Phytochemistry, Vol. 25, No. 7, pp. 1603–1606, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

INFLUENCE OF PHOSFON D AND CYCOCEL ON GROWTH AND ESSENTIAL OIL CONTENT OF SAGE AND PEPPERMINT*

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(*Received* 25 *October* 1985)

Key Word Index—*Salvia officinalis*; sage; *Mentha piperita*; peppermint; Lamiaceae; metabolism; essential oils; monoterpenes; growth retardants; Phosfon D; Cycocel.

Abstract—Foliar application of Phosfon D at 50–100 ppm stimulates the growth of *Salvia officinalis* (sage) and moderately retards the growth of *Mentha piperita* (peppermint), while increasing the essential oil yield of both species by 50–70%. Phosfon D increases the proportions of (−)-3-isothujone and (+)-3-thujone in sage oil and decreases the level of (−)-β-pinene and (+)-camphor, whereas this growth retardant increases the proportions of (+)-isomenthone and (+)-neoisomenthol in peppermint oil and decreases the level of (−)-menthone and (−)-menthol. Foliar application of Cycocel at 250–500 ppm slightly stimulates growth and essential oil formation in peppermint, and retards growth of sage with little effect on oil yield. The influence of Cycocel on sage oil composition was the opposite of that of Phosfon, with a tendency to increase the level of (−)-β-pinene and decrease the level of (−)-3-isothujone under severe stunting. The effect of Cycocel on the composition of peppermint varied with concentration. The influence of growth retardants on essential oil composition and yield are most readily explained by alterations in the levels or activities of the relevant enzymes.

INTRODUCTION

Growth retardants, such as Phosfon D and Cycocel, exert their effect at the level of gibberellin metabolism [1, 2], and are also known to influence the accumulation or depletion of other isoprenoids, including sesquiterpenes, diterpene resins, triterpenes and higher terpenoids [3–7].

Relatively little attention has been directed toward the influence of growth retardants on the production of essential oils and their monoterpene constituents. Cycocel, although stunting the growth of rose geranium (*Pelargonium graveolens*), was reported to increase essential oil yield and alter the proportion of the acyclic monoterpenoid constituents [8]. The same compound had little effect on essential oil production in Davena (*Artemisia pallens*, Wall.) [9]. Application of Phosphon D as a soil drench with peppermint (*Mentha piperita*, L.) stimulated growth at low concentration and reduced growth at higher concentrations. However, the effect of this regulator on essential oil production was not examined [10].

The present study was undertaken to examine the influence of two common growth retardants (Phosphon D and Cycocel) [11–13] on essential oil formation in peppermint and sage (*Salvia officinalis*, L.) grown under controlled conditions. These two commercially important oil producing species were chosen for this work because the oil composition of both is well known [14–16] and because the biosynthetic origins of most of the relevant monoterpene constituents have been recently deciphered [17, 18].

RESULTS

Influence of Phosfon D on sage

Three weekly foliar applications of Phosfon D at 50 ppm significantly increased plant growth, with a 30–35% increase in fresh and dry weight (reflected in both stem and leaves) and a 10–15% increase in height. At 100 ppm Phosfon D, the treated plants were indistinguishable from the controls, although dry weight remained elevated by 10–20%. At 200 ppm Phosfon D, stunting was severe with a 50% reduction in height and a 35–40% reduction in fresh and dry weight. Chlorosis of the leaves was evident at 200 ppm Phosfon D, whereas at lower concentrations green colouration was more intense.

Phosfon D at the 50 and 100 ppm levels increased the essential oil content of sage on both a fr. wt and per plant basis compared to the controls (Table 1). At 2000 ppm of this growth retardant the fr. wt yield was maximum (170% of controls) but the yield on a per plant basis was indistinguishable from controls. In terms of oil composition, Phosfon D had a pronounced effect in increasing the levels of the ketones (−)-3-isothujone and (+)-3-thujone, and decreasing the level of the ketone (+)-camphor (Table 1). The levels of β-pinene and of the sesquiterpene olefins humulene and caryophyllene were also reduced. However, the levels of the monoterpene

*This is Scientific Paper No. 7391, Project 0268, College of Agriculture Research Center, Washington State University, Pullman, Washington, 99164-6340, U.S.A. This investigation was supported in part by Department of Energy Contract DE-AM06-76RL02221, Agreement DE-AT06-82ER12027, and by grants from the Washington Mint Commission and Mint Industry Research Council.

