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Reproductive phenology of *Vaccinium floribundum* Kunth (Ericaceae) and codification according to the BBCH scale based on evidence from the volcano Chimborazo paramo (Ecuador)

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ABSTRACT

Vaccinium floribundum Kunth (Ericaceae) is a native Andean species with shrubby habit and edible fruits. It is mostly distributed in the paramos of the Andes, from Venezuela to Bolivia, where it is commonly known as "mortiño". In this study, we describe the reproductive phenological growth stages of *V. floribundum* in the paramo of the Chimborazo volcano in Ecuador, using an adaptation of the BBCH scale system, and according to semi-monthly observations conducted in three localities from January 2017 to June 2018 (18 months). In addition, a linear regression analysis was performed to detect any potential influence of climatic parameters on the reproductive phenology of “mortiño”. Throughout the monitoring period, different stages of reproductive growth such as bud, flower and fruit were simultaneously found among individuals and, sometimes, even in the same plant. The reproductive phenology of sampled individuals from sites 1 and 2 (Mindala Loma and Polylepis, respectively) were relatively synchronized, whereas individuals from site 3 (Mechahuasca) showed a different phenological pattern. A significant, apparently quick, loss of flowers was evident in all localities during the study period. The patterns observed could reflect physiological constraints imposed by the extreme weather conditions of the paramo.

1. Introduction

Paramos are high-altitude humid mountain environments, where high number of species co-exist and show homoplasy traits adapted for the survival in this environment characterized by volcanic orography and extreme climatic conditions (Gentry, 1988; Luteyn, 2002). One emblematic species of the paramos is *Vaccinium floribundum* Kunth (Ericaceae), locally known as "mortiño". This species is particularly common in the Andes from Venezuela to Bolivia, and has been used since immemorial times. Since the syncretism between the Catholic faith and the indigenous culture, "mortiño" became the main ingredient of a traditional, relatively thick, beverage called “colada morada”, that is prepared to commemorate “All Souls’ Day” (Torres et al., 2010). The widespread use of "mortiño" is a healthy custom because its fruits have relatively high concentrations of sugar, antioxidants such as vitamin C and vitamin B complex, and minerals such as potassium, calcium, and phosphorus (Freire, 2004; Santamaría et al., 2012). Yet, over the years, human consumption of "mortiño" in Ecuador has decreased, as well as its availability in the local markets, probably because of the decimation of its natural populations, the technical difficulties to cultivate it, and the lack of knowledge about its health benefits (Freire, 2004). Indeed, "mortiño" populations are being continuously fragmented by anthropogenic processes such as deforestation, productive land reconversion, and overexploitation (Torres et al., 2010).

Reproductive phenology tends to respond to the local climate, which assures that gene flow occurs between individuals from the same or nearby populations (Jianwu et al., 2016). Furthermore, depending on the local climate variability, plant species can adopt different reproductive phenological strategies to guarantee the maintenance of their genetic diversity (Körner et al., 2016). In tropical regions, the variability and intensity of rain is generally considered as an environmental signal and evolutionary force that explains the variation of phenological events

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among species (Morellato et al., 2006; Martínez-Adriano et al., 2016; Medina-Cano et al., 2019). In Ecuador, phenological studies of paramo species has been historically relegated, despite the importance of these complex ecological systems in terms of the ecosystem services they provide (Jianwu et al., 2016). Ideally, assessing the variation of the reproductive growth stages (flowering and fruiting) of each species in different paramos would allow the design of effective repopulation or management plans (Prado and Valdebenito, 2000; Caiza, 2011).

The genus Vaccinium includes a group of species to which the "mortiño" and blueberry belong. Reproductive phenology and growth developmental stages of both species are quite similar (Prado and Valdebenito, 2000), but it has not been evaluated or described to date in the ecological conditions of Ecuadorian central Andes.

For this species, information on phenological studies has been obtained. Gómez (2004) described some reproductive and growth developmental stages as leaf sprouting, floral bud, open flower, anthesis, fruit set and green and mature fruits. Other studies even reported up to six different stages of berry ripening (Buitrago et al., 2015). Furthermore, the peasant communities of Guarne (Antioquia, Colombia) revealed that the "agraz" (*Vaccinium meridionale*) yielded biannual fruiting because of the bimodal rain pattern in the area (Medina et al., 2019). For the area of the Antilles, flowering occurred from the beginning of winter in the Ericaceae family. This reproductive stage of development extended throughout the spring until the beginning of summer. Likewise, the fruiting stage lasted from the beginning of fall, continuing through the winter, and to reach its peak during the spring-summer period. These plants showed abundant flowering and fruiting all over the year (Berazaín, 2006).

