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Agronomic traits and essential oil profiles of *Humulus lupulus* L. cultivated in southern Italy

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Original

Agronomic traits and essential oil profiles of *Humulus lupulus* L. cultivated in southern Italy / Gresta, F., Calvi, A., Santonoceto, C., Strano, T., Ruberto, G.. - In: JOURNAL OF ESSENTIAL OIL RESEARCH. - ISSN 1041-2905. - (2022), pp. 1-11. [10.1080/10412905.2022.2103190]

Availability:

This version is available at: <https://hdl.handle.net/20.500.12318/131747> since: 2023-01-06T14:46:51Z

Published

DOI: <http://doi.org/10.1080/10412905.2022.2103190>

The final published version is available online at: <https://www.tandfonline.com/doi/full/10.1080/10412905>.

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***AGRONOMIC TRAITS AND ESSENTIAL OIL PROFILES OF HUMULUS LUPULUS L.
CULTIVATED IN SOUTHERN ITALY***

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DOI: [10.1080/10412905.2022.2103190](https://doi.org/10.1080/10412905.2022.2103190)

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1 ***AGRONOMIC TRAITS AND ESSENTIAL OIL PROFILES OF HUMULUS***
2 ***LUPULUS L. CULTIVATED IN SOUTHERN ITALY***

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14 **Abstract**

15 **BACKGROUND:** *Humulus lupulus* L. is a dioecious herbaceous perennial plant with a
16 climbing habit, whose female inflorescences, commonly known as cones, produce and
17 accumulate bitter substances and essential oils. The present study aimed to assess the
18 adaptability of some American hop varieties (Cascade, Chinook, Comet) in the
19 Mediterranean environment of the Calabria region (Italy) through the evaluation of the
20 morpho-biological and productive characteristics and the characterization of the aromatic
21 traits of the inflorescences.

22 **RESULTS:** Cascade emerged as the earliest variety. Different morphological traits were
23 ascertained among the studied varieties. Comet proved to be the most productive variety,
24 with a dry cone production of 0.35 t ha⁻¹, while chinook the earliest. Essential oil was
25 obtained by hydrodistillation and analysed by a combination of GC-FID and GC-MS.
26 Myrcene, β-caryophyllene and α-humulene were the main components.

27 **CONCLUSION:** The combination of leaf and cone dimensions could be adopted as useful
28 tools together with the determination of the aromatic profiles to discriminate varieties.
29 From the agronomic point of view Comet was the most productive variety, while Chinook
30 emerged as the earliest one; concerning the essential oils Comet and Chinook showed
31 similar profiles, Comet was different especially for the sesquiterpenes content.

32
33 **Keywords:** *Humulus lupulus*, morphological traits, cone yields, essential oi
34

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35 INTRODUCTION

36 Hop plant belongs to the genus *Humulus* comprising three different species: *H. lupulus*,
37 *H. japonicus* and *H. yunnanensis*¹. The genus belongs to the *Cannabaceae* family, which
38 was included in the *Rosales* order since 2003.² Plants show consistent differences in
39 distinct parts of the world, therefore several subspecies have been identified.¹

40 *Humulus lupulus* L. is a dioecious herbaceous perennial plant with a climbing
41 habit, whose female inflorescences, commonly known as cones or strobiles, produce and
42 accumulate bitter substances and essential oils.³ Its unique bittering and flavouring
43 properties have led to the plant becoming a widely cultivated crop,⁴ mainly in Germany
44 and the USA.² As well as α - and β -acids contribute to the bitterness of the beer balancing
45 the sweetness of the malt, volatile compounds give the beer its characteristic hoppy
46 aroma.⁵ Different chemical substances contribute in various and complementary ways to
47 developing aromatic traits in beer: aldehydes give grassy flavours, esters give citrusy
48 flavours, etc.⁶ Each cultivar has a characteristic α -, β -acids and essential oils profile, but
49 variations are expected due to the specific environmental conditions.⁷ Indeed, factors
50 affecting hop yield and quality characteristics include temperatures and soil water
51 content;⁸ in particular, accumulation of resins is inhibited by high temperatures.⁷ In a
52 study carried out in the Czech Republic, Mozny et al.⁹ found that an increase in air
53 temperature leads to changes in the timing of the phenological stages which are earlier
54 onset, resulting in shorter intervals between them.

