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Science and Technology
Meri-Rastilantie 3 B, FI-00980
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Journal of Food, Agriculture & Environment Vol.11 (3&4): 1323-1327. 2013 www.world-food.net

Phenological growth and development stages of the native Patagonian fruit species *Berberis buxifolia* Lam.

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Received 18 July 2013, accepted 20 October 2013.

Abstract

This work studied the phenological growth and development stages of *Berberis buxifolia*, a native Patagonian species, whose purple berries are of economic value. Data were recorded from a population of *B. buxifolia* adult plants, growing naturally near Ushuaia city, 54º48´SL, 68º19´WL (Tierra del Fuego, Argentina). The BBCH-scale has been adopted in this study, and the phenological stages were coded using two digits. Nine principal growth and development stages were recognized for the phenological description of *B. buxifolia*, starting with vegetative and mixed bud development (stage 0). The following four of them were assigned to the vegetative growth, which described leaf development on shoots (stage 1), shoot elongation (stage 3), development of plant propagation organs (stage 4) and partial senescence, beginning of dormancy (stage 9). The last four principal growth stages describe flower emergence (stage 5), flowering (stage 6), fruit development (stage 7) and maturity of fruit (stage 8).

Key words: BBCH-growth stages, morphology, calafate, barberry, Argentina.

Introduction

Different aerial vegetative and reproductive organs present throughout the growing cycle of a plant show different aspects according to the season. These changes are called phenological phases and the study of the succession in time of these phases is called phenology [1], which is closely regulated by climate and seasonal changes. Precise knowledge of each stage of a species can anticipate possible adverse effects caused by weather or erroneous crop handling. In addition, phenological stages can predict a likely pest outbreak, the need for a specific fertilisation, the application of a hormonal product, etc. [2]. Phenology is not a new science. The best known information comes from Robert Marsham who began recording phenological stages of species during spring and special events, back in 1736 on his family estate near Norwich, Norfolk England. These data provided unique records on spring evolution of that period and how they related to the weather, showing us just how responsive spring events are to temperature, and warning us that not all species respond at the same rate [3]. Such is the importance of phenological records in the USA that a calendar was created. William Felker has studied phenology in Ohio since 1973 and now he publishes the "Poor Will's Almanack", a phenological calendar for farmers (http://www.poorwillsalmanack.com).

In spite of the well known importance of wild flora as source of food and medicinal substances, only a few species have been evaluated for their agronomic and curative potential [4]. Hence, more studies on wild flora diversity and on the agronomic and therapeutic latent value of these plant species are still needed [5]. Areas with indigenous flora offer non-domesticated plants [6] like *Berberis* L. genus in Patagonia [7]. *Berberis* L. is well represented by 16 species of native evergreen or semi-evergreen shrubs [8], with a large distribution in Argentina from Neuquén to Tierra del Fuego [9]. Species of this genus belong to the so-called group of minor or under-utilized fruit tree species that are of relevant value for diversification of agrofood production. At present, commercial *Berberis* orchards are being planned: this crop has economic potential, not only for flavour and taste of the fruits, but also because of its putative high antioxidant properties. In fact, the black-blue berries are now consumed fresh, in marmalades and jams, in non-alcoholic beverages and in ice creams [10, 11]. *B. buxifolia* Lam., commonly called "calafate" in Spanish and barberry in English, is an evergreen shrub that may be semi-evergreen where winters are particularly cold and harsh, as occurs in Tierra del Fuego. It is a spiny and erect shrub up to 4 m high, often growing in coastal scrub, *Nothofagus* forest margins and clearings, moister areas in grass steppes, and along streams and rivers [12]. *B. buxifolia* is considered a non timber forest product [13] that can be propagated through seeds, rhizomes and *in vitro* culture [10]. Shoot growth and fruit production were studied in a natural population of this species [14], and recently floral biology, fruit development and the assessment of morphological variability were described [15]. No specific description of the phenological stages of the whole biological cycle is available for *B. buxifolia*, and until now floral phases have been defined using the Fleckinger scale [16]. Therefore, it is important to define comprehensively the bud, leaf, shoot, flower and fruit development processes for this species. In this paper we present the BBCH-scale (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) for the description of the phenological growth of *B. buxifolia* "calafate" according to the growth stage identification keys for monocotyledonous and dicotyledonous plants [17].

