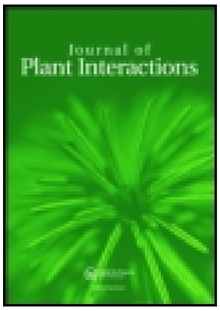
This article was downloaded by: [Universidad de Buenos Aires]
On: 23 July 2013, At: 15:32
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Plant Interactions
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tjpi20

Changes in Eucalyptus camaldulensis essential oil composition as response to drought preconditioning

Silvia R. Leicach [a], Ana M. Garau [b], Ana B. Guarnaschelli [b], Margarita A. Yaber Grass [a], Norberto D. Sztarker [a] & Analia Dato [a] [b]

[a] Química de Biomoléculas, Facultad de Agronomía (FA), Universidad de Buenos Aires (UBA), Ciudad Autónoma de Buenos Aires, Argentina

[b] Dasonomía, Facultad de Agronomía (FA), Universidad de Buenos Aires (UBA), Ciudad Autónoma de Buenos Aires, Argentina

Published online: 10 May 2010.

To cite this article: Silvia R. Leicach , Ana M. Garau , Ana B. Guarnaschelli , Margarita A. Yaber Grass , Norberto D. Sztarker & Analia Dato (2010) Changes in Eucalyptus camaldulensis essential oil composition as response to drought preconditioning, Journal of Plant Interactions, 5:3, 205-210, DOI: 10.1080/17429145.2010.483744

To link to this article: http://dx.doi.org/10.1080/17429145.2010.483744

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Journal of Plant Interactions
Vol. 5, No. 3, September 2010, 205–210

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Changes in *Eucalyptus camaldulensis* essential oil composition as response to drought preconditioning

Silvia R. Leicach[a]*, Ana M. Garau[b], Ana B. Guarnaschelli[b], Margarita A. Yaber Grass[a], Norberto D. Sztarker[a] and Analia Dato[a,b]

[a]*Química de Biomoléculas, Facultad de Agronomía (FA), Universidad de Buenos Aires (UBA), Ciudad Autónoma de Buenos Aires, Argentina;* [b]*Dasonomía, Facultad de Agronomía (FA), Universidad de Buenos Aires (UBA), Ciudad Autónoma de Buenos Aires, Argentina*

(Received 2 March 2010; final version received 5 April 2010)

Water deficit, a common constraint in forestry, is the main cause of plant stress during plantation. The survival and growth of seedlings is also compromised by herbivory during establishment. The potential of nursery preconditioning to enhance survival chances of future trees by reducing palatability or attracting beneficial insects as a result of changes in chemical defences may be an answer to overcome this situation. Changes in essential oil production and composition were evaluated by GC and GC-MS in *Eucalytus camaldulensis* seedlings submitted to drought during four weeks at the last stage of nursery period (20 weeks). Significant changes in essential oil relative composition were found. Seedlings (young leaves) submitted to drought developed a terpenoid blend, which has been previously associated to mature leaves and related to their higher resistance towards herbivory. Total amount of non-oxygenated terpenes decreased by 44%, whereas some oxygenated sesquiterpenes (globulol, epiglobulol and ledol) were doubled, and 1,8-cineole content was enhanced by 28.3%.

Keywords: *Eucalyptus camaldulensis*; essential oil; nursery stage; water stress

Introduction

A considerable increase in the area of *Eucalyptus* plantations occurred in Argentina during the last six decades, mainly because they constitute one of the most important wood resources. More than 700,000 acres are currently exploited to develop several *Eucalyptus* species, which have been particularly chosen because of their capacity to achieve high growth rates over a reasonable range of site conditions. They provide wood of high quality and commercial value that is used in pulp, hardboard, particleboard, and sawmill industries.

