JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Essential Oils from Mediterranean Lamiaceae as Weed Germination Inhibitors

LUCIANA G. ANGELINI,*,† GIOVANNA CARPANESE,§ PIER LUIGI CIONI,§ IVANO MORELLI,§ MARIO MACCHIA,† AND GUIDO FLAMINI§

Dipartimento di Agronomia and Gestione dell'Agroecosistema, Università di Pisa, Via S. Michele degli Scalzi 2, 56124 Pisa, Italy, and Dipartimento di Chimica Bioorganica and Biofarmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy

The essential oils obtained from rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), and savory (*Satureja montana* L.) and the four monoterpenes that are their major constituents have been analyzed by GC and GC-MS and tested for their allelopathic properties on the seeds of three different annual weeds (*Chenopodium album*, *Portulaca oleracea*, and *Echinochloa crus-galli*) and three crops (*Raphanus sativus*, *Capsicum annuum*, and *Lactuca sativa*), with the aim to evaluate in vitro their potential as germination inhibitors. The essential oil composition varied with the species, thymol being the main constituent (44%) of thyme and carvacrol (57%) that of savory oil. Differences in essential oil composition were observed within two different rosemary ecotypes, type A, with α-pinene (37%) and 1,8-cineole (23%), and type B, characterized by a 2-fold content of 1,8-cineole (47%). This latest essential oil inhibited completely the germination of weeds while concurrently displaying little effect on pepper. The other two oils showed less selective action. *S. montana* essential oil, with 57% carvacrol, is the most active compound, completely inhibiting germination both of crops and weeds. Borneol, one of the main constituents of the oil of rosemary type B, showed an activity comparable to that of the whole oil. Crop and weed seeds treated with 1,8-cineole showed germination values that were not significantly different from controls, even if a slowing of the germination process expressed in terms of a significant increase in mean germination time was observed. Monoterpene compounds also present in the essential oils mainly represented the volatile fraction released from the crops and their residues into the soil.

**KEYWORDS:** ***Rosmarinus officinalis*; *Thymus vulgaris*; *Satureja montana*; Lamiaceae; essential oil composition; terpenes; allelopathy; weeds; crops; germination inhibitors; *Chenopodium album*; *Portulaca oleracea*; *Echinochloa crus-galli*; *Raphanus sativus*; *Capsicum annuum*; *Lactuca sativa***

## INTRODUCTION

Environmental constraints of crop production systems have stimulated interest in alternative weed management strategies. In fact, the continued use of synthetic herbicides may threaten sustainable agricultural production and has resulted in serious ecological and environmental problems, such as the increased incidence of resistance in weeds to important herbicides and increased environmental pollution and health hazards (*1*, *2*). Allelopathy offers potential for selective biological weed management through the production and release of allelochemicals from the leaves, flowers, seeds, stems, and roots of living or decomposing plant materials (*3*). The potential for using allelopathy in weed management has been well documented (*4*−*7*). Under appropriate conditions, allelochemicals may be released in quantities suppressive to developing weed seedlings (*8*). A variety of allelochemicals have been identified, including essential oils that inhibit seed germination and plant growth (*9*). Among the Lamiaceae family many species release phytotoxic monoterpenes that hinder the development of herbaceous species (*10*−*15*) among which the common ones are α- and β-pinene, camphene, limonene, α-phellandrene, *p*-cymene, 1,8-cineole, borneol, pulegone, and camphor (*16*). Although the hydrosolubility of the monoterpene hydrocarbons was quite low, monoterpene ethers, ketones, and alcohols show an unexpected solubility in water, which can justify their presence in solutions circulating in the soil and therefore their activity in affecting the germination of seedlings and their growth. In this respect Reynolds (*17*) reported phytotoxic effects on the germination of lettuce seeds of the aqueous extracts of open-chain monocyclic and bicyclic monoterpenes. Open-chain alcohols (nerol, geraniol, and linalool), as well as monocyclic ones (terpinen-4-ol, α-terpineol, and borneol), have been shown to be significantly more active than hydrocarbons (myrcene, limonene,

* Author to whom correspondence should be addressed (e-mail angelini@agr.unipi.it; telephone +39 050 599242; fax +39 050 540633).
† Dipartimento di Agronomia and Gestione dell'Agroecosistema.
§ Dipartimento di Chimica Bioorganica and Biofarmacia.

10.1021/jf0210728 CCC: $25.00 
Published on Web 09/09/2003

α-terpinene, phellandrene, *p*-cymene, and α- and β-pinene). Allelopathic inhibition typically results from the combined action of a group of allelochemicals that interfere with several biochemical interactions among plants, including those mediated by soil microorganisms.

The objectives of this study were (1) to evaluate in vitro the allelopathic activity of thyme, rosemary, and savory oils on the germination of some weed and crop species, (2) to determine the chemical basis for the differential allelopathic activities among the three species and two rosemary ecotypes, and (3) to identify and quantify the volatile fraction released from the crops and their residues into the soil.

## MATERIALS AND METHODS

**Plant Material and Growing Conditions.** Cuttings of two *Rosmarinus officinalis* L. ecotypes were collected in two divergent Italian hilly environments: from cultivated plants grown on calcareous sandy soil under a more humid climate in the inner part of Pisa Province (Cevoli of Lari 200 m above sea level) (sample A) or from wild specimens grown on the coastal strip of the protected area of Montemarcello (La Spezia Province) on the Caprione rocky promontory where the Mediterranean scrub grows (sample B). *Satureja montana* L. cuttings were collected from wild plants on the hilly part of Val of Vara (150 m above sea level, La Spezia Province).

S.A.I.S. Seed Co. supplied a single strain of *Thymus vulgaris* L. seeds.

They were planted in small pots filled with sand, peat, and garden soil (20:30:50%) and regularly irrigated. After 2 months, the plants were transplanted to the field in April in double rows with 1.5 m interdouble row and rows 0.7 × 0.5 m apart (for rosemary) or single rows 0.5 × 0.2 m (for savory). The seeds of thyme were sown directly in pots and the plants transplanted from April to May in the field in single rows 0.5 × 0.3 m apart. Trials were carried out at the Experimental Centre of Rottaia of the Department of Agronomy (Pisa, central Italy, 43° 40′ N, 10° 19′ E) on a deep silt loam soil (clay, 17%; silt, 54%; sand, 29%; total nitrogen, 1.08%; organic matter, 2.23%; pH 7.7). Fertilizer was applied at soil preparation, at rates of 50/100/100 kg $ha^{-1}$ of N/P/K. An additional amount of 50 kg $ha^{-1}$ of nitrogen was supplied during springtime. Plots were kept weed-free by hand hoeing.

The upper 20-cm aerial parts were harvested manually at flowering when plants were ∼1 year old and dried in a ventilated chamber to constant weight.

