# Integrated Overview of Olive Reproductive Bud Dormancy and Biennial Bearing

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To the memory of Shimon Lavee

**Keywords:** dormancy onset, dormancy release, explants method, floral transition, *Olea europaea* L., paradormancy, winter rest

***Abstract***

**Observations of olive bud initiation, growth and dormancy and new experiments with olive shoot explants have broadened our understanding of bud dormancy in potentially reproductive buds, the role of chilling, and biennial bearing. Shoot apex and leaf removal at different times and the effect of temperature on reproductive budburst were tested on shoots and shoot explants sampled from ON (high cropping) and OFF (low cropping) trees. Overall the results indicate and/or confirm: 1) a long period of dormancy onset in the axillary buds during vegetative shoot growth; 2) floral transition of buds from undefined to reproductive is inhibited by the presence of fruits; 3) dormancy becomes generalized in all axillary buds (winter rest) with autumn low temperatures; 4) leaves play a major role in triggering and maintaining dormancy in axillary buds; 5) chilling accumulation releases potential reproductive buds from dormancy; 6) reproductive bud development is morphologically initiated after winter rest; and 7) similar dormancy release dynamics occur in potential reproductive buds on explants from shoots sampled from ON and OFF trees and in shoots remaining on those trees. Regarding the significance of cold temperature, we hypothesize that chilling-imposed winter rest impedes the initiation of growth of potentially reproductive buds in winter, promoting synchronized budburst and standardizing the timing of inflorescence and flower development in buds formed over extended time the previous year.**

## INTRODUCTION

Winter rest is an annual developmental phase in deciduous polycarpic plants, characterized by bud dormancy and the absence of leaves. Winter rest allows fruit trees to survive cold temperatures and promotes synchronized budburst once chilling accumulation removes bud dormancy and spring temperature is favorable to bud growth and development (Saure, 1985, Westwood, 1993, Faust et al., 1997, Campoy et al., 2011). In the olive (*Olea europaea* L.), an evergreen fruit tree originated and cultivated in the Mediterranean Basin (a region with mild winter temperature), winter rest is characterized by the absence of growth and generalized bud dormancy. Budburst is progressively promoted by favorable temperatures for bud growth once sufficient chilling to overcome dormancy has been accumulated (Rallo and Martin, 1991; Rallo and Cuevas, 2010).

Observations of olive bud initiation, growth and dormancy and new experiments with olive shoot explants (Ramos, 2000; Rubio-Valdés, 2009) and potted trees (Rubio-Valdés, 2009) have broadened our understanding of bud dormancy in potentially reproductive buds, and the roles of chilling, and biennial bearing. This communication summarizes our main results, validates the explant method for determining the dormancy release period, and advances an integrative hypothesis of the role of chilling during winter rest.

## MATERIAL AND METHODS

The experiments reviewed here characterize the initial growth of olive buds and subtending leaves, the onset and maintenance of dormancy in axillary buds and the dynamic of release from dormancy of potential reproductive buds after winter rest.

### Experiments to determine bud initial growth and dormancy

We studied the growth and differentiation of olive axillary buds initiated at two different times: early (5 April) and late (31 May) spring, also monitoring the growth of their subtending leaves. The experiment used current-year shoots of three OFF adult olive trees 'Arbequina' selected on those two dates, each shoot containing an apical node with a reference leaf blade length of 1 cm ± 1 mm long (reference size for leaf appearance date, LAD). Periodic measurements were made of the initially marked leaves, their axillary buds, and shoot elongation distal to that initial node.

### Experiments to determine factors affecting winter rest and dormancy release.

Different factors that might affect the buds' ability to overcome winter rest, that is chilling accumulation, bearing status of the trees, response of budburst to spring temperature, shoot apex and leaf removal at different times and the effect of temperature on reproductive budburst, were tested on shoots from ON (high cropping) and OFF (low cropping) trees using either shoot explants (Rallo and Martin, 1991, Rallo et al., 1994, Ramos, 2000; Rubio-Valdés, 2009) or potted trees (Rubio-Valdés, 2009), in 'Manzanilla de Sevilla' or 'Arbequina', two prominent cultivars worldwide. It was considered particularly important to test the explant method, frequently used to represent whole tree behaviour in investigating flower induction and dormancy.

### Anatomical observations

Sequential anatomical observations using the methods developed by Rapoport and co-workers (De la Rosa et al., 2000; Ramos, 2000; Castillo-Llanque 2003; Rubio-Valdés, 2009), described axillary bud development from initiation, until budburst the following season. The anatomical observations complement the periodical bud growth data from the previously cited explant experiments (Rallo and Martin, 1991; Rallo et al., 1994; Ramos, 2000; De la Rosa et al., 2000; Rubio-Valdés, 2009).

