Industrial Crops & Products 118 (2018) 20–25

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Biomass yield of resin in adult *Pinus elliottii* Engelm. trees is differentially regulated by environmental factors and biochemical effectors

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ARTICLE INFO

Keywords:
Pinus elliottii
Biomass
Terpene resin
Seasonal
Benzoic acid
Regenerated forest

ABSTRACT

Biomass of pine resin finds several applications in the chemical, pharmaceutical, biofuel and food industries. Resin exudation after injury is a key defense response in Pinaceae since this complex mixture of terpenes has insecticidal, antimicrobial and wound repair properties. Resin yield is increased by effectors applied on the wound area, including phytohormones and metal cofactors of terpene synthases. The interaction of resinosis mechanism effectors is not fully understood, particularly in adult forest setups under natural environmental variations. The aim of this work was to determine how resin exudation by wounded trunks of adult *P. elliottii* responded to combined chemical effectors involved in different regulatory pathways of resinosis (metal cofactors of terpene synthases, benzoic acid and plant growth regulators) and whether seasonal and tree distribution variations affected these responses. Symmetrically planted and scattered trees regenerated from the seed bank had similar resin biomass yields, suggesting that the homogeneity in development and spatial arrangement were not significant factors in resin yield. This new finding is of practical importance with the used tapping system since costs of implanting forests by regeneration can be advantageous compared to planting. In addition, it was shown for the first time that the salicylic acid precursor benzoic acid and the auxin naphthalene acetic acid promoted resin exudation when individually applied to wound sites. Both these adjuvants are two orders of magnitude less costly compared to the conventionally used ethylene precursors, besides facing less environmental and health restrictions for use. Most adjuvant-treated trees showed higher resin flow in the second year, indicating mechanisms of response build up. Overall, temperature was more important than rainfall as environmental parameter affecting resin biosynthesis, which was higher in the warmer months of spring and summer. The combination of resinosis stimulant effectors from different signaling pathways showed no significant synergistic or additive effect, suggesting possible converging signaling pathways and/or limitation of common intermediate transducing molecules.

1. Introduction

Pines occupy highly diverse environments, over a range of temperatures, water and nutrient availabilities, irradiance levels and photoperiods, being able to effectively face attacks from diverse herbivore and pathogen guilds. The success of conifers is linked to their complex terpene biochemistry hosted by specialized secretory cells. The terpenoid resin synthesized by *Pinus* spp. is one of the main mechanisms of defense of these trees, particularly against bark beetles and the fungi they carry (Fett-Neto and Rodrigues-Corrêa, 2012). Pine resin biomass is essentially composed of a monoterpene and sesquiterpene-rich turpentine and diterpenoid-rich rosin fraction, both finding numerous industrial applications as non-wood forest products (Rodrigues-Corrêa et al., 2012).

Molecules capable of modulating different signaling pathways have been identified as resin yield stimulators, including sulfuric acid (extends wound damage), 2-chloroethylphosphonic acid (CEPA, a synthetic ethylene precursor), paraquat (free radical generator), yeast extract (mimics attack by pathogens), salicylic acid (pathogen signaling molecule), auxin (promotes ethylene biosynthesis and resin canal differentiation), jasmonic acid (signals mechanical damage and promotes secondary metabolism) and metal ions, such as potassium, iron and manganese (cofactors of terpene synthases in conifers) and copper (a component of ethylene receptors) (Clements, 1970; Conrath et al., 2002; Fett-Neto and Rodrigues-Corrêa, 2012; Hudgins and Franceschi, 2004; Lewinsohn et al., 1994; Martin et al., 2002; Popp et al., 1995;

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https://doi.org/10.1016/j.indcrop.2018.03.027
Received 12 December 2017; Received in revised form 9 March 2018; Accepted 13 March 2018
0926-6690/

Table 1
Resinosis effectors, concentrations and functional rationale.

Chemical adjuvant	Concentration	Activity	Recent source
Potassium sulfate (K_2SO_4)	500 mM	K: activation of terpene synthases	Rodrigues et al. (2011), Savage et al. (1994)
Copper sulfate ($CuSO_4$)	10 mM	Cu: part of ethylene receptor	Rodrigues et al. (2011), Rodriguez et al. (1999)
Naphthaleneacetic acid	1 mM	increases ethylene biosynthesis and resin canal differentiation	Chae and Kieber (2005), Fahn (1982)
Benzoic acid	10 mM	phytoalexin and precursor of salicylic acid, a defense phytohormone	Franich et al. (1986), Sha (2003)
Chloroethylphosphonic acid (CEPA)	3% (w/v)	releases ethylene, a defense-related phyhormone	Chae and Kieber (2005)

Rodrigues et al., 2011; Rodrigues et al., 2008; Rodrigues and Fett-Neto, 2009; Stubbs et al., 1984). Research efforts aiming at finding lower cost resin stimulant adjuvants with equivalent or superior performance of CEPA (most used currently) are of interest to the tapping industry. Although synergistic responses in wound-related resin production by supplying combined metal cofactors of terpene synthases were not observed (Rodrigues-Corrêa and Fett-Neto, 2013), simultaneous treatments with effectors acting in different signaling pathways (for example, metal ions and auxin) have not been previously examined. In addition, it is not clear how wound-related resin biosynthesis and accumulation under field conditions in adult trees of subtropical introduced slash pine plantations is affected by climate factors, namely temperature and precipitation.