**Essential Oil Analyses.** The essential oils were obtained by hydrodistillation of the dried ground material in a Clevenger-like apparatus for 2 h. The GC analyses were accomplished with an HP-5890 series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60 °C for 10 min, ramp of 5 °C/min to 220 °C; injector and detector temperatures, 250 °C; carrier gas, nitrogen (2 mL/min); detector, dual FID; split ratio, 1:30; injection, 0.5 μL. The identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons.

The relative proportions of the essential oil constituents were percentages obtained by FID peak area normalization, all relative response factors being taken as 1.

GC-EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness, 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature, programmed from 60 to 240 °C at 3 °C/min; carrier gas, helium at 1 mL/min; injection, 0.2 μL (10% hexane solution); split ratio, 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their LRI relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (*18*−*23*). Moreover, the molecular weights of all of the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

**Analyses of the Soil Subtending the Crops.** Samples of soil (∼2 kg each) were taken up to 20 cm under the same number of plants; one reference sample was taken from an area lacking in plants. All of the samples were washed twice with 2 L of water (40 °C), and then the liquids were filtered. The liquids were hydrodistilled in a Clevenger-like device for 2 h, and the volatiles were trapped in 0.5 mL of hexane. The hexane solutions were analyzed by GC and GC-MS as described above for essential oils.

**Weed and Crop Germination Bioassays.** Mature seeds of the annual weeds lambsquarters (*Chenopodium album* L.), little hogweed (*Portulaca oleracea* L.), and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] were collected from parent plants in October in Pisa (43° 41′ N, 10° 26′ E). Weed seeds were dried in a glasshouse and then rubbed gently over a sieve to remove excess chaff, which, together with empty seeds, was removed with a seed blower. Dry seeds were stored at 10 °C and 35% relative humidity until germination testing began. Certified seeds with recommended germination and purity characteristics of radish (*Raphanus sativus* L.), pepper (*Capsicum annuum* L.), and lettuce (*Lactuca sativa* L.) were used kindly provided by S.A.I.S. Seed Co.

A first set of trials was carried out to compare four different essential oils and two kinds of controls, represented by deionized water and a Tween 20 water solution, on seed germination and mean germination time of the six weed and crop species mentioned above. The deionized water control was used to obtain a parameter of maximum seed germination, as in this medium no inhibition was expected. Tween 20 was used for its surface activity to better dissolve oil in water, and a control was made to verify the absence of inhibitory activity of this compound on seed germination. The seeds were treated with an aqueous solution of essential oils (500 mg $L^{-1}$) and Tween 20 (100 mg $L^{-1}$). In the second set of experiments we evaluated the effects of the spreading seeds with the main compounds of the essential oils on the germination process of the same six species. Carvacrol, thymol, borneol, and 1,8-cineole aqueous solutions (250 mg $L^{-1}$) were mixed with Tween 20 solution (100 mg $L^{-1}$) and used to spread seeds. In all cases, five replicates of 50 seeds each were placed in plastic Petri dishes on 9 cm seed test paper moistened with 4.4 mL of essential oil solutions or single pure compound solutions. The same quantity was used for controls with deionized water or Tween 20 solution.

Petri dishes were incubated at 20/30 °C (±1 °C), alternating temperature (16 h/8 h) for *R. sativus*, *C. annuum*, *C. album*, *P. olearacea*, and *E. crus-galli*, or at constant temperature (20 ± 1 °C) for *L. sativa* in the light (24 h) (cool white Osram L18 W/20; 50 μmol of photons $s^{-1}$ $m^{-2}$ photosynthetic active radiation). Dishes were sealed to reduce evaporation, and no more additional water was required during the tests. All tests were conducted following the recommendations of the International Seed Testing Association (*24*). Normal and abnormal seedlings were counted daily for 15 days, following the ISTA rules for seedling evaluation suggested for lettuce, radish, and pepper (*24*−*26*). To evaluate the possible phytotoxic effects of the essential oils and their main compounds on seed properties, germination and mean emergence time (*24*) were recorded. Growth inhibition was measured by radicle elongation and seedling length (on 20 seedlings) measured for the controls and the treatments at the first count, on the first day of germination. Mean germination time (MGT, expressed as days) was calculated using the Ellis and Roberts (*27*) formula. When there is no germination, then the germination time calculation was not considered. Dead and ungerminated fresh seeds were recorded at the end of the experiments.

**Experimental Design and Data Analysis.** A randomized complete block design with five replications was adopted for the germination trials.

Data were submitted to analysis of variance after arcsin transformation (for percentage values), and the means were separated using Duncan's multiple-range test (DMRT) at $P \leq 0.05$ significance level (*28*).

When no germination values were observed in each treatment (germination zero in all five replications with no variability within data),

**Table 1.** Composition and Yield of the Four Essential Oils by GC Analyses

| constituent[a] | LRI[b] | *Thymus vulgaris* | *Satureja montana* | *Rosmarinus officinalis* A | *Rosmarinus officinalis* B |
|---|---|---|---|---|---|
| α-pinene | 941 | 1.2[c] | 0.8 | 37.2 | 13.5 |
| camphene | 955 | 1.2 | 0.2 | 7.2 | 3.9 |
| β-pinene | 981 | | 0.2 | 1.3 | 4.7 |
| myrcene | 992 | 1.9 | 1.9 | 1.3 | 1.3 |
| α-terpinene | 1020 | | 2.0 | 0.6 | 0.6 |
| *p*-cymene | 1028 | 21.0 | 9.7 | 0.9 | 1.0 |
| limonene | 1033 | 0.5 | 0.4 | 3.8 | 2.3 |
| 1,8-cineole | 1035 | 0.6 | 0.3 | 22.6 | 46.8 |
| γ-terpinene | 1064 | 10.5 | 13.2 | 0.7 | 0.7 |
| linalool | 1099 | 2.5 | 0.3 | 2.1 | 0.6 |
| camphor | 1144 | 0.7 | | 7.1 | 4.1 |
| borneol | 1167 | 2.0 | 0.7 | 4.6 | 12.9 |
| 4-terpineol | 1178 | | 0.1 | 0.8 | 1.0 |
| α-terpineol | 1190 | | | 1.6 | 3.6 |
| geraniol | 1256 | 0.7 | | | |
| bornyl acetate | 1286 | 0.9 | | 1.4 | |
| thymol | 1291 | 44.1 | 0.3 | 1.8 | |
| carvacrol | 1299 | 2.6 | 56.8 | | |
| β-caryophyllene | 1420 | 1.8 | 3.6 | 1.8 | 0.7 |
| germacrene D | 1482 | | 0.4 | | |
| yield[d] (% w/w) | | 0.6 ± 0.02 | 0.9 ± 0.03 | 0.9 ± 0.02 | 0.6 ± 0.01 |

[a] Only constituents ≥0.1% are reported. [b] Linear retention indices (HP-5 column). [c] Percentages obtained by FID peak area normalization, all relative response factors being taken as 1 (HP-5 column). [d] Mean value ± standard error.

only data with variability were included in the statistical analysis in order to not alter error omoschedasticity.