## RESULTS AND DISCUSSION

### Bud Initial Growth and Dormancy

Both leaves and buds reached their final size 4-6 weeks after LAD (Figures 1 and 2), showing well-coordinated timing. In contrast shoot elongation showed high variability. From that time until winter rest no further changes were observed in bud size or structure (Figure 2). Bud destiny was confirmed on similarly marked reference OFF shoots, for which most of the buds became inflorescences the following spring. This information sets a background for better exploring the processes and timing of flower induction and dormancy establishment in olive buds.

### Dormancy maintenance and reproductive budburst: the role of leaves.

Experimental defoliation and shoot decapitation from summer to winter showed the inhibiting role of each bud's subtending leaf in budburst. Prior to sufficient accumulated chilling, defoliation of the subtending leaf promoted vegetative budburst. As chilling accumulated budburst shifted progresively from vegetative to reproductive, becoming fully reproductive by the end of winter rest. These data suggest a dual role localized in the leaves: 1) a regulatory role maintaining dormancy of axillary buds (paradormancy) before chilling completion and 2) a nutritional role in the development of reproductive buds once chilling requirements are fullfilled (Rallo and Martin,1991).

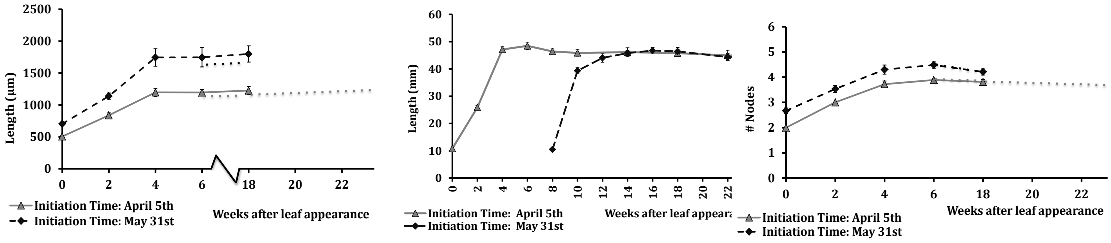


**Figure 1**. Length of subtending leaves (left) and buds (center), and number of bud nodes (right) sampled at different weeks after the two leaf appearance dates (5 April and 31 May) (From Rubio-Valdés, 2009).

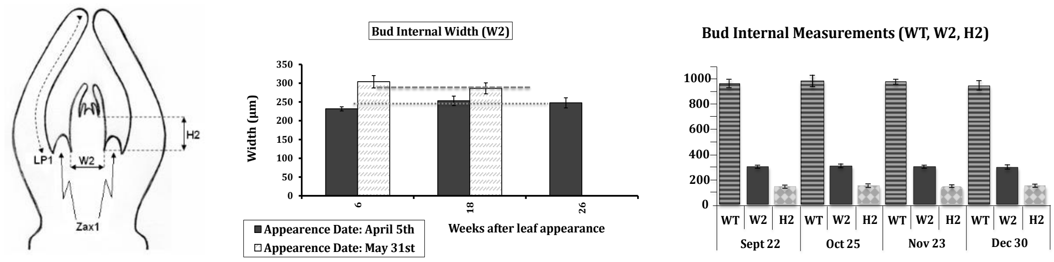


**Figure 2**. Bud internal measures (left) recorded in experiments, data from internal width (W2) along weeks after two Leaf Appearance Dates (5 April and 31May) (center) and bud internal dimensions (WT, W2 and H2) on different sampling dates in buds selected at LAD (20 May) (right) (From Rubio-Valdés, 2009).

**Floral transition to reproductive bud development: the role of fruits**

The inhibiting action of developing fruits on return bloom is well established as a major factor in olive tree biennial bearing (Almeida, 1940; Lavee et al., 1986; Dag et al., 2010; Rallo and Cuevas, 2010). Currently the absence of fruits after full bloom is considered a favorable signal for floral induction, i.e. the first step in the transition of buds from undefined to potential reproductive. (Stute and Martin, 1986; Lavee et al., 1986; Fernández-Escobar et al., 1992), (See also Amnon Habem et al. in this symposium). We hypothesize that this signal would be prior to bud dormancy onset. Based on this assumption, a long period of floral induction would begin previously to the progressive onset of dormancy in the successively initiated buds (Figure 5).