Eucalyptus camaldulensis Dehnh. (Myrtaceae) is commonly grown in temperate central areas of Argentina; nevertheless, plantations can also be found in the north subtropical areas. Several thousand seedlings produced every year under nursery conditions, are planted during spring when chances of drought are likely to occur. Their establishment is commonly hindered by both biotic and abiotic factors. Water deficit is, in particular, a very common constraint in forestry and the main cause of plant stress during plantation (Burdett 1990; Grossnickle and Folk 1993). During establishment, seedlings survival and growth is also compromised by herbivory damages caused mainly by leaf-cutting ants in Argentina (Folgarait et al. 1996).

It has been demonstrated that plants can develop physical, chemical, and/or phenological defences; however, a full range of them is rarely present in a single species. Some species defend themselves by developing physical barriers like thorns or increasing their tissues hardness by lignification, others by chemical means producing toxins such as alkaloids, glucosinolates, hydroxamic acids, tiophenes, and cyanogenic glycosides, among others. Glandular trichomes containing bioactive substances, a paradigmatic example of joint physico-chemical mechanism, have been demonstrated to be one of the most effective defences against noxious organisms (Hammerschmidt and Schultz 1996). Studies with labelled (^{14}C) precursors demonstrated that terpenes are biosynthetized within these structures (Gershenzon and Croteau 1990). Mixtures of structurally related terpenes, which are responsible for plant odors and fragrances, are found in many species including *Eucalyptus*.

Eucalyptus species contain high concentrations of monoterpenic and sesquiterpenic compounds in their foliage that can be obtained from plant fresh tissues by steam distillation as part of their essential oils. Essential oil yield and chemical composition have shown significant variations between species corresponding to the same genus and even between individuals within a population of the same species (Nicole et al. 1998; Chalchat et al. 2000; Pagula et al. 2000; Pappas and Sheppard-Hanger 2000). Leaf age is also a determining factor (Silvestre et al. 1997;

---

*Corresponding author. Email: leicach@agro.uba.ar

ISSN 1742-9145 print/ISSN 1742-9153 online

DOI: 10.1080/17429145.2010.483744
http://www.informaworld.com

Wildy et al. 2000), higher oil concentrations were found in *E. camaldulensis* when leaves were fully expanded, but not yet fully lignified (Doran and Bell 1994). Significant differences in essential oil yield and composition were detected between young and mature leaves from *E. globulus* (Chennoufi et al. 1980; Silvestre et al. 1997), and juvenile, mature, and senescent leaves of *E. citriodora* (Batish et al. 2006a, 2006b). Environmental factors contribute to modulate the level at which secondary metabolism occurs in plant species (Einhellig 1989), and in particular that related to biosynthesis of terpenic derivatives. Biotic stresses such as disease or herbivory and abiotic stresses such as nutrient deficiency and drought can affect essential oil yield and composition (Tang et al. 1995; Leicach et al. 2008).

Mixtures of terpenic compounds have proved to be responsible for eucalypts' particular ability towards bacterial and fungal attack, and also towards herbivory (Batish et al. 2008). Herbivore performance is linked to leaf quality, which depends only in part on leaf age (Lill and Marquis 2001). Metcalf and Luckman (1994) reported that drought preconditioning affected plant material turning it into an inadequate food source for herbivore insects. Environmental restrictions occurring during establishment are also modulating factors that modify the levels of herbivore damage on improved cultivars. It has been reported that damage level produced by leaf cutting ants on *E. globulus* depends on its leaves anatomical and physical characteristics related to its provenance (Guarnaschelli et al. 2000).

Nursery conditions can affect leaf quality by modulating their chemical composition, which can be a determinant feature, besides tissue hardness, regarding herbivory. It has been demonstrated that defoliation level due to the beetle *Anoplognathus* sp. can be affected by the 1,8-cineol proportion in terpenoids mixture (Edwards et al. 1993). In order to develop a more sustainable commercial forestry minimizing chemical input, studies have been performed to obtain more resistant seedling to begin with. Controlled drought conditions at particular nursery stages may be one of the possible ways to achieve it, particularly, if they prove to enhance the chemical defensive potential of seedlings. However, literature related to drought effects on chemical composition of essential oil from eucalypt at seedling stage is scarce. The aim of this work was to evaluate changes in essential oil yield and composition from *E. camaldulensis* seedlings as a response to controlled drought preconditioning during the last stage of nursery period.

