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Herbicidal Activity of Cineole Derivatives

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Essential oils and their constituents have potential as ecologically acceptable pesticides that may also have novel modes of action. In this work hydroxy and ester derivatives of the naturally occurring monoterpenoids 1,8-cineole **3**, the main component in most eucalyptus oils, and 1,4-cineole **4** were prepared and their pre-emergence herbicidal activity against annual ryegrass (*Lolium rigidum*) and radish (*Raphanus sativus* var. Long Scarlet) investigated in laboratory-based bioassays. 1,8-Cineole, eucalyptus oil and all derivatives showed a dose-dependent herbicidal activity against annual ryegrass and radish with many of the derivatives showing improved herbicidal activity relative to 1,8-cineole and high-cineole eucalyptus oil. Increased activity of cineole ester derivatives compared to their associated hydroxy-cineole and carboxylic acid was not observed. No relationship between lipophilicity of the carboxylic acid portion of cineole ester derivatives and herbicidal activity was observed. The results indicate that these cineole derivatives could be environmentally acceptable herbicides.

KEYWORDS: Cineole; eucalyptus oil; herbicidal; pre-emergence; germination; ryegrass; radish; bioactivity; phytotoxicity

INTRODUCTION

A major impact of weeds in agricultural systems is reduced crop yield as a result of strong competition for space, nutrients and sunlight (*1*, *2*). Thus weed management is an important aspect of agricultural practice. Chemical control has been a significant part of management strategies since the 1940s when synthetic chemicals were introduced for this purpose, but in recent decades public concerns over the use of synthetic chemicals and the development of resistance of weeds to synthetic chemicals have led to increased investigation of the herbicidal activity of plant-derived secondary metabolites (*3*). Such natural products not only may be more environmentally acceptable than synthetic pesticides but also may have novel mechanisms of action compared to the current suite of herbicides to which weeds are developing resistance (*4*).

It is not surprising that there are natural products with phyotoxicity. Many organisms have evolved compounds to inhibit growth, or facilitate attack and digestion of plants that are potential competitors. The triketone, bialaphos and glufosinate herbicides, derived from natural products, have novel phytotoxic mechanisms not previously seen in synthetic herbicides (*5*). The bleaching triketone herbicide mesotrione **1** was developed from the allelochemical leptospermone **2** found in the roots of the bottle brush *Callistemon citrinus* (*6*).

A wide range of classes of volatile monoterpenes, including oxygenated monoterpenes such as 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane) **3**, inhibit plant growth (*7*−*9*). Muller et al. (*10*) demonstrated in field studies that volatile monterpenes released by *Salvia leucophylla* gave greatest inhibition during seedling development and establishment. A major component in the essential oils of *Salvia* species is 1,8-cineole, which has been shown by Halligan (*11*) to be one of the most potent allelochemicals released by *Artemisia* species and also by Kumar and Motto (*12*) in *Eucalyptus* species. However, Angelini et al. (*13*) found no significant germination inhibition by 1,8-cineole of a number of crop and weed species, and some field tests indicated that 1,8-cineole has low herbicidal activity (*11*, *14*).

1,4-Cineole (1-isopropyl-4-methyl-7-oxa-bicyclo[2.2.1]heptane) **4**, a less abundant naturally occurring structural isomer of 1,8-cineole, also inhibits seed germination and plant growth (*9*, *15*, *16*). Vaughn and Spencer (*9*) showed that 1,4-cineole completely inhibited the germination of wheat, large crabgrass, redroot pigweed and ryegrass while 1,8-cineole completely inhibited the germination of corn, wheat, alfalfa, large crabgrass, redroot pigweed and annual ryegrass.

Romagni et al. (*16*, *17*) also examined the effects of 1,4-cineole, cinmethylin **5** and 1,8-cineole on lettuce seedlings. Cinmethylin, the *o*-methylbenzyl ether of racemic 2-*exo*-hydroxy-1,4-cineole **6**, has been used as a pre-emergence herbicide. The concentration at which there was 50% inhibition in root growth was an order of magnitude lower for the natural monoterpene **4** than for its derivative cinmethylin. Romagni and co-workers also found that the lowest concentration needed to give maximum phytotoxic effect was an order of magnitude higher for cinmethylin than for 1,4-cineole. Their original results suggested that on uptake by a plant cinmethylin was metabolically cleaved to give a hydroxylated cineole and a benzyl ether portion. The herbicidal activity of cinmethylin is postulated to be due primarily to the hydroxylated cineole portion with the benzyl portion having little role in any bioactivity. The benzyl ether derivative **5** is manufactured to give a compound with increased molecular weight and reduced volatility compared to 1,4-cineole. If 1,4-cineole were applied in the field,

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it would evaporate before a sufficient amount was taken in by target plants.

While allelopathic and herbicidal activity of 1,8-cineole against some plant species has been established, there is clearly a range of activities, and there are no reports of either derivatization of 1,8-cineole for the purposes of reducing its volatility or subsequent testing of the herbicidal activity of derivatives. Low herbicidal activity found in some field tests may be due to the high volatility of 1,8-cineole causing reduced uptake by plants. 1,8-Cineole and other phytochemicals offer the potential to produce new herbicides with novel modes of action, and while modification of their structures may be needed to improve efficacy, their environmental impact is likely to be less than that of synthetic herbicides.

One aim of this work was to prepare derivatives of 1,8-cineole with reduced volatility but equivalent or increased phytotoxicity. The study investigated whether ester derivatives of 1,8-cineole and 1,4-cineole would have higher phytotoxicity than their corresponding hydroxylated cineoles and carboxylic acids, and whether phytotoxicity would increase as the nonpolar carboxylic acid portion of the esters increased in size and hence lipophilicity. Pre-emergence herbicidal activity of the cineole derivatives and the corresponding carboxylic acids was assessed, and the cineole isomers were compared.

MATERIALS AND METHODS

Instruments and Chemicals. Unless otherwise stated, ^{1}H and ^{13}C NMR spectra were measured at 300 and 75 MHz respectively, on a Bruker Avance DPX-300 spectrometer, for solutions in deuterochloroform ($CDCl_3$) with internal standard tetramethylsilane (TMS) (^{1}H, ^{13}C, δ 0.00) and residual chloroform (^{1}H, δ 7.26; ^{13}C, δ 77.0). The signals in the ^{13}C spectra were assigned with the aid of DEPT experiments, and assignment of signals with the same superscripts are interchangeable. All coupling constants are given in hertz. Infrared spectra were recorded on a Nicolet 850 series III FTIR, as thin films between KBr disks for oils, and using a diffuse reflectance unit for solids. High resolution mass spectra were obtained on a V.G. Autospec high resolution mass spectrometer at the University of Western Australia, Perth, Australia. All chemicals and reagents were purchased from standard commercial suppliers.

Synthesis of 1,8-Cineole Derivatives. 3-Hydroxy-1,8-cineole ((±)-*exo*-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-5-ol) **7** was synthesized in an adaptation of the method of de Boggiatto et al. (*18*) (**Figure 1**). The ester derivatives **8a**−**e** were prepared using well established reaction methods. The acetate **8a** was prepared by reaction of **7** with acetic anhydride and dry pyridine, and esters **8b**−**e** were prepared by reaction of **7** with the appropriate acid chloride.