55 The crop requires areas with moderate temperatures and rainfalls, conditions that
56 are generally found between 45° and 50° latitude.¹⁰ Long and warm days are needed to
57 ensure a successful flowering and thus a suitable cones yield, as well as winter
58 temperatures of approximately 4 °C or less are necessary to satisfy the cold requirements
59 of the crop, which is variety dependent, for a period of one to two months.¹¹ Although the
60 utilisation of hop strobiles is almost entirely related to the brewing industry,² the earliest
61 uses were for culinary (young shoots) and medicinal purposes¹² due to their counteracting
62 effects on microbes and viruses.¹³ In the modern brewing industry, hop has become a
63 crucial element for the characterization of brewing products. As reported by Van Holle et
64 al.,¹⁴ hop brewing quality and value is associated both with cultivation area and variety.
65 Indeed, there is a growing interest in the impact that the growing area may exert on the
66 biochemical composition of the female inflorescences. The term terroir, which can be

67 defined as the set of biotic and abiotic factors related to environment influencing the crop
68 traits in a certain zone,¹⁵ commonly related to wine, cocoa, coffee, is therefore gaining
69 interest in the hop industry.¹⁴

70 Hop plant is spontaneous in many environments of Southern Italy, anyway it has
71 never been extensively cultivated in this area, and this is probably due to a less beer
72 consumption, strong competition with grape widely cultivated in all Italy and high
73 irrigation requirement of hop.¹⁶ However, nowadays the situation has changed and hop
74 cultivations are growing everywhere both for research purposes and for short-chain
75 brewing industry. As a consequence of the development of the craft beer movement
76 worldwide, the cultivation of hops has also spread to new regions far from the traditional
77 areas. The same happened in Italy, where there is still a lack of knowledge and experience
78 in the management of this crop in new environments.¹⁶ The craft beer sector has also
79 established itself in Italy as one of the most enterprising and constantly developing
80 phenomena, despite dependence on foreign countries for the supply of raw materials,
81 including hops. Since 2016, the area involved in hop cultivation in Italy has increased by
82 80 hectares, with the prevalence of small farms.¹⁷ With this in mind, the present study
83 aimed to assess the adaptability of three American hop varieties (Cascade, Chinook,
84 Comet) in the Mediterranean environment of the Calabria region (Italy) through the
85 evaluation of the morpho-biological and productive characteristics and the
86 characterization of the aromatic traits of the inflorescences.

87

88 **MATERIALS AND METHODS**

89 **Plant material and experimental design**

90 The experimental trial was conducted in 2018 in Gioiosa Ionica (Reggio Calabria,
91 Southern Italy, 38°21'12.59 "N; 16°20'42.03 "E; 195 m a.s.l.) on a sandy loam soil with
92 a good content of phosphorus, potassium and organic matter, which main characteristics
93 are reported in Table 1. Three different American hop varieties were studied: Cascade,
94 Chinook and Comet (Table 2). The hop plants were purchased from the online company
95 MRHOPS, which is specialised in potted hop seedlings. In March, the soil was milled
96 burying 6.5 t ha⁻¹ of mature manure. A 4 m high supporting structure was created,
97 consisting of wooden poles with steel wires placed at the top, forming three rows.
98 Irrigation was supplied when needed with a drip system. Seedlings were transplanted on

