH) scale, a system for uniformly coding phenologically similar growth stages among all monocotyledonous and dicotyledonous plant species.

The BBCH scale has been used to uniformly code the phenological growth stages of different fruiting species, namely, grapevine (Lorenz et al. 1994) and pear (Martinez-Calvo et al. 1999), persimmon (García-Carbonell et al. 2002), apricot (Pérez-Pastor et al. 2004), guava (Salazar et al. 2006), kiwi (Salinero et al. 2009) and mango (Hernández-Delgado et al. 2011) trees.

The BBCH scale has also been used in two studies of *Annona* species. A two-digit scale describing seven principal growth stages was proposed for *A. cherimola* Mill. (Cautín and Agustí 2005), and a three-digit scale describing eight principal growth stages was proposed for *A. squamosa* L. (Liu et al. 2014).

Temperature is one of the most important climatic factors affecting plant growth rates (Stewart et al. 1998), and its effects on plant development may be estimated using heat sums, such as degree-days (DD) (Warrington and Kanemasu 1983).

Calculating the accumulated DD (ADD) is very important for determining the duration of crop phenological cycles because variations that depend on meteorological conditions may only be observed if the temporal scale is considered (Oliveira et al. 2012). In addition, ADD are an important tool for decreasing climatic risks because knowing the temperature requirements of a given crop contributes to the planning of adequate crop management practices and can be used to determine the climatic potential of a region for improved crop production.

Although the BBCH scale has been previously applied to *A. squamosa*, that study was performed in a region located in the Northern hemisphere and employed a three-digit numerical system. The aim of the present study was to apply the BBCH scale to sugar-apple using a two-digit numerical system and to determine the temperature requirements of the plant for two crop cycles under irrigated conditions in the Brazilian semiarid region.

MATERIALS AND METHODS

CHARACTERIZATION OF THE EXPERIMENTAL AREA AND PLANT MATERIAL

The experiment was performed in a commercial sugar-apple orchard with adult plants (seven years old) spaced 4 x 4 m apart. The orchard is located in the municipality of Janaúba, Minas Gerais, Brazil at 15°47’50”S latitude and 43°18’31”W longitude and an altitude of 516 m. The region’s climate is Aw (Köppen), i.e., a typical savanna with dry winters and a mean air temperature in the coldest month above 18°C; the annual mean temperature is approximately 23.7°C. The soil is a clayey Eutrophic Red Latosol (Embrapa 2006). Irrigation was applied by microsprinkler, and crop handling was performed according to crop requirements (Pereira et al. 2011).

Plants were pruned twice during the year on 08/22/2013 (winter) and 03/28/2014 (autumn), and in addition to pruning, manual deleafing was performed to stimulate the vegetative growth of buds in the leaf axils. Ten uniform, healthy and vigorous plants were selected. Four branches were labeled in each plant, one in each quadrant (north, south, east and west), and an intermediate bud was selected from each branch.

CHARACTERIZATION USING THE BBCH SCALE AND DETERMINATION OF TEMPERATURE REQUIREMENTS

Phenology and temperature requirements were determined in two consecutive harvests (two crop cycles) using the same plants in the experimental area. The main meteorological data were monitored throughout the experiment (August 2013 to September 2014) (Figure 1).

After pruning, phenological stages were characterized through visual observations two to three times per week from stage 00 until the beginning of stage 91. Phenological behavior was evaluated through photographs and the dates of observation, and the phenological stages were classified based on the BBCH scale (Hack et al. 1992). A two-digit numerical system was used with the first digit corresponding to the principal growth stages and the second to the secondary growth stages. In addition, the durations of the principal growth stages over the two crop cycles were calculated.

To quantify plant temperature requirements, the heat sum in DD was determined for the two crop cycles for the following phenological sub-stages: (1) stages 00 to 09: closed leaf buds to visible, 5-mm leaf tips; (2) stages 10 to 19: first leaves to fully expanded first leaves; (3) stages 31 to 39: shoot at 10% of the final length to shoot at final length; (4) stages 50 to 59: floral buds closed and covered with light scales to closed flowers with petals forming a long corolla; (5) stages 60 to 69: beginning of petal opening to end of flowering; (6) stages 71 to 79: fruit development, i.e., the beginning of ovary growth to fruit of characteristic size for the species; and (7) stages 81 to 89: appearance of mature fruit color to full fruit ripening.