2-*endo*-Hydroxy-1,8-cineole ((1*R*,6*R*)-1,3,3-trimethyl-2-oxabicyclo-[2.2.2]octan-6-ol) **9** was obtained as the primary metabolite of a novel bacterium grown on 1,8-cineole as sole carbon source. The bacteria were isolated by inoculating liquid growth medium containing 1,8-cineole as carbon source with aliquots of deionized water in which eucalyptus leaves had been stirred.

Synthesis of 1,4-Cineole Derivatives. Synthesis of the 1,4-cineole esters is outlined in **Figure 2**. 2-*exo*-Hydroxy-1,4-cineole ((±)-*exo*-4-isopropyl-4-methyl-7-oxabicyclo[2.2.1]heptan-2-ol) **10** was prepared as described by Payne (*19*) and then converted to esters **11a**−**e** in the same manner as for the 1,8-cineole esters. Cinmethylin **5** was prepared from alcohol **10** as described by Silvestre et al. (*20*).

Proton NMR, carbon-13 NMR and mass spectral data for compounds are provided in **Tables 1**, **2**, **3**, **4** and **5**.

(±)-**1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-one 6** was recovered in a yield of approximately 30%, and its ^{1}H NMR spectrum was consistent with published spectra (*18*, *21*).

(±)-***exo*-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-ol 7** was recovered in a yield of approximately 98%, and its ^{1}H NMR spectrum was consistent with published spectra (*18*, *21*).

Esters **8a**−**e** were recovered in yields ranging from 47 to 95%.

(±)-***exo*-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl ethanoate 8a** was recovered in 95% yield as a colorless oil.

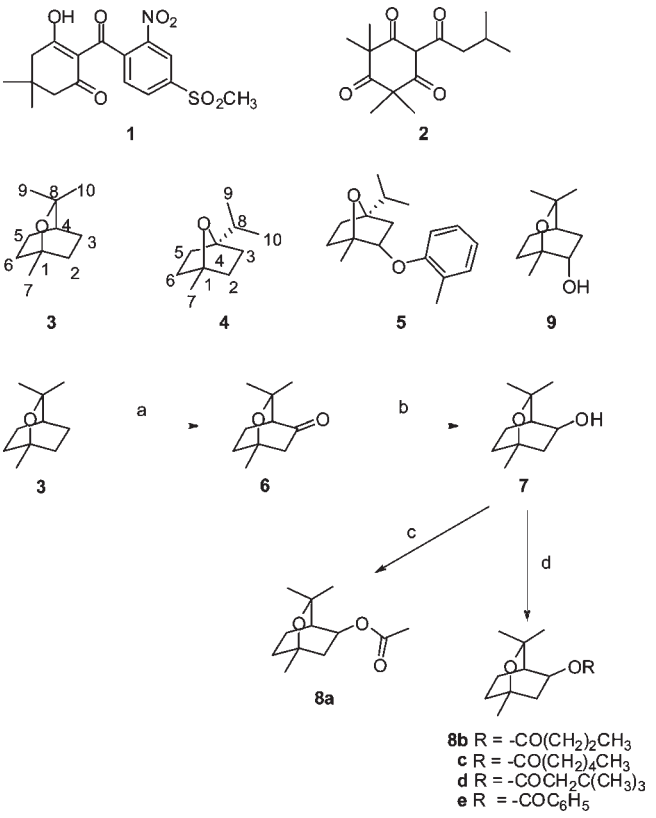

Figure 1. Synthesis of 1,8-cineole esters **8a**−**e**. Reagents and conditions: (a) (*18*) CrO_3, $CH_3COOH/(CH_3CO)_2O$, 4 °C, 48 h, rt, 10 h; (b) (*18*) $NaBH_4$, dry EtOH, rt, 2 h, reflux, 5 h; (c) dry pyridine, $(CH_3CO)_2O$, dry CH_2Cl_2, reflux, 22 h; (d) dry pyridine, RCOCl, dry CH_2Cl_2, reflux, 5 h.

(±)-***exo*-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl butanoate 8b** was recovered in 47% yield as a very pale yellow oil. (Found: $(M + 1)^+$, 241.1791, $C_{14}H_{25}O_3$ requires (M + 1), 241.1804.) IR (cm^{-1}) 2968, 2932 (C−H, str), 1734 (OCO ester).

(±)-***exo*-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl hexanoate 8c** was obtained as a clear oil in a yield of 63%. (Found: $(M - H)^+$, 267.1947, $C_{16}H_{27}O_3$ requires (M − H), 267.1960.) IR (cm^{-1}) 2964, 2931, 2869 (C−H str), 1734 (OCO ester).

(±)-***exo*-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl 3,3-dimethyl-butanoate 8d** was recovered as white needles after recrystallization from ethyl acetate in a yield of 95% (mp 54−55 °C). (Found: $(M + 1)^+$, 269.2117, $C_{16}H_{29}O_3$ requires (M + 1), 269.2117.) IR (cm^{-1}) 2961, 2934, 2866 (C−H, str), 1725 (OCO ester).

(±)-***exo*-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl benzoate 8e** was recovered as white crystals in a yield of 68% (mp 75−77 °C). (Found: $(M + 1)^+$, 275.1662, $C_{17}H_{23}O_3$ requires (M + 1), 275.1647.) IR (cm^{-1}) 3065, 3012 (Ar−H), 2992, 2964, 2924 (C−H str), 1713 (OCO ester) 1601, 1582 (aromatic).

(±)-***exo*-4-Isopropyl-1-methyl-2-(2-methylbenzyloxy)-7-oxabicyclo-[2.2.1]heptane 5** was recovered as a pale yellow oil in a yield of 59%.

(±)-***exo*-4-Isopropyl-4-methyl-7-oxabicyclo[2.2.1]heptan-2-ol 10** was recovered as white crystals in a yield of 68% after recrytallisation from hexane (mp 83−86 °C).

(±)-***exo*-4-Isopropyl-1-methyl-7-oxabicyclo[2.2.1]heptan-2-yl ethanoate 11a** was recovered as a pale yellow oil in a yield of 58%. (Found: M^+, 212.1412, $C_{12}H_{20}O_3$ requires M, 212.1412.) IR (cm^{-1}) 2964, 2877 (C−H, str), 1740 (OCO, ester).

(±)-***exo*-4-Isopropyl-1-methyl-7-oxabicyclo[2.2.1]heptan-2-yl butanoate 11b** was recovered as a pale yellow oil in a yield of 74%. (Found: M^+, 240.1729, $C_{14}H_{24}O_3$ requires M, 240.1725.) IR (cm^{-1}) 2964, 2877 (C−H, str), 1734 (OCO ester).

(±)-***exo*-4-Isopropyl-1-methyl-7-oxabicyclo[2.2.1]heptan-2-yl hexanoate 11c** was recovered as a nearly colorless oil in a yield of 75%. (Found: M^+, 268.2044, $C_{16}H_{28}O_3$ requires M, 268.2038.) IR (cm^{-1}) 2959, 2873 (C−H, str), 1734 (OCO ester).

