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Unveiling winter dormancy through empirical experiments

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ABSTRACT

Temperate woody perennials enter into a dormant status during winter in order to survive low temperatures. However, dormancy is not just a survival strategy, since cold winter temperatures are required for proper flowering. Global warming is having an impact on the phenology of woody perennials; warmer temperatures during dormancy may lead to an erratic reproductive behaviour due to the lack of chilling accumulated during winter. Although the relevance of dormancy for the adaptation of temperate woody perennials is well known, the biological processes behind dormancy remain unclear. In this work, we review how shoot and seedling experiments have contributed to the current knowledge on dormancy in woody perennials from the early discovery of the role of cold temperatures for adequate flowering to the latest knowledge on dormancy physiology and genetics. The information available has been organised in seven sections: (i) Climate change and winter dormancy in woody perennials; (ii) Discovering the importance of cold and the establishment of dormancy bases; (iii) Experiments to estimate the dormancy period; (iv) Exploring the physiology of dormancy; (v) Looking for biological markers for the dormancy status through histochemical techniques; (vi) Molecular biology of bud dormancy and (vii) Conclusions and perspectives.

1. Introduction: climate change and winter dormancy in woody perennials

One of the significant effects of global warming is the change in the phenology of woody perennials (Cleland et al., 2007). In temperate and boreal regions, rising temperatures during late winter and spring have caused earlier vegetative and reproductive timing, which continued into advance (Schwartz et al., 2006). However, warmer winters have unpredictable effects (Morin et al., 2010); mild temperatures during this period may lead to an erratic bud burst and blooming due to the lack of accumulated cold temperatures during winter dormancy. Temperate woody perennials enter a dormancy status during winter in order to survive low temperatures (Perry, 1971). However, dormancy is not just a survival strategy, since cold winter temperatures are required for proper flowering (Rohde and Bhalerao, 2007; Kurokura et al., 2013). Although the relevance of dormancy for the adaptation of temperate woody perennials is well known, the biological processes behind dormancy remain unclear (Considine and Considine, 2016).

Understanding dormancy is becoming a major issue for several scientific disciplines. From a biological point of view, the regulation of dormancy by temperatures could be involved in different processes, such as reproduction and flowering (Hedhly et al., 2009), photosynthesis (Gunderson et al., 2010; Tanino et al., 2014) and transport of nutrients (Schrader et al., 2004), since the timing of these processes needs to be adjusted to maximise the survival potential of the plant. Furthermore, changes in winter temperatures could affect the ecology from individual trees to whole ecosystems, since the timing of vegetative and reproductive phases is crucial to optimise seed set for individuals and populations (Hedhly et al., 2009). Changes in dormancy breaking may affect ecosystem stability since the cycle of some species may be altered. As a consequence, blooming periods may not be coincident, impeding adequate pollination and causing the emergence of new situations of competence for recourses (Cleland et al., 2007). Rising temperatures could extend the growing season in temperate and boreal forests (Menzel and Fabian, 1999) and disturb ecosystem-level carbon uptake (Stinziano and Way, 2017). In addition, fruit production is compromised by rising winter temperatures since the requirements of some cultivars are not being fulfilled (Campoy et al., 2011). In fruit tree species, the lack of a clear marker for the identification of breaking dormancy hampers the determination of chilling requirements of commercial cultivars, and hence the prediction of their adaptation to particular areas of cultivation, and the phenotyping of the progenies in those breeding programs whose objectives include the adaptation of cultivars to climate change conditions.

Although dormancy can be approached from different points of view (biology, ecology and agriculture), a common methodology for the

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determination of the dormancy status is the use of shoots, seedlings, rooted shoots and young potted trees, which are usually used in experiments to observe whether buds recover their capacity to grow after a certain period of chilling. Dormancy has been studied in a wide number of tree species, depending on the purpose of each study. For agricultural studies, dormancy and chilling requirements are studied at the genotype level, due to the clonal propagation of the commercial cultivars (Atkinson et al., 2013). On the other hand, in forestry, dormancy is mainly studied at the population level (Way and Montgomery, 2015). Temperature conditions are highly variable among dormancy studies, since regions of interest include temperate and cold latitudes and climates, ranging from the mild winters of the Mediterranean regions (Gannouni et al., 2017) to the long freezing period of boreal areas (Man et al., 2016). This has led to a wide variety of experimental conditions, resulting in a large amount of information available concerning dormancy in woody perennials, which is highly dispersed and therefore impedes the integration of the results.

In recent years, different reviews on dormancy in woody perennials under a climate change context have been reported, focusing on agriculture (Atkinson et al., 2013; Campoy et al., 2011), forestry (Delpierre et al., 2016) and the molecular mechanisms involved in the process (Cooke et al., 2012). However, our understanding of dormancy is fragmented, and the biology behind dormancy remains elusive. In this work, we review how shoot and seedling experiments have contributed to current knowledge of dormancy in woody perennials from the early discovery of the role of cold temperatures for adequate flowering to the latest knowledge about dormancy physiology and genetics.