99 17 March, with a planting pattern of 3 m between rows and 1 m between plants, adopting
100 a randomized block experimental design with 3 replications. Weeds were managed by
101 hand. Ropes were arranged for each plant in a V-shaped structure to provide support for
102 the growth of the climbing plants. Three to four shoots per plant were trained on each
103 rope, while those in excess were not pruned. The plants did not undergo any pruning of
104 excess shoots during the growing season. The ripe cones were harvested manually in a
105 staggered manner between mid-July and mid-September, therefore dried in a desiccator
106 at 50 °C, vacuum-packed and stored at 5°C. A One-way ANOVA model with multiple
107 mean comparisons according to Tukey's (HSD) test was performed to determine
108 differences between varieties, using DSAASTAT v. 1.1 software.¹⁸ To ensure normality,
109 percentage values were previously arcsin square root transformed; in tables, percentage
110 data were reported. Meteorological data relating to the cultivation period were also
111 acquired from an ARPACAL (Regional Agency for Environmental Protection of
112 Calabria) data logger located near the experimental field. During the growing season, the
113 temperature trend increased from February to July, at which the maximum temperature
114 was recorded (37.2 °C) (Figure 1), temperature similar to the average of the maximum
115 temperatures (37.6 °C) recorded in the two decades 1999-2018. The minimum
116 temperature was instead recorded in March (6.1 °C). From March to August, rainfall was
117 equal to 161 mm, basically lower than that recorded in the two decades 1999-2018, in the
118 same period (184 mm). For what has been said above, frequent irrigation was used to
119 ensure an optimal water supply to the hop plants, a plant notoriously demanding of water.

120 **Field measurements**

121 The following measurements were carried out on plants and inflorescences of each
122 replication during the entire crop cycle: date of the main phenological phases (start of
123 flowering, appearance of the first cones and cones ripening), plant height (cm), leaf length
124 (cm) and width (cm), number of leaves per plant (on three plants for each replication),
125 number of shoots per plant, length (cm) and width (cm) of cones, fresh and dry weight of
126 the harvested cones ($t\ ha^{-1}$). Morphological measurements on leaves and cones were
127 carried out on 10 leaves and cones for each replication. Cones were picked at maturity,
128 when flowers presented a deep green colour, with a paper-like consistency to the touch,
129 well-developed yellow lupulin glands and a strong and distinct aroma.

130 **Essential oil extraction**

131 Dry plant material of each sample (50 – 100 g) has been finely ground, placed in distilled
132 water and submitted to hydrodistillation by a Clevenger apparatus as reported in the
133 European Pharmacopoeia.¹⁹ Three hydrodistillations lasting about three hours until there
134 was no significant increase in the volume of oil collected have been carried out obtaining
135 the following yields as % v/w: Cascade Gioiosa 0.80%, Comet Gioiosa 1.33%, and
136 Chinook Gioiosa 1.24%. Each oil has been treated with sodium sulphate to eliminate any
137 trace of water and stored at -20 °C until their analyses.

138 **Essential oil analysis**

139 Gas chromatographic (GC) analyses were run on a Shimadzu gas chromatograph, Model
140 17-A equipped with a flame ionization detector (FID), and with an operating software
141 Class VP Chromatography Data System version 4.3 (Shimadzu). Analytical conditions:
142 SPB-5 capillary column (15 m x 0.10 mm x 0.15 µm), helium as carrier gas (1mL/min).
143 Injection in split mode (1:200), injected volume 1 µL (4% essential oil/CH₂Cl₂ v/v),
144 injector and detector temperature 250 e 280 °C, respectively. Linear velocity in column
145 19 cm/sec. The oven temperature was held at 60 °C for 1 minute, then programmed as
146 reported previously.²⁰ Percentages of compounds were determined from their peak areas
147 in the GC-FID profiles.

148 Gas-chromatography-mass spectrometry (GC-MS) was carried out in the fast
149 mode on a Shimadzu GC-MS mod. GCMS-QP5050A, with the same column and the
150 same operative conditions used for analytical GC-FID, operating software GCMS
151 solution version 1.02 (Shimadzu). Ionization voltage 70 eV, electron multiplier 900 V,
152 ion source temperature 180 °C. Mass spectra data were acquired in the scan mode in *m/z*
153 range 40-400. The same oil solutions (1 µL) were injected with the split mode (1:96).

154 **Identification of Components of Essential Oils**

155 The identity of components was based on their GC retention index (relative to C₉-C₂₂ *n*-
156 alkanes on the SPB-5 column), computer matching of spectral MS data with those from
157 NIST MS libraries,²¹ the comparison of the fragmentation patterns with those reported in
158 the literature²² and, whenever possible, co-injections with authentic samples.