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Table 1. Effect of growth retardants on yield and composition of sage oil

Treatment	ppm	Oil yield		Oil composition (%)						
		% fr. wt	mg/plant	α-pinene and camphene	β-pinene	1,8-cineole	isothujone	thujone	camphor	sesqui-terpenes
Control		0.16	36.7	7.2	11.7	9.9	34.8	3.6	13.4	11.7
Phosfon D	50	0.19	52.8	7.6	12.3	11.8	34.7	4.8	11.7	7.9
Phosfon D	100	0.24	53.2	6.3	10.6	8.8	47.6	6.5	9.7	6.3
Phosfon D	200	0.27	37.7	7.6	9.9	9.8	46.2	5.4	10.7	4.1
LSD (0.05)		0.02	5.8	NS*	1.0	1.3	7.0	1.2	2.3	2.0
Control		0.15	33.0	8.7	8.3	9.4	40.1	5.3	16.4	9.3
Cycocel	250	0.15	32.8	7.6	8.1	9.7	41.1	5.5	13.1	9.4
Cycocel	500	0.15	28.1	8.9	8.0	10.9	41.9	5.5	11.2	9.6
Cycocel	1000	0.12	22.1	10.8	9.3	10.7	33.7	6.2	11.8	12.2
Cycocel	2000	0.11	21.2	8.9	12.3	11.1	28.9	4.7	13.0	8.2
LSD (0.05)		0.02	4.6	NS	1.9	NS	7.9	NS	2.6	2.4

*NS: Not significant.

olefins α-pinene and camphene, and of the cyclic ether 1,8-cineole, were essentially uneffected.

Influence of Phosfon D on peppermint

At the 50 ppm application rate of Phosfon D, the treated plants were indistinguishable from controls, but stunting was marked at the 100 and 200 ppm levels. At 100 ppm, plant fresh weight was reduced by 20% and plant height by 25%. At 200 ppm, fresh weight and plant height were reduced by over 60%, with a corresponding decrease in internode length. Leaves were moderately to severely chlorotic and greatly reduced in size.

Foliar application of Phosfon D at all concentrations resulted in significant increase in fr. wt yield, but at the highest application rate oil yield per plant was greatly reduced (Table 2). Major effects on oil composition were a decrease in the levels of menthone and menthol, and an increase in the proportion of isomenthone, neoisomenthol and 1,8-cineole.

Influence of Cycocel on sage

Foliar application of Cycocel at 250 ppm had essentially no effect on the growth of sage. However, in the 500–2000 ppm application range stunting was pronounced. At the 500 ppm level, plant height was reduced by 55% and fr. wt by 20%, whereas at the 2000 ppm level, plant height was reduced by 70% and fr. wt by 50%. At all concentrations of Cycocel the leaves were intensely green.

Unlike the result observed with Phosfon D, oil yield on both fr. wt and per plant bases decreased as stunting under the influence of Cycocel increased (Table 1). Compositional effects with Cycocel were also the opposite of those observed with Phosphon D, notably an increase in the proportion of β-pinene and a decrease in the proportion of isothujone. The camphor level decreased under the influence of both growth retardants.

Influence of Cycocel on peppermint

Cycocel at the 250 ppm level had a slight but significant growth stimulating effect on peppermint, increasing both

Table 2. Effect of growth retardants on yield and composition of peppermint oil

Treatment	ppm	Oil yield		Oil composition (%)				
		% fr. wt	mg/plant	menthone	isomenthone	menthol	neoisomenthol	1,8-cineole
Control		0.25	38.1	39.8	17.7	20.2	2.7	4.5
Phosfon D	50	0.41	47.8	40.2	17.8	19.7	4.1	4.1
Phosfon D	100	0.45	46.1	32.4	20.6	16.6	5.9	4.1
Phosfon D	200	0.43	26.2	29.3	20.9	15.1	6.6	4.3
LSD (0.05)		0.05	5.4	6.0	NS*	1.7	0.7	NS
Control		0.24	36.6	36.2	14.6	21.6	4.1	5.4
Cycocel	250	0.27	44.6	36.1	15.3	21.2	3.8	6.2
Cycocel	500	0.22	36.2	43.7	14.2	18.7	4.7	5.9
Cycocel	1000	0.17	29.2	28.3	9.6	18.3	4.9	4.9
LSD (0.05)		0.04	5.1	9.2	1.9	NS	NS	NS

*NS: Not significant.

fr. wt and plant height by roughly 10%. Plants treated with 500 ppm Cycocel were morphologically indistinguishable from the controls, but the leaves were darker green in colour as was the case with all the Cycocel treatments. At the highest application level (1000 ppm) growth was reduced, but by less than 10% in plant height and weight.

Oil yield was slightly increased at 250 ppm Cycocel, but decreased at higher concentrations of growth retardant in spite of the minimal influence on growth. Compositional effects as a result of Cycocel treatment were minor, with the only discernible trend being an increase and subsequent decrease in the proportion of the ketones menthone and isomenthone (Table 2).

DISCUSSION

Foliar application of low concentrations of Phosfon D (50 ppm) stimulates the growth of sage, yet at the 200 ppm level of this growth retardant stunting is severe. Peppermint was generally more sensitive to the dwarfing influence of Phosfon D than was sage. At low concentrations of Phosfon D, where stunting was negligible to moderate, oil yield for both species was significantly increased on both fr. wt and per plant bases. Under conditions of severe stunting, oil yield per plant declined sharply although yield on a fr. wt basis remained high. Since a moderate degree of stunting did not influence plant maturity, as determined by time to flowering, and since microscopic examination of control and treated plants evidenced no observable difference in leaf oil gland numbers, the effects of the growth retardants on oil yield and composition were ascribed to a direct influence on the enzymes of monoterpene metabolism.