Material and methods

Plant material

Pre-germinated seeds of *Eucalyptus camaldulensis* (Lake Coorong provenance; provided by Kylisa Seeds, Australia) were sown during fall, one per pot (plastic containers 20 cm height, 6.5 cm diameter) filled with a grounded mixture of coco fiber and composted pinebark (1:1, v/v). Seedlings were maintained in a glasshouse located in the experimental field of the School of Agronomy, University of Buenos Aires (34°35′27″ S, 58°29′47″ W). To insure adequate growth conditions they were watered every day and received nutrient solution [6 ml/l NO_3K 1M, 4 ml/l $(NO_3)_2Ca$ 1M, 2 ml/l $PO_4H_2NH_4$ 1M, 1 ml/l $SO_4Mg.7H_2O$, 1 ml/l micronutrients, 1 ml/l Fe-EDTA] twice a week during the first 16 weeks.

Drought preconditioning period

Thirty seedlings were selected randomly and separated in two groups: (i) Control (C) seedlings receiving daily irrigation, and (ii) Water stressed (WS) seedlings that were irrigated every three days. Differential treatments were applied during the last four weeks of nursery period. Fifteen replications were performed for each treatment. At the end of this period leaves were harvested and essential oil was extracted, for each seedling.

Acquisition of essential oils

Essential oils were obtained from freshly collected leaves by hydrodistillation using a modified Clevenger-type apparatus. Each leaf sample (150 g) was separately treated for 3 h, and the corresponding essential oil recovered by decantation and dried over anhydrous sodium sulphate. Essential oil samples were stored at −18°C until chromatographic analysis. Total oil content was calculated for each repetition ($n = 15$) and expressed as% (w/w, fresh weight).

Chromatographic conditions

GC and GC-MS were performed to evaluate qualitative and quantitative changes in essential oil composition as a response to water deficiency.

GC analysis

1 μ of each sample, dissolved in CH_2Cl_2 (1:100 v/v), was injected. Relative abundances of essential oil components were determined by GC using a Hewlett Packard 5890A Gas Chromatograph equipped with a flame ionization detector (FID) using a HP-1 (30 m × 250 mm × 0.25 mm) capillary column. Nitrogen was used as carrier gas (flow rate: 1 ml/min) and hexadecane at a concentration of 1 g/10 ml as internal standard. Temperature program: 60°C (2 min), 60–280°C at 8°C/min; injector and detector temperature: 250°C.

Quantification of oil components was computed by the normalization method from the GC peak areas, calculated by means of three injections from each oil.

Qualitative identification of the different constituents was determined from their GC retention indices, relative to a homologous series of *n*-alkanes C_7- C_{25}, which were compared with literature data (Adams 1995; Fadel et al. 1999; Karioti et al. 2003).

Results were confirmed by co-chromatography with authentic samples of some mayor components. Response factors were determined for standard commercial samples of limonene, α-pinene, 1,8-cineol, and globulol (Sigma Aldrich-Argentina), and as expected when FID is used, they did not differ significantly from unity.

GC-EIMS analysis

Determination of individual terpenoids was achieved by Capillary GC electron impact mass spectrometry (EIMS) with a Hewlett-Packard GC-HP 6890 coupled to a Mass Spectrometer, MSD 5973, with the same column and identical operating conditions to those previously used for GC. The ionization voltage applied was 70 eV, mass range m/z 40–400 a.m.u.

Unknown peaks were tentatively identified by comparing their mass spectra with those corresponding to NIST Mass Spectral Database 98.L, and confirmed by comparison with literature data or with those of authentic reference compounds (Sigma Aldrich-Argentina).

