What's old and new about phase change and propagation©

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INTRODUCTION

Physiological aging (cyclophysis) is an important factor associated with propagation. It is important for determining the time to first flowering and therefore seed set. Equally important is the relationship between a plant's physiological age and the ability to regenerate adventitious organs (like rooting in some woody perennial cuttings). The relationship between plant age and the ability of cuttings to form adventitious roots has been known since the early 1900s and in a landmark study, Gardner (1929) established the role juvenility on rooting in cuttings from 21 tree species. Juvenility was also the subject of one of the first IPPS presentations made by F.L. O'Rourke in 1950 and published in Volume 1 of the proceedings (O'Rourke, 1950). Although there has been recent work on the mechanisms controlling phase change (especially the transition from the juvenile to reproductive maturity), many of the concepts related to the impact of juvenility on propagation have not changed in the past 70 years (Klaehn, 1962; Preece, 2003). The objective of this paper is to revisit physiological aging and discuss current methods of plant manipulation related to phase change and cutting propagation.

PLANT LIFE CYCLE

Plants transition from embryo to a reproductive mature plant through several qualitative phases (Figure 1). These have been designated as embryonic, juvenile, transition and mature (adult) phases (Davies et al., 2018; Hackett, 1985). The embryonic phase begins with sexual gamete fertilization leading to zygote formation and finally seed maturation. The juvenile phase begins with seed germination and seedling establishment. During the subsequent juvenile growth phase, the plant is not able to respond to environmental signals that would ordinarily induce flowering. This is also the phase where the plant has the highest growth rate and capacity for adventitious organ regeneration (i.e. de novo shoot or root initiation). This is followed by a transition phase that can be a few days in some herbaceous plants or decades for some woody perennials. The final phase of ontogenetic development is the mature or adult phase where the plant attains the ability to flower. However, not all plant characteristics of an adult habit appear simultaneously. The transition phase is characterized as a time where several morphological characteristics for development change asynchronously prior to the plant finally attaining a mature reproductive phase. This is most obvious in those plants with heteromorphic leaf development associated with phase transition (Figure 2). In these plants, juvenile phase leaves are distinctly different in size and shape compared to mature phase leaf morphology. However, this abrupt transition in leaf morphology usually occurs prior to attaining the ability to flower. Other important but less morphologically obvious features also change in the transition phase. Most notable is the loss or reduction in adventitious organ regeneration capacity that impacts the rooting ability in woody perennial cuttings.

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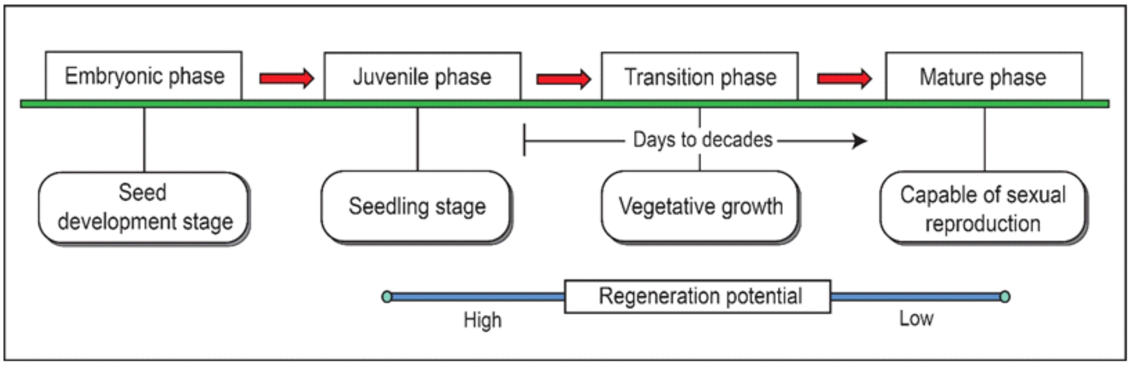

Figure 1. Schematic representation of the phase change during a plant's life cycle.

