ISSN : 0974 - 7508 Volume 6 Issue 4

Trade Science Inc.

Natural Products

An Indian Journal

Review

NPAIJ, 6(4), 2010 [151-155]

Understanding variation and ecology of foliar terpenes in *Eucalyptus*

V.K.Varshney*, Sanjay Naithani

Chemistry Division, Forest Research Institute, P.O. New Forest, Dehradun - 248 006, (INDIA)

E-mail : varshney2000@yahoo.com; varshneyvk@icfre.org

Received: 22nd August, 2010 ; Accepted: 1st September, 2010

ABSTRACT

Foliar terpenes in *Eucalyptus* have multiple ecological functions and also show a great deal of variation between and within species. Variability in these chemicals make them significant from ecology, taxonomy and industrial utilization point of view. The present review summarizes the available knowledge on terpene biosynthesis and its relevance in understanding the variation in terpene concentration, terpene profile and terpene composition, and ecology of these secondary metabolites.

KEYWORDS

Eucalyptus;
Foliar terpenes;
Ecology;
Terpene biosynthesis.

INTRODUCTION

One of the most distinctive features of *Eucalyptus* is their high content of foliage essential oils which are stored in the lysogenic oil glands. These oils are complex mixtures of mono-(C_{10}) and sesquiterpenes (C_{15}) hydrocarbons and their oxygenated derivatives which are volatile, have distinct aromas and contribute towards characteristics scent of the *Eucalyptus* trees.

1,8-cineole is the main dominant monoterpene of *Eucalyptus*, particularly, in high oil yielding species[1] while in low oil yielding species α-pinene is usually the predominant monoterpene. Other monoterpenes that are known as major constituents of the foliar oils are piperitone, citronellal, α-phellendrene, p-cymene and terpinen-4-ol. The main sesquiterpene hydrocarbons in *Eucalyptus* are aromadendrene, β-caryophyllene, and bicyclogermacrene, and the major terpene alcohols are globulol, spathulenol and eudesmols[1,2]. Triterpenes with ursane skeleton have been characterized in eucalyptus foliage where ursolic acid predominates[3]. In addition to the simple terpenes, polyketides called Formylated Phloroglucinol Compounds (FPCs), occur frequently in *Eucalyptus* foliage, as conjugates of terpenes, and two main types-macrocarpals and euglobals can be distinguished.

Terpenes in *Eucalyptus* have either an external (ecological) or internal (either physiological or metabolic) role. Plant feeding studies with radio labelled CO_2, mevalonic acid and terpenes have shown that essential oils may provide a metabolic pool for synthesis of indispensable plant components such as pigments, sugars, amino acids, and respiratory co-enzymes[4]. Terpenes are significant as component of cell membranes (sterol type triterpenes), as components of photosynthesis I and II (carotenoid tetraterpenes and diterpenoid phytol chains of chlorophylls) and as phytohormones [gibberellins (of a diterpenoid origin) and absisic acid (of tetraterpenoid origin). The terpene involved in above metabolic processes are generally non volatile containing C_{20} or more carbon atoms, and account for maintaining intracellular structure and assimilative and regulative processes. While specialized biochemical studies are to be conducted, it may be speculated that eucalyptus oils also have a metabolic role to play within the plant. Seasonal variation in concentration might then be partially explained in terms of a dynamic balance between increase in concentration due to synthesis of terpenes and their chemical alteration and utilization within the foliage or plant. For example, monoterpenes have been shown to have a significant effect in increasing the thermo tolerance of photosystem II[5]. Therefore the function of a monoterpene within a plant could be essential to maintain photosynthesis in summer, whereas in the cooler months, when there is little need for thermo protection, it may serve mainly as a deterrent to herbivores.

What are terpenes?

Terpenes is a generic term originated from the first isolation of this class of natural product from turpentine oil. The basic building block of the terpenes is the isoprene unit [(C_5H_8), 2-methyl-1,3-butadiene] called hemiterpene, union of which gives rise to other terpenes classified as monoterpenes (2 isoprene units, $C_{10}H_{16}$), sesquiterpenes (3 isoprene units, $C_{15}H_{24}$), diterpenes (4 isoprene units, $C_{20}H_{32}$), triterpenes (6 isoprene units, $C_{30}H_{48}$), and tetraterpenes (8 isoprene units, $C_{40}H_{64}$). Within each of the main classes, categories such as acyclic, monocyclic and polycyclic terpenes consisting of hydrocarbons, alcohols, ketones, aldehydes, acids, esters, lactones are also recognized. Although terpene can occur as simple compounds, they are also found as components of more complex structures such as complex with acetate and other carboxylic acids, iridoids[6], simple sugars[7] and polyketides[8,9].