Data analysis

Data corresponding to essential oil yield and to compounds relative abundances were submitted to analysis of variance (ANOVA) using InfoStat/Professional, Version 1.1, 2002. Means were compared using Tukey's test ($p < 0.05$). Principal Component Analysis (PCA) was also performed ($p < 0.05$) on terpenoids relative abundances.

Results and discussion

Oil yield

Water stress did not cause significant changes in oil yield. Total oil yield was 0.79 ± 0.05 (%, FW) in C plants, and 0.80 ± 0.04 (%, FW) in WS plants.

Essential oil quantitative analysis

Essential oil composition of control samples is given in Table 1. Mean relative abundances corresponding to *E. camaldulensis* essential oil components from control (C) and water-stressed (WS) treatments are shown in Table 1.

Differences in essential oil composition between treatments were analyzed. Due to the high differences in their relative abundances, major components (Figure 1) (abundances $>1\%$) were represented separated from minor components (Figure 2) (abundances $<1\%$).

Significant changes were found in essential oil relative composition. 1,8-cineole was the major constituent, representing 45.7% of total leaf oil in control seedlings (Table 1). The other oxygenated monoterpenes include α-terpineol (4.7%) and 4-terpineol (2.5%), and the oxygenated sesquiterpene globulol (1.5%) (Figure 1). Among non-oxygenated monoterpenes, limonene (9.7%) was the most abundant, followed by γ-terpinene (8.4%), and α-pinene (7%). Other oil constituents such as β-pinene, α-terpinolene, β-myrcene, α- and β-phellandrene, linalool, epiglobulol, and ledol were present in much lower amounts (Figure 2).

Terpenic profiles for WS seedlings resulted quite differently to those for C ones. Most non-oxygenated terpenes, except for α-pinene that did not show significant differences in its relative abundance, lowered their total relative abundance under drought conditions (Figure 1).

Among them, α-phellandrene and terpinolene almost disappeared (Figure 2). At the same time most oxygenated terpenes exhibited significant increments in their relative abundances. 1,8-cineole increased its content by 28.3% (Figure 1), sesquiterpenoids globulol (Figure 1), epiglobulol and ledol (Figure 2) also showed significantly higher abundances. There was a striking five-fold increment in linalool content; while ledol doubled its abundance and epiglobulol enhanced its own in 52%, followed by *trans*-pinocarveol showing an increment of 40% (Figure 2).

Biplot representation of Principal Component Analysis that grouped terpenes by their abundance showed clear differences between C and WS treatments, associating non-oxygenated compounds to the first treatment and oxygenated ones to the second one (Figure 3).

The first principal component accounted for 70% of total variation, and clearly separated C and WS treatments. 1,8-Cineole was the most important variable associated to first component exhibiting the highest positive value, followed by α-pinene, globulol, linalool, and ledol, with lower positive values, showing that most oxygenated terpenes enhanced their abundances in WS treatment. On the contrary, higher negative values corresponded to γ-terpinene, and limonene, followed by lower negative values for other non-oxygenated terpenes, strongly associating these components to C treatment.

The second principal component accounted for the rest of total variation (30%). Most important variable associated to the second component was sesquiterpene epiglobulol, which exhibited the highest positive value, followed by aromadendrene, while the highest negative values related with WS samples corresponded to ledol, β-phellandrene and *trans*-pinocarveol.

Eucalypt essential oils are mainly bioactive terpenoids blends with antimicrobial and antifungal activity, some of them being used in different countries as efficient fumigants and contact insecticides in integrated pest management of stored products (Batish et al. 2008). Plants natural ability to produce allelochemicals is currently revised to

Table 1. Mean relative abundances of *E. camaldulensis* essential oil components in control (C) and water-stressed (WS) treatments.