## RESULTS AND DISCUSSION

**Chemical Analyses.** The GC and GC-MS analyses of the essential oil of *T. vulgaris* (**Table 1**) permitted the identification of 15 principal constituents, with thymol (44.1%), *p*-cymene (21.0%), and γ-terpinene (10.5%) among the main ones. This is the typical composition of the phenolic chemotype of thyme; the presence of *p*-cymene and γ-terpinene is in agreement with the biosynthetic pathway that leads to thymol.

Carvacrol (56.8%) was the main component of the essential oil of savory, followed by γ-terpinene (13.2%) and *p*-cymene (9.7%) (**Table 1**).

The essential oil of rosemary type A contained mainly α-pinene (37.2%), 1,8-cineole (22.6%), camphor (7.1%), camphene (7.2%), and borneol (4.6%), whereas the oil obtained from type B was characterized by a lesser amount of α-pinene (13.5%), a 2-fold concentration of 1,8-cineole (46.8%), and 3 times as much borneol (12.9%) (**Table 1**). The latter sample, consequently, is in conformity with the chemotype with a high content of 1,8-cineole (typical of Italy, Morocco, and Tunisia), whereas the former resembled the essential oils from Spain and the former Yugoslavia, with a lesser amount of 1,8-cineole and a higher amount of α-pinene (*29*).

**Germination Trials.** Data from the germination trials in both crop and weed seeds treated with essential oils are shown in **Tables 2**−**4**.

The essential oil of rosemary type A was found to be the least active. In fact, in pepper and the two weeds *C. album* and *P. oleracea* this oil induced germination values that were not different as compared to controls. In radish, lettuce, and *E. crus-galli* it caused a significant decrease in germination percentage. Furthermore, in *L. sativa* and *E. crus-galli* this inhibitory activity was accompanied by a slowing of the germination process expressed in terms of an increase in MGT (**Tables 2** and **3**) and by a significant reduction in seedling and radicle length (**Table 4**).

**Table 2.** Germination Tests of *R. sativus*, *C. annuum*, and *L. sativa* with Seeds Treated with Thyme, Savory, and Rosemary Essential Oils in Comparison with Controls[a]

| treatment | germination (%) | abnormal seedlings (%) | dead seeds (%) | ungerminated fresh seeds (%) | MGT (days) |
|---|---|---|---|---|---|
| | | | *Raphanus sativus* | | |
| distilled water | 61.3 **a** (4.8) | 5.3 (1.3) | 28.0 **b** (2.3) | 5.3 **b** (3.5) | 2.22 **c** (0.06) |
| Tween 20 | 62.7 **a** (4.8) | 4.0 (2.3) | 30.7 **b** (3.5) | 2.7 **b** (1.3) | 2.29 **c** (0.14) |
| *T. vulgaris* | 8.0 **c** (2.3) | 14.7 (7.4) | 52.0 **ab** (6.9) | 25.3 **a** (2.7) | 6.78 **a** (0.22) |
| *S. montana* | 0 (0.0) | 17.3 (7.1) | 62.7 **a** (9.3) | 20.0 **a** (2.3) | |
| *R. officinalis* B | 17.3 **bc** (5.8) | 12.0 (4.0) | 37.3 **b** (5.3) | 37.3 **a** (5.3) | 5.29 **b** (0.41) |
| *R. officinalis* A | 20.0 **b** (4.6) | 21.3 (5.81) | 32.0 **b** (7.4) | 26.7 **a** (7.4) | 3.10 **c** (0.23) |
| significance[b] | *** | ns | ** | *** | *** |
| | | | *Capsicum annuum* | | |
| distilled water | 60.0 (2.3) | 12.0 (2.3) | 1.3 (1.3) | 26.6 **d** (1.3) | 14.38 **b** (0.04) |
| Tween 20 | 61.3 (1.3) | 8.0 (2.3) | 4.0 (1.1) | 26.6 **d** (2.7) | 14.97 **ab** (0.23) |
| *T. vulgaris* | 0 (0.0) | 0 (0.0) | 0 (0.0) | 100 **a** (0.01) | |
| *S. montana* | 0 (0.0) | 0 (0.0) | 0 (0.0) | 100 **a** (0.01) | |
| *R. officinalis* B | 53.3 (5.3) | 9.3 (7.4) | 0 (0.0) | 37.3 **c** (5.8) | 16.50 **a** (0.83) |
| *R. officinalis* A | 50.7 (4.8) | 1.3 (1.3) | 0 (0.0) | 48.0 **b** (6.1) | 15.64 **ab** (0.07) |
| significance | ns | ns | ns | ** | * |
| | | | *Lactuca sativa* | | |
| distilled water | 38.7 **a** (3.5) | 13.3 **c** (3.5) | 32.0 **a** (6.1) | 16.0 **d** (6.1) | 4.00 **b** (0.00) |
| Tween 20 | 46.7 **a** (2.7) | 10.7 **c** (1.3) | 38.7 **a** (3.5) | 4.0 **e** (2.3) | 4.14 **b** (0.08) |
| *T. vulgaris* | 0 (0.0) | 28.0 **b** (4.0) | 32.0 **a** (6.9) | 40.0 **b** (8.0) | |
| *S. montana* | 0 (0.0) | 20.0 **bc** (4.6) | 6.7 **b** (3.5) | 73.3 **a** (3.5) | |
| *R. officinalis* B | 0 (0.0) | 46.6 **a** (9.3) | 34.7 **a** (7.4) | 18.7 **cd** (2.7) | |
| *R. officinalis* A | 22.6 **b** (2.7) | 14.6 **bc** (3.5) | 26.7 **a** (4.8) | 36.0 **bc** (2.3) | 5.05 **a** (0.24) |
| significance | ** | ** | ** | *** | ** |

[a] Values are means ± SE (in parentheses) of five replicates of 50 seeds each. Within each species and for each character means followed by a common letter are not significantly different at the 5% level by Duncan's multiple-range test (DMRT); ***, **, *, ns = $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, and not significant, respectively. [b] Statistical analyses were carried out on arcsin-transformed data, and results were extrapolated to original data. In order not to alter error omoschedasticity, only data with variability were included in the statistical analysis.

The allelopathic effect of rosemary type A was mainly manifested through a significant increase in the percentage of fresh ungerminated seeds (i.e., seeds well conformed and vital but unable to germinate) in radish, pepper, lettuce, *E. crus-galli*, and *C. album*. The abnormal seedlings observed had malformations preferentially involving the root system. Such root malformations preclude seedling survival; therefore, abnormal seedlings cannot be considered in the calculation of the number of germinated seeds. The most widespread abnormalities induced by this species consisted of smaller-sized radicles and/or the absence of root capillitum. Radish seeds treated with rosemary type A oil gave a percentage of abnormal seedlings 4-fold higher than controls but with similar length.

