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INFLUENCE OF HERBICIDES AND GROWTH REGULATORS ON THE GROWTH AND ESSENTIAL OIL CONTENT OF SAGE*

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Key Word Index *Salvia officinalis*; Lamiaceae; sage; metabolism; essential oils; monoterpenes; herbicides; growth regulators.

Abstract Foliar application of a wide range of herbicides provided suggestive evidence for a direct correlation between growth and essential oil production in sage (*Salvia officinalis*) grown under controlled environmental conditions. Conversely, foliar application of the growth regulators AMO-1618 and DCPA indicated that moderate stunting of growth was associated with an increase in essential oil yield. Quantitative changes in the principle monoterpenes of sage oil, β-pinene, 1,8-cineole, 3-isothujone and camphor, were also observed in response to the various treatments. However, attempts to correlate the changes in oil composition with alteration in growth or oil yield also failed to establish any definitive relationships. Since the four monoterpene components examined arise by independent routes from a common precursor, the changes in oil composition observed indicate that the applied bioregulators exert a direct effect on terpene metabolism by a means independent of growth or development.

INTRODUCTION

Weeds are a serious problem in the cultivation of aromatic plants since they can impart undesirable colour and odour to the derived essential oil, as well as decrease the oil yield [1–4]. For these reasons the application of both pre-emergence and post-emergence herbicides has become a common practice in the commercial production of aromatic plants, a practice which has consequently stimulated interest in the influence of these compounds on aromatic plants themselves. Such investigations are numerous, and a variety of effects on herbage and oil yield have been reported, depending on the nature of the herbicide and the timing and rate of application [5–12]. Since many of these studies were carried out under field conditions it often was difficult to distinguish the effects of weed control from more direct influences on essential oil composition and yield.

We have initiated a series of systematic studies on the influence of growth regulators and bioregulators on essential oil-bearing plants raised under controlled environmental conditions [13–15], and have observed significant changes in both oil yield and composition. Compositional changes are most notable in those species, such as sage and peppermint, which produce complex oils containing multiple monoterpene components, and in some instances it has been possible to correlate such changes in monoterpene content with alterations in monoterpene metabolism [13–15]. In this communication we extend this work to describe the influence of a number of herbicides and growth regulators on growth and essential oil production in common sage (*Salvia officinalis* L.). Sage was chosen as a model for these studies because the essential oil produced contains four key cyclic monoterpene components [(−)-β-pinene, 1,8-cineole, (−)-3-isothujone, and (+)-camphor] which are readily analysed by gas–liquid chromatography and, more importantly, which are known to arise by independent pathways from the common acyclic precursor geranyl pyrophosphate [16], thus providing a sensitive probe for alterations in monoterpene metabolism.

RESULTS

A series of 17 pre-emergence and post-emergence herbicides were screened at the 100–400 ppm foliar application level for their influence on growth and oil production in sage (*Salvia officinalis*) and were shown to comprise three general categories with regard to effect: those which slightly stimulated, or had little effect, on growth and oil production; those which produced moderate stunting of growth and a concomitant decrease in oil yield; and those which severely stunted growth and significantly decreased oil yield. Although a range of effects on growth and oil yield were observed, compositional changes were surprisingly uniform. Table 1 presents the results obtained with three representative herbicides: Bensulide, Linuron and Benefin. Growth parameters are reported as a percentage of control plants which typically weighed 4–6 g, were 15–20 cm in height, and bore leaves with an average surface area of 7–8 cm^2.

Bensulide at the 100–400 ppm application rate (four

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Table 1. Effect of herbicides and growth regulators on growth and essential oil of sage

