The influence of sampling from different canopy positions on the evaluation of flower bud anomalies and dormancy in apricot (*Prunus armeniaca* L.)

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Abstract — Introduction. For some species such as *P. armeniaca*, overcoming dormancy may be problematic when cold requirements are not satisfied. Moreover, the different positions of flower buds on the tree canopy and on the apical and basal portions of twigs can influence their development. Sampling problems have not been extensively studied. Therefore, the effect of different sectors (west and east), of different heights (top and bottom) of the canopy and of twig portions (medium-apical and medium-basal) on the break of dormancy in the apricot tree was evaluated. Attention was also focused on floral anomalies in relation to the flower bud position on the twig, in order to identify the least variable portion of twigs and thus provide useful information for optimization of periodic bud sampling. **Materials and methods**. Two cultivars of *P. armeniaca* with different chilling requirements were chosen: 'San Castrese' and 'Orange Red'. The end of endodormancy was evaluated from December to blooming time by traditional biological approaches, such as increase in flower bud fresh weight and evolution of the bud phenological stage, before and after 7 d under forcing conditions. In addition, the percentage of floral anomalies was considered in relation to the flower bud position on the twig. **Results and conclusion**. The results showed that canopy and twig positions exerted different effects on the breaking of the flower bud endo-dormancy, and some practical applications can be drawn from them for the best sampling design inside the tree structure: the west orientation of the canopy and the medium-apical portion of the twig were the best positions to obtain an earlier active growth of buds, allowing the prediction of endo-dormancy breaking. The analysis of flower anomalies showed considerable differences depending on the position of the bud on the twig. High percentages of anomalous buds were observed in the medium-basal portion of the twig, which could distort the results on the evaluation of overcoming dormancy, due to a weaker growth trend of flower buds.

Italy / *Prunus armeniaca* / methods / sampling / plant developmental stages / flowering / dormancy breaking / site factors

Influence de l'échantillonnage en différents points de la frondaison pour l'évaluation d'anomalies du bourgeon floral et de la dormance chez l'abricotier (*Prunus armeniaca* L.).

Résumé — Introduction. Pour certaines espèces telles que l'abricotier, la sortie de dormance peut être problématique quand des besoins en froid ne sont pas satisfaits. Par ailleurs, l'emplacement des bourgeons floraux dans la frondaison de l'arbre et sur les parties apicales et basales des rameaux peut influencer leur développement. Les techniques d'échantillonnage méritaient d'être mieux étudiées. L'effet, sur la sortie de la dormance des bourgeons, de différents secteurs échantillonnés dans l'arbre (ouest et est), de la hauteur des échantillonnages (haut et bas) et de la partie du rameau portant les bourgeons floraux observés (moitié supérieure et moitié inférieure) a donc été évalué chez l'abricotier. Par ailleurs, les anomalies florales ont été étudiées également par rapport à la position du bourgeon floral sur le rameau afin d'en identifier la partie la moins variable et de disposer ainsi d'informations utiles pour la détermination de la sortie de la dormance. **Matériel et méthodes**. Deux cultivars de *P. armeniaca* avec différents besoins en froid ont été choisis : 'San Castrese' et 'Orange Red'. La fin de l'endodormance a été évaluée, de décembre jusqu'au moment de la floraison, par des approches biologiques traditionnelles, telles que l'augmentation du poids frais du bourgeon floral et l'évolution du stade de développement phénologique de ce bourgeon avant et après 7 j en conditions de forçage. De plus, le pourcentage des anomalies florales a été considéré par rapport à la position du bourgeon floral sur le rameau. **Résultats et conclusion**. Les résultats ont prouvé que la position du bourgeon floral dans la frondaison et sur le rameau avait différents effets sur la rupture de l'endodormance du bourgeon floral et quelques applications pratiques peuvent être proposées pour une meilleure conception de l'échantillonnage à l'intérieur de la structure arborescente : la partie ouest de la frondaison et la moitié apicale du rameau se sont révélées être les plus aptes à permettre une croissance active plus précoce des bourgeons, permettant la prévision de la rupture de l'endodormance. L'analyse des anomalies florales a montré d'importantes différences selon la position du bourgeon sur le rameau. Les pourcentages élevés de bourgeons anormaux observés dans la moitié inférieure du rameau pourraient biaiser les résultats sur l'évaluation de la sortie de dormance du fait d'une tendance à une plus faible croissance des bourgeons floraux.