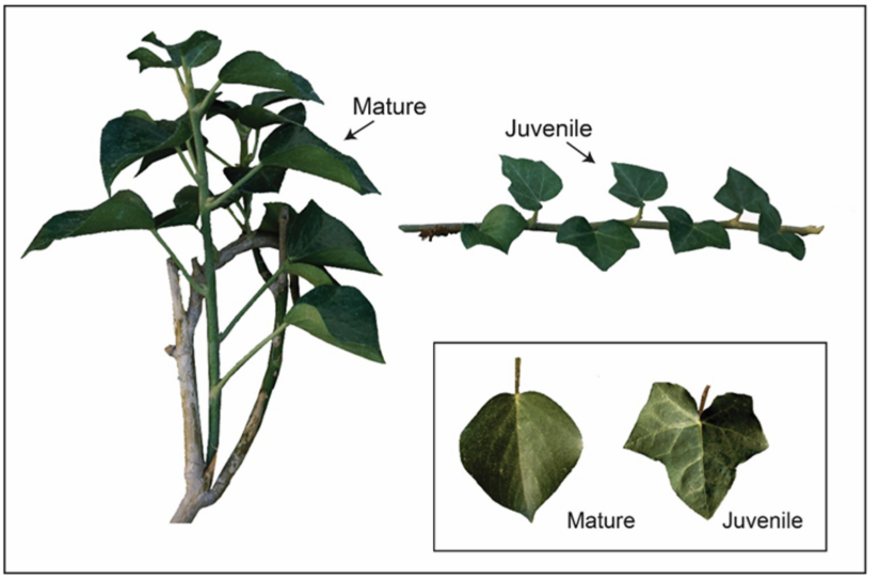

Figure 2. Leaf dimorphism related to phase change in English ivy (*Hedera helix*). English ivy has been used as a model system to study adventitious root formation because of the easily distinguished easy-to-root juvenile and difficult-to-root mature phases.

Paradox of aging in woody perennials

Although the life cycle phases of plant development appear to progress in a strict chronology related to the plant's age, chronological age does not completely describe the physiological age of all plant tissues in the plant at any given time. Therefore, in woody perennials, it is better to consider the physiological age of a specific plant tissue rather than its chronological age. In general, tissue closest to the root/shoot junction retains a more juvenile state than tissue near the distal growing shoots. This is the "cone-of-juvenility" (Figure 3). The paradox of plant age relative to position on the plant indicates that tissue that is chronologically oldest (tissue formed soon after seedling emergence) retains a physiologically juvenile state, while seasonal new growth at the top of a tree that has just recently formed would be physiologically "old" and behave as mature tissue (capacity to flower). Therefore, position on the plant is more important than strict chronological age when considering juvenility as it relates to organ regeneration potential.

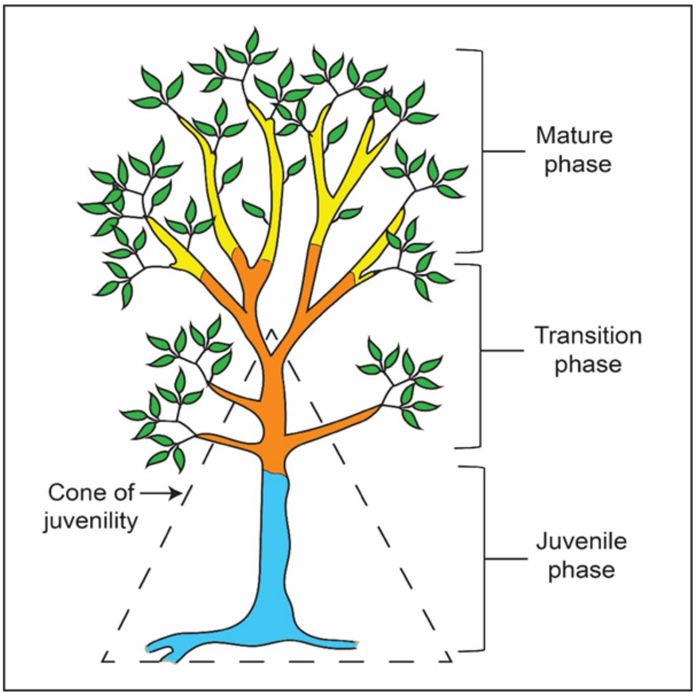

Figure 3. Representation of the cone-of-juvenility.

Juvenile phase retention

Tissue within the cone-of-juvenility naturally retain juvenile phase characteritics, but these usually remain undeveloped during the plant's growth cycle. However, some plants produce specialized structures within this juvenile zone that afford the plant certain ecological advantages usually associated with shoot regeneration. These naturally occurring juvenile tissues include epicormic shoots, sphaeroblasts, lignotubers and root suckers. Epicormic shoots develop or emerge from latent buds under the bark on the main trunk usually after biotic or abiotic stress. Sphaeroblasts are specialized tissues on the lower trunk of some tree species with a high capacity to form epicormic shoots. Lignotubers are similar to sphaeroblasts and occur at or below the soil surface at the root/shoot junction. Lignotubers are an ecological adaptation in fire prone species designed to have a high regeneration capacity and provide a cache of buds for quick reestablishment. Root suckers are adventitious shoots arising from roots. These naturally occur in plants that form clonal colonies. It is highly presumed that roots remain juvenile so shoots arising from roots retain a relatively juvenile phase character.