Meanwhile, the "mortiño" also played an important and ecological role in the high Andes. Because of its high regenerative capacity, the species was vital for vegetation preservation in the paramos following man-made destructive fires (Ramsay and Oxley, 1996). And despite its commercial interest, cultural and ecological significance, "mortiño" remains as an endangered wild species due to the ongoing fragmentation of its natural habitat (Coba et al., 2012).

Therefore, the main goal of the current study was to describe the reproductive phenological growth stages (flowering and fruiting) of *Vaccinium floribundum* Kunth, as observed in a natural paramo conditions in the Andes and through an adaptation of the BBCH extended scale. To ensure the accuracy of observations in the scale development, 18 months of observations on a semi-monthly basis, from January 2017 to June 2018, were taken in three paramo areas of the Chimborazo volcano in central Ecuador, South America.

2. Materials and methods

2.1. Study area

Three different localities with presence of *V. floribundum* were sampled in the paramo of the Chimborazo volcano (Sierra, 1999) in the central Andes of Ecuador. The localities where to observe *V. floribundum* were selected based on verbal information from park rangers. In each locality, different adult plants were randomly selected. More information about each locality is shown in **Tables 1 and 2** and **Fig. 1**.

Table 2
Monthly maximum, minimum and mean air temperatures (°C) and precipitation (mm) for the three monitored sites (Mindala Loma, Polylepis and Mechahuasca). Climatic data was provided by the meteorological station of the R. P. F. Chimborazo from January 2017 to June 2018.

Month	Air temperature (°C) Max.	Min.	Mean	Precipitation(mm)
2017				
January	4.9	3.5	4.2	56.7
February	5.7	3.1	4.4	22.9
March	5.2	2.9	4.1	114.5
April	4.6	3.5	4.1	163.8
May	4.8	3.3	4.0	45.7
June	3.63	2.1	2.9	70.7
July	4.0	2.2	3.1	8.7
August	4.0	2.3	3.1	4.4
September	3.6	2.1	2.8	42.2
October	3.9	2.7	3.3	66.1
November	4.2	2.9	3.6	25.0
December	3.4	2.1	2.1	81.5
2018				
January	3.5	2.3	2.9	94.1
February	3.4	2.2	2.8	137.6
March	3.1	2.1	2.6	235.9
April	3.8	2.7	3.3	108.1
May	4.1	2.7	3.4	90.3
June	3.4	2.3	2.8	88.2

2.2. Plant material

Phenological observations and measurements were recorded on 47 adult and healthy plants in three different localities (Table 1) belonging to the paramo of Chimborazo volcano (Ecuador). *Vaccinium floribundum* is considered a wild plant species in Ecuador that grows in the highlands of the mountain range. The adaptation zone of "mortiño" are the Andean Paramos located at altitudes ranging from 1000 to 4500 m.a.s.l. But the truth is that few of them have enough plant populations to carry out *in situ* studies. Because of the extension of the agricultural areas, this plant species has been relegated to paramo areas between the 3400 and 3500 up to 4500 m.a.s.l. (MAGAP, 1998).

This deciduous perennial shrub is extraordinarily resistant to drought and frost. It grows successfully in tundra-like ecosystems, commonly known as Paramos (Coba et al., 2012). And since the "mortiño" is a wild and endemic fruit species of the high Andes, more studies on basic biology and ecology are necessary to establish efficient conservation programs for sustainable agriculture (Cobo et al., 2016).

2.3. Monitoring of reproductive phenology

The monitoring of the different reproductive phenological growth stages of *V. floribundum* was conducted from January 2017 to June 2018 (18 months). The reproductive phenological phases were monitored in order to ensure that the phenological information would cover the entire period of manifestation of the characteristic, from the beginning to the decline. This was achieved through semi-monthly visits to the different localities. In each visit, the number of reproductive phenological organs in each individual was counted, using the following categories: flower bud, flower, green immature fruit, red maturing fruit and black mature fruit. Using this quantitative information, a semi-qualitative scale following the BBCH system was adaptively constructed, focusing on the

Table 1
Localities where the reproductive phenology of *Vaccinium floribundum* "mortiño" was monitored on a semi-monthly basis from January 2017 to June 2018 (18 months) in the paramo of the Chimborazo volcano, Ecuador.

