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Chemical Defenses in Eucalyptus Species: A Sustainable Strategy Based on Antique Knowledge to Diminish Agrochemical Dependency

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1. Introduction

A large number of tree species from different genera have being used world over for their timber resources. Most of them produce roundwood for sawmill and commercial valuable derivatives such as those related to pulp and paper, hardboard and particleboard industries (FAO 2011b). Species within Fabaceae, Pinaceae, Myrtaceae, Cupressaceae, Araucariaceae, Meliaceae, Fagaceae, and Proteaceae families are exploited by those industries.

Wood is characterized by a quite heterogeneous structure based on cell walls mainly composed by cellulose (41-43%), hemicellulose (20-30%), and lignin (27%). Phenylpropanoid derivatives are also contained within lignocellulosic wood structure (Baucher et al 2003, Boerjan et al 2003).

Besides timber uses, some wood particular components are considered adequate resources for other kind of industries. Cellulose derivatives are currently used as a source for natural adhesives. New hydrocolloids are being obtained from cellulose derivatives; some of them have been applied to improve cohesion of wound bandages. Lignins have also industrial applications in fiber-board and paste applications in plywood (Otten et al 2007).

Different wood properties are considered as key characteristics depending on the industrial utilization of forest species. Wood density is the most useful parameter when measuring wood quality. For solid wood purposes wood density is positively correlated to mechanical strength and shrinkage; other properties related to solid wood uses or structural applications are modulus of rupture, modulus of elasticity, percentage of tension, dimensional stability, grain and texture (Hoadley 2000, Wiedenhoeft 2010).

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Current concerns about environmental stability and conservation of natural resources have increased most countries interest in integrated exploitation of forestry species encouraging the use of non-timber products; they have proved to be the source of a wide range of bioactive chemicals. Most of them are secondary metabolites that can be used for nutraceutical, pharmaceutical, and medicinal purposes, also exhibiting potential in integrated pest management to diminish agrochemicals use or even as agrochemicals substitutes (Willfor et al 2004, Krasutsky 2006, Fernándes and Cabral 2007, Domingues et al 2011).

Timber plantations can produce besides bioactive chemicals, many highly valuable non-timber products, including an array of goods like seeds, nuts, oils and fragrances. Some of them also produce other industrial materials such as latexes, tannins, gum exudates, dyes and resins (Wong et al 2001, Jones and Lynch 2007, FAO 2011a).Timber trees producing economically valuable non-timber products have been named 'timber plus trees' (Mull 1993).

Acacia, *Pinus* and *Eucalyptus* genera include some of the most commonly cultivated species in the tropics and South America. Non-timber tropical forest products have been grouped into four categories: (i) fruits and seeds, with plant parts harvested mainly for fleshy fruit bodies, nuts and seed oil; (ii) plant exudates such as latex, resin and floral nectar; (iii) vegetative structures such as apical buds, bulbs, leaves, stems, barks and roots, and (iv) small stems, poles and sticks harvested for housing, fencing, fuel wood, and craft and furniture materials such as carvings and stools (Cunningham 1996, Dovie 2003).

Gums, resins, and latexes have been the most widely used categories of non-wood forest products (Copper et al. 1995; Willfor et al., 2004; Krasutsky, 2006; Fernandes and Cabral, 2007). Species within *Acacia*, *Sterculia*, *Ceratonia*, *Prosopis*, *Agathis,* and *Shorea* genera are among the most important supply sources for such industries. Gums and resins are employed for a wide range of food and pharmaceutical purposes. Pharmaceutical industry uses gums as binding agents for tablets production, and as emulsifying agents in creams and lotions. Food and printing industries have also taken advantage of their thickening, emulsifying, and stabilizing characteristics (Mbuna and Mhinzi 2003, Gentry et al 1992, Anderson 1993). Rosin derivatives from pine trees are being used alone or in combination with acrylic resin solutions and emulsions to produce better quality water-based flexographic inks and varnishes (Vernardakis 2009). Resins are used for paper, wood paints production, and also to prepare varnishes and lacquers, whereas soft resins and balsams are used to produce fragrances (Mbuna and Mhinzi 2003, Messer 1990). Latexes have been early used in the chewing gum industry (Williams 1963).

From an ecological standpoint, non timber products are considered more valuable than timber ones as they can be harvested through the years without cutting down the trees, with almost no perturbation on ecosystems. Moreover, plantations may provide environmental benefits across diverse areas including the atmosphere (replacing ozone-depleting substances), agriculture (land rehabilitation, phytoremediation), and carbon sequestration (Barton 1999). From a social point of view integrated exploitation of forest resources has been considered within the group of multiple-use strategies, which increases the range of income generating alternative options for forest-dependent communities, while avoiding some of the ecological costs of timber cutting (Olajide et al 2008).

2. Eucalypts

Eucalyptus species, belonging to Myrtaceae family, are native to Australia although there are only a few native species from Papua New Guinea, Indonesia and the Philippines. Eucalypts represent a very important group of tree species in Australia, playing a dominant role in continental vegetation and being extremely important for biodiversity conservation. There are more than 800 species, divided in 13 subgenera, and hybrids (Boland et al 2006, Brooker et al 2006). Classification of the genus has been made based on a wide range of attributes. Among genetic, anatomical and chemical features, leaf surface characteristics and the presence of specific secondary metabolites, particularly terpenoids and phenolic derivatives (i.e., renantherin is present in most *Monocalyptus* section), have been recognized as fundamental in eucalypts interaction with specific groups of insects and mammals.

Eucalyptus species grow in natural forests and have been successfully introduced worldwide, being cultivated as one of the main biomass sources under different environmental conditions, depending on the species. Most of them are commercially used to obtain timber and fiber. Eucalypts can play useful environmental roles in natural forests, plantations being also useful for ornamentation and shade purposes in cities green spots, being also useful in lowering water tables. They represent one the most important fiber sources for pulp and paper production in European, African, and Asian countries, and in South America, particularly in Argentina, Brazil, and Chile. Even when the genus is rich in secondary metabolites only a few species are used for essential oil production.

Eucalyptus species grow faster than most other commercially exploited tree species, usually producing high yields (Binkley and Stape 2004). Their abilities to grow rapidly when environmental conditions are suitable and to survive and recover easily from fire and other damaging factors are related to the bud system characterizing their trees. They do not need resting period during winter, growing whenever water is available and temperatures are mild, regardless the time of the year. If they do stop growing (due to a summer drought or a winter cold period) they are able to resume growth whenever stressful factors disappear (Williams and Woinarski 1997, Wei and Xu 2003, Florence 2004).

Eucalypts show better growth performance outside their native environment, it has been suggested that at their natural habitats tree regeneration is affected by insects and diseases commonly absent in other environments. Trees seem to have less access to light, water and nutrients due to neighboring competition in their native land, where soil conditions may have became less adequate and chances of fire higher.

Outside native habitat volume yields can be quite different depending on each particular species genetic potential, resources availability and plantation strategy, i.e. mean annual increments varying from 10 to 90 m^3/ha/year have been obtained (depending on the use of wood, and whether clear-cut or thinning is used) in sample plots as a result of rotation periods from 5 to 25 years (Eldridge et al 1994, Wei and Xu 2003). One of the most important advantages of the genus is its capacity to coppice which allows a new production of wood from the same plantation. *E. grandis*, *E. camaldulensis*, *E. globulus*, *E. tereticornis*, *E. urophylla*, *E. dunni*, and *E. nitens* are among the most successful species. Iberian countries use around 1.29 million ha for timber plantations, predominantly *E. globulus*, whereas in South America *E. grandis*, the hybrid *E. urograndis* and *E. globulus* are among the preferred species, being cultivated in approximately 6.64 million ha (Iglesias-Trabado at al 2009). It has been estimated that pulp production by both these regions may currently represent more than 80% *Eucalyptus* spp. pulp produced worldwide (BRACELPA 2009).

