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EVALUATING CHEMICAL AND THERMAL WEED SUPPRESSION IN LEMON BALM (*Melissa officinalis* L.) CULTIVATION

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ABSTRACT

The usefulness of 3 herbicides and flame weeding in lemon balm sown directly into the field was assessed in a two-year experiment. Glufosinate-ammonium (600 g·ha^{-1}) and flame weeding (90 kg propane·ha^{-1}) were applied 12–13 days after lemon balm sowing, i.e. shortly after weed emergence and 4 days before crop emergence. Bentazon (960 g·ha^{-1}) and fluazifop-P-butyl (150 g·ha^{-1}) were sprayed approximately 3 weeks after sowing, during the emergence of lemon balm, and when the weeds were in the cotyledon – 2–4 true leaves stage. All of the studied weed control methods significantly reduced the number and fresh weight of weeds growing 4 weeks after lemon balm sowing. The most effective method was spraying with glufosinate-ammonium, which controlled 69–76% of weeds. The efficiency of flame weeding was slightly lower. Bentazon caused slight, temporary chlorosis of some lemon balm cotyledons. Content of essential oil (1.9–2.1%), its composition and content of rosmarinic acid (2.08–2.44%) in lemon balm leaves, as well as content of total nitrogen (2.18–2.55%), phosphorus (0.30–0.32%), potassium (2.94–3.22%), calcium (1.02–1.60%), and magnesium (0.30–0.32%) in lemon balm raw material were independent of the weeding method. Content of essential oil, phosphorus, potassium and calcium were significantly higher in the dryer year. Studied weed control methods proved useful in the cultivation of lemon balm from direct sowing into the field. Methods with total action (flaming and glufosinate-ammonium) were more effective than those with selective herbicides (bentazon and fluazifop-P-butyl).

Key words: bentazone, fluazifop-P-butyl, glufosinate-ammonium, flame weeding, essential oil, rosmarinic acid, macroelements

INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is a perennial plant from the Lamiaceae family reaching 1 m in height, native to the Mediterranean Basin and Western Asia, and now cultivated worldwide [Seidler-Łożykowska et al. 2015]. Valued in medicine, cuisine, and the perfume and cosmetics industry today [Nurzyńska-Wierdak 2013], it was used already by ancient Greeks and Romans over 2000 years ago [Koch-Heitzmann and Schultze 1988]. In Poland, lemon balm was probably introduced by Italian monks in the Middle Ages [Rumińska 1983]. It is currently cultivated on an area of 5000 ha [Seidler-Łożykowska et al. 2015] and is among the most important herbs in the country [Newerli-Guz 2016]. Lemon balm has adapted well to the Polish climate, although it can freeze in snowless and cold winters [Seidler-Łożykowska et al. 2015]. The yield of lemon balm fresh herb depends on the environmental conditions, cultivation method, and age of plantation. In Poland, in the first year of growing, it usually ranges from approximately 700 to

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1500 g·m^{-2} [Politycka and Seidler-Łożykowska 2009, Seidler-Łożykowska et al. 2015]. In an experiment conducted by Dzida et al. [2015], lemon balm cultivated from transplants and harvested at the beginning of flowering reached an average height of 28.3 cm, and produced an average yield of fresh herb of 830 g·m^{-2}. In Turkey, an average plant height of 11 different populations of lemon balm grown from transplants, measured at the beginning of flowering just before every cutting during two- or three-year cultivation period, varied depending on the habitat conditions from 10.6 to 55.8 cm, and the yield of fresh herb ranged from 5.4 to 343.3 g·m^{-2} [Sari and Ceylan 2002].

Lemon balm is seeded directly to the field, or grown from transplants [Rumińska 1983]. Direct seeding is an easier and less energy consuming method, however weed control in such a crop requires considerably more work [Sadowska 2019]. The seeds are seeded in April, and germination takes up to 2–3 weeks, making the crop very susceptible to infestation with early germinating weeds [Król 2018]. In an experiment conducted by Kucharski and Mordalski [2007], coriander (*Coriandrum sativum* L.), summer savory (*Satureja hortensis* L.), and sweet basil (*Ocimum basilicum* L.), which, like lemon balm, had long period of emergence, completely died out in the absence of weeding. Little information is still available concerning weed control in the cultivation of lemon balm. In Poland, several selective post emergence herbicides of different mode of action are recommended for this purpose [Korbas 2020], among others bentazon controlling many broadleaved weeds, and fluazifop-P-butyl which controls only grasses [Krawiec et al. 2019]. Bentazon is a foliar applied and readily absorbed herbicide with minimum basipetal translocation. Established plants also absorb the herbicide from the soil into the roots, and translocate it throughout the plant [Senseman 2007]. Its mode of action is inhibition of photosynthesis at photosystem II [HRAC 2020] and its average field half-life is 12–45 days depending on the environmental conditions [Praczyk and Skrzypczak 2004]. In a two-year field experiment conducted by Krawiec et al. [2019], bentazon applied at a dose of 960 g·ha^{-1} in the second year of lemon balm cultivation was effective against annual dicotyledonous weeds, and selective for the grasses as well as for the crop. Fluazifop-P-butyl is rapidly absorbed into the leaves, then slowly translocated in the symplasm and finally accumulated in meristematic regions of the root and shoot [Senseman 2007]. It inhibits the action of acetyl-CoA carboxylase [HRAC 2020] and its average field half-life is less than 4 weeks [Praczyk and Skrzypczak 2004]. In a field experiment carried out by Kordana et al. [2002], fluazifop-P-butyl applied at doses of 112.5–225.0 g·ha^{-1} and 375.0–450.0 g·ha^{-1} was very effective in controlling barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) up to the tillering stage, and couch grass (*Elymus repens* (L.) Gould) at the 4–6 leaves stage, respectively, as well as selective towards pale purple coneflower (*Echinacea pallida* Nutt.), ribwort plantain (*Plantago lanceolata* L.), summer savory (*Satureja hortensis* L.), and variegated thistle (*Silybum marianum* Gaertn.). Similarly, Krawiec et al. [2019] stated its good effectiveness at a dose of 150 g·ha^{-1} in the control of barnyardgrass and couch grass, as well as its selectivity against annual meadow grass (*Poa annua* L.) and two-year old lemon balm plants.

