Quantification of Endodormancy in Seeds of Woody Plants

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Endodormancy (ED) of woody plants has many controlling and modifying factors: temperature, thermoperiod, light, photoperiod, promoters, inhibitors, seedcoats, bud scales, and immature embryos and growth apices. Since these factors vary somewhat from year to year, ED also varies slightly from year to year (Thevenot, 1982). Seasonal variables influence ED during induction, transition, and/or release. In addition, several types of dormancy may coincide. For instance, chilling apple seeds (6 °C) in coat, with coat removed, and with coat and endosperm removed results in 50% germination (25 °C) in 64, 42, and 28 days (Visser, 1954). Paradormancy (PD) removal allows ED expression. When PD effects are not removed, evaluation of the data must recognize the PD effects of testa and endosperm. This paper will focus on quantification of ED of seeds of *Malus* and *Prunus* species, and will be limited to the ED transition facilitated by chilling at temperatures between -2 and 15 °C.

Endodormancy development's first phase, induction, begins in fruit trees in mid- to late summer or early fall. It may be partially transduced by decreasing growth promoter concentrations concurrent with high inhibitor concentrations. Little is known about ED induction. Much more is known about the ED transition leading to dormancy release. The ED and anthesis phenomena are thermally driven processes that have characteristic temperature response curves (del Real-Laborde, 1989; Richardson et al., 1974, 1975; Seeley and Damavandy, 1985). Endodormancy intensity (EI) increases in the fall and decreases during late fall and winter (Hatch and Walker, 1969; Walser et al., 1981). Release occurs when the chilling requirement nears completion. Vegetative and flower bud break and seed growth initiation are gradual during the late ED transition (Seeley, 1968; Seeley and Damavandy, 1985) and may be due to the ebbing ED influence accompanied by increasing growth capability (Hanninen, 1990) or simultaneous changes in both, depending on prevailing climatic or stratification conditions.

Temperature response curves for the ED transition are not new. Hansel (1953) described one for Petkus winter rye (*Secale cereale* L.) and Schander (1955) described one for apple (*Malus domestica* Borkh.). We described one for peach [*Prunus persica* (L.) Batsch.] flower buds in 1974 in the original Utah Chill Unit model (the curve was published later by Anderson et al., 1986) (Fig. 1). Seed dormancy transition studies in several orchard species have shown the general characteristics of the temperature response curve of the ED mechanism. In general, the temperature response occurs above the freezing point of plant tissues, increasing to an optimum between 5 to 10 °C, depending on species and locale, and then decreasing to a compensation point (Abbott, 1955) at ≈ 15 °C. The transition is sigmoid with time at the optimum or equivalent temperatures ranging from ≈ 400 to 2000 h. Above 15 °C, negation of some previous chilling can occur. These ED temperature response studies were followed by similar studies that resulted in a more universal climate ED model for apple (del Real-Laborde, 1989). In these models, the initiation point for chill unit accumulation of vegetative and flower buds was the point of maximum negative chill unit accumulation. However, seeds are ED after maturation in the parent fruit; therefore, in nature, their chilling would begin with the advent of low temperatures. With manipulated seeds, the stratification time and temperature can be easily determined.

In seeds, ED is induced and probably conditioned by influences occurring during seed maturation. Come (1980-81) and Thevenot (1982) suggested that relationships between various parts of the seed

change during chilling to cause the dormancy transition. Similar changes may also cause induction. In apples, there is some evidence that embryo axis dormancy is partially controlled by the cotyledons. Cotyledon removal results in increased germination of isolated, dormant embryos (Thevenot, 1982). This evidence confirms that ED is a multifaceted phenomenon that includes a modifying PD influence. However, since only $\approx 50\%$ of the embryos grew with the most severe cotyledon removal, it is evident that other control mechanisms are also present.

Care must be taken with "ED breaking" treatments. In most cases, we have not adequately determined whether a treatment really breaks ED (the controlling mechanism of which we do not know) or if the treatment forces growth by some other mechanism (also unknown). Judgment concerning this is crucial in ED research.

In the obvious unknown ED milieu, how can we measure its intensity (EI), its transition (ET), or its release. Assuming that ED is present, the most direct method of measuring the ET is to monitor the mechanism and the parallel variations of the processes and influencing variables involved. Unfortunately, we cannot do this for we do not know the mechanism.

In the absence of direct methods, indirect methods must be used to quantify dormancy in seeds of woody orchard species. Some of them are listed below.

