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Effects of biodynamic production on growth and essential oil content in basil

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Abstract – The effects of a biodynamic sowing calendar on the growth (plant height, fresh herb yield, nodes number) and quality (percentage of leaf mass, essential oil content) of three basil species, *Ocimum americanum* L., *Ocimum* × *hybrida* and *Ocimum basilicum* L., represented by the cultivars 'Rosso' and 'Eco Genovese', were tested. Statistical analyses showed that the species had greater impact on the observed parameters than either the sowing date or the species and sowing date in combination. The species showed a significant influence on all five tested parameters, while sowing date and interaction of both factors significantly influenced plant height, leaf percentage and essential oil content. The best yield per plant was obtained for *O.* × *hybrida* and *O. basilicum* 'Eco Genovese'. The lowest species *O.* × *hybrida* produced the highest amount of essential oil. »Nodes number« parameter most clearly separated the species, but not the cultivars. Even though *O. americanum* gave the tallest plants, it did not yield either the highest amount of fresh herb or essential oil. This species' height was most consistent, considering the significant impact of biodynamic rhythm. Sowing date was not crucial for basil fresh yield; however if there is a need for taller plants with a higher percentage of leaf mass and more essential oil, sowing date needs to be controlled.

Key words: biodynamic production, essential oil, *Ocimum americanum* L., *Ocimum basilicum* 'Eco Genovese', *Ocimum basilicum* 'Rosso', *Ocimum* × *hybrida*, sowing calendar

Introduction

The genus *Ocimum* belongs to the large botanical family Lamiaceae which includes a high number of aromatic plants, well-known herbs and ornamental plants grown worldwide. Sweet basil (*Ocimum basilicum* L.) is the most widely grown *Ocimum* species in the world for the fresh market, but also for essential oil production (Zheljazkov et al. 2008). Sweet basil essential oil is used in medicine, for aromatherapy, and the cosmetic and food industries (Putievsky and Galambosi 1999), and as an insecticide (Umerie et al. 1998, Keita et al. 2001, Pascual-Villalobos and Ballesta-Acosta 2003). Due to its insecticide effects, sweet basil is also used in intercropping, for example with cotton (Shader et al. 2005). After more than 200 analyses of essential oils isolated from *O. basilicum* L., Lawrence (1988) classified essential oils of sweet basil into four main chemotypes: methyl chavicol-rich, linalool-rich, methyleugenol-rich, methyl cinnamate-rich, and also found numerous subtypes. *O. americanum* is not often used as a culinary herb, unlike the related *O. basilicum*, but more often as a medicinal plant (Vieira et al. 2003). The essential oils in this species have strong fungicidal activity (Dubey 1991), and the leaves have been used as an insecticide against postharvest damage (Weaver et al. 1991).

On the global market of agricultural products, organic, safe, high quality agricultural products, including herbs and spices, achieve the best prices. Organic agricultural production implies, in general, production without easily soluble fertilizers and chemical pesticides. According to EC Reg. 834/2007 it combines best environmental practices, a high level of biodiversity, the preservation of natural resources and the application of high animal welfare standards. The objectives of organic plant production are to work within natural systems and cycles, to maintain soil fertility, use renewable resources and to produce safe, nutritious, wholesome food (Mikkelsen 2008).

The concept of biodynamic agricultural production, devised by Rudolf Steiner (1924), respects the principles of organic production, supplemented by the use of biodynamic

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preparations (Demeter International Production Standards 2005) and implies the use of a strict biodynamic sowing and planting calendar (Wistinghausen et al. 1998). Namely, it has been proven that time of sowing has a significant effect on plant growth or yield (Ejimofor Ogbonna et al. 2012, Ehsanullah et al. 2014). Biodynamic preparations include strictly prescribed herbal and mineral additives and extracts for plant conditioning and compost activation, biostimulants and organic fertilizers, manures and composts (Wistinghausen et al. 1998). The use of biodynamic preparations in organic production is also allowed by EU regulation (Council Reg. 834/2007).