Tolerances to abiotic stresses such as drought, cold, and salinity have been reported for different species. *E. camaldulensis* seems to be a successful one in arid and semi-arid regions, also exhibiting a better performance under moderate to severe soil salinity. *E. nitens* is a cold adaptable species while *E. globulus* grows better in soils characterized by slight to moderate salinity, mainly under temperate climates, and *E. grandis* is mainly cultivated in subtropical and regions (Florence 1996, Teulieres et al 2007).

Several research and breeding programs have been successful improving timber yields, obtaining significant changes in fast growing eucalypt plantations that incremented production volume in more than 40 m^3/ha/year (Binkley and Stape 2004, Shani et al 2003). They have reported the development of 25 transgenic lines in *E. camaldulensis*, *E. grandis* and its hybrids with the ability to produce considerably higher biomass amounts.

Wood quality traits, particularly those needed to produce solid wood products have been investigated by different research groups. Pilodyn penetration is an indirect method for determining wood basic density, Muneri and Raymond (2000) have analyzed genetic parameters and genotype-by-environment interactions for basic density, pilodyn penetration, and stem diameter in *E. globulus*. Genomics and markers linked to wood properties were also studied by other authors, Barros et al (2002), Moran et al (2002), and Thamarus et al (2004).

Even when commercial exploitation of transgenic eucalypts has not been developed in a significant degree, research work has been performed in order to obtain genetically improved clones producing higher yields of high quality woods, also exhibiting higher levels of resistance to environmental stress (Girijashankar 2011). Eucalypt fibers are particularly appreciated for manufacturing high-quality grades of tissue paper, writing and printing papers (Foelkel 2009). Advances have been made related to microfibrillar orientation of secondary cell wall in *E. grandis* (Thumma et al 2005, Spokevicius et al 2007) and *E. nitens* (MacMillan et al 2010), and a reduction of lignin content has been achieved in *E. grandis* x *E. urophylla* (Tournier et al 2003) and in *E. camaldulensis* modified clones (Kawaoka et al 2006, Chen et al 2001). Improved plant raw materials for pulp and paper purposes have been obtained by modifying lignin biosynthetic pathway; these changes can affect lignin content, composition, or both (Baucher et al 2003).

Water deficit, a common constraint in forestry, is the main cause of plant stress during eucalypts establishment, when seedling growth can be also compromised by herbivory and disease. Genetic changes to improve plant survival chances enhancing resistance levels towards environmental adversities would contribute to obtain higher yields in forestry plantations. The ability to successfully grow under different kinds of abiotic stress represents an important trait in eucalypts plantations. Transgenic lines in several eucalypt species with tolerance to salinity, low temperatures and drought were obtained by Yamada-Watanabe et al (2003) and Navarro et al (2011).

Higher resistance levels to plagues and herbicides have been obtained in transgenic *E. camaldulensis* (Harcourt et al 2000) and *E. urophylla* (Shao et al 2002), commercial potential of this feature being an important management option during establishment period.

Although successful development of most species depends on their ability to compete for resources, it has been also related to their production of bioactive defensive chemicals. Research on genetic control of secondary metabolites production by forest trees has been increased in recent years. Several studies performed on *E. globulus* (Freeman et al 2008, O´Reilly-Wapstra et al 2011, Külheim et al 2011) and *E. nitens* (Henery et al 2007) allowed the identification of the quantitative trait loci for terpenes and formylated phloroglucinol derivatives, compounds considered to be the most significant defensive treats against herbivory. Essential oils have been accepted as the main defensive trait in eucalypts. Research work on genetic modulation of essential oil production has been performed by Ohara et al (2010). These authors developed a transgenic *E. camaldulensis* clone introducing genes associated to limonene synthesis; they demonstrated that plastidic and cytosolic expression of PFLS (Perilla frutescens limonene synthase) resulted in 2.6 to 4.5-fold increments in limonene concentrations, also emphasizing a synergistic increase in 1,8-cineole and α-pinene biosynthesis.

High hemicellulose content represents a desirable pulp characteristic for paper production, since hemicellulose levels are positively correlated to pulp resistance; surprisingly it seems to be also related to essential oil production. Correlations between leaf volatile organic compounds and pulp properties may be useful in genetic improvement programs. Positive correlations between leaf volatiles and hemicellulose content in pulp have been previously reported by Zini et al (2003) for 14 eucalypt clones.

3. Plants natural defenses

While primary metabolism has been related to biomass development and reproduction, exhibiting very slight differences among living organisms; secondary metabolism includes a wide range of chemical families, making it possible to classify plants in different species through chemical taxonomy, according to each particular profile.

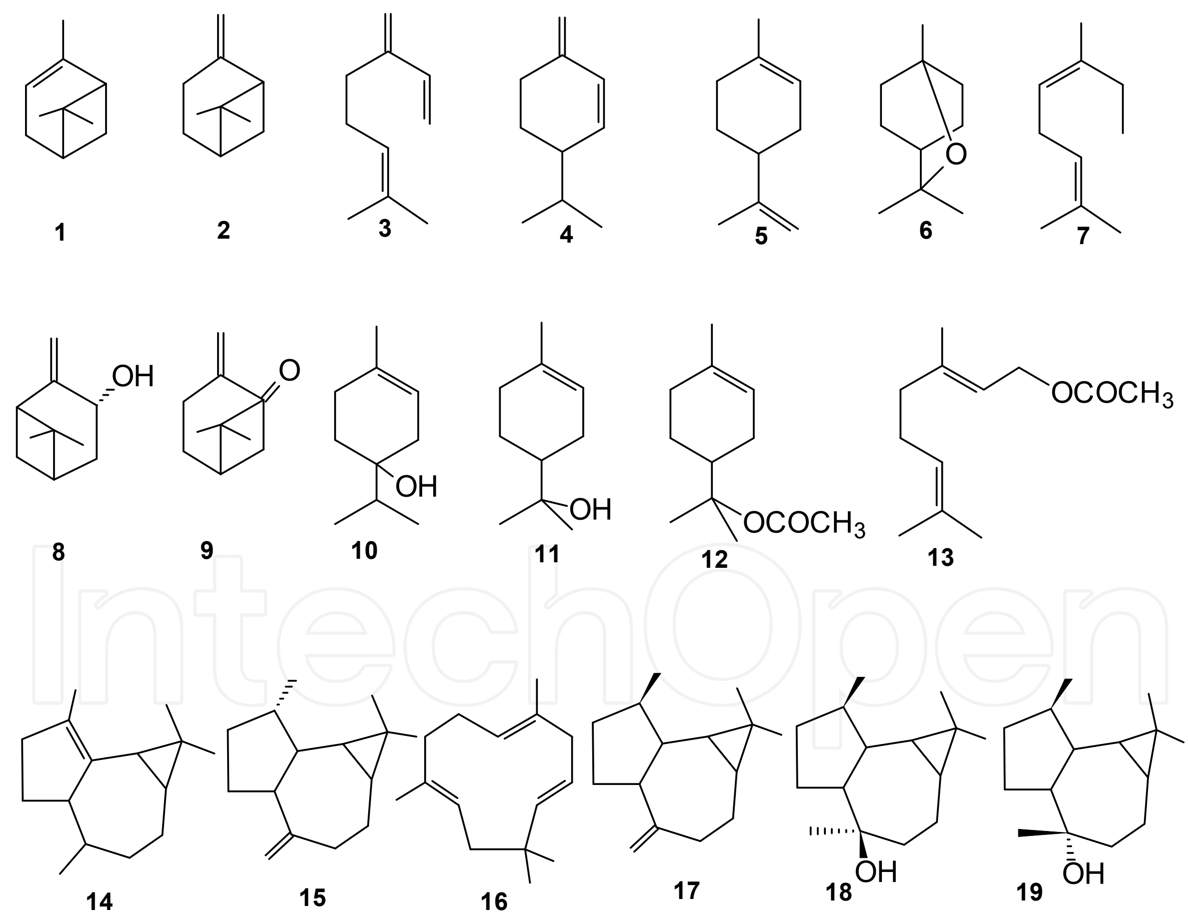
During evolution, terrestrial plants have developed new biosynthetic pathways to produce flavonoids, terpenoids, alkaloids, cyanogenic glycosides, glucosinolates, and numerous phenolic compounds (including polymers such as lignins and tannins), secondary metabolites that have provided advantages to the producing species allowing their successful survival despite invasive organisms and other environmental stresses.