Essential oil	KI	Relative abundance		*P*	Identification
Major components		Control	Water stress		
α-pinene	941	6.99±3.17	12.13±3.62	0.0397	MS, KI, St
β-myrcene	996	1.25±0.06	0.60±0.05	<0.0001	MS, KI
limonene	1035	9.71±1.49	5.31±0.41	0.0004	MS, KI, St
1,8-cineole	1037	45.76±6.01	58.77±2.71	0.0030	MS, KI, St
γ-terpinene	1064	8.42±2.07	2.07±2.95	0.0051	MS, KI
4-terpineol	1193	2.48±0.48	1.92±1.66	0.4501	MS, KI, St
α-terpineol	1198	4.67±1.08	4.98±0.64	0.5252	MS, KI
globulol	1597	1.45±0.55	3.06±0.62	0.0448	MS, KI, St
Minor components					
2-(*E*)-hexenal	857	0.12±0.04	traces	<0.0001	MS, KI
β-pinene	985	0.72±0.15	0.59±0.01	0.0728	MS, KI, St
α-phellandrene	1015	0.42±0.07	traces	0.0056	MS, KI, St
β-phellandrene	1039	0.36±0.04	0.15±0.02	<0.0001	MS, KI
terpinolene	1092	0.50±0.12	traces	0.0074	MS, KI, St
linalool	1099	0.17±0.05	0.97±0.12	<0.0001	MS, KI
trans-pinocarveol	1142	0.69±0.12	0.97±0.12	0.0065	MS, KI
2-hidroxycineole-acetate	1362	0.35±0.03	0.47±0.11	0.5327	MS
aromadendrene	1476	0.60±0.09	0.40±0.06	0.0603	MS, KI
epiglobulol	1535	0.48±0.05	0.73±0.08	0.1565	MS, KI
ledol	1568	0.42±0.12	0.91±0.12	0.0032	MS, KI

Notes: Relative abundances given as mean value±SD (standard deviation), *n* = 15. Identification by: MS (NIST 98.L data base), KI (literature data), St (authentic reference compounds).

enhance the number of cultivated species, varieties, or clones with enriched chemical defences in order to diminish agrochemicals dependency. Selection of populations or individuals with particular morphological and/or chemical characteristics making them less susceptible to biotic or abiotic stress is one of the possible ways to achieve it (Gershenzon and Dudareva 2007).

Environmental conditions modulate the rate at which secondary metabolism occurs, and drought is one of the abiotic factors that can affect it (Einhellig 1989). Different irrigation regimes at nursery stage are commonly used to modify eucalypt seedlings quality through short- and long-term physiological and morphological changes that might allow plants to overcome stress conditions during establishment (Zine El Abidine et al. 1994; Guarnaschelli et al. 2003). Manipulation of nursery environment can also alter plant chemical composition, particularly its essential oil. However, the published reports about a possible relationship between water deficiency and essential oil quality are not consistent. It has been observed that water restriction decreased essential oil production in several eucalypt species (Muller da Silva et al. 2006). In contrast, other researchers reported that neither total terpenoids yield or 1,8-cineole proportion were modified in the foliage of mature *E. camaldulensis* trees by water deficiency (Stone and Bacon 1994).

Our results suggesting that water stress did not change essential oil yield in *E. camaldulensis* seedlings are in agreement to those obtained by Stone and

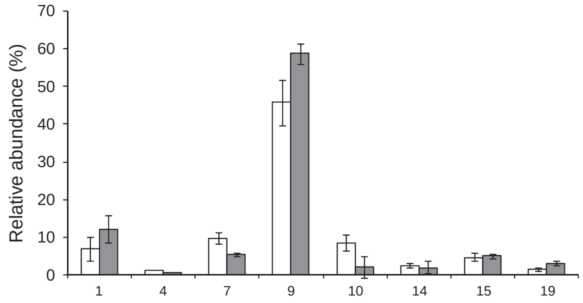


Figure 1. Changes in *E. camaldulensis* essential oil major components as response to water stress. 1: α-pinene, 4: β-myrcene, 7: limonene, 9: 1,8-cineole, 10: γ-terpinene, 14: 4-terpineol, 15: α-terpineol, 19: globulol. White bars: control treatment (C), grey bars: water-stressed treatment (WS).