METHODS FOR QUANTIFYING SEED DORMANCY

Models

The chilling requirement and temperature response curves of seeds and buds of many species and cultivars have been determined experimentally. From these characterized responses, the dormancy transition of stratified seeds can be estimated from the temperatures (chill unit accumulation compared to the chill unit requirement) to which they are exposed. Models currently available, the Degree Growth Stage model (Fuchigami et al., 1982), the dynamic model of Fishman et al. (1987), and the Chill Unit model of the Utah group (del Real-Laborde, 1989), allow quantification of ED progression of various plants. The Degree Growth Stage model requires forcing of plant material to determine ED status and thus is not predictive, while the other models require calibration of each cultivar in regard to its specific ED transition temperature requirement. Prediction of ED status from climatic data is reasonably accurate in mid- to northern temperate zone climates with the Dynamic and Chill Unit models. These models have not performed as well in warm climates where extensive chill negation and uncertain climatic signals are common. Extension and refinement of these models to include ED induction and release components will increase their value.

Seed germination

Seed germination is an indirect method of determining EI and ET. Germination is a measurement of growth not dormancy. Nevertheless, a germination curve can be constructed for a stratified population of dormant seeds. The results of such experiments reflect the underlying EI and ET, but they are limited by the fact that germination is at least partially an "after the fact" indicator. Paradormancy influences may be present and must be recognized.

Germinability. This term refers to radicle growth capability following the completion of chilling and preceding the initiation of radicle elongation. Germinability may or may not mark the end of the dormant transition phase. In some cases, germination will take place without subsequent growth of the epicotyl. Partial or complete radicle ED release has occurred, but epicotyl ED may remain. Hence, germinability sometimes fails to be a reliable indicator of ED.

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Germination *sensu stricto*. This feature is present when seeds are chilled in the fruit. It is a process delimited by the completion of chilling and the initiation of radicle elongation (Tissaoui and Come, 1975); it may also mark the end of ED and the beginning of growth. However, germination *sensu stricto* is not identical with germinability. Chilling seeds in the fruit does not completely release dormancy. When seeds that have been chilled in the fruit are placed on their side with one cotyledon in contact with a medium they will germinate readily at 10 or 20 °C, but germination is much slower at 25 °C and markedly inhibited at 30 °C. With embryos having only the distal cotyledon ends in the medium, germination is partially inhibited at 20 °C and completely stopped at 25 °C. When compared with germination *sensu stricto*, chilled, fully imbibed, stratified seeds germinated rapidly at all temperatures between 15 and 35 °C (Thevenot et al., 1983). Chilling within the fruit, with limited hydration, partially breaks ED, but such seeds require a short intermediate-temperature treatment before they will germinate (Thevenot et al., 1983). Thus, whatever affects germination, may or may not also be affecting ED. In light of these facts, it is incumbent on the investigator to detail germination studies accurately and interpret results carefully. In spite of the problems involved, germinability is probably one of our best ED status indicators when done with care.

Forcing. Forcing of seeds and buds has been a common practice to determine EI. The degree growth stage model of Fuchigami et al. (1982) relies on this ability. However, in some plant materials, forcing is impossible during the period of deepest EI. Forcing is also fraught with difficulty when inappropriate temperatures are used. Almost universally, growth chamber, glasshouse, and laboratory forcing has been done at optimum growth temperatures from 22 to 27 °C. The problem with such forcing arises in plants that have the capability of further chilling or chill unit negation with low or high temperatures. Concurrent chilling or negation during forcing confounds results, especially when long intervals are involved. An appropriate forcing experiment requires knowledge of the chilling and negation response curves and the compensation point (Abbott, 1955), the forcing temperature at which no chilling or negation occurs. Few experiments have strictly adhered to these protocols.

Emergence

Growth measurements subsequent to germination, such as emergence—the eruption of the plumule from the seed or above the germination medium—shoot growth, foliation, seedling growth and abnormalities, and foliage or stem composition as indicators of ED status are further removed from ED and result in more variation being introduced when they are used. However, in some cases, analysis of ED status requires quantification of growth subsequent to germination.

Germination is most often associated with radicle growth. But, in some cases, radicle growth is not a good indicator of dormancy status. That is, entirely dormant seeds can be made to germinate without any subsequent growth. Epicotyl growth is just as, if not more, important and is governed by a different set of controls. Germination may occur, for instance, with very little subsequent epicotyl growth, with an additional chilling period being necessary to release ED of the shoot. In some experiments, we have seen epicotyl growth patterns that differed from radicle growth patterns. In one, for example, with identical chilling, epicotyl emergence occurred more rapidly than radicle growth (Frisby and Seeley, 1993).

Physiological dwarfs

Growth measurements beyond emergence have been used to determine ED in pome and stone fruit seeds. Extent of growth, while it introduces greater variation, may be required in some cases to accurately determine ED. For instance, in peach, physiological dwarfs arise due to insufficient chilling or chilling at certain temperatures. Temperature responses for physiological dwarfing at isothermal temperatures have been developed from seed chilling experiments. Chilling of peach seeds at 2 and 14 °C resulted in physiological dwarfs even after 60 days of chilling. Physiological dwarfing occurred until 35 to 40

days of stratification had been given at 0, 4, 6, 8, or 10 °C, with a subsequent linear release of physiological dwarfing symptoms with chilling at these temperatures beyond 40 days. These results indicate that the return to normal growth is connected with chilling and that the anomalous conditions are the result of ED, since physiologically dwarfed plants return to normal after being chilled.