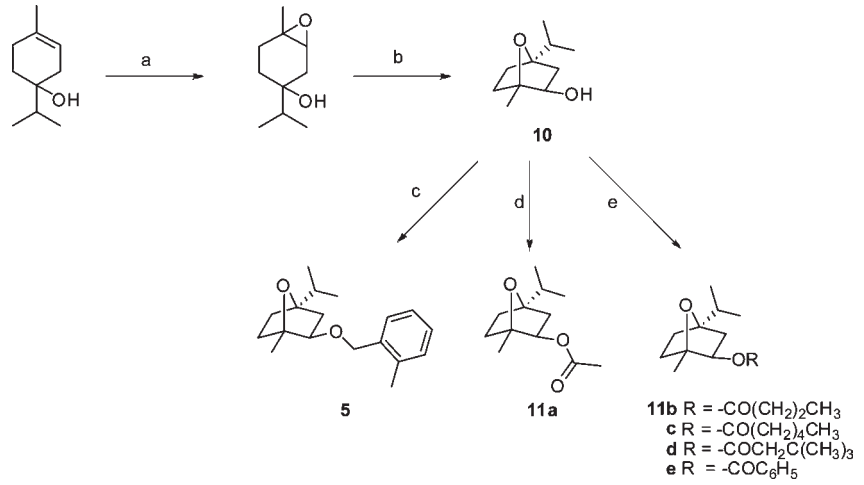

Figure 2. Synthesis of cinmethylin, **5**, and 1,4-cineole esters **11a**−**e**. Reagents and conditions: (a) (*19*) *t*-butyl hydroperoxide, CH_2Cl_2, $VO(AcAc)_2$, reflux, 2 h; (b) (*19*) *p*-TSA, reflux, 1.5 h; (c) (*20*) $CH_3C_6H_4CH_2Cl$, NaH, dry THF, N_2, reflux, 15 h; (d) dry pyridine, $(CH_3CO)_2O$, dry CH_2Cl_2, reflux, 20 h; (e) dry pyridine, RCOCl, dry CH_2Cl_2, reflux, 5 h.

Table 1. Proton NMR Data for 1,8-Cineole Derivatives

proton	6	7	8a	8b
2-H_2	2.12−2.24, m	1.98−2.09, m 2.06, dd, *J* = 10.3, 13.8	1.69−1.78, m 2.10, dd, *J* = 10.6, 14.1	1.59−1.77, m 2.11, dd, *J* = 10.6, 14.1
3-H		4.15, ddd, *J* = 2.0, 6.2, 10.3	4.98, ddd, *J* = 2.2, 6.0, 10.6	5.00, ddd, *J* = 2.2, 6.0, 10.5
4-H	2.38, dd, *J* = 2.9, 18.9	1.69, ddd, *J* = 3.2, 6.1, 13.8	1.69−1.78, m	1.59−1.77, m
5-H_2	1.54−1.70, m 1.72−1.86, m	1.33−1.42, m 1.52−1.62, m	1.37−1.53, m 2.02−2.13, m	1.40−1.53, m 2.02−2.10, m
6-H_2	1.72−1.86, m 2.12−2.24, m	1.33−1.42, m 1.52−1.62, m	1.37−1.53, m 1.54−1.68, m	1.40−1.53, m 1.59−1.77, m
7-H_3	1.11, s	1.11, s	1.12, s	1.11, s
9-H_3	1.20, s	1.24, s	1.24, s	1.24, s
10-H_3	1.27, s	1.44, s	1.35, s	1.35, s
other		2.14 (br s, 1H, OH)	2.05 (s, 3H, $COCH_3$)	0.96 (t, 3H, *J* = 7.4, 4′-H_3), 1.66 (sept, 2H, *J* = 7.4, 3′-H_2), 2.28 (t, 2H, *J* = 7.5, 2′-H_2)

proton	8c	8d	8e	9[a]
2-H_2	1.54−1.76, m 2.11, dd, *J* = 10.6, 14.1	1.55−1.76, m 2.13, dd, *J* = 10.6, 14.1	1.85−1.95, m 2.25, dd, *J* = 10.5, 14.2	3.68−3.78, m
3-H	4.99, ddd, *J* = 2.2, 6.0, 10.5	5.00, ddd, *J* = 2.1, 6.1, 10.5	5.26, ddd, *J* = 2.1, 6.1, 10.5	1.26−1.37, m 2.45−2.58, m
4-H	1.54−1.76, m	1.55−1.76, m	1.85−1.95, m	1.46−1.61, m
5-H_2	1.41−1.53, m 2.01−2.13, m	1.38−1.54, m 2.02−2.15, m	1.59−1.72, m 2.08−2.19, m	1.84−2.03, m 2.04−2.08, m
6-H_2	1.41−1.53, m 1.54−1.76, m	1.38−1.54, m 1.55−1.76, m	1.43−1.57, m 1.59−1.72, m	1.46−1.61, m 1.84−2.03, m
7-H_3	1.11, s	1.11, s	1.16, s	1.10, s
9-H_3	1.24, s	1.24, s	1.28, s	1.20, s
10-H_3	1.35, s	1.36, s	1.48, s	1.28, s
other	0.90 (t, 3H, *J* = 6.9, 6′-H_3), 1.28−1.38 (m, 4H, 4′-H_2, 5′-H_2), 1.54−1.76 (m, 3′-H_2), 2.29 (t, 2H, *J* = 7.7, 2′-H_2),	1.04 (s, 9H, $C(CH_3)_3$), 2.19 (s, 2H, 2′-H_2)	7.45 (t, 2H, *J* = 7.7, 3′-H, 5′-H), 7.54−7.66 (m, 1H, 4′-H), 8.05 (d, 2H, *J* = 7.8, 2′-H,6′-H)	1.46−1.61 (m, OH)

[a] Compound **9** has 1 H atom on C2 and 2 H atoms on C3 while **7** and **8a**−**e** have 2 H atoms on C2 and 1 H atom on C3.

(±)-***exo*-4-Isopropyl-1-methyl-7-oxabicyclo[2.2.1]heptan-2-yl 3,3-dimethylbutanoate 11d** was recovered in a yield of 59% as a pale yellow oil. (Found: M^+, 268.2039, $C_{16}H_{28}O_3$ requires M, 268.2038.) IR (cm^{-1}) 2960, 2874 (C−H str), 1732 (OCO ester).

(±)-***exo*-4-Isopropyl-1-methyl-7-oxabicyclo[2.2.1]heptan-2-yl benzoate 11e** was obtained in a yield of 92% as white crystals after recrystallization from ethanol (mp 77−79 °C). (Found: $(M + 1)^+$, 275.1643, $C_{17}H_{23}O_3$ requires (M + 1), 275.1647.) IR (cm^{-1}) 3067 (Ar−H), 2968, 2942, 2874 (C−H, str), 1712 (OCO ester), 1600, 1581 (aromatic).

Seed Sources. Annual ryegrass seeds (*Lolium rigidum*) were obtained from the Wongan Hills Research Station 2EA, Western Australia, in November 2002, and radish seeds (*Raphanus sativus* var. Long Scarlet) were a commercially available variety (Mr Fothergill's Seeds Pty Ltd.).

Seed Treatment. Seeds were surface sterilized in 2% sodium hypochlorite solution for 10 min, rinsed 3 times with sterile deionized water and then imbibed for approximately 15 h in sterile deionized water.