Treatment	ppm	Growth parameters: Plant height	Plant wt	Leaf area	Oil yield: % fr. wt	mg/plant	Oil composition (%): β-Pinene	1,8-Cineole	Isothujone	Camphor
Control		100	100	100	0.16	39.5	3.6	8.9	43.3	15.8
Bensulide	100	142	111	121	0.16	49.5	5.3	8.7	42.9	16.7
Bensulide	200	144	128	134	0.17	48.3	5.5	8.2	41.8	17.4
Bensulide	400	124	118	116	0.18	47.8	6.3	7.3	38.4	19.6
LSD (0.05)		8	12	5	NS	9.9	2.2	NS	NS	3.1
Linuron	100	99	82	93	0.14	28.6	4.3	7.3	35.7	15.8
Linuron	200	89	76	104	0.13	27.9	5.0	6.6	33.8	20.8
Linuron	400	62	58	63	0.12	13.0	6.9	4.9	32.0	22.8
LSD (0.05)		8	17	10	0.03	12.0	1.8	2.1	4.6	4.1
Benefin	100	61	58	70	0.15	24.2	5.7	6.7	37.2	19.4
Benefin	200	24	36	33	0.12	8.2	8.2	5.2	36.7	22.9
Benefin	400	17	25	24	0.11	5.2	9.7	4.1	25.8	23.0
LSD (0.05)		8	17	9	0.03	12.2	1.8	1.9	4.8	4.7
Control		100	100	100	0.17	41.8	4.2	9.1	41.4	16.2
AMO 1618	12.5	100	91	89	0.23	47.1	4.1	9.0	41.0	16.6
AMO 1618	25	99	85	86	0.22	51.9	4.6	8.7	40.2	18.5
AMO 1618	50	90	82	85	0.23	61.7	4.7	8.1	40.5	22.4
AMO 1618	100	85	77	83	0.24	50.1	5.1	7.7	36.6	25.7
AMO 1618	200	81	75	84	0.21	36.5	5.6	7.4	32.7	30.1
LSD (0.05)		9	12	6	0.04	10.3	NS	NS	4.3	2.8
DCPTA	250	104	91	81	0.17	41.4	4.3	8.7	49.9	13.7
DCPTA	500	104	89	73	0.21	43.4	4.8	8.6	52.6	11.9
DCPTA	1000	92	88	73	0.21	50.1	4.8	8.8	47.2	17.8
DCPTA	2000	90	72	54	0.23	46.7	5.0	9.2	43.6	21.0
DCPTA	4000	72	51	44	0.26	32.7	4.6	9.1	37.9	26.5
LSD (0.05)		9	15	7	0.05	9.8	NS	NS	4.3	3.5
Malazide	75	60	66	68	0.17	24.7	4.5	9.0	37.7	16.8
Malazide	125	59	60	74	0.14	20.5	4.3	8.8	32.5	15.2
Malazide	250	40	58	131	0.08	10.2	5.1	7.4	27.8	14.7
Malazide	500	38	57	141	0.07	7.9	6.0	6.8	25.0	13.1
Malazide	1000	30	46	142	0.05	5.7	6.0	6.2	24.7	11.4
LSD (0.05)		8	13	8	0.02	6.1	NS	2.1	3.6	2.8

sprays at weekly intervals) resulted in a significant increase in vegetative growth as indicated by plant height, plant fresh weight and average leaf area (Table 1). Oil yield on a fresh weight basis was increased slightly [significant at LSD (0.10) = 0.12], and was increased by roughly 25% on a per plant basis. Regarding oil composition, the proportions of both β-pinene and camphor increased, while the proportions of 1,8-cineole and 3-isothujone decreased slightly [significant at the LSD (0.10) level]. Similar effects on growth, oil yield and oil composition were observed with Sinbar, Methabenzthiazuron, Simazine, Kerb and Aminophon at the 200 ppm application level.

Two applications of Linuron at the indicated concentrations resulted in a significant decrease in growth at the 200 and 400 ppm levels (Table 1). Oil yield decreased moderately (by about 25% at the highest application rate) on a fresh weight basis, and was reduced by about 60% on a per plant basis. Compositional changes observed under the influence of Linuron were similar to those resulting from Bensulide treatment, notably an increase in β-pinene and camphor content and a decrease in the level of 1,8-cineole and (−)-3-isothujone. Similar results on growth, oil yield and oil composition were observed with Goal 2EC, Aresin, Bromacil and Prometryne at the 200 ppm application level.

Benefin was applied only twice, since at the 400 ppm level necrosis of the leaf margins was significant (about 25% of the plants) and additional applications or higher rates (600 ppm) were severely toxic. Overall growth was significantly retarded, and oil yield on a fresh weight basis was reduced by over 30% (Table 1). Oil yield on a per plant basis was decreased greatly to a level only 13% of the control value. Although yield reduction was severe, the compositional effects noted were similar to those observed with Bensulide and Linuron (i.e. an increase in the percentages of β-pinene and camphor, and a decrease in the percentages of 1,8-cineole and 3-isothujone). Even at concentrations of Benefin below 25 ppm (two applications), at which effects on growth were negligible, the influence on oil composition persisted (β-pinene = 5.4%, 3-isothujone = 38.5%). Similar results on growth, oil yield and oil composition were observed on comparing Benefin to Aminotriazole, Carbethamid, Mecoprop, Treflan and Diquat at comparable application rates (2 × 200 ppm).