Italie / *Prunus armeniaca* / méthode / échantillonnage / stade de développement végétal / floraison / levée de dormance / facteur lié au site

1. Introduction

In temperate zones, woody species growth ceases in response to shortening daylength and/or lower temperatures, setting the buds for the next year's crop. Research has shown that acclimation and dormancy are under genetic control and that they are distinct processes, induced by plant hormones and regulated by environmental factors and abiotic stresses [1–3].

For some species such as apricot, overcoming dormancy may be problematic when cold requirements are not satisfied; as a consequence of this phenomenon, high flower bud anomalies and drop may be found [4–7]. However, the climatic conditions have not always succeeded in correlating the extent of the anomalies with chilling requirement satisfaction [8, 9].

Several methods have been proposed to define the end of bud dormancy and, therefore, the heat and chilling requirements. Recently, procedures based on biochemical approaches have been introduced, but the most widely used are the biological techniques that consist of following the increase in flower bud weight and the rate of blooming after forcing [5, 10–15]. These traditional methods to determine the end of dormancy after forcing are very simple but they are still destructive and indirect ways. Furthermore, factors such as the canopy position of buds and the periodicity of bud sampling can influence the evaluation of the break of dormancy [16].

In this perspective, many studies on temperate fruit plants have shown that different positions in the canopy and the apical and basal portions of twigs can influence flower bud development [17, 18]. Peach and walnut exhibit considerable variability in break of dormancy among buds collected at the same time on the same tree, but in different positions. In particular, a relation has been found between twig elongation dynamics and the dormancy level of buds growing on the given twig [19]. In apple, the effect of twig length and position on the tree and its angle of insertion on the vertical was observed [20]. Other authors [21] investigating apricot (cv. Rouge de Roussillon) have observed a different flower bud ontogenesis from the base to the top of annual long shoots: flower buds are bigger in the medium-apical zone of the twig, but, at blooming time, flower quality no longer depends on bud position on the tree [22]. From a methodological point of view, flower bud quality can influence the responses in terms of overcoming dormancy. Consequently, the bud sampling method can be determinant to establish the end of dormancy and the chilling requirement of a genotype.

This study was designed to identify the best sampling method for estimating and predicting early the break of dormancy in the flower buds. In particular, flower bud collection from different canopy positions was taken into account.

Our attention was also focused on floral anomalies in relation to their position on the twig, in order to identify the least variable portion of a twig and thus provide useful information for optimization of periodic bud sampling. In certain years or environmental conditions, floral anomalies, generally originated from genetic or physiological effects, may reach such high percentages as to distort the dynamic of the bud weight increase, a fundamental index to define the end of dormancy.

2. Material and methods

2.1. Experimental site and plant material

Trials were carried out in an experimental field in Venturina (Livorno), Tuscany, Italy (altitude 6 m, lat. 43° 02' N, long. 10° 36' E) during the years 1998–1999. The trees were 7 years old, grafted onto Myrabolan rootstock, and trained to the free palmette system with rows oriented east-west.

We used two cultivars characterized by different chilling requirements: 'San Castrese' (low chilling requirement), an Italian variety of Vesuvian origin, characterized by considerable adaptability to different

Table I.
Experimental design adopted for twig sampling of *Prunus armeniaca* L. according to canopy positions (sectors and tree height) and flower bud sampling according to twig position.

Twig position		Flower bud position on the twig
Tree sector	Tree height	
West (TS_w)	Top (TH_t)	Medium-apical (FB_a)
East (TS_e)	Bottom (TH_b)	Medium-basal (FB_b)

The eight resulting combinations are:
1. [$TS_w \times TH_t \times FB_a$], 2. [$TS_w \times TH_t \times FB_b$], 3. [$TS_w \times TH_b \times FB_a$], 4. [$TS_w \times TH_b \times FB_b$], 5. [$TS_e \times TH_t \times FB_a$], 6. [$TS_e \times TH_t \times FB_b$], 7.[$TS_e \times TH_b \times Fb_a$] and 8. [$TS_e \times TH_b \times FB_b$].

pedo-climatic conditions; and 'Orange Red' (high chilling requirement), a United States variety obtained from 'Lasgerdi Mashad' × ['Scout' × 'Mc Clure'], characterized by inconstant productivity and poor adaptability to Tuscan climatic conditions [23].

