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Ethylene evolution by juvenile and adult developmental phases of *Sequoia sempervirens* shoots cultured in vitro

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Abstract. Cultures of juvenile and rejuvenated *Sequoia sempervirens* shoots generated more ethylene than those of adult shoots. But the higher phytohormone production was only indirectly related to the developmental phase. The juvenile and rejuvenated shoots also grew more rapidly; thus, when measured on a per gram tissue basis the rates of ethylene evolution were the same for tissues of both developmental phases, and even higher for one of the adults. The investigation did not establish whether the faster growth of juvenile and rejuvenated shoots was caused by the ethylene; on the other hand, there was no evidence of inhibitory effects.

Keywords: Ethylene; Gas chromatograph; Phase change; *Sequoia sempervirens*; Tissue culture.

Introduction

Several biochemical markers can now be employed to distinguish the juvenile from the mature or adult developmental phases of certain plants, e.g., differences in peroxidase and esterase isozymes (Brand and Lineberger, 1992; Huang et al., 1996) and in protein phosphorylation (Kuo et al., 1995). Furthermore, the phytohormones gibberellin (Frydman and Wareing, 1973; Rogler and Hacket, 1975a), abscisic acid (Rogler and Hackett, 1975b) and cytokinin (Bouriquet et al., 1985; Mullins et al., 1979) have been shown to be intimately involved in phase change. Gibberellin and cytokinin treatments can cause rejuvenation, whereas abscisic acid can stabilize the adult phase. This investigation was undertaken to determine whether the ubiquitous phytohormone ethylene might also have a role in phase change, inasmuch as it has been associated with diverse growth processes (Buddendorf-Joosten and Woltering, 1994; Dimasi-Theriou and Economou, 1995; Magdalita et al., 1997; Nour and Thorpe, 1994). We measured its production by aseptic cultures of juvenile, adult and rejuvenated shoots of the coastal red-wood tree, *Sequoia sempervirens*.

Materials and Methods

Shoots for the investigation were obtained from stock cultures established in vitro. Stocks of juvenile shoots were initiated from seedlings germinated in vitro; they were identified by the abbreviation SS, for *Sequoia* seedling. The adult stocks were established by culturing shoot tips excised from trees that were at least 60 years old. One stock, AS, was initiated from shoots isolated in 1976, and another, AST, was started from shoots obtained in 1994. Stocks of rejuvenated shoots, RS and 5XRT, were derived from AS and AST, respectively. The rejuvenation was achieved by repeatedly grafting the shoot tips from adult stocks onto freshly rooted segments of SS shoots in vitro. Five successive grafts resulted in the reappearance of selected juvenile morphogenetic characteristics, namely high rooting competence and rapid shoot elongation, in the RS and 5XRT shoots. The grafting procedure, previously described by Huang et al. (1992), consisted of transferring ca. 1.5 cm long adult shoot terminals onto 1 cm long, rooted and decapitated juvenile shoots. Scions were left on rootstocks after each graft for 2 months. The new growths that emerged were then severed and their shoot tips re-grafted onto fresh rootstocks.

For ethylene analysis, 2 cm long stem terminals were subcultured in 25- × 150-mm glass tubes, each containing 20 ml nutrient medium. The medium contained MS (Murashige and Skoog, 1962) salts, 3% sucrose, 0.2% Gelrite™, and in μM: 555 *i*-inositol, 3 thiamine HCl, 2.4 each of nicotinic acid and pyridoxine HCl, and 26.6 glycine. After adjusting its pH to 5.7, diluting to volume, and adding and dissolving the Gelrite, prescribed quantities of medium were dispensed into culture tubes. Tubes were capped with Bellco polypropylene closures and autoclaved 10 min at 1.05 kg cm^{-2}. The closures were not tight-fitting, so they enabled some gas exchange between the interior and exterior of tubes.

Experiments were performed at least 5 times. Ten cultures were employed for each of SS, AS, RS, AST, and 5XRT, with each culture containing one shoot. Three days prior to obtaining gas samples, the propylene closures were replaced by air-tight, serum vial caps to cause accu-

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mulation of the evolving ethylene. Gas samples of 0.5 ml per culture were obtained and analyzed for ethylene, following the method of Lin et al. (1999), and using a Shimadzu GC-8A gas chromatograph equipped with a flame ionization detector.

Results and Discussion

Earlier studies established that *Sequoia sempervirens* shoots cultured in vitro were extremely well suited for morphological, physiological and molecular biology investigations of developmental phase change of trees (Huang et al., 1992 and 1995). The initial ethylene analyses showed a direct relationship between the developmental phase of *Sequoia* shoots and the evolved ethylene level of their cultures (Figure 1). Cultures of juvenile (SS) and rejuvenated (RS and 5XRT) shoots produced significantly more ethylene per shoot than those of adult (AS and AST) shoots. When cultures were monitored through an entire passage, the difference in ethylene levels, especially between AS and juvenile or rejuvenated shoots, was evident as early as a week after the start of the passage. The difference became progressively magnified with further progress of cultures (Figure 2). At the end of the passage, or after 22 days, cultures of both adult shoots, AS and AST, displayed considerably lower ethylene levels per culture than juvenile (SS) or rejuvenated shoots (RS and 5XRT).

