

Study on the morphological evolution of bud break in *Vitis vinifera* L.

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Summary

The aims are to evaluate morpho-anatomical bud development during dormancy and to compare the heat requirement needed to start bud break in several grapevine cultivars characterised by different geographic origins. A detailed description is presented of the bud growth stages of *Vitis vinifera* cultivars to contribute to the standardisation of national and international testing systems of fruit growing. Based on the general BBCH-scale, the codes describe the first stages of bud-break in several cultivars with different geographical origins. Dormancy release was evaluated both under natural and forcing conditions, appropriate method to establish the budbreak of deciduous species. The cultivars were characterised in relation to the achievement of complete bud scale opening stage (03 of BBCH scale) which is suggested to consider as an early and indicator of budbreak.

Key words: dormancy, phenology, *Vitis vinifera* L. genotype, forcing condition.

Introduction

In temperate climates, the buds of deciduous fruit trees are dormant during the autumn and winter. It is commonly assumed that this rest period consists of an endodormancy phase, followed by an ecodormancy phase (LANG *et al.* 1987). Endodormancy involves growth-controlling perception events that are entirely within the floral bud. Chilling temperatures, during autumn and winter, are perceived by the flower bud and cumulative chilling effects (chilling requirement) are generally considered to be the main factor in the endodormancy-breaking process (TROMP 2005). Following release from endodormancy, the ecodormancy phase is associated with unsuitable environmental conditions for active growth of the floral bud, due to low temperatures, nutrient deficiency, or water stress. In standard orchard practices, the cumulative effects of moderate and high temperatures during winter and spring (heat requirement) are generally considered to be the main factors that determine the active growth phase (budbreak), resulting in flower bud opening.

The Italian germplasm of *Vitis Vinifera* L. is characterized by a high genomic diversity, resulting from natural and human selection which established a strict relationship between the cultivar and the environment. The consequence

is the presence of a considerable number of cultivars characterised by a different physiological and morphological behaviour.

In grapevine, buds become dormant as a result of prolonged exposure to short days and / or low temperatures. They will not resume growth even when apical dominance is removed when they are exposed to warm temperatures (DOKOOZLIAN *et al.* 1995). After wood maturation budburst becomes more difficult, even with optimal light and temperature conditions. In fact, the time required for them to sprout quickly increases from 8 up to 200 d, when the buds are considered in deep dormancy (POUGET 1963, 1967). Compared to other deciduous fruit species, to complete the dormancy period, grapevines require relatively short exposure to chilling, ranging between 50 and 400 h at temperatures < 7 °C (POUGET 1963). Moreover, NIGOND (1970) suggested that the range of suitable temperatures for dormancy release, could be very broad, though with variable effects over time. The number of sprouting buds usually improves with increased exposure to chilling temperatures (DOKOOZLIAN 1999), while at the end of the rest period, budbreak intensity is related to warm temperatures and characterised by heat requirement. For *Vitis vinifera* L. a base temperature of 10 °C is widely accepted, below which vegetative growth is inhibited (OLIVEIRA 1998).

In all grapevine cultivars, a hierarchy of buds is present in each leaf axil. The 'prompt bud' sometimes opens to become a 'lateral' shoot growing concurrently with the main shoot during the spring and summer. The 'dormant' or 'winter' or 'latent' bud rarely opens during its formation season, but in the next spring (LAVEE and MAY 1997, LEBON 2004). The bud system of grapevines, the so-called 'compound-bud', is made up of three meristematic apices, where the central meristem is the primary bud that opens during the next spring following its formation (MAY 2000).