Materials and Methods

Location and plant material: Data were recorded from a population of *Berberis buxifolia* adult plants (n = 20) growing naturally in a representative area located near Ushuaia city, 54º48´SL, 68º19´WL (Tierra del Fuego, Argentina). Even though the phenological phases have been registered asynchronously over the last 15 years, most of the data and photographs presented in this work were taken during the 2010 and 2011 years. Climatic data were collected for maximal, minimal, and mean air daily temperatures (ºC), mean ambient relative humidity (%), and rainfall (mm). Data were recorded by a Meteorological Station located at the Centro Austral de Investigaciones Científicas (CONICET, Argentina) from June 2010 to May 2011.

Scale: Developmental stages and morphological characteristics were recorded as per the extended BBCH-scale, a system for uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species [17]. Representative shoot sections were coded as per primary and secondary phenological growth stages and photographed for recording information. Nine recognisable and distinguishable principal growth stages were determined of ten of general BBCH-scale. This scale started with bud development (stage 0) and ended with partial senescence and beginning of dormancy (stage 9). Each principal growth stage was subdivided into secondary stages which described shorter developmental intervals in the major growth stage. The secondary stages were numbered 0 to 9 and they described the related ordinal or percentile values of growth.

Results and Discussion

Climatic conditions during the trial period: During the period from June 2010 to May 2011 (12 months), the mean air temperature during winter, spring, summer and autumn were 2.6, 7.1, 9.7 and 4.0 ºC, respectively (Fig. 1A). The minimum air temperatures were reached during June and July (-1.3 and -1.2 ºC, respectively) (Fig. 1A), the maximum air temperatures on February and January (15.6 and 13.9 ºC, respectively) (Fig. 1A), December was a month with unusual low mean air temperature (7.1 ºC) (Fig. 1A). The mean relative air humidity during the analyzed period was 70.4% (Fig. 1B), while the months with the highest rainfall were December and January with 80.8 and 73.6 mm, respectively (Fig. 1B), being July and October the months with the lowest rainfall. The registered values are comparable with those described earlier [10].

BBCH-scale for calafate: The adaptation of the BBCH-scale for calafate (Table 1) begins with a description of bud development (stage 0) and finishes with partial senescence and beginning of dormancy (stage 9). Among the nine growth stages, the first one corresponds to the bud development of vegetative and mixed buds (stage 0); the following four of them were assigned to the vegetative growth, which described leaf development on shoots (stage 1), shoot elongation (stage 3), development of plant propagation organs (stage 4) and partial senescence, beginning of dormancy (stage 9). The last four principal growth stages