Degree-days were calculated following Villa-Nova et al. (1972) for a base temperature of 10°C (Da Silva et al. 2006).

$$DD = (Tm - Tb) + (TM - Tm) / 2, \text{ when } Tm > Tb;$$

$$DD = (TM - Tb)2 / 2(TM - Tm), \text{ when } Tm < Tb;$$

$$DD = 0, \text{ when } Tb > TM$$

where DD = degree days; TM = maximum daily temperature (°C); Tm = minimum daily temperature (°C); and Tb = base temperature (°C).

RESULTS

A detailed description of the principal sugar-apple growth stages in the Brazilian semiarid region was obtained using the BBCH scale with a two-digit numerical system for eight out of ten possible principal growth stages (Figures 2, 3 and 4).

PRINCIPAL GROWTH STAGE 0: BUD DEVELOPMENT

- 00: Buds closed and covered by brown scales (CLB).
- 01: Bud swelling, beginning of leaf bud expansion.
- 03: End of leaf bud expansion, brown scales slightly separated.

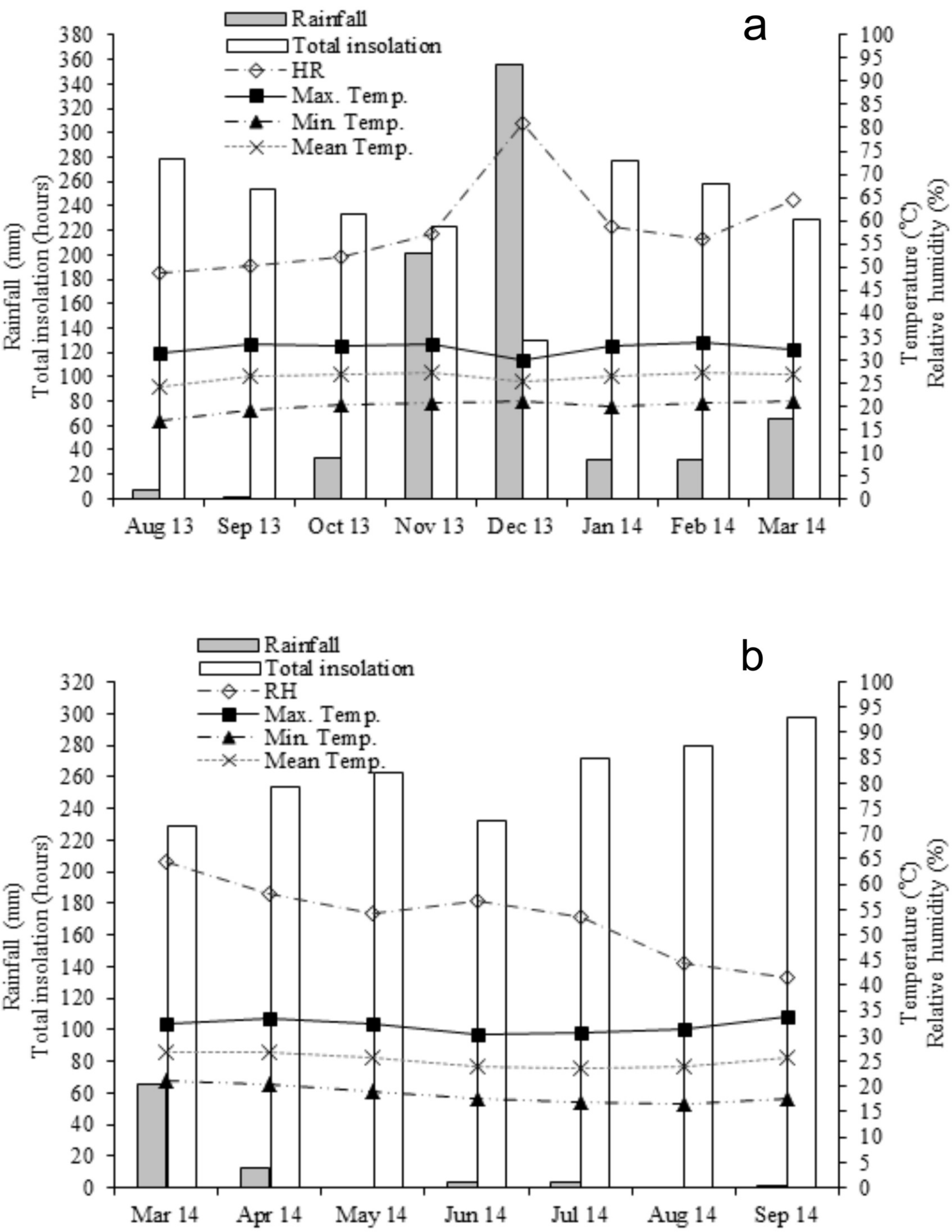

Figure 1 - Rainfall, total insolation, relative humidity and maximum, minimum and mean temperature during the (**a**) first and (**b**) second sugar-apple (*A. squamosa* L.) crop cycles in Janaúba, Minas Gerais, Brazil, 2013/2014.