Ultrastructural indicators

Ultrastructural changes occur in apple seeds during the ED transition. Rough endoplasmic reticulum (RER) appeared increasingly arranged in stacks as a function of time at 0 °C. These stacks were rare upon stratification at 12 °C and did not appear at all in seeds stratified at 20 °C (Bouvier-Durand et al., 1981).

Ultrastructural changes in flower buds correlate well with the end of the chilling period, with changes from smooth to rough endoplasmic reticulum, maturing of golgi bodies, disappearance of heterochromatin patches, and appearance of lysosomes coinciding with the end of the chilling requirement in 'Elberta' peach flower bud tissue (Feng et al., 1974).

Growth rate of roots

Chilling has a positive effect on the growth rate of the root, with the effect increasing with duration of chilling (Bulard, 1985). 'Golden Delicious' apple embryos, isolated from fruits stored for 15 days at 0 °C, were cultured with the radicle end in the medium in mineral agar containing sucrose at 20 g·L⁻¹. They were either directly placed at 23 °C in light or treated first at 5 °C in darkness for 3, 6, 9, or 12 weeks. Root growth increased to a maximum at 9 weeks of chilling (1512 chill units) and then decreased, probably because shoot growth began to dominate.

Increase in gibberellins

Sinska et al. (1973) determined that GA₄ in apple embryos increases rapidly to a peak at 4 weeks of chilling and then drops to a low value after 8 weeks. Bianco and Bulard (1980) found high levels of GA₄ in apple cotyledons after 4 weeks, which then decreased to prechilling levels, followed by high bound GA₄ cotyledon concentration at 8 weeks. Free axis GA₄ levels rose to 900 ng per 1600 embryos at 8 weeks and then decreased. Gianfagna and Rachmiel (1986) concluded that GA-like activity in peach... "is a part of the germination process controlling the rate of radicle and shoot emergence, as well as internode length, rather than a part of the process which triggers or permits germination to take place." They found increases in GA occurring after 35 days of chilling at 5 °C. Previous to this period, GA substitution did not result in increased germination. Percentage germination increased linearly with chilling in these seeds after the 28-day

Fig. 1. Chilling temperature response curves for seeds of Petkus winter rye (Hansel, 1953), apple (Schander, 1955), and peach (Seeley and Damavandy, 1985).

“initiation” period. Bradbeer (1988) concluded that the gibberellin synthesizing apparatus was elaborated during chilling in hazel (*Corylus avellana* L.) seeds and that subsequent higher temperatures were necessary for GA synthesis to force growth.

Gibberellin substitution compared well with chilling in breaking ED of apple seeds (Bulard, 1986), but even the highest concentration could not fully reproduce the optimum chilling treatments. In Bulard's studies, cotyledon greening, germination percentages, and extent of growth were used to determine the extent of ED. Gibberellins (GA₄₊₇) can be used to measure ED of apple embryos as measured by germination during the first 42 days of stratification at 4 °C (Rudnicki et al., 1971). After 42 days, gibberellin reversed the effects of added ABA but had no effect alone.

Sensitivity to ABA

EI may be estimated from the amount of ABA required to block germination. Sensitivity of apple embryos to ABA decreases during stratification (Rudnicki, 1969). Concentrations of ABA used to block germination ranged from 0 to 4 µg·mL⁻¹, with the higher concentration being effective even at 84 days of stratification at 2 to 4 °C. The study by Kaminski et al. (1971) using seedling growth for 6 or 12 weeks showed the same dose-response curve.

Respiration

Pear seed cytochrome oxidase respiration increases linearly over time during stratification at 5 °C but not at 25 °C. The increased growth capacity is exhibited by growth at higher growth range temperatures (Alscher-Herman et al., 1981). Cherry (*Prunus cerasus* L.) seed embryonic axis and leaf primordia respiratory capacity and respiration also increase linearly with time at 5 °C. Growth capacity also increases linearly but only after a 4-week lag. Growth capacity is accompanied by translocation of material from the cotyledons to the embryo (Pollock and Olney, 1959). Results with peach were not as conclusive. Respiration of peach seeds with testa removed increased 400% to 500% during the first 900 h of stratification, but it was not related to the bud chilling requirement (Chang and Werner, 1984). This increase might be used as a rough indicator of seed dormancy in some seeds. Respiration rates in seeds without testas were significantly higher than rates for intact seeds. After a respiratory peak at 900 h of stratification at 4 °C, seeds of all cultivars experienced a respiratory decrease followed by subsequent respiration at higher levels.

SUMMARY

ED quantification in seeds remains limited by the necessity of using indirect measurements. However, models of the phenomenon are getting better. Germinability, emergence, and subsequent growth give rough ED estimates on which the models have been built. Ultrastructural indicators and hormone measurements and manipulations have been used. And, respiration and phenological variables, such as cotyledon greening capacity, have also been suggested.

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