Organic production in Croatia is a small, but growing, branch of food production; it occupies about 2.5% (Ministry of Agriculture, 2013) of all farmland and is subsidized by the Ministry of Agriculture. Biodynamic production, however, is not yet recognized as very promising. The aim of this study was to test the effect of four sowing dates recommended in the biodynamic sowing and planting calendar (Thun and Thun 2011) on the growth and essential oil content of three basil species (including two cultivars of one species) grown in pots for fresh seasoning herb. For that purpose we screened: 1) plant height, 2) number of nodes, 3) yield of fresh herb, 4) percentage of leaves mass, and 5) essential oil content of *O. basilicum* 'Rosso', *O. basilicum* 'Eco Genovese', *O.* × *hybrida* and *O. americanum*.

Material and methods

The practical experiment was conducted in summer 2012 with different basil species/cultivars (X): two cultivars of sweet basil, *O. basilicum* 'Rosso' and 'Eco Genovese', bushy basil *O.* × *hybrida*, and the citral type basil *O. americanum* L. The experiment was conducted in a plastic tunnel and on an open area of the Institute of Agriculture and Tourism in Poreč. The organic seeds used in the experiment were supplied for *O. basilicum* 'Rosso' and 'Eco Genovese' by Amarant (Slovenia) and for *O.* × *hybrida* and *O. americanum* by Reinsaat (Austria).

Four species/cultivars were tested and each one was sown on four different sowing dates. Sowing was done under the usual climatic conditions for this geographic area and period (monthly average temperatures were for June 23.1 °C, July 26.1 °C, August 25.1 °C; total monthly precipitation was for June 18.6 L m^{-2}, July 2.2 L m^{-2}, August 3.8 L m^{-2}) (Croatian Meteorological and Hydrological Service, 2012). Basil was sown in containers filled with standard substrate, Klasmann 1 (Producer: Klasmann-Deilmann, Germany) on four different sowing dates (Y) chosen according to the biodynamic rhythm sowing scheme (Thun and Thun 2011), recommended for leafy plants in the Mediterranean climatic zone: (1) June 7th 2012 after 1.00 pm – suitable for root plants; (2) June 8th 2012 after 6.00 pm – suitable for sowing of flowering plants; (3) June12th 2012 after 8.00 am – suitable for sowing of leafy plants; (4) June 14th 2012 after 5.00 pm – suitable for sowing of fruity plants. From the scientific viewpoint, these sowing periods will be evaluated as different sowing dates with minimal timings.

At the 4 true leaf stage, basil plants were transplanted into pots (644 mL volume) in Klasmann 2 (Producer: Klasmann-Deilmann, Germany) standard substrate. Pot basil was placed in an open area. Irrigation with tap water was done daily, fertilisation twice after transplantation with 2.0 dL per pot of 1.0% standard organic fluid fertiliser Bio Plantella (Producer: Unichem Agro Ltd). Every treatment was represented in three repetitions with 15 pots per repetition.

Four species/cultivars (X) were tested and each one was sown in four different sowing dates. Species and cultivars with the same sowing date began to flower homogenously, with only 1–2 days difference. The duration of the cultivation was considered as a period in days from sowing to harvest, where the first day was the sowing day and the last day was the day of harvest. It lasted on average between 44 and 54 days, depending on the sowing date (Tab. 1). The shortest-lasting cultivation period was for the basil sown on June 7th and June 8th, and the longest for the basil sown on June 12th and 14th.

Tab. 1. Biodynamic calendar of basil.

Sowing date	Germination (days after sowing)	Transplanting into pots (days after sowing)	Flowering beginning (days after sowing)	Harvesting date
June 7th	4	22	44	July 23rd
June 8th	4	23	45	July 23rd
June 12th	4	20	52	August 8th
June 14th	4	22	54	August 8th

The plant height, number of nodes per main stem, fresh yield per plant, leaf mass percentage and the essential oil content were measured. The harvest of basil was done at the beginning of the flowering period, at the time of the appearance of the first flowers in 10% of plants. Measurements of fresh herb were made just before harvesting. Fresh herb was then air dried in a shady and airy place in standard room conditions. After drying, leaves were separated from the stems; the leaf-to-stem ratio presents the value obtained from the mass of dried leaves divided by the mass of dried stems. Plant height was defined as the length of the main stem in cm and the number of nodes was determined by counting from the first fully developed leaves in the basal part of the main stem until the last fully developed and visible nodes. Leaf mass percentage was determined as the percentage of leaves in the whole mass of dried herb. Essential oil content was expressed in ml per 100 g of leaf dried weight.