Locality Name	Province	No. of individuals sampled	Coordinates	Altitude (m)	Vegetation type
1. Mindala Loma	Bolívar	17	01°27'45″ S 78°56'05″ W	4261	herbaceous paramo
2. Polylepis	Chimborazo	19	01°32'41″ S 78°53'05″ W	4076	herbaceous paramo
3. Mechahuasca	Tungurahua	11	01°25'26″ S 78°47'53″ W	4370	herbaceous paramo

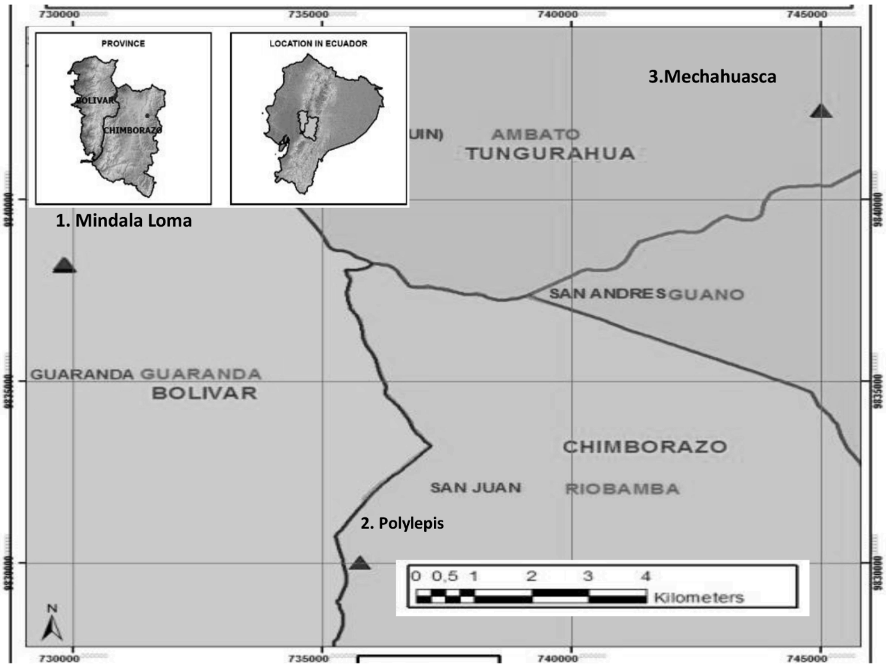

Fig. 1. The three paramo locations where "mortiño" reproductive phenology was monitored and described on the Chimborazo volcano area (Ecuador).

reproductive stages only.

For each reproductive phenological growth stage, we then elaborated a description accompanied by a photographic record and a graphical summary of the presence or absence of the different stages during the monitoring period. Once the monitoring of the selected individuals was completed, the best photographs were chosen to illustrate the main developmental stages of "mortiño" and, then, to successfully codify its reproductive phenology (Sakar et al., 2019).

2.4. Local climatic patterns

Climatic data from January to December 2017 and from January to September 2018 was obtained from a temporary weather station that was installed near the study sites for the purpose of a different research project (**Table 2**). Note that the phenological monitoring period of this study lasted from January 2017 to June 2018. The cumulative rainfall during the period from January to December 2017 was 702.2 mm, while from January to September 2018 was 794.4 mm. In 2017, average monthly rainfall (12 months) was 58.5 mm, with only two months, March and April, showing a cumulative monthly rainfall >100 mm (114.5 and 163.8 mm, respectively). In 2018, the average monthly rainfall (9 months) was 88.3 mm, with monthly rainfall >100 mm occurring in February, March and April (138.4, 235.1 and 108.8 mm, respectively). In both years, the period from July to September tended to be relatively dry, with an average monthly rainfall of <20 mm. Relative humidity during a day-night cycle can vary quickly—in any case, daily relative humidity average (24 h) ranged from 77.3% to 92.4% in 2017, and from 82% to 92.8% in 2018; although a few hourly records <20% existed, the great majority of hourly records were >80%. The average daily temperature was around 4 °C, with relatively low maximum to minimum temperature variation.

