Oregano and its Potential Use as Bioherbicide

G. De Mastro, M. Fracchiolla, L. Verdini and P. Montemurro
Dipartimento di Scienze delle Produzioni Vegetali, University of Bari, Italy.

Keywords: Biological control, sustainable agriculture, weeds.

Abstract

Widespread use of synthetic herbicides in weed control could result in negative impact on human health and on the environment. Natural compounds could be successfully used as bioherbicides because they are potentially more environmental friendly and safe. Plants are an important source of active compounds. In particular, many species belonging to the Labiate family produce essential oils containing compounds that could act as natural herbicides. In this paper we report on preliminary studies about the effects on seed germination and plant growth of an oregano hybrid (*Origanum vulgare* L. ssp. *virilidum* x O. *vulgare* L. ssp. *hirtum* (Link) Iestwart). Experiments were done both in pots and in the field. Increasing amounts of chopped leaves and stems of oregano were added to the soil. In the pots, several weed species were sown, whereas naturally occurring infestation was evaluated in the field. In comparison with the control, a reduced number of weeds was observed where oregano biomass was added. The above-mentioned trend was more visible in the pots than in the field. The results suggest that the hybrid of oregano used in the trial could be an interesting source of natural compounds effective against weeds. Hence, further studies with this plant are likely to be successful.

INTRODUCTION

The Rationale to Investigate the Potentials of Natural Compounds in Weed Control

The concept of “sustainable agriculture” involves many themes, such as environment protection, food quality and safety, economic profitability. Pest management is one of the most important topics to be considered in order to attain these goals. For weed control, effective strategies, both in crops and in gardens or in non-arable lands, require the use of synthetic herbicides whose potential risk for human health and environment is generally regarded as a problem. For this reason, public concern has induced most developed countries (including Europe) to promote the re-registering of older pesticides according to more stringent guidelines (Dayan et al., 1999). At the same time, chemical companies find difficulty and low profitabily making new active ingredients available in the market due to high discover and registration costs. This overall trend is causing in many crops, especially minor use crops, the reduction of chemical herbicides able to solve weed problems; hence, new solutions need to be found (Charudattan, 2001). Another problem related to the chemical weeding is that many weeds have become resistant to several herbicides: 304 resistant biotypes, 182 species (109 dicots and 73 monocots) in over 270,000 fields throughout the world (source: weedscience.com).

The confluence of these several factors is leading the interest of researchers and technicians toward alternative strategies. In this context, the study of natural products with herbicidal potentials is one of the most promising research field so that the future of weed management will probably be significantly affected by this matter (Dayan et al., 1999). There are several advantages deriving from the discovery of new phytotoxic natural products. Most of their valuable properties are related to chemical features. For example, naturally occurring products rarely contain heavy atoms, such as halogens (Cl, F, Br) and have few sulphur and phosphate groups but are often rich in oxygen and nitrogen. Moreover, they normally are more structurally complex than synthetic herbicides (Henkel et al., 1999). One of the most important benefits of the above

Proc. Ist IC on Labiatae
Eds.: B. Ruffoni et al.
Acta Hort. 723, ISHS 2006

mentioned chemical composition is that these compounds are rapidly degraded in the environment; hence, they are supposed to be more environmental friendly (Duke, 1986; Abbas and Duke, 1987; Dayan et al., 1999). In some small market crops, the use of "natural pesticide" could appeal the consumer, whereas in organic systems, where synthetic herbicide are not allowed, these compounds could provide a valuable alternative tool (Duke et al., 2002). In addition, due to their structural features, natural phytotoxic metabolites tend to have different mechanisms of action than synthetic herbicides (Duke et al., 2000a). The research of new alternative modes of actions is currently an important issue for agrochemicals companies. For example, in the USA, pesticide registration does not consider the toxicological potentials of a single pesticide, but rather evaluates the effects on human health and environment of aggregate exposure to an entire group of active ingredients with the same mode of action (Dayan et al., 1999). Moreover, commercial herbicides currently used act on only very few target-sites (Rapparini, 1986), a factor that inevitably leads to the selection of herbicide-resistance weed populations (Kudsk and Streibig, 2002; Heap, 1997). For these reasons, even if a compound is not suitable for direct use as herbicide, it could provide the identification of a novel target-site and could be used as template for new active ingredients (Duke et al., 2002; Duke et al., 2000b).