**Dormancy (winter rest) becomes generalized in all axillary buds with autumn low temperatures**

In all our experiments the onset of dormancy progressed acropetally along the shoot in buds formed in successively initiated shoot nodes. Once shoot growth ceased with mid-autumn low temperatures, dormancy became generalized in all axillary buds, as reported in deciduous fruit trees (Saure, 1985; Westwood, 1993; Faust et al., 1997; Campoy et al., 2011). We have confirmed that generalized onset of bud dormancy by low temperature by decreasing the forcing temperature from 20-22ºC to 10ºC in growth chambers experiments (Rubio-Valdés, 2009).

**Morphogenesis of reproductive buds during dormancy release**

Following the end of winter rest changes in the bud third-node (Lp3) axils were observed only in previous year OFF buds (Figure 3): On January 9 (Fig.3A), at the start of dormancy release, those buds developed a “shell-zone” of cell activity, followed on 7 February (Fig.3C), at the end of dormancy release, by a developing inflorescence branch (circle). In buds from previous ON trees (Fig. 3B and D), no morphological activity was visible (Ramos, 2000). On 9 January, when only few buds have fulfilled their chilling requirements, reproductive differentiation occurs at 20ºC explant-forcing temperature but is delayed at 30º C, a temperature that nullifies chilling accumulation. In contrast, on 7 February, when all buds have fulfilled their chilling requirements, inflorescence development proceeds faster at 30ºC than at 20ºC (Ramos, 2000; Rubio-Valdés 2009). Our data agree with previous observations that olive bud reproductive differentiation only occurs after winter rest (Almeida, 1940; Hackett and Hartmann, 1963; Fabri and Alerci, 1999; De la Rosa et al., 2000).

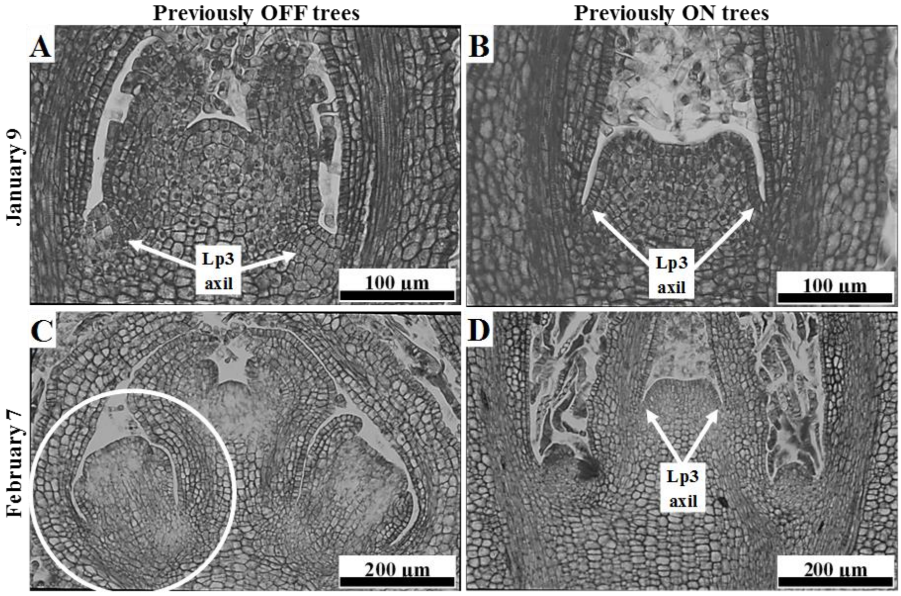


Figure 3. Differential morphological olive buds central sections from previously OFF (left) and ON (right) trees on 9 January (top) and 7 February 7 (bottom), (Toluidine Blue. Stain)

**Winter rest and release of dormancy**

The explant experiments confirm the role of developing fruits in inhibiting floral induction and support the proposed role of chilling in releasing potential reproductive buds from dormancy (Rallo and Martin, 1991; Rallo et al., 1994). Thus reproductive budburst in field tree explants sampled sequentially from mid-autumn to mid-winter and forced four weeks at 20ºC increased and ensued faster with greater chilling accumulation, with final budburst percentage much higher in OFF than in ON explants (Ramos, 2000; Rubio Valdés, 2009). Additional observations of forced explants showed that 12.5ºC was an efficient temperature for chilling accumulation and also allowed slow budburst once enough chilling was accumulated (Rallo and Martín, 1991; Ramos, 2000). In contrast, forcing at 30ºC annulled natural chilling accumulation except for samples taken very late and abundant chilling could be accumulated (Ramos, 2000; Rubio-Valdés, 2009). Dormancy release dynamics were similar for buds on explants from shoots sampled from ON and OFF trees and on shoots remaining on those trees (Rubio-Valdés, 2009).