2.5. Statistical analysis

Linear regression analysis was carried out to detect any possible influence of climatic parameters on the reproductive phenology of the "mortiño". XLSTAT Premium 2016 software (Addinsoft, New York, NY, USA) and Statgraphics Plus (version 3.1, Statistical Graphics Corp., Rockville, MA, USA) were used for this analysis.

3. Results

3.1. BBCH scale codification for vaccinium floribundum reproductive phenology

Bleiholder et al. (1991) and Hack et al. (1992) proposed the BBCH scale (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie), which describes a uniform coding system for the phenological identification of the growth stages of all species of mono- and dicotyledonous plants. It uses a decimal code that is basically divided into principal and secondary plant growth stages, including those reproductive ones.

The BBCH scale for mono and dicotyledonous plants considers 10 principal growth stages, numbered from 0 to 9 (Hack et al., 1992). In addition to the principal growth stages, secondary stages are defined using a second digit from 0 to 9, which represent short developmental steps that occur within the principal stages of development. For the *V. floribundum* BBCH scale, as adapted for this study, only the principal stages corresponding to the reproductive phenology were used, starting at flower development (stage 5) and ending at the ripening or maturity of fruit (stage 8). For the secondary stages, we did not always use all the digits from 0 to 9, but instead defined secondary stages that better matched the official descriptions of the BBCH scale (Hack et al., 1992). **Fig. 2** portrays, using photographs, representative reproductive phenological stages observed in 47 individuals of *V. floribundum* from the three different localities studied.

Below we describe each reproductive phenological growth stage, and the period in the year at which each stage was observed.

Principal growth stage 5: Inflorescence emergence

51: Inflorescence buds swelling: buds closed, light brown scales visible (Fig. 2).

This stage represents the beginning of the inflorescence and basically occurs throughout the year in localities 1 and 2 (Figs. 3 and 4). However, in locality 3, it was only observed from May to August (Fig. 5).

53: Bud burst: green sepals tips enclosing flowers visible.

This stage is similar to the 51 stage, except that the inflorescence shows a higher degree of maturity. It occurred at more or less the same time as stage 51.