Rosemary type B oil showed phytotoxic activity to seeds of the species analyzed greater than that of type A. Thus, it completely inhibited germination in the three weed species and in lettuce. In radish, while not completely blocking germination, it caused a significant decrease in germination percentage and a roughly 28% increase in MGT. On the other hand, pepper germination percentage did not differ as compared to controls, but a significant increase of ungerminated fresh seeds as well as a roughly 13% lengthening of MGT was recorded. This oil caused also a significant reduction in radicle length (**Table 4**).

From the practical application point of view, the activity of this oil was significant and of interest on the weed species in which germination was completely inhibited.

**Table 3.** Germination Tests of *E. crus-galli*, *C. album*, and *P. oleracea* with Seeds Treated with Thyme, Savory, and Rosemary Essential Oils in Comparison with Controls[a]

| treatment | germination (%) | abnormal seedlings (%) | dead seeds (%) | ungerminated fresh seeds (%) | MGT (days) |
|---|---|---|---|---|---|
| | | *Echinocloa crus-galli* | | | |
| distilled water | 46.7 **a** (3.5) | 8.0 **bc** (4.0) | 0 (0.0) | 45.3 **d** (4.8) | 4.24 **b** (0.08) |
| Tween 20 | 48.0 **a** (6.1) | 12.0 **bc** (2.3) | 1.3 (1.1) | 38.7 **d** (4.8) | 4.50 **b** (0.26) |
| *T. vulgaris* | 13.3 **c** (5.3) | 36.0 **ab** (2.3) | 0 (0.0) | 50.7 **cd** (5.8) | 9.33 **a** (1.30) |
| *S. montana* | 0 (0.0) | 8.0 **bc** (4.6) | 0 (0.0) | 92.0 **a** (4.6) | |
| *R. officinalis* B | 0 (0.0) | 20.0 a**b** (4.6) | 0 (0.0) | 80.0 **b** (4.6) | |
| *R. officinalis* A | 26.7 **b** (1.3) | 5.33 **c** (2.7) | 0 (0.0) | 68.0 **bc** (2.3) | 5.94 **a** (0.83) |
| significance[b] | ** | *** | | *** | ** |
| | | *Chenopodium album* | | | |
| distilled water | 78.7 **a** (3.5) | 0 (0.0) | 6.7 (3.5) | 14.7 **d** (3.5) | 9.33 **b** (0.36) |
| Tween 20 | 84.0 **a** (2.3) | 0 (0.0) | 2.7 (1.3) | 13.3 **d** (3.5) | 9.37 **b** (0.27) |
| *T. vulgaris* | 21.3 **b** (21.3) | 29.3 **b** (10.9) | 0 (0.0) | 49.3 **b** (10.9) | 11.44 **a** (3.81) |
| *S. montana* | 0 (0.0) | 0 (0.0) | 0 (0.0) | 100 **a** (0.2) | |
| *R. officinalis* B | 0 (0.0) | 72.0 **a** (4.6) | 1.3 (1.3) | 26.7 **cd** (3.5) | |
| *R. officinalis* A | 64.0 **a** (4.6) | 0 (0.0) | 0 (0.0) | 36.0 **bc** (4.6) | 10.52 **ab** (0.62) |
| significance | * | * | ns | *** | * |
| | | *Portulaca oleracea* | | | |
| distilled water | 70.7 (8.1) | 2.7 **b** (2.7) | 0 (0.0) | 26.7 **c** (5.8) | 3.67 (0.05) |
| Tween 20 | 74.7 (5.3) | 1.3 **b** (1.3) | 0 (0.0) | 24.0 **c** (4.0) | 3.38 (0.06) |
| *T. vulgaris* | 0 (0.0) | 2.6 **b** (1.3) | 6.7 (3.5) | 90.7 **b** (2.7) | |
| *S. montana* | 0 (0.0) | 0 (0.0) | 0 (0.0) | 100 **a** (0.01) | |
| *R. officinalis* B | 0 (0.0) | 73.3 **a** (1.3) | 0 (0.0) | 26.7 **c** (1.3) | |
| *R. officinalis* A | 84.0 (10.5) | 0 (0.0) | 0 (0.0) | 16.0 **c** (10.6) | 4.36 (0.37) |
| significance | ns | *** | | *** | ns |

[a] Values are means ± SE (in parentheses) of five replicates of 50 seeds each. Within each species and for each character means followed by a common letter are not significantly different at the 5% level by Duncan's multiple-range test (DMRT); ***, **, *, ns = $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, and not significant, respectively. [b] Statistical analyses were carried out on arcsin transformed data, and results were extrapolated to original data. In order to not alter error omoschedasticity, only data with variability were included in the statistical analysis.

**Table 4.** Radicle Elongation and Seedling Length of Crop (Left) and Weed (Right) Species after Treatment with Thyme, Savory, and Rosemary Essential Oils in Comparison with Controls[a]

| treatment | radicle length (mm) | seedling length (mm) | treatment | radicle length (mm) | seedling length (mm) |
|---|---|---|---|---|---|
| *Raphanus sativus* | | | *Echinocloa crus-galli* | | |
| distilled water | 7.6 | 14.7 | distilled water | 22.1 **a** | 33.4 **a** |
| Tween 20 | 7.9 | 14.0 | Tween 20 | 25.7 **a** | 36.1 **a** |
| *T. vulgaris* | 9.5 | 18.7 | *T. vulgaris* | 9.0 **b** | 17.9 **b** |
| *S. montana* | | | *S. montana* | | |
| *R. officinalis* B | 7.3 | 14.8 | *R. officinalis* B | | |
| *R. officinalis* A | 8.2 | 14.4 | *R. officinalis* A | 9.8 **b** | 18.4 **b** |
| significance | ns | ns | significance | ** | ** |
| *Capsicum annuum* | | | *Chenopodium album* | | |
| distilled water | 17.2 **a** | 25.1 **a** | distilled water | 14.3 | 28.8 |
| Tween 20 | 16.7 **a** | 24.1 **ab** | Tween 20 | 14.0 | 24.9 |
| *T. vulgaris* | | | *T. vulgaris* | 14.0 | 28.7 |
| *S. montana* | | | *S. montana* | | |
| *R. officinalis* B | 10.1 **b** | 16.6 **b** | *R. officinalis* B | | |
| *R. officinalis* A | 15.0 **a** | 22.0 **ab** | *R. officinalis* A | 8.4 | 17.4 |
| significance | * | * | significance | ns | ns |
| *Lactuca sativa* | | | *Portulaca oleracea* | | |
| distilled water | 16.3**a** | 26.5**a** | distilled water | 5.1 | 10.3 **a** |
| Tween 20 | 18.7 **a** | 28.5 **a** | Tween 20 | 5.2 | 9.1 **b** |
| *T. vulgaris* | | | *T. vulgaris* | | |
| *S. montana* | | | *S. montana* | | |
| *R. officinalis* B | | | *R. officinalis* B | | |
| *R. officinalis* A | 10.8 **b** | 17.9 **b** | *R. officinalis* A | 4.3 | 7.3 **c** |
| significance | ** | ** | significance | ns | ** |

[a] Measurements done on the first day in which germination occurred. Values are means of five replicates of 25 seedlings each. Means followed by the same letter within each column are not different at the 5% level of probability (Duncan's multiple-range test, DMRT); **, *, ns = $P \leq 0.01$, $P \leq 0.05$, and not significant, respectively.