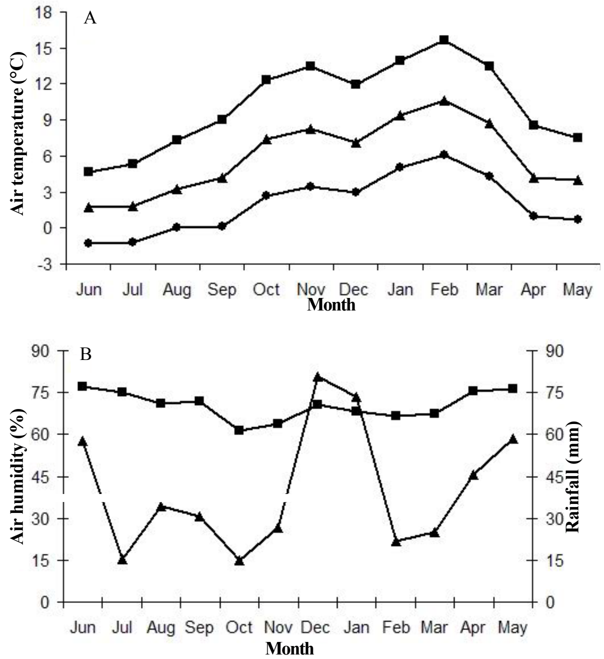

Figure 1. Climatic data for the experimental region near Ushuaia city, 54º48´ SL, 68º19´ WL (Tierra del Fuego, Argentina).
A. Maximal ( ), minimal ( ) and mean ( ) air daily temperatures,
B. Mean ambient relative humidity ( ) and rainfall ( ) were recorded from June 2012 to May 2011.

describe flower emergence (stage 5), flowering (stage 6), fruit development (stage 7) and maturity of fruit (stage 8). The scale did not include descriptions for the principal growth stage 2 (formation of side shoot/tillering), since the growth of early shoots is not a regular process in all the plants.

Principal growth stage 0 - Bud development: The scale starts at stage 00 which corresponds to the dormancy period. *B. buxifolia* is a perennial shrub that behaves as semi-evergreen probably due to the cold and harsh winters in Tierra del Fuego. The vegetative and mixed buds are closed and covered by reddish brown scales in this period (stage 00, Fig. 2). When mean air temperatures are close to 3-4 ºC, the buds begin to swell (stage 01, Fig. 2). Then the buds end swelling (stage 03, Fig. 2) and the stage finishes with the bud break (stage 09).

Principal growth stage 1 - Leaf development: Once buds break, the leaves start to unfold (stage 11, Fig. 2). Later on, more leaves of the rosette (short shoot) continue unfolding (stage 15) and the first leaves acquire their full size (November 1st fortnight) (stage 17) earlier than the other ones.

Principal growth stage 3 - Shoot development: During the beginning of shoot growth, the axes of developing shoots turn visible (stage 31, Fig. 2). This phase is accomplished after flowering occurred. Shoots are about 15-20% of final length on December (1st fortnight) (stage 32, Fig. 2). When the fast shoot elongation ends, shoots are about 80% of final length (January 1st fortnight). End of shoot elongation (90 to 95% of final length) occurs on February 1st fortnight (stage 39, Fig. 2).

Table 1. BBCH-scale proposed for *Berberis buxifolia*.