Principal growth stage 6: Flowering

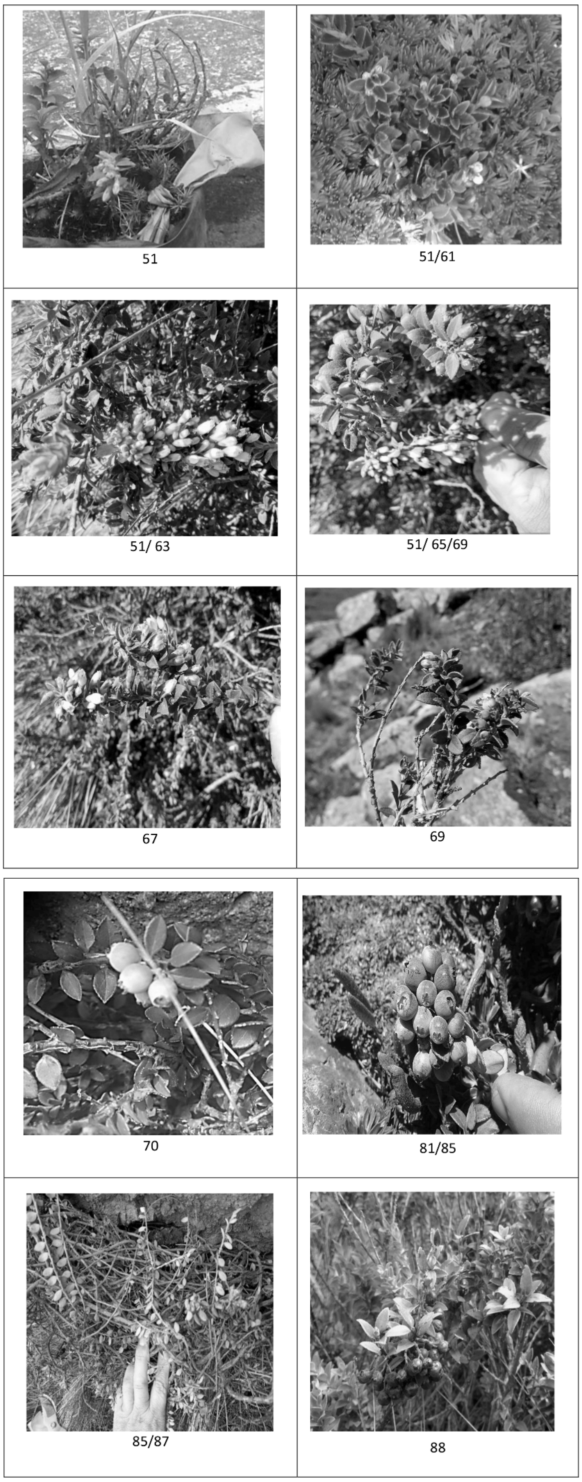

Fig. 2. Representative photographs portraying the primary and secondary reproductive growth stages observed in 47 *Vaccinium floribundum* individuals, according to the BBCH-scale and focusing only on the reproductive stages. Note that it is common that in a given individual more than one stage is occurring at the same time.

61: Beginning of flowering: about 10% flowers open (Fig. 2).

This stage is present in very few individuals. In locality 1, it was observed in August 2017 and from February to March 2018 (Fig. 3); while in locality 2, it was only observed in January 2018 (Fig. 4). This stage was not observed in locality 3 (Fig. 5).

62: About 20% of flowers open.

This stage was only observed in locality 1 during May 2017 (Fig. 3), and in the month of March 2018 in locality 2 (Fig. 4). This stage was not observed in locality 3 (Fig. 5).

63: About 30% of flowers open.

This stage was monitored on only a few plants (Fig. 2). It was observed in locality 1 in April and June 2017 (Fig. 3), and in March 2017 in locality 2 (Fig. 4). This stage was not observed in locality 3 (Fig. 5).

64: About 40% of flowers open.

This stage was rarely observed. It was only observed in locality 2 in February 2018 (Fig. 4).

65: Full flowering: at least 50% of flowers open, many visible styles and stigmas (Fig. 2).

This stage was rarely observed. It was detected in locality 2 in January 2017 and March 2018 (Fig. 4).

66: Full flowering: at least 60% of flowers open, many visible styles and stigmas. This stage was rarely observed. It was only detected in locality 2 in December 2017 (Fig. 4).

67: Full flowering: at least 70% of flowers open, many visible styles and stigmas (Fig. 2). This stage was observed in locality 2 in May and October 2017 and in January 2018 (Fig. 4); while in locality 3, it was only detected in June 2017 (Fig. 5). This stage was not observed in locality 1 (Fig. 3).

69: End of flowering: numerous fruits have already set (Fig. 2).

In locality 2, this stage was observed almost throughout the study period (Fig. 4). In locality 3, this stage was also registered during many months, from April to October 2017 (Fig. 5). There is no record of this stage in locality 1 (Fig. 3).

Principal growth stage 7: Fruit development

70: Immature fruits visible (Fig. 2).

In localities 1 and 2, this stage was commonly observed throughout the monitoring period (Figs. 3 and 4). In locality 3, it was observed sporadically, from January to March 2017, and from August to October 2017 (Fig. 5).

Principal growth stage 8: Ripening or maturity of fruit

81: Beginning of maturation and color change: fruit skin appears orange. Approximately 10% of the final color (Fig. 2).

In localities 1 and 2, this stage was commonly observed throughout the monitoring period (Figs. 3 and 4). In locality 3, it was observed from January to March 2017, and from September 2017 to January 2018 (Fig. 5).

85: Coloring advanced: first fruits ripe black (Fig. 2).

This stage was not commonly observed in the three localities. In locality 1, it was observed in the months of February, June-July and December 2017 (Fig. 3). In locality 2, it was observed in November 2017 (Fig. 4); while in locality 3, it was observed in January and November 2017 (Fig. 5).

87: Beginning of softening of the fruits (Fig. 2).

This stage was observed almost throughout the monitoring period in localities 1 and 2 (Figs. 3 and 4). It was not observed in locality 3 (Fig. 5).

88: Beginning of the decrease of the consistency of the fruits (Fig. 2).

This stage is similar to the 87 stage, except that the fruit shows a higher degree of maturity. It occurred at more or less the same time as stage 87.