The essential oil was distilled with the Neo-Clevenger apparatus, using the method described in the European Pharmacopeia 5.0 (2005). Each sample, represented by three repetitions, was distilled twice.

Experimental data were statistically analysed using factorial (two way) ANOVA and post hoc Tukey test for multiple comparisons between groups with $p \leq 0.05$.

Tab. 2. Impact of analysed factors (species/cultivar (X) and sowing date (Y)) and their interaction (X×Y) on quantitative and qualitative plant parameters (two-way ANOVA). The numbers denote squared total value of the parameter variation caused by species/cultivar, sowing date or their interaction. *significant with p ≤ 0.05, DF – degrees of freedom.

Source of variation	DF	Mean square (s^2)				
		Plant height (cm)	Nodes number	Yield (g per plant)	Percentage of leaves mass	Essential oil content (mL per 100 g DW)
Species/cultivar (X)	3	5320.2*	60.4*	245.1*	245.9*	2.3*
Sowing date (Y)	3	1704.5*	1.3	22.6	197.3*	0.1*
X×Y	9	342.5*	3.2	26.0	120.0*	0.2*
Error	222	24.5	2.0	18.4	1.8	0.0

Results

Statistical analysis of tested factors throughout all observed parameters showed that the largest source of variation in all parameters was the species, which is the factor with the largest impact (Tab. 2). Plant height, percentage of leaf mass and essential oil content were significantly affected not only by the species/cultivar, but also by the sowing date (Y) and by the interaction between the species/cultivar and the sowing date (X×Y).

Shortly before flowering, basil formed on average between 8.0 and 10.2 nodes per main stem, depending on the species and cultivar (Tab. 3). The highest number of nodes was observed in *O.* × *hybrida,* which formed significantly more nodes than other species/cultivars tested. The highest number of nodes and the lowest plant height were found in *O.* × *hybrida*, indicating its very short internodes i.e. its compact, bushy habitus. The lowest number of nodes was found in *O. basilicum* cultivars. The number of nodes was not influenced by sowing date, or by interaction of species/cultivar and sowing date.

The fresh yield of basil ranged between 12.5 and 17.2 g/plant. *O.* × *hybrida* had a significantly higher yield than *O. americanum* or *O. basilicum* 'Rosso' (Tab. 3). The second best cultivar in terms of fresh yield was *O. basilicum* 'Eco Genovese'. The yield per plant was not influenced by sowing date or by interaction of species/cultivar and sowing date.

In Fig. 1, the parameters influenced by both species/cultivars and sowing date, as well as their interaction, are presented.

The height of basil plants at the beginning of flowering was between 18.1 and 38.3 cm (Fig. 1). This large range is a result of the genetic potential of species/cultivars and the different sowing dates, but is also, as the statistical analysis showed, impacted by the interaction between these two factors (Tab. 2). *O. americanum* dominated in growth. At the last sowing date, it was significantly higher than all the other species and cultivars. Additionally, at all sowing dates, *O. americanum* was significantly taller than *O.* × *hybrida.*

Leaf proportion in dried herbs ranged between 45.6 and 73.1% (Fig.1). Significantly the highest leaf mass proportion was found in *O. basilicum* 'Rosso' sown on June 7th, and the lowest in 'Eco Genovese' sown on June 8th.

The most pronounced differences among species/cultivars were found for essential oil content (Fig. 1), which was predominantly influenced by the species (Tab. 2), and to a lower extent by the sowing date, and by the interaction of both factors. The essential oil content of *O.* × *hybrida* was significantly higher than that of any of the other species/cultivars with the same sowing dates, while the lowest essential oil content was found in *O. basilicum* cultivar 'Rosso'. The *O.* × *hybrida* of the last sowing date reached maximum essential oil content.

Tab. 3. Influence of analysed factors (species/cultivar (X) and sowing date (Y)) and their interaction (X×Y) on quantitative plant parameters. *significant with p ≤ 0.05. Different letters in the same column indicate significant difference. SD – standard deviation.