	Code	Description
0 Bud development	00	Dormancy: vegetative and mixed buds are closed and covered by reddish brown scales (Fig. 2)
	01	Beginning of vegetative and mixed bud swelling: bud scales begin to separate (Fig. 2)
	03	End of vegetative and mixed bud swelling: scales are completely separated, light green buds emerged (Fig. 2)
	09	Vegetative and mixed bud break
1 Leaf development	11	First leaves are unfolded (Fig. 2)
	15	More leaves are unfolded but not yet at full size (Fig. 2)
	17	First leaves are fully expanded (November 1st fortnight) (Fig. 2)
	19	All leaves are completely unfolded and expanded
3 Shoot development	31	Beginning of shoot growth: axes of developing shoots are visible (Fig. 2)
	32	Shoots are about 15-20% of final length (December 1st fortnight) (Fig. 2)
	38	Completion of fast shoot elongation. Shoots are about 80% of final length (January 1st fortnight)
	39	End of shoot elongation. Shoots are about 90-95% of final length (February 1st fortnight) (Fig. 2)
4 Development of plant vegetative propagation organs	40	Vegetative propagation organs (rhizomes) begin to develop (Fig. 2)
	42	Vegetative propagation organs (rhizomes) growing (Fig. 2)
	49	Vegetative propagation organs (rhizomes) reach final size, become lignified and are ready to be harvested for plant propagation (Fig. 2)
5 Flower emergence	53	Mixed bud break: green flower button appears at the apical position of the leaf rosette (October 1st fortnight) (Fig. 2)
	54	Green flower button with elongating pedicel (Fig. 2)
	59	Yellow flower button with the elongated pedicel (October 2nd fortnight) (Fig. 2 and 3)
6 Flowering	60	First flowers open (Fig. 3)
	61	Beginning of flowering: about 10% of flowers open. Sepals and petals are open outside exposing the stigma
	65	Full flowering: 50% of flowers are open. Sepals are arranged at an angle of 90 degrees respect to petals (end of October, beginning of November) (Fig. 3)
	67	Flowers fading: majority of petals fallen or dry
	68	End of flowering: 20% of flowers show fading and petals fallen (Fig. 3)
7 Fruit development	71	Fruit set: beginning of ovary growth. Small fruits lacking perianth. Beginning of first phase of fast fruit growth (November 1st fortnight to December 2nd fortnight) (Fig. 3). Purple colour appearing on fruit green surface
	75	Lag phase of fruit growth (January 1stfortnight). Fruits become round (Fig. 3)
	76	Beginning of second phase of fast fruit growth (January 2nd to February 2nd fortnights)
8 Maturity of fruit	80	Physiological maturity: fruits are fully developed. The percentage of skin surface having purple color reaches 50%
	83	Advances ripening: increase of fruit sugars and purple intensity
	86	Fruit color fully developed: the percentage of skin surface having purple color reaches 100% (Fig. 3). Fruit ripe for consumption, with correct firmness and typical taste
	89	Fruit overripe
9 Partial senescence, beginning of dormancy	91	Shoot growth is completed; most of leaves are fully expanded and dark green
	93	Older leaves begin to yellowing and fall (partial senescence) (April to May)
	97	Plant enters to winter rest period

Principal growth stage 4 - Development of plant vegetative propagation organs: The rhizome system begins its growth in the spring (stage 40, Fig. 2) and continues elongating (stage 42, Fig. 2) to become lignified at the end of the growing season (April to May) (stage 49, Fig. 2).

Principal growth stage 5 - Flower emergence: Once the scales of the mixed buds are separated and the bud break occurs, green flower button appears at the apical position of the leaf rosette (short shoot). This stage is observed during the first half of October when the pedicel elongates quickly (stage 53, Fig. 2). During stage 54, the green flower button elongates its pedicel (Fig. 2). When the floral structure reaches 90% of the total size (stage 59, Fig. 2 and Fig. 3), the pedicel stops elongation, the flower button turns yellow and it is close to entering the pre-anthesis stage (Fig. 3). This stage is observed during the second half of October, and had already been described by Arena *et al.* [15] as stage E according to the scale of Fleckinger [16].

Principal growth stage 6 - Flowering: Flowering begins with the anthesis step (stage 60, Fig. 3). *B. buxifolia* shows pendulous flowers with yellowish and yellowish orange sepals and petals, respectively. During anthesis both structures move from the center towards outside, leaving exposed the pistil [15]. Afterwards, sepals move to a position of 90º with respect to the floral axis whereas the petals surround partly the reproductive organs. Stamen moves as well as petals. Anthesis phase was previously recognized as F1 and F2 (Fig. 3) [15], according to the scale of Fleckinger [16]. Flowering (stage 61) begins when 10% of flowers opens. During spring of 2010, and from a careful count of flowers, this event occurred on October 27 [15]. Suddenly, the full of flowering was registered with 50% of flowers open (stage 65, Fig. 3). The duration of flower blooming is directly related to weather conditions. The higher daily temperature recorded make short the life of flowers. Flower fading is evidenced by a color change and loss of turgidity of the petals. Petals change from yellowish orange to brownish with white borders (stage 67). End of flowering was considered when 20% of flowers showed fading, petals fall [15] and the swollen ovary can be seen. This step corresponds to stage 68 of BBCH-scale (Fig. 3).