159

160 **RESULTS AND DISCUSSION**

161 **Agronomic traits**

162 *Crop cycle*

163 Among the studied varieties, since the time of planting (17 March), Cascade showed the
164 earliest vegetative growth with well-developed buds; Comet had a few hints of buds,
165 while Chinook developed them exclusively at ground level. In the following period until
166 mid-May, Cascade and Comet showed almost constant development. In contrast,
167 Chinook plants exhibited uneven and slow growth. First inflorescences have been
168 detected on Chinook variety (26 May) and in a later period on the remaining two varieties
169 (01 July). Due to the different vegetative development and consequently to the different
170 timing of strobiles differentiation, the harvest period resulted staggered among varieties
171 starting from mid-July, with Chinook being the earliest, as stated above. The other
172 varieties were harvested one month later Chinook. The varieties have shown times of
173 development and maturation of the inflorescences contrasting with what is reported in the
174 literature, probably due to the environmental conditions very different from the
175 environments in which they are traditionally grown.

176 *Morphological traits*

177 Comet and Cascade showed the greatest and the lowest plant height development
178 respectively, the former reaching an average height of 3.7 m and the latter 2.2 m (Figure
179 2). Chinook plants were in an intermediate position, reaching an average height of 3.0 m.
180 Comet showed a fast growth rate, developing the highest plant biomass due to the
181 numerous side shoots broadened by the plants.

182 On average, Cascade developed the higher number of stems per plant (8)
183 measured at the end of the growth cycle, despite not being the most productive variety.
184 Chinook and Comet, on the other hand, developed an average of 5 stems per plant.

185 Comet showed the highest number of leaves (78) per stem, followed by Cascade
186 (62) and Chinook (62), following the height development of the plants.

187 The values of leaf length and width showed some variability. Chinook plants
188 presented on average the highest values for both parameters, with a maximum value of
189 7.8 cm for length and 7.6 cm for width. Comet and Cascade plants, on the other hand,
190 presented lower values and were quite similar. In the light of the results described here,
191 it can be stated that these values are strongly related to the characteristics of the varieties.

192 At harvest, Chinook variety showed the longest average length (3.17 cm) and the
193 widest average width (1.92 cm) of flower cones (Figure 3). The lowest values were found
194 in the Cascade variety, with an average length of 2.37 cm and an average width of 1.42

195 cm. Comet developed cones with an average length of 3.14 cm and an average width of
196 1.50 cm.

197 These data are in agreement with those reported by Mongelli et al.²³, who, in a
198 characterization of 23 wild genotypes of hop from Northern Italy, found a cone length
199 ranging from 2.04 to 3.62 cm and a cone width ranging from 1.54 to 2.62 cm.

200 Considering the cone length/width ratio, the Comet variety presented the highest
201 value (2.06), while the Cascade and Chinook varieties showed a very similar average
202 value for this parameter of approximately 1.6. This ratio is an index of the shape of the
203 flower cones, where a high value corresponds to tapered cones and a lower value to squat
204 cones. Little information is reported in the literature, anyway, the three studied varieties
205 showed a different combination of the cone and leaf dimensions allow us to hypothesize
206 to use these data as useful tools to discriminate varieties.

207 *Productive traits*

208 Cone yield varied among varieties (Fig. 3). Comet proved to be the most productive
209 variety, with a dry cone production of 0.35 t ha⁻¹, as the plants developed numerous lateral
210 shoots on which numerous inflorescences were formed. The remaining varieties gave a
211 similar yield of about 0.19 t ha⁻¹.

212 It should be noted that the productions obtained are relative to the first year of
213 cultivation and that the plant increases its productivity in the years to come. Furthermore,
214 in order to carry out the morphological surveys, the plant was left to grow freely without
215 pruning the secondary stems, a circumstance that tends to reduce the vigour of the main
216 ones.