Natural compounds could be also used in mixture with herbicides in order to improve their efficacy and/or reduce the application rate, as shown by Vurro et al. (2001).

Sources of Natural Compounds

Nature is a huge source of new compounds. Many living organisms, such as bacteria, fungi, insects, lichens and plants, can provide natural bioactive compounds (Duke, 1986; Fracchiolla, 2003).

Plant pathogens are well known as producers of phytotoxic metabolites. Most of them are effective against a wide group of hosts, even if the pathogen is able to infect only few species (Duke et al., 2002). Another important group of organisms are bacteria. Bialophos is a metabolite from the actinomycete *Streptomyces viridochromogenes*. This product is a proherbicide that, in the target plant, is converted in phosphinothricin, metabolite with herbicidal activity. This last compound is currently synthetically produced and it is the most important example of commercial success in the discovery of naturally based herbicides (Duke et al., 2002; Fisher and Bellus, 1983).

Plant Products

Plants produce a large number of secondary metabolites with herbicidal potentials. Bell (1980) suggests that, in the course of evolution, millions of compounds have been synthesized by different species. In the present flora we have the compounds conferring to the producer a selection advantage; hence, from an ecological standpoint, these secondary metabolites are involved in the plant-plant and plant-animal relationships and make the producer able to survive and spread in new environments (Hierro and Callaway, 2003). This phenomenon, with respect to plant-plant relationship, was called by Molish (1937) "allelopathy" and was described by Rice (1984) as "*the effect of one plant (including microorganism) on another plant through the release of a chemical compound into the environment*". Allelochemicals can be released by several mechanisms such as volatilization from leaves, exudation from roots, leaching from leaves or as consequence of dead plant decomposition (Vyvyan, 2002). The herbicidal potentials of the allelochemicals from a large number of plants belonging to many botanical families have been described (Vyvyan, 2002; Duke et al., 2002; Duke et al., 2000b; Mallik, 2000; Birkett et al., 2001).

Allelochemicals from Essential Oils

Many allelochemicals are present in essential oils, whose main components are volatile terpenes (Dudai et al, 1999; Sangwan et al., 2001). Allelopathic effects caused by essential oils and pure terpenes have been highlighted by many authors. For example, among twenty-five oils tested by Tworkoski (2002), those from red thyme (*Thymus vulgaris*), summer savory (*Satureja hortensis*), cinnamon (*Cinnamomun zeylanicum*), and clove (*Syzgium aromaticum*), were highly phytotoxic on Johnson grass (*Sorghum halepense*), common lambsquarters (*Chenopodium album*) and common ragweed (*Ambroisia artemisiifolia*). Assays about germination have been done by Mao et al. (2004); the authors report that vetiver essential oils are able to inhibit germination of redroot pigweed (*Amaranthus retroflexus*), common lamsquarters (*Chenopodium album*), giant ragweed (*Ambroisia trifida*), pitted morninglory (*Ipomea lacunosa*) and velvetleaf (*Abutilon theophrasti*). Mucciarelli et al. (2001) assessed the ability of essential oil from peppermint (*Mentha x piperita*) and its main components to interfere with respiratory functions of cucumber (*Cucumis sativus*). Essential oil inhibited 50% of root and mitochondrial respiration whereas the most phytotoxic pure component was pulegone followed by menthone in its inhibitory effects. Nishida et al. (2005) evaluated the activity of several monoterpenoids produced by *Salvia leucophylla*. Camphor, 1,8-cineole and β-pinene inhibited germination of *Brassica campestris* seeds. In addition, according to morphological and biochemical analysis, the authors suggest that *S. leucophylla* is able to inhibit cell proliferations in the root apical meristem. Essential oils extracted from 32 aromatic plants were evaluated for allelopathic properties by Dudai et al. (1999). The results obtained by the authors show that germination of seeds of several weeds (*Amaranthus blitoides, Amaranthus palmieri, Euphorbia hirta*, *Sinapis nigra*) was strongly reduced by essential oils when they were applied at 20-80 ppm. Germination was also inhibited when oils were mixed with soil; the activity depended on the type of soil and was greater in loam or loess than in clay soil.