Pre-emergence Bioassays. Water agar was prepared by autoclaving (103.4 kPa, 121 °C, 30 min) 4.0 g of agar (BBL Agar, grade A) in 500 mL of deionized water containing calcium and boron at concentrations of 0.05 mol L^{-1} and 0.001 mol L^{-1}, respectively. Under sterile conditions, the agar was poured into 55 mm plastic Petri dishes (or sterile Pyrex dishes for chloroform solutions) to a depth of approximately 2 mm and allowed to

Table 2. Proton NMR Data for 1,4-Cineole Derivatives

proton	5	10	11a	11b
2-H	3.54, dd, J = 2.4, 6.7, 12.1	3.70−3.81, m	4.88, dd, J = 2.6, 7.3	4.88, dd, J = 2.6, 7.2
3-H_2	1.40−1.64, m	1.34−1.61, m	1.45−1.64, m	1.43−1.73, m
	1.95, dd, J = 6.7, 12.1	2.10−2.15, m	2.07−2.21, m	2.02−2.21, m
5-H_2	1.40−1.64, m	1.34−1.61, m	1.45−1.64, m	1.43−1.73, m
6-H_2	1.40−1.64, m	1.34−1.61, m	1.45−1.64, m	1.43−1.73, m
7-H_3	1.47, s	1.42, s	1.39, s	1.38, s
8-H	2.11, sept, J = 6.9	2.10−2.15, m	2.07−2.21, m	2.02−2.21, m
9-H_3	0.99, d, J = 6.9	0.96, d, J = 6.9	0.97, d, J = 6.9	0.96, d, J = 6.8
10-H_3	0.97, d, J = 6.9	0.97, d, J = 6.9	0.98, d, J = 6.8	0.98, d, J = 6.8
other	2.32 (s, 3H, Ar CH_3), 4.36 + 4.54 (AB system, 2H, J = 12.4, 1′-H), 7.11−7.20 (m, 3H, Ar4−H, Ar5−H, Ar6−H), 7.30−7.35 (m, 1H, Ar3−H)	1.89 (br d, 1H, J = 8.8, OH)	2.07 (s, 3H, 2′-H)	0.95 (t, 3H, J = 7.4, 4′-H), 1.43−1.73 (m, 2H, 3′-H_2), 2.31 (t, 2H, J = 7.3, 2′-H)

proton	11c	11d	11e
2-H	4.87, dd, J = 2.6, 7.2	4.83, dd, J = 2.6, 7.2	5.10, dd, J = 2.4, 7.2
3-H_2	1.55−1.66, m	1.44−1.53, m	1.54−1.71, m
	2.16, dd, J = 7.2, 13.2	2.17, dd, J = 7.2, 13.2	2.27, dd, J = 7.2, 13.0
5-H_2	1.42−1.53, m	1.55−1.65, m	1.54−1.71, m
	1.55−1.66, m		
6-H_2	1.42−1.53, m	1.55−1.65, m	1.54−1.71, m
	1.55−1.66, m		
7-H_3	1.38, s	1.40, s	1.48, s
8-H	2.10, sept, J = 6.9	1.93−2.14, m	2.15, sept, J = 6.9
9-H_3	0.96, d, J = 6.9	0.96, d, J = 6.9	1.00, d, J = 6.9
10-H_3	0.97, d, J = 6.9	0.97, d, J = 6.9	1.00, d, J = 6.9
other	0.89 (t, 3H, J = 6.9, 6′-H), 1.26−1.35 (m, 4H, 4′-H_2, 5′-H_2), 1.55−1.66 (m, 2H, 3′-H_2), 2.32 (t, 2H, J = 7.7, 2′-H)	1.03, s, C(CH_3)$_3$), 2.22 (s, 2H, 2′-H)	7.43 (t, 2H, J = 7.6, 3′,5′-H), 7.56 (t, 1H, J = 7.2, 4′-H), 8.03−8.12 (m, 2H, 1′,6′-H)

Table 3. Carbon-13 NMR Data for 1,8-Cineole Derivatives

carbon	8b	8c	8d	8e
C1	70.00	69.99	70.04	70.08
C2	40.42	40.40	40.57	40.46
C3	72.53	72.53	72.29	73.33
C4	37.55	37.52	37.72	37.71
C5	21.09	21.07	21.17	21.16
C6	30.15	30.13	30.17	30.16
C7	26.77	26.75	26.77	26.77
C8	73.14	73.12	73.18	73.10
C9	30.17	30.15	30.57	30.11
C10	30.52	30.51	30.57	30.88
C═O	173.28	173.46	172.01	166.17
other	13.71 (C-4′), 18.39 (C-3′), 36.72 (C-2′)	13.87 (C-6′), 22.29 (C-5′), 24.56 (C-4′), 31.30 (C-3′), 34.76 (C-2′)	29.72 (C(CH_3)$_3$), 30.87 (C-3′), 48.37 (C-2′)	128.41 (C-3′, 5′), 129.53 (C-2′, 6′), 130.42 (C-1′), 132.94 (C-4′)

solidify. A solution (1 mL) of the test compound in the required organic solvent (**Table 6**) was introduced into the Petri dishes using a micropipet, and the dishes were left open in a laminar flow cabinet for 3 h to allow evaporation of the organic solvent. Pre-emergence bioassays were carried out at solution concentrations of 1, 0.1, 0.01, 1×10^{-3} and 1×10^{-4} mol L^{-1}.

Filter paper bioassays were used for 1,8-cineole and eucalyptus oil. Filter papers (Whatman number 4) were autoclaved, oven-dried and placed into autoclaved pyrex Petri dishes (55 mm) under sterile conditions. 1,8-Cineole solution or eucalyptus oil solution (1 mL) was transferred onto the filter paper using a micropipet, the lid placed on the Petri dish and the dish sealed with plastic food wrap. The 1,8-cineole and eucalyptus oil were prepared in aqueous solution with 3.4×10^{-4} g mL^{-1} of the nonionic surfactant Tween 80 (polyoxyethylene (20) sorbitan monooleate). Concentrations of 1,8-cineole solutions were at the molar concentrations stated above, and the high-cineole eucalyptus oil solutions were (in g mL^{-1}) 0.154, 1.54×10^{-2}, 1.54×10^{-3}, 1.54×10^{-4} and 1.54×10^{-5}. These concentrations were chosen to give a 1,8-cineole concentration in the eucalyptus oil solutions that approximately matched its concentrations in the 1,8-cineole solutions. The deionized water/Tween 80 solution, containing calcium and boron as above, was autoclaved prior to preparation of the 1,8-cineole and eucalyptus oil solutions. All glassware used in the preparation of these solutions was washed with 2% sodium hypochlorite solution and then rinsed with sterile deionized water.

For pre-emergence bioassays, ten seeds were placed in each Petri dish and then sealed with plastic food wrap. At each concentration and for controls, five replicates were used (i.e., 10 seeds in each of 5 Petri dishes). The Petri dishes were placed randomly in a tray with Styrofoam supports to angle the dishes at approximately 70° to the horizontal. Angling Petri dishes gave straighter root and shoot growth than placing them flat. Petri dishes with 1,8-cineole and eucalyptus oil were placed flat. The tray was incubated under light (135 to 195 μE m^{-2} s^{-1} photosynthetic active radiation) at 25 °C for 72 h. Two controls, one with and one without solvent, were used for each experiment. For solvent controls, solvent (1 mL) was pipetted onto the surface of the agar and the Petri dish left open in a laminar flow cabinet for three hours. The nonsolvent control consisted of the same agar solution in Petri dishes that were similarly left open in a laminar flow cabinet for three hours.