Benzoic and cinnamic acids and their phenolic derivatives, flavonoids, and long chain hydrocarbon compounds including derivated alcohols, carbonylic compounds and fatty acids, are among the most common defensive chemicals in plant kingdom. Alkaloids, terpenoids, glucosinolates, hydroxamic acids, tiophenes, cyanogenic glycosides, disulfures and sulfoxides are less distributed and restricted to particular genera, (Leicach et al, 2009a, Leicach et al 2010, Yaber Grass et al 2011).

Most of them are natural products also known as **allelochemicals**, which were originally defined as secondary metabolites involved in plant-plant and plant-microorganism interactions, until International Allelopathy Society (IAS) extended in 1998 its definition to every natural product playing a role in plant-environment interactions (Leicach et al 2009b). They have proved to provide protection towards competition by weeds and other plants and to avoid detrimental action of herbivores, fungi, bacteria, and viruses. Insects and mammals feeding on leaves have co-evolved in such a way that some secondary metabolites also play important roles in host selection by herbivores (Matsuki et al 2011). Secondary metabolites seem to represent the chemical language in plant-environment interactions, continuously growing in number and changing as co-evolution takes place.

Terpenoids are physiologically, ecologically, and commercially important, protecting plants against herbivores and pathogens and having important roles in allelopathic interactions, nutrient cycling and attraction of pollinators. Different woody species produce volatile mixtures of terpenoids known as essential oils that are responsible for their particular odours and fragrances. They are produced by species within different families such as Asteraceae (*Matricaria*), Labiatae (*Mentha*), Myrtaceae (*Eucalyptus*), Pinaceae (*Pinus*) and Rutaceae (*Citrus*) and contain structurally related terpenes as main components (Harborne 1998, Leicach et al 2009a). Essential oils are usually obtained from non-woody parts of the plant, particularly foliage, through steam distillation also known as hydrodistillation. They are complex mixtures of monoterpenes (C_{10}) and sesquiterpenes (C_{15}), with minor abundances of phenylpropanoids, and acyclic hydrocarbon derivatives such as oxides, ethers, alcohols, esters, aldehydes, and ketones.

Figure 1 shows the chemical structure of most distributed essential oil mono and sequiterpenic compounds.



1-α–pinene, **2**- β–pinene, **3**- myrcene, **4**- α–phellandrene, **5**- limonene, **6**- 1,8-cineole, **7**- *cis*-ocymene, **8**- *trans*-pinocarveol, **9**- pinocarvone, **10**- 1-terpinen-4-ol, **11**- α–terpineol **12**- α-terpinyl acetate, **13**- geranyl acetate. Sesquiterpenes: **14**- α-gurjenene, **15**- aromadendrene, **16**- β–humulene, **17**- allo-aromadendrene, **18**- globulol, **19**- epiglobulol.

Fig. 1. Essential oils most common terpenic derivatives

Essential oils are commonly contained in glandular trichomes, a paradigmatic example of joint physicochemical mechanism that has demonstrated to be one of the most effective defences against noxious organisms (Hammerschmidt and Schultz 1996, Bowers et al 2000). Enzymes involved in the different metabolic steps associated to these terpenes biosynthesis are located within these structures (Gershenzon and Croteau 1990, Gershenzon and Dudareva 2007).

Essential oil blend depends on the relative amounts of their components, a feature that characterizes each species. Table 1 shows essential oil most important components for some *Eucalyptus* species (Juan et al 2011, Gilles et al 2010).

Oil constituents	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
α-Thujene		0.6		0.3	0.2					3.1		0.5	
α-Pinene	4.3	5.4	2.3	1.2	10.1	9.3	5.6	8.3		0.4	5.5	5.6	52.7
Camphene		1.6	0.3		0.3	23.1	0.3						
β-pinene	25.3	0.1	1.7	0.7	2.1	2.7		2.5	6.2				
Myrcene		0.2	0.6		0.4		1.7	1.3		1.1		0.5	
α-Phellandrene				7.2	1.2		2.3			17.4	1.4	7.6	
limonene	4.6	5.4		2.6	6.4	5.1	10.1		3.5				
β-Phellandrene										2.8			
1,8-Cineole	5.2	58.9	1.2	35.7	57.7	44.3	61.3			0.7	48.5	26.7	18.7
β-ocimene				0.1	4.4					0.3			
γ-Terpinene	1.2	2.8	0.3	2.8	0.2			0.8		0.8	13.0	3.0	5.0
para-cymene	7.4	2.1				1.6	7.2	28.6	27.3	8.5	4.4	13.6	9.7
Terpinolene										2.4			
citronellal			72.7										
Linalool	0.4		0.1		2.5	0.3				0.9			
β-caryophyllene	4.3		2.6	0.2		2.2							
Terpin-4-ol	1.7			1.2		0.2		1.7		4.7	3.6	1.8	1.1
α-Terpineol	6.2	2.7	0.7	1.4	1.3	0.3	3.1	5.6	6.3	1.0	3.6	1.5	5.7
Piperitone										40.5			
cryptone		1.1		25.4	0.4	1.3	3.7	17.8					
α–Terpinyl acetate		2.1	1.5			1.2		0.2		0.3	5.2		
citonellol	2.3		6.3			0.1							
Arommadendrene	1.7	2.1		1.3		1.3			0.3		3.4	2.3	
alloaromadendrene													
epiglobulol												7.5	
Spathulenol	4.1			0.2				1.8	1.1				
globulol	2.4	1.6		3.1	4.4	7.3	0.3	0.5			4.2	0.1	

1-*E. alba,* ***2****- E. camaldulensis,* ***3****- E. citriodora,* ***4****- E. deglupta,* ***5****- E. urophylla,* ***6****- E. globulus,* ***7****- E. saligna,* ***8****- E. tereticornis,* ***9****- E. robusta,* ***10****- E. dives,* ***11****- E. dunnii,* ***12****- E. gunnii,* ***13****- E. grandis.*

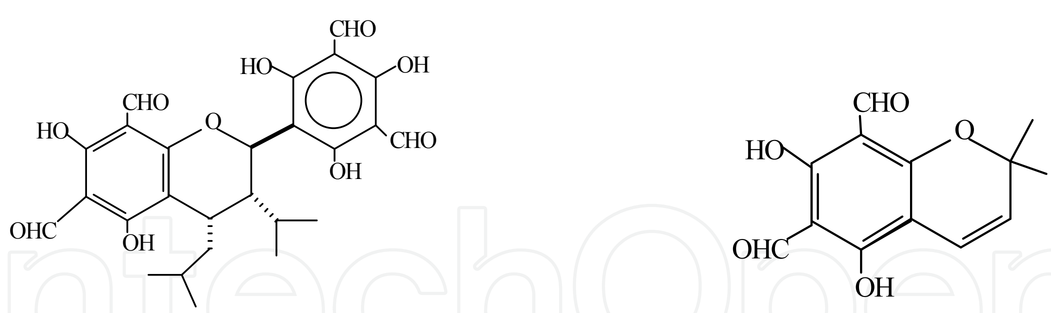
Table 1. Essential oil components for some *Eucalyptus* species

1,8-cineole, also known as eucalyptol, is one of the most characteristic chemical features in *Eucalyptus* species, being the main component in many of their essential oils, with relative abundances varying from 20 to 70%, depending on the species. Some of them (*E. alba*, *E. camaldulensis*, *E. urophylla E. globulus* and *E. saligna*) produce high amounts of 1,8-cineole, the latter being the one with the higher relative abundance in Table 1. *E. sideroxylon* represents an extreme example as its essential oil can contain up to 90% 1,8-cineole (Alzogaray et al 2011).