Weeds usually germinate earlier than lemon balm and many other herbs. Therefore, weed killing just before crop emergence using total herbicides e.g. diquat [Kucharski and Mordalski 2007], glufosinate-ammonium, or glyphosate [Kordana et al. 1997] is of great importance. Among the mentioned preparations, glufosinate-ammonium is much less toxic than diquat [Praczyk and Skrzypczak 2004] and does not raise such controversy as glyphosate [Myers et al. 2016]. It is a nonselective foliar herbicide of limited movement in the xylem or phloem [Senseman 2007], inhibiting the action of glutamine synthetase [HRAC 2020]. Little to no glufosinate-ammonium is absorbed through the roots under field conditions because of rapid microbial breakdown [Senseman 2007] and its average field half-life varies from 6 to 23 days [Praczyk and Skrzypczak 2004]. In a three-year study conducted by Kordana et al. [1997], glufosinate-ammonium used at a dose of 600 g·ha^{-1} during weed emergence and before lemon balm emergence controlled weeds very well, and caused no damage to the crop. Moreover, its residues in lemon balm raw material were less than 0.01 mg·kg^{-1}, and did not exceed the level specified by the standards.

Herbicides influence many physiological and biochemical processes in plants [Ashton and Crafts 1981, HRAC 2020], although little information is still available concerning their influence on the production and composition of essential oils produced by oil-bearing plants. According to Zheljazkov et al. [1996], herbicides may increase plant secondary metabolites synthesis and accumulation in some crops. Pank [1992] stated no effect of 6 herbicides on the content of geranial, neral and d-citronellal in lemon balm oil. Similarly, Kucharski and Mordalski (2007) reported no effect of linuron on the content of essential oil in the herb of summer savory and in the fruit of coriander, as well as no effect of diquat on the content of essential oil in the herb of sweet basil. Other authors have shown varied effects of herbicides depending on their type as well as the species of herbal plant [Pank 1992, Zheljazkov et al. 1996, Singh et al. 2011, Zhalnov and Zheljazkov 2016]. No such information is still available regarding bentazon, fluazifop-P-butyl, and glufosinate-ammonium.

An alternative method to kill weeds just before emergence of slowly germinating medicinal plants under organic cropping systems is flame weeding [Carrubba and Militello 2013, Knežević 2016]. The direct effect of flames on the exposed plant tissues involves denaturation and aggregation of cell membrane proteins, causing an increase in cell permeability, and finally death [Ascard 1995]. In the studies conducted by Ascard [1995], weed species with unprotected growing points and thin leaves such as common lambsquarter (*Chenopodium album* L.), common chickweed (*Stellaria media* (L.) Vill.), and annual nettle (*Urtica urens* L.) were killed completely when the plants had 0–4 true leaves, at propane doses of 20–50 kg·ha^{-1}. Species with protected growth points such as shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) and pineappleweed (*Chamomilla suaveolens* (P.) Rydb.) were tolerant due to regrowth after flaming, and they could be completely killed only in the early stages. Annual meadow grass could not be completely killed with a single flame treatment, regardless of the developmental stage or propane dose. Propane doses of 10–40 kg·ha^{-1} were required to achieve 95% control of plant numbers for sensitive species with 0–4 true leaves, whilst plants with 4–12 leaves required 40–150 kg·ha^{-1}. In the studies carried out by Knežević et al. [2009, 2014a, 2014b], propane doses of 60–80 kg·ha^{-1} provided over 90% control of major broadleaf species at early growth stages, among others common lambsquarter and redroot pigweed (*Amaranthus retroflexus* L.), as well as 80% control of grasses, among others barnyardgrass, green foxtail (*Setaria viridis* (L.) Beauv.) and yellow foxtail (*Setaria glauca* (L.) Beauv.). According to Storeheier [1994], round burners under a shield are more effective than flat and open ones. So far, flaming proved to be a promising method in cultivation of 2 perennial (*Lavandula officinalis* L., *Salvia* L. sp.) [Martini 1996] and 3 annual (*Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Plantago psyllium* L.) [Carrubba et al. 2009, Carrubba and Militello 2013] herbal plant species.

The pharmacopoeial raw material of lemon balm is the leaf (*Melissae folium*) and the herb (*Melissae herba*), harvested at the beginning of flowering, and dried in natural conditions or in a drying room at 35°C. The main biologically active component of the dried raw material is essential oil [URPL 2017]. The biosynthesis and quantitative composition of the oil depend among others on the genetic characteristics of the plant [Sari and Ceylan 2002], the environmental conditions in which it is grown [Sari and Ceylan 2002, Patora et al. 2003, León-Fernández et al. 2008], distillation method [Khalid et al. 2009], and particularly on drought stress [Abbaszadeh et al. 2009, Radácsi et al. 2016, Gorgini Shabankareh et al. 2017]. The results of research conducted in Poland showed large fluctuations in content of essential oil in lemon balm raw material. In a two-year study carried out in 3 places by Klimek et al. [2000], the content of this component in dry lemon balm leaves harvested at different stages of plant development ranged from 0.08 to 0.22 ml·100 g d.w. (dry weight). In studies by Patora et al. [2003], the content of essential oil in sixteen samples of lemon balm dry herb or dry leaves harvested before or after blooming, originated from different parts of Poland, ranged from 0.060 (herb) to 0.233 (leaves) ml·100 g d.w. In a two-year field experiment conducted by Politycka and Seidler-Łożykowska [2009], the dry herb without stems obtained from one- and two-year old lemon balm plants cultivated from transplants in the central-western part of the country contained an average of approximately 0.22% of essential oil. In another two-year field experiment conducted by Dzida et al. [2015] in the central-eastern part of Poland, content of essential oil in dried leaves of lemon balm cultivated from transplants was in a range of 0.20–0.21%.

The mean content of essential oil in the dry herb of two- and three-year-old plants harvested in the full flowering period under organic and conventional cultivation in four locations during a three-year field experiment by Seidler-Łożykowska et al. [2015] ranged from 0.120 to 0.150 ml·100 g^{-1} d.w., whereas the differences were non-significant.

León-Fernández et al. [2008] identified 73 compounds in oil produced by plants grown in the field, whereas the most abundant compounds included geranial (39.0%), neral (30.4%), caryophyllene oxide (12.1%), geranyl acetate (5.3%), 6-methyl-5-hepten-2-one (3.8%), linalool (1.6%), (*E*)-caryophyllene (1.5%), trans-p-mentha-1(7),-8-dien-2-ol (1.2%), geraniol (1.0%), and 64 other compounds, each representing less than 1.0% of the oil. Before that, Pino et al. [1999] found 28 compounds in oil obtained from plants grown in the same natural conditions. The major compounds of this oil were also geranial (41.0%) and neral (29.9%), with caryophyllene oxide (5.3%), geranyl acetate (4.4%), 6-methyl-5-hepten-2-one (2.5%), citronellol (0.8%), citronellal (0.2%), 14-hydroxy-9-epi-(*E*)-caryophyllene (0.1%), and 10 other identified compounds present in much smaller amounts. In studies conducted by Khalid et al. [2009], essential oil produced by lemon balm plants cultivated in a greenhouse contained 46 compounds with citronellal constituting: 23.3% of the oil, citronellol – 16.0%, linalool – 12.2%, α-terpinene – 9.7%, limonene – 9.3%, (*E*)-rose oxide – 6.7%, nerol – 4.1%, α-phellandrene – 2.6%, 3-octanol – 1.75%, nerol – 1.1%, geraniol – 1.0%, and 35 other components, each constituting less than 1.0% of the oil on an average for 2 harvests and 3 distillation methods. Lemon balm oil is characterized by a high ratio of total monoterpene aldehydes (geranial, neral, citronellal) to total monoterpene alcohols (geraniol, nerol, and citronellol), and its composition is subjected to significant fluctuations caused by many factors [Klimek et al. 2000, Patora et al. 2003]. A specific ingredient present in small amounts in natural lemon balm oil is 6-methyl-5-hepten-2-one [Patora et al. 2003].