217 The varieties were not very productive when compared with the typical yields of
218 the same varieties where they are traditionally cultivated (1.5-2 t ha⁻¹).^{7,16,24,25} This should
219 not cause any perplexity, as these are productions relating to the first year of cultivation.
220 Generally, full production is obtained in the third year for European cultivars, while good
221 results are obtained in the first or second year for American ones.²⁶ Moreover, it should
222 be noted that numerous variables can affect the yield. In fact, various studies and
223 experiments conducted in Italy have shown how cultivars, cultivation techniques, soil,
224 and climatic conditions can significantly influence production.²⁶ Furthermore, it should
225 be remembered that deliberately, in order to detect the morphological characteristics, the
226 plants did not undergo any pruning of the excess stems, which certainly contributed to

227 reducing the vigour of the main stems. In fact, referring to the common management of a
228 hops grove, it is a consolidated practice to choose two or three stems for each support
229 wire, so as to promote an optimal growth density in order to obtain a high cone yield and
230 a good quality.¹

231 Experimental trials carried out in a Mediterranean environment on the agronomic
232 and qualitative traits revealed that plant productivity is strongly influenced by the cultivar
233 in a trial carried out on four varieties in the USA,^{7,27} found yields ranging from 0.55 to
234 4.68 t ha⁻¹, in relation to different deficit irrigations. On the other hand Ceh (2014) 28
235 recorded yields from 0.95 to 1.51 t ha⁻¹. Anyway, our data are in agreement with those of
236 some varieties reported by Rossini et al.³ in a trial carried out in central Italy.

237 **Essential oils**

238 Table 3 lists the 92 compounds fully characterized in the three essential oils, whereas
239 Figure 4 reports the GC profiles of the three oils. For an easier comparison of the oils the
240 components have been grouped into five classes: monoterpene hydrocarbons, oxygenated
241 monoterpenes, sesquiterpenes, diterpenes and others (not terpenoidic compounds). From
242 a general point of view concerning the essential profiles obtained in this study the hop
243 cultivated in Southern Italy is quite aligned with the hop samples cultivated in other
244 Italian areas.^{29,30}

245 In all samples monoterpene hydrocarbons is the main class ranging between 43
246 and 66 % *ca.*, with only 9 components. Myrcene is largely the main compound in all
247 cases, the other components are below 1% with the sole exception of β -*E*-ocimene which
248 reaches 2% *ca.* in the Comet sample.^{31,32} Very low is the number and quantity (8 and <
249 2%, respectively) of oxygenated monoterpenes.

250 Sesquiterpenes show a more complex and variegated composition: 31 compounds
251 have been detected and their total amount ranges between 26 and 45%, being
252 complementary to that observed for monoterpenes The cyclic sesquiterpenes β -
253 caryophyllene and α -humulene are normally the main components of hop essential oils
254 (together with the monoterpene hydrocarbon myrcene as previously mentioned). β -
255 Caryophyllene is probably the most widespread sesquiterpene in Nature, α -humulene is
256 present in many plants but not as widespread as the previous one, however, in the same
257 essential oil hop samples it can reach almost half of the total oil.^{29,33} In the case here
258 examined β -caryophyllene is one of the main components in all samples (range 5 – 10 %

259 *ca.*); much higher is the concentration of α -humulene (range 12 – 20% *ca.*), placing itself
260 in second place for quantity among all components, however, in the Comet sample this
261 compound does not reach 1%, therefore, then it can be counted a low humulene variety.³³
262 Always among the sesquiterpenes appreciable amounts are showed by β - ed δ -selinene,
263 which in Comet reach about 5%, whereas in the other two samples their content ranges
264 between 1 and 2%, respectively. Another interesting group of sesquiterpenes is
265 represented by γ - δ - and α -cadinene, which in the Chinook sample reaches appreciable
266 amounts (Table 3), whereas in the other two samples remain below 1%.

267 Finally, the class denominated ‘others’, namely, the not terpenoidic compounds,
268 ranges between 5 and 8 % of the total amount, despite being the most numerous with 42
269 components, most of them below 1%.

270 Given the important and essential role of hop in beer production, either for the
271 bitter substances and for the aromatic compounds, several researches have been carried
272 out to understand the different aromatic profiles of the hop varieties, but also to valorize
273 a particular territory and/or production area.^{32,34} The data until now collected, included
274 those coming from the present study, do not allow to establish yet if Southern Italy may
275 be considered a particularly favourable area, however, some aspects emerged also from
276 this study leave hope for the future.