Factor	Nodes number ± SD	Yield of fresh herb (g per plant) ± SD
Species/cultivar (X)		
O. basilicum 'Rosso'	8.3±0.6 c	12.5±1.0 c
O. basilicum 'Eco Genovese'	8.0±0.9 c	15.8±0.9 ab
O. × *hybrida*	10.2±1.0 a	17.2±0.8 a
O. americanum	9.5±0.7 b	14.6±0.6 b
Sowing date (Y)		
June 7th 2012 after 3.00 pm	9.1±1.2 a	15.1±1.1 a
June 8th 2012 after 8.00 pm	9.1±0.6 a	14.1±1.0 a
June 12th 2012 after 9.00 pm	8.9±0.9 a	15.5±2.0 a
June 14th 2012 after 5.00 pm	8.9±1.0 a	15.4±0.8 a
ANOVA, Tukey test		
X	*	*
Y		
X×Y		

Discussion

Conventional farming is being progressively replaced by more ecology-friendly and sustainable organic agricultural production. Basil is grown worldwide for seasoning and for essential oil extraction.

For culinary purposes, fresh basil leaves are used; harvesting takes place at the period with the best leaf/stem proportion (over 1.0), mostly reached shortly before or at the beginning of flowering. For essential oil extraction, basil is harvested in full bloom, because then it has the highest

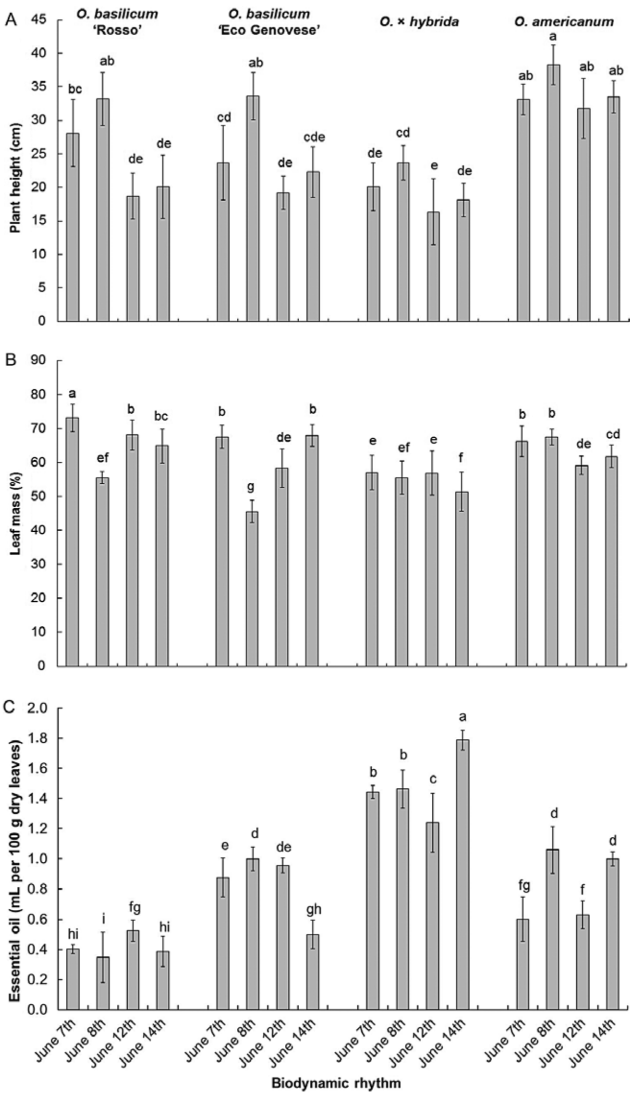

Fig. 1. Plant parameters influenced by both species/cultivar and sowing date, as well as their interaction: A) height, B) leaf mass and C) essential oil. Data are the means of three replicates ± SD (standard deviation). The means labelled by different letters are significantly different (two way ANOVA and Tukey's test), $p \leq 0.05$.

essential oil content (Sifola and Barbieri 2006). Sifola and Barbieri (2006) investigated the cultivation of three basil cultivars, which lasted from the end of May to mid-June. They harvested basil 49 days after transplanting, in the full bloom stage. The full bloom stage, as mentioned above, is considered to be the most appropriate for commercial harvesting of basil for essential oil production. These two authors report the branching of basil plant, a parameter comparable to the number of nodes per main stem. Higher bud and node numbers can be an indicator of branching formation potential and yield. In their experiment, basil formed between 9.3 and 9.8 branches. This is in conformity with our determined numbers of nodes of between 8.0 and 10.2 per main stem in pot basil (Tab. 3). The range in numbers of nodes in pot basil is affected mostly by the characteristics of the species/cultivar, which is also in accordance with our result. Additionally, we observed that node number significantly depended on the tested species, but not on the cultivars.