Since earlier studies with growth retardants had indicated that oil yield was increased under conditions of moderate stunting [14], unlike the above results with herbicides, the influence of two additional growth retardants (AMO 1618 and Malazide) and an additional bioregulator (DCPTA) were examined for comparative purposes. Four applications of AMO 1618 (which alters growth by interfering with gibberellin biosynthesis [17]) at the indicated concentrations (Table 1) resulted in moderate stunting of growth based on all the parameters, while producing generally significant increases in oil yield on both fresh weight and per plant bases. Leaf number per plant was unchanged except at the highest concentration of AMO 1618 (decreased *ca* 10%) at which a decrease in oil yield per plant was also observed (Table 1); leaves were darker in colour at all the applied concentrations. Oil composition was generally unaffected except at the higher concentrations where there was a significant decrease in the concentration of isothujone and a concomitant rise in the level of camphor.

DCPTA, a bioregulator known to influence carotenoid metabolism [18], had relatively little effect on plant height or weight, except at the 2000 and 4000 ppm levels, whereas leaf size was reduced significantly even at the lowest application rate (Table 1). Oil yield on both bases was only marginally influenced. At the highest application rate the percentage oil yield was highest while the yield per plant was lowest. Since leaf number was unaffected, this combination of results is most likely due to the severely diminished leaf size plus prominent chlorosis at this application rate. DCPTA had no perceptible influence on the content of β-pinene or 1,8-cineole of the oil. Curiously, increasing concentrations of DCPTA first increased, then decreased, the level of isothujone, while having the opposite influence on the level of camphor (i.e. a decrease followed by a significant increase in the level of camphor).

Malazide very markedly reduced plant height and weight at all the application levels (two sprays at 75–1000 ppm); however, at the higher application rates of this substance the leaves were substantially increased in size and were much darker in colour than were untreated controls. Malazide severely decreased oil yield on one or both bases (Table 1), and it was necessary to decrease the Malazide level to below 20 ppm before effects on growth and oil yield were not significantly different from controls. The 80–85% oil yield reduction per plant at the higher application rates (500 and 1000 ppm) was due in part to a reduction in leaf number (by *ca* 30%); however, neither this nor the 60–70% oil yield reduction on a weight basis could be fully rationalized by a decrease in leaves. The β-pinene content of sage oil was unaffected by treatment with Malazide, whereas the levels of 1,8-cineole, isothujone and camphor were reduced. The reduction in 1,8-cineole, isothujone and camphor content was accompanied by an increase in the levels of numerous other monoterpenes, including α-pinene, camphene, limonene, thujone and borneol, as well as of the sesquiterpenes humulene and caryophyllene.

DISCUSSION

The results obtained on examining the influence of herbicides on sage were suggestive of a loose correlation between growth and essential oil formation, oil yield roughly paralleling growth parameters (Table 1). On the other hand, studies with the bioregulator DCPTA and the growth retardant AMO 1618 (Table 1), as well as earlier work with the growth regulators Phosfon D and Cycocel [14], indicated that moderate stunting of plant growth need not result in diminished oil yield, and more often is associated with a significant increase in oil production. Additionally, cytokinins have been shown to increase oil yield without a sensible effect on growth or development [15]. It should be noted in this regard that the primary sites of monoterpene biosynthesis are the epidermal secretory glands [19], fully exposed structures likely to be more sensitive to the effects of foliar applied chemicals than most other plant parts. In the present study we screened nearly 30 foliar herbicides, growth regulators and bioregulators for their influence on growth and essential oil production in sage [13–15] and, with the exception of oil yield reduction under severe stunting [as with Malazide (Table 1)], we observed no direct relationship between tissue growth and essential oil formation in this species. Similar, but less extensive, studies with peppermint [13–15] forced the same conclusion.

Corresponding attempts to correlate changes in sage oil composition with alteration in growth or oil yield also failed to establish any definitive relationships. While there is a general tendency for the isothujone content to diminish with decreasing yield, there are clear exceptions [14], and the corollary, that high yields are associated with high levels of isothujone, is invalid [13]. That the contents of (−)-3-isothujone and (+)-camphor, the two major components of the oil, exhibit a reciprocal relationship is not unexpected.