An essential oil is a mixture of several pure components whose activity can potentially interact. This important aspect explaining the overall essential oil activity was examined by Vokou et al. (2003) who estimated the effects of 47 monoterpenoids on seed germination and growth of *Lactuca sativa* seedlings. Twenty-four compounds showed strong effects on seedling growth and five on seed germination. When the compounds were tested in pairs, authors found both synergistic and antagonistic interactions. Based on these results, they argue that the participation of one or more strongly active compounds is an important prerequisite to make the oil active; however, the total activity of the mixture could be increased by other, less active components.

Exploiting Allelopathy for Weed Control

Different strategies have been proposed for the application of allelopathy in alternative weed control methods, such as the use of crude extract from tissues of plants or the spreading of pure compounds (Duke et al, 2002). Another strategy is exploiting the capability of some crop cultivars to release allelochemicals that inhibit weed growing (Khanh et al., 2005; Bhowmik and Inderjit, 2003; Wu et al., 1998); allelopathic plant residues can be also used for mulching (Khanh et al., 2005; Bhowmik and Inderjit, 2003).

We have already mentioned the potentials of allelochemicals as templates for synthetic herbicides. Duke et al. (2000b) report some important commercial results. Among these, there is the herbicide “cinmethylin,” an analogue of 1,4-cineole, whose commercial formulation was developed by Shell Chemicals. Specifically, the related synthetic compound is less volatile than cineole ring; hence, the compound is more suitable to be used as herbicide (Duke et al., 2000b). Moreover, a compound produced by *Callistemon citrinus* (leptospermone), has been used by Syngenta Crop Protection as a template for the active ingredient mesotrione, whose trade name in over 30 countries is Callisto. This compound belongs to “triketones”, a new class of herbicides whose target site is an enzyme involved in plastoquinone synthesis (Cornes, 2005).

The Research Proposal

In an earlier work (De Mastro et al., 2004) we reported preliminary observations about the inhibitory effects on germination and growth of some weeds caused by leaf extracts of oregano hybrid ‘Carva’ (*Origanum vulgare* L. ssp. *virilidum* x O. *vulgare* L.

ssp. *hirtum* (link) Iestwart), a cultivar characterized by high content of carvacrol, a component of essential oils produced by numerous aromatic plants (De Vincenti, 2004). After having assayed the activity of the above-mentioned component on seed germination in some weed species, the aim of this work was to obtain further information about possible strategies to exploit the properties of this oregano varieties for biological weed control.

MATERIALS AND METHODS

Evaluation of the Activity of Carvacrol on *Lolium perenne* and *Amaranthus retroflexus* Seed Germination

Lolium perenne L. and *Amaranthus retroflexus* L. seeds were used as test plants. These species were chosen because of their easy germination and because they are widespread in southern Italy fields. Carvacrol was purchased from Sigma-Aldrich and solutions were prepared by adding the pure compound to distillated water at the concentrations of 3×10^{-1}, 2×10^{-1}, 1×10^{-1}, and 2×10^{-2} µL mL^{-1}. Seeds were placed in plastic Petri dishes lined with filter paper wetted with 2 mL of solution. Dishes were sealed with Parafilm and put in a thermostatically controlled growth chamber with a light intensity equal to 6000 lux and at the temperature of 25°C. Each of the treatments was replicated 4 times, using 50 seeds for single replication. A control, consisting of distillate water, was also tested in the experiment. Only seeds with visible radicles and cotyledons were considered as germinated. They were counted daily and the experiment was concluded only when, for three consecutive days, no germinated seeds were detected in the control dishes. Data were subjected to analysis of variance according to a completely randomized experimental design; the significance of differences between treatments was determined by Duncan's multiple range test. Data in the table are presented as percentage of inhibition respect to the control; they are calculated according to the following formula: $(N_c - N_t)/ N_c * 100$, where N_c and N_t are the number of germinated seeds in the control and in the treated dishes respectively.

Evaluation of the Activity of Oregano Biomass on *Lolium perenne* and *Amaranthus retroflexus* Seed Germination and Growth

The effects on seed germination of different doses of oregano fresh and dried-frozen biomass mixed to the soil were evaluated.