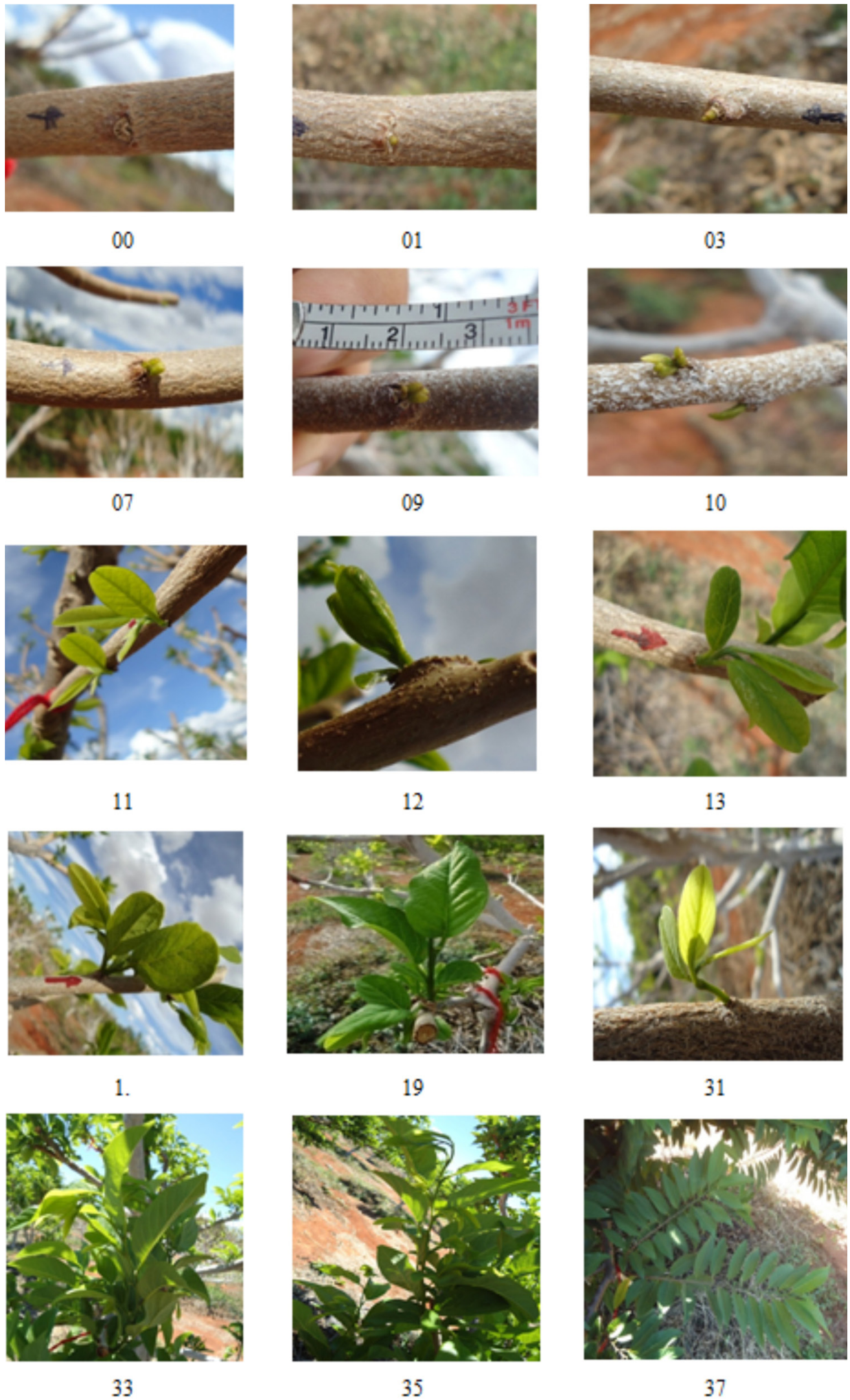

Figure 2 - Principal growth stages (00-37) of sugar-apple (*A. squamosa* L.) as described by the BBCH scale in Janaúba, Minas Gerais, Brazil.

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

09: Green leaf tips of approximately 5 mm in size emerging from bud scales (LT5).

PRINCIPAL GROWTH STAGE 1: LEAF DEVELOPMENT

10: First leaves separating and green scales slightly open (SFL).

11: First leaves visible and unfolded.

12: Second leaf emergence, not yet fully expanded.

13: Third leaf emergence, not yet fully expanded.

1.: Continuation of leaf growth.

19: First leaves fully expanded (EFL).

PRINCIPAL GROWTH STAGE 3: SHOOT/BRANCH DEVELOPMENT

31: Beginning of shoot growth; developing shoots visible at 10% of the final length (S10L).

33: Shoot at 30% of final length.

35: Shoot at 50% of final length.

37: Shoot at 70% of final length.

38: Shoot at 80% of final length.

39: Shoot at final length, i.e., the maximum length for the species (SFL).

PRINCIPAL GROWTH STAGE 5: INFLORESCENCE EMERGENCE

50: Floral buds closed and covered by whitish-green scales (CFB).

51: Beginning of floral bud swelling.

53: Bud burst, first floral tips visible.

54: Sepals visible, and petals begin to elongate.

55: Petal elongation.

57: Flowers closed, petals elongating.

59: Flowers closed with fully elongated petals forming a long corolla (FCPL).

PRINCIPAL GROWTH STAGE 6: FLOWERING