Other valuable ingredients of lemon balm raw material are polyphenols, including phenolic acids [Carnat et al. 1998, Radácsi et al. 2016, Papoti et al. 2019]. A major phenolic component characterized by several beneficial therapeutical properties is rosmarinic acid, accounting for 2 to 4% of lemon balm dry weight [Radácsi et al. 2016]. Leaves sampled by Tóth et al. [2003] in Slovakia from a three-year-old culture contained from 3.50% (just before flowering) to 3.91% (full flowering phase) of rosmarinic acid, whereas the differences were non-significant. A similar amount (3.65%) of this compound was found by Shekarchi et al. [2012] in aerial parts of flowering lemon balm plants cultivated in Iran. In France, lemon balm leaves collected in different regions by Lamaison et al. [1991] contained an average of 4.7% of rosmarinic acid, with the highest content reaching 6.5%, while the inflorescences studied by Lamaison et al. [1990] contained 2.5% of this compound. A considerably lower quantity of rosmarinic acid (0.78%) was determined by Benedec et al. [2015] in the lemon balm aerial parts harvested in the blossom period in Romania.

An increase in interest in lemon balm and other herbs as a natural source of easily digestible minerals necessary for the proper functioning of the human body has been observed in recent years [Özcan 2004, Özcan et al. 2008, Raczuk et al. 2008, Dzida et al. 2015, Papoti et al. 2019]. According to Rumińska [1983], lemon balm herb is characterized by a high content of potassium (3.4% d.w.) and nitrogen (2.18% d.w.), and a low content of phosphorus (0.32% d.w.). In studies curried out by Dzida et al. [2015], air-dry lemon balm herb harvested at the beginning of flowering contained 1.79–3.21% of total nitrogen, 0.11–0.23% of phosphorus, 1.45–1.79% of potassium, 0.55–1.20% of calcium, and 0.13–0.38% of magnesium. Content of these elements was significantly higher in the year with more rainfall. Raczuk et al. [2008] determined 1.02–1.30% of calcium and 0.74–1.06% of magnesium in dry lemon balm leaves obtained from 3 herbal companies and 2 field crops located in different regions of Poland. Aerial parts of lemon balm provided by a local bazaar in southern Turkey contained among others 0.57% d.w. of phosphorus, 2.07% d.w. of potassium, 1.02% d.w. of calcium and 0.32% d.w. of magnesium [Özcan et al. 2008]. In earlier studies conducted in this country, the content of macroelements in dry lemon balm leaves and flowers was considerably lower, amounting to 0.001% – N, 0.14% – P, 0.66% – K, 1.17% – Ca, and 0.56% – Mg [Özcan 2004]. No information is still available regarding the effect of weeding methods on the content of minerals in herbs.

The objective of this study was to evaluate and compare the effect of 3 herbicides and flame weeding on weed suppression in the first year of growing of lemon balm sown directly into the field. Moreover, the quantity of lemon balm herb as well as its quality expressed as content of major components were also evaluated in the context of the tested weed control methods.

MATERIAL AND METHODS

The experiment was carried out in the years 2014–2015 in the Felin Experimental Farm, University of Life Sciences in Lublin, Poland (215 m above sea level, 51°23'N latitude, 22°56'E longitude) on podsolic soil developed from dusty medium loam containing 2.6% of organic matter and with pH (in 1 M KCl) of 6.9. On April 24th (2014) and on April 29th (2015) the field was fertilized with the following doses of mineral fertilizers: 30 kg $N \cdot ha^{-1}$ (ammonium nitrate), 60 kg $P_2O_5 \cdot ha^{-1}$ (superphosphate), and 90 kg K_2O (potassium salt), and then tilled by means of a cultivator with a surface roller. On the following day, lemon balm (*Melissa officinalis* L.) seeds produced by the Polish seed company PNOS based in Ożarów Mazowiecki were seeded 0.5 cm deep in 5 rows 4 m long on each plot with 40 cm distance between rows (8 m^2 plot area), maintaining the seeding rate of 5.0 $kg \cdot ha^{-1}$. The field experiment was laid out in a completely randomized block design with four replications. Two weeks later, a soil sample was collected from the 20 cm soil layer and the following average content (mg) of available potassium (flame photometry method), phosphorus (spectrophotometric method), and magnesium (flame atomic absorption spectrometry – FAAS method) in 100 g of the soil was determined by the Regional Chemical–Agricultural Station in Lublin, accredited by the Polish Centre for Accreditation: P – 20.3, K – 32.6, and Mg – 5.5.

Glufosinate-ammonium (600 $g \cdot ha^{-1}$) and flaming (90 kg $propane \cdot ha^{-1}$) were applied on May 8th, 2014, and on May 12th, 2015, which is 13 and 12 days after lemon balm sowing, respectively. The treatment was performed 4 days before the emergence of lemon balm when weeds were at the cotyledon and first true leaves stage. Bentazon (960 $g \cdot ha^{-1}$) was sprayed on May 16th (2014) and May 18th (2015), at an air temperature of 20°C when lemon balm was at the emergence-cotyledon stage, and weeds were at the cotyledon-up to 2–4 true leaves stage. Fluazifop-P-butyl (150 $g \cdot ha^{-1}$) was sprayed on May 18th (2014) and May 20th (2015), i.e. when barnyard grass had 3 small leaves, and couch grass was at the 3–4 leaves stage. Herbicides were applied using a back-pack sprayer mounted with an XR TeeJet® nozzle at 1.5 bars pressure and 300 L of $water \cdot ha^{-1}$. Flaming was carried out by hand using a universal flamer mounted with an open tubular burner with a diameter of 50 mm at 2.5 bar pressure. Weeds were counted by species in four 20 × 50 cm frames placed randomly in interrow spaces on each plot on May 25th (2014) and May 27th (2015), i.e. 33 and 29 days after sowing, respectively. During the counting, weeds were pulled out, and their fresh weight was determined. On the following day, the plots were weeded by hand, and any later germinating weeds were removed till the end of lemon balm cultivation. The number of emerged lemon balm seedlings was counted in 10 running meters on each plot on June 6th (2014) and June 12th (2015), i.e. 45 days after lemon balm sowing. The percentage of soil covering by lemon balm plants was visually assessed in late June and early August. On August 18th, the height of 30 randomly chosen plants was measured on each plot. Next day, plants starting flowering were cut at the soil surface, and their fresh weight was measured. Then, the plants were dried in natural conditions, in a shaded and well ventilated place at 32°C. Air-dried constant weight plant material was crushed or ground, depending on the planned method of analytical research.

