Methyleugenol in *Ocimum basilicum* L. Cv. Genovese Gigante

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Ocimum basilicum cv. Genovese Gigante is the basil cultivar used the most in the production of a typical Italian sauce called pesto. The aromatic composition of plants at different growth stages was determined. Plants from different areas of northwestern Italy were analyzed at 4 and 6 weeks after sowing and showed methyleugenol and eugenol as the main components. The content of these compounds was correlated with plant height rather than plant age. Particularly, methyleugenol was predominant in plants up to 10 cm in height, whereas eugenol was prevalent in taller plants. These results are important in the evaluation of risk to human health posed by dietary ingestion of methyleugenol contained in pesto.

Keywords: *Ocimum basilicum cv. Genovese Gigante; sweet basil; methyleugenol; eugenol; toxicity*

INTRODUCTION

Basil is an annual plant of the *Ocimum* genus belonging to the Lamiaceae family. The *Ocimum* genus offers, among its >50 species, a wide diversity in growth characteristics, leaf size, flower color, physical appearance, and aroma (*1*). Among them, *Ocimum basilicum*, or sweet basil, has long been prized, whether fresh, dried, or processed, for its foliage, which adds a distinctive flavor to many foods. In its processed form, basil is employed in the production of pesto, a typical Italian sauce known for its unmistakable aroma. In the past decade the spread of pesto consumption has made basil an increasingly popular culinary herb all over the world. It is an economically important crop in several countries, particularly in Italy, where its cultivation covers ∼80 ha, mostly in Liguria, situated in the northwest of the country (*2*). *O. basilicum* cv. Genovese Gigante is considered one of the best for pesto production by both local producers and national industries.

The aromatic character of each type of basil is determined by genotype and depends on the major chemical compounds of essential oils primarily consisting of monoterpenes and phenylpropanoids (*3*, *4*). Some phenylpropanoids cited in this paper are reported in Figure 1. Chemotypes are generally classified on the basis of prevalent compounds (*5*) or components >20% (*6*) in adult plants. Although the literature concerning the aromatic composition in basil is significant, very few data are available on the Genovese Gigante cultivar and all refer to the adult or flowering stage (35−40 cm in height). A study of 10 Italian cultivars of *O. basilicum*, analyzed at the beginning of the flowering stage, showed that the principal essential oil components were linalool,

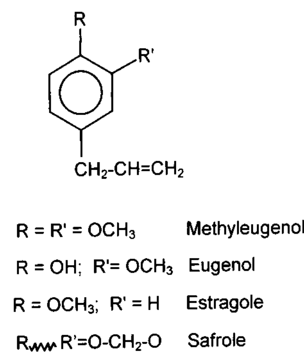

Figure 1. Structures of phenylpropanoids.

estragole, and cineole, depending on the cultivar (*4*). Among them, the Genovese Gigante cultivar showed linalool as the main component. In Liguria only young basil plants (10−12 cm in height) are considered to have organoleptic properties especially suitable for a dainty pesto: small and young leaves are relished, whereas plants close to flowering possess a strong, less refined taste. Because the quality of essential oil is largely influenced by the vegetative stage of the plant, we analyzed Genovese Gigante plants at the stage at which they are purchased from local markets and used for pesto preparation (*7*). Methyleugenol was found to be the prevalent component (58.7%) of the essential oil. Methyleugenol belongs to the phenylpropanoid group, an important natural constituent of a large number of herbs, spicee, and vegetables (*8*−*10*). Although methyleugenol is approved for commercial use as a flavoring agent in food and as fragrance in perfumes, creams, and detergents, human exposure to this compound is of toxicological concern because of its structural resemblance to known carcinogenic phenylpropanoids such as estragole and safrole (*11*). Intake of methyleugenol with the human diet is usually considered to be very low, but the consumption of pesto made with very short basil may lead to the intake of non-negligible doses of methyleugenol. As methyleugenol was not found in full-

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Table 1. Northwestern Italy Localities of Cultivation and Their Position with Respect to Genova Prà

sample	locality	distance from 1 (km)	direction from 1	altitude (m)
1	Genova-Prà			sea level
2	Albenga	80	west	sea level
3	Carcare	60	west	80
4	Ventimiglia	150	west	sea level
5	Ovada	50	north	300
6	Casale Monferrato	110	north	10
7	Vercelli	130	north	10
8	Soriso	180	north	400
9	Genova-Granarolo	10	east	80
10	Recco	20	east	sea level
11	Casarza Ligure	50	east	100

grown plants (*4*) we inferred that there is a large variation in the production of this compound during the growth cycle. The latter consideration induced us to undertake a project aimed at determining the content of methyleugenol and other phenylpropanoids in the Genovese Gigante cultivar at different stages of growth. The influence of environmental conditions on the production of these compounds was also considered.