One-year-old mixed twigs (20–30) were periodically collected from December 1998 to February 1999 on the basis of sectors of the tree (west and east) and height on the tree (top and bottom). Flower buds were collected according to their position on the twigs (medium-apical and medium-basal sector) (*table I*).

2.2. Biological observations

2.2.1. Fresh weight of flower buds

Fresh weight was recorded on 25 whole flower buds. The weight was determined: (1) on the date of collection from the orchard, and (2) on the date of the end of forcing. Forcing was conducted on twigs kept in water in a climatized chamber for 1 week under the following environmental conditions: 23 °C, 60% relative humidity, photoperiod 12 h of light at 300–400 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Dormancy was considered broken when the fresh weight of the flower buds increased by at least 30% after 1 week of forcing.

2.2.2. Phenological stage of flower buds

On the same sampling dates the flower bud phenological stage was also recorded. After forcing conditions, the end of dormancy was defined as the transition of almost 30% of the flower buds from bud stage A to B [16].

2.2.3. Percentage and types of floral anomalies

Samples of 25 flower buds were used for observations of floral anomalies. The buds were collected from the same twigs utilized for the fresh weight analysis, supposing that damaged tissues were present. Each flower bud was dissected and observed under a stereomicroscope to classify the anomalies as necrosis or browning of different flower organs.

2.2.4. Field observations

From autumn to spring, the following data were recorded under field conditions:

– The twig length and number of flower buds per node on 10 medium-vigor twigs (60–80 cm *length*): these parameters were detected in October to calculate the “fertility Index” = [number of flower buds/cm].

– The flower bud drop (in January and at the blooming time) and blooming

Table II.
Conversion of sampling dates into number of days after the day where 50% of leaf drop was observed and accumulated chill units according to Richardson *et al.* [24] (*Prunus armeniaca* L., winter 1998–1999).

Sampling date	Days after 50% of leaf drop (DLD)	Chill unit (CU)
December 15	35	521
January 4	55	790
January 12	63	912
January 19	70	1027
January 25	76	1127
February 2	84	1209
February 9	91	1288

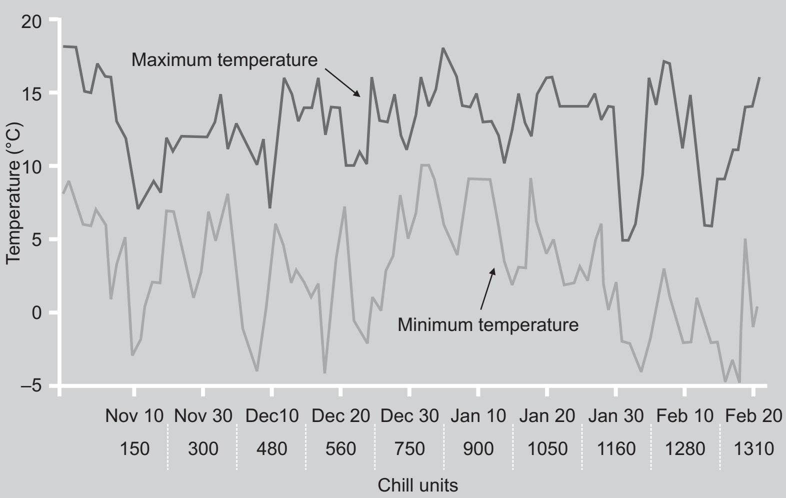

Figure 1.
Minimum and maximum daily temperatures recorded from November 10 (50% leaf drop) to February 20 (winter 1998–1999). The corresponding accumulated chill units according to Richardson *et al.* [24] are reported.

percentage: these data were recorded on several twigs labeled in early autumn, with a total of at least 500 flower buds.