Two months after harvest, content of total nitrogen (titration method), phosphorus (spectrophotometric method), potassium (flame photometry), calcium (flame photometry), and magnesium (FAAS) in air-dried lemon balm raw material was determined by the Regional Chemical-Agricultural Station in Lublin.

At the same time, essential oil was isolated from air-dried leaves by hydro-distillation in the Dering's apparatus according to the method recommended by the European Pharmacopoeia 5 [Council of Europe 2005]. In 2015, its qualitative composition was determined by the Central Research Laboratory of the University of Life Sciences in Lublin accredited by Polish Centre for Accreditation. The composition was determined with a gas chromatograph Varian Chrompack CP-3800 coupled with mass detector Varian 4000 GC/MS/MS and flame ionization detector (FID) using VF column – 5 ms (DB-5 equivalent). Temperature was raised up to 50°C during 1 minute, followed by its increase from 50°C to 250°C (4°C per 1 min.) and then maintained at 250°C for 10 minutes. Helium (He) was used as carrier gas, at a constant flow of 0.5 ml·min.$^{-1}$. The injector temperature was 250°C and the split was 1 : 100; 1 µl of the solution was injected (1 µl of sample per 1000 µl hexane). A Varian 4000 GC/MS/MS detector was employed at a registration range of 40–1000 m/z and scan rate of 0.8 sec. per scan. The Kovats retention indices were determined based on a range of n-alkanes from C10 to C40 [Van Den Dool and Kratz 1963]. The qualitative analysis was based on MS spectra which were compared with the obtained data and the identity of the compounds was confirmed by their retention indices available in the literature [Adams 2007]. Content of rosmarinic acid was determined at 25°C by the reverse phase HPCL method using a Merck LaChrom liquid chromatograph with a DAD diode detector (L-7450), a quaternary pump (L-7100), a solvent degasser (L-7612), a 20 µl dosing loop, a thermostat (L-7360), a Rheodyne sample injector, and a LiChrospher 100 RP C 18 column with dimensions of 250 × 4 mm filled with a stationary phase of 5 µm particle size. The mobile phase was an acetonitrile + water (20 + 20 v/v) solution with 1% (v/v) acetic acid [Nollet 2000, Najda and Dyduch 2004]. The flow rate was 1.0 ml·min.$^{-1}$, and the injection volume was 20 µl. Rosmarinic acid was identified by comparing its retention time (t_R) with the standard, and determining spectroscopically its UV spectrum. The acid content was calculated from the calibration curve. Sigma-Aldrich standards were used in the analysis.

The obtained results were analyzed statistically by means of analysis of variance (ANOVA) involving a model for orthogonal data, while the differences between means were estimated by Tukey's test at a level of significance of $p = 0.05$.

RESULTS

The course of air temperature (Tab. 1) and distribution of precipitation (Tab. 2) differed considerably over the years of research. The sum of precipitation in June 2014 was equal to the average long-term sum of precipitation for that month, while in 2015 it was lower by half. Precipitation during the first two decades of August 2014 was higher than the average long-term total rainfall, while in 2015, total absence of precipitation coincided with very high air temperatures in this period. In 2014, during the cultivation of lemon balm (third decade of April – second decade of August), a total of 246 mm more rain fell than in 2015. During both years, the average monthly air temperatures were higher than or equal (June 2014) to the long-term averages.

Weed emergence began 5 days after sowing of lemon balm seeds. Three weeks later, 23 weed species, typical for the region, were identified, of which 19 were annual (Tab. 3). Perennial weeds represented by black bent (*Agrostis gigantea* Roth), couch grass, creeping thistle (*Cirsium arvense* (L.) Scop.), and creeping yellowcress (*Rorippa sylvestris* (Leyss) Bess.) occurred sporadically. Monocotyledonous weeds were slightly more abundant, and constituted 54.9% of all weeds

Table 1. Decade, monthly and long-term (1951–2010) air temperatures (°C) in Felin Experimental Farm in the years 2014–2015

Month	2014				2015				Long-term
	decade			monthly	decade			monthly	
	1st	2nd	3rd		1st	2nd	3rd		
April	7.4	7.9	14.1	9.8	4.4	8.9	12.5	8.6	7.4
May	10.6	12.9	17.4	13.6	13.1	13.4	13.3	13.3	13.0
June	17.4	16.0	15.4	16.3	19.1	17.9	17.1	18.0	16.3
July	19.4	19.8	21.8	20.3	21.4	19.8	20.7	20.6	17.9
August	22.5	18.1	14.4	18.3	25.1	22.0	20.6	22.6	17.2

Table 2. Decade, monthly and long-term (1951–2010) precipitation sums (mm) in Felin Experimental Station in the years 2014–2015

Month	2014				2015				Long-term
	decade sums			monthly sum	decade sums			monthly sum	
	1st	2nd	3rd		1st	2nd	3rd		
April	3.2	15.3	26.2	44.7	12.7	2.8	25.3	40.8	40.2
May	32.7	88.4	72.5	193.6	26.8	8.9	76.2	111.9	57.7
June	11.2	3.0	63.9	78.1	0.8	8.1	3.2	12.1	65.7
July	14.2	38.4	30.6	83.2	9.4	23.5	10.7	43.6	83.5
August	24.9	32.8	44.5	102.2	0.0	0.0	7.6	7.6	68.6
Total	501.8				216.0				315.7

present in the experiment. The dominant species were barnyardgrass, constituting 45.6% of the total weed population on average, shepherd's purse – 21.5%, hairy galinsoga (*Galinsoga ciliata* (Raf.) S.F. Blake) – 12.8%, annual meadow grass – 8.4%, redroot pigweed – 3.5%, and common lambsquarter – 3.2%. The share of the remaining weeds ranged from 0.3 to 0.9%. Weed infestation was significantly higher in the second year of the study.

