

# Plant Preservative Mixture™ Can Affect Shoot Regeneration from Leaf Explants of Chrysanthemum, European Birch, and Rhododendron

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**Abstract.** Plant Preservative Mixture™ (PPM), a relatively new, broad-spectrum preservative and biocide for use in plant tissue culture, was evaluated as an alternative to the use of conventional antibiotics and fungicides in plant tissue culture. Concentrations of 0.5 to 4.0 mL·L<sup>-1</sup> were tested with leaf explants of chrysanthemum (*Dendranthema ×grandiflora* Kitam), European birch (*Betula pendula* Roth), and rhododendron (*Rhododendron catawbiense* Michx.). PPM had little effect on the percentage of explants forming shoots and the number of shoots formed per explant in birch and rhododendron, but dramatically reduced both responses in chrysanthemum. Therefore, the effects of PPM must be evaluated for each species of interest prior to use.

Microbial contamination is the single most important reason for explant loss in plant tissue culture (Boxus and Terzi, 1987; Cassells, 1991). Plant Preservative Mixture™ (PPM) (Plant Cell Technology, Washington, D.C.) is a relatively new, broad-spectrum preservative and biocide for use in plant tissue culture. The active ingredients are 5-chloro-2-methyl-3(2*H*)-isothiazolone and 2-methyl-3(2*H*)-isothiazolone. PPM is effective against both bacteria and fungi, is heat stable, and, unlike conventional antibiotics, can be autoclaved in the media. These characteristics of PPM make it an attractive alternative to using conventional antibiotics and fungicides in plant tissue culture.

We were interested in using PPM in our laboratory for the following two purposes: 1) to eliminate *Agrobacterium tumefaciens* in transformation experiments involving European birch and two rhododendron cultivars; and 2) to prevent or reduce contamination in classroom demonstrations of shoot regeneration from chrysanthemum leaves. We conducted the following experiments to determine if adding PPM to media affected shoot regeneration from leaves of these plants.

## Materials and Methods

The first two or three fully expanded leaves adjacent to actively growing shoot tips of chrysanthemum (*Dendranthema ×grandiflora*

ends of the leaves were removed and a transverse cut was made across the midrib. Typical explant size was 1 cm<sup>2</sup>. Chrysanthemum and birch explants were placed with the adaxial surface in contact with the appropriate shoot regeneration medium (described below). For the rhododendrons, leaf explants were placed with the abaxial side in contact with the medium. Leaf explants were cultured in 120-mL baby food jars fitted with Magenta-B caps. Five explants for chrysanthemum and birch or six explants for rhododendron were placed in each jar. Three replicate jars were used for each PPM treatment, and all experiments were repeated twice. Explants were placed in a Hoffman germinator (model SG30S; Hoffman Manufacturing, Albany, Ore.) at 25 °C with a 16-h photoperiod and an average of 40 μmol·m<sup>-2</sup>·s<sup>-1</sup> photosynthetically active radiation (PAR) provided by fluorescent lamps. Birch explants were first placed in the dark at 25 °C for 2 weeks before being placed in the germinator.

The effect of PPM on shoot regeneration from leaves was tested by adding 0, 0.5, 1, 2, or 4 mL·L<sup>-1</sup> PPM to the media. The medium for chrysanthemum leaves contained 0.3 μM benzyladenine (BA) and 11.5 μM indole-3-acetic acid (IAA) (Trigiano and May, 1992). Birch medium was supplemented with 15 μM BA (Leege and Tripepi, 1993). Rhododendron medium was woody plant medium (WPM) (Lloyd and McCown, 1980) containing 4.9 μM indole-3-butyric acid (IBA) and 73.8 μM N<sup>6</sup>

Kitam.) ‘Iridon’, European birch (*Betula pendula* Roth), and two rhododendron (*Rhododendron catawbiense* Michx.) cultivars, ‘Album’ and ‘America’, were excised from microshoots. Both the tip and petiole