60: First flowers open: pre-female stage (BPO).

61: Beginning of flowering: female stage and pollination.

65: Full flowering, 50% of flowers open: male stage.

67: Flowers fading, majority of petals fallen or dry.

69: End of flowering, fructification visible and all petals fallen or dry (EF).

PRINCIPAL GROWTH STAGE 7: FRUIT DEVELOPMENT

71: Beginning of ovary growth; ovaries green, sometimes with dry petals (FOG).

72: Fruits approximately 20% of final size.

73: Fruits approximately 30% of final size.

74: Fruits approximately 40% of final size.

75: Fruits approximately 50% of final size.

76: Fruits approximately 60% of final size.

77: Fruits approximately 70% of final size.

78: Fruits approximately 80% of final size.

79: Fruits with final size characteristic of the species: physiological maturity (harvest) (IRH).

PRINCIPAL GROWTH STAGE 8: FRUIT MATURATION

81: Fruit color appears; beginning of carpel separation and intercarpel tissue yellowish-green (harvest) (BFC).

89: Fruits ripe and detach easily (FRC).

PRINCIPAL GROWTH STAGE 9: SENESCENCE AND BEGINNING OF DORMANCY

91: Branches fully grown, and foliage remains green.

The number of days and temperature requirements necessary to complete each phenological sub-stage differed between the two pruning times (Table I).

Figure 3 - Principal growth stages (38-71) of sugar-apple (*A. squamosa* L.) as described by the BBCH scale in Janaúba, Minas Gerais, Brazil.