277

278

279 **CONCLUSION**

280 The results obtained from the experimental trial conducted on the three varieties of
281 American hops, *Humulus lupulus* L., allowed us to draw some conclusive considerations
282 regarding their morphological, phenological and chemical characteristics displayed in
283 cultivation in the Calabrian environment. First of all, it is possible to state how hop
284 introduced into cultivation for the first time in the Calabrian coastal area showed excellent
285 adaptability to the tested environment.

286 As for the studied characteristics, a certain variability was found regarding the
287 morphological traits and the yield in flowering cones. Cone and leaf dimensions seem to
288 be interesting criteria to discriminate varieties. The pedoclimatic conditions of the chosen
289 area have certainly influenced the development of the plants, especially if we compare
290 the typical yields generally obtained in the countries of traditional cultivation of the crop

291 with those obtained in this experimental test, the latter being basically lower. In particular,
292 Comet was the most productive variety, while Chinook emerged as the earliest variety,
293 followed by Comet and Cascade.

294 The promising results obtained in this first experience on the introduction into
295 cultivation of hop in Southern Italy, laying the foundation for a widespread of cultivation,
296 and develop the craft industry with high traceability and zero km hop.

297

298 **CONFLICT OF INTEREST**

299 There are no conflicts of interest to declare.

300

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Table 1. Soil main physical and chemical traits of the experimental field.

Trait	value
Gravel >2 mm (%)	26.4
Sand (2–0.05 mm) (%)	64.5
Silt (0.05–0.002 mm) (%)	22.7
Clay (<0.002 mm) (%)	12.7
ECe (dS m ⁻¹)	0.153
pH	6.8
Total CaCO ₃ (g kg ⁻¹)	0
Active CaCO ₃ (g kg ⁻¹)	0
Organic matter (g kg ⁻¹)	22.5
Total N (g kg ⁻¹)	0.8
C/N	16.3
Available P as P ₂ O ₅ (ppm)	98
Exchangeable K as K ₂ O (ppm)	248
Exchangeable Ca as CaO (ppm)	2515
Exchangeable Mg as MgO (ppm)	288
Exchangeable Na (ppm)	5
Mg/K (meq/100 g)	2.71
CEC (meq/100 g)	17.8
BS (%)	62
ESP (%)	0.12

Table 2. Maturation, use and provenance of the studied varieties.

Variety	Maturation	Use	Origin
Cascade	Medium	Bittering/aroma	USA
Chinook	Medium-late	Bittering/aroma	USA
Comet	Medium-late	Bittering/aroma	USA

Table 3. Chemical Composition of the Three Hop Essential Oils.