3.2. *Chronology and timing of phenological events*

The reproductive phenology of sampled individuals from sites 1 and 2 (Mindala Loma and Polylepis, respectively) were relatively synchronized, whereas individuals from site 3 (Mechahuasca) showed a different phenological pattern. Though the distance that separates the three sampled sites is 15 km at most (Fig. 1), site 3 reproductive phenology differed from the others since it is a special ecological niche surrounded by a huge lagoon.

A statistical analysis was performed using a linear regression model to detect potential influence of climatic parameters (independent variables) on the appearance frequency of each monitored reproductive

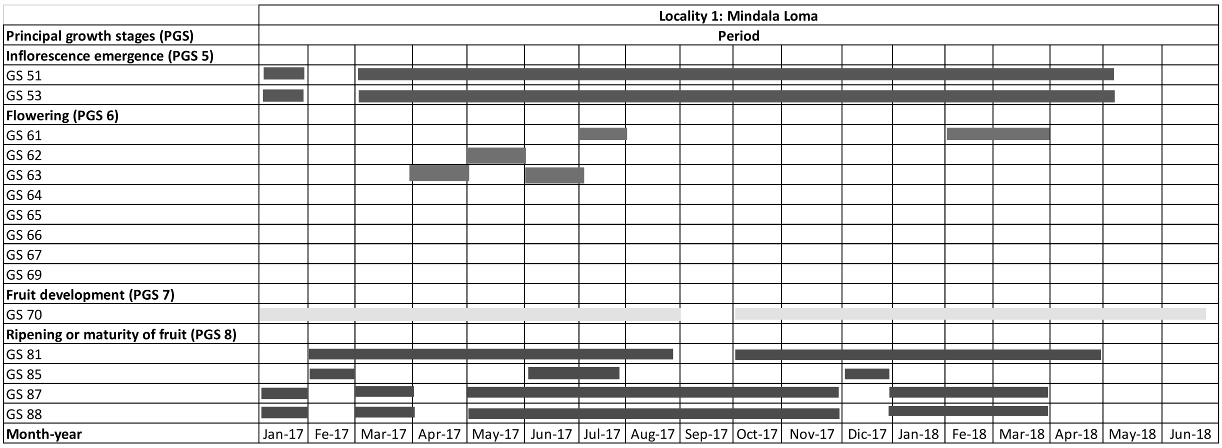

Fig. 3. Schematic representation of the chronological progression of reproductive phenological growth stages of *V. floribundum* ("mortiño") in Mindala Loma locality, paramo of the Chimborazo volcano.

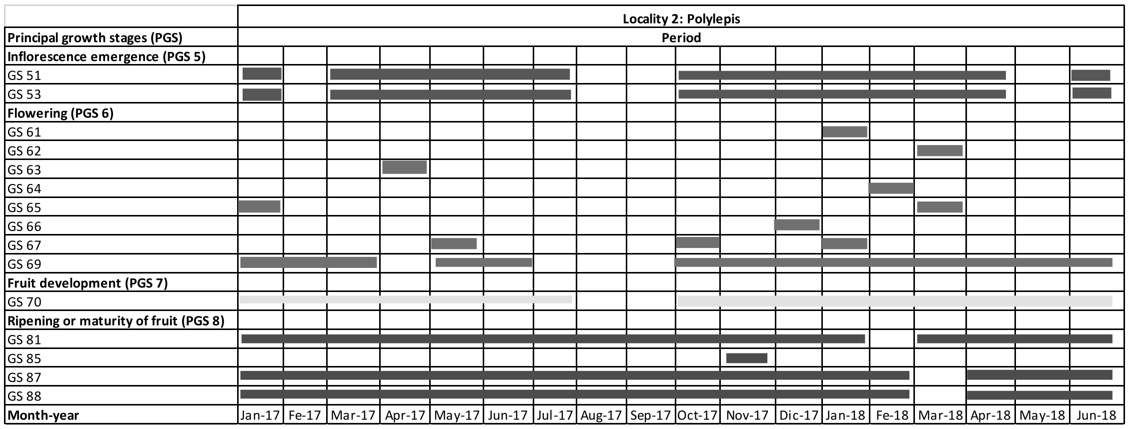

Fig. 4. Schematic representation of the chronological progression of reproductive phenological growth stages of *V. floribundum* ("mortiño") in Polylepis locality, paramo of the Chimborazo volcano.

