From Forest Nursery Notes, Winter 2011

197. © A technique for field collection of woody plants for micropropagation.
Westhuizen, A. and Fischer, L. International Plant Propagators' Society, combined proceedings, 2009, 59:190-191. 2010.

A Technique for Field Collection of Woody Plants for Micropropagation©

Annemarie vd Westhuizen and Lorna Fischer
Shaw Research Centre, Sappi Forests, P.O. Box 473, Howick, 3290 South Africa
Email: annemarie.van.der.westhuizen@sappi.com

One of the biggest obstacles with the initiation of woody plants in tissue culture is contamination. Even when keeping the mother material in sheltered areas, pruning to encourage the growth of new material and treating regularly with broad-spectrum fungicides, it is not uncommon to observe contamination percentages greater than 90% during initiation.

Sterilization protocols for the majority of woody plants require the use of mercuric chloride ($HgCl_2$), but this it is not an environmentally friendly option. Calcium hypochlorite [$Ca(OCl)_2$] can be used as an alternative but is not as effective. Using a sterilization protocol adapted from other woody plants, which includes the use of both calcium hypochlorite and sodium hypochlorite (NaOCl) in an attempt to initiate *Eucalyptus* species into culture resulted in 100% contamination. After applying a technique on pre-sterilization storage for 48 h in the dark developed by Watt et al. (2003) explant contamination was reduced to 9%. Although the trials were conducted only on *Eucalyptus* it is a technique that has the potential for use in initiation of other woody species in tissue culture. The aim of this study was to assess the adaptability of a reported eucalypt-field-collection technique on the successful decontamination of eucalypt hybrid material.

INTRODUCTION

Eucalyptus micropropagation is used for the mass propagation of improved genetic material used in clonal programme activities in forestry. Tissue culture techniques can be used by the forestry industry for the propagation of selected genotypes in the breeding programmes, bulking up of hybrid genotypes, or replacement of nursery hedge stock (Watt et al., 2003). For effective in vitro multiplication a process of direct organogenesis from axillary buds was used as described in Jones and Van Staden (1997).

MATERIALS AND METHODS

Surface Sterilization for Micropropagation. For eucalypts hedge plants are normally used as stock material for micropropagation, because they can be kept in optimal growing conditions. In order to harvest the best quality material, the hedges must be exposed to optimal temperature, irrigation, and fertilization regimes, determined by the specific growing conditions of the clone. A strict fungicide regime must be employed to reduce the prevalence of endogenous pathogens causing the development of contamination after initiation in vitro (Watt et al., 2003).

After new developing shoots (coppice) are harvested from hedges, they are surfaced sterilized before being paced onto initiation medium. New shoots arising from the axillary buds are then harvested and transferred onto a multiplication medium. Surface sterilization techniques are not 100% effective, but a success rate of 5%–10% can be expected with woody plants. For the surface sterilization of eucalypts a