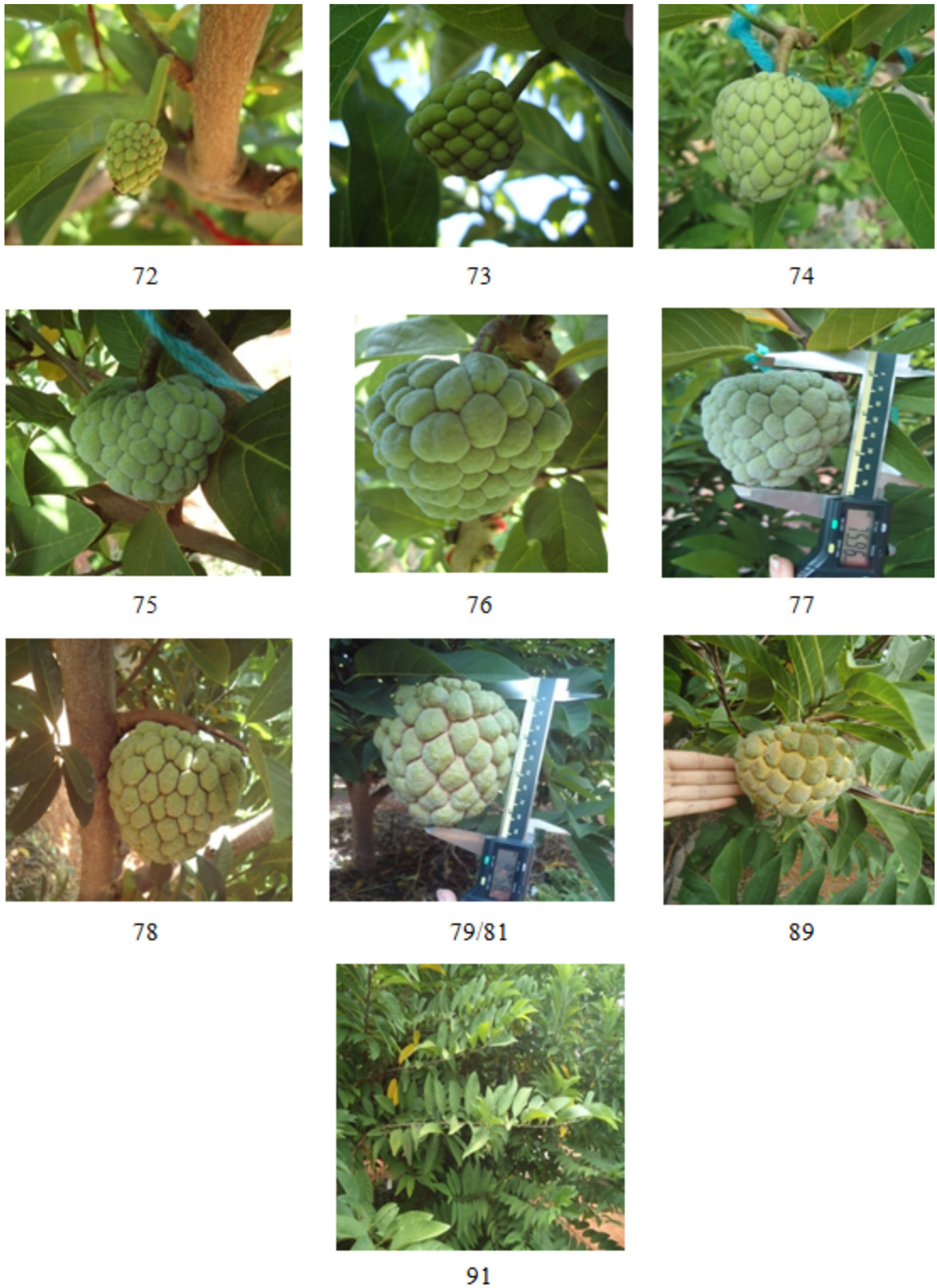

Figure 4 - Principal growth stages (72-91) of sugar-apple (*A. squamosa* L.) as described by the BBCH scale in Janaúba, Minas Gerais, Brazil.