1. Biomass Source. An oregano hybrid characterized by high content of carvacrol was used (*Origanum vulgare* L. ssp. *virilidum x O. vulgare* L. ssp. *hirtum* (link) Iestwart). Plants, grown in a field located in Policoro (Lucania – Southern Italy), were harvested in May, at the beginning of bloom by cutting the stems 4 cm above the surface of the soil. Essential oil yield was 7.9% (Table 1). After harvesting, plants were frozen at the temperature of -20°C and stored for 1 month.

2. Essential Oil Content. 5 g air-dried leaf-flower fraction of each single plant were submitted to water distillation in a Clevenger type-apparatus with a flask of 500 mL and 200 mL water for 60 minutes.

3. Essential Oil Composition. The distilled essential oil of experiment passed gas chromatography/mass spectrometry on a HP 6890 coupled with a HP 5972 MSD and fitted with a HP 30 m x 0.25 mm capillary column coated with HP-5MS (0.25 (µm film thickness). The analytical conditions were: Helium as carrier gas, injector temperature 250°C, split ratio 50:1, temperature programme 60-110°C with 2°C/min and 110-220°C with 10°C/min. The analysed components were then identified by the system HP Enhanced ChemStation G1701BA Version B.00.00 (Hewlett Packard) by comparing their mass spectra with the data in the literature (John Wiley & Sons), and confirmed by their gas chromatographic retention indices (Adams, 1995).

4. Substrate. Loam soil was put in ventilated oven at the temperature of 60°C for 24 hours in order to kill seeds present in the soil. After this treatment, 3 samples were prepared by mixing the soil with fresh chopped biomass (17, 50, and 100 g kg^{-1} of soil).

The other 3 samples were prepared by mixing the same quantities of biomass that had been freeze-dried. Soil without any other addition was used as blank test. Trays were filled with the substrate prepared as described above.

5. Experiment Set-up and Procedures. *L. perenne and A. retroflexus* seeds were sown in trays filled with substrate at the different concentration of oregano biomass. For each tray, 500 seeds of *L. perenne* and 200 seeds of *A. retroflexus* were sown. Trays were put in a growth chamber under a light intensity of 6000 lux for 12 hours and at a constant temperature of 25°C. They were periodically watered by adding an equal amount of water for each tray. Each treatment was replicated four times.

6. Assessments. Both for *L. perenne* and for *A. retroflexus*, the number of emerged plants and their dry weight were determined 30 days after the sowing. Data are shown in the tables as percentage of decrease respect to the control, using the same formula cited for the previous experiment.

Field Evaluation of the Activity of Oregano Biomass for Weed Control in Processing Tomato Field

The trial was designed to obtain first results about the use of oregano biomass to control weeds in low input systems. In July, processing tomato (cultivar *Diaz* P55517) was transplanted in double rows (30 cm within double-rows, 60 cm between double-rows, and 30 cm for plants along the row) after the harvesting of durum wheat. Before transplanting, a rotary tillage was made only on the double rows in order to incorporate oregano fresh biomass into the soil top layer (10 cm). Between the double rows, weeds were mowed without moving the soil. The amount of biomass was 1.7 or 3.5 kg m^{-2} and no treated plots were used as control. A randomized block experimental design with four replications was used; plot size was 0.72 m^2. Crop was irrigated with drip system and grown according to the standard practices of the area. The experimental field was located in Policoro (Lucania – Southern Italy) on a silty clay soil. 30 days after transplanting, the number of weeds per m^2, height and phytotoxicity symptoms on tomato plants were assessed. Phytotoxicity symptoms were evaluated using the increasing scale of symptoms severity, ranging from 1 to 9, as proposed by the European Weed Research Society. according to this scale, 1= no symptoms and 9 = dead plant (Vercesi, 1983). At the harvesting (20th of October), fruit yields were measured in each plot. Data were processed by analysis of variance and the significance of differences between treatments was determined by Duncan's multiple range test.