How terpenes are produced in foliage?

In the *Eucalyptus* different classes of terpenes require different substrate and enzymes for their biosynthesis. They are all biosynthesized from two independent and compartmentally separated pathways-called mevalonic acid pathway (MAV) operated in the cytoplasm and deoxyxylulose phosphate pathway (DXP) localized in the plastid[10]. The former uses acetyl co- enzyme A from the Krebs cycle, via mevalonic acid while the latter uses glyceraldehyde phosphate from the Calvin cycle, via deoxyxylulose phosphate. Both the MVA and DXP pathways lead to the production of isopentenyl diphosphate (IDP), the simplest common precursor, and its isomer dimethyl allyl diphosphate (DMADP) which form prenyl diphosphates (PDPs) such as geranyl diphosphate (GDP) used as a precursor to the monoterpenes, farnesyl diphosphate (FDP) used in the biosynthesis of sesquiterpenes and triterpenes, and geranylgeranyl diphosphate (GGDP) used in the biosynthesis of di- and tetraterpenes. Specific ratios of IDP and DMADP are responsible for the synthesis of the individual PDPs[11], for example-GDP synthases utilize IDP and DMADP in 1:1 ratio and FDP synthases use the two isomers in a 2:1 ratio, respectively. The GGDP synthases require three IDP molecules for every DMADP molecules. To obtain the optimal ratio in each specific tissue, isopentenyl diphosphate isomerases (IDPI) catalyze the conversion of IDP to DMADP. Thus, it has been found that variation in these genes affect the overall composition of foliar terpenes[12]. All the above described processes, incorporating different compounds, compartments and biosynthetic pathways end in the synthesis of a terpene skeleton. This process is catalyzed by a single family of enzymes, the terpene synthases (TPS), irrespective of the specific substrate used or the organellar localization of the reaction.

Although monoterpenes such as α-pinene and 1,8-cineole and sesquiterpenes such as bicyclogermacrene, β-caryophyllene, globulol, farnesol and α-humulene are produced directly by TPS while production of monoterpene- piperitone is the result of post enzymatic modification of the primary structure. Production of some terpenes is also possible without enzymatic catalysis. These involve spontaneous conversion of several monoterpenes containing polyunsaturated cyclohexane rings into p-cymene in the presence of atmospheric oxygen through natural processes such as leaf ageing but also during steam distillation and solid phase micro extraction[13,14]. The majority of the tricyclic structures such as aromadendrene, globulol and some bicyclic sesquiterpenes such as eudesmols are likely to be acid solvolysis products of macrocyclic structures[15]. Bicyclogermacrene is known to form artifact during steam distillation and in the injector and / or the column of a GC[11]. Similar changes may appear in the leaf during ageing due to exposure to high temperature and UV radiation[16,17].

Chemical variability in foliar terpenes

Eucalyptus terpenes show significant variability, between and within species, in terpene concentration, terpene profile and terpene composition. This variation may be attributed to the four major factors such as genetics, type and age of leaf, environment and technique of extraction and analysis. Understanding the cause of terpene variation is of interest to ecologists, taxonomists and natural products industries. Variation in the total concentration of terpenes is the most important type of variation relevant to the essential oil industry. This variation is influenced by environmental and genetic factors, and the processes leading to an increase or decrease in foliar terpene concentration may arise at several stages of the metabolic pathway. Changes in the availability of enzymes of terpene biosynthesis affect all oil components. This may result from changes in the regulation of the DXP and MVA pathways[1].

Presence or absence of individual components, irrespective of overall terpene content or the ratios of the components to each other, constitutes the foliar oil profile. Variation in terpene profile defines a chemical form or chemotype. Chemotypes are plants in naturally occurring population which can not be distinguished on the basis of morphological characters but which are readily distinguished by marked difference in their oil profile. A number of chemotypes have been identified in many eucalyptus species such as five in *E. dives*, six in *E. radiata*, four in *E. citriodora*, five in *E. racemosa*, three in *E. elata* and two in *E. piperita*[18] as well as distinct variants in *E. camphora*, *E. ovata*[19] and *E. camaldulensis*[20]. In case of *E. radiata* where presence or absence of piperitone distinguishes two of its six known Chemotypes[1,21]. The ketone group in piperitone is not likely to result from reactions directly catalyzed by TPS. Therefore, formation of a ketone (piperitone) must result from conversion of either a terpene hydrocarbon (α-phellendrene) or a terpene alcohol (piperitol) catalyzed by separate enzymes. This is supported by the co-occurrence of α-phellendrene and piperitone in Chemotype 2[1], and the presence of an intermediate chemotype characterized by high piperitol content in mosaic *E. radiata* individuals[22]. Based on the analogy of the conversion of limonene to (-) –trans-carveol, and piperitol to piperitone in mint[23], the transformation of α-phellendrene to piperitol and piperitol to piperitone may likely to be catalyzed by a p450 monoxygenase and NAD-dependent dehydrogenase enzymes, respectively. Both the enzymes are substrate specific and if the preferred substrate at any of these steps is shifted due to genetic change, the oil profile corresponding to the changed chemotype would result. Following this is interesting to speculate that variation in terpene -FPC adduct in *E. globulus* and *E. melliodora* is attributed to the similar mechanism[24] if the presence or absence of macrocarpals is determined by the function of enzymes catalyzing FPC-terpene addition. Since macrocarpals and euglobals and other FPC-terpene adducts are non volatile constituents of the foliage and such biochemical processes may also have direct influence on the foliar oil profile by removing individual components from the pool of volatile terpenes[11].

Variation in the terpene composition i.e. the proportion of the individual compounds present is observed due to variation in the terpene synthases since these genes often have overlapping product profile. In certain cases, the ratio of entire chemical classes such as sesquiterpenes and monoterpenes can vary between chemotypes as for example in chemotype 1 and 2 of *E. camaldulensis*[1]. Chemotype 1 is rich in monoterpenes (dominated by 1,8-cineole) while chemotype 2 is rich in sesquiterpenes and this may be influenced by the IDP:DMADP ratio as determined by the activity and expression of IDPI.

In *Eucalyptus* chemical variability in terpenes appears to be a common and significant characteristics of individual species and this variation may be brought about by sequence variation of multiple genes involved with terpene biosynthesis pathway. Considerable research into the biochemical pathways leading to the formation of foliar volatiles has been conducted and contributed significantly in industrially important species. Thus understanding the cause of chemical variation in foliar terpenes has ecological, taxonomical and industrial significance. Such findings can help in selection of the right species for a site under land rehabilitation programme. Once chemical variation to gene sequence variation is linked, genetic markers could be developed and used to screen the individuals for optimal oil yield and quality, and disease and pest resistance in natural population and trials. This means that screening could be carried out before planting and the maturation of the foliar chemotype, and that subsequent generation can further be screened to maintain the optimal characteristics in spite of open pollination. This knowledge could be utilized by aroma industries, and paper and pulp industries which are dependent on *Eucalyptus* plantations[11].

Chemoecology of eucalyptus foliar terpenes

Terpenes play an important role among plants and between plants and animals and variability in these chemicals make them ecologically significant. Terpene mediated ecological interactions are seen in *Eucalyptus*. They have role as attractant / repellent to vertebrate herbivores[25], feeding and reproductive deterrents to insect herbivores[26], allelopathic[27] and antifungal agents[28], attractants for parasitoids and pollinators[29], cues that indicate the presence of other toxic constituents[30] and determinants of leaf litter decomposition rates[31]. Terpenes are also thought to influence variation in soil mineralization rates and understory biodiversity as well as significantly contributing to atmospheric hydrocarbons[32].

The survival potential of the *Eucalyptus* genus in relatively harsh Australian environments could be attributed to the contributions of their essential oils owing to their leaf eating insects repellant properties. This is further supported by the resistance of the *Eucalyptus*, introduced in areas outside Australia, to the locally adapted herbivores. A relationship between foliar oil concentration and level of insect herbivory[26] has been shown. Phytochemical studies of *Eucalyptus* growing in Tasmania and feeding bioassays have shown that host tree selection by paropsine chrysomelid defoliators is affected by the quantitative and qualitative mix of monoterpene hydrocarbons. 1,8-cineole, α-pinene and α-phellendrene have been demonstrated to significantly influence the ovipositional preference and rates of larval feeding and survival[1].