The oil of thyme completely inhibited germination in lettuce and pepper as well as in *P. oleracea*. In radish, the germination percentage of treated seeds was very low and MGT was 3 times as high as in controls. Germination percentage was likewise drastically reduced in *E. crus-galli* and *C. album*, with an increase in abnormal seedlings and a marked rise in MGT. In *E. crus-galli* a significant decrease in radicle and seedling lengths was also observed. These results suggest that the allelopathic activity of thyme oil is elevated but devoid of selectivity in relation to weeds.

The essential oil of savory was active on all species, completely blocking germination of both crop and weed species. On all seeds tested, a significant increase of ungerminated fresh seed compared to controls was observed. In pepper, *C. album*, and *P. oleracea* this essence led to 100% of ungerminated fresh seeds.

Because in some cases the above tested essences inhibited weed seed germination without damaging some crops, we conducted tests with some of their main components to determine which component might be responsible, in total or in part, for the activity of essential oils. The components assayed were thymol for thyme (44.1%) and carvacrol for savory (56.8%). In addition, for rosemary, borneol and 1,8-cineole were chosen to test the hypothesis that these components might be responsible for its activity. The ingredient α-pinene was not used for these tests, as it was found to be present in 3-fold greater quantities in the essence of rosemary type A—which proved to have poor activity—as compared to type B. Because the various compounds used for these tests rarely accounted for >50% of the total composition of the oils, the compounds were used at half the concentration utilized for oils (250 ppm).

Data obtained in this series of tests were also subjected to analysis of variance, and the results are shown in **Tables 5**−**8**.

Carvacrol completely inhibited germination in all species except radish, showing a behavior similar to that of savory essence. This component led to a significant increase of ungerminated fresh seeds in all weed species (100% in *P. oleracea*, 98.7% in *E. crus-galli*, and 72% in *C. album*) and in pepper (100%). In *C. album* a significant increase in dead seed percentage was also observed.

Thymol showed a significant inhibiting activity on *E. crus-galli* (∼90% of reduction in comparison with controls); furthermore, the few seeds that did germinate presented poor seedling development with values lower than controls (**Table 7**). In other species, with the exception of radish, germination was completely impaired, as shown by an increase in the number of malformed seedlings (in lettuce and in all weed species). Like carvacrol, thymol was totally ineffective on radish germination, causing only a slight reduction in radicle and hypocotyl growth, which did not compromise seedling survival. Nevertheless, a slowing of the germination process expressed in terms of an increase in MGT (**Table 5**) was observed.

With regard to 1,8-cineole, only in the case of *C. album* did this compound inhibit seed germination at a significant level in

**Table 5.** Germination Tests of *R. sativus*, *C. annum*, and *L. sativa* with Seeds Treated with Monoterpene Pure Compounds in Comparison with Controls[a]

| treatment | germination (%) | abnormal seedlings (%) | dead seeds (%) | ungerminated fresh seeds (%) | MGT (days) |
|---|---|---|---|---|---|
| | | | *Raphanus sativus* | | |
| distilled water | 61.3 (4.8) | 5.3 (1.3) | 28.0 (2.3) | 5.3 (3.5) | 2.22 **d** (0.06) |
| Tween 20 | 62.7 (4.8) | 4.0 (2.3) | 30.7 (3.5) | 2.7 (1.3) | 2.29 **d** (0.14) |
| thymol | 66.7 (4.8) | 12.0 (4.0) | 8.0 (2.3) | 13.3 (7.0) | 6.18 **ab** (0.31) |
| carvacrol | 37.3 (10.9) | 21.3 (5.8) | 33.3 (13.5) | 8.0 (2.3) | 3.40 **c** (0.16) |
| borneol | 66.7 (15.4) | 10.7 (5.3) | 9.3 (3.5) | 13.3 (9.6) | 6.96 **a** (0.46) |
| 1,8-cineole | 60.0 (6.1) | 16.0 (4.6) | 12.0 (8.3) | 12.0 (2.3) | 5.44 **b** (0.31) |
| significance[b] | ns | ns | ns | ns | ** |
| | | | *Capsicum annuum* | | |
| distilled water | 60.0 **a** (2.3) | 12.0 (2.3) | 1.3 (1.3) | 26.6 **c** (1.3) | 14.38 **c** (0.04) |
| Tween 20 | 61.3 **a** (1.3) | 8.0 (2.3) | 4.0 (0.0) | 26.6 **c** (2.7) | 14.97 **c** (0.23) |
| thymol | 0 (0.0) | 0 (0.0) | 0 (0.0) | 100 **a** (0.1) | |
| carvacrol | 0 (0.0) | 0 (0.0) | 0 (0.0) | 100 **a** (0.1) | |
| borneol | 38.7 **b** (5.3) | 8.0 (2.3) | 0 (0.0) | 53.3 **b** (5.8) | 19.05 **a** (0.19) |
| 1,8-cineole | 53.3 **a** (5.3) | 8.0 (4.0) | 2.7 (2.7) | 36.0 **c** (6.9) | 15.96 **b** (0.33) |
| significance | * | ns | ns | *** | ** |
| | | | *Lactuca sativa* | | |
| distilled water | 38.7 (3.5) | 13.3 **b** (3.5) | 32.0 (6.1) | 16.0 (6.1) | 4.00 **b** (0.00) |
| Tween 20 | 46.7 (2.7) | 10.7 **b** (1.3) | 38.7 (3.5) | 4.0 (2.3) | 4.14 **b** (0.08) |
| thymol | 0 (0.0) | 41.3 **a** (2.7) | 46.7 (5.8) | 12.0 (8.3) | |
| carvacrol | 0 (0.0) | 37.3 **a** (3.5) | 45.3 (5.8) | 17.3 (5.3) | |
| borneol | 0 (0.0) | 42.7 **a** (10.7) | 37.3 (3.5) | 20.0 (8.3) | |
| 1,8-cineole | 49.3 (6.7) | 13.3 **b** (5.81) | 28.0 (4.0) | 9.3 (3.5) | 4.74 **a** (0.05) |
| significance | ns | ** | ns | ns | ** |

[a] Values are means ± SE (in parentheses) of five replicates of 50 seeds each. Within each species and for each character means followed by a common letter are not significantly different at the 5% level by Duncan's multiple-range test (DMRT); ***, **, *, ns = $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, and not significant, respectively. [b] Statistical analyses were carried out on arcsin transformed data, and results were extrapolated to original data. In order to not alter error omoschedasticity, only data with variability were included in the statistical analysis.