RESULTS

Effects of Carvacrol on *Lolium perenne* and *Amaranthus retroflexus* Germination

Carvacrol completely inhibited the germination both of *Lolium perenne* and *Amaranthus retroflexus* seeds at the concentration of 3 x 10^{-1} µL mL^{-1} (Table 2). The concentration of 2 x 10^{-1} µL mL^{-1} gave significantly lower inhibition rates equal to 62.8% and 72.7 % for *L. perenne* and *A. retroflexus*, respectively.

Effects of Oregano Biomass on Emergence and Growth of *Amaranthus retroflexus*

An amount of 100 g per kg of soil gave the 93.7% of dry weight decrease and the 77.5% of seed emergence inhibition (Table 3). For the dry weight, the above-cited rate was significantly higher than those obtained y mixing 50 and 17 g of fresh biomass per kg of soil. The number of emerged plants at 100 g and 50 g of fresh biomass per kg of soil gave values statistically higher than those obtained with 17 g of fresh oregano biomass (15.5 %). At 20 and 10 g of dried-frozen oregano biomass per kg of soil, decreasing dry weight (93.7% and 77.5%) and number of emerged plants (85.7% and 64.3%) were observed (Table 3). These data were statistically higher than those measured for 17 g per kg, 55.9% for the dry weight and 15.5% for the number of plants.

Effects of Oregano Biomass on Emergence and Growth of *Lolium perenne*

Table 4 shows that, when 100 g of fresh biomass per kg of soil were used as substrate, *L. perenne* dry weight decrease was 52.3%. This value was statistically different only from the one obtained with 17 g per kg (27.4%). The number of emerged plants was lower compared to the control of the 30%. Significantly lower decreasing rates were obtained with 50 and 17 g per kg of soil (18.2% and 9.6% respectively). With regard to the effects of the lyophilized biomass (Table 4), both dry weight (59.5%) and number of emerged plants decrease (35.2%) was statistically higher for the highest amounts of biomass added to the soil.

Field Evaluation of the Activity of Oregano Biomass for Weed Control in Processing Tomato Field

Besides durum wheat plants coming from germinated seeds of the previous crop, the main weeds detected in the plots of the control were *Portulaca oleracea* and *Amaranthus graecizans* (Table 5). In the treated plots the above-mentioned species were not detected. Regarding the effects of oregano biomass on tomato plants, Table 6 shows that average plant height was significantly lower in the plots where 3.5 kg m^{-2} of biomass were buried in the soil. In the same plots phytotoxicity was statistically the highest and equal to 7 as well as it was equal to 4 in the plots treated with the lower amount of biomass. Fruits yield was significantly higher in plots treated with 1.7 kg m^{-2} of biomass (122.0 t ha^{-1}) than in the other plots (Table 6).

DISCUSSION

Carvacrol at 0.3 µL mL^{-1} completely inhibited the germination of *A. retroflexus* and *L. perenne* seeds. Also concentrations of 0.2 µL mL^{-1} carvacrol generally inhibited germination of the target species. Lower rates of carvacrol were less effective, especially on *L. perenne* seeds. Hence, a concentration ranging from 0.1 to 0.3 µL mL^{-1} could be considered to control seed germination of the tested species. Oregano biomass mixed with soil was able to inhibit both germination and plant growth. For *A. retroflexus* acceptable effectiveness was detected using 50 g of fresh biomass or 10 g of freeze dried biomass per kg of soil; for *L. perenne*, sensible results were obtained using the higher amount of biomass. In both cases, freeze dried biomass showed an activity comparable to the fresh biomass. When fresh biomass was buried on the double row of tomato, an amount of 1.7 kg per m^2 was able to totally control weeds for at least 30 days. Afterwards, weeds that eventually emerged were suppressed by tomato plants and their growth was stopped (data not reported). The higher amount of biomass (3.5 kg m^{-2}) was phytotoxic for tomato plants so that the marketable fruit yield was decreased. That decrease seemed to be due to a delayed ripening and in fact the green fruit yield was higher in the plots treated with the highest amount biomass (data not shown). In contrast, even though 1.7 kg m^{-2} was slightly phytotoxic during the first growth stages, crops was able to recover from the injury. Based on these results, an amount ranging around 1.7 kg m^{-2} of biomass could be suggested to obtain an effective weed control.