All studied methods of weed control significantly reduced the number and the fresh weight of weeds growing at 4 weeks after lemon balm sowing. In both years, the most effective method was spraying with glufosinate-ammonium, which controlled 69–76% of weeds growing on plots about 2 weeks after treatment. The number and fresh weight of weeds growing on the plots treated with this herbicide was significantly lower than on control plots and plots sprayed with other herbicides, and did not differ significantly from that on the flame weeded plots. Flame weeding and glufosinate-ammonium showed similar effectiveness, with glufosinate-ammonium controlling annual grasses considerably better. Both of compared methods were selective for lemon balm plants. Bentazon controlled approximately 65% of weeds, proving effective against annual dicotyledonous species, and selective for grasses. It caused a slight temporary chlorosis of some lemon balm seedlings, and more distinct chlorosis of the leaves of creeping thistle and creeping yellowcress. Fluazifop-P-butyl controlled about 55% of weeds, being very effective against barnyard grass, and selective to annual meadow grass. It was also selective for lemon balm plants.

Lemon balm emergence began 17–18 days after sowing. It was very uneven, and lasted for 4 consecutive weeks. An average number of seedlings growing in 1 running meter 45 days after sowing was 43 in 2014 and 50 in 2015, with the differences being significant. The emergence was independent of the method of weeding (Tab. 4). Directly after emergence, the seedlings were very small and grew slowly. The extension of emergence over time resulted in a large difference in plant growth. In the middle of June, the highest plants were reaching a height of 5–10 cm, and began to form side shoots. At the end of the month, lemon balm covered about 15% of the soil surface, and the entire surface was not covered until the beginning of August. In 2014, the average height of lemon balm plants measured at the beginning of flowering (middle of August) was 44.3 cm, i.e. significantly higher than 32.9 cm in 2015. Weeding methods had no effect on this trait (Tab. 4). Similarly, the average yield of fresh herb was significantly higher in 2014 (1426 $g \cdot m^{-2}$) than in 2015 (968 $g \cdot m^{-2}$), and was independent of the weeding method (Tab. 4).

Content of essential oil in air-dried lemon balm leaves ranged from 1.9 to 2.1%. It was significantly higher in 2015, and independent of the weeding method (Tab. 4). Content of rosmarinic acid ranged from 2.08 to 2.44%. It was independent of both the year of research and the method of weeding (Tab. 4).

Seventy compounds were detected in lemon balm essential oil, of which 63 were identified. The main ingredients of the oil were geranial, with content varying depending on the weeding method from 29.59 to 33.69%, followed by neral (25.78–35.20%), caryo-

Table 3. Effect of weed control methods on number (pcs·m^{-2}) and fresh weight (g·m^{-2}) of weeds growing in plots, in the years 2014–2015

Weed species	Control			Flame weeding			Bentazon 960 g·ha^{-1}			Fluazifop-P-butyl 150 g·ha^{-1}			Glufosinate-ammonium 600 g·ha^{-1}		
	2014	2015	mean	2014	2015	mean	2014	2015	mean	2014	2015	mean	2014	2015	mean
Agrostis gigantea Roth	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0
Amaranthus retroflexus L.	9	15	12	1	2	2	1	4	3	8	19	14	3	3	3
Capsella bursa-pastoris (L.) Medik.	64	83	74	9	23	16	19	24	22	59	77	68	8	26	17
Chenopodium album L.	8	13	11	0	2	1	2	6	4	9	15	12	0	2	1
Chenopodium glaucum L.	2	1	2	0	0	0	0	0	0	1	0	1	0	0	0
Cirsium arvense (L.) Scop.	1	0	1	0	0	0	1	0	1	1	1	1	0	0	0
Conyza canadensis (L.) Cronq.	4	0	2	0	0	0	0	0	0	3	0	2	0	0	0
Echinochloa crus-galli (L.) P. Beauv.	134	179	157	78	148	113	137	163	150	7	9	8	54	68	61
Elymus repens (L.) Gould	2	1	2	1	3	2	1	1	1	0	0	0	1	0	1
Galinsoga ciliata (Raf.) S. F. Blake	36	51	44	1	7	4	8	14	11	39	47	43	1	4	3
Geranium pusillum L.	0	1	1	0	1	0	0	0	0	0	2	1	0	0	0
Gnaphalium uliginosum L.	4	1	3	0	0	0	0	0	0	4	0	2	0	0	0
Lamium amplexicaule L.	1	3	2	1	0	0	0	1	1	2	3	3	0	0	0
Matricaria chamomilla L.	1	0	1	0	0	0	0	0	0	1	0	1	0	0	0
Poa annua L.	9	48	29	7	31	19	10	52	31	6	45	26	3	19	11
Polygonum persicaria L.	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0
Rorippa sylvestris (Leyss) Bess.	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0
Senecio vulgaris L.	2	1	2	0	0	0	0	0	0	2	0	1	0	0	0
Sinapis arvensis L.	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0
Stellaria media L.	2	1	2	0	1	1	0	1	1	2	1	1	0	2	1
Trifolium arvense L.	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0
Urtica urens L.	2	2	2	0	1	1	0	1	1	2	1	2	0	1	1
Veronica persica Poir.	2	1	2	0	0	0	0	1	1	1	0	1	0	0	0
Total number	284	404	344	98	219	159	179	270	225	150	221	186	70	125	98
Fresh weight	32.8	45.3	39.1	11.2	24.7	18.0	21.1	30.4	25.8	17.9	25.8	21.9	7.9	14.0	11.0

Means for the years; number of weeds: 156.2 (2014) and 247.8 (2015); fresh weight of weeds: 18.2 (2014) and 28.0 (2015)

Table 4. Effect of weeding method on lemon balm emergence, plant height, yield of fresh herb and content of essential oil and rosmarinic acid in dry herb

Weeding method	No. of seedlings ($pcs \cdot m^{-1}$)			Plant height (cm)			Yield of fresh herb ($g \cdot m^{-2}$)			Content of essential oil (% d.w.)			Content of rosmarinic acid (% d.w.)		
	2014	2015	mean	2014	2015	mean	2014	2015	mean	2014	2015	mean	2014	2015	mean
Control	48	52	50	44.1	32.8	38.5	1487.0	940.0	1213.5	0.19	0.20	0.20	2.08	2.30	2.19
Flame weeding	40	48	44	44.0	32.1	38.1	1398.0	952.0	1175.0	0.20	0.21	0.21	2.15	2.43	2.29
Bentazon 960 $g \cdot ha^{-1}$	39	59	49	44.5	33.6	39.1	1358.0	987.0	1172.5	0.20	0.21	0.21	2.09	2.36	2.23
Fluazifop-P--butyl 150 $g \cdot ha^{-1}$	45	47	46	45.1	33.2	39.2	1481.0	1040.0	1260.5	0.19	0.20	0.20	2.24	2.28	2.26
Glufosinate--ammonium 600 $g \cdot ha^{-1}$	41	45	43	43.9	32.7	38.3	1406.0	921.0	1163.5	0.19	0.21	0.20	2.17	2.44	2.31
Mean	43	50	46	44.3	32.9	38.6	1426.0	968.0	1197.0	0.19	0.21	0.20	2.15	2.36	2.26
$LSD_{0.05}$	treatments (A): n.s. years (B): 6.7 A × B: n.s.*			A: n.s. B: 8.14 A × B: n.s.			A: n.s. B: 347.20 A × B: n.s.			A: n.s. B: 0.018 A × B: n.s.			A: n.s. B: n.s. A × B: n.s.		