Grapevine bud phenological stages were first described by BAGGIOLINI (1952) in terms of stages between 'budburst' and 'setting'. In the Baggiolini-scale, the "woolly" bud (stage B) is the first morphological change and its main limitation, even after further revision (BAILLOD and BAGGIOLINI 1993), was the lack of intermediate stages, which are necessary for an accurate and comprehensive identification of grapevine development. Subsequently EICHHORN and LORENZ (EICHHORN and LORENZ 1977, LORENZ *et al.* 1994) made a more complete and effective scheme similar to the extended BBCH scale (HACK *et al.* 1992). The new BBCH General Scale method unifies previous codes that were specific to different botanical families, into a scale appli-

cable to all plants (MEIER 2001). Different developmental stages are identified by a decimal code system (from 00 to 09 stages). On the other hand, the BBCH-scale introduces some important weak changes occurring between the dormant and wool bud stages, corresponding to various sub-phases, which as yet have not been studied accurately and which could be early signals of bud break. A standard definition of bud break is essential as a standard reference for comparison under different pedo-climatic conditions. The growing cycle depends on plant genotypes as well as on climatic conditions. To manage grapevines effectively, it is crucial to know the phenological cycle. Several phenological indicators are used to monitor and evaluate plant development (SALAZAR *et al.* 2006). Several authors (COMBE 1995, DOKOOZLIAN 1999, KOVACS *et al.* 2003) agree that the “greentip” bud stage (C for BAGGIOLINI; 4 for EICHORN-LORENZ modified, 07 for extended BBCH-scales) is indicative of ‘budbreak’ (IPGRI, UPOV, OIV, 1997), however it is a difficult stage to identify, because the period can be very short (LAVEE 1997). A recent study identified the complete bud scale opening, corresponding to stage 03, as a possible early indicator of budbreak in *Vitis vinifera* (ANDREINI *et al.* 2007).

The purpose of this paper is to evaluate morpho-anatomical bud development during dormancy to contribute to the standardization of national and international codes that describe the first stages of budbreak. The heat requirement needed to start bud break in several grapevine cultivars characterised by different geographic origins was determined.

Material and Methods

Plant material consisted of eight cultivars of *Vitis vinifera* characterized by different geographical origins (Table): ‘Cabernet Sauvignon’, ‘Sangiovese’, ‘Aleatico’, ‘Vermentino’, ‘Ansonica’, ‘Ciliegiolo’ (CALÒ *et al.* 2006), ‘Durella

gentile’, ‘Nera 1 Pi’ (SCALABRELLI and DODI 1998). Three 5-year-old plants of each cultivar (grown in 0.05 m³ pots) were used for observations. Plants were located in the experimental station of the Department of Fruit Science and Plant Protection of Woody Species (Pisa-University) in the Tuscan coastal area (Italy; altitude 6 m, lat. 43.02 N, long. 10.36 E).

Observations on bud development under field conditions were made during 2006 and hourly temperatures were recorded by automatic data-loggers, Tynitag Plus ®, West Sussex, UK, 2003). To determine the effective amounts of chill and heat, temperatures were transformed into Chilling Units (CU) and Growing Degree Hours (GDH) according to RICHARDSON *et al.* (1977) using a modified Asymkur program (PITACCO *et al.* 1992). This method was based on the accumulation of the effective chilling hours during the winter season. One Chilling Unit is equal to one hour of exposure below to 6.1 °C and the chill contribution becomes less as the temperatures rise above or fall below this threshold. When the chilling requirement is satisfied, buds become sensitive to warm temperatures with the resumption of their active growth. This model is considered appropriate under the environmental conditions of Mediterranean areas (BARTOLINI *et al.* 2006).

The determination of CU began at the end of leaf fall while calculation of GDH started on 30th Julian Day (JD 1= 1st January). This was based on the assumption that early heat is not effective in promoting budbreak (TOMASI *et al.* 2002).

Under the forcing and field conditions, phenological stages, according to the extended BBCH-scale were recorded (30 buds/per cultivar) and their morphological characteristics were observed at two-day intervals from 30 to 99 JD. The forcing test allow to evaluate the bud break in relation to the only genetic traits of each cultivar by removing the environmental factors (solar radiation, temperature variations, water availability and soil), which influence the process in field conditions.