CONCLUSIONS

The observations support the view that carvacrol has the ability to control weed. Fresh or dried-frozen biomass of an oregano variety characterized by high content of carvacrol was also effective in weed control. Oregano biomass showed good control of weeds in tomato crop when combined with mowing. In this case, the advantages of reduced tillage were added to the positive effects due to the application of "no-chemical herbicides." Many other applications can be proposed for this material. In organic systems, the herbicide effects shown by the oregano cultivar could be a promising tool for weed control. Also, in conventional systems, the integrated application of natural and chemical herbicides could reduce the quantity and/or increase the number of the controlled species. In this last case, another advantage could derive from alternating different weed control tools that is one of the most recommended strategies to avoid the growing of herbicide resistant weed populations (Sattin et al., 2001).

However, in this paper we report only preliminary results about the cited potentials. Although these results are remarkable and amazing, they are still far from supplying techniques or tools ready to be applied by farmers. For these purposes, many questions need to be answered. It is important to know the performance with a larger number of weeds. Moreover, a too large amount of biomass was used in order to obtain high effectiveness. Hence, further studies are required to decrease these quantities. For example, it could be possible to find other species or cultivars that produce higher amounts of carvacrol or to improve growing techniques to increase the yield of carvacrol in the essential oils.

The use of the pure compound or plant essential oil could be another promising technique to exploit allelopathic effects for weed control. However, studies regarding their chemical formulations are required to solve problems related to volatility, low water solubility and aptitude for oxidation.

Moreover, it is likely that other compounds contained in the essential oils could have synergic or additive activity with carvacrol, an effect demonstrated by Vokou et al. (2003) for several monoterpenoids.

For all the reasons set out above, the base for further researches should be a study about the mode of action of the compounds involved in the process and their chemical features.

Literature Cited

Adams, R.P. 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing Corp., Carol Stream, IL, USA.

Abbas, H.K. and Duke, S.O. 1997. Plant pathogens and their phytotoxins as herbicides. In: K. Rajeev Upadhyay and K.G. Mukerji (Eds.). Toxins in Plant Disease Development and Evolving Biotechnology. Oxfrord and IBH Publishing Co. Pvt. Ltd, New Daly, Calcutta. pp. 1-20.

Bell, E.A. 1980. The possible significance of secondary compounds in plants. In: Secondary Plants Products. E.A. Bell and B.V. Chalwood, eds. Springer-Verlag Berlin. pp. 11-21.

Bhowmik, P.C. and Inderjit. 2003. Changele and opportunities in implementing allelopathy for natural weed management. Crop Protection 22:661-671.

Birkett. M.A., Chamberlain K., Hooper, A. and Pickett, J.A. 2001. Does allelopathy offer real promise for practical weed management and for explaining rhizosphere interactions involving higher plants? Plant and Soil 232:31-39.

Charudattan, R. 2001. Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. BioControl, 46:229-260.

Cornes, D. 2005. Callisto: a very successful maize herbicide inspired by allelochemistry. In: J.D.I Harper, M. An, H. Wu and J.H. Kent (Eds.). Proceedings of the 4th World Congress on Allelopathy. Charles Sturt University, Wagga Wagga, NSW, Australia. August 2005. International Allelopathy Society.

Dayan, F., Romagni, J., Tellez, M., Rimando, A. and Duke, S. 1999. Managing weeds with natural products. Pesticides Outlook (October):185-188.

De Mastro, G., Fracchiolla, M., Brunetti, G. and Verdini, L. 2004. Bioerbicidi: Nuove opportunità di valorizzazione delle produzioni officinali (abstract). I Convegno Nazionale sulle Piante Mediterranee. Agrigento, 7-8 Ottobre. 155 p.

De Vincenti, M., Stammati, A., De Vincenti, A. and Silano, M. 2004. Constituents of aromatic plants: carvacrol. Fitoterapia 75:801-804.

Dudai, N., Mayer, A.M., Poljako-Mayber, A., Putievsky, E. and Lerner, H.R. 1999. Essential oils as allelochemicals and their potential use as bioherbicides. Journal of Chemical Ecology 25:1079 - 1089.