Other species do not include 1,8-cineole in their essential oils (*E. tereticornis* and *E. robusta*) or produce it in much lower amounts. *E. grandis* essential oil contains α-pinene as the main component (52.7%) being 1,8-cineole the second one in relative abundance (18.7%), *E. alba* produces β–pinene (25.3%) as the main component and only 5.2% 1,8-cineole and *E. citriodora* characterized by a high amount of citronellal in its essential oil (72.7%), contains only 1.2% 1,8-cineole (Cimanga et al 2002).

Some *Eucalyptus* species have been studied by our research group. Essential oils obtained from fresh leaves of four species grown in Argentina within Agronomy School experimental field (34º 37′ S, 58º 20′ W, Buenos Aires University) were obtained by hydro-distillation using a Clevenger device to be further analyzed by GC and GC-MS. *E. globulus* showed the highest (1.5% fw) essential oil yield, followed *E. sideroxylon* (1.1% fw), *E. tereticornis* (0.9% fw), and *E. camaldulensis* (0.8%), adult leaves from the latter containing the highest 1,8-cineole relative abundance (60%), almost two-fold the value found in the other three species (unpublished results).

Eucalyptus species produce, besides essential oils, secondary metabolites that are ubiquitously distributed in plant kingdom such as hydrolyzable and condensed tanins, flavonoids, and others less distributed such as phloroglucinol derived compounds and cyanogenic glycosides (Moore et al 2004).



Sideroxylonal A loxophlebene

Fig. 2. Eucalypts formylated phloroglucinol compounds

Foley and coworkers have reported that there are species within *Eucalyptus* subgenera (e.g. *Monocalyptus*) lacking formylated phloroglucinols while others (e.g. *Symphyomyrtus*) contain a wide variety of them. They have also reported that there is a positive correlation between formylated phloroglucinols concentrations and those corresponding to terpenes in trees foliage, allowing koalas and other marsupial species to make food choices based on the way leaves smell (Foley et al 2009). Formylated phloroglucinol compounds are characterized by at least one fully substituted phenolic ring with one or two aldehyde groups. Phloroglucinol sesquiterpene derivatives have been identified in *E. globulus* leaves (Osawa et al 1996).

Dimeric phloroglucinol compounds such as sideroxylonals, have also been found in leaves and flower buds of several *Eucalyptus* species (Eschler and Foley 1999).

Recently Sidana et al. (2011) have reported the isolation of two new formylated phloroglucinols, loxophlebal B and loxophlebene in *E. loxophleba* ssp. *Lissophloia* leaves.

Cyanogenic glycosides, on contrary, are not common chemical features in eucalypts, however there are few species containing them. *E. cladocalyx* allocates up to 20% of leaf nitrogen to cyanogenic glycosides production, being prunasin the most representative compound (Gleadow et al 1998, Gleadow and Woodrow 2000). Gleadow et al. (2008) have described 18 cyanogenic species and one subspecies (e. g. *E. acaciiformis* Deane & Maiden, *E. leptophleba* F. Muell., *E. nobilis* Johnson & Hilld).

Most cyanogenic species produce mainly (*R*)-prunasin, although its epimer, sambunigrin, has been also detected. The diglycoside amygdalin has been reported to be produced by *E. camphora* (Neilson et al 2006).

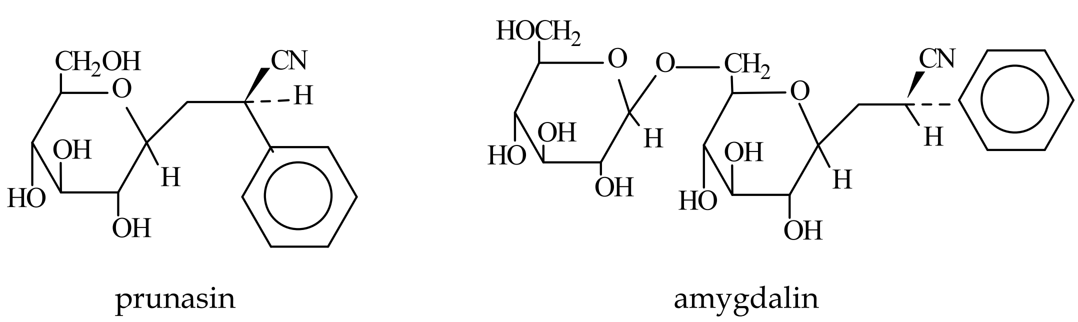


Fig. 3. Cyanogenic glycosides in some eucalyptus

4. Allelochemicals production

The amount and structural characteristics of biosynthesized defensive chemicals vary with the species and depend on each individual physiological and ontogenic state, also being strongly modulated by environmental conditions (Einhellig 1989, Einhellig 1995, Leicach 2009c). Essential oil yield and composition have demonstrated to change for a particular species depending on individual features as much as on geographical location, climate conditions and particular characteristics of soil. They can differ between individuals from the same species, even within a small population.

4.1 Individual features

The amount and structural characteristics of biosynthesized defensive chemicals vary with age in each species, and may dramatically change with changes in each individual physiological condition (Leicach 2009d). Even when it has been speculated that immature plant tissues lack of defensive chemicals since their production would be constrained by the lack of the corresponding enzymatic machinery, it has been demonstrated that concentration of some secondary metabolites (particularly low molecular weight phenolics, cyanogenic glycosides, terpenes, and alkaloids) reaches the highest value during early stages of seedling growth and leaf expansion, being only synthesized in young tissues (Herms and Mattson 1992). Most of them, including essential oil components, are powerful biocides that play a protective role against noxious organisms at this particularly sensitive stage of plant development. $^{14}CO_2$ incorporation rate values indicated maximum levels of camphor biosynthesis during *Salvia officinalis* leaf expansion (3-4 weeks), which declined to almost undetectable ones after 6 weeks (Genshenzon and Croteau 1990).

Essential oil yield and chemical composition have shown to be related to leaf age in *Eucalyptus* species (Silvestre et al 1997, Wildy et al 2000). Higher essential oil yields have been obtained from *E. camaldulensis* fully expanded but not fully lignified leaves (Doran and Bell 1994). Significant differences in essential oil yield and composition were also detected between *E. globulus* young and mature leaves (Chennoufi et al 1980, Silvestre et al 1997), and also among juvenile, mature, and senescent leaves from *E. citriodora* (Batish et al 2006).

We have studied differences in essential oil yield and composition between adult leaves and the new ones harvested three months after submitting *E. camaludulensis* trees to mechanical damage. We have found that young leaves produced higher yields, 66.3% more essential oil than adult ones. Non oxygenated terpenes were particularly abundant in young leaves, whereas higher relative abundances in many oxygenated terpenes, including 1,8-cineole, were found in adult leaves. Table 2 shows relative abundances of *E. camaludulensis e*ssential oil components exhibiting significant differences between adult and young leaves (unpublished results).

Terpene	**Adult leaves**	**Young leaves**
α-Pinene	4.4 ± 0.5	7.3 ± 0.6
Camphene	0.3 ± 0.09	0.1 ± 0.05
β-Pinene	3.1 ± 0.4	2.3 ± 0.2
Myrcene	0.7 ± 0.22	2.7 ± 0.5
α-Phellandrene	0.1 ± 0.04	0.5 ± 0.1
α-Terpinene	0.09 ± 0.02	0.4 ± 0.17
1,8 Cineole	59.6 ± 3.7	48.6 ± 3.3
β-Ocymene	0.5± 0.1	0.3 ± 0.1
γ-Terpinene	3.9 ± 0.3	5.7 ± 0.8
α–Terpinolene	traces	1.0 ± 0.2
Linalool	0.23 ± 0.08	0.55 ± 0.1
α–Fenchol	0.55 ± 0.2	0.31 ± 0.1
Trans-Pinocarveol	2.12 ± 0.70	0.52 ± 0.15
Pinocarvone	0.86 ± 0.14	0.55 ± 0.16
α-terpineol	8.35 ± 0.69	5.80 ± 0.61
geranil acetate	traces	0.43 ± 0.15
Isolongifolene	traces	0.33 ± 0.05
β–Humulene	0.67 ± 0.3	0.99 ± 0.24
Globulol	2.47 ± 0.63	4.25 ± 0.60
Epiglobulol	1.24 ± 0.47	3.65 ± 0.35
Oxigenated terpenes	80.27 ± 0.35	69.47 ± 0.46
Non-oxigenated terpenes	16.71 ± 0.17	24.44 ± 1.82

Table 2. Mean relative abundances of *E. camaldulensis* essential oil components in adults and young leaves. Data given as mean ± standard deviation.