– Climatic parameters: rainfall, relative humidity, minimum and maximum daily temperatures: temperatures were recorded by means of a Tinytalk System (range sensor: – 40 °C to + 125 °C, accuracy: ± 0.2 °C). Temperatures were transformed into "chill units" (CU) according to Richardson *et al.* [24], by a modified Asymkur program [25]. Calculation of CU began on November 10 at 50% of leaf drop. Sampling dates were also converted into DLD measured by the number of days after the day where 50% of leaf drop was observed (DLD = 1 for November 10).

Data were statistically analyzed and the differences between means were separated by the Student's test procedure.

3. Results and discussion

3.1. Climatic parameters

A good amount of chill units was accumulated (1000 CU on January 14; *table II*) during the winter of 1998–1999. The last frosts were recorded during the first fortnight of February, when roughly 1200 CU were accumulated (*figure 1*).

3.2. Weight and phenological stage of flower buds

Considering the flower bud samplings from different canopy positions all together, in the 'San Castrese' cultivar, the analysis of the evolution of the mean fresh weight without forcing revealed the first significant weight increase on January 19 when 1027 CU were accumulated (*figure 2*). In forcing conditions, the first sign of endodormancy breaking was represented by an increase (≥30%) in flower bud weight with respect to the weight recorded before forcing. In this cultivar, a weight increase of 45% was detected when 790 CU were accumulated on January 4. Moreover, under forcing conditions, the transition of at least 30% of the flower buds from phenologic stage A to B was recorded later, on January 12 at 912 CU (*figure 2*).

On the other hand, the 'Orange Red' cultivar showed a more delayed response (*figure 2*). Under field conditions, resumption of active growth was detected only at the last sampling on February 9. Under forcing conditions, more than a 30% weight increase was observed when 1200 CU were accumulated on February 2. At the same time, phenologic stage B occurred in 42% of the flower buds.

In the early blooming San Castrese cultivar, the forcing method confirmed the ability to establish the end of endo-dormancy considerably in advance. The other parameter utilized, namely, the phenologic stage transition (from stage A to stage B), confirmed the breaking of endo-dormancy but only slightly preceded the response of flower buds in field conditions. In the late-blooming Orange Red cultivar, the results obtained by forcing (considering both the fresh weight increase and phenologic stage methods) only slightly preceded weight increase under natural conditions. This result showed that, in this cultivar, the chill requirement is much higher than in San Castrese, confirming previous studies done on the same cultivars [26]. The fresh weight and the phenologic stage appeared to be linked in the cultivar with the greatest chilling requirement, while no relation was observed in the cultivar with the lesser chilling requirement. In this latter case, the end of endo-dormancy is delayed when the phenologic stage method is adopted, in accordance with Guerriero *et al.* [26].

By examining the percent weight increase of flower buds taken from different separate canopy and twig positions after forcing, at the time of endo-dormancy breaking, the influence of the different sampling positions was evident (*table III*). In San Castrese, where the end of endo-dormancy was recorded on January 4, 790 CU, the buds collected from the east orientation and from the medium-basal position on the twig did not reach the 30% threshold. This threshold was reached and exceeded in all other positions. In Orange Red, where the end of endo-dormancy was recorded on February 2, 1200 CU, the positive influence of west orientation and medium-apical position of the twig was even more pronounced, showing that the apical tissues are more responsive to environmental conditions [27]. Moreover, it has been demonstrated that floral morphogenesis is influenced by environmental parameters, e.g., light irradiance and/or annual light integral [28]. In this cultivar, as in San Castrese, the east and medium-basal positions delayed the endo-dormancy breaking of buds.