Table 4. Carbon-13 NMR Data for 1,4-Cineole Derivatives

carbon	11a	11b	11c	11d	11e
C1	84.4	84.42	84.47	88.81	88.86
C2	78.4	78.08	78.14	78.22	78.98
C3	43.2	43.23	43.26	43.26	43.06
C4	88.7	88.73	88.79	84.32	84.69
C5	33.4	33.50	33.53	33.62	33.68
C6	31.3	31.38	31.43	31.72	31.71
C7	16.3	16.32	16.37	16.59	16.54
C8	32.5	32.45	32.49	32.46	32.45
C9	17.9	17.91[a]	17.96[a]	18.00[a]	18.04[a]
C10	18.1	18.12[a]	18.17[a]	18.13[a]	18.13[a]
C=O	170.7	173.38	173.66	172.15	166.24
other	21.1 (C-2′)	13.62 (C-4′), 18.47 (C-3′), 36.30 (C-2′)	13.91 (C-6′), 22.32 (C-5′), 24.72 (C-4′), 31.32 (C-3′), 34.46 (C-2′)	29.69 (C(CH_3)$_3$), 30.79 (C-3′), 47.94 (C-2′)	128.32 C-3′, 5′), 29.68 C-2′, 6′), 130.32 (C-1′), 132.96 (C-4′)

[a] Assignment of signals with the same superscript in the spectra is interchangeable.

Table 5. Mass Spectral Data for 1,8-Cineole and 1,4-Cineole Derivatives

compound	MS data *m/z* (rel int)
8b	240 (M^+, 17%), 239 (16), 226 (12), 225 (84), 223 (22), 153 (100), 152 (35), 151 (23), 147 (16), 137 (37), 135 (55), 11 (11)
8c	269 (M + 1, 100%), 268 (M, 11), 267 (18), 254 (13), 253 (78), 252 (10), 251 (31), 175 (36)
8d	269 (M + 1, 100%), 268 (M, 10), 254 (11), 253 (73), 251 (15), 175 (20), 153 (95), 152 (36), 151 (19), 137 (73), 135 (57)
8e	275 (M + 1, 98%), 274 (M^+, 11), 259 (50), 181 (24), 155 (22), 154 (73), 153 (85), 152 (27), 139 (16), 138 (33), 137 (100), 136 (50), 135 (36), 124 (22), 123 (25)
11a	208 (M-4, 10%), 170 (13), 166 (58), 154 (15), 153 (62), 152 (34), 151 (29), 137 (30), 127 (25), 125 (32), 124 (27), 112 (37), 111 (26), 110 (26), 109 (100), 97(20), 95 (27)
11b	240 (M, 6%), 170 (21), 154 (13), 153 (91), 152 (70), 137 (34), 127 (12), 125 (22), 124 (38), 123 (12), 112 (23), 111 (13), 109 (100), 107 (13)
11c	268 (M, 4%), 170 (14), 153 (61), 152 (45), 137 (22), 125 (13), 124 (28), 109 (61), 99 (100)
11d	268 (M, 6%), 170 (21), 154 (18), 153 (100), 152 (65), 137 (30), 125 (20), 124 (45), 109 (83), 99 (98)
11e	275 (M + 1, 77%), 274 (M, 17), 153 (100), 152 (41), 124 (11)

Table 6. Solvents Used for Bioassays of Test Compounds

compound	solvent
acetic acid	water
benzoic acid	trichloromethane (chloroform)
butanoic acid	water
hexanoic acid	hexane
t-butylacetic acid	hexane: chloroform; 99:1
1,8-cineole **3**	Tween 80 in water (0.34 g L^{-1})
eucalyptus oil	Tween 80 in water (0.34 g L^{-1})
cinmethylin **5**	hexane
3-oxo-1,8-cineole **6**	hexane
3-*exo*-hydroxy-1,8-cineole **7**	hexane
3-*exo*-acetoxy-1,8-cineole **8a**	hexane
3-*exo*-butoxy-1,8-cineole **8b**	hexane
3-*exo*-hexoxy-1,8-cineole **8c**	hexane
3-*exo*-*t*-butylacetoxy-1,8-cineole **8d**	chloroform
3-*exo*-benzoxy-1,8-cineole **8e**	chloroform
2-*endo*-hydroxy-1,8-cineole **9**	hexane:chloroform, 99:1
2-*exo*-hydroxy-1,4-cineole **10**	hexane:chloroform, 9:1
2-*exo*-acetoxy-1,4-cineole **11a**	hexane
2-*exo*-butoxy-1,4-cineole **11b**	hexane
2-*exo*-hexoxy-1,4-cineole **11c**	hexane
2-*exo*-*t*-butylacetoxy-1,4-cineole **11d**	hexane:chloroform, 9:1
2-*exo*-benzoxy-1,4-cineole **11e**	chloroform

Experimental Design and Data Analysis. At each concentration and for controls, five replicates were used and Petri dishes were placed in a completely randomized manner in the support tray. After 72 h the number of seeds germinating was counted and the lengths of their shoots and roots were measured.

Data were subjected to one way analysis of variance (ANOVA), using the SPSS 15.0 statistics package (SPSS Inc., 2007). Means were considered to be statistically different at $P = 0.05$.

RESULTS AND DISCUSSION

Pre-emergence Bioassays. Data from pre-emergence bioassays for radish and ryegrass seeds with both cineole compounds, their derivatives and the carboxylic acids corresponding with the ester derivatives are shown in **Tables 7**, **8** and **9**. To facilitate comparisons between the substances their activity relative to 1,8-cineole was determined (**Table 9**).

A dose−response was observed toward the radish for all compounds and the eucalyptus oil showing suppression of germination, and root and shoot growth increasing with concentration. **Figures 3** and **4** give typical dose−response curves for selected compounds (see Supporting Information for remaining dose−response curves). A one-way ANOVA gave the concentration at which suppression of germination, and root and shoot lengths were significant (at $P = 0.05$) (**Table 7**).

No trends are apparent in the toxicity of these substances against radish. The results for the pre-emergence bioassays on radish do not support the hypothesis that the cineole esters would have improved phytotoxicity compared to the corresponding hydroxylated cineole and carboxylic acid, nor do they support the hypothesis that increasing lipophilicity of the carboxylic acid portion of the ester would improve herbicidal activity.

All the carboxylic acids suppressed radish germination, root and shoot growth at 0.1 mol L^{-1} or less (**Table 7**) and gave complete inhibition above 0.1 mol L^{-1} (**Table 8**). Eucalyptus oil and 1,8-cineole both suppressed germination, root and shoot growth at similar concentrations (**Table 7**) but at the higher concentration of 0.1 mol L^{-1} 1,8-cineole was more active (**Tables 8** and **9**). When considering the concentration at which suppression of germination, root and shoot growth first occur, of the 1,8-cineole derivatives 3-oxo-1,8-cineole was the most effective against radish with the acetate being the most