The differences in quantities of ethylene produced by juvenile or rejuvenated and adult shoot cultures were re-

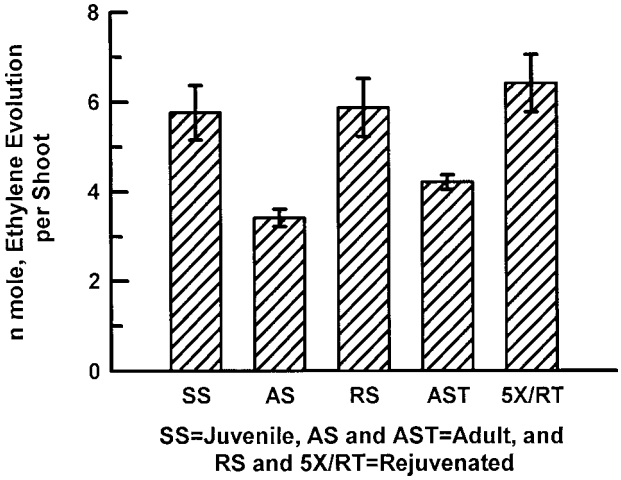

Figure 1. Ethylene evolution by *Sequoia sempervirens* shoots after 27 days of culture. SS = juvenile, AS = adult, RS = rejuvenated AS, AST = more recently established adult shoot culture, and 5XRT = rejuvenated AST.

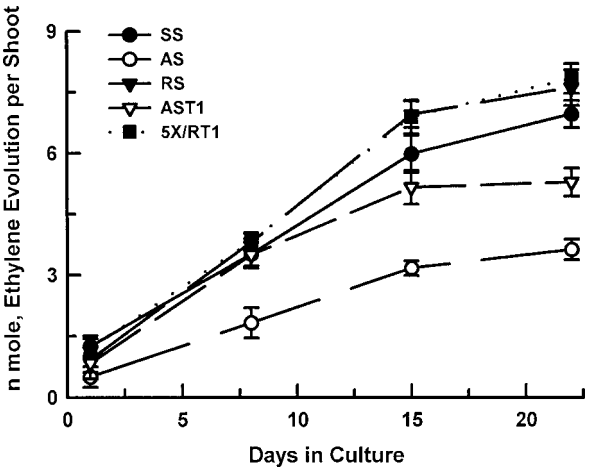

Figure 2. Ethylene evolution during the course of culture of *Sequioa* shoots of different developmental phases. Tissue labeling same as in Figure 1.

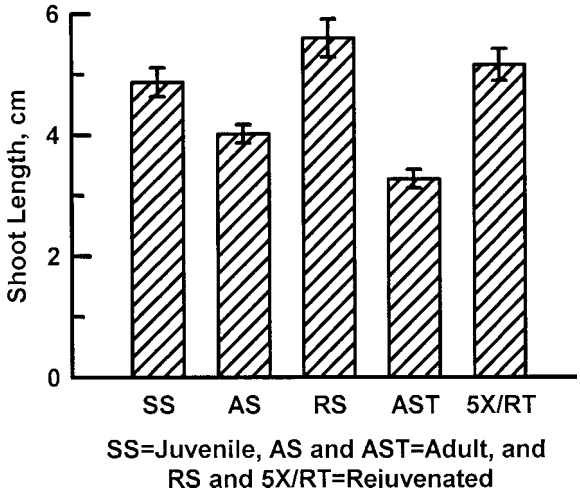

Figure 3. Growth based on shoot elongation of juvenile, adult and rejuvenated *Sequoia* shoots after 27 days in culture. See Figure 1 for tissue labeling.

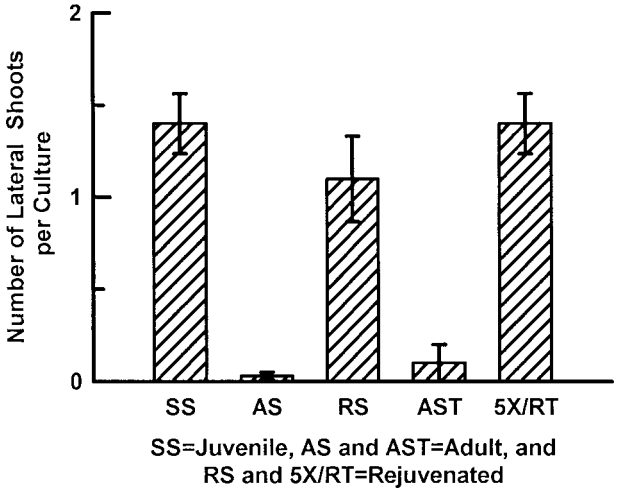

Figure 4. Number of lateral shoots produced per juvenile, adult and rejuvenated *Sequoia* shoot after 27 days in culture. Tissue labeling as in Figure 1.