Table

Origin, diffusion area and time of bud break of the cultivars examined

Cultivar	Origin	Diffusion area	Time of budbreak
Ansonica	Sicily	Sicily and Tuscany	2nd decade of April
Vermentino	Spain or Middle East	Liguria, Tuscany and Sardinia, Southern France, Corsica	1st - 2nd decade of April
Durella gentile	Area Lombardo-veneta	Asolo (Tr), Schio (Vi)	1st - 2nd decade of April
Aleatico	Tuscany	Tuscany (Island of Elba), Latium, Apulia, Corsica, Central Asia	1st - 2nd decade of April
Nera 1 Pi	Tuscany	Tuscany	1st - 2nd decade of April
Sangiovese	Tuscany (or Calabria)	Widespread distribution in Italy and foreign countries	1st - 2nd decade of April
Cabernet Sauvignon	France (Bordeaux)	Widely diffused in temperate areas	Late 2nd - 3rd decade of April
Ciliegiolo	Italy (Uncertain region)	Tuscany and rarely Central Italy	1st - 2nd decade of April

Forcing conditions: At 30 JD, cane cuttings (replication $n = 3$) containing 10 nodes per cultivar were collected. Forcing was conducted on two-node segments maintained in water for 18 days (repeating the cut twice) in a heat chamber in the following environmental conditions: $25\text{ }^{\circ}\text{C} (\pm 1)$, 60 % relative humidity, photoperiod 12 h of light at $300\text{-}400\ \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These forcing conditions insure the best bud development to study all growth stages. The heat requirement for growth after rest was calculated using the following formula: $\text{GDH} = 20\text{ }^{\circ}\text{C} \times n$, where n is the number of hours during which the cuttings were forced, $20\text{ }^{\circ}\text{C}$ is the maximum efficacy temperature to stimulate budbreak (RICHARDSON *et al.* 1977).

Morpho-anatomical observations (10 for each cultivar) were made on randomly distributed buds to eliminate potential variations due to the position of the nodes. Hand-cut longitudinal and transversal sections of bud tissue were immediately observed under a stereo-microscope at 10-40x magnification (Nikon HFX-II) and representative selected sections were photographed with a digital camera (Olympus C-2000 z). A comparison of the GDH accumulation in the cultivars was made using the ANOVA-test ($p \leq 0.05$).

Results and Discussion

Climatic conditions: During the 2005-06 winter period, under the environmental conditions of the Tuscan coastal area, temperatures led to a satisfactory amount of Chill Units (CU): 800 CU were recorded on 23 JD, 1000 CU on 36 JD and 1200 CU on 51 JD (Fig. 1). The climatic trend and the relative CU amount fell within a seasonal mean over several years in the same environmental area (GUERRIERO and MONTELEONE 1988, GUERRIERO *et al.* 2002).

Description of the phenological stages of the grapevine: Field observations enabled us to describe the bud evolution phenological stages (Fig. 2). Stage 00: In all cultivars, a conic shape, rounded at the base, with the brown bud scales strictly closed, characterizes the complex system of the 'dormant bud'. The 'winter bud' consists of three distinct growth points, visible in longitudinal section (Fig. 3a) and protected by internal wool and external rigid bud scales. Stage 01: At

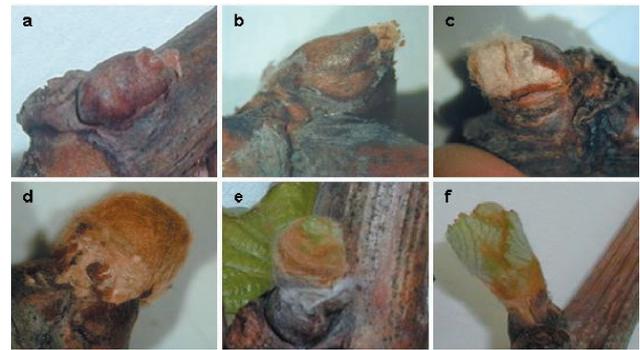


Fig. 2: Phenological stages of bud evolution: 00 winter bud (a), 01 start of swelling (b), 03 bud scale opening (c), 05 woolly bud (d), 07 green tip (e), 09 bud opening (f).