Table 7. Concentration (mol L^{-1}) above Which Suppression of Radish and Ryegrass Occurred (at $P = 0.05$)

	radish			ryegrass		
compound	root	shoot	germination	root	shoot	germination
acetic acid	0.1	0.1	0.1	0.01	0.01	0.1
benzoic acid	0.01	0.01	0.1	0.0001	0.0001	0.01
butanoic acid	0.01	0.01	0.1	0.01	0.1	0.01
hexanoic acid	0.001	0.1	0.1	0.0001	0.0001	0.01
t-butylacetic acid	0.01	0.1	0.1	0.001	0.001	0.01
1,8-cineole **3**	1	1	0.1	0.1	0.1	0.1
eucalyptus oil[a]	0.154	0.154	0.0154	0.0154	0.0154	0.0154
3-oxo-1,8-cineole **6**	0.01	0.01	0.01	0.1	0.0001, 0.1 not 0.001 and 0.01	0.1
3-*exo*-hydroxy-1,8-cineole **7**	0.01	0.1	0.1	0.1	0.01	0.1
3-*exo*-acetoxy-1,8-cineole **8a**	0.01	1	0.01	0.1	0.1	0.1
3-*exo*-butoxy-1,8-cineole **8b**	0.1	0.1	0.1	0.01	0.01	0.1
3-*exo*-hexoxy-1,8-cineole **8c**	0.01	1	0.01	0.01	0.01	0.1
3-*exo*-*t*-butylacetoxy-1,8-cineole **8d**	not suppressed	not suppressed	0.1	0.01	0.001	0.1
3-*exo*-benzoxy-1,8-cineole **8e**	0.01	0.1	1	0.01	0.1	0.1
2-*endo*-hydroxy-1,8-cineole **9**	0.01	0.1	0.01	0.1	0.01	0.1
cinmethylin **5**	0.0001	0.01, 0.1 but not 1	1			
2-*exo*-hydroxy-1,4-cineole **10**	0.1	0.1	0.1	0.01	0.1	0.1
2-*exo*-acetoxy-1,4-cineole **11a**	0.01	0.01	0.01	0.01	0.01	0.1
2-*exo*-benzoxy-1,4-cineole **11e**	0.01	0.01	not suppressed	0.0001	0.0001	0.1
2-*exo*-hexoxy-1,4-cineole **11c**	0.1	0.1	0.1 but not 1			
2-*exo*-butoxy-1,4-cineole **11b**	0.1	0.1	0.1	0.001	0.01	0.1
2-*exo*-*t*-butylacetoxy-1,4-cineole **11d**	0.01	0.01	0.1	0.01	0.1	0.1

[a] Concentration of eucalyptus oil is in g mL^{-1} with 0.0154 g mL^{-1} giving a 1,8-cineole concentration of approximately 0.1 mol L^{-1}.

Table 8. Pre-emergence Herbicidal Activity of Compounds Tested against Radish and Ryegrass at 0.1 mol L^{-1} (Ordered on Germination Inhibition of Radish)

	percentage of control					
	radish			ryegrass		
compound	RL[a]	SL[a]	germ[a]	RL[a]	SL[a]	germ[a]
acetic acid	0	0	0	0	0	0
benzoic acid	0	0	0	0	0	0
butanoic acid	0	0	0	0	0	0
hexanoic acid	0	0	0	0	0	0
t-butylacetic acid	0	0	0	0	0	0
2-*exo*-butoxy-1,4-cineole **11b**	0	0	0	6.7	9	9.3
3-*exo*-hydroxy-1,8-cineole **7**	26.8	46.5	2.4	16.6	10.2	2.6
2-*endo*-hydroxy-1,8-cineole **9**	6.8	0	2.6	39.5	15.4	51.5
2-*exo*-hydroxy-1,4-cineole **10**	9.9	41.7	2.6	6.1	23.1	21.7
2-*exo*-acetoxy-1,4-cineole **11a**	2.7	0	4.5	0	0	0
3-oxo-1,8-cineole **6**	20.5	29.3	10	18.9	18.2	33.3
3-*exo*-acetoxy-1,8-cineole **8a**	29.2	*64.3*[b]	18.9	7.6	21.3	34.1
3-*exo*-*t*-butylacetoxy-1,8-cineole **8d**	*72.5*	*90.9*	31.4	64.9	44.8	*97.8*
1,8-cineole **3**	*37.1*	*64.7*	34.1	22.4	*28.4*	30.2
2-*exo*-*t*-butylacetoxy-1,4-cineole **11d**	*55.8*	*71.2*	42.9			
3-*exo*-hexoxy-1,8-cineole **8c**	39.8	*73.3*	47.2	16	10.2	2.3
3-*exo*-butoxy-1,8-cineole **8b**	26.7	63.4	51.4	7.9	11.1	14.6
2-*exo*-hexoxy-1,4-cineole **11c**	44.4	44.3	58.1	10.9	9.2	4.3
eucalyptus oil[c]	*47.7*	*80.4*	58.8	*26.2*	*23.8*	4.9
2-*exo*-benzoxy-1,4-cineole **11e**	54.7	64	*82.9*	28	23.7	46.3
cinmethylin **5**	36.5	62.5	*85.7*			
3-*exo*-benzoxy-1,8-cineole **8e**	42.4	61	*98*	10.9	41.6	*82.2*

[a] RL = root length; SL = shoot length; germ = germination. [b] Italicized font = not significant. [c] Concentration of eucalyptus oil is 0.0154 g mL^{-1} giving 1,8-cineole concentration of approximately 0.1 mol L^{-1}.

Table 9. Pre-emergence Herbicidal Activity of Test Compounds against Radish and Ryegrass at 0.1 mol L^{-1} Relative to 1,8-Cineole (CI = Complete Inhibition) (Ordered on Germination Inhibition of Radish)

	activity relative to 1,8-cineole					
	radish			ryegrass		
compound	RL[a]	SL[a]	germ[a]	RL[a]	SL[a]	germ[a]
acetic acid	CI	CI	CI	CI	CI	CI
benzoic acid	CI	CI	CI	CI	CI	CI
butanoic acid	CI	CI	CI	CI	CI	CI
hexanoic acid	CI	CI	CI	CI	CI	CI
t-butylacetic acid	CI	CI	CI	CI	CI	CI
2-*exo*-butoxy-1,4-cineole **11b**	CI	CI	CI	3.3	3.2	3.2
3-*exo*-hydroxy-1,8-cineole **7**	1.4	1.4	14.2	1.3	2.8	11.6
2-*endo*-hydroxy-1,8-cineole **9**	5.5	CI	13.1	0.6	1.8	0.6
2-*exo*-hydroxy-1,4-cineole **10**	3.7	1.6	13.1	3.7	1.2	1.4
2-*exo*-acetoxy-1,4-cineole **11a**	3.5	CI	7.5	CI	CI	CI
3-oxo-1,8-cineole **6**	1.8	2.2	3.4	1.2	1.6	0.9
3-*exo*-acetoxy-1,8-cineole **8a**	1.3	1.0	1.8	2.9	1.3	0.9
3-*exo*-*t*-butylacetoxy-1,8-cineole **8d**	0.5	0.7	1.1	0.3	0.6	0.3
1,8-cineole **3**	1.0	1.0	1.0	1.0	1.0	1.0
2-*exo*-*t*-butylacetoxy-1,4-cineole **11d**	0.7	0.9	0.8			
3-*exo*-hexoxy-1,8-cineole **8c**	0.9	0.9	0.7	1.4	2.8	13.1
3-*exo*-butoxy-1,8-cineole **8b**	1.4	1.0	0.7	2.8	2.6	2.1
2-*exo*-hexoxy-1,4-cineole **11c**	0.6	1.5	0.6	2.1	3.1	7.0
eucalyptus oil[b]	0.8	0.8	0.6	0.9	1.2	6.2
2-*exo*-benzoxy-1,4-cineole **11e**	0.7	1.0	0.4	0.8	1.2	0.7
cinmethylin **5**	0.8	1.0	0.4			
3-*exo*-benzoxy-1,8-cineole **8e**	0.8	1.1	0.3	2.1	0.7	0.4