Systems for inducing and maintaining juvenility (rejuvenation)

One of the important aspects of phase change in propagation is loss of rooting capacity in cuttings as plants transition from juvenile to mature phases. Rooting recalcitrance in some woody plant species requires that they be propagated by seeds, grafting or micropropagation. However, it is possible to induce or reacquire juvenility in stock plants for cutting production that is more amenable to rooting. Most of these manipulations involve induction of adventitious shoots from tissue near the root/shoot junction.

Practices designed to rejuvenate stock plants that have commercial implications include (1) severe pruning or hedging; (2) induction of epicormic shoots; (3) shoots from lignotubers; (4) second generation cuttings from tissue culture; and (5) embryogenesis.

1. Severe pruning or hedging.

Severe pruning can have a dramatic rejuvenation effect on mature phase plants that are pruned near the root/shoot junction to induce stump sprouts. This is not a long-term stock plant management strategy for cuttings, but has been used to recover rooting potential in individual plants (Schreiber and Kawase, 1975). However, severe cut-back pruning is the basis for mound layering systems where plants are cut to the root/shoot junction each year to induce new shoots that root under stool bed conditions (Davies et al., 2018).

Severe pruning to produce hedged stock blocks for cutting production is the more common stock plant management system used to maintain high rooting potential. It has been recognized since the 1950s that hedging reduces or retards the progression toward a mature phase state (Libby et al., 1972). Hedging has the additional advantages of producing more cuttings per stock plant, reducing or eliminating flowering stems, and producing upright stems that produce regenerated plants with less tendency for horizontal, plagiotropic growth (topophysis), especially in conifers. Hedging remains the most important stock plant management tool for sustained production of cuttings with high rooting potential.

2. Induction of epicormic shoots.

Large severed limbs from certain hardwood species have the ability produce epicormic shoots when placed under a proper environment. These shoots when used as cuttings have demonstrated high rooting success in certain recalcitrant species like oaks, white ash, maple, and honeylocust (Preece and Read, 2007). For example, 10-cm diameter branch segments from mature red maple trees produced 6.5 shoots per hardwood stem segment and softwood cuttings taken from those shoots showed 59% rooting when treated with auxin and placed under mist (Henry and Preece, 1997).

3. Lignotubers.

Although lignotubers naturally occur in only a few species, they have played an important part in establishing stock plants for clonal *Eucalyptus* production (Assis, 2011). *Eucalyptus* are clonally propagated from “mini-hedge” stock plants that are established initially from juvenile shoots growing from lignotubers. Mini-hedge stock plants are grown using a modified hydroponic system to optimize nutrition, and cuttings are consistently removed (hedged) to keep cutting wood from maturing. This procedure has been termed “minicuttings” and they result in vigorous rooted cuttings that have better root systems compared to traditional cuttings (Cliffe, 2010). *Eucalyptus* has served as a model to develop systems to root additional hardwood species that do not make lignotubers (Chinnaraj and Malimuthu, 2011). The key aspect to adapting a mini-hedge system to a new species is to initiate stock plants from a clonal, rejuvenated source such as stump sprouts, epicormic shoots, sphaeroblasts or root suckers.

4. Second generation cuttings.

It has been well established that woody species that are difficult-to-propagate from cuttings can often be successfully micropropagated. The ability of microcuttings to form adventitious roots has been attributed to a transient reversion to a more juvenile state or “invigoration”. This rooting capacity can carryover in micropropagated plants as second-generation cuttings. Commercial propagation for second-generation cuttings has become a common way to cost effectively use plants from micropropagation, but rooting potential retention appears to be transient. Establishment of managed stock plants from second-generation cuttings would appear to be worth further investigation.

5. Zygotic and somatic embryogenesis.

As stated earlier, the natural reestablishment of the juvenile phase in a plant’s life cycle occurs during seed formation. Therefore, seedling stock plants tend to produce cuttings with high rooting potential. However, the plant characters of most interest to forestry and nursery producers such as growth habit or flowering are not evident in seedling populations. One scenario to produce clonal material with superior characteristics would be to select seedling populations with high rooting potential that are maintained as hedged stock plants or maintained in cryopreserved storage. Clonal selection can then take place as the progeny matures and superior plant characteristics become evident. This type of reverse selection has been successful for several oak species like *Quercus lyrata* and *Q. phellos* (Drew and Dirr, 1989).

Somatic embryogenesis mimic zygotic embryogenesis, but the originating tissue is vegetative rather than from reproductive gametes. Somatic embryogenesis would also reestablish the juvenile phase. Somatic embryo formation from mature plants offers the potential for clonal rejuvenation or multiplication of planned crosses from parents with elite genetics. Somatic embryo-derived seedlings can be used to establish hedged or mini-hedged stock blocks to produce clonal cuttings. This system has been developed for a few crops including conifers for clonal forestry systems (Bonga, 2014; Smith, 1999) and for mini-cuttings in coffee (Georget et al., 2017).

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