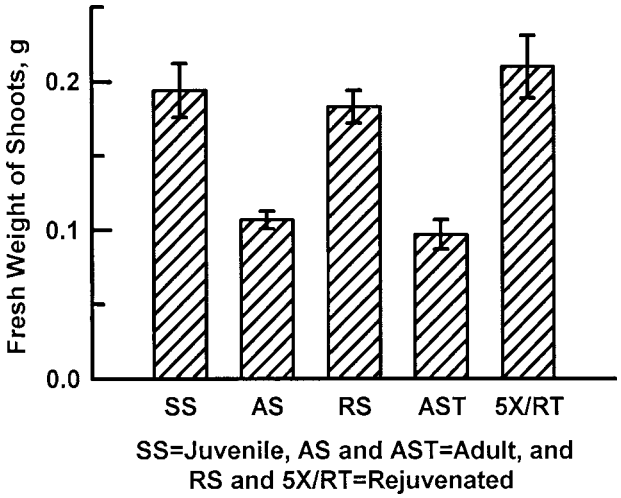

Figure 5. Fresh weights after 27 days in culture of juvenile, adult and rejuvenated *Sequoia* shoots. Labeling as in Figure 1.

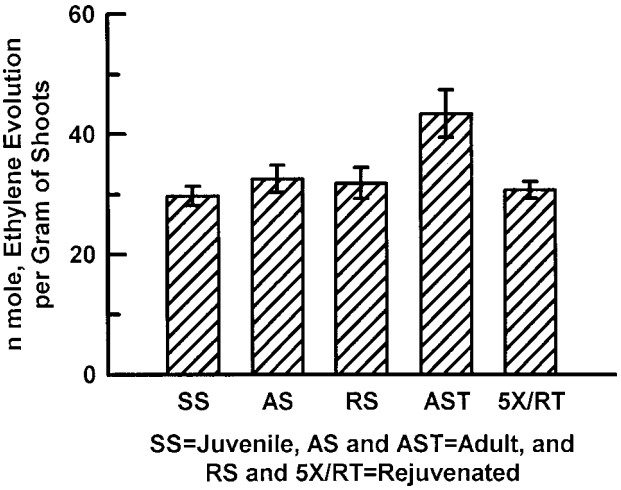

Figure 6. Ethylene evolution per unit fresh weight of juvenile, adult and rejuvenated *Sequoia* shoots after 27 days of culture. Tissue labeling same as in Figure 1.

lated to the greater stem elongation (Figure 3), as well as lateral shoot formation (Figure 4), of the former. However, when shoots were harvested and the evolved ethylene levels were expressed on a per gram fresh weight basis, the rates of phytohormone release were the same for juvenile, rejuvenated, and adult tissues (Figures 5-6). In fact, AST, or the adult shoots established in culture in 1994, showed more ethylene generated per gram of tissue than juvenile and rejuvenated shoots.

The phytohormone ethylene has been observed as having both growth promoting and inhibiting effects in vitro. Kumar et al. (1987) reported that accumulation of endogenous ethylene caused a de-differentiation to occur in otherwise organogenetic *Pinus radiata* cotyledon cultures. Similarly, Magdalita et al. (1997) attributed the poor shoot growth and early leaf senescence of *Carica papaya* nodal segment cultures to ethylene accumulation. On the other hand, Kevers et al. (1992) found that the multiplication and growth of *Rosa hybrida* shoots in vitro were enhanced by endogenous and applied ethylene. The explanation for these discrepancies has probably been provided by Lai et al. (1998). Lai et al. found that the effects of ethylene on *C. papaya* shoot cultures were temporally determined. High levels of ethylene during the initial 1-2 wk period, followed by low levels during the remaining culture period, enabled the largest number of and most vigorously developing shoots. Continuous exposure to either high or low ethylene levels produced fewer and less vigorous shoots. Although growth and proliferation of our *S. sempervirens* shoot cultures were correlated with levels of endogenously generated ethylene, we were unable to establish a cause and effect relationship. Clearly, the ethylene release rate is not a useful marker of developmental phase change of *Sequioa sempervirens.*

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試管紅杉 (*Sequoia sempervirens*) 不同生長期之乙烯分析

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幼年期（juvenile phase）之試管紅杉苗生長快速，乙烯產生量高，成齡期（adult or mature phase）之老株試管苗生長緩慢，乙烯產生量低，但老株返老還輕復幼（rejuvenation）後，植株又恢復年輕株之生長勢，乙烯產生量亦隨之升高，故植物生長相轉變時，生長勢之強弱與乙烯產生成正比，若以每克細胞鮮重為單位評估，則乙烯產生沒有差別，且成齡老樹每克細胞釋放之乙烯量高，但因生長緩慢，乙烯產生總量低。本試驗著重於植物生長相轉變時與乙烯之關係，但乙烯產生量之高低是否影響植物生長快慢，則不能直接證明。

關鍵詞：乙烯；氣相分析；生長相之轉變；紅杉；組織培養。