**Table 6.** Germination Tests with Seeds of *E. crus-galli*, *C. album*, and *P. oleracea* Treated with Monoterpene Pure Compounds in Comparison with Controls[a]

| treatment | germination (%) | abnormal seedlings (%) | dead seeds (%) | ungerminated fresh seeds (%) | MGT (days) |
|---|---|---|---|---|---|
| | | | *Echinochloa crus-galli* | | |
| distilled water | 46.7 **a** (3.5) | 8.0 **bc** (4.0) | 0 (0.0) | 45.3 **bc** (4.8) | 4.24 **c** (0.08) |
| Tween 20 | 48.0 **a** (6.1) | 12.0 **b** (2.3) | 1.3 (1.3) | 38.7 **cd** (4.8) | 4.50 **c** (0.26) |
| Thymol | 5.3 **b** (2.7) | 32.0 **a** (2.3) | 0 (0.0) | 62.7 **b** (3.5) | 10.95 **a** (5.48) |
| carvacrol | 0 (0.0) | 1.3 **c** (1.3) | 0 (0.0) | 98.7 **a** (1.3) | |
| borneol | 0 (0.0) | 1.3 **c** (1.3) | 0 (0.0) | 98.7 **a** (1.3) | |
| 1,8-cineole | 64.0 **a** (8.0) | 8.0 **bc** (2.3) | 0 (0.0) | 28 **d** (6.1) | 6.15 **b** (5.00) |
| significance[b] | ** | *** | ns | *** | ** |
| | | | *Chenopodium album* | | |
| distilled water | 78.7 **ab** (3.5) | 0 (0.0) | 6.7 **c** (3.5) | 14.7 **d** (3.5) | 9.33 **b** (0.36) |
| Tween 20 | 84.0 **a** (2.3) | 0 (0.0) | 2.7 **c** (1.3) | 13.3 **d** (3.5) | 9.37 **b** (0.27) |
| Thymol | 0 (0.0) | 18.7 **a** (3.5) | 29.3 **b** (3.5) | 52.0 **b** (0.0) | |
| carvacrol | 0 (0.0) | 0 (0.0) | 28.0 **b** (4.0) | 72.0 **a** (4.0) | |
| borneol | 0 (0.0) | 8.0 **b** (2.3) | 62.7 **a** (2.7) | 29.3 **c** (3.5) | |
| 1,8-cineole | 70.7 **b** (2.7) | 0 (0.0) | 1.3 **c** (1.3) | 28.0 **c** (2.3) | 9.96 **a** (0.36) |
| significance | * | * | *** | *** | * |
| | | | *Portulaca oleracea* | | |
| distilled water | 70.7 (8.1) | 2.7 **c** (2.7) | 0.0 (0.0) | 26.7 **c** (5.8) | 3.67 **b** (0.05) |
| Tween 20 | 74.7 (5.3) | 1.3 **c** (1.3) | 0.0 (0.0) | 24.0 **c** (4.0) | 3.38 **b** (0.06) |
| thymol | 0 (0.0) | 21.3 **b** (3.5) | 0.0 (0.0) | 78.7 **b** (3.5) | |
| carvacrol | 0 (0.0) | 0 (0.0) | 0.0 (0.0) | 100 **a** (0.01) | |
| borneol | 0 (0.0) | 77.3 **a** (2.7) | 0.0 (0.0) | 29.3 **c** (2.7) | |
| 1,8-cineole | 70.7 (8.1) | 1.33 **c** (1.3) | 0.0 (0.0) | 28.0 **c** (6.9) | 5.96 **a** (0.44) |
| significance | ns | *** | | *** | ** |

[a] Values are means ± SE (in parentheses) of five replicates of 50 seeds each. Within each species and for each character means followed by a common letter are not significantly different at the 5% level by Duncan's multiple-range test (DMRT); ***, **, *, ns = $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, and not significant respectively. [b] Statistical analyses were carried out on arcsin transformed data, and results were extrapolated to original data. In order to not alter error omoschedasticity, only data with variability were included in the statistical analysis.

comparison with Tween 20 control. In all species studied, 1,8-cineole induced a significant increase in MGT and a decrease in seedling length (**Table 7**). Therefore, while compromising seedling growth, 1,8-cineole cannot be the sole ingredient responsible for the allelopathic activity shown to be exerted by rosemary type B.

Borneol completely impaired germination in all weed species tested, whereas among cultivated species it negatively affected lettuce and pepper germination. Furthermore, even when germination occurred, all resulting seedlings proved to be abnormal and therefore cannot be considered in the calculation of germination percentages. In pepper, borneol induced a decrease in germination percentage (≈30%) as compared to controls and an increase in MGT values. In radish, this compound was devoid of activity and only a significant decrease in germination energy was observed. However, the allelopathic effect was also manifested in these two cultivated species by a decrease in root and hypocotyl elongation as compared to controls (**Table 7**).

Comparison between the activity of essential oils and that of their pure constituents (**Table 8**) suggests that both the essential oil of rosemary type B and its constituent borneol have inhibiting activity on all weed species tested in the present trials. With regard to crop species, the essential oil of rosemary type B negatively affected radish and lettuce germination. Among pure ingredients of this essence, only borneol had a significant adverse effect on pepper and lettuce germination. The oil of savory exerted total damage on both cultivated and weed species and therefore acted nonselectively. Carvacrol proved to be more selective, inhibiting germination in all species with the exception of radish.

The oil of thyme inhibited germination in all species, although inhibition was not complete on radish, *E. crus-galli*, and *C. album*. Thymol, like its isomer carvacrol, was found to be more selective as it did not inhibit radish germination.

Lorber and Muller (*30*) investigated the mechanism of action of terpene compounds in previous studies. By means of microscopic analysis of *Cucumis sativus* tips treated with volatile substances of *Salvia leucophylla*, these authors observed severe cell damage affecting organelle membranes, in particular mitochondrial membranes. Results from another study of the same authors on *Allium cepa* L. root tips (*31*) suggested that the same constituents responsible for blocking mitosis were also the cause of reduced root tip development, due to interference with glycidic metabolism following mitochondrial damage.