* n.s. – not significant

phyllene oxide (7.31–11.57%), geranyl acetate (3.08–4.77%), citronellal (2.84–4.20%), α-cadinol (1.46–4.62%), (*E*)-caryophyllene (1.41–2.47%), bornyl acetate (0.22–2.91%), methyl citronellate (0.76–1.12%), himachalol (0.35–1.46%), 6-methyl-5-hepten-2-one (0.65–0.77%), methyl geranate (0.58–0.71%), IR 1598 unidentified compound (0.00–1.60%), and epoxy-linalooloxide (0.18–1.29%). Mean content of each of the remaining 53 compounds was less than 0.5% (Tab. 5).

Air-dried lemon balm raw material contained 2.18–2.55% of total nitrogen, 0.30–0.41% of phosphorus, 2.94–3.22% of potassium, 1.02–1.60% of calcium, and 0.30–0.32% of magnesium. Content of phosphorus, potassium, and calcium was significantly higher in the drier year 2015, while content of total nitrogen was significantly higher in the more wet year 2014. Magnesium content did not differ significantly in both years of the study. The weed control methods did not affect the content of the studied elements (Tab. 6).

DISCUSSION

The soil and weather conditions recorded in the experiment are considered as suitable for the cultivation of lemon balm in Poland [Król 2018]. Nevertheless, the emergence of lemon balm, originating from a warmer region, started quite late, was very uneven, and stretched over time. Moreover, the emerged seedlings were very small, and grew slowly which favored the competition of fast growing native weeds. This shows the importance of protection of lemon balm from weeds during the emergence period, and confirms the opinion of Król [2018] on the subject. Further plant growth was also slow, and weeds posed a threat to the crop until the soil surface was completely covered by lemon balm in early August. In the absence of weeding, lemon balm would probably die out, as well as coriander, garden savory, and sweet basil in the study of Kucharski and Mordalski [2007]. It should be noted that even several large weeds, such as, for example, common lambsquarter or redroot pigweed, can significantly contaminate the raw material. All this allows lemon balm grown from direct sowing into the field to be classified as a plant very sensitive to weed competition.

The effectiveness of the compared weed control methods depended on the scope of their effect on weeds. Flame weeding and glufosinate-ammonium destroyed the aerial part of all weeds, and were much more effective than the selective for grasses bentazon, and selective for dicotyledonous weeds fluazifop-P-butyl (Tab. 3). The most effective treatment was

Table 5. Effect of weed control method on composition of lemon balm essential oil (% share of total components) in the year 2015

Name of the compound	Control			Flame weeding			Bentazon 960 $g \cdot ha^{-1}$			Fluazifop-P-butyl 150 $g \cdot ha^{-1}$			Glufosinate-ammonium 600 $g \cdot ha^{-1}$		
	IR*	%	E.u.%**	IR	%	E.u.%	IR	%	E.u.%	IR	%	E.u.%	IR	%	E.u.%
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
(*E*)-2-Hexenal	868	0.25	±0.00	868	0.19	±0.00	868	0.28	±0.00	868	0.26	±0.00	868	0.52	±0.01
1-Octen-3-ol	978	0.34	±0.00	978	0.38	±0.00	978	0.45	±0.00	978	0.39	±0.00	978	0.43	±0.00
6-Methyl-5--hepten-2-one	983	0.66	±0.00	983	0.70	±0.00	983	0.65	±0.01	983	0.67	±0.00	983	0.77	±0.00
Myrcene	987	0.09	±0.00	988	0.06	±0.00	988	0.10	±0.00	988	tr.	±0.00	987	0.08	±0.00
p-Cymene	1023	tr.***	±0.00	1023	tr.	±0.00	1023	tr.	±0.00	1023	0.05	±0.00	1023	tr.	±0.00
Limonene	1027	0.05	±0.00	1027	tr.	±0.00	1028	0.05	±0.00	1028	tr.	±0.00	1028	tr.	±0.00
Benzene acetaldehyde	1043	0.15	±0.01	1042	0.16	±0.02	1043	0.13	±0.02	1043	0.07	±0.00	1043	0.09	±0.01
(Z)-β-Ocimene	1033	tr.	±0.00	1033	0.05	±0.05	1033	tr.	±0.00	1033	tr.	±0.00	1033	tr.	±0.00
(*E*)-β-Ocimene	1044	0.15	±0.00	1044	0.12	±0.01	1044	tr.	±0.00	1044	0.05	±0.00	1051	tr.	±0.00
Bergamal	1051	0.13	±0.01	1051	tr.	±0.00	1051	0.08	±0.00	1051	0.13	±0.00	1051	tr.	±0.00
Linalool	1097	0.23	±0.05	1097	0.30	±0.01	1097	0.21	±0.00	1097	0.24	±0.05	1097	0.26	±0.00
α-Pinene oxide	1103	0.22	±0.02	1103	0.16	±0.00	1103	0.26	±0.04	1103	0.21	±0.02	1103	0.18	±0.01
cis-Rose oxide	1108	0.06	±0.01	1108	0.05	±0.00	1108	0.11	±0.00	1108	0.07	±0.01	1107	0.08	±0.00
n.i.****	1132	0.14	±0.01	1133	0.13	±0.01	1131	0.12	±0.00	1133	0.09	±0.00	1132	0.10	±0.00
trans-Thujone	1137	0.11	±0.01	1137	0.14	±0.00	1137	tr.	±0.00	1137	0.10	±0.01	1137	0.14	±0.02
Isophorone	1139	tr.	±0.00	1139	0.16	±0.01	1139	0.92	±0.03	1139	0.06	±0.00	1139	0.68	±0.02
n.i.	1142	0.17	±0.02	1142	0.17	±0.02	1142	0.26	±0.00	1142	0.17	±0.01	1142	0.19	±0.01
cis-Limonene oxide	1148	0.45	±0.01	1148	0.39	±0.00	1148	0.41	±0.00	1148	0.46	±0.00	1147	0.45	±0.00
Citronellal	1151	3.07	±0.10	1151	3.21	±0.06	1151	2.84	±0.01	1152	4.20	±0.06	1151	3.96	±0.01
cis-Chrysan-thenol	1160	0.25	±0.05	1161	0.21	±0.01	1161	0.20	±0.03	1161	0.25	±0.01	1160	0.26	±0.01
Rosefuran epoxide	1169	0.16	±0.00	1169	0.10	±0.00	1169	tr.	±0.00	1169	0.06	±0.00	–	–	–
Lavandulol	1175	0.14	±0.00	1175	0.12	±0.00	1176	0.20	±0.00	1176	0.16	±0.01	1175	0.17	±0.00
trans-p-Mentha -1(7), 8-dien-2-ol	1180	0.48	±0.02	1180	0.46	±0.02	1180	0.44	±0.01	1180	0.58	±0.00	1180	0.51	±0.00
Methyl salicylate	1192	0.15	±0.00	–	–	–	1191	tr.	±0.00	1191	0.07	±0.00	1192	tr.	±0.00
Citronellol	1221	0.20	±0.01	1221	0.20	±0.00	1222	tr.	±0.00	1222	0.14	±0.03	1221	0.21	±0.00
n.i.	1232	0.39	±0.00	1232	0.39	±0.01	1232	0.18	±0.00	1232	0.27	±0.01	1232	0.32	±0.02
Neral	1241	32.37	±0.28	1242	35.20	±0.17	1240	25.78	±0.07	1242	32.89	±0.21	1241	35.03	±0.05
Geraniol	1251	0.20	±0.01	1252	0.24	±0.00	1252	0.22	±0.01	1252	0.25	±0.01	1251	0.18	±0.01
Piperitone	1255	0.11	±0.01	1255	0.12	±0.00	1255	tr.	±0.00	1256	0.24	±0.02	1255	0.05	±0.00
Methyl citronellate	1258	0.76	±0.02	1258	0.94	±0.01	1258	1.01	±0.15	1258	1.12	±0.02	1258	0.84	±0.00
Geranial	1272	31.34	±0.04	1273	33.69	±0.00	1270	29.59	±0.23	1273	32.85	±0.09	1271	33.17	±0.29
Epoxy--linalooloxide	1280	0.20	±0.00	1280	0.25	±0.00	1280	1.29	±0.03	1280	0.18	±0.01	1280	1.22	±0.06
Bornyl acetate	1285	0.32	±0.02	1286	0.26	±0.00	1285	2.91	±0.03	1286	0.22	±0.02	1285	1.56	±0.08
Methyl geranate	1322	0.69	±0.01	1322	0.58	±0.01	1322	0.71	±0.01	1322	0.63	±0.01	1321	0.59	±0.03
n.i.	1336	0.34	±0.01	1336	0.33	±0.00	1336	tr.	±0.00	1336	0.48	±0.02	1336	0.24	±0.01
Terpin hydrate	1340	tr.	±0.00	1341	0.11	±0.01	1341	0.28	±0.02	1341	0.14	±0.02	1340	0.27	±0.10
n.i.	1372	0.40	±0.00	1372	0.43	±0.02	1373	0.13	±0.00	1373	0.62	±0.00	1372	0.28	±0.01