A strong relationship between foliar concentrations of the commercially valuable terpene-1,8-cineole and FPC (macrocarpals in the case of *E. viminalis* and sideroxylonals in the case of *E. Melliodora*) has been found[33]. The FPCs act as powerful antifeedant against insects and marsupial folivores[34]. This correlation is important ecologically because marsupial folivores use the concentration of the cineole as a cue to the concentration of the FPC i.e. if high concentration of cineole is detected, the folivores will eat little of the foliage, and low concentration of the cineole will suggest to the folivore that the foliage is palatable[30]. This findings suggested that any selection towards increasing the concentration of essential oils will also result in the improvement in the yield of FPCs, where these two compounds do in fact co-occur. The FPCs have been found to be associated with a wide range of biological activities including potency as antifouling agents, tumour suppression and antifungal, antibacterial, antiviral and enzyme inhibitory properties[35].

Increased level of α-phellendrene and p-cymene in the foliage has been found to be associated with the early cold resistance in seedlings of several eucalypt species grown in Florida, USA[36]. Allelopathic effects of the terpenes leached from the leaves of *Eucalyptus* on the forest floor has been reported to inhibit the growth of associated vegetation[37-39]. The likely mechanism of allelochemical release could be attributed to aqueous leaching of foliage and litter - an apparent incongruity with the reputed insolubility of monoterpenes. Aqueous solubility of many bioactive monoterpenes has been determined by gas chromatography (GC) in support of this hypothesis. This was established that hydrocarbons were of low solubility (< 35 ppm) while oxygenated monoterpenes exhibited solubilities one or two orders of magnitude higher with ranges of 155-6990 ppm for ketones and of 183-1360 ppm for alcohols[40]. Many monoterpenes were phytotoxic in concentration under 100 ppm, well below the saturated aqueous concentration of oxygenated solutions of monoterpenes. Therefore, even dilute unsaturated solutions of monoterpenes occurring naturally in plant tissues and soil solution, may act as potent biological inhibitors.

ACKNOWLEDGEMENTS

We are thankful to Director, Forest Research Institute, Dehra dun for his keen interest, encouragement and necessary facilities to initiate work on chemical ecology of *Eucalyptus*.

REFERENCES

[1] D.J.Boland, D.Brophy, A.P.N.House; 'Eucalyptus Leaf Oils', Iukata Press, Sydney, **(1991)**.

[2] J.J.Brophy, I.A.Southwell; 'Eucalyptus Chemistry', In: J.J.W.Copper Ed.; Eucalyptus: The Genus Eucalyptus, Taylor and Francis, London, 102-160 **(2002)**.

[3] G.G.Santos, J.C.N.Alves, J.M.L.Rodilla, A.P.Duatte, A.M.Lithgow, J.G.Urones; Phytochemistry, **44**, 1309 **(1997)**.

[4] W.F.Erman; 'Chemistry of the Monoterpenes', In: P.G.Gassman Ed.; 'Studies in Organic Chemistry', Marcel Dekker, New York, **2A and B**, **(1985)**.

[5] L.O.Copolovici, I.Filella, J.Liusia, V.Niinemets, J.Penueles; Plant Physiology, **139**, 485 **(2005)**.

[6] M.Wiuk; Phytochemistry, **64**, 3 **(2003)**.

[7] J.Lucker, M.K.El Tamer, W.Schwab, F.W.A.Verstapper, L.H.W.Vander Plas, H.J.Bo- uwmeester, H.A.Vethoeven; The Plant Journal, **27**, 315 **(2002)**.

[8] Y.Yamakoshi, M.Murata, A.Shimiju, S.Homma; Bioscience, Biotechnology and Biochemistry, **56**, 1570 **(1992)**.

[9] E.P.M.de Meijet, K.M.Hammond; Euphytica, **145**, 189 **(2005)**.

[10] W.Eisenreich, M.Schwarz, A.Cartayrade, D.Arigoni, M.H.Zenk, A.Bacher; Chemistry and Biology, **5**, 221 **(1998)**.

[11] A.Keszei, C.L.Brubaket, W.J.Foley; Australian Journal of Botany, **56**, 197 **(2008)**.