[a] RL = root length; SL = shoot length; germ = germination. [b] Concentration of eucalyptus oil is 0.0154 g mL^{-1} giving a 1,8-cineole concentration of approximately 0.1 mol L^{-1}.

active 1,4-cineole derivative (**Table 7**). However, at 0.1 mol L^{-1} the most active of the cineole compounds against radish was 2-*exo*-butoxy-1,4-cineole, being completely inhibitory (**Table 8**). At this concentration the most effective pre-emergence compounds (that is, compounds preventing germination) were 2-*exo*-butoxy-1,4-cineole, 3-*exo*-hydroxy-1,8-cineole, 2-*endo*-hydroxy-1,8-cineole, 2-*exo*-hydroxy-1,4-cineole and 2-*exo*-acetoxy-1,4-cineole, with germination suppressed by approximately 97% as compared to 66% suppression by 1,8-cineole (**Tables 8** and **9**). 2-*endo*-Hydroxy-1,8-cineole was the most active of the 1,8-cineole derivatives at 0.1 mol L^{-1}, being completely inhibitory of shoot growth and second only to 2-*exo*-butoxy-1,4-cineole in its suppression of root growth.

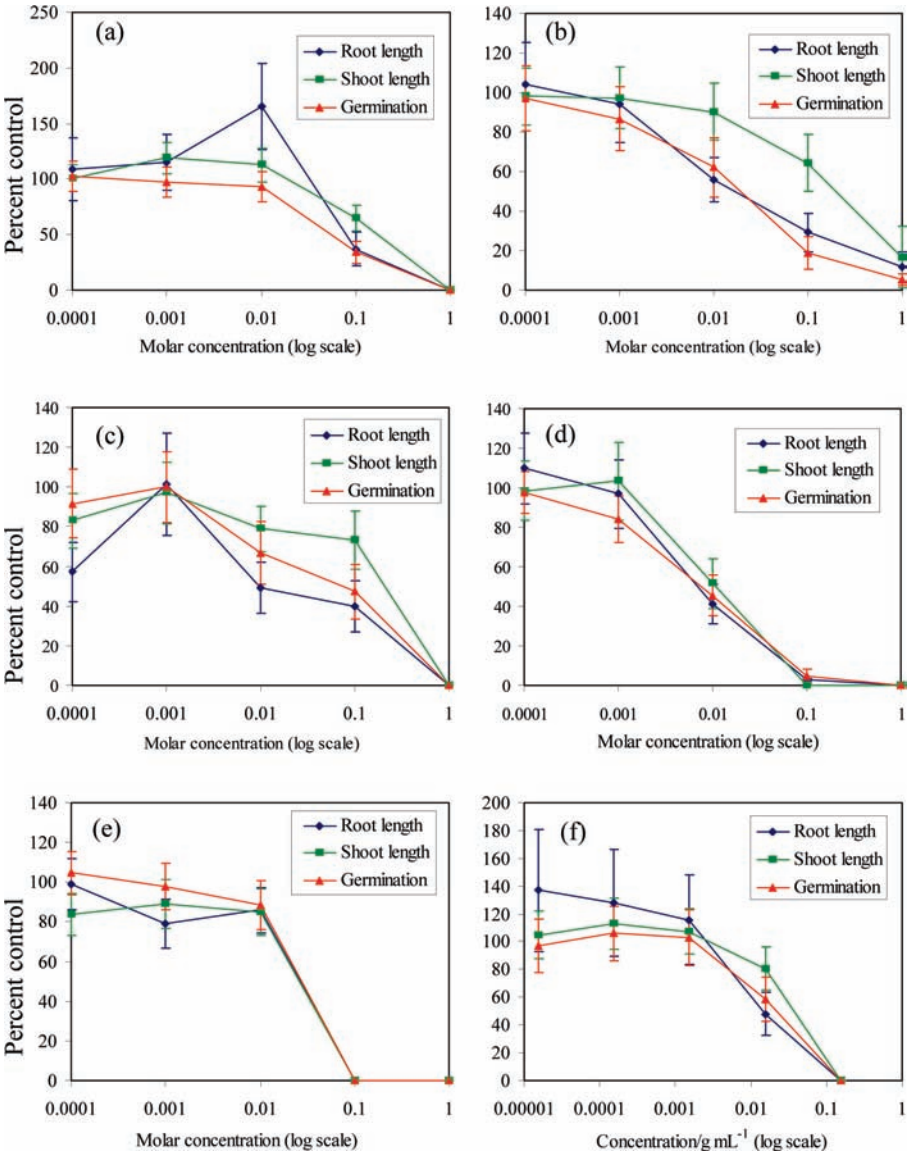

Figure 3. Effects of (**a**) 1,8-cineole, (**b**) 3-*exo*-acetoxy-1,8-cineole, (**c**) 3-*exo*-hexoxy-1,8-cineole, (**d**) 2-*exo*-acetoxy-1,4-cineole, (**e**) 2-*exo*-butoxy-1,4-cineole and (**f**) eucalyptus oil on root growth, shoot growth and germination of radish 72 h after exposure. Bars = ±SE.

In general the 1,8-cineole derivatives were more effective against radish roots than shoots while 1,4-cineole derivatives showed no particular trend in their activity against roots versus shoots (**Tables 7** and **8**). Considering germination and root and shoot suppression together, 3-*exo*-acetoxy-1,8-cineole was the most active of the 1,8-cineole esters, although only the hexanoate ester gave complete suppression of germination (at 1 mol L^{-1}) (**Figure 3**). Although the butoxy ester of 1,4-cineole gave complete inhibition at 0.1 mol L^{-1}, 2-*exo*-acetoxy-1,4-cineole gave suppression at a lower concentration and overall was the most effective of the 1,4-cineole esters (**Figure 3**).

Ryegrass also showed a dose response toward all compounds and eucalyptus oil in the pre-emergence bioassays, with suppression of germination, root growth and shoot growth increasing with concentration. A one way ANOVA gave the concentration at which suppression of germination, and root and shoot lengths were significant (at $P = 0.05$) (**Table 7**).

As for radish, there are no trends in the toxicity of these substances against ryegrass. The phytotoxicity of the esters is no better than that of the corresponding hydroxylated cineole and carboxylic acid in the ryegrass pre-emergence bioassays. Neither did increasing the lipophilicity of the carboxylic acid portion of the ester improve herbicidal activity against ryegrass.

Again, as for the radish, ryegrass germination, root and shoot growth were suppressed by the acids at 0.1 mol L^{-1} or less (**Table 7**) and germination was completely inhibited by the acids above 0.1 mol L^{-1} (**Table 8**).