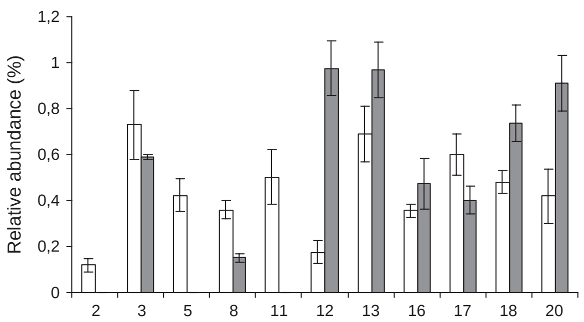


Figure 2. Changes in *E. camaldulensis* essential oil minor components as response to water stress. 2: 2-(*E*)-hexenal, 3: β-pinene, 5: α-phellandrene, 8: β-phellandrene, 11: terpinolene, 12: linalool, 13: *trans*-pinocarveol, 16: 2-hidroxycineole-acetate, 17: aromadendrene, 18: epiglobulol, 20: ledol. White bars: control treatment (C), grey bars: water-stressed treatment (WS).

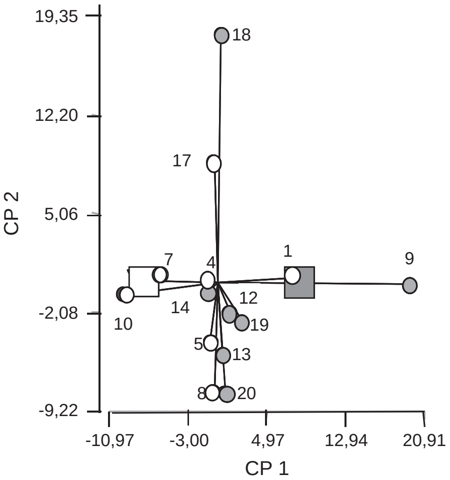


Figure 3. Principal Component Analysis ($p < 0.05$) of *E. camaldulensis* essential oil components. 1: α-pinene, 4: β-myrcene, 7: limonene, 9: 1,8-cineole, 10:γ-terpinene, 14: 4-terpineol, 19: globulol, 5:α-phellandrene, 8:β-phellandrene, 12: linalool, 13: *trans*-pinocarveol, 17: aromadendrene, 18: epiglobulol, 20: ledol. □: Control treatment (C), ■: Water-stress treatment (WS). ○: Non-oxigenated terpenes, ●: Oxigenated terpenes

Bacon (1994) when they studied mature trees of the same species.

We also found that drought triggered a significant increase in several oxygenated terpenes production, particularly in linalool and 1,8-cineole, both terpenoids known to repel different defoliator species. Stone and Bacon (1994) suggested that genetic selection of trees with high 1,8-cineole content would probably reduce insect herbivory. The role of linalool in plant defences against herbivory has also been demonstrated in bioassays with transgenic *Arabidopsis thaliana* producing larger amounts of linalool, which significantly repelled *Myzus persicae* aphids than non-modified samples (Gershenzon and Dudareva 2007).

Higher abundances of oxygenated terpenes seem to be associated to mature leaves in *Eucalyptus* species, Silvestre et al. (1997) demonstrated that non-oxygenated terpenes were the major components in *E. globulus* young leaves, while oxygenated ones, particularly 1,8-cineole, were more associated to mature ones. Our results show that young leaves (from five-month seedlings) submitted to water deficiency at the end of nursery period developed a particular terpenoid blend, which seems to be more associated to mature leaves than to young ones.