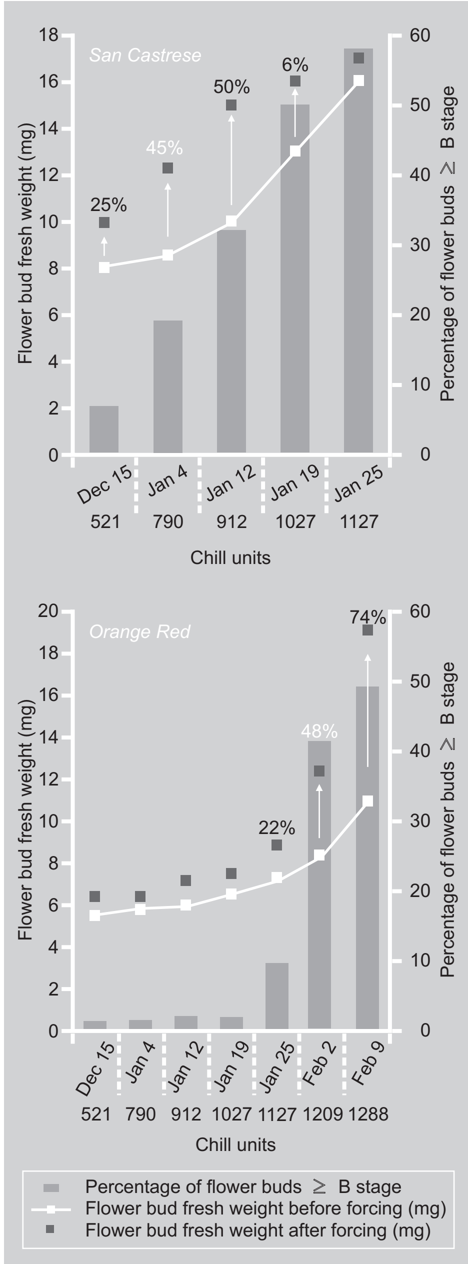

Figure 2.
Cvs. San Castrese and Orange Red (*Prunus armeniaca* L.): evolution of the mean flower bud fresh weight (mg) recorded before and after forcing with the corresponding percentages in weight increase with respect to natural conditions, i.e., "before forcing". Bars indicate the percentage of flower buds that were at the B phenological stage after forcing.

In regard to the different combinations of canopy and twig position (*table IV*), the results show that, in San Castrese, when the east sector and the medium-basal position

Table III.
Percentage of fresh weight increase (%) recorded on flower buds of *Prunus armeniaca* L. taken from different canopy and twig positions, after forcing conditions at the end of endo-dormancy: January 4 (790 chill units) for 'San Castrese', and February 2 (1200 chill units) for 'Orange Red', winter 1998–1999). Chill units were calculated according to Richardson *et al.* [24].

Flower bud position		San Castrese	Orange Red
According to the tree sector	West	60	81
	East	26	27
According to the tree height	Top	52	56
	Bottom	37	34
Flower bud position on the twig	Medium-apical	66	77
	Medium-basal	21	16

For each type of position (west/east, top/bottom, medium-apical/medium-basal), the values were separated by the Student's test procedure and the means were significant at $p \leq 0.01$.

are analyzed together [(east, bottom, medium-basal) and (east, top, medium-basal) combinations], the delayed flower bud growth is much more evident. In fact, a greater number of CU (912) was necessary to reach a 30% weight increase. Buds from other positions seemed to reveal no difference in regard to dormancy breaking, with weight increase occurring equally at 790 CU. In no case was the phenologic stage influenced by the different parts of the canopy: in all combinations the transition from stage A to stage B in 30% of flower buds was observed when 912 CU were accumulated. In Orange Red, a more variable response was observed in relation to different combinations and no differences were detected in phenologic stage transition, as in the case of San Castrese.

3.3. Floral anomalies

Flower bud anomalies appeared to be unrelated to climatic conditions from December to early February, since the conditions were favorable for flower bud development.

In general, in cv. San Castrese (*figure 3*), more than 10% of anomalies was observed as early as the first sampling, at 500 accumulated CU. This percentage gradually increased, to more than 40% in mid-January

Table IV.
Chill units (according to Richardson *et al.* [24]) recorded at the moment of the flower bud fresh weight increase and at the onset of stage B in at least 30% of flower buds after forcing of two cultivars (San Castrese and Orange Red) of *Prunus armeniaca* L.

Combination of canopy and twig position			San Castrese variety		Orange Red variety	
Tree sector	Tree height	Flower bud position	Fresh weight increase	Stage B	Fresh weight increase	Stage B
West	Bottom	Medium-apical	790	912	1209	1209
West	Bottom	Medium-basal	790	912	1209	1209
West	Top	Medium-apical	790	912	1127	1209
West	Top	Medium-basal	790	912	1209	1209
East	Top	Medium-apical	790	912	1127	1209
East	Bottom	Medium-apical	790	912	1209	1209
East	Top	Medium-basal	912	912	1127	1209
East	Bottom	Medium-basal	912	912	1209	1288

(900 CU), but subsequently decreased, as a result of damaged bud drop. For this cultivar, the percentage of bud dropped during winter was very high, about 40% (*table V*). In contrast, cv. Orange Red always showed low percentages of flower bud anomalies. Only at blooming time did marked flower bud drop occur and, therefore, the flowering percentage (*table V*) was low (14%), while in San Castrese the flowering percentage was much higher (32%). In this cultivar, the fertility index was high, particularly in the medium-apical sector of the twig: this characteristic may influence the flowering entity, in spite of high flower bud anomalies.