For the first crop cycle, for which pruning was performed on 08/22/2013, the duration of each sub-stage and the respective temperature requirements were 25 days and 282.7 DD for CLB-LT5; 10 days and 121.5 DD for SFL-EFL; 166 days and 1905.4 DD for S10L-SFL; 25 days and 315.4 DD for CFB-FCPL; 20 days and 230.2 DD for BPO-EF; 99 days and 1116.2 DD for FOG-IRH; and 21 days and 252.6 DD for BFC-FRC. The period from pruning until the end of flowering lasted 59 days with a temperature requirement of 678.3 ADD, and the time from pruning until full fruit ripening lasted 156 days with a temperature requirement of 1764.3 ADD (Table I).

For the second crop cycle, for which pruning was performed on 03/28/2014, the duration of each sub-stage and respective temperature requirements were 28 days and 346.4 DD for CLB-LT5, 24 days and 282.5 DD for SFL-EFL, 162 days and 1764.8 DD for S10L-SFL, 36 days and 409.7 DD for CFB-FCPL, 32 days and 368.15 DD for BPO-EF, 115 days and 1231.3 DD for FOG-IRH, and 27 days and 316.6 DD for BFC-FRC. The period from pruning until the end of flowering lasted 70 days with a temperature requirement of 803.8 ADD, and the time from pruning until full fruit ripening lasted 171 days with a temperature requirement of 1863.3 ADD (Table I).

DISCUSSION

In the present study in the Brazilian semiarid region, *A. squamosa* was observed to present eight out of the ten principal growth stages described in the BBCH scale. The tillering stage (stage 2) and the development of vegetatively propagated organs (stage 4) were not observed.

This result is consistent with Liu et al. (2014), who applied the BBCH scale with a three-digit numerical system to sugar-apple in a similar experiment in Southern China and described only eight of the ten principal growth stages; stages 2 and 4 were also not observed.

The vegetative growth of sugar-apple in the semiarid region included bud development (00-09),

TABLE I
Duration (days) and temperature requirements (degree-days) for the phenological sub-stages of sugar-apple following different pruning times (1 and 2) in Janaúba, Minas Gerais, Brazil, 2013/2014.

Phenological sub-stages		Pruning times							
		(1) 08/22/2013				(2) 03/28/2014			
Beginning	End	ND	AND	DD	ADD	ND	AND	DD	ADD
CLB	LT5	25	25	282.7	282.7	28	28	346.4	346.4
SFL	EFL	10	27	121.5	306.8	24	38	282.5	446.5
S10L	SFL	166	191	1905.4	2175.8	162	183	1764.8	2013.7
CFB	FCPL	25	47	315.5	553.3	36	55	409.7	634.8
BPO	EF	20	59	230.2	678.3	32	70	368.15	803.8
FOG	IRH	99	149	1116.2	1684.5	115	164	1231.3	1786.7
BFC	FRC	21	156	252.6	1764.3	27	171	316.6	1863.3

ND: number of days; AND: accumulated number of days following pruning; DD: degree-days; ADD: accumulated degree-days following pruning; CLB: closed leaf buds; LT5: leaf tips at 5 mm; SFL: separation of first leaves; EFL: first leaves fully expanded; S10L: shoots at 10% of final length; SFL: shoots at final length; CFB: floral buds closed and covered with light-colored scales; FCPL: flowers closed, petals forming long corolla; BPO: beginning of petal opening, pre-female stage; EF: end of flowering; FOG: fructification, beginning of ovary growth; IRH: fruit at final size typical of species, ideal ripeness for harvest; BFC: appearance of mature fruit color, harvest; FRC: fruit ripe for consumption.

leaf development (10-19), shoot development (31-39) and the end of shoot growth (91). Shoot growth varied with the pruning time; the final shoot length (39) was reached at 142 days, and shoot growth ended at 191 days. For the second crop cycle, final shoot length started to be reached at 92 days, and shoot growth ended at 183 days.