EXPERIMENTAL PROCEDURES

Plant Material. Seeds of *O. basilicum* cv. Genovese Gigante purchased from SIAS (Società Italiana Agricola Sementi) were sown in 22 pots at the end of July 1998 and distributed in 11 different sites in northwestern Italy. Table 1 shows the position and distance of these sites with respect to Genova-Prà, where the soil was obtained: localities situated to the east and west belong to the Liguria region, and localities situated to the north belong to the Piedmont region; Liguria is known to possess a milder climate with respect to that of Piedmont.

All of the pots contained the same soil, provided by Sacco (Genova-Prà), and were exposed to the sun from the southwest. Eleven pots, each with six to eight plants of *O. basilicum* cv. Genovese Gigante, were recovered 4 weeks after sowing (recovery I), and the mean height for each pot was calculated. The remaining 11 pots were recovered 2 weeks later (recovery II), and the same measurements were performed.

Aromatic compounds from fresh leaves were obtained by distillation and analyzed by GC-MS.

Distillation. In a 500 mL flask, 8 g of leaves was added to 150 mL of water and the mixture was hydrodistilled until 50 mL was recovered. The distillate was extracted three times with freshly distilled ethyl ether. The solvent was then removed at room temperature, and the essential oil was diluted with ethyl acetate to 5 mg/mL. An aliquot was injected into the chromatograph.

GC-MS Analysis. Capillary GC-MS measurements were carried out on an HP-5MS (0.25 mm × 30 m) column coupled directly to a quadrupole MS. Conditions: carrier gas, He; flow rate, 1 mL/min; split, 1:49; injection point, 250 °C; oven program, initial temperature of 60 °C for 4 min, ramped at 5 °C min^{-1}, final temperature of 210 °C; electron energy, 70 eV. Quantitative data were obtained from normalized area values. Each analysis was performed in duplicate.

Methyleugenol: RT, 20.08 min; 178 $[M]^+$ (100), 163 (25), 152 (10), 147 (24), 135 (9), 115 (11), 107 (18), 103 (23), 91 (26), 77 (11), 65 (8).

Eugenol: RT, 18.85 min; 164 $[M]^+$ (100), 149 (33), 137 (19), 131 (26), 121 (13), 107 (5), 103 (29), 91 (24), 77 (22), 65 (8).

Linalool: RT, 11.42 min; 154 $[M]^+$.

Cineole: RT, 9.25 min; 154 $[M]^+$.

Camphene: RT, 14.16 min; 136 $[M]^+$.

Cadinene: RT, 25.78 min; 204 $[M]^+$.

RESULTS

The mean height of plants contained in each pot was calculated just before leaves were collected for steam distillation and the following GC-MS analysis. Table 2 shows the plants' mean height and the percentage of the main components obtained for all 22 pots. Mean height varied from 3.0 to 13.8 cm and from 2.7 to 16.7 cm in recoveries I and II, respectively. The mean height of all the plants collected 4 weeks after sowing (8.18 ± 3.70 cm) and the mean height of all the plants collected 2 weeks later (8.22 ± 4.68 cm) were not significantly different (unpaired *t* test).

Table 2. Mean Height and Essential Oil Composition of Basil Plants Analyzed 4 Weeks (I) and 6 Weeks (II) after Sowing