In San Castrese, a high percentage of browning at the base of the pistil was observed from January 26 onwards, after resumption of bud weight, when 1127 CU were accumulated. The highest percentage of pistil necrosis was not observed until February 2. These results suggest that the flower bud tissues became brown in the first step, while, in the second step, the browning tissue evolved into necrosis, followed by bud drop. Thus, it is possible that anomalous flower buds do not drop, but remain on the twig until the next observation. These anomalies cannot be attributed to frost which occurred in February (*figure 1*), after the end of endo-dormancy in San Castrese.

The analysis of results in terms of bud position on the tree (*table VI*) showed no appreciable or statistically significant differences attributable to canopy position (west/east or top/bottom). On the other hand, a greater percentage of anomalies was observed in the medium-basal position of the twig. This percentage of anomalies was particularly noticeable in the San Castrese cultivar. The greater percentage observed in the medium-basal position of the twig is in accordance with the response obtained by the forcing test, which revealed a delay in weight increase in flower buds belonging to this position. These flower buds were small, they rarely broke and, at blooming time, even though they still appeared dormant, they were all dead. These flower buds are probably in worse nutritional condition compared with those growing on the apical portion at higher light irradiance levels [29]. The difference in competition for nutrients could explain the different growth trend of buds on long shoots [21].

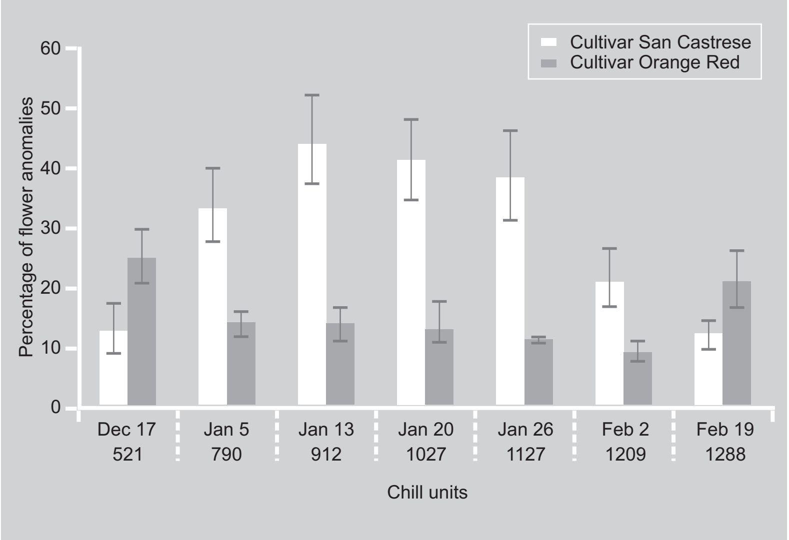

Figure 3.
Percentages of anomalies recorded in cvs. San Castrese and Orange Red (*Prunus armeniaca* L.), from December 1998 to February 1999 Standard deviation of means is indicated.

Table V.
Fertility index [number of flower buds / cm], percentage of winter flower bud drop recorded in January and percentage of blooming measured for the cultivars San Castrese and Orange Red (*Prunus armeniaca* L.).

Characteristics of flowering		San Castrese	Orange Red
Fertility Index	Total	1.2	0.7
(number of flower buds per cm)	Medium-apical	1.5	0.9
	Medium-basal	0.8	0.6
Bud drop (%)		42	19
Blooming (%)		32	14

Table VI.
Percentage of anomalies (mean of periodical samplings) recorded in the different canopy and twig positions for two cultivars (San Castrese and Orange Red) of *Prunus armeniaca* L.