The major effect of Phosfon D on the composition of sage oil was to increase the production of the epimeric ketones (−)-3-isothujone and (+)-3-thujone (Table 1), which arise by alternate stereospecific reductions of the double bond of sabinone [19]. Since none of the intermediates leading to isothujone/thujone were significantly increased (sabinene, *cis*-sabinol and sabinone are present at trace levels to a few percent), the retardant must serve to increase the rate of the slowest step of the reaction sequence, presumably the cyclization of geranyl pyrophosphate to sabinene [19]. Both (−)-β-pinene and (+)-camphor content decrease under the influence of Phosfon D and, since both monoterpenes arise *via* alternate cyclizations of geranyl pyrophosphate, the simplest explanation would be a diminution of the levels or activities of the relevant cyclases [20, 21]. Borneol, the immediate precursor of camphor [20], was only a trace oil constituent under all circumstances. Phosfon D treatment also significantly reduced the level of the sesquiterpene olefins, humulene and caryophyllene, in sage oil (Table 1), and the effect on caryophyllene production was the greatest. Thus, the oil from control plants contained humulene and caryophyllene in a ratio of about 4:1, whereas the ratio increased to 10:1 in the oil of Phosfon D-treated plants suggesting separate origins of these olefins from farnesyl pyrophosphate [22].

Phosfon D served to decrease the level of (−)-menthone and (−)-menthol in peppermint oil, while increasing the level of the epimeric ketone and alcohol, (+)-isomenthone and (+)-neoisomenthol, respectively (Table 2). Since (−)-menthone and (+)-isomenthone arise by alternate stereospecific reductions of the isopropylidene double bond of (+)-pulegone [23, 24], Phosfon D must alter the proportion of the responsible reductases. The level of (+)-pulegone in the oil (~ 2%) was not significantly influenced by Phosfon D treatment, and the precursors of pulegone (limonene, isopiperitenol, isopiperitenone, etc.) [25], remained at trace or very low levels (< 2%) under all circumstances. The differing levels of (−)-menthol and (+)-neoisomenthol reflect the levels of the corresponding ketones from which these alcohols arise via the same dehydrogenase [i.e. reduction of (−)-menthone yields (−)-menthol, whereas (+)-isomenthone yields (+)-neoisomenthol] [26].

Sage was more sensitive to the stunting effect of Cycocel than was peppermint. Oil yield in sage was unaffected by low concentrations of Cycocel, but decreased as stunting increased. Oil composition in sage was influenced by Cycocel in a manner opposite to that of Phosfon D, but in a manner similar to that previously observed with the growth regulators Daminozide and Ethephon [27]. The increase in (−)-β-pinene content and decrease in the level of (−)-isothujone and (+)-camphor can best be explained by alteration in the levels or activities of the relevant cyclases [19–21].

Cycocel slightly stimulated growth of peppermint and increased oil yield at the 250 ppm level. Stunting increased and oil yield decreased with progressively higher concentrations of retardant. Compositional effects were insignificant except under severe stunting where the level of ketones was diminished and the proportion of a number of minor components increased (Table 2).

The results of this study indicate that both sage and peppermint can be moderately stunted with significant increase in oil yield and with little change, or slight improvement in oil quality [28] (e.g. increase in isothujone and decrease in olefins in sage). The results also indicate that growth retardants can influence essential oil formation by a direct effect on monoterpene metabolism.

EXPERIMENTAL

Plant material. Sage (*Salvia officinalis*, L.) was grown from seed and peppermint (*Mentha piperita*, L.) was propagated from single-node cuttings of etiolated rhizomes. The plants were grown in peat moss in a growth chamber with a 14 hr photoperiod (900 ± 100 fc, fluorescent/incandescent), 29° day/25° night temp. cycle and relative humidity of 62 ± 12%. Plants were watered as needed and fed 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 Phosfon D (tributyl[2,4-dichlorobenzyl]phosphonium chloride) or Cycocel (Chlormequat chloride, chlorocholine chloride, [2-chloroethyl]trimethyl ammonium chloride).

Treatments. Each experiment was conducted in randomized complete block design with three replications totalling 60 plants per treatment. Growth retardants 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. Two additional applications were made at weekly intervals, and the plants were harvested one week after the last treatment. 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. Controls sprayed without growth regulator were included in each experiment.

Oil analysis. A minimum of three representative 10 g samples of fresh tissue were steam distilled using a simultaneous steam

distillation–extraction apparatus (J & W Scientific), employing as internal standards (+)-isomenthone for sage and (+)-fenchone for peppermint. On completion of distillation (1 hr) the essential oil collected (in pentane) was dried over anhydrous 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 standards. Yield and relative percent of major oil constituents were also determined. Data were statistically analysed [29], and the LSD (0.05) of the means are reported. Identifications of oil components based on RR_t were confirmed by GC-MS comparison of retention times and mass spectra to authentic standards.

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