#a	RI ^b	RI ^c	Class/Compound	CASCADE		CHINOOK		COMET	
				%	SD	%	SD	%	SD
Monoterpene hydrocarbons (#9)				64.16	1.32	43.42	2.32	66.13	2.11
6	934	930	α -Pinene	0.18	0.02	0.15	0.00	0.24	0.01
8	957	954	Camphene	0.04	0.00	-	0.04	0.00	
10	976	979	β -Pinene	1.03	0.06	0.62	0.01	0.93	0.02
11	993	991	β-Myrcene	62.04	1.33	41.89	2.29	61.76	2.25
12	1001	1003	α -Phellandrene	0.04	0.00	0.06	0.00	0.07	0.00
16	1029	1029	Limonene	0.59	0.01	0.56	0.04	0.80	0.02
17	1037	1035	β -Z-Ocimene	0.03	0.00	0.03	0.00	0.10	0.02
18	1048	1050	β -E-Ocimene	0.17	0.04	0.07	0.01	2.14	0.27
20	1059	1060	γ -Terpinene	0.04	0.02	0.03	0.00	0.05	0.02
Oxygenated monoterpenes (#10)				1.87	0.08	1.59	0.04	1.79	0.09
23	1098	1097	Linalool	0.37	0.01	0.34	0.01	0.56	0.02
25	1115	1119	1-Myrcenol	0.11	0.01	0.16	0.01	0.02	0.00
28	1184	1177	Terpinen-4-ol	0.04	0.01	0.02	0.00	-	
33	1240	1238	Neral	-	0.04	0.01	t		
34	1275	1267	Geranial	0.06	0.00	0.05	0.00	0.04	0.00
39	1327	1324	Methyl geranoate	0.42	0.03	0.56	0.01	0.45	0.05
44	1384	1381	Geranyl acetate	0.32	0.02	0.04	0.01	0.21	0.01
51	1474	1477	Geranyl propionate	0.16	0.02	0.05	0.01	0.34	0.02
60	1519	1515	Geranyl isobutanoate	0.39	0.04	0.33	0.02	0.17	0.01
Sesquiterpene hydrocarbons (#21)				26.61	0.13	41.50	0.22	23.72	0.15
40	1345	1351	α -Cubebene	0.03	0.00	0.02	0.00	-	
42	1377	1375	α -Ylangene	0.09	0.00	0.19	0.01	0.05	0.00
43	1381	1377	α -Copaene	0.13	0.01	0.67	0.03	0.06	0.01
45	1426	1419	β-Caryophyllene	5.28	0.26	7.97	0.50	9.47	0.68
46	1430	1432	β -Cubebene	t	0.31	0.03	t		
47	1434	1434	α -E-Bergamotene	0.12	0.01	0.04	0.00	0.05	0.00
49	1440	1456	β -E-Farnesene	0.15	0.00	0.02	0.00	0.03	0.01
50	1462	1455	α-Humulene	12.12	0.85	19.41	1.44	0.48	0.04
52	1478	1476	<i>E</i> -Cadina-1(6),4-diene	t	0.19	0.01	t		
53	1481	1480	γ -Muurolene	0.72	0.04	1.82	0.29	0.97	0.08
54	1485	1484	α -Amorphene	0.06	0.00	0.15	0.01	0.05	0.00
55	1492	1490	β -Selinene	1.78	0.10	1.43	0.04	4.79	0.39
56	1494	1495	Cadina-1,4-diene	0.25	0.07	t	t		
57	1500	1492	δ -Selinene	2.16	0.16	2.13	0.37	5.08	0.42
58	1507	1500	α -Muurolene	0.12	0.03	0.36	0.16	0.37	0.04
59	1512	1506	β -Bisabolene	0.26	0.06	0.21	0.05	0.41	0.02
61	1524	1513	γ -Cadinene	0.05	0.01	1.65	0.10	0.15	0.01
62	1526	1522	7-epi- α -Selinene	-	0.07	0.00	-		
63	1528	1523	δ -Cadinene	0.81	0.08	3.34	0.19	0.37	0.03
64	1542	1538	α -Cadinene	0.32	0.05	1.64	0.01	1.27	0.09
65	1549	1546	Selina-3,7-(11)-diene	0.27	0.04	1.39	0.01	1.15	0.10
Oxygenated sesquiterpenes (9)				3.31	0.05	2.93	0.04	1.99	0.02
66	1578	1572	Caryolan-8-ol	0.13	0.01	0.11	0.00	0.13	0.01
67	1589	1583	Caryophyllene oxide	0.67	0.06	0.21	0.08	0.14	0.01
68	1616	1608	Humulene epoxide	1.01	0.18	0.48	0.15	t	
69	1621	1619	1,10-di-epi-Cubenol	0.08	0.01	0.10	0.01	0.15	0.01