Principal growth stage 7 - Fruit development: Once the fruit set occurs and the ovary growth begins, the first phase of fast fruit growth takes place; at the same time purple colour appears on the fruit green skin (stage 71, Fig. 3). The size of the fruit at the end of this phase is highly variable (from 23 to 79% of the final size),

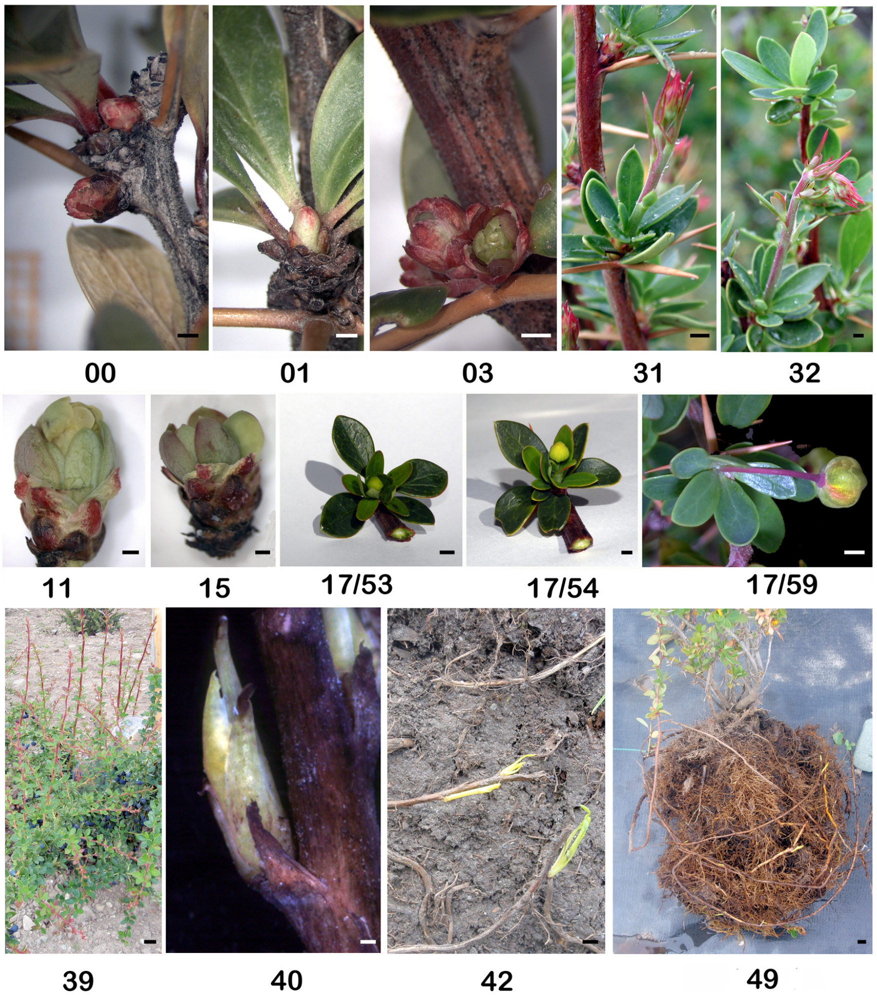

Figure 2. Phenological growth and development stages of *Berberis buxifolia* recorded in Tierra del Fuego, Argentina. Illustration of principal growth stage 0, 3, 4 and 5 with corresponding phenophase codes. Bar 00 – 15 and 40 = 1 mm; 17/53 – 17/59 = 2 mm; 39; 42 and 49 = 10 mm.