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
α-Copaene	1375	0.15	±0.03	1375	0.14	±0.00	1376	tr.	±0.00	1376	0.14	±0.00	1376	0.15	±0.01
Geranyl acetate	1378	3.08	±0.02	1378	3.40	±0.02	1378	4.77	±0.02	1379	3.11	±0.07	1378	4.33	±0.05
(*E*)-Caryophyllene	1421	1.41	±0.01	1422	2.47	±0.01	1421	1.83	±0.02	1422	1.46	±0.02	1421	1.99	±0.01
cis-Muurola--3,5-diene	1454	0.11	±0.00	–	–	–	1453	0.10	±0.00	1454	0.10	±0.00	1453	0.09	±0.00
α-Humulene	1462	0.07	±0.00	1462	0.16	±0.01	–	–	–	1460	0.09	±0.00	1462	0.05	±0.00
allo-Aroma-dendrene	1467	0.23	±0.01	1467	0.11	±0.00	1467	0.20	±0.00	1467	0.18	±0.00	1467	0.09	±0.00
γ-Muurolene	1483	0.09	±0.00	1483	tr.	±0.00	1484	0.11	±0.00	1484	0.10	±0.00	–	–	–
(*E*)-β-Ionone	1488	0.21	±0.01	1488	0.19	±0.00	1489	0.28	±0.00	1489	0.24	±0.02	1488	0.30	±0.05
n.i.	1493	0.09	±0.00	1492	tr.	±0.00	1492	0.13	±0.00	1492	0.12	±0.13	1491	0.06	±0.00
epi-Cubebol	1506	0.55	±0.02	1506	0.16	±0.00	–	–	–	1506	0.34	±0.00	1506	tr.	±0.00
α-Muurolene	1508	0.23	±0.01	1509	0.07	±0.00	1509	0.16	±0.01	1509	0.21	±0.01	–	–	–
γ-Cadinene	1522	0.29	±0.02	1522	0.08	±0.00	1522	0.37	±0.07	1522	0.33	±0.01	1522	tr.	±0.04
10-epi-Cubebol	1524	0.35	±0.01	1524	0.06	±0.01	1524	0.36	±0.13	1524	0.25	±0.02	1526	0.26	±0.03
α-Cadinene	1526	0.18	±0.00	1526	0.09	±0.00	1525	0.12	±0.00	1527	0.16	±0.02	1526	0.15	±0.00
trans-Calamenene	1530	tr.	±0.00	–	–	–	1530	0.05	±0.00	1530	0.05	±0.00	1530	tr.	±0.00
α-Calacorene	1547	0.08	±0.00	1548	tr.	±0.00	1548	0.11	±0.01	1548	0.10	±0.00	–	–	–
epi-Longipinanol	1556	0.61	±0.02	1557	0.34	±0.02	1557	0.33	±0.01	1557	0.30	±0.01	1556	0.39	0.02
n.i.	1561	0.20	±0.01	1561	0.09	±0.01	1561	0.12	±0.00	1561	0.09	±0.00	1561	tr.	±0.00
Ledol	1574	0.16	±0.01	–	–	–	1573	0.09	±0.00	1574	0.07	±0.00	1573	0.17	±0.00
Longicam-phenylone	–	–	–	1578	0.11	±0.03	1578	tr.	±0.00	1578	0.12	±0.01	1578	0.07	±0.00
Caryophyllene oxide	1585	7.71	±0.53	1585	8.08	±0.16	1585	11.57	±0.02	1585	7.31	±0.04	1584	8.02	±0.06
Viridiflorol	1596	0.92	±0.02	1596	0.34	±0.02	1596	0.40	±0.04	1596	0.23	±0.00	1595	0.36	±0.02
n.i.	1598	1.60	±0.03	1598	0.57	±0.01	1598	0.64	±0.01	1598	0.35	±0.01	–	–	–
n.i.	1606	0.70	±0.02	1606	0.25	±0.00	1607	0.32	±0.02	1606	0.20	±0.01	1607	0.36	±0.02
α-Humulene epoxide II	1612	0.51	±0.04	1612	0.48	±0.02	1613	0.38	±0.04	1613	0.42	±0.01	1612	0.39	±0.01
1-epi-Cubenol	1633	0.29	±0.02	–	–	–	1634	0.16	±0.00	1634	0.20	±0.00	1633	tr.	±0.00
allo-Alloaro--madendrene epoxide	1644	0.20	±0.02	1644	0.21	±0.01	1645	0.29	±0.01	1644	0.19	±0.01	1644	0.22	±0.01
α-Muurolol	1650	0.41	±0.03	1650	0.19	±0.05	1650	0.66	±0.02	1650	0.52	±0.03	1651	0.07	±0.10
Himachalol	1652	0.85	±0.01	1652	0.35	±0.07	1652	1.46	±0.02	1652	0.94	±0.03	1665	0.89	±0.96
n.i.	1655	0.17	±0.01	1655	0.08	±0.02	1656	0.27	±0.01	1656	0.18	±0.00	–	–	–
α-Cadinol	1665	3.22	±0.07	1665	1.46	±0.31	1666	4.62	±0.14	1666	3.06	±0.03	1965	2.01	±0.16
(*E*)-epi-9-hydroxy--14 Caryophyllene	1682	0.43	±0.10	1683	0.42	±0.01	1683	0.59	±0.04	1683	0.36	±0.08	1682	0.42	±0.06
Total (68) identified		99.87%			99.90%			100.00%			99.89%			99.86%	