The role of mono- and sesqui- terpenes in plant defence has been thoroughly discussed. For plant species with a wide range of enemies, a diverse combination of terpenic derivatives may help to achieve simultaneous protection against numerous predators, parasites and competitors. Complexity of essential oils mixtures may increase the chance for individuals within a population to develop unique and more effective defensive blends, which may lessen the ability of natural enemies to evolve resistance. Their lipophilic nature may also synergize more polar toxins effects by facilitating their passage through membranes. Moreover, low-molecular-weight terpenes that are good conveyors of information over distance are involved in mutually beneficial interactions, their messages being very specific as a result of their structural variety. Development of a novel terpenoid mixture may have defensive value against herbivores already adapted to circumvent this kind of chemical defences prevalent in a given population. Moreover, individual components might be able to synergize the deterrence capacity or toxicity of other components of the mixture (Gershenzon and Dudareva 2007). It has been suggested that there are particular thresholds over which essential oil concentration in foliage may influence insect herbivory (Morrow and Fox 1980).

However, it is still to be established, if the particular 1,8-cineole concentration, and terpenoid blend developed by our experimental WS seedlings is adequate to minimize a particular herbivore attack. Potential of nursery pre-conditioning to enhance survival chances of future trees by reducing palatability or attracting beneficial insects as a result of changes in chemical defences seems to be promising. Whether these changes can affect leaf-cutting ants or other possible predators in Argentina, is still to be determined.

Acknowledgments

The authors wish to thank Universidad de Buenos Aires (Argentina) for grants UBACYT G055 and G067 that financially supported this work.

References

Adams RP. 1995. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, IL: Allured Publishing Corporation.

Batish DR, Harminder PS, Kohli RK, Kaur S. 2008. *Eucalyptus* essential oil as a natural pesticide. Forest Ecol Manag. 256(12):2166–2174.

Batish DR, Harminder PS, Setia N, Kaur S, Kohli RK. 2006a. Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. Z. Naturforsch 61c:465–471.

Batish DR, Harminder PS, Setia N, Kaur S, Kohli RK. 2006b. Chemical composition and inhibitory activity of essential oil from decaying leaves of *Eucalyptus citriodora*. Z. Naturforsch. 61c:52–56.

Burdett AN. 1990. Physiological processes in plantation establishment and the development of specification for forest planting stock. Can J For Res. 20:415–427.

Chalchat J, Garry R, Sidibe L, Harama M. 2000. Aromatic plants of Mali (V): Chemical composition of essential oils of four eucalyptus species implanted in Mali: *Eucalyptus camaldulensis, E. citriodora, E. toreliana* and *E. tereticornis*. J Essent Oil Res. 12(6):695–701.

Chennoufi R, Morizur J, Richard H, Sandret F. 1980. Study of *Eucalyptus globulus* essential oils from Morocco (young and adult leaves). Riv Ital EPPOS. 62:353–357.

Doran J, Bell R. 1994. Influence of non-genetic factors on yield of monoterpenes in leaf oils of *Eucalyptus camaldulensis*. N For. 8:363–379.

Edwards PB, Wanjura WJ, Brown WV. 1993. Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. Oecologia 95(4):551–557.

Einhellig FA. 1989. Phytochemical ecology: Alelochemicals, mycotoxins, and insect pheromones and allomones. In: Chou CH, Waller GR, editors. Interactive effects of allelochemicals and environmental stress. Taipei. Academia Sinica Monograph Series 9. pp 101–118.

Folgarait P, Dyer L, Marquis R, Braker E. 1996. Leaf cutting ant preferences for five native tropical plantation tree species growing under different light conditions. Entomol Exp Appl 80:521–530.

Gershenzon J, Croteau R. 1990. Biochemistry of the mevalonic pathway to terpenoids. In: Towers GHN, Stafford HA, editors. Regulation of monoterpene biosynthesis in higher plants. New York: Plenum Press. pp 99–160.

Gershenzon J, Dudareva N. 2007. The function of terpene natural products in the natural world. Nat Chem Biol. 3:408–414.

Grossnickle SC, Folk RS. 1993. Stock quality assessment: Forecasting survival or performance on a reforestation site. Tree Planter's Note 44:113–121.

Guarnaschelli AB, Lemcoff JH, Prystupa P, Basci SO. 2003. Responses to drought preconditioning in *Eucalyptus globulus* Labill. provenances. Trees 17:501–509.