Duke, S.O., Dayan, F.E., Rimando, A.M., Schrader, K.K., Aliotta, G., Oliva, A. and Romagni, G.J. 2002. Chemicals from nature for weed management. Weed Science, 50:138-151.

Duke, S.O. 1986. Microbially produced phytotoxins as herbicides - a perspective. In: *The Science of Allelopathy*. Alan Putman and Chung-Shih Tang, eds. John Wiley & Sons, Inc., New York. pp. 287-304.

Duke, S.O., Dayan, F.E. and Rimando, A.M. 2000a. Natural products and herbicide discovery. In: A.H. Cobb and R.C. Kirkwood (Eds.). Herbicides and their Mechanisms of Action. Academic Press, Sheffield, U.K. pp. 105-1333.

Duke, S.O., Fayadan, F.E., Romagni, J.G. and Rimando, A.M. 2000b. Natural products as sources of herbicides: current status and future trends. Weed Research, 40:99-111.

Fisher, H.P. and Bellus, D. 1983. Phytotoxicans from microorganism and related compounds. Pesticide Science: 334-346.

Fracchiolla, M. 2003. Biological control of grass weeds by using bioherbicides. Ph.D. Thesis, Crop Science Department, University of Bari, Italy (in Italian).

Heap, I.M. 1997. The occurrence of herbicide-resistant weed worldwide. Pesticide Science 51:235–243.

Henkel, T., Brunne, R.M., Muller, H. and Reichel, F. 1999. Statistical investigation into the structural complementaritiy of natural products and synthetic compounds. Angewandte Chemie International Edition 38:643-647.

Hierro, L.J., Callaway, R.M. 2003. Allelopathy and exotic plant invasion. Plant and Soil 256: 29–39.

Khanh, T.D., Chung, M.I., Xuan, T.D. and Tawata, S. 2005. The exploitation of crop Allelopathy in sustainable agricultural production. Journal of Agronomy and Crop Science 191:172-184

Kudsk, P. and Streibig, J.C. 2002. Herbicides – a two-edged sword. Weed Research 43:90-102.

Mao, L., Henderson, G. and Laine, R.A. 2004. Germination of various weed species in response to vetiver oil and nootkatone. Weed Technology 18:263-267.

Mallik, A.U. 2000. Challenges and opportunities in allelopathy research: a brief overview. Journal of Chemical Ecology 26(9): 2007-2009.

Molisch, H. 1937. Der Einfluss einer Pflanz auf die andere-Allelopathige. Fischer, Jena, Germany.

Mucciarelli, M., Camusso, W., Bertea, C.M., Bossi, S. and Maffei, M. 2001. Effect of (+)-pulegone and other oil components of *Mentha* x piperita on cucumber respiration. Phytochemistry 57:91-98.

Nishida, N., Tomotsu, S., Nagata, N., Saito, C. and Sakai A. 2005. Allelopathic effects of volatile monoterpenoids produced by Salvia leucophylla: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. Journal of Chemical Ecology 31(5):1187-1203.

Rapparini, G. 1996. Il diserbo delle colture. Edizioni “L’informatore Agrario”,Verona, 495 p.

Rice, E.L. 1984. *Allelopathy*. Academic Press, Orlando, FL.

Sangwan, N.S., Farooqi, A.H.A., Shabih, F. and Sangwan, R.S. 2001. Regulation of essential oil production in plant. Plant Growth Regulation 34:3–21.

Sattin, M. and Gasparetto, M.A. 2001. Situation and management of *Avena sterilis*_spp. *ludoviciana* and Phalaris paradoxa resistant to ACCase inhibitors in Italy. Proc. The BCPC Conference. pp. 755-762.

Tworkoski, T. 2002. Herbicide effects of essential oils. Weeds Science 50:425-432.

Vercesi, B. 1983. Diserbanti e loro impiego. Edizioni Agricole. 491 p.

Vyvyan, J.R. 2002. Allelochemicals as leads for new herbicides and agrochemicals. Tetrahedron 58:1631-1646.

Vokou, D., Douvli, P., Blionis, G.J. and Halley, J.M. 2003. Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. Journal of Chemical Ecology 29(10):2281-2310.