sample	mean height (SD)[a] (cm)	methyl-eugenol (%)[b]	eugenol (%)[b]	OC[c] (%)[b]
1^{I}	12.0 (2.19)	39.4	51.7	8.9
2^{I}	7.3 (1.27)	78.1	21.9	0.0
3^{I}	12.8 (2.92)	55.5	43.2	1.3
4^{I}	13.8 (3.47)	10.6	75.0	14.4
5^{I}	5.5 (0.55)	75.0	18.1	6.9
6^{I}	3.7 (0.37)	100.0	0.0	0.0
7^{I}	8.8 (0.73)	80.2	16.4	3.4
8^{I}	7.7 (2.01)	52.0	46.0	2.0
9^{I}	5.2 (0.36)	20.6	74.8	4.6
10^{I}	10.2 (3.10)	44.9	55.1	0.0
11^{I}	3.0 (0.55)	91.4	2.4	6.2
1^{II}	12.0 (2.53)	5.5	73.0	21.5
2^{II}	7.3 (1.50)	79.5	20.5	0.0
3^{II}	16.7 (3.65)	11.8	88.2	0.0
4^{II}	13.8 (4.01)	10.7	74.3	15.0
5^{II}	5.3 (0.18)	86.6	10.3	3.1
6^{II}	2.7 (0.37)	100.0	0.0	0.0
7^{II}	6.0 (0.55)	99.8	0.2	0.0
8^{II}	6.0 (1.26)	100.0	0.0	0.0
9^{II}	5.3 (0.73)	16.5	80.3	3.2
10^{II}	12.3 (1.82)	16.6	78.9	4.5
11^{II}	3.0 (0.32)	89.3	10.7	0.0

[a] Mean height of plants contained in each pot (standard deviation shown in parentheses). [b] Percentage by GC. [c] Other components: linalool, cineole, camphene, and cadinene.

GC-MS analyses showed that methyleugenol and eugenol were the main compounds, whereas linalool and other terpenes such as cineole, camphene, and cadinene were also found in some samples. The main components varied as follows: methyleugenol from 10.6 to 100% and eugenol from 0 to 75.0% in recovery I; the former from 5.5 to 100% and the latter from 0 to 88.2% in recovery II. The mean percentages of these compounds were also not statistically different (unpaired *t* test) in the two recoveries: methyleugenol was 58.89 (± 28.75) and eugenol 36.78 (± 26.67) in recovery I, whereas methyleugenol was 56.30 (± 42.72) and eugenol 39.67 (± 38.25) in recovery II.

The chromatograms pictured in Figure 2 show the composition of essential oil from samples 5^{II}, 3^{I}, and 4^{II} and are representative of all analytical profiles.

With regard to phenylpropanoids, a particularly negative correlation was found between methyleugenol and plant height, whereas eugenol was positively correlated with it (Figures 3 and 4). These correlations were statistically significant with $p < 0.05$ and $p < 0.01$ in recoveries I and II, respectively. The other components were not considered in our statistical analyses because they were detected in only a few samples.

DISCUSSION

The effect of environmental conditions on the growth stage and on the percentage of aromatic components was considered 4 and 6 weeks after sowing. At the sites with a mild climate the plants were well developed and

