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Efficient oleoresin biomass production in pines using low cost metal containing stimulant paste

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ABSTRACT

Oleoresin biomass production by trees of *Pinus elliottii*, which is a source of terpenes for the chemical and pharmaceutical industries, was investigated. Trees were individually analyzed for oleoresin yield using the bark streak method of wounding for stimulation of resin flow. Controls included plain wounding and wounding followed by application of commercial stimulant paste, based on sulfuric acid and synthetic precursors of the phytohormone ethylene. Metal cofactors of terpene synthases and ethylene receptors applied locally as adjuvants of the oleoresin stimulant paste on wounded bark tissue improved resin yields. The use of potassium, copper, and iron did not affect significantly the composition of semiochemical monoterpenes, which are involved with pine - bark beetle interactions, and are important as oleoresin-derived products. Some adjuvants, particularly potassium, were capable of supporting oleoresin yields equivalent to those obtained with current commercial stimulant paste, even with removal of ethylene precursor, the most expensive adjuvant.

1. Introduction

The increasing need for wood, cellulose pulp, natural chemicals, fiber, and bioenergy has prompted efforts in developing high yielding forest plantations, as well as a search for alternative plant sources for these products [1–3]. Non-timber forests products have also been drawing considerable attention, including as a means to replace fossil-fuel-derived materials [4]. In this scenario, pine oleoresin seems to be a copious, readily available, and profitable biomass alternative, which can be extracted for several years throughout tree life.

Pine oleoresin is made of a complex mixture of terpenoids, consisting of turpentine (monoterpene, C_{10}, and sesquiterpene, C_{15}) and rosin (diterpene, C_{20}) fractions [4,5]. The potential of hydrocarbons and turpentine derived from oleoresin as biofuel is also relevant [4], as exemplified by the development of efficient methods of obtaining very high density biofuels from selective dimerization of pinenes [6], major components of pine oleoresin. Tapping pine trees to obtain terpene biomass is an activity well suited to countries with a standing resource of pines, leading to many economic and social benefits [7]. Brazil has extensive areas of tree plantations (around 7 million ha), which, in addition to wood products, yield resin, rosin and gum turpentine [8]. The national resin yield in 2009 was approximately 83 400 t, and the average price was 470.22 $ t^{-1} [9].

The pine rosins are abundant natural chemicals that have many industrial applications including synthetic rubber,

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coatings, paper sizing, polymerization emulsifiers, adhesive tackifiers, printing ink resins, and waterproofing material [10]. The pine turpentine has been traditionally employed as a solvent or cleaning agent for paints and varnishes or in the pharmaceutical industry [11]. Turpentine can be fractionally distilled to provide its major constituents, α- and β-pinene in pure form. Alpha and β-pinene are an important source of intermediates and ingredients for flavors and fragrances [12]. This industry consumes approximately 30 kt y^{-1} of pinenes, which are used to produce a diverse range of products [13,14].

Commercial resin tapping is normally carried out on the trunk of fully-grown trees. The bark is mechanically removed exposing the under-wood surface. In the "bark streak" system [10], 2.5 cm wide strips of pine bark are removed from about one-third of the diameter of the tree. The area from which bark is removed during the production season is called a face [15]. Resin extrudes from resin channels and drips into an open plastic bag, placed on the wound base and attached to the tree by a metal wire (Fig. 1). To increase resin flux and to extend the extruding period, a chemically active paste containing sulfuric acid is applied to the exposed fresh surface. The extrusion of resin peaks immediately after the cutting, decreasing with time. New bark removals are made in the trunk in an area immediately above the previous cutting, at intervals of 15 days [16]. Since the 1980s, 2-chloroethylphosphonic acid (CEPA) (CAS No. 82375-49-3), an ethylene-releasing compound [17], is also commonly present in the commercial stimulant paste [15,18]. In temperate zones, this activity is seasonal and extends from spring to autumn, the growing seasons [12,13]; however, the mild winter in southern Brazil can be a productive season, improving yearly yields by approximately 20% in relation to the total oleoresin biomass produced from spring to autumn [19].

Fig. 1 – Resin collection system.

Resin terpenes are derived from isopentenyl diphosphate (IPP) produced by the mevalonate pathway in the cytosol-endoplasmic reticulum or by the deoxi-xylulose-5-phosphate pathway in the plastids [5]. The enzymes relevant to resin terpene biosynthesis include monoterpene synthases, sesquiterpene synthases and diterpene synthases. Although these enzymes lead to a variety of different products, some of their biochemical properties are shared due to a partial conservation of catalytic mechanisms. All three enzyme classes require divalent cations for catalysis. Pine monoterpene synthases require divalent cations (Mg^{+2}, Mn^{+2}, or Fe^{+2}) and activity is improved by K^{+} [20].

Members of the genus *Pinus* constitutively produce and store copious amounts of resin [5]. Pine resin production from species growing in tropical and subtropical regions has been less studied than those from temperate regions [21]. In the former regions, however, bark beetles, that cause massive perforation of the bark and vector pathogenic fungi, are not a major threat as in North America [5].

A large field experimental study on oleoresin tapping yields of southern Brazil was carried out with approximately 3000 28-year old *Pinus elliottii* var. *elliottii* (slash pine) trees. This study aimed at identifying chemical adjuvants, based on terpene synthases and ethylene receptor metal cofactors, for reducing costs and improving the stimulant paste commercially used in the tapping practice. Effects of the use of these adjuvants in stimulant pastes were also examined in relation to the composition of major monoterpene components of the resin, not only because of their value for the chemical industry, but also due to their importance as semiochemicals in the interaction with bark beetles where these occur.

Experiments were carried out in a totally randomized layout, using trees of internal areas of pine plantations to avoid forest border effects. This layout was chosen on the basis of homogeneity of trees (similar size, age and genetic origin) and soil properties within forest sites. A total of 40–50 randomly selected trees were used per treatment, and, to further ensure reproducibility of results, experiments were independently replicated in two different sites (totalizing 3320 trees).

2. Materials and methods

2.1. *Plant material and resin tapping operation*

Resin tapping was done by the "bark streak" system in 28 year-old slash pine (*P. elliottii* Engelm. var. *elliottii*) trees, grown in Rio Grande do Sul (southernmost state of Brazil), city of São José do Norte (approximately 32° of south latitude and 52° of west longitude) [21]. All forests used in the study had not been tapped for resin prior to the experiments. Stimulant pastes produced with different chemical adjuvants (Table 1) were applied on the wounded tissue. Every experiment had two control treatments: a negative control consisting of bark streak without paste application, and a positive control using the current commercially exploited stimulant paste (a mixture of pyrogenic silica, emulsifier, and active ingredients