2. Discovering the importance of cold and the establishment of dormancy bases

Dormancy and the effects of chilling winter temperatures were discovered in the late 18th century to the early 19th century. Different experiments and approaches, in which potted plants or shoots of temperate woody perennials were exposed to warm temperatures during the winter period, showed unexpected behaviour of plants, attracting the attention of scientists and allowing the establishment of the early bases of dormancy.

The interest of T. Knight in the ascent of sap in woody plants in of the late 18th century led him to place potted trees of different temperate woody plant species commonly cultivated in England, such as apple (*Malus x domestica* Borkh.), pear (*Pyrus communis* L.) and vine (*Vitis vinifera* L.), under warm conditions. By monitoring changes in the phenology, he first established the theory that woody perennial plants had to abscise their leaves and be exposed to a certain period of cold for proper bud burst (Knight, 1801). Despite of the importance of these observations, it was not until more than a century later that dormancy studies acquired more importance. During the early 20th century, the fact that chilling was a prerequisite for flowering was more or less common knowledge, as revealed by Rosendahl (1914) in his attempt of obtaining flowers from herbaceous perennials during winter for his botanical classes at the University of Minnesota. His studies also showed the differences in chilling requirements between species and the role of dormancy in the adaptation of species to different latitudes (Rosendahl, 1914).

The first work specifically designed for the study of dormancy was performed in Washington by F. Coville (1920a), (1920b). After failure in obtaining two reproductive cycles per year in a blueberry (*Vaccinium corymbosum* L.) breeding program, he observed that warm temperatures during winter were unsuitable for bud burst and that the dormancy status was brought by cold temperatures. Based on this, Coville designed experiments with other species, such as grouseberry (*Viburnum americanum* Mill.), tamarack [*Larix laricina* (Du Roi) K. Koch] and crab [*Malus coronaria* (L.) Mill], by submitting seedlings, potted trees and shoots to different temperature conditions and by applying different treatments such as girdling, notching or rubbing, searching for substituting the effects of chilling. These experiments not only put in relevance the process of dormancy in temperate fruit production, allowed establishing that dormancy was a prerequisite for proper flowering and subsequent fruit setting (Coville, 1920a, 1920b). These experiments established a general method for further work.

After chilling fulfilment and dormancy breaking, a certain period under mild temperatures is required for growth resumption (heat requirements) (Perry, 1971). Modelling relating warm temperatures and phenological development was first established in the early 18th century (de Reaumur, 1735). Heat requirements were further incorporated to dormancy models for the prediction of bud burst (Richardson et al., 1974). The lack of a clear biological factor associated with the dormancy status has resulted in many terms and definitions of the process (Considine and Considine, 2016; Doorenbos, 1953; Lang et al., 1987; Samish, 1954; Vegis, 1964). Nowadays, the most used terms are those proposed by Lang et al. (1987): endodormancy, referred to when the regulation of dormancy is triggered by physiological factors; ecodormancy refers to dormancy regulated by environmental factors; paradormancy is used when growth inhibition arises from another part of the plant (e.g. apical dominance) (Lang et al., 1987). Recently, other terms have been proposed, taken in account other biological processes. Thus, Rohde and Bhalerao (2007) define dormancy as “the inability to initiate growth from meristems under favourable conditions”. Also, quiescence is referred to as a condition of repressed cell division, but growth would be resuming without delay under proper conditions. In this context, dormancy would represent a state of quiescence of meristematic or embryonic organs, in which growth is not resumed even under favourable conditions until after sufficient entrainment by environmental cues (Bewley, 1997; Considine and Considine, 2016).

The relevance of dormancy for forest and crop tree species has resulted in a number of experiments focused on the effect of temperatures on phenology and dormancy overcome.

3. Experiments to estimate the dormancy period

The general approach to determine when dormancy has overcome is based on evaluating phenology and bud growth in relation to time spent at low temperatures. Potted trees or shoots are transferred at different moments along winter to growth chambers with warmer temperatures. The date of breaking of endodormancy is established when bud growth is detected after several weeks under the respective growing conditions. This approach is time-consuming, and the delay in obtaining results is not suitable for many purposes, since the date of dormancy release is obtained several weeks after it occurs. Thus, this methodology has been combined with different mathematical models that quantify chilling temperatures along winter in order to determine the chilling requirements of particular genotypes for the prediction of dormancy release in other years.

Early experiments were performed using rooted shoots of pear (Bennett, 1949; Erez and Lavee, 1971) and later in peach (*Prunus persica* (L.) Batsch) (Couvillon et al., 1975) and blueberry (Mainland et al., 1977; Spiers, 1976), in which the breaking of dormancy was established when vegetative bud burst was observed after a period in the growth chamber. Bennett (1949) indicated that the requirements for dormancy release were a temperature between freezing and about 7.2 °C (45 °F), with a period of continuous exposure of two to three months. Other studies monitored flower buds rather than vegetative buds, most of them using peach shoots. In these experiments, the date of breaking of endodormancy was established when the flower buds, after several weeks in the growth chamber, increased significantly in weight (Brown and Kotob, 1957) or showed phenological development (Bennett, 1949).

To determine which temperatures are effective under field conditions, different models have been proposed to predict the response of buds of woody plants to chilling. Models for quantifying chilling accumulation are based on the effects of different temperatures on