Eucalyptus oil and 1,8-cineole first suppressed ryegrass germination, root and shoot growth at similar concentrations of 0.0154 g mL^{-1}/0.1 mol L^{-1} (**Table 7**) and while their suppression of shoot growth and root growth were similar, eucalyptus oil was approximately six times more effective at suppressing germination than 1,8-cineole (**Tables 8** and **9**), a contrast to what was observed for the radish where 1,8-cineole was more active. Overall, when considering the concentration at which suppression first occurs, 3-*exo*-hexoxy-1,8-cineole was the most active 1,8-cineole derivative against ryegrass but of the 1,8-cineole derivatives, 3-*exo*-*t*-butylacetoxy-1,8-cineole suppressed ryegrass shoot growth at a lower concentration than the other 1,8-cineole derivatives. Its suppression did not go above 60% until complete inhibition at 1 mol L^{-1} (**Figure 4**). Of the 1,4-cineole derivatives, the most effective germination inhibitor was 2-*exo*-acetoxy-1,4-cineole. Although 2-*exo*-benzoxy-1,4-cineole only inhibited germination at 1 mol L^{-1}, it suppressed ryegrass root and shoot growth at a lower concentration than any of the cineole derivatives

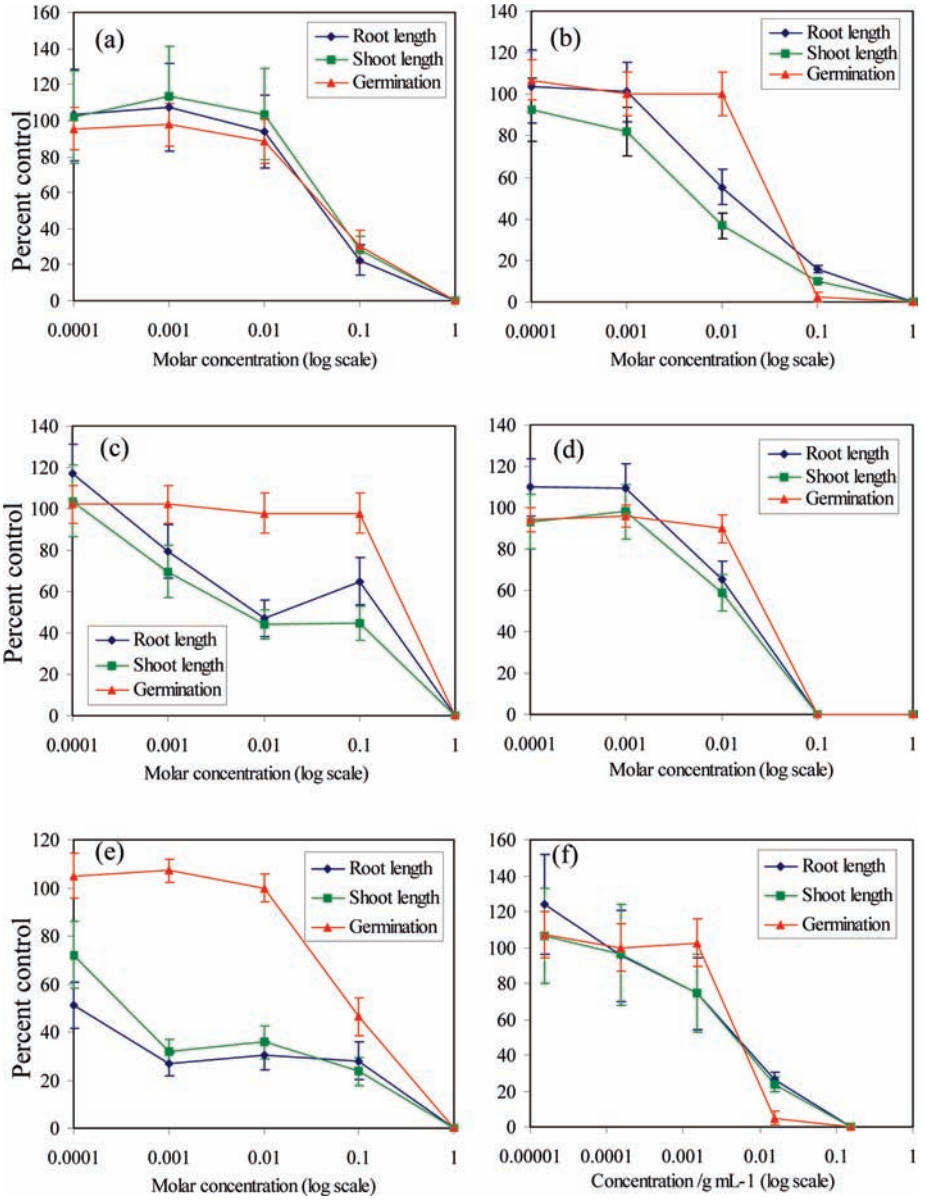

Figure 4. Effects of (**a**) 1,8-cineole, (**b**) 3-*exo*-hexoxy-1,8-cineole, (**c**) 3-*exo*-*t*-butylacetoxy-1,8-cineole, (**d**) 2-*exo*-acetoxy-1,4-cineole, (**e**) 2-*exo*-benzoxy-1,4-cineole and (**f**) eucalyptus oil on root growth, shoot growth and germination of ryegrass 72 h after exposure. Bars = ±SE.

(**Figure 4**). In general, all the substances were more effective at suppressing ryegrass root and shoot growth than they were at suppressing its germination but there was no clear trend in the sensitivity of ryegrass roots compared to ryegrass shoots (**Table 7**).

At 0.1 mol L^{-1}, 2-*exo*-acetoxy-1,4-cineole was the most effective of the cineole compounds as a pre-emergence herbicide against ryegrass giving complete inhibition of germination (**Table 8**). In contrast to radish, 2-*endo*-hydroxy-1,8-cineole was less active against ryegrass roots, shoots and germination than 3-*exo*-hydroxy-1,8-cineole.

For radish, root sensitivity was higher than shoot sensitivity for most of the tested substances at 0.1 mol L^{-1}, but there was no strong trend in sensitivity of ryegrass roots versus ryegrass shoots. A comparison of the germination inhibition at 0.1 mol L^{-1} does not indicate a greater sensitivity of one species over another, but ryegrass roots and shoots were generally more sensitive to the cineole compounds at 0.1 mol L^{-1} than were the radish roots and shoots. No effect of functionalizion of the cyclohexane ring at position 2 compared to position 3 was observed for either species at 0.1 mol L^{-1}. In radish, the 2- and 3-hydroxy-cineole compounds had similar germination suppression at 0.1 mol L^{-1} while for ryegrass 2-*endo*-hydroxy-1,8-cineole was less effective against germination than the other hydroxy-cineole compounds. Likewise, a comparison of 3-*exo*-hydroxy-1,8-cineole, 2-*endo*-hydroxy-1,8-cineole and 2-*exo*-hydroxy-1,4-cineole shows there was no effect of position of functionalization on activity at concentrations when initial suppression was observed (**Table 7**).

In conclusion, hydroxy and ester derivatives of 1,8-cineole and 1,4-cineole have a dose-dependent herbicidal activity against annual ryegrass and radish germination and root and shoot growth, although no trends were observed between lipophilicity of ester derivatives and herbicidal activity. In addition many of the derivatives have improved phytotoxicity relative to 1,8-cineole. A likely contributor to their phytotoxicity is hydrolysis of the esters on uptake by the plants to produce the hydroxy-cineole and carboxylic acid. The carboxylic acid may be phytotoxic due to a generalized pH effect. Weak organic acids with a pH between 5 and 8 can disturb photosynthetic processes by disrupting the hydrogen ion concentration gradient across the

two sides of the thylakoid membrane (*22*). Future experiments should focus on the bioactivity of compounds relative to 1,8-cineole in field tests as well as experiments to assess whether these esters hydrolyze on uptake by plants.

Supporting Information Available: Additional dose−response curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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