Marzoug et al (2011) have also reported higher amounts of oxygenated derivatives in *E. oleosa* adult leaves (43.2%) compared to young ones.

Secondary metabolites can be found in almost all plant organs, their concentrations and relative abundances being characteristic of each kind. Particular chemical families are in general distributed in different proportions depending on the plant organ and tissue. Marzoug et al 2011 analyzed essential oil yield and composition in different *E. oleosa* organs, their results for most abundant components shown in table 3.

Oil component (%)	Stems	Adult leaves	Fruits	Flowers
α-Pinene	5.2	1.7	2.6	2.2
p-Cymene	6.8	10.6	9.0	9.2
Limonene	4.2	1.5	0.7	1.6
1,8-Cineole	31.5	8.7	29.1	47.0
cis-Sabinol	3.1	4.2	2.5	1.0
trans-Pinocarveol	9.9	-	0.1	0.1
Pinocarvone	3.5	1.8	1.0	0.3
Verbenone	2.1	3.7	1.4	0.8
α-Selinene	-	0.5	10.0	2.1
Spathulenol	3.5	16.1	3.4	-
γ-Eudesmol	5.6	15.0	16.4	12.5
Essential oil yields (%)	0.52	0.45	1.12	0.53

Table 3. Essential oil yield and composition in different *E. oleosa* organs.

Essential oils bioactivity depends on their particular composition, which changes from organ to organ; different biological activities have been reported for essential oils obtained from eucalypts different organs. Essential oils from *E. globulus* Labill leaves have been widely used as antiseptic and for the relief of cough symptoms, colds, sore throat and other infections (Kumar et al 2007). It has been early reported that the main compound of its fruit oil was 1,8-cineole (Basias and Saxena 1984), however aromadendrene was reported almost two decades later by other authors to be the main constituent for the same species (Pereira et al 2005); differences in environmental conditions might be responsible for the lack of agreement in the results. Mulyaningsih and coworkers (2010) have suggested that aromadendrene may significantly contribute to antimicrobial activity of its fruit oil. Combinations of aromadendrene and 1,8-cineole showed additive effects in most cases, but also synergistic behavior. *E. globulus* fruit essential oil has been proved to display a pronounced antimicrobial effect towards multidrug-resistant bacteria.

4.2 Environmental conditions

Biotic stresses (disease, herbivory and/or the presence of competitors) as much as abiotic stresses such as nutrient deficiency and drought can affect chemical defence's production, in particular essential oil yield and composition. Potential of nursery preconditioning to enhance survival chances of future trees by reducing their palatability or by attracting beneficial insects as a result of changes in chemical defenses may be an answer to overcome environmental adversities (Leicach et al 2010). It can affect leaves quality by modulating their chemical composition, a determinant feature, besides tissues hardness, towards herbivory. It has been demonstrated that a higher 1,8-cineol relative abundance in essential oil can lower defoliation level due to *Anoplognathus* beetles (Edwards et al 1993). In order to develop a more sustainable commercial forestry minimizing chemical input, studies have been performed by our research group to obtain more resistant seedlings to begin with. Controlled drought conditions at particular nursery stages have proved to be one of the possible ways to achieve it, particularly when they enhance seedlings chemical defensive potential (Leicach el al 2010).

4.2.1 Abiotic factors

Studies performed in the last fifty years confirmed that most important abiotic factors affecting allelochemicals biosynthesis are water, soil quality, light, and temperature, several pathways within secondary metabolism being often increased when plants are exposed to growth condition different from their optimal requirements. Spatial and temporal variation in resources availability may influence the relative magnitude of defence benefits in plants, each particular class of compounds within them being able to respond in different ways to changes in environmental conditions.

Soil degradation has proved to increase allelochemicals production in different species. We have previously reported data related to the modulating effect of soil deterioration on secondary metabolites production by two widespread weed species, *Chenopodium album* and *Senecio grisebachii*. *C. album* samples grown in continuously cultivated (deteriorated) Argentinean Rolling Pampa soils have demonstrated to produce higher levels of long chain hydrocarbon derivatives (known to inhibit plant germination) than those grown in pristine soil (Leicach et al 2003). We have also demonstrated that the invasive weed *S. grisebachii* produced higher levels of toxic pyrrolizidine alkaloids in soils with higher deterioration degree. Significant differences were found when reproductive organs were studied, inflorescences from samples grown in less deteriorated soil showing lower relative abundances of toxic alkaloids (Yaber Grass et al 2011).

Water deficiency has proved to be a usual stress affecting agriculture and forestry, drought conditions being able to change allelochemicals abundances, including essential oil yield and composition. As a result of previous studies we have reported qualitative changes in essential oils obtained from young *E. camaldulensis* leaves after submitting seedlings to drought during nursery period. We did not find significant changes in essential oil yield, but we found significant changes in essential oil relative composition. Leaves from seedlings submitted to drought developed an essential oil composition that has been previously reported to be characteristic of mature leaves (Silvestre et al 1997) and related to their higher resistance to herbivory. Total amount of oxygenated terpenes were significantly increased as a result of water deficiency; globulol, epiglobulol, and ledol abundances were doubled, and 1,8-cineole content was enhanced by 28.3%, whereas total amount of non-oxygenated terpenes was significantly decreased (44%) (Leicach et al 2010).

Light quality and nutrient availability are also determining factors. Gleadow and Woodrow (2002) have reported that *E. cladocalyx* cyanogenic glycoside concentration was increased in near 70% in fully expanded leaves in response to moderate water stress; another research group has demonstrated that light deficiency caused the opposite effect on this species, enhancing cyanogenic glycoside production (Burns et al 2002). In both reports total phenolics and condensed tannins remained unaffected. Studies related to fertilization effects showed that *E. globulus* seedlings reduced their condensed tannins content while significantly enhancing essential oil production when nutrients were added (O´Reilly-Wapstra et al 2005).

4.2.2 Biotic factors

Damage caused by biotic factors such as herbivory have often proved to enhance secondary metabolism, however response depends on the particular species. We have previously reported changes in quinolizidine alkaloids abundances in two *Lupinus* species (*L. albus* and *L. angustifolius*) that responded in different ways to mechanical damage and herbivory (Vilariño et al 2005, Chludil et al 2009).

Many plants species respond to wounding, herbivory or pathogens attack by increasing endogenous synthesis and releasing jasmonic acid and methyl jasmonate, starting in damaged organs. These volatile signals have proved to activate defence responses in intact neighbours from the same species. Even when *Eucalyptus* species produce a complex array of constitutive chemical defenses, no significant changes have been found in essential oils, polyphenolics and foliar wax related compounds from trees following foliar-chewing insect damage (Rapley et al 2007). These authors reported that only foliar tannins seemed to be affected, increasing their concentration three months after larval feeding. However they did not find such differences in wounded trees five months later, suggesting that increase of tannins production was most likely a rapid response, which proved to diminish further larval survival and branch defoliation. They have suggested that foliar tannins may operate as toxins and/or anti-feedants to *Mnesampela privata* larval feeding.

5. Ecological significance of natural defenses of plants

Both volatile and non-volatile secondary metabolites from eucalypts have been associated to a wide spectrum of roles associated to defensive strategies.