Our results suggest a model based on the role of chilling as the driving factor for removing bud winter rest dormancy, followed synchronous budburst and inflorescence development under favorable temperatures for growth once overcoming winter rest (Figure 4). This model is supported by the best-fitted models proposed to forecast budburst (Cesaraccio et al., 2003) and bloom (De Melo-Abreu et al. 2004) times and may explain the observed asynchronous reproductive budburst in warm winter areas likely associated with insufficient winter chilling (Aybar et al. 2013), as in deciduous fruit trees (Saure, 1985, Westwood, 1993, Faust et al., 1997, Campoy et al., 2013).

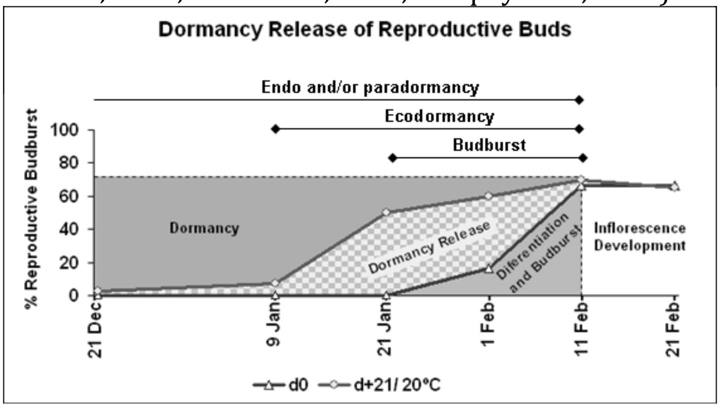


Figure 4.Periods of dormancy, budburst and differentiation for reproductive bud population; limits determined by field evaluation (d0) and after 21 d at 20º forcing conditions (d+21/20ºC)

## CONCLUSIONS

Integrating new and previous data we hypothesize a biennial reproductive cycle (Figure 5) in which: 1) axillary buds formed over a long time the first year progress from undefined to reproductive buds before the onset of dormancy unless this transition is inhibited by the presence of fruits; 2) the initial onset of dormancy in axillary buds is closely related to the development of the subtending leaves; 3) chilling-imposed winter rest generalizes dormancy to all axillary buds and impedes the reinitiating of growth of potentially reproductive buds in winter; 4) completion of chilling requirements triggers the release of potential reproductive buds from dormancy and promotes synchronized reproductive bud differentiation, thus standardizing the timing of inflorescence and flower development to provide a short and synchronized blooming period

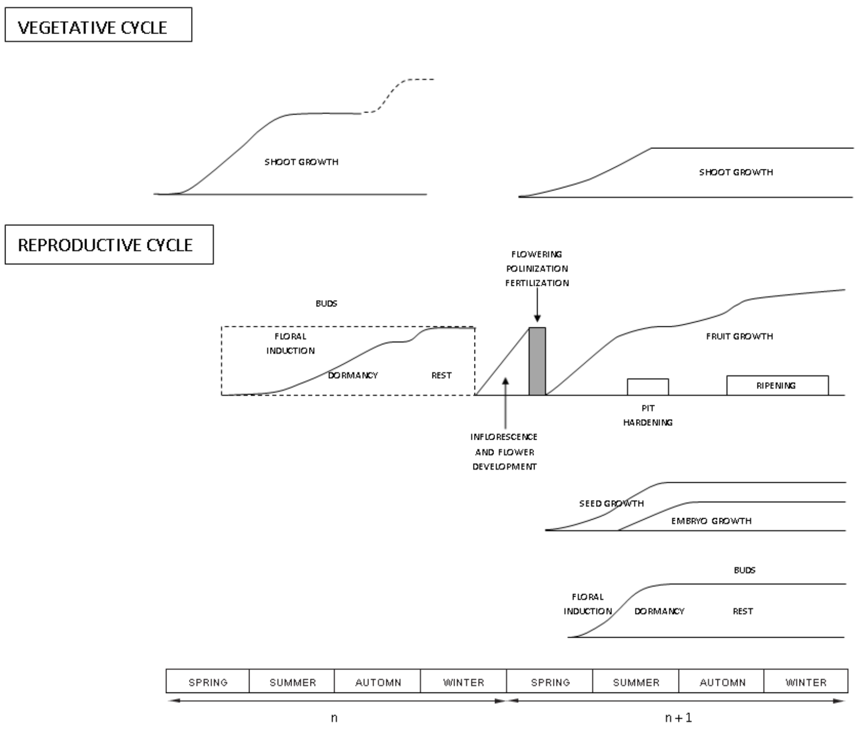


Figure5. Proposed vegetative annual cycles and reproductive biennial cycle in successive OFF (n) and ON (n+1) years

**AKNOWLEDGEMENT**

We are in debt to Diego Cabello for technical and computational assitance.

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