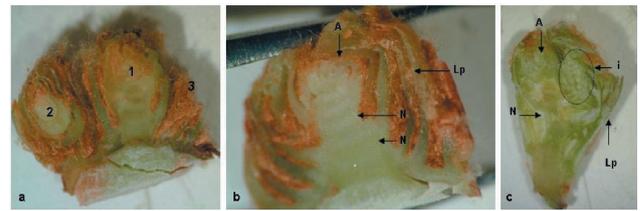


Fig. 3: Longitudinal sections of the winter bud (a). Longitudinal sections of primary buds in winter (b) and spring (c). Primary (1), secondary (2), tertiary (3) buds. Apex (A), nodes (N), leaf primordium (Lp), inflorescence (I).

the end of the rest period, a swelling of the bud and the consequent appearance of a white wool tip is the first morphological change. Stage 03: A swelling of the bud then causes the bud scales opening as a consequence, the bud enclosed by the white wool is observed. At this point, the primary and secondary buds are of a similar dimension. A longitudinal section shows the close sequence of nodes at the foliar primordia axil and the vegetative apex (Fig. 3 b). Stage 05: The bud appears enclosed by brown wool and is characterised by a complete opening of the bud scales, an increase in length of the primary bud axis, while, under normal conditions the secondary bud stays quiescent. Stage 07: This stage, the so-called "greentip" bud stage, is generally considered as the indicator of 'budbreak': the green leaf tips break through the brown wool and are just visible. In comparison to the previous stage no significant morphological changes have occurred, the bud axis only

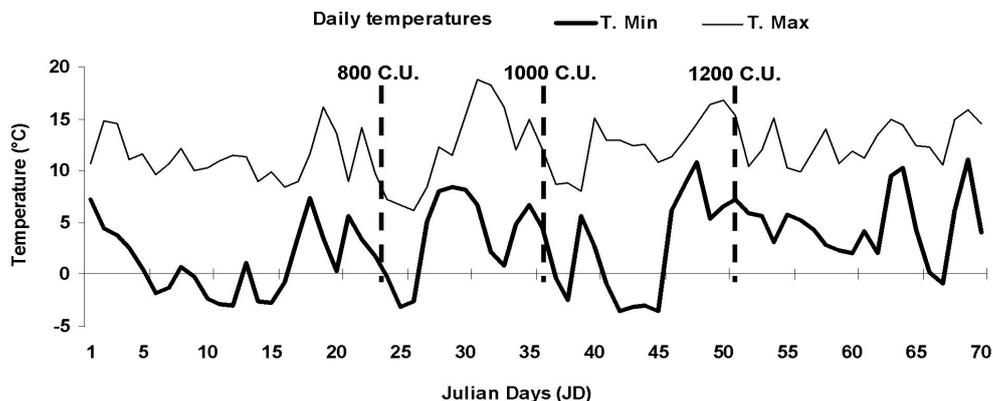


Fig. 1: Daily (minimum and maximum) temperatures recorded during winter 2006 in Pisa. The number of Julian Days (JD 1 = 1st January) for accumulation of 800 CU (23JD), 1000 CU (36 JD) and 1200 CU (51 JD) is reported.

increases to a limited extent. In all cultivars stage 07, is a difficult phase to identify, because it may be very short. Stage 09: The last phase of bud development is complete bud opening, during which the new leaves are clearly visible, the new sprout continues to grow, and in longitudinal sections (Fig. 3c) the inflorescences are evident.

Comparison of different genotypes under field and forcing conditions: Identification of the phenological stages between the dormant (00) and wool bud stage (05), was very important in order to characterise the bud development of different genotypes, under both field and forcing conditions.