Host location by insects has proved to be related to leaves chemical composition, secondary metabolites involved in those interactions are known as **semiochemicals**. Research on semiochemicals has grown since the 1950s, parallel to that related to allelochemicals, in terms of isolation and identification of responsible secondary metabolites; the final goal in both areas being development of solutions for agriculture and forestry problems through applied research. Sex pheromones and kairomones represent the most important groups of chemical signals in intra and interspecies communication, having proved to affect host choice by a particular insect species, i.e. mountain pine beetles feed on pines avoiding alder trees as a result of their ability to detect kairomones of appropriate hosts and non-host species. Volatile organic compounds playing the role of kairomones, are detected by insects through olfactory receptors located usually in antennae hairs.

Similarities in secondary metabolites between *Eucalyptus* species and those belonging to other genera have proved to trigger utilization of novel hosts by insects feeding on leaves. Electroantennogram studies (EAG) have demonstrated that *M. privata* female responds to ubiquitous eucalypt monoterpenes using them as host location and assessment cues (Steinbauer et al 2004). These authors also suggested that epicuticular waxes can be used as a leaf age indicator. *M. private*, also known as autumn gum moth, seems to take oviposition decisions based on both nonstructural epicuticular wax and foliar monoterpene cues. They may lay their eggs on novel hosts if foliar chemistry resembles that of the primary host (Ostrand et al 2008), new expansion hosts sharing many terpenes with natural ones (Paine et al 2011). Behavioral assays showed that natural and novel hosts with high amounts of α-pinene received fewer eggs than those with lower amounts; the opposite occurred with α-terpineol that have shown to enhance *M. private* oviposition in eucalypts containing it higher concentrations (Ostrand et 2008). De Little (1989) has also suggested that similarities in foliar terpenes may partly explain host expansion of leaf beetles *Chrysophtharta bimaculata* onto *E. nitens* in Tasmania. Steinbauer and Wanjura (2002) have discussed the preferential selection by Christmas beetles (*Anoplognathus montanus* and *A. pallidicollis*) of exotic peppercorn trees (*Schinus molle*) over neighbouring previous eucalypt hosts, suggesting that it could be related to the lack of 1,8-cineole in *S. molle* combined with the presence of other attractant monoterpenes, since 1,8-cineole is a main constituent in eucalypt species resistant to Christmas beetles.

Comparative GC-EAG studies have shown that several volatile compounds, including 3-hydroxy-2-butanone, 3-methyl-1-butanol, ethyl 3-methylbutanoate, (*Z*)-3-hexen-1-ol, α-pinene, β-pinene, *p*-cymene, 1,8-cineole, limonene, guaiene, α-terpinene, and linalool are be detected as hosts signals by the woodborer *Phoracantha semipunctata* (Barata et al 2002). These authors suggested that α-cubebene and (*E*)-4,8-dimethylnone-1,3,7-triene may act as cues for avoidance of unsuitable hosts.

Eucalypts extracts contain an array of defensive allelochemicals exhibiting various biological effects, antibacterial, antioxidant, and antihyperglycemic, among them (Takahashi et al 2004), with essential oils playing a central role in many of these biological functions. Most essential oils, including those obtained from eucalypts, have shown to display some degree of antimicrobial activity that is in general related to the presence of terpenoid and phenylpropanoid compounds that have proved to exhibit individual antimicrobial effects. Biological activity of essential oils starts most likely, affecting cell membrane structure and functions. Because of their lipophilicity, many of their components can accumulate in membranes being able to disrupt its structure and to affect transport processes and other membrane-associated events such as signal transmission, and ATP synthesis particularly in microorganisms (Einhellig 1995).

Essential oils bioactivity has been often related to the presence of 1,8-cineole in eucalypts, however it has been suggested that several other components may eventually act additively or synergistically. Citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α–terpineol, alloocimene, and aromadendrene are among them (Duke 2004, Batish et al 2006; Su et al 2006, Liu et al 2008). *E. camaldulensis* and *E. urophylla* antibacterial properties have been described by Cimanga et al (2002), their antifungal activities have been confirmed by Su (2006). Strong inhibiting effects on *S. aureus* and *E. coli* were described by Bachir Raho and Benali (2008) for *E. globulus* and *E. camaldulensis* essential oils. Another *Eucalyptus* species, *E. oleosa*, produces essential oils with proven antibacterial activity against Gram-positive bacteria, and inhibitory effect on yeast-like fungi. Both activities have been related to the presence of oxygenated monoterpenes, 1,8-cineole in particular (Marzoug et al 2011).

Different assays were also performed by our research group to determine *Eucalyptus* essential oil biological activities. Antifungal activity of four *Eucalyptus* species (*E. camaldulensis*, *E. globulus*, *E. sideroxylon, E. tereticornis*) was tested against common pathogens affecting crops production (*Aspergillus flavus, Aspergillus niger, Cladosporium cucumerinum*). Antifungal activity (8 μl/dot) was tested by means of bioautographic assay (Homans and Fuchs 1970), *E. camaldulensis* being more active than *E. globulus*, *E. sideroxylon* and *E. tereticornis*.

A. flavus and *C. cucumerinum* susceptibilities to essential oil decreased in the following order: *E. camaldulensis* > *E. globulus* > *E. sideroxylon* > *E tereticornis*, being *C. cucumerinum* the most affected species. At the same dose *A. niger* also showed differential susceptibility between four species essential oil. *E. camaldulensis* essential oil exhibited almost two-fold *E. globulus* activity and more than two-fold *E. tereticornis* activity, but it was only 25% more effective than *E. sideroxylon* essential oil. The fact that *E. camaldulensis* essential oil was the one containing higher amounts of oxygenated terpenoids may suggest a possible correlation to its higher activity (Marzoug et al 2011).

Eucalyptus essential oil components have also been associated to allelopathic activities, most of them inhibitory; few species have been able to develop successfully near these trees in natural habitats (Liu et al 2008).

Other *Eucalyptus* defensive chemicals such as phenolic derivatives have also proved to sensitize phospholipids bi-layer, increasing cell membrane permeability and leakage of vital intracellular constituents and also impairing microbial enzymes activity (Moreira et al 2005).

A different class of phenolic derivatives, formylated phloroglucinols, only produced by some *Eucalyptus* species have shown to display a wide range of ecologically significant biological activities, particularly as anti-feedant (Lawler et al 1998, Sidana et al 2010).

Ecological significance of formylated phloroglucinol derivatives in *Eucalyptus* species has been associated to marsupial folivores feeding behaviour (Eschler et al 2000, Marsh et al 2003, Sidana et al 2010). Interactions between eucalypts and mammalian herbivores that feed on them have also been investigated by other authors (Lawler et al 2000, Close et al 2003). O'Reilly-Wapstra et al (2002) analyzed the role of plant genotype in *E. globulus* resistance to browsing by a generalist marsupial folivore, the common brushtail possum. Formylated phloroglucinol compounds (Eschler et al 2000) seem to play a significant defensive leaf trait conferring resistance in *E. globulus* juvenile coppice foliage (O'Reilly-Wapstra et al 2004).

6. Potential uses

Secondary metabolites produced by forestry species might be used in Integrated Pest Management either as repellents or attractants. *Eucalytpus* species have shown not only to reduce atmospheric carbon dioxide levels and provide timber biomass, but also to perform a variety of indirect services through their chemical defenses, essential oils being their most characteristic defensive treat with a wide spectrum of biological effects.

Plant extracts and isolated natural compounds represent a wide range of possibilities to replace or at least diminish the use of synthetic products to control pests and diseases affecting plants, animals and/or human beings. Bioactive natural products should also be seriously considered as they have proved to be more specific in most of their biological activities.

Forestry-derived industries have been focused during last decades on development of breeding techniques to produce trees with higher timber yields and enhanced wood quality. An undesired feature observed in most improved cultivars was an enhancement of susceptibility to diseases and predators, suggesting that they were investing less metabolic energy in chemical defence (Wallis et al 2010). This fact and the development of pesticide resistance in previously controlled herbivores may explain the increment in pesticide amounts further used in forestry plantations. Continuous agrochemicals overuse seriously affected environmental sustainability also triggering pest resurgence and development of resistance/cross-resistance (Brunherotto and Vendramim 2001). Similar practices in agriculture associated to food demands of a permanently growing population, strongly contributed to enhance environmental degradation; soil and water pollution, and losses in biodiversity being the main deterioration symptoms faced by most countries in the world.