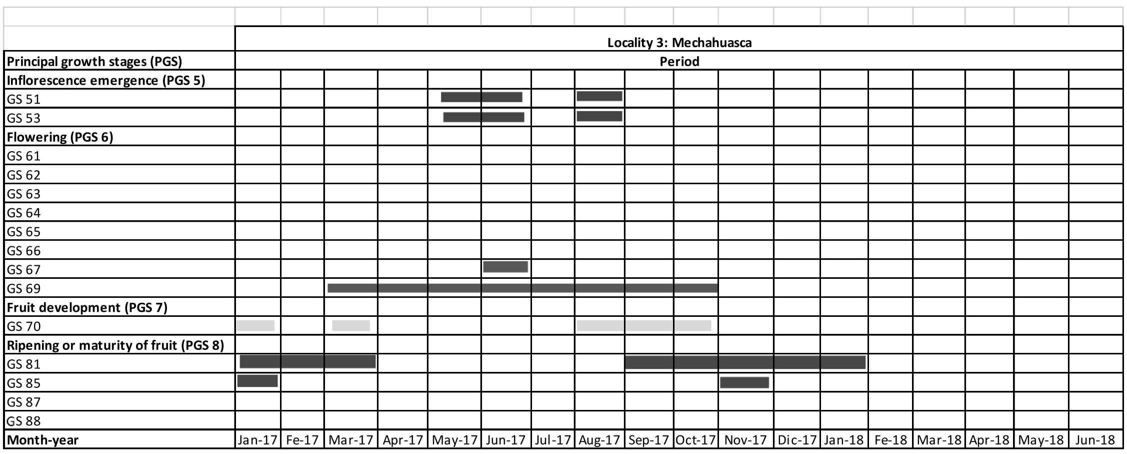

Fig. 5. Schematic representation of the chronological progression of reproductive phenological growth stages of *V. floribundum* ("mortiño") in Mechahuasca locality, paramo of the Chimborazo volcano.

growth stage (dependent variable). There was no significant influence of any climatic variable on the reproductive phenological patterns described (Figs. 3–5).

The chronological evolution of principal growth stages for the reproductive phenology of "mortiño" is shown in Figs. 3–5. Localities 1 and 2 (Mindala Loma and Polylepis, respectively) practically showed similar phenograms (Figs. 3 and 4), being the growth stages (GS) 51, 53, 70, 81, 87 and 88 the most frequent ones. This could be attributed to the fact that both locations are very close within the same area. In contrast, Mechahuasca (locality 3) showed a different trend since it is much further away and because the type of vegetation (alpine grasses) differed from sites 1 and 2 (herbaceous paramos). Here, the reproductive growth stages were rather irregular (Fig. 5).

4. Discussion

The BBCH codification scale has been used in different fruit crops, more commonly in northern latitudes (Legua et al., 2013; Hernández et al., 2015; Sánchez-Salcedo et al., 2017; Kishore, 2018). In the Neotropics, the BBCH scale was applied to *Vaccinium meridionale* in the Colombian paramos (Medina et al., 2019) and for *Vaccinium floribundum* in the Ecuadorian paramos (Mendoza, 2018; Rivera, 2019). However, it is difficult to compare the *V. floribundum* studies with the present study because of the different, or convoluted, approaches used in them. In this sense, it is important to realize that not all growth stages that are usually easily distinguishable in a crop, grown under semi-controlled conditions, are also observable or perceptible when a wild species in its natural setting is monitored, as is the case for this study. Another confounding factor, for the direct application of the BBCH scale on *V. floribundum* individuals monitored in this study, could be that they are growing at very high altitudes (>4000 m.a.s.l.), which could enforce particular physiological constraints imposed by the extreme weather conditions. On the other hand, in other studies with *Vaccinium spp.* (Medina et al., 2019; Mendoza, 2018; Rivera, 2019), observations were made at lower altitudes (3500–3600 m.a.s.l.).