During bud dormancy release, stage 03, corresponding to bud scale separation, was the stage that allowed us best to identify all varieties with a high discriminatory power. In fact, during stage 03, it was possible to characterise three statistically different groups of cultivars (early, intermediate, late) in relation to the different GDH accumulation in field conditions (Fig. 4a). ‘Vermentino’ and ‘Sangiovese’, were the earliest, reaching stage 03 with less than 3,000 GDH accumulated; ‘Ansonica’, ‘Aleatico’ and ‘Cabernet Sauvignon’ were the latest, requiring more than 5,500 GDH to achieve bud scale separation; whereas ‘Durella

gentile’, ‘Nera 1 Pi’ and ‘Ciliegiolo’ have an intermediate heat requirement, ranging from 3,500 to 4,000 GDH. In correspondence of stage 05, the GDH accumulation differences were less marked, thus it was only possible to separate the cultivars into two statistically different groups (Fig. 4b). ‘Cabernet Sauvignon’, ‘Aleatico’, ‘Ansonica’ and ‘Durella gentile’ achieved the bud wool stage with over 6,000-7,000 GDH, while ‘Vermentino’, ‘Sangiovese’, ‘Ciliegiolo’ and ‘Nera 1 Pi’ reached this stage when they had accumulated about 5,000 GDH.

Under forcing conditions, the bud development showed a different behaviour in comparison with natural conditions (Fig. 5). ‘Sangiovese’ and ‘Vermentino’ cultivars, characterised by relatively low heat requirements, under forcing conditions required more GDH accumulation to reach the same 03 stage, as also observed in ‘Ciliegiolo’ (intermediate). On the other hand, ‘Cabernet Sauvignon’ and ‘Aleatico’, cultivars that under natural conditions needed more GDH, and ‘Durella gentile’ and ‘Nera 1 Pi’, characterised by intermediate GDH requirements, under forcing reached the same phenological stage with less heat. Only the ‘Ansonica’ cultivar (late) showed a similar bud break in both experimental conditions and the 03 phenological stage was reached with the same amount of heat. These different re-