The fact that synthetic pesticides have proved to represent a significant hazard to mammals and mankind directly consuming plant material or derived foodstuff has been thoroughly confirmed. Current concerns about their presence contaminating grains, fruits, and vegetables, has limited the number of permitted synthetic fumigants, encouraging the search for friendlier pest control alternatives. Natural products represent one of the most important alternatives, to control pests and diseases that affect plants and animals without deleteriously effecting environmental safety (Isman 1997, Men and Hall 1999, Tripathi et al 2008). Plants natural ability to produce allelochemicals is currently revised to 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, Yaber Grass et al 2011).

Chinese folk medicine has used *Eucalyptus* species for centuries, hot water extracts of dried leaves from *E. citriodora* have been, and are still used to prepare anti-inflammatory, analgesic and antipyretic formulas for respiratory infections, such as sinus congestion and flu. Essential oils are easily biodegraded and have proved to exhibit low toxicity against vertebrates also playing an important role as bioherbicide for weed management (Barton 1999, Batish et al 2007, Batish et al 2008).

E. camandulensis essential oil has also proved to be useful for pharmaceutical purposes. It has been used to treat lung diseases and cough in medicines like expectorants, also taking advantage of its antituberculosis, antibacterial, and antifungal properties.

The significant negative effects of *E. camaldulensis* and *E. urophylla* essential oils on *S. aureus* and *E. coli* development contribute to point out the potential of both *Eucalyptus* species for antiseptic, microbiostatic, or as disinfectant activities (Bachir Raho and Benali 2008).

Eucalyptus essential oils have been preferred over those obtained from other forestry exploited species because they have proved to be useful in perfumery, pharmaceutical and other industries playing multipurpose roles (FAO 1995). They have also proved to negatively affect virus development, Schnitzler et al (2001) reported *in vitro* activity against antiherpes virus. *E. globulus* essential oil components, alone or in combination with other antibacterial agents, may provide a promising new scheme in phytotherapy.

Besides essential oil components, other eucalypts defensive treats may also be used taking advantage of their biological effects. Formylated phloroglucinols have also proved to be therapeutically and/or pharmacologically significant (EBV inhibitory (Takasaki et al 1990), anti-bacterial (Satoh et al 1992), HIV-RT inhibitory (Nishizawa et al 1992), aldose reductase inhibitory (Satoh et al 1992), anti-protozoal (Bharate et al 2006). The dimeric derivative, sideroxylonal A, has proved to act as a potent marine anti-fouling agent comparable to the most active compound 2,5,6-tribromo-1-methyl-gramine (Singh et al 1996).

Chemical preservatives used during last decades to avoid losses in crops production and spoilage of packed, canned and bottled foodstuff, have proved to be responsible for several kinds of residual toxicity, carcinogenic and teratogenic effects, among them. *Eucalyptus* essential oil may represent a possible alternative to replace, at least partially, some agrochemicals currently used to control crops diseases and plagues, and as chemical preservatives in foodstuff. They have proved to be efficient as fumigants and contact insecticides in the control of stored-product insects (Batish et al 2008).

Eucalyptus essential oils have been early included in the list of Generally Regarded as Safe category by Food and Drug Authority of USA and classified as non-toxic (USEPA, 1993), and European countries have also accepted them as flavoring agents in foodstuff.

Eucalyptus oil and 1,8-cineole oral and acute LD_{50} have been reported to be 4440 mg/kg bodyweight (BW) and 2480 mg/kg BW (Regnault-Roger 1997), respectively, to rats, values that demonstrated they could be less toxic than pyrethrins (LD_{50}: 350–500 mg/kg BW; USEPA 1993) and even technical grade pyrethrum (LD_{50} values 1500 mg/kg BW) (Casida and Quistad, 1995). *Eucalypts* leaf extracts have also been approved as natural food additives because of their antioxidant properties and included in the List of Existing Food Additives in Japan (Amakura et al 2002, Tyagi and Malik 2011); some of them are used in cosmetic formulations (Takahashi et al 2004, Gilles et al 2010).

Fresh and dried *E. globulus* leaves are commonly used in Africa to control insects feeding on crops. *Eucalyptus* leaves have been also used in Brazilian grain stores to deter *Sithophilus zeamais* and *Rhysopertha dominica*. It has been demonstrated that secondary metabolites produced by *Eucalyptus* and other closely related species displayed high levels of repellency towards a variety of invertebrates (Thacker and Train 2010).

Some *Eucalyptus* essentials oils containing high 1,8-cineole amounts have also proved to be effective to control mites. They could be used as a natural acaricides, as they have shown to be effective against varroa mite, *Varroa jacobsoni*, an important parasite of honeybee, *Tetranychus urticae* and *Phytoseiulus persimilis* (Choi et al 2004) and *Dermatophagoides pteronyssinus* (Saad et al 2006).

Essential oils and their major constituents have shown toxicity against a wide range of microbes including bacteria and fungi, both soil-borne and post-harvest pathogens. Su et al. (2006) demonstrated the antifungal activity of essential oils from *E. grandis, E. camaldulensis*, and *E. citriodora* against the mildew and wood rot fungi viz, *Aspergillus clavatus, A. niger, Chaetomium globosum, Cladosporium cladosporioides, Myrothecium verrucaria, Penicillium citrinum, Trichoderma viride, Trametes versicolor, Phanerochaete chrysosporium, Phaeolus schweinitzii*, and *Lenzites sulphureus*.

Several authors have described *Eucalyptus* essential oils bioactivity against pathogenic and food spoilage bacteria and yeast (Papachristos and Stanopoulos 2002, Sartorelli et al 2007). It has been demonstrated that *E. globulus* essential oils can display significant bactericidal and bacteriostatic effects on *E. coli* (Moreira et al 2005). Citronellal, major constituent of *E. citriodora*, has been successfully used to control development of two fungal pathogens affecting rice crops, *Rhizoctonia solani* and *Helminthosporium oryzae*. Ramezani et al (2002) have reported the complete inhibition of both funguses by *E. citriodora* essential oil, emphasizing that citronellal was even more effective.

Tzortzakis (2007) has reported an alternative use of *E. globulus* essential oil, suggesting that its vapors represent a good choice when trying to maintain strawberry and tomato postharvest freshness and firmness during storage and transit. It was demonstrated that no changes occurred in their sweetness, organic acid and total phenolic content, after exposing fresh strawberries and tomatoes to oil vapors.

S. oryzae is one of the most distributed species deleteriously affecting stored grains. We have performed bioassays to analyze fagorrepellency, obtaining data on differential capacity of *E. camaldulensis* essential oils depending on leaves age. *E. camaldulensis* essential oils obtained by hydro-distillation from adult and young fresh leaves were tested at two doses (10 and 20 μl/dot) on *S. oryzae* as target insect. Essential oil higher repellency effects were observed for both kinds of leaves in the first 20 min; being always more active those obtained from adult leaves (50 y 60 %) compared to young ones (30-35%). After that period, essential oils from both, adult and young leaves, showed lower repellency values, with no significant differences between doses by the time bioassay was ending (unpublished results). The higher repellency levels of adult leaves essential oil might be related to its higher proportion of oxygenated terpenoids, as it has been previously suggested for other of its biological activities (Marzoug et al 2011). The following figure represents repellency percentages of *E. camaldulensis* adult and young leaves essential oils towards *S. oryzae*.