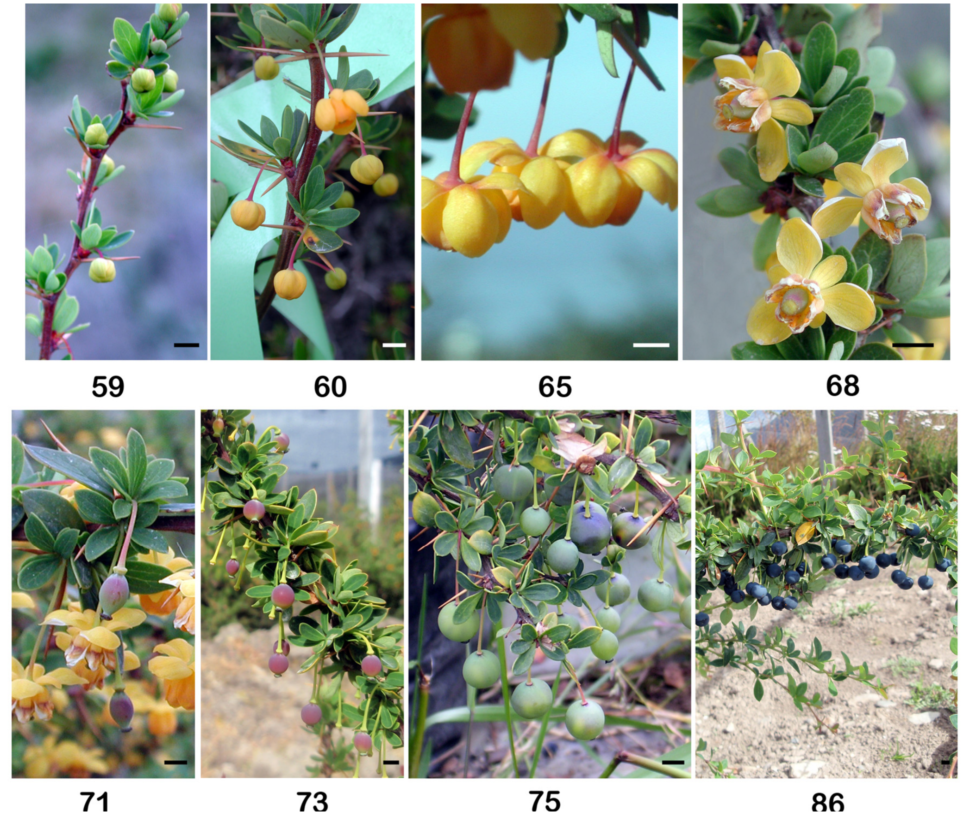

Figure 3. Phenological growth and development stages of *Berberis buxifolia* recorded in Tierra del Fuego, Argentina. Illustration of principal growth stage 6-8 for *Berberis buxifolia* with corresponding phenophase codes. Bar = 5 mm.

depending of the climatic conditions of the growing season [10, 11]. Successively the fruit growth shows a short lag phase, and the fruits become round (stage 75, Fig. 3). Afterwards, the second phase of fast fruit growth begins (stage 76) and the fruits attain the 100% of the final growth.

Principal growth stage 8 - Maturity of fruit: When the second phase of rapid fruit growth begins, the fruit, showing the 50% of skin surface of purple color, acquires the physiological maturity. The fruit then begins to accumulate sugars and the intensity of purple color increases to attain the 100% of the fruit surface with purple color (stage 86, Fig. 3).

Principal growth stage 9 - Partial senescence, beginning of dormancy: When shoot growth is completed, the leaves are fully expanded and dark green. Later on, the older leaves begin to yellowing and fall (partial senescence). Plant enters to the winter rest period.

Conclusions

The proposed BBCH-scale for *Berberis buxifolia* is easy to use for studying different growth stages and includes all the subtle details of calafate growth and development. The exhaustive study of the principal growth stage 4 indicates the time when the rhizome system is ready to be harvested for clonal propagation i.e. at the end of the growing season (April to May), before soil freezing and snowing. Growth stage 8 indicates the precise time when the fruit is ripe for consumption, with correct firmness and typical taste. In the context of phenology related studies, the adoption of a uniform phenological scale could be useful for comparative behaviour of this species at different locations, or among different species of the genus, as well as for studies of impact of global warming and climatic change on plants of high latitudes as occurs with *Berberis* genus in Patagonia.

Acknowledgements

This work received funds from the projects PIP 0263 and PIP 0223 (CONICET).

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