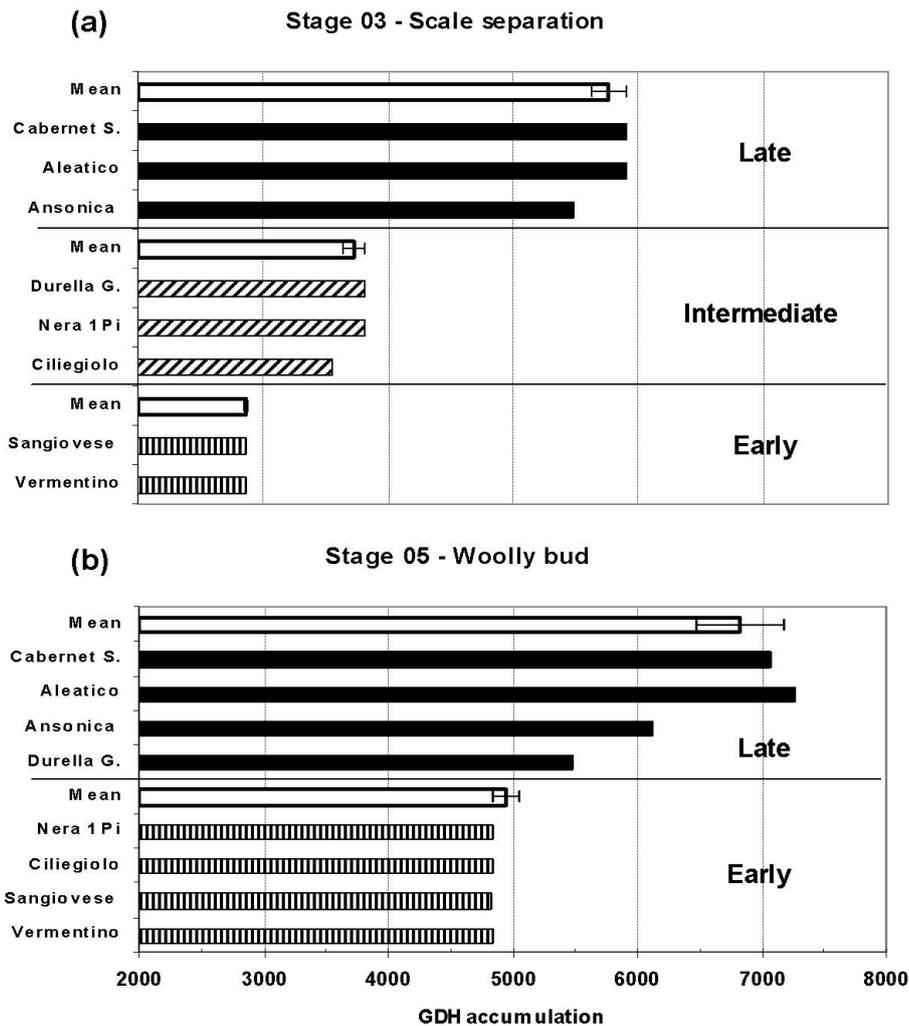


Fig. 4: Different groups of cultivars (early, intermediate, late) in relation to different GDH accumulated in field conditions to reach stage 03 (a) and stage 05 (b).

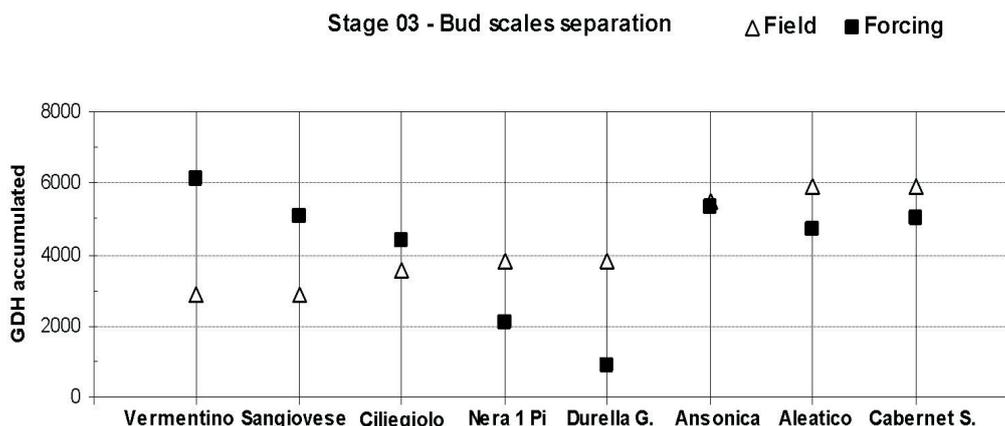


Fig. 5: Different GDH cumulated to reach stage 03 (bud scale separation) recorded under field (\circ) and forcing (\blacksquare) conditions in several cultivars.

sponses to heat under natural and forcing conditions could be attributed to the important role of roots and their phytohormones during the bud break process.

Conclusions

The forcing test enabled us to study the budbreak process in *Vitis vinifera* because remove the environmental factors influencing the bud development. No significant relation between forcing test and observation under natural conditions could be found as the important role of roots and their phytohormones can not be neglected.

This study enabled us to describe morphological bud evolution when the cultivars examined were dormant and differences in bud break between genotypes were observed. Usually the stage 07 is defined an important stage to indicate the time of bud break when at least the 50 % of the buds are at this stage (IPGRI, UPOV, OIV, 1997). Our study showed that the green tip stage can be very fast and hardly to observe. On the contrary, the stage 03, corresponding to bud scale opening showing white wool, was the stage where genotype behaviour could most easily be differentiated during the early bud break phases. Therefore at this stage 03 not always follow the regular development, if the bud is damaged. Under field conditions, when the bud regularly growth, we recommend to consider the stage 03 as the reference stage for bud break.

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