Although these studies have provided little of predictive value regarding the relationship between growth, oil yield and oil composition, they consequently have provided clear evidence that growth regulators and bioregulators can influence oil yield and composition, presumably by direct action on terpene metabolism and by means independent of growth and development. In the present instance, changes in both relative proportions and absolute levels of the four cyclic monoterpenes 1,8-cineole, (−)-β-pinene, (−)-3-isothujone and (+)-camphor were observed (Table 1). Since each product arises independently by an alternative mode of cyclization of the common precursor geranyl pyrophosphate [16], the results can best be explained by differential changes in the levels or activities of the various cyclase enzymes present. Foliar application of cytokinins has been shown to increase the levels or activities of such cyclases in sage and peppermint as determined by *in vitro* assay [15]. Presumptive evidence for alteration in levels of dehydrogenases and reductases responsible for the subsequent transformations of the parent cyclic products has also been obtained [13, 14], but not yet confirmed by *in vitro* assay. The diversity of effects on essential oil formation observed in response to foliar applied chemicals [13–15] is not surprising when considering the great structural diversity of the compounds applied and the numerous possible means by which they may operate. In no instance is the mechanism yet understood by which any such bioregulator exerts an influence on the enzymes of monoterpene metabolism.

EXPERIMENTAL

Plant material. Salvia officinalis L. (sage) was grown from seed in peat moss in a growth chamber with a 14 hr photoperiod (900 ± 100 fc, fluorescent:incandescent), 29° day / 25° night temp cycle and a relative humidity of 62 ± 12%. Plants were watered as needed and fertilized weekly with a complete fertilizer (N:P:K, 20:20:20, with microelements and iron chelate). Plants were thinned and allowed to grow for 5 weeks before treatment with herbicides and growth regulators.

Treatments. Herbicides and growth regulators at the indicated concns were prepared in distilled H_2O containing 0.1% Tween 20 and sprayed to the point of run-off with a hand sprayer. Expts with Bensulide [*S*-(*O*,*O*-diisopropyl) phosphorodithioate of *N*-(2-mercaptoethyl)benzene sulphonamide], Linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], Benefin (*N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine), AMO 1618 [2'-isopropyl-4'-(trimethylammonio)-5'-methylphenylpiperidine-carboxylate chloride], DCPTA [2-(3,4-dichlorophenoxy)triethylamine] and Malazide (maleic hydrazide) were conducted in randomized complete block design with three replications totalling 60 plants per treatment. A total of four applications of Bensulide, AMO 1618 and DCPTA were made at weekly intervals, and the plants were harvested 2 weeks after the last treatment. A total of two applications of Linuron, Benefin and Malazide were made at weekly intervals, and the plants were harvested 4 weeks after the last treatment. Experiments with Sinbar, Methabenzthiazuron, Simazine, Kerb and Aminophon (4 weekly sprays at 200 ppm with harvest after 2 weeks) and with Goal 2EC, Aresin, Bromacil, Prometryne, Aminotriazole, carbethamid, Mecoprop, Treflan and Diquat (2 weekly sprays at 200 ppm with harvest after 4 weeks) were conducted in randomized complete block design with a single replication totalling 40 plants per treatment. Controls sprayed without growth regulator or herbicide were included in each expt. Data on growth characteristics (fr. wt of plant, leaves, stem and total branches; length, width and number of leaves; length of stem; number of internodes and internode length) were collected and subjected to analysis of variance with comparison of means by least significant differences at $P = 5\%$ (LSD 0.5) [20].

Oil analysis. A minimum of three representative 10 g samples of fresh tissue were steam-distilled using a simultaneous steam distillation solvent extraction apparatus (J. & W. Scientific), employing (+)-isomenthone as internal standard. On completion of distillation (1 hr) the essential oil collected (in pentane) was dried (Na_2SO_4) and kept under N_2 in a sealed glass tube in the dark at −20° until analysis. Oil analysis (1 μl samples) was performed by capillary GC (FID at 230°, 100:1 injection split at 220°) on a 25 m Carbowax 20 M WCOT column operated at 4 ml/min H_2 and programmed from 45° (5 min hold) to 180° at 10°/min. FID output was electronically integrated, and fr. wt yield and per plant yield were calculated based on the internal standard. The yield and relative percentage of major oil constituents (> 2%) were also determined. Data were statistically analysed as before [20], and the LSD (0.05) of the means is reported. Identifications of oil components based on *RR*, were confirmed by GC/MS comparison of retention times and mass spectra to authentic standards.

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