70	1635	1628	1- <i>epi</i> -Cubenol	0.05	0.00	0.13	0.01	0.05	0.00
71	1638	-	N.I.S. ^d	0.07	0.01	0.16	0.01	0.11	0.01
72	1648	1640	<i>epi</i> - α -Cadinol	0.19	0.03	0.48	0.04	0.13	0.01
73	1658	1646	α -Muurolol	t	0.05	0.00	0.10	0.01	
74	1663	-	N.I.S. ^e	1.11	0.16	1.21	0.03	1.18	0.08
			Diterpenes (#2)	0.22	0.07	0.10	0.05	0.03	0.00
75	1950	1955	<i>m</i> -Camphorene	0.03	0.00	0.05	0.02	-	
77	1974	1986	<i>p</i> -Camphorene	0.19	0.06	0.05	0.01	0.03	0.00
			Others (#27)	3.33	0.41	6.53	0.33	2.85	0.17
1	855	855	2- <i>E</i> -Hexenal	0.04	0.00	-	-		
2	867	875	2-Methylbutyl acetate	-	0.02	0.00	0.05	0.01	
3	899	905	Heptanal	0.03	0.00	0.02	0.00	0.03	0.00
4	911	911	Isobutyl isobutyrate	0.03	0.00	0.09	0.01	0.04	0.00
5	923	927	Methyl hexanoate	0.03	0.00	0.06	0.01	0.03	0.00
7	939	939	Allyl isovalerate	t	-	0.03	0.00		
9	970	966	Isoamyl propionate	0.13	0.02	0.49	0.07	0.17	0.01
13	1010	1009	Isoamyl isobutyrate	0.07	0.00	0.32	0.02	0.06	0.01
14	1014	1015	2-Methylbutyl isobutyrate	0.16	0.01	0.92	0.07	0.20	0.01
15	1023	1025	Methyl heptanoate	0.19	0.01	0.14	0.01	0.17	0.01
19	1054	1063	2,3,6-trimethyl-1,5-Heptadiene	0.04	0.00	0.02	0.00	0.02	0.00
21	1086	1087	methyl 6-Methylheptanoate	0.13	0.02	0.41	0.08	0.25	0.02
22	1092	1090	2-Nonanone	0.19	0.02	0.03	0.01	0.12	0.00
24	1101	1100	Nonanal	0.18	0.03	0.17	0.01	0.09	0.03
26	1124	1127	Methyl octanoate	0.11	0.01	0.16	0.02	0.10	0.01
27	1179	1192	2-Decanone	0.05	0.00	0.03	0.00	0.08	0.03
29	1192	1229	Methyl nonanoate ^f	0.06	0.01	0.04	0.01	0.27	0.01
30	1195	1192	Dodecane	0.03	0.01	0.31	0.05	t	
31	1204	1201	Decanal	0.03	0.00	0.02	0.00	-	
32	1225	1223	Methyl 4-nonenoate	0.09	0.01	0.13	0.00	0.11	0.00
35	1280	1271	2- <i>E</i> -Decen-1-ol	0.06	0.01	0.05	0.00	0.11	0.00
36	1312	1289	Methyl 4-decenoate ^f	0.42	0.08	0.92	0.12	0.62	0.06
37	1290	1294	2-Undecanone	0.04	0.01	-	0.08	0.01	
38	1313	1323	Methyl caprate	0.03	0.01	0.20	0.05	0.03	0.00
41	1367	1383	2-Dodecanone	0.04	0.00	0.06	0.02	0.02	0.00
48	1440	1410	3- <i>Z</i> -Decen-1-ol acetate ^f	0.15	0.03	t	0.06	0.01	
76	1952	1959	Hexadecanoic acid	0.21	0.04	0.08	0.01	-	
78	2650	2682	Lupulon	0.20	0.12	1.03	0.22	0.11	0.01
			<i>α-Humulene/β-Caryophyllene</i>	2.29	0.09	2.43	0.03	0.05	0.00

^aThe numbering refers to elution order, and values (relative peak area percent) represent averages of 9 determinations (t = trace, < 0.05%), ^b Retention index (RI) relative to standard mixture of n-alkanes on SPB-5 column; ^c Literature Retention Index (RI); ^d N.I.S. = Not Identified Sesquiterpene MW: 222 C₁₅H₂₆O (m/z = 43, 67, 79, 93, 109, 135, 164, 204); ^e N.I.S. = Not Identified Sesquiterpene MW: 222 C₁₅H₂₆O (m/z = 43, 59, 81, 105, 119, 133, 161, 179, 204); ^f tentatively identified.

Figure captions

Figure 1. Meteorological trend of 2018 at the experimental station of Gioiosa Jonica (RC).

Figure 2. Plant height, number of stems per plant, number of leaves per plant, leaf length and leaf width (\pm Standard Error) of the three studied varieties during the crop cycle.

Figure 3. Flower cones length (Cm), width (Cm), length/width ratio and yield ($T\ ha^{-1}$) of the studied varieties. Different letters indicate significative differences ($P < 0.01$) between varieties.

Figure 4. GC profiles of Cascade, Chinook and Comet Hop essential oils (For numbering see [Table 3](#)).