Vurro, M., Zonno, M.C., Evidente, A., Andolfi, A. and Montemurro, P. 2001. Enhancement of efficacy of *Ascochyta caulina* to cControl *Chenopodium album* by use of phytotoxins and reduced rates of herbicides. Biological Control 21:182–190.

Wu, H., Pratley, J., Lemerle, D. and Haig, T. 1999. Crop cultivars with allelopathic capability. Weed Research 39:171-180.

Tables

Table 1. Essential oil quali-quantitative component (% v/v) of the oregano hybrid used in the experiments.

Compounds	RT*	Composition (%)
α-thujene	12.93	0.85
α-pinene	13.24	0.35
camphene	14.00	0.06
sabinene	15.42	0.05
octen - 3 - ol	16.08	0.17
β-myrcene	16.41	1.01
α-phellandrene	17.03	0.16
δ-3-carene	17.31	0.05
α-terpinene	17.67	0.68
p-cymene	18.14	3.28
β-phellandrene	18.32	0.23
β-ocimene	18.90	0.04
γ-terpinene	19.91	3.25
cis-sabinene-hydrate	20.46	0.61
linalool oxide	21.55	0.05
linalool	22.17	0.23
borneol	25.49	0.16
terpinen-4-ol	26.00	0.34
α-terpineol	26.71	0.11
carvacrol-methyl-ether	29.12	0.06
thymol	31.79	0.34
carvacrol	**32.26**	**83.95**
trans-caryophyllene	36.78	2.61
α-humulene	38.21	0.22
β-bisabolene	40.41	0.36
caryophyllene oxide	43.44	0.15
total		99.37

** Ritention time (min.)*

Table 2 - Effects of carvacrol on Lolium perenne and Amaranthus retroflexus seed germination.

Carvacrol ($\mu L\ mL^{-1}$)	Inhibition of germination (%)	
	Lolium perenne	*Amaranthus retroflexus*
$3\ 10^{-1}$	100.0 a	100.0 a
$2\ 10^{-1}$	62.8 b	72.7 b
$1\ 10^{-1}$	8.7 c	27.3 c
$2\ 10^{-2}$	9.8 c	23.9 c

Values with no letter in common are statistically significant at 0.05 (Duncan's test).

Table 3 - Effects of oregano biomass on emergence and growth of Amaranthus retroflexus.

Oregano biomass ($g\ kg^{-1}$ of soil)	*Amaranthus retroflexus*	
	dry weight (%)	number of plants (%)
fresh		
100	93.7 a	77.5 a
50	85.7 b	64.3 a
17	55.9 c	15.5 b
dried-frozen		
20	86.0 a	67.6 a
10	79.0 a	65.2 a
3	64.3 b	32.7 b

Values with no letter in common are statistically significant at 0.05 (Duncan's test).

Table 4 - Effects of oregano biomass on emergence and growth of Lolium perenne.

Oregano biomass ($g\ kg^{-1}$ of soil)	*Lolium perenne*	
	dry weight (%)	number of plants (%)
fresh		
100	52.3 a	30.0 a
50	42.8 a	18.2 b
17	27.4 b	9.6 b
dried-frozen		
20	59.5 a	35.2 a
10	33.3 b	6.7 b
3	3.6 c	0.0 b

Values with no letter in common are statistically significant at 0.05 (Duncan's test).

Table 5 - Effects of oregano fresh biomass on weeds in transplanted tomato.

Oregano biomass ($kg\ m^{-2}$)	Weeds ($n.\ m^{-2}$)		
	Portulaca oleracea	*Amaranthus graecizans*	*Triticum durum*
1.7	0.0 a	0.0 a	0.0 a
3.5	0.0 a	0.0 a	0.0 a
Control	4.2 b	3.2 b	8.8 b

Values with no letter in common are statistically significant at 0.05 (Duncan's test).

Table 6 - Effect of oregano biomass on tomato yield and plant growth.

Oregano biomass (kg m^{-2})	Plant height (cm)	Phytotoxicity (rank)	Fruit yield (t ha^{-1})
1.7	29.2 a	4.0 b	122.0 a
3.5	17.0 b	7.0 a	113.4 b
Control	27.1 a	0.0 c	110.8 b

Values with no letter in common are statistically significant at 0.05 (Duncan’s test).