Because experimental data show that essential oils of rosemary, thyme, and savory are endowed with significant inhibiting activity on in vitro seed germination, we conducted a further series of trials to determine whether root secretions or residues of above-ground vegetation of these species could lead to the presence of terpene compounds in soil subtending the crops, where such compounds might then exert allelopathic

**Table 7.** Radicle Elongation and Seedling Length of Crop (Left) and Weed (Right) Species after Treatment with Monoterpene Pure Compounds in Comparison with Controls[a]

| treatment | radicle length (mm) | seedling length (mm) | | radicle length (mm) | seedling length (mm) |
|---|---|---|---|---|---|
| *Raphanus sativus* | | | *Echinocloa crus-galli* | | |
| distilled water | 7.6 **ab** | 14.7 **a** | distilled water | 22.1 **b** | 33.4 **a** |
| Tween 20 | 7.9 **a** | 14.0 **a** | Tween 20 | 25.7 **a** | 36.1 **a** |
| thymol | 6.7 **ac** | 12.1 **b** | thymol | 9.0 **d** | 22.0 **b** |
| carvacrol | 7.9 **a** | 15.0 **a** | carvacrol | | |
| borneol | 5.7 **c** | 11.0 **b** | borneol | | |
| 1,8-cineole | 6.5 **b** | 12.1 **b** | 1,8-cineole | 16.3 **c** | 22.9 **b** |
| significance | * | ** | significance | ** | ** |
| *Capsicum annuum* | | | *Chenopodium album* | | |
| distilled water | 17.2 **a** | 25.1 **a** | distilled water | 14.3 | 28.8 **a** |
| Tween 20 | 16.7 **a** | 24.1 **ab** | Tween 20 | 14.0 | 24.9 **a** |
| thymol | | | thymol | | |
| carvacrol | | | carvacrol | | |
| borneol | 10.0 **b** | 17.0 **c** | borneol | | |
| 1,8-cineole | 10.3 **b** | 19.6 **b** | 1,8-cineole | 11.3 | 19.0 **b** |
| significance | * | * | significance | ns | ** |
| *Lactuca sativa* | | | *Portulaca oleracea* | | |
| distilled water | 16.3 | 26.5 **a** | distilled water | 5.1 **a** | 10.3 **a** |
| Tween 20 | 18.7 | 28.5 **a** | Tween 20 | 5.2 **a** | 9.1 **b** |
| thymol | | | thymol | | |
| carvacrol | | | carvacrol | | |
| borneol | | | borneol | | |
| 1,8-cineole | 15.9 | 21.7 **b** | 1,8-cineole | 3.4 **b** | 5.9 **b** |
| significance | ns | * | significance | ** | ** |

[a] Measurements done on the first day in which germination occurred. Values are means of five replicates of 25 seedlings each. Means followed by the same letter within each column are not different at the 5% level of probability (Duncan's multiple-range test, DMRT); **, *, ns = $P \leq 0.01$, $P \leq 0.05$, and not significant, respectively.

**Table 8.** Summary of the Allelopathic Properties of the Essential Oils and Their Main Constituents on the Germination Process[a]

| | *Rosmarinus officinalis* | | | | *Satureja montana* | | *Thymus vulgaris* | |
|---|---|---|---|---|---|---|---|---|
| | essential oil | | | | essential | | essential | |
| species | A | B | 1,8-cineole | borneol | oil | carvacrol | oil | thymol |
| *Raphanus sativus* | < | < | ns | ns | 0 | ns | < | ns |
| *Capsicum annuum* | ns | ns | ns | < | 0 | 0 | 0 | 0 |
| *Lactuca sativa* | < | 0 | ns | 0 | 0 | 0 | 0 | 0 |
| *Echinochloa crus-galli* | < | 0 | ns | 0 | 0 | 0 | < | < |
| *Chenopodium album* | ns | 0 | <ns | 0 | 0 | 0 | < | 0 |
| *Portulaca oleracea* | ns | 0 | ns | 0 | 0 | 0 | 0 | 0 |

[a] ns = germination percentage mean values not statistically different from controls. < = germination percentage mean values statistically lower than controls. 0 = no germination percentage.

activity. Analyses confirmed this hypothesis: although the control soil sample contained no volatile substances, samples taken from around the above-described plants were found to contain mono- and sesquiterpene compounds (**Table 9**).

Analysis of the volatile fractions showed that some monoterpenes found in soil were also present in the essential oils, whereas others did not appear in the respective aromatic species (**Table 1**). Such differences may be explained by a concomitance of factors such as different volatilities of the individual compounds, soil adsorbent power, root secretion, microbial decomposition, chemical transformation, and leaching.

**Table 9.** Volatile Constituents Extracted from the Soil under Each Cultivated Species Analyzed by GC

| constituent[a] | LRI[b] | *Thymus vulgaris* | *Satureja montana* | *Rosmarinus officinalis* A | *Rosmarinus officinalis* B |
|---|---|---|---|---|---|
| α-pinene | 941 | 19.3 | 6.1 | 3.2 | 32.0 |
| camphene | 955 | | | | 0.6 |
| β-pinene | 981 | | | | 1.5 |
| myrcene | 992 | 6.1 | 2.9 | 1.5 | 12.9 |
| sabinene | 978 | | | | 1.5 |
| 3-carene | 1012 | | | | 0.8 |
| *p*-cymene | 1028 | 1.8 | 1.0 | | 3.7 |
| limonene | 1033 | 3.6 | 2.0 | 0.8 | 8.9 |
| borneol | 1167 | 7.0 | | | |
| 4-terpineol | 1178 | 3.2 | | | |
| α-terpineol | 1190 | 10.6 | 3.3 | 6.9 | |
| verbenone | 1205 | 3.0 | | | |
| thymol | 1291 | 1.0 | | | |
| carvacrol | 1299 | 1.4 | | | 0.4 |
| α-cedrene | 1411 | | 0.5 | | |
| β-caryophyllene | 1420 | | 29.7 | 34.6 | 14.4 |
| α-humulene | 1455 | | 0.6 | | |
| (*E*)-β-farnesene | 1459 | | 6.4 | 6.9 | 2.4 |
| germacrene D | 1482 | | | 9.2 | |
| (*E*)-β-ionone | 1485 | | | 0.4 | |
| δ-cadinene | 1524 | | 9.2 | 1.7 | |
| cedrol | 1597 | | 4.7 | | |

[a] Only constituents ≥0.1% are reported. [b] Linear retention indices (HP-5 column). [c] Percentages obtained by FID peak area normalization, all relative response factors being taken as 1 (HP-5 column).

Release of terpene compounds into soil probably represents an attempt by the plant to create around itself an environment that is unfavorable to the development of other species. Consequently, this would ensure the plant more advantageous conditions in the struggle for survival.

A preliminary in vitro test, using the rinsing water obtained by washing soil samples taken from beneath the plants in question, provided support for this hypothesis. Thus, although the rinsing water was highly diluted, it induced a slowing in MGT of lettuce seeds in all four treatments.

In addition, because β-caryophyllene was present in elevated concentrations in rinsing water from soil samples beneath rosemary type B and savory, we also performed a germination inhibition test on lettuce seeds (belonging to the same lot of seed used before) using this compound. Results showed complete inhibition of germination, in agreement with findings obtained with rinsing water (data not shown).