Guarnaschelli AB, Mantese A, Barañao JJ, De Haro AM, Lemcoff JH. 2000. The tree. In: Quentin I, editor. Anatomical leaf characteristics related to herbivory in *Eucalyptus globulus* subesp. *maidenii* seedlings; Montreal, Canada: IQ Collectif Institut de Recherche en Biologie Végétale. pp 59–63.

Hammerschmidt R, Schultz JC. 1996. Phytochemical diversity and redundancy in Ecological interactions. Recent Advances in Phytochemistry 30. Chapter 5, Multiple defenses and signals in plant defense against pathogens and herbivores; New York and London: Plenum Press. pp 121–154.

Fadel H, Marx F, El-Sawy A, El-Ghorab A. 1999. Effect of extraction techniques on the chemical composition and antioxidant activity of *Eucalyptus camaldulensis* var. *brevirostris* leaf oils. Z. Lebensm Unters Forsch A. 208:121–216.

Karioti A, Skaltsa H, Demetzos C, Perdetzoglou D, Economakis CD, Salem AB. 2003. Effect of nitrogen concentration of the nutrient solution on the volatile constituents of leaves of *Salvia fruticosa* Mill. in solution culture. J Agric Food Chem. 51:6505–6508.

Leicach SR, Garau A, Guarnaschelli AB, Sztarker N, Dato A. 2008. Changes in *Eucalyptus camaldulensis* essential oils with water stress. Conference presented at 5th World Congress on Allelopathy, 4. Allelopathy in Forest Systems. September 24, 2008. Saratoga Springs, NY, USA.

Lill J, Marquis R. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia. 126:418–428.

Metcalf RL, Luckman WH. 1994. Introduction to insect pest management. New York: John Wiley & Sons. p 650.

Morrow PA, Fox LR. 1980. Effects of variation in *Eucalyptus* essential oils yield on insect growth and grazing damage. Oecologia. 45:209–219.

Muller da Silva P, Brito J, da Silva F. 2006. Potential of eleven *Eucalyptus* species for the production of essential oils. Sci Agric. 63:85–89.

Nicole D, Dunlop PJ, Bignell CM. 1998. A study of the variation with time of the compositions of the essential leaf oils of 16 *Eucalyptus* species. Flavour Fragance J. 13:324–328.

Pagula P, Baser K, Kürkcüoglu M. 2000. Essential oil composition of *Eucalyptus camaldulensis* Dehn. From Mozambique. J Essent Oil Res. 1:333–335.

Pappas R, Sheppard-Hanger S. 2000. Essential oil of *Eucalyptus camaldulensis* Dehn. From South Florida: A high crytone/low 1,8-cineole eucalyptus. J Essent Oil Res. 12:383–384.

Silvestre AD, Cavaleiro JAS, Delmond B, Filliatre C, Bourgeois G. 1997. Analysis of the variation of the essential oil composition of *Eucalyptus globulus* Labill. from Portugal using multivariate statiscal analysis. Ind Crops Prod. 6:27–33.

Stone C, Bacon PE. 1994. Relationships among moisture stress, insect herbivory, foliar 1,8-cineole content and the growth of river red gum *Eucalyptus camaldulensis*. J Appl Ecol. 31(4):604–612.

Tang C, Cai W, Kohl K, Nishimoto RK. 1995. Allelopathy: Organisms, processes and applications. In: Inderjit, Dakshini KMM, Einhellig FA, editors. Chapter 11, Plant stress and allelopathy. Washington, DC: ACS Symposium Series 582, American Chemical Society. pp 348–362.

Wildy D, Pate J, Bartle J. 2000. Variations in composition and yield of leaf oils from alley-farmed oil mallees (*Eucalyptus* spp) at a range of contrasting sites in the Western Australian wheatbelt. Forest Ecol Manag. 134:205–217.

Zine El Abidine A, Bernier PY, Stewart JD, Plamondon AP. 1994. Water stress preconditioning of black spruce seedlings from lowland and upland sites. Can J Bot. 72:1511–1518.