The observed variations in vegetative plant growth between the two crop cycles can be partly explained by the climatic conditions during the crop cycle; temperatures were milder and rainfall was lower during the second crop cycle (Figure 1). Decreases in shoot vegetative growth are common during periods of lower temperatures with branches reaching final lengths up to 40 cm, but if plants are subjected to higher temperatures and higher rainfall, branches can reach between 70 and 100 cm (Santos et al. 2014, São-José et al. 2014).

For reproductive development, the stages of inflorescence emergence (50-59), flowering (60-69), fruit development (71-79) and fruit ripening with color change (81 and 89) were described. During flowering, protogynous dichogamy was observed with the maturation of female and male organs separated in time. Flowers presented a pre-female (60) and a female (61) stage, during which the stigma was receptive and ideally suited to artificial pollination. Flowers reached the functional pistillate phase at stage 65, when pollen was released.

Differences in the reproductive stages were also observed between the two crop cycles. For the first crop cycle, a 41-day interval was observed between pruning and pollination (61), and there were 94 days between pollination and fruit ripening (81) for a total of 135 days from pruning to harvest. For the second crop cycle, an interval of 39 days was observed between pruning and pollination, and there were 105 days between pollination and fruit ripening for a total of 144 days from pruning to harvest.

The nine-day increase in the period between pruning and harvest from the first to the second crop cycle was likely due to the higher temperatures in the first crop cycle. This has been reported previously, and temperature is considered the determining factor in the speed of reproductive development in *A. squamosa* (A.S. Nogueira, unpublished data, Dias et al. 2003).

Fruits were considered physiologically mature and ready for harvest at stage 81. Sugar-apple fruits should be harvested all at once, and the time of harvest is determined by the separation of carpels and the yellow-green color of the intercarpel tissue (Araújo-Filho et al. 1998). Characteristics similar to those in stage 81 were also observed in stage 79 with fruits reaching the characteristic size for the species, the occurrence of color changes and the separation of carpels. Under the edaphoclimatic and geographical conditions evaluated in the present study, sugar-apple may be harvested from stages 79 to 81, which corresponded to 135 days after pruning for the first crop cycle and 144 days for the second crop cycle.

Commercial harvest is performed when fruit maturation is complete, which occurred at 149 and 164 days for the first and second crop cycles, respectively, so it is possible, through adequate crop management and irrigation, to produce two annual harvests in the study region. Fruit ripe for consumption (stage 89) was characterized by fruit flavor and firmness typical for the species.

Except for the sub period from pruning until budding, the second crop cycle presented overall higher temperature requirements (DD) and stage durations (number of days). The DD concept was introduced to overcome the inadequacies of the conventional calendar, allowing the phenological events of crops to be predicted. Temperature accumulation is widely used to determine the duration of phenological events and the adaptability of hybrids to certain geographical locations (Warrington and Kanemasu 1983).

Comparing the results of the present study to those of Liu et al. (2014) and accounting for the relevant differences in geographical and climatic factors and the use of different numerical systems for the same scale, both studies obtained very similar results. Although the two studies were performed in different hemispheres, sugar-apple presented the same phenological development profile, which indicates strong genetic determination of the characters associated with the different phenological stages and can be very important for the development of highly adaptive cultivars.

Understanding the phenology of a given species and determining its temperature requirements are essential for developing management practices to expand commercial cultivation. The integrated use of this information will increase temporal accuracy and consequently improve the efficiency of pruning, fertilization, plant health management, pollination and harvest of sugar-apple. The dissemination of these studies will also allow for producers to better organize labor and plan intercropping seasons with the goals of decreasing production costs, mitigating environmental impacts and increasing the rentability of agricultural activities.

CONCLUSIONS

The BBCH scale can be used to identify the phenological stages of sugar-apple by dividing them into eight out of ten possible principal stages.

The phenological cycle of sugar-apple from the closed leaf buds to the fruit ripening stage lasted 149 and 164 days with temperature requirements of 1684.5 and 1786.7 DDs for the first (end of winter) and second (mid-autumn) crop cycles, respectively, under irrigated conditions in the Brazilian semiarid region.

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