Future experiments, involving both essential oils and each of the terpene compounds found to give interesting results in vitro, could focus on the possible effects of the length of time during which such compounds are present in soil, possible structural modifications with consequent loss or acquisition of activity, and allelopathic action on weed seeds in field conditions. In addition, if residues of the aromatic species investigated in the present study return to the soil as crop residues, this would not only provide organic matter soil enrichment but also allow assessment of their degree of germination inhibition on weeds that are damaging to crops. Finally, a system of crop rotation could be devised that would include aromatic species in the long-term succession of crops, alternating aromatic species with crops than are not affected by volatile terpenes.

## ACKNOWLEDGMENT

We thank Lucia Ceccarini, Elisabetta Campeol, and Sabrina Tozzi for technical assistance.

## LITERATURE CITED


(1) Narwal, S. S. Allelopathy in weed management. In *Allelopathy Update Vol. 2: Basic and Applied Aspects*; Narwal, S. S., Ed.; Science Publisher: Enfield, NH, 1999; pp 203−254.

(2) Heap, I. The International Survey of Herbicide Resistant Weeds; June 25, 2001; available at www.weedscience.com.

(3) Weston, L. A. Utilization of allelopathy for weed management in agroecosystems. *Agron. J.* **1996**, *88*, 360−366.

(4) Putnam, A. R. Allelochemicals from plants as herbicides. *Weed Technol.* **1988**, *2*, 510−518.

(5) Nelson, C. J. Allelopathy in cropping systems. *Agron. J.* **1996**, *88*, 991−996.

(6) Wu, H.; Pratley, J.; Lemerle, D.; Haig, T. Crop cultivars with allelopathic capability. *Weed Res.* **1999**, *39*, 171−180.

(7) An, M.; Pratley, J. E.; Haig, T. Allelopathy: from concept to reality. In *Proceedings of the 9th Australian Agronomy Conference*; Michalk, D. L., Pratley, J. E., Eds.; Australian Agronomy Society: Wagga Wagga, Australia, 1998; pp 563−566.

(8) Wu, H.; Haig, T.; Pratley, J.; Lemerle, D.; An, M. Biochemical basis for wheat seedling allelopathy on the suppression of annual ryegrass (*Lolium rigidum*). *J. Agric. Food Chem.* **2002**, *50*, 4567−4571.

(9) Neori, A.; Reddy, K. R.; Císková-Koncalová, H.; Agami, M Bioactive chemicals and biological-biochemical activities and their functions in rhizospheres of wetland plants. *Bot. Rev.* **2000**, *66*, 350−378.

(10) Muller, C. H. The role of chemical inhibitors (allelopathy) in vegetational composition. *Bull. Torrey Bot. Club* **1966**, *93*, 332−351.

(11) Elakovich, S. D.; Stevens, K. L. Volatile constituents of *Lippia nodiflora*. *J. Nat. Prod.* **1985**, *48*, 504−506.

(12) Katz, D. A.; Sneh, B.; Friedman J. The allelopathic potential of *Coridothymus capitatus* L. (Labiatae): Preliminary studies on the roles of the shrub in the inhibition of annuals germination and/or to or to promote allelopathically active actinomycetes. *Plant Soil* **1987**, *98*, 53−66.

(13) Gonzalez de la Parra, M.; Anaya, A. L.; Espinosa, F.; Jimenez, F.; Castillo, R. Allelopathic potential of *Piqueria trinervia* (Compositae) and piquerols A and B. *J. Chem. Ecol.* **1981**, *7*, 509−515.

(14) Nishimura, H.; Kaku, K.; Mizutani, J. Allelopathic substances, (±)-*p*-methane-3,8-diols isolated from *Eucalyptus citriodora* Hook. *Agric. Biol. Chem.* **1982**, *46*, 319−320.

(15) Alsaadawi, I. S.; Arif, M. B.; AlRubeaa, A. J. Allelopathic effects of *Citrus aurantium* L. II. Isolation, characterization, and biological activities of phytotoxins. *J. Chem. Ecol.* **1985**, *11*, 1527−1534.

(16) Fisher, N. H. The function of mono and sesquiterpenes as plant germination and growth regulators. In *The Science of Allelopathy*; Putnam, A. R., Tang, C. S., Eds.; Wiley: New York, 1986; pp 203−218.

(17) Reynolds, T. Comparative effects of alicyclic compounds and quinones on inhibition of lettuce fruit germination. *Ann. Bot.* **1987**, *60*, 215−223.

(18) Massada, Y. *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*; Wiley: New York, 1976.

(19) Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Chromatography*; Academic Press: New York, 1980.

(20) Davies, N. W. Gas chromatographic retention indexes of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr.* **1990**, *503*, 1−24.

(21) Swigar, A. A.; Silverstein, R. M. *Monoterpenes*; Aldrich Chemical Co.: Milwaukee, WI, 1981.

(22) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography Mass Spectroscopy*; Allured Publishing: Carol Stream, IL, 1995.

(23) Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids*; Chapman and Hall: London, U.K., 1991; Vol. 2, pp 1447.

(24) International Seed Testing Association. International Rules for Seed Testing 1996. *Seed Sci. Technol.* **1996**, *24* (Suppl.).

(25) International Seed Testing Association. International Rules for Seed Testing 1985. *Seed Sci. Technol.* **1985**, *13*, 299−355.

(26) International Seed Testing Association,. International Rules for Seed Testing. Annexes 1985. *Seed Sci. Technol.* **1985**, *13*, 356−513.

(27) Ellis, R. H.; Roberts, E. H. The influence of temperature and moisture on the seed viability period in barley (*Hordeum disticum* L.). *Ann. Bot.* **1980**, *45*, 31−37.

(28) SAS Institute. *SAS User's Guide: Statistics*; Statistical Analytical System Institute: Cary, NC, 1991.

(29) Flamini, G.; Cioni, P. L.; Morelli, I.; Macchia, M.; Ceccarini, L. Main agronomic-productive characteristics of two ecotypes of *Rosmarinus officinalis* L. and chemical composition of their essential oils. *J. Agric. Food Chem.* **2002**, *50*, 3512−3517.

(30) Lorber, P.; Muller, W. H. Volatile growth inhibitors produced by *Salvia leucophylla*: effects on seedling root tip ultrastructure. *Am. J. Bot.* **1976**, *63*, 196−200.

(31) Lorber, P.; Muller, W. H. Volatile growth inhibitors produced by *Salvia leucophylla*: effects on cytological activity in *Allium cepa*. *Comp. Physiol. Ecol.* **1980**, *5*, 60−67.


**Received for review October 23, 2002. Revised manuscript received August 1, 2003. Accepted August 1, 2003. We are thankful to CNR ABSOV Project for having partially supported this project.**

JF0210728