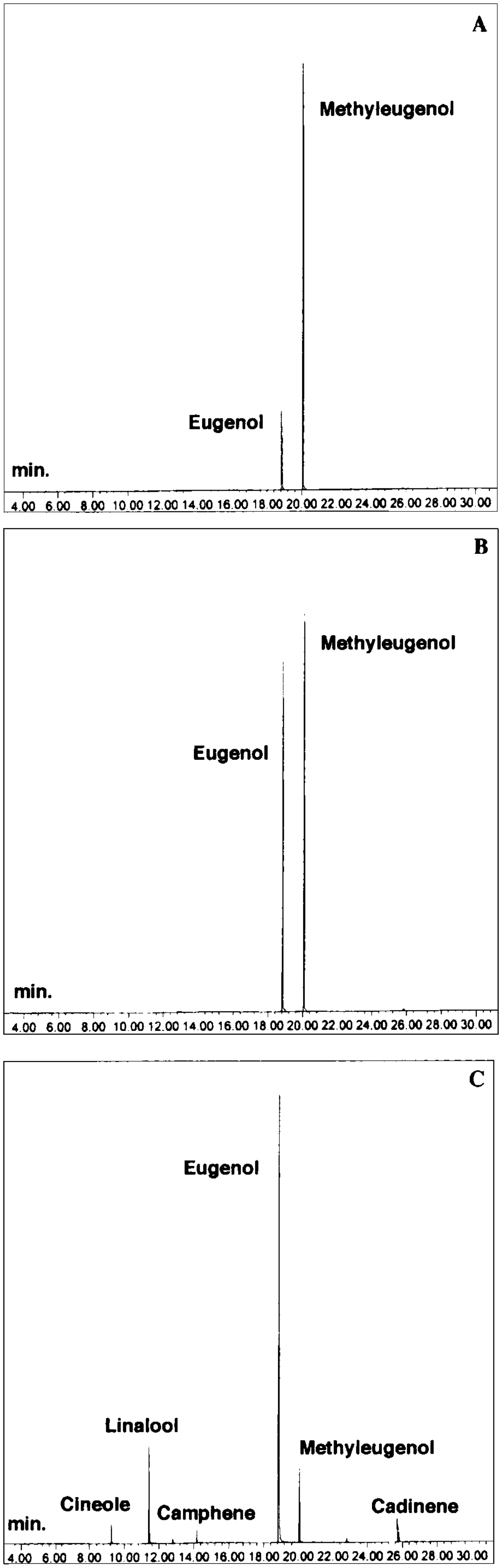

Figure 2. Chromatograms of samples 5^{II} (A), 3^{I} (B), and 4^{II} (C).

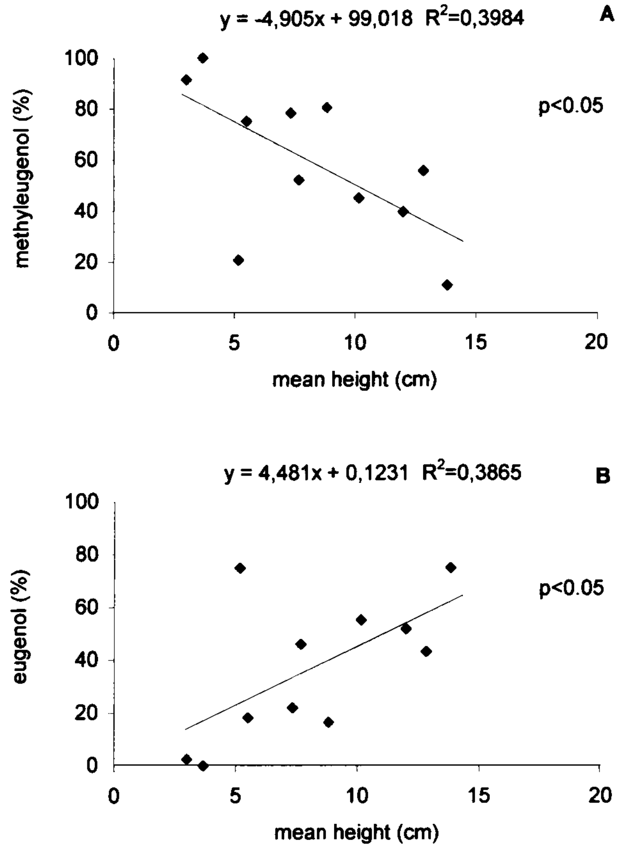

Figure 3. Correlation between mean height of plants in each pot and methyleugenol (A) and eugenol (B) 4 weeks after sowing.

eugenol was prevalent in the essential oil (samples 1, 3, 4, and 10). On the contrary, plants grown in northern localities presented a reduced development and were rich in methyleugenol (samples 5−8). Nevertheless, the environmental conditions were not unequivocal. In fact, although samples 2, 9, and 11 were grown in Liguria, they were very short; moreover, sample 9, although ∼3 cm, contained a high level of eugenol and a low level of methyleugenol.

The lack of significant differences in plants' mean height and essential oil composition between recoveries I and II could be explained by the fact that pots with taller plants were chosen for the first analysis. Plants of the second recovery seemed to possess, generally, a stage of maturity similar to the first one: such a stage of maturity is, for us, correlated to the presence of other components (OC) in Table 2. In this regard, sample 1^{II}, which has the same height as 1^{I}, seemed to have an increased maturity stage, indicated by the high percentage of OC as well as a low level of methyleugenol.

Although the analyzed plants showed a large variability in heights and chemical characteristics, our results showed that the major aromatic components contained in *O. basilicum* cv. Genovese Gigante were methyleugenol and eugenol, at least in the range of investigated heights.

Recently Wang et al. (*12*) reported that in *Clarkia breweri*, methyleugenol derived from eugenol by a specific methylation involving an *S*-adenosylmethionine-dependent *O*-methyltransferase. The strict correlation between methyleugenol and eugenol found in basil could be explained by a similar pathway. The activity of the invoked enzyme decreases when plants grow.