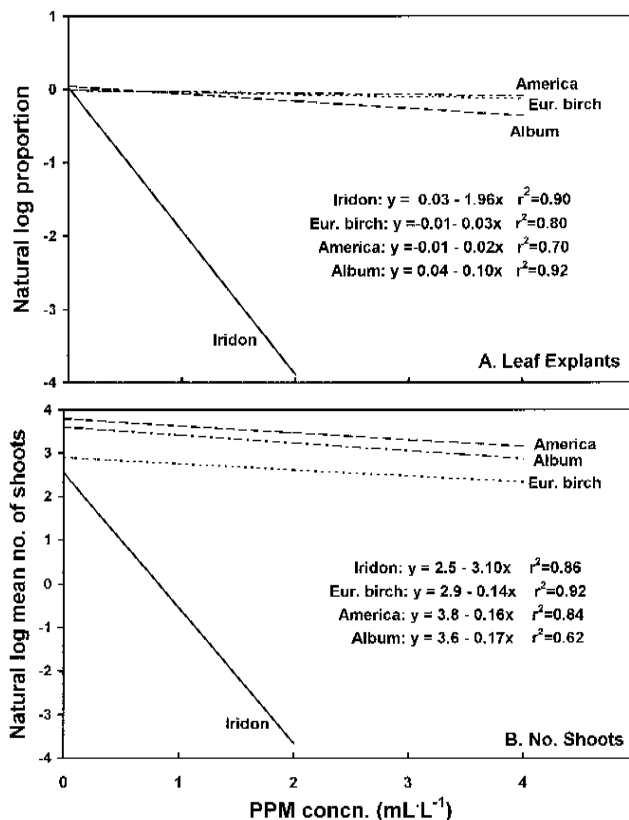


Fig. 1. Linear regression equations relating (A) the proportion of explants producing shoots or (B) the mean number of shoots per regenerating explant to the PPM concentration in the media. In the equations, y = natural log of the mean and x = PPM concentration. For (A), only the slopes for European birch, ‘Album’ and ‘Iridon’ chrysanthemum were significant at  $P \leq 0.05$ . For (B), all parameters except the slope for ‘Album’ were significant at  $P \leq 0.05$ .

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[2-isopentenyl]adenine (2iP) (Iapichino et al., 1992). In a second part of the study, only chrysanthemum leaf explants were tested for shoot regeneration on medium containing 0, 0.1, 0.2, 0.3 or 0.4 mL·L<sup>-1</sup> PPM.

The number of explants that formed shoots and the number of shoots formed per regenerating explant were recorded after 4, 6, and 10 weeks for chrysanthemum, birch, and rhododendron, respectively. A linear regression model was used as an approximation to examine the effects of PPM in the media on shoot regeneration. For the analyses, trials were combined for each species; the two separate PPM concentration experiments with chrysanthemum were also combined for the analysis. Since they were not normally distributed, the data were log transformed. The analyses of the mean number of shoots per regenerating explant were weighted by the number of regenerating explants.

### Results and Discussion

PPM had the greatest effect on shoot regeneration from chrysanthemum leaves (Fig. 1). Shoot regeneration was completely inhibited by 2.0 mL·L<sup>-1</sup> PPM. At 0.1 mL·L<sup>-1</sup> PPM, the mean number of shoots produced was reduced at least 35%. Although the number of shoots produced on leaf explants treated with 0.1 mL·L<sup>-1</sup> would be sufficient for classroom demonstration purposes, this concentration may be too low to control microbial contamination. The recommended concentration of PPM to reduce or eliminate airborne contamination in media is 0.5 to 1.0 mL·L<sup>-1</sup> (Plant Cell Technology, 1998).

As the level of PPM in the media increased, the proportion of rhododendron and birch explants forming shoots and the mean number of shoots formed per regenerating explant decreased (Fig. 1). As the concentration increased from 0 to 4.0 mL·L<sup>-1</sup> the percentage of explants regenerating shoots decreased from 97% to 87%, 67% and 88%; and the mean number of shoots per regenerating explant decreased 49%, 47%, and 53% for birch, and for 'Album' and 'America' rhododendron, respectively. The highest PPM levels used in these experiments were those recommended for eliminating *Agrobacterium tumefaciens* in transformation experiments (Plant Cell Technology, 1998). Whether or not the number of shoots produced at these levels is sufficient for transformation of European birch and of rhododendron needs to be determined. Our experiments also demonstrated that for European birch and rhododendron, PPM could be present in the medium continuously at the higher levels without seriously affecting shoot regeneration from leaves.

PPM controls microbes by penetrating the microbial cell wall and inhibiting several key enzymes in the citric acid cycle and electron transport chain (Plant Cell Technology, 1998). It may also inhibit transport of monosaccharides and amino acids from the medium into the microbial cells. The complexity of the plant cell wall apparently prevents the PPM molecules from having the same effect on plant tissues (Plant Cell Technology, 1998). However, PPM's effect on metabolic or transport pathways apparently can also affect the ability of leaf explants from certain species to

form adventitious shoots as demonstrated by the significant decrease in the regenerative ability of chrysanthemum explants. Therefore, the effects of PPM on shoot regeneration from leaves of desired species should be tested before using it in experiments or commercial production.

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