Flower bud position		San Castrese	Orange Red
According to the tree sector	West	30	18
	East	28	13
	Significance	ns	ns
According to the tree height	Top	31	17
	Bottom	26	14
	Significance	ns	ns
Flower bud position on the twig	Medium-apical	18	11
	Medium-basal	40	20
	Significance	**	**

Mean values within each column (west/east), top/bottom, medium-apical/medium-basal) were separated by the Student's test procedure. Ns: not significant, **: significant at $p \leq 0.01$.

4. Conclusion

After comparing two different biological methods to determine chilling requirements, the technique based on the bud weight increase after forcing was found to be the best. The evaluation of the phenologic stage transition provided results coinciding with, or only slightly later than, the weight increase method.

Concerning the bud sampling procedure, these results showed that canopy and twig positions exerted different effects on the breaking of the flower bud endo-dormancy. Although the two varieties considered have different chilling requirements, it was found that the west orientation of the canopy and medium-apical position of the twig were the best positions to obtain an earlier active growth of buds, allowing the prediction of endo-dormancy breaking. The top of canopy position was less determinative, although it did have a positive effect on bud response.

The analysis of flower anomalies showed considerable differences depending on the position of the bud on the twig. Thus, buds growing on the medium-apical portion of the twig were bigger and had a low percentage of anomalies, while they were predominant in the medium-basal portion. The buds that developed on the first nodes at the base of the twig were small and often presented irregular growth.

Taking into account all these results, some practical applications can be drawn from them for the best sampling design inside the tree structure to evaluate the dynamic of flower bud dormancy. From the canopy, the twigs could be collected from the west orientation and in the top position. From the twigs, the flower buds could be sampled on the medium-apical position, that was the most appropriate. Moreover, the high percentages of anomalous buds observed in the medium-basal position on the twigs could distort the results on the evaluation of overcoming dormancy, due to a weaker growth trend of these flower buds.

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Influencia del muestreo en diferentes puntos de la copa para la evaluación de anomalías de la yema floral y de la latencia en el albaricoque (*Prunus armeniaca* L.).

Resumen — Introducción. Para algunas especies como el albaricoquero, la salida de latencia puede ser problemática cuando no se satisfacen las necesidades de frío. Por otra parte, la disposición de las yemas florales en la copa del árbol y en las partes apicales y basales de las ramas puede influir en su desarrollo. Las técnicas de muestreo deberían estudiarse mejor y, por ello, se evaluó en albaricoquero el efecto, en la salida de la latencia de los brotes, de distintos sectores muestreados en el árbol (oeste y este), de la altura de los muestreos (arriba y abajo) y de la parte de la rama que contenía las yemas florales observadas (mitad superior y mitad inferior). Por otro lado, las anomalías florales se estudiaron también con relación a la posición de la yema floral en la rama para identificar la parte menos variable y disponer así de información útil para la optimización del seguimiento de la floración. **Material y métodos**. Se eligieron dos cultivares de *P. armeniaca* L. con diferentes requerimientos de frío: 'San Castrese' y 'Orange Red'. Se evaluó el final de la endolatencia, de diciembre hasta el momento de la floración, mediante enfoques biológicos tradicionales, como el aumento del peso fresco de la yema floral y la evolución de la fase de desarrollo fenológico de este brote antes y después de 7 d en condiciones de forzado. Además, se estudió el porcentaje de anomalías florales con relación a la posición de la yema floral en la rama. **Resultados y conclusión**. Los resultados demostraron que la posición de la yema floral en la copa y en la rama tenía diferentes efectos en la ruptura del endolatencia de la yema floral y que se podían proponer algunas aplicaciones prácticas para una mejor concepción del muestreo dentro de la estructura arborescente: la parte oeste de la copa y la mitad apical de la rama se mostraron como las más aptas para permitir un crecimiento activo más precoz de los brotes, permitiendo la previsión de la ruptura de la endolatencia. El análisis de las anomalías florales mostró importantes diferencias según la posición del brote en la rama. Los altos porcentajes de brotes anormales observados en la mitad inferior de la rama podrían sesgar los resultados sobre la evaluación de la salida de latencia debido a una tendencia a un crecimiento más débil de las yemas florales.

Italia / *Prunus armeniaca* / métodos / muestreo / etapas de desarrollo de la planta / floración / salida de la latencia / características del sitio