Based on the general BBCH phenological scale (Meier, 1997), and along with the observations of mortiño reproductive growth phenology over the 18-months period and across the three monitored sites, we established and extended BBCH scale specific to *Vaccinium floribundum*. The use of the BBCH scale is very appropriate for the study of the phenological behavior of a plant species under different ecological conditions (Leather, 2010). The specific scale, obtained to detail the reproductive phenology of mortiño, definitely agreed with the general key proposed by Finn et al. (2007) for tree and woody plants description.

Morellato et al. (2006 and 2013) stated that rainfall is usually considered as an environmental signal responsible for the phenological variation of plant growth stages in tropical zones. And according to Mendoza et al. (2017), the most determining factor on plant phenology in high-altitude areas is certainly temperature. But in the tropics it is really the periodicity between dry and rainy periods. In our study, in fact, the occurrence and duration of the reproductive phenological growth stages, monitored over 18 months, did not show a clear relation with the precipitation pattern (i.e., with the strong wet season from March to April, and with the strong dry season around July-August) (Figs. 3–5). And this does not mean that climate could have an influence on the intensity or abundance of reproductive structures. Future studies should attempt to assess this possible relationship. In addition, it can be observed that throughout the monitoring period, there was a constant production of flowers and fruits.

Zapata (2002) reported that fruiting of *V. floribundum* occurs in eastern Antioquia in two main periods: from April to June and from September to December. However, Gómez (2004) found that in natural populations fruiting occurred throughout the year, with percentages ranging from 17% to 39%. In our study, we observed fruits throughout the whole monitoring period (18 months), but not necessarily at the time when the rainy season occurred, from February-March to April-May.

Regarding the time span that different reproductive organs can last (in days) during the reproductive phase of *V. floribundum*, we could gain some insight from the studies conducted in Colombia with *V. meridionale*. In this species, Chamorro and Nates-Parra (2015), in Guachetá (Cundinamarca) and San Miguel de Sema (Boyacá), found that the time it takes from a flower bud to become a full open flower was 18 days; and six days later, the senescence of this organ started. In addition, Chamorro (2014) stated that flowers only lasted for six to ten days, which was already considered a long floral longevity for the Ericaceae family (Primack, 1985). In the future, it will be worthwhile to embark in detailed floral biology studies for the *V. floribundum* populations of Ecuador, in order to allow comparisons with the numerous studies in Colombia.

Regarding the relative flower vs. fruit production, Rathcke (2003), Torres-Díaz et al. (2011), Chamorro (2014) and Chamorro and Nates-Parra (2015) found that *V. meridionale* produces a large number of flowers but low fruit set, which could be evidence of a selective abortion process of self-pollinated fruits. Though in our study we did find fruit production throughout the monitoring period, particularly in locations 1 and 2, while flowers were just sporadically observed because of a relatively fast flower loss.

5. Conclusions

For the first time, the reproductive phenology specific to "mortiño" (*Vaccinium floribundum*) was established according to BBCH phenological scale, based on evidence from the volcano Chimborazo paramo of Ecuador. The reproductive growth stages (GS) can be described in more detail using this specific scale. And based on the phenological observations carried out in the three monitored areas, we conclude that in this paramo there was not an ordered chronological progression of the reproductive phenology of "mortiño", probably because of the adverse and continuously changing climatic conditions. Furthermore, it was relatively common to observe different growth stages concomitantly occurring in the same individual.

The main limiting factor for the cultivation and production of "mortiño" on a large scale is the lack of knowledge about its particular ecology, climatic requirements, soils and rhizosphere. This leads to a lack of domestication of the Andean fruit crop and, then, limiting its significant nutritional potential. And because of that, it is necessary to learn more about the conditions in which this fruit species grows in order to domesticate it for agro-industrial purposes. In addition, "mortiño" can also be used as biofuel and for regeneration and reforestation of paramos devastated by the great fires.

The results reported in this study can provide insight for: (i) climate change studies for preservation of the species on fragile habitats, (ii) adaptation studies of cultivars to different environmental conditions, (iii) propagation trials of the species under quasi *in situ* conditions, (iv) development of germoplasm collections for programing agronomic practices and (v) establishment of programs for "mortiño" commercial breeding.

CRediT authorship contribution statement

Jorge M. Caranqui-Aldaz: Data curation, Investigation, Writing – original draft, Writing – review & editing. **Hugo Romero-Saltos:** Software, Writing – original draft, Writing – review & editing. **Francisca Hernández:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Rafael Martínez:** Methodology, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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