[12] M.R.Wildung, R.B.Croteau; Transgenic Research, **14**, 365 **(2005)**.

[13] F.Sefidkon, M.Dabiri, A.Rahimi –Bidgoly; Flavor and Fragrance Journal, **14**, 405 **(1999)**.

[14] D.Zabaras, S.G.Wyllie; Journal of Separation Science, **25**, 685 **(2002)**.

[15] C.P.Cornwell, N.Reddy, D.N.Leach, S.G.Wyllie; Flavor and Fragrance Journal, **15**, 352 **(2001)**.

[16] H.Fadel, F.Matx, A.El-Sawy, A.El-Ghorab; Food Research and Technology, **208**, 212 **(1999)**.

[17] J.Hardet, S.Foss; Geomicrobiology Journal, **16**, 295 **(1999)**.

[18] A.R.Penfold, J.L.Willis; Nature, **17**, 883 **(1953)**.

[19] D.Simmons; 'Geographic Variation in the Morphology and Leaf oils of Some Eucalyptus Species', Ph.D. Thesis, La Trobe Univ., Victoria, **1**, **(1974)**.

[20] J.C.Doran, J.J.Brophy; New Forests, **4**, 25 **(1990)**.

[21] P.C.Jonstone; Chemosystematic and Morphometric Relationships of Population of Some Peppermint Eucalyptus', Ph.D. Thesis, Monash University, Melbourne, **(1984)**.

[22] A.R.Penfold, F.R.Morrison; Journal and Proceedings of the Royal Society of New South Wales, **70**, 375 **(1937)**.

[23] S.Lupien, M.R.B.Wildung, R.Croteau; Archives of Biochemistry and Biophysics, **368**, 181 **(1999)**.

[24] B.W.Moore, I.R.Wallis, J.Pala-paul, J.J.Brophy, R.H.Willis, W.J.Foley; J.Chem.Ecol., **30**, 1743 **(2004)**.

[25] I.A.Southwell; 'Essential Oil Content of Koala Food Trees', In: T.J.Bergin Ed.; The Koala Proceedings of the Taronga Symposium, Royal Zoological Society of NSW, Sydney, 62-74 **(1978)**.

[26] P.A.Morrow L.R.Fox; Oecologia, **45**, 209 **(1980)**.

[27] M.D.C.Alves, S.Medeiros, R.Innecco, S.B.Torres; Pesquisa Agropecuaria Brasilrira, **39**, 1083 **(2004)**.

[28] A.Eyles, N.W.Davies, Z.Q.Yuan, C.Mohammed; Forest Pathology, **33**, 317 **(2003)**.

[29] A.Giamakis, O.Kretsi, I.Chinou, O.G.Spyropow-los; Phytochemistry, **58**, 351 **(2001)**.

[30] I.R.Lawler, J.Stapley W.J.Foley, B.M.Escher; J.Chem-Ecol., **25**, 401 **(1999)**.

[31] A.Molina, M.J.Reigosa, A.Cerballeita; J.Chem. Ecol., **17**,147 **(1992)**.

[32] G.Iason; Proceedings of the Nutrition Society, **64**,123 **(2005)**.

[33] I.R.Lawler, W.J.Foley, B.M.Eschier; Ecology, **81**, 1327 **(2000)**.

[34] B.M.Eschier, D.M.Pass, R.Willis, W.J.Foley; Biochem.Syst.Ecol., **28**, 813 **(2000)**.

[35] J.Sidana, R.K.Rohilla, N.Roy, R.A.Barrow, W.J.Foley, I.P.Singh; Fitoterapia (In Press).

[36] J.Y.Shimiju; Variation in Frost Resistance and Monoterpene Composition in Eucalyptus Species, M.Sc., Thesis, Univ.of Florida, **(1974)**.

[37] A.H.Al-Mousawi, F.A.G.Al-Naib; J.Univ.Kuwait (Sci.), **2**, 59 **(1975)**.

[38] R.Del Moral, C.H.Muller; Bull.Torrey.Bot.Club, **96**, 467 **(1969)**.

[39] D.R.Batish H.P.Singh, R.K.Kohli, S.Kaur; Forest Ecology and Management, **256(12)**, 2166 **(2008)**.

[40] J.Q.Weidenhames, F.A.Macias, N.H.Fisher, G.B.Williamson; J.Chem.Ecol., **19(8)**, 1799 **(1993)**.