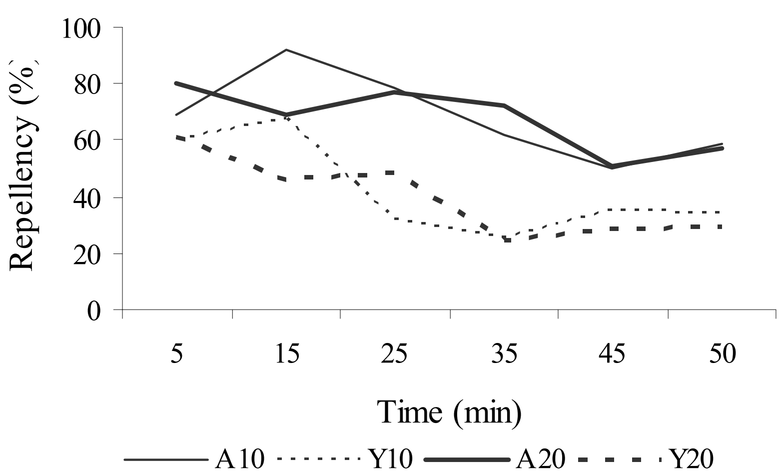


Fig. 4. Repellency of *E. camaldulensis* leaves essential oils towards *S. oryzae*. Adult leaves (A) 10 and 20 μl/dot, Young leaves (Y) 10 and 20 μl/dot.

Eucalyptus essential oil has been also used as antifeedant, particularly in formulas against biting insects (Chou et al 1997). Su et al (2006) have described insecticidal properties including larvicidal and mosquito repellent of different members of botanical Myrtaceae family. Trigg (1996) has earlier reported that products based on eucalypts essential oils used as insect repellent, can protect humans from biting insects up to 8 h depending on the concentration of the essential oil. Lucia et al (2007) have also demonstrated that *E. globulus* essential oil displays toxic effects on *Aedes aegypti* larvae, these authors determined its LC_{50}=32.4 ppm. In relation to this activity, Seyoum et al (2003) have reported that burning of *E. citriodora* leaves represents a cheap and effective method of household protection against mosquitoes in Africa.

Plant-parasitic nematodes represent another major plant plague that infesting different food crops such as vegetables and fruit plantations. They cause considerable economic losses related to reduced yields and unmarketable production features. *Eucalyptus* essential oils have also been shown to possess nematicidal activity. Pandey et al (2000) demonstrated that essential oils (at 250 ppm) obtained from *E. citriodora* and *E. hybrida* resulted highly toxic to *Meloidogyne incognita*, inhibiting growth of root-knot nematode at 250 ppm. More recently, Ibrahim et al (2006) confirmed those results as they reported that eucalypts essential oil demonstrated to be toxic to second-stage juveniles (J2s) of root-knot nematode *M. incognita*.

Native insects that became pests on *Eucalyptus* species in plantations outside Australia, are either highly polyphagous or have native trees belonging to Myrtaceae family as natural hosts. Insects in the latter group may be pre-adapted to shift hosts depending on host chemical composition (Kliejunas et al 2001, Wingfield et al 2008). Chemical identification of secondary metabolites used by insects as signals to select host trees can be useful in association to genetic programs to obtain clones lacking these substances, in order to turn them less attractive to them (Hall and Menn 1999). Potential of several essential oil components to diminish impact of most deleterious plagues should be considered in such programs.

Eucalyptus species produce, as other tree species, kairomones that have proved to modulate behavior of insects feeding on leaves. Semiochemicals, particularly host kairomones, can be useful tools to disrupt the location of food crops by pests or to design baited traps for monitoring programs (Thacker and Train 2010).

Traditional methods to detect pests in forest plantations were usually based on visual signs of damage, when plantation production was almost lost and there was not enough time to apply control methods to avoid it. Static traps have been developed in the last decades in order to prevent such losses in plantations yields. Baited traps, incorporating kairomone lures have proved to attract a range of insect families being able to detect the presence of target species even when populations are small, as in developing outbreaks or new incursions (Miller 2006). They can be applied to monitor periodical outbreak of established pests, their spread into new areas, and the emergence of new folivores.

As it was mentioned before, drought represents a common stress during eucalypts establishment and water stress has proved to diminish levels of resistance to insect attack in host trees. There are many reports on tree mortality caused by stem borers in mid-rotation *Eucalyptus* plantations that have been correlated with drought events. Damage by stem-boring insects has proved to kill or degrade pruned trees so severely that they cannot be used at all or can only be suitable for pulpwood (Bashford 2008).

Static traps containing *Eucalyptus* essential oil components have been developed for early detection of stem-boring insects in *Eucalyptus* plantations, they have been used to detect the presence of low (pre-outbreak) populations of stem-borers in *Eucalyptus* plantations where a range of species emerge at different times during summer months. Several host tree volatiles known to attract stem-boring beetle species have been studied by Bashford 2008. Eucalypt volatiles such as 1,8-cineole, phellandrene, α-pinene y α/β pinene have been successfully used to monitor almost all present stem borers in intercept panel traps. They were used to survey ambrosia beetles and other Scotylidae presence in *Eucalyptus* plantations in Brazil (Flechtmann et al 2000).

Phytotoxicity has been mentioned above as a characteristic biological activity of some eucalypts chemical components. *E. citriodora* essential oil has proved to be more effective towards broad-leaved (dicot) weeds than to grassy (monocot) ones (Singh et al 2006) most likely because citronellal, the main component of its essential oil, has proved to be more toxic towards to broad-leaved weeds. Though several authors have evaluated phytotoxic effects of eucalypts oils taking advantage of their herbicidal potential against weeds, there are several constraints mostly related to oil yield variability with season and climate, among many other environmental factors. Volatility and lipophilicity of oil components, difficulties in plant uptake, affectivity under field conditions and toxicity towards non-target plants are some other issues to be clarified before their use as commercial herbicides is accepted (Batish et al 2006).

The same constrains effecting their application as herbicide can be extended to the wide spectrum of other biological activities. The low amount of commercialized products based on eucalypts essential oils in spite of the huge scope and market for natural pesticides, is basically caused by the strict market regulations including toxicological evaluation against non-target organisms, need of product standardizations, demands related to the lack of reproducibility in plant material quality and regulatory approvals limiting their use.

Even when there is still much work to do to overcome those constrains, *Eucalyptus* essentials oils, which has proved to exhibit an environment friendly nature, can be considered a potential sustainable alternative for pest management in urban areas, homes and other sensitive areas such as schools, restaurants and hospitals. Moreover, farmers involved in organic crops and greenhouse production systems and those from developing countries who cannot afford costly synthetic pesticides, could also take advantage of their bioactivity using them as natural pesticides (Isman 2006).

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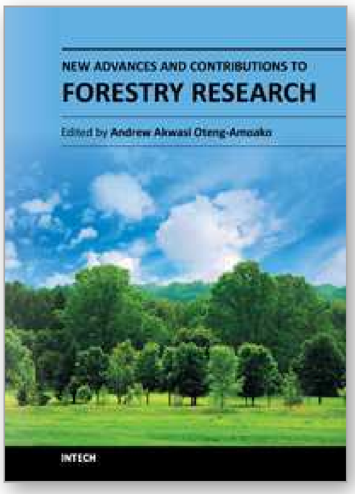

New Advances and Contributions to Forestry Research

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New Advances and Contributions to Forestry Research consists of 14 chapters divided into three sections and is authored by 48 researchers from 16 countries and all five continents. Section Whither the Use of Forest Resources, authored by 16 researchers, describes negative and positive practices in forestry. Forest is a complex habitat for man, animals, insects and micro-organisms and their activities may impact positively or negatively on the forest. This complex relationship is explained in the section Forest and Organisms Interactions, consisting of contributions made by six researchers. Development of tree plantations has been man's response to forest degradation and deforestation caused by human, animals and natural disasters. Plantations of beech, spruce, Eucalyptus and other species are described in the last section, Amelioration of Dwindling Forest Resources Through Plantation Development, a section consisting of five papers authored by 20 researchers. New Advances and Contributions to Forestry Research will appeal to forest scientists, researchers and allied professionals. It will be of interest to those who care about forest and who subscribe to the adage that the last tree dies with the last man on our planet. I recommend it to you; enjoy reading it, save the forest and save life!

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