In the study of Frąszczak et al. (2011) on two basil cultivars grown in small pots in different daily light regimes and different temperatures, a significant influence of the cultivar, temperature and photoperiod on the monitored parameters was found. These results are in accordance with ours, where the species, i.e. genetic factor, was confirmed as the main significant factor that influenced the monitored parameters. In our experiment, the differences between species/cultivars of basil were much more expressed, thus clarifying the intensity of species/cultivar influence on the monitored parameters. Strong cultivar influence on the yield of basil was also confirmed by Politycka and Gołcz (2004), who confirmed that the yield of dry leaves of green basil cultivars was up to 100% higher than that of cultivars with anthocyanin.

We further detected that sowing date itself, as well as the interaction of species/cultivar and sowing date, significantly affected the plant height, leaf share and the essential oil content of basil, but not the number of nodes and overall yield (Tab. 2).

The quality of herbs is, among other factors, influenced by the proportion of leaves and stems (Werker et al. 1993, Ioannidis et al. 2002), as well as by essential oil content (Rey and Saez 2002). A higher proportion of leaves than stems represents a higher quality, as the concentration of oil glands is the highest in mature leaves (Werker et al. 1993, Ioannidis et al. 2002) and in basil, leaves and calyxes have the highest concentration of oil glands (Putievsky and Galambosi 1999). The leaf proportion in our experiment is analogous to the leaf-to-stem ratio used by Sifola and Barbieri (2006). The leaf-to-stem ratio in their investigation varied between 0.7 and 1.2. All values over 1.0 mean that the amount of leaves is over 50%. The percentage of leaf mass in our investigation depended on the species/cultivars, but it was also influenced by the sowing date and the interaction of species/cultivar × sowing date (Tab. 2). Only in *O. basilicum* 'Eco Genovese', which was sown on June 8th (second sowing date), was the percentage of leaves under 50%, while all other combinations of species/cultivars and sowing dates had leaf percentages of over 50%.

Essential oil content, as the second quality aspect, in four basil species and cultivars, ranged between 0.4 and 1.8 mL per 100 g dried leaves, depending on the species/cultivar and sowing date (Fig. 1). The species with the highest amount of essential oil in this study was *O.* × *hybrida*, while *O. basilicum* 'Rosso', as expected, had the lowest amount. Interestingly, even though *O.* × *hybrida* had the least plant height, it contained the highest amount of essential oil, indicating that internodes negligibly contribute to essential oil production. *O. basilicum* 'Rosso' is a cultivar for decorative purposes; it is purple basil with high anthocyanidin content. Compared to the essential oil content in *O. basilicum* cultivars from the experimental garden in Prague (Klimánková et al. 2008), our *O. basilicum* 'Rosso' is most similar to the Czech cultivar V, while *O. basilicum* 'Eco Genovese' is more similar to the Czech cultivars I–IV.

Based on the obtained data we conclude the following: 1) species had greater impact on the observed parameters than sowing date or species and sowing date in combination; 2) three parameters – plant height, percentage of leaf mass and essential oil content – were also significantly affected by the sowing date and the interaction between species/cultivars and sowing date; 3) the best yield per plant was given by *O.* × *hybrida* and *O. basilicum* 'Eco Genovese'; 4) the highest production of essential oil was in the lowest species *O.* × *hybrida* indicating that *Ocimum* stem negligibly contributes to the essential oil production. The lowest essential oil content was confirmed in the *O. basilicum* purple cultivar 'Rosso'; 5) the percentage of leaf mass was over 50.0% in all cases, except in *O. basilicum* 'Eco Genovese', sown on June 8th, with 45.6%; 6) the most clear species separation was according to the »number of nodes« parameter, while the cultivars did not differ; 7) even though *O. americanum* gave the tallest plants, it did not yield the highest amount of fresh herb or essential oil; 8) the species with the most consistent height, considering the significant impact of biodynamic rhythm, was *O. americanum*; 9) finally, if we were interested in yield only, then the sowing date would not be crucial, however if we needed higher plants with a higher percentage of leaf mass and more essential oil (i.e. more leaves) then the sowing date should be controlled.

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