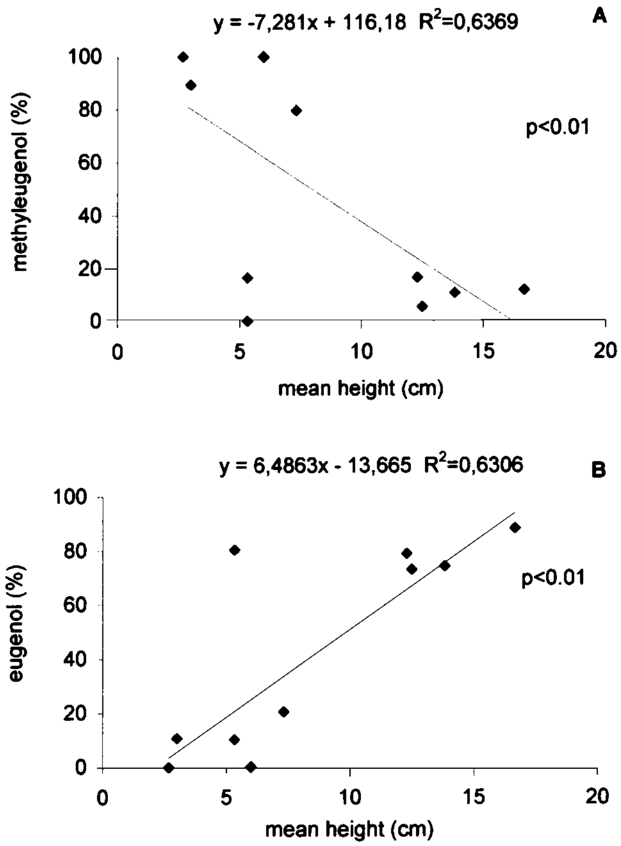

Figure 4. Correlation between mean height of plants in each pot and methyleugenol (A) and eugenol (B) 6 weeks after sowing.

Methyleugenol has been shown to be genotoxic and carcinogenic in several systems (*13*−*15*).

The National Toxicology Program (NTP), following a recent 2-year study, reported clear evidence of carcinogenic activity of methyleugenol in rats and mice based on the increased incidence of liver neoplasms and neuroendocrine tumors (*16*).

Although methyleugenol and eugenol are structurally similar, their metabolism and activity against biological molecules are extremely different. Methyleugenol is unreactive itself and, like safrole and estragole, must undergo metabolic activation to produce electrophilic intermediates that enable it to interact with biomolecules. A major metabolite is the 1′-hydroxy derivative, which shows greater carcinogenic potency than the parent compound. 1′-Hydroxymethyleugenol is further sulfated, yielding a sulfoxy metabolite which decomposes spontaneously in an aqueous environment to electrophilic carbocations that bind covalently to DNA and other cellular macromolecules, including proteins (*17*).

In contrast, eugenol, which presumably is conjugated readily to its 4-hydroxy group, did not show any genotoxic and carcinogenic activity when assayed in the same system as methyleugenol (*14*). This biological inactivity of eugenol was consistent with the essentially negative findings reported in the studies conducted by the NTP and IARC, which did not classify it as a carcinogenic compound (*18*).

Our data are of importance in the assessment of a possible risk to humans exposed to methyleugenol. The rodent lifetime carcinogenicity bioassay undertaken by NTP is generally considered to overestimate the risk to human health posed by dietary ingestion of methyleugenol, which is thought to be on the order of a few micrograms per kilogram per day (*17*).

In the case of pesto consumers, intake of methyleugenol could be much higher than that reported above. Pesto is in fact traditionally prepared with basil that is 10−12 cm in height, when the percentage of methyleugenol in the essential oil is generally >40%. Considering that at this stage of growth the amount of essential oil in *O. basilicum* cv. Genovese Gigante corresponds to ∼0.5% (data not shown) and that one portion of pesto contains ∼10 g of basil, the resulting intake of methyleugenol could reach 250 μg/kg/meal in adults and 500 μg/kg/meal in children.

Further studies are necessary to evaluate whether a real risk is associated with the consumption of pesto prepared with basil rich in methyleugenol. The results would not be of only local significance because the worldwide consumption of pesto has increased due to recent interest in Mediterranean cuisine, which is considered to be healthful and valuable even if it is sometimes lacking of suitable controls.

Genovese Gigante is by far the most popular basil cultivar in Liguria and is most commonly used for pesto production. The aromatic composition of this cultivar is largely influenced by plant height. Particularly, plants 3.5−6.5 cm high contain methyleugenol as the prevalent component, whereas in taller plants eugenol is largely predominant.

In conclusion, for the time being we suggest that pesto be prepared with basil taller than 16 cm, when eugenol is the main component and methyleugenol is greatly reduced.

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