* IR – retention index [Van Den Dool and Kratz 1963]
** E.u.% – expanded uncertainty
*** tr. – traces
**** n.i. – not identified

Table 6. Content of macroelements in lemon balm herb in dependence on weeding method (% d.w.)

Weeding method	Nitrogen			Phosphorus			Potassium			Calcium			Magnesium		
	2014	2015	mean	2014	2015	mean	2014	2015	mean	2014	2015	mean	2014	2015	mean
Control	2.48	2.53	2.51	0.39	0.32	0.35	3.18	3.14	3.16	1.40	1.12	1.26	0.30	0.30	0.30
Flame weeding	2.32	2.44	2.38	0.40	0.31	0.36	3.21	2.94	3.08	1.31	1.33	1.32	0.30	0.31	0.31
Bentazon 960 g·ha^{-1}	2.49	2.49	2.49	0.41	0.33	0.35	3.22	2.94	3.08	1.41	1.21	1.31	0.30	0.32	0.31
Fluazifop-P-butyl 150 g·ha^{-1}	2.23	2.46	2.35	0.39	0.30	0.34	3.19	3.05	3.12	1.60	1.02	1.31	0.30	0.30	0.30
Glufosinate-ammonium 600 g·ha^{-1}	2.18	2.55	2.37	0.41	0.33	0.34	3.20	3.05	3.13	1.32	1.10	1.21	0.31	0.31	0.31
Mean	2.34	2.49	2.42	0.40	0.32	0.35	3.20	3.01	3.11	1.41	1.16	1.28	0.30	0.31	0.31
$LSD_{0.05}$	treatments (A): n.s. years (B): 0.096 A × B: n.s.*			treatments (A): n.s. years (B): 0.019 A × B: n.s.			treatments (A): n.s. years (B): 0.138 A × B: n.s.			treatments (A): n.s. years (B): 0.081 A × B: n.s.			treatments (A): n.s. years (B): n.s. A × B: n.s.		

* n.s. – not significant

spraying with glufosinate-ammonium about 2 weeks after lemon balm sowing, and 4 days before crop emergence, when most weeds were at the cotyledon and first true leaves stage. As a foil applied, contact, total herbicide with no residual activity [Praczyk and Skrzypczak 2004, Senseman 2007], it killed all annual weeds in about 4 days, but did not prevent their later emergence. It also destroyed the leaves of couch grass, but soon new ones were growing from the underground rhizomes. The herbicide did not affect lemon balm plants. The obtained results are consistent with those by Kordana et al. [1997], and evidence good usefulness of glufosinate-ammonium for weed control directly before lemon balm emergence under conventional cultivation.

Flame weeding, applied at the same time as glufosinate-ammonium, was characterized by slightly lower effectiveness in grass control. Weeds that emerged about 2 weeks after lemon balm sowing were completely destroyed during flaming, but new emergence, especially of barnyardgrass, was soon observed. After 2 more weeks, 98 and 219 weeds·m^{-2} grew on the flamed plots in 2014 and 2015, respectively, with the weed flora composition different from that observed in the control treatment. The abundance of hairy galinsoga and common lambsquarters was lower for both years by an average of 91%, the abundance of redroot pigweed was lower by 83%, the abundance of shepherd's purse – by 78%, the abundance of annual meadow grass – by 34%, and abundance of barnyardgrass – by 28% (Tab. 3). These results confirm greater tolerance to flaming for grasses than broad-leaved weeds. They are largely in line with data obtained by several other authors [Ascard 1995, Knežević et al. 2009, 2014a, 2014b]. According to Ascard [1995], flame weeding does not reduce subsequent weed emergence, and may even increase the germination of some species. Nevertheless, in the discussed experiment, flaming effectively protected lemon balm from weeds during the critical period of emergence. Gas consumption was high due to the use of a general purpose unshielded burner, as well as slow and thorough manual burning of weeds. An unshielded burner made it possible to follow the course of the weed destruction process, but also increased the consumption of gas [Storeheier 1994] which was in the high dose ranges assessed by Ascard [1995] and Knežević et al. [2009, 2014a, 2014b]. No effect of flame weeding on lemon balm was observed in the experiment. Flaming is cheaper than hand weeding, but more expensive than spraying with herbicides. It should be therefore treated as a supplement to other weed control methods [Knežević

2016]. Flame weeding as an accepted non-chemical method can be particularly useful in organic cultivation of herbs [Carrubba and Militello 2013].

In the case of flaming and glufosinate-ammonium, the timing of the treatment is very important. The later it is done, the more weeds will emerge, and the more effective it will be. However, an unexpected long-lasting precipitation within the time of the planned treatment, followed by the emergence of lemon balm during this period, may make it impossible to perform the treatment over the entire field surface, and possibly only in the inter-rows, after installing shields on the burner or sprayer nozzles.

The date of application of bentazon and fluazifop-P-butyl was adjusted to the phase of weeds' greatest sensitivity to their action. At that time, lemon balm was in the emergence period, during which plants are usually more susceptible to foliage applied herbicides. Like several other dicotyledonous herbal plants under first year of cultivation studied by Kordana et al. [2002], in the experiment, lemon balm was resistant to fluazifop-P-butyl applied at a dose of 150 g·ha^{-1}. However, it was slightly sensitive to bentazon used at a dose of 960 g·ha^{-1}, and an air temperature of 20°C. The herbicide caused transient chlorosis which did not affect the further growth or yielding of lