



## CHAPTER 1

### INTRODUCTION

Many allelopathic effects studied indicate that the phenomenon is a possible means by which weeds could influence crops, crops could influence weeds, or one crop influence another which follows it. Table 1 and 2 summarise much of the experimental evidence showing weed inhibition of crop development and vice versa. Their effects on shrub-or tree-weeds, however, have not been subjected to investigated yet. Only a relatively few studies monitoring the effects against shrubs and trees have been investigated. For example, there are reports that ericaceous shrubs (Brooks, 1951) or even apple trees (Schneiderhan, 1927) would be killed if they are planted too close to the walnut plants. Should the allelochemicals from E. camaldulensis Dehn. leaves also affect an aggressive shrub-weed like M. pigra Linn., they might profitably be used for suppressing or eradicating this aggressive weed. Thus, the aim of the present work was to determine:

1. The germination and growth inhibitory activity of extracts from green leaves, fallen leaves and bark of E. camaldulensis against M. pigra.

2. The identities of inhibitors from green leaves by simultaneous chromatography with five chemicals known to be allelochemicals in E. camaldulensis leaf litter : gallic, ferulic caffeic, p-coumaric and chlorogenic acids.

Table 1 Examples of weeds exerting allelopathic effects on crop plants

Weed	Crop	Type of effect	Source of inhibitors	Reference
<u>Polygonum hydropiper</u> L.	<u>Oryza sativa</u> L.	Inhibition of root growth	Extract of whole mature plant	Harada and Yano, 1983
<u>Setaria faberii</u> Herrm.	<u>Zea mays</u> L.	Inhibition of growth and accumulation of dry and fresh weight	Exudates of whole mature plant residue	Bell and Koeppel, 1972
<u>Cyperus esculentus</u> L.	<u>Zea mays</u> L.	Inhibition of growth and accumulation of dry weight	Tuber residue	Drost and Doll, 1980
<u>Abutilon theophrasti</u> Medic.	<u>Brassica rapa</u> L.	Inhibition of germination	Seed extract	Elmore, 1980
<u>Abutilon theophrasti</u> Medic.	<u>Glycine max</u> (L.) Merr.	Inhibition of seedling growth	Fresh leaf extract	Colton and Einhellig, 1980
<u>Abutilon theophrasti</u> Medic.	<u>Lycopersicum esculentum</u> L.	Inhibition of germination	Seed extract	Gressel and Holm, 1964
<u>Galium aparine</u> L.	<u>Lactuca sativa</u> L.	Inhibition of germination and seedling growth	Seed extract	Komai, Iwamura and Ueki, 1983

Table 2 Examples of crop plants exerting allelopathic effects on weeds

Crop	Weed	Type of effect	Source of inhibitor	Reference
<u>Eucalyptus camaldulensis</u> (River red gum)	<u>Bromus rigidus</u>	Inhibition of growth	Leaves	Del Moral and Muller, 1970
<u>Eucalyptus citriodora</u> (Lemon-cented gum)	<u>Setaria viridis</u>	Inhibition of growth and germination	Leaves	Nishimura, Nakamura and Mizutani, 1984
<u>Cucumis sativus</u> (Cucumber)	<u>Panicum miliaceum</u> (Proso millet)	Inhibition of growth	Leachates from whole plant	Putnam, 1974
<u>Helianthus annuus</u> (Sunflower)	<u>Brassica kaber</u> (wild mustard)	Inhibition of growth	Stem and leaf leachates	Leather, 1983
<u>Hordeum vulgare</u> (Barley)	<u>Stellaria media</u> (Chickweed)	Inhibition of growth and germination, flower production	Intact whole plant	Overland, 1966
<u>Phaseolus vulgaris</u> (bean)	<u>Ipomoea hederifolia</u> <u>Biden Pilcoa</u> <u>Amaranthus dubius</u>	Inhibition of germination	Leaves	Altieri and Doll, 1978
<u>Psidium guajava</u> (Guava)	<u>Setaria verticillata</u> (bristly foxtail)	Inhibition of growth	Root exudates	Brown, Tang and Nishimoto, 1983
<u>Festuca arundinacea</u> (fescue)	<u>Lotus corniculatus</u>	Inhibition of growth	Root exudate	Peters, 1968
<u>Avena spp.</u>	<u>Brassica kaber</u> (wild mustard)	Inhibition of growth. severe chlorosis, stunting and twisting	Intact whole plant	Fay and Duke, 1977

3. The inhibitory activity of 13 phenolic acids known to be allelochemicals in E. camaldulensis and some other plants on the growth of M. pigra and Oryza sativa seedlings.

#### Literature Review.

##### 1. Eucalyptus camaldulensis Dehn. and Allelopathy.

Eucalyptus (gum tree) is a member of the family Myrtaceae. There are 600 or so species and varieties of eucalyptus in the world. Almost all of them are endemic to Australia. Extensive eucalypt plantations have recently been established in Thailand to meet the increasing demand of reforestation, raw material for pulp and charcoal productions. The total area in Thailand for planting them is more than 200,000 rai now albeit just passed a period of experimental planting. Since the most rapid growing of eucalypts tested is Eucalyptus camaldulensis Dehn. almost all of the eucalypts grown in Thailand is it.

E. camaldulensis is the most widespread member of its genus in Australia, where it is primarily a riparian species (Hillis, 1966) leading to a common name, river red gum. It can grow rapidly as an exotic plant because there is usually a lack of insects being so prevalent on them in their natural Australian conditions. It is one of the most aggressively allelopathically active plants in dry climatic area. This allelopathic effect is due to some monoterpenes (especially 1 : 8 cineole and some phenolic acids such as gallic, p-coumaric, caffeic, ferulic, and chlorogenic acids (del Moral & Muller, 1970). In fact, monoterpenes and phenolic acids and their

derivatives are the most common growth inhibitors produced by many allelopathically active plants (Whittaker & Feeny 1971). Some compounds of these two categories and examples of the natural sources in which they are reported to have allelopathic properties are summarized in Table 3 and 4.

## 2. Inhibitor Emission from *E. camaldulensis*

As mentioned above, there are two categories of inhibitors, i.e. monoterpenes and phenolic acids, in *E. camaldulensis*. The fact that extracts of a plant are inhibitory to other plants, however, does not indicate that this plant exerts allelopathic action against other plants. An essential part of allelopathy is the movement of the potential allelopathic agent into the environment. Ways in which inhibitors get out of plants depend on the nature of the inhibitors. Monoterpenes, volatile substances, get out of plant by means of volatilization. Phenolic acids, rather polar substances, may escape from the plant synthesizing them by the action of rain or dew (leaching).

### 2.1 Volatilization.

Many volatile inhibitors such as ethylenes and terpenes emanated from aerial plant parts may have an effect upon plant growth. The monoterpenes 1:8 cineole,  $\alpha$ -phellandrene,  $\beta$ -phellandrene, myrcine, and geranial were reported to be present in the essential oil of *E. camaldulensis* (Guenther, 1950; del Moral & Muller, 1970; Abou-Dahab & Abou-Zeid, 1973). 1 : 8 Cineole and camphor were shown to be released from *Salvia leucophylla* into the California

Table 3 Allelopathic phenolic acids and their occurrence

Compounds	Natural occurrence	Reference
Benzoic acid	<u>Populus tremuloides</u>	Hubbes, 1969
Salicylic acid	<u>Cyperus esculentus</u>	Jangaard, Sckerl, and Schieferstein, 1971
Caffeic acid	<u>Kochia scoparia</u>	Lodhi, 1979
Gallic acid	<u>Eucalyptus globulus</u>	del Moral and Muller, 1969
Gentisic acid	<u>Celtis laevigata</u>	Lodhi and Rice, 1971
p-Coumaric acid	<u>Celtis laevigata</u>	Lodhi and Rice, 1971
Ferulic acid	<u>Kochia scoparia</u>	Lodhi, 1979
Chlorogenic acid	<u>Platanus occidentalis</u>	Al-Naib and Rice, 1971
Isochlorogenic acid	<u>Platanus occidentalis</u>	Al-Naib and Rice, 1971
p-Hydroxybenzoic acid	<u>Cyperus esculentus</u>	Sanchez, Gesto, and Vieitez, 1973
Protocatechuic acid	<u>Cyperus esculentus</u>	Sanchez, Gesto, and Vieitez, 1973
Vanillic acid	<u>Pteridium aquilinum</u>	Glass and Bohm, 1969
Vanillin	<u>Oryza sativa</u>	Kato and Takahashi, 1981

Table 4 Allelopathic monoterpenoids and their occurrence

Compounds	Natural occurrence	Reference
Camphene	<u>Salvia apiana</u>	Muller, 1965
Camphor	<u>Salvia leucophylla</u>	Muller, 1965
1 : 8 Cineole	<u>Eucalyptus globulus</u>	Nishimura and Calvin, 1979
$\alpha$ - Phellandrene	<u>Sassafras albidum</u>	Gant and Clebsch, 1975
$\alpha$ - Pinene	<u>Salvia leucophylla</u>	Muller, 1965
$\beta$ - Pinene	<u>Eucalyptus radiata</u>	Nishimura, Paton and Calvin, 1980

desert soil and inhibit root growth of understory plants (Muller & Chou, 1972).

Del Moral et al. (1970) found that, during the dry summer, several monoterpenes especially 1:8 cineole and  $\alpha$ -pinene produced from E. camaldulensis leaves were volatilized from the leaves and then adsorbed onto the soils under the trees' canopy in concentration high enough to completely stop germination of Bromus rigidus seeds. They also found that these volatile oils were adsorbed and remained in dry soils in large amounts until rains brought into activity of soil micro-organisms which degraded them. The adsorbed monoterpenes can be transported through the waxy coating of seeds or roots and eventually reach sites of inhibition and thus, play a role in allelopathy (Muller & del Moral, 1966).

Indeed, the amount of volatile oils in Eucalyptus species is so high that Nishimura & Calvin (1979) and Nishimura, Paton & Calvin (1980) decided to elucidate which species of Eucalyptus plant produces the largest amount of volatile oils and whether it can be regarded as an alternative source of hydrocarbon fuel or an additive to gasoline for automobiles. They found that, of the five juvenile Eucalyptus species investigated (as shown in Table 5), E. radiata, consisting of 4.2% (w/w) volatiles in totals, was the best candidate. From the work of Abou-Dahab et al. (1973) on E. camaldulensis as shown in Table 6, it appears that during the winter months (November till March), the lowest volatile oil percent was observed, followed by a steady rise through the summer months (May till September) with the highest percent (1.5%) in September.



Table 5 Average volatile oil percent in the fresh samples of some Eucalyptus species.

Species	Plant part	Collection time	Collection place	Reference	Method	Volatiles (%)
<u>E. radiata</u>	Fresh juvenile leaves & branches	End of November, 1978	New South Wales, Australia	Nishimura et al., 1980	Acetone extraction	4.2
<u>E. dives</u>	Fresh juvenile leaves & branches	End of November, 1978	New South Wales, Australia	Nishimura et al., 1980	Acetone extraction	2.7
<u>E. polybractea</u>	Fresh juvenile leaves & branches	End of November, 1978	New South Wales, Australia	Nishimura et al., 1980	Acetone extraction	2.3
<u>E. pulverulenta</u>	Fresh juvenile leaves & branches	End of November, 1978	New South Wales, Australia	Nishimura et al., 1980	Acetone extraction	2.0
<u>E. globulus</u>	Fresh juvenile leaves & branches	16 <sup>th</sup> June, 1977	University of California	Nishimura et al., 1980	Acetone extraction	1.2
<u>E. camaldulensis</u>	Fresh adult leaves	November, 1969	Cairo University	Abou-Dahab et al., 1973	Distillation	0.92
<u>E. polyanthemos</u>	Fresh adult leaves	November, 1969	Cairo University	Abou-Dahab et al., 1973	Distillation	0.56

Table 6 Average volatile oil percent (w/w) in the E. camaldulensis fresh leaves collected throughout the year (adapted from Abou-Dahab et al., 1973)

Months	Oil Percent
September (1969)	1.50
October	1.34
November	0.92
December	0.76
January (1970)	0.73
February	0.87
March	0.91
April	-
May	1.03
June	1.20
July	1.32
August	1.51

From those works mentioned above, however, it appears that the amount of volatile oil in E. camaldulensis is nowhere near as high as that of E. radiata which is sensible in terms of renewable fuel (Table 5).

## 2.2 Leaching.

Large quantities of a diverse variety of organic and inorganic metabolites are leached from above-ground parts of plants by rain and dew action (Tukey, 1966). Fog and mist are also important in certain area (del Moral et al. 1969, 1970). In addition to metabolically important nutrients, growth regulatory substances such as 4-glucoside of 1, 4, 5-trihydroxynaphthalene (Bode, 1958), gibberellin (Whyte & Luckwill, 1966; Kozel & Tukey, 1968), canavanine (Wilson & Bell, 1978) and inhibitory phenolic acids (del Moral et al. 1969, 1970; McPherson, Chou & Muller, 1971) also can be leached, thus, play a role of allelopathy.

Del Moral et al. (1970) found numerous toxins in the artificial leachate from leaf litter of E. camaldulensis. The toxins found including gallic, p-coumaric, caffeic, ferulic and chlorogenic acids and five unidentified inhibitors. They also demonstrated in several ways that these inhibitors leached into the soil were, at least in parts, related to the scarcity of the understory.

Regardless of the mode of emission, intoxication of plants can occur only after uptake of an allelochemical, usually by the root. The fact that toxic substances do move out of the producers is not sufficient to prove the existence of allelopathic effects the producers exert against their neighboring species (Grümmer, 1953).

Naturally, the phytotoxins have to be present in sufficient concentration and their effects are not eliminated by soil adsorptions or interaction with microorganisms (Grümmer, 1953). This is why allelopathic phenomena are particularly difficult to study. Even today, not all plant ecologists believe that the actual existence of allelopathy has been proved adequately, although now there are probably more than 1000 scientific papers published on this subject (Putnam, 1983).

### 3. Mechanisms of Action of Inhibitors .

The most interesting group of allelopathic compounds in terms of their mode of action are the nonprotein amino acids since the allelopathically active plants synthesizing them are able to discriminate against these potentially toxic compounds (Bell, 1976). Fowden (1963) reported that azetidine-2-carboxylic acid, the lower homologue of proline, was present in high concentrations in the rhizome of Polygonatum multiflorum without dire effects, although one-fiftieth of the concentration was lethal to Phaseolus aureus, a plant that does not synthesize the acid. Two years later, Peterson & Fowden (1965) found that this difference is due to the prolyl-s-RNA synthetase (prolyl-transfer RNA synthetase) of Polygonatum multiflorum, unlike that of Phaseolus aureus, is able to discriminate between the two metabolites. Thus, azetidine-2-carboxylic acid is not incorporated into proteins in Polygonatum multiflorum tissues, whereas, when supplied to Phaseolus aureus, replacement of proline occurs in the bean protein resulting in anomalous protein molecules.

Monoterpenes and phenolic acids found in E. camaldulensis and a variety of other plant species, however, are less specific than the allelochemical mentioned above. Some other plants, and/or the producers themselves are affected by these inhibitors. Moreover, these allelochemicals can act on several sites within a plant cell.

### 3.1 Mechanisms of Action of Phenolic Acids .

Albeit numerous studies have demonstrated the germination- or growth-inhibition properties of phenolic acids, the surface has barely been scratched in determining the mechanisms by which these toxins exert their inhibitory actions.

#### 3.1.1 Influence of Phenolic Acids upon Ion Uptake .

Ion uptake is certainly of basic importance in the growth and reproduction of organisms. Considerable evidence indicates that many phenolic acids can alter the rate of ion uptake by plants (eg. Tilberg, 1970; Glass 1973, 1974; McClure, Gross & Jackson, 1977; Harper & Blake, 1981). Tilberg (1970) found that the phenolic compound salicylic acid at concentrations of  $10^{-6}$  -  $10^{-3}$  M. decreased the uptake of phosphate ions by the alga, Scenedesmus, but t-cinnamic acid and abscisic acid had no effect. Various phenolic acids including both cinnamic acid and benzoic acid derivatives, caused a significant reduction of phosphate (Glass, 1973) and potassium ion (Glass, 1974) uptake by excised barley (Hordeum vulgare L. cv. Karlsberg) roots. McClure et al. (1977) reported that ferulic acid inhibited phosphate absorption by excised and intact root of soybean (Glycine max (L.) Merrill) but not nearly to the extent reported by Glass for excised barley roots. Harper & Blake (1981) extended the work of Glass (1973)

and McClure (1977) and reported that the genetic potential for differential response to phenolic acids may exist among important crop species and may be important in expression of allelopathy by phenolic acids.

### 3.1.2 Inhibition of GA-or IAA-Induced Growth.

IAA, the plant growth hormone, is present in plants in both active and inactive form and is broken down enzymically by IAA oxidase. Lee & Skoog (1965) and Tomaszewski & Thimann (1966) found that monohydroxybenzoic acid stimulated IAA oxidase, which accelerates IAA breakdown but 3, 4-dihydroxybenzoic acid and ferulic acid strongly inhibited it. Consequently, Tomaszewski & Thimann (1966) concluded that phenolic compounds may play a role in regulating IAA activity in plants. Moreover, Geissman & Phinney (1972) found that cinnamic acid as well as tannins and coumarin showed some inhibition of gibberellin-induced growth of dwarf pea seedlings.

### 3.1.3 Retardation of Photosynthesis.

Patterson (1981) found that, at concentration  $10^{-3}$  M caffeic, t-cinnamic, p-coumaric, ferulic, gallic and vanillic acids severely reduced the net photosynthetic rates of single leaflets of Glycine max (L.) Merr. 'Tracy' seedlings. These chemicals also severely caused stomata, the tiny opening in a plant's outer surface, to close, but not clear whether this is a cause or a result of photosynthetic inhibition.

### 3.2 Mechanisms of Action of Monoterpenes

Albeit monoterpenes are commonly identified toxins produced by higher plants and involved in allelopathy, only a relatively few studies monitoring the mode of action of these inhibitors have been researched.

#### 3.2.1 Inhibition of Cell Division and Elongation

Muller (1965) reported that mitotic cell division and cell elongation in root of Cucumis sativus (cucumber) were inhibited by volatile terpenes, the large constituent of which were cineole and camphor, emanated from leaves of Salvia leucophylla. Moreover, he found that these volatile terpenes also inhibited cell division of 32 of 44 bacterial isolates obtained from soil in and around Salvia stands.

#### 3.2.2 Inhibition of Respiration

Muller et al. (1969) reported that two volatile terpenes, cineole and diterpene, emanated from leaves of Salvia leucophylla markedly reduced respiration in mitochondria isolated from Avena fatua and Cucumis sativus (cucumber). The inhibition appeared to be localized in that part of the Krebs's cycle where succinate is converted to fumarate or fumarate to malate (Muller et al., 1969).

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