To better understand the roles of phytohormones and some of their precursors, receptor components and metal cofactors of terpene biosynthetic enzymes on wound response defense resin flow in relation to climate, this study investigated the following hypotheses: a) resin biomass stimulators involved in different signaling response pathways are more effective combined than alone; b) stimulant effect of resin flow stimulators is season dependent; c) temperature is more significant in modulating resin production than rainfall. The study examined adult 16-year-old slash pine forests derived from planted seedlings or regenerated from the seed bank, submitted to one of 9 treatments, for 2 years, and involved over 1600 individually tracked trees.

2. Materials and methods

2.1. Plant material, wounding procedure, resin harvest and quantification

Slash pine (*P. elliottii* Engelm. var. elliottii) trees (16 years old), grown in two forest stands in northeastern Rio Grande do Sul, city of Balneário Pinhal (approximately 30.19° south latitude and 50.36° west longitude) were used in the experiments. The forests of similar genetic composition were in the installations of Celulose Irani S.A. One of the stands was established by regeneration of individuals from the seed bank previously deposited by the last felled forest, whereas the second tree stand originated from planted seedlings. The effect of different individual or combined effectors that operate in diverse physiological pathways (benzoic acid, plant growth regulators, co-factor metals of terpene synthases) was evaluated on resinosis. Potential synergism between pathways on resin production was examined. Randomly distributed trees (a total of 1620, being 900 trees in the regenerated forest and 720 trees in the planted forest) with circumference at breast height ranging from 65 to 90 cm were used. Trees were evaluated for two years and the experiments started in winter (2013). All trees used in the study had not been damaged or explored for resin prior to the experiments and shared the same previous history, except for the mode of stand establishment (regenerated or planted). A total of 80 and 100 trees from planted and regenerated forest, respectively, were used in each treatment.

Bark strips approximately 2.5 cm wide and 2–5 mm high were removed biweekly with a bark shaving tool, corresponding to approximately one-third of the tree circumference, exposing the sapwood surface. Seasonal resin production was evaluated for a period of two years (2013–2015). At the end of each season (winter, June–August; spring, September–November; summer, December–February; fall, March–May, approximately 90 days each), accumulated resin in plastic bags belted under the wounding panel was harvested and weighed on a field digital balance after careful removal of the upper layer of rainwater, which minimized resin volatile loss during the experiments (Rodrigues et al., 2008). Plastic bags were regularly checked and replaced as required to avoid any resin leak.

2.2. Treatments

Nine treatments were evaluated in each forest type. Pastes produced with different chemical effectors alone or in combination were applied on the wound panel right after each bark stripping (Table 1).

Each treatment paste contained 20% sulfuric acid in aqueous solution as basal active constituent and rice husk powder as an inert substrate to optimize paste consistency and residence time on the wound line (Fuller et al., 2016). Single modifications in the composition of the resinosis paste were done by including: K (500 mM potassium sulfate); BA (10 mM benzoic acid); Cu (10 mM copper sulfate); NAA (1 mM Naphthaleneacetic acid); K + BA (500 mM potassium sulfate + 10 mM benzoic acid); K + BA + Cu (500 mM potassium sulfate + 10 mM benzoic acid + 10 mM copper sulfate); K + BA + Cu + NAA (500 mM potassium sulfate + 10 mM benzoic acid + 10 mM copper sulfate + 1 mM naphthaleneacetic acid); CEPA (3% 2-chloroethylphosphonic acid, which is the most frequently used resinosis effector, herein used as a positive control), negative control (only streaking without paste application).

2.3. Resin composition analysis

Resin samples were taken from the trunks of five randomly selected trees belonging to each of the following treatments: potassium sulfate, NAA, CEPA or only bark streak (as described on section 2.2). Each sample consisted of a mixture of equal proportions of freshly flowing resin. The trees used in this study had been previously tapped for resin production for one year with the respective stimulant paste. Samples were collected in the fall of 2014. Immediately after harvest, all samples were frozen in liquid nitrogen and kept as such until storage at −80 °C. The preparation of samples for terpene analysis was performed as previously described (Wang et al., 1997). Extractions and analyses were done in triplicate. Quantitative and qualitative analyses of resin were done by gas chromatography (GC) and GC-Mass Spectrometry, respectively, as previously described (Rodrigues et al., 2011).

2.4. Weather and soil data

Weather data (temperature and rainfall) were obtained from the nearest meteorological station, located 40.5 km from the forest (city of Tramandaí). Potential Evapotranspiration (PET) was calculated using the Thornthwaite monthly water-balance program (McCabe and Markstrom, 2007). The soil in the area is classified as Hydromorphyc Planosoil, Eutrophic Solodic (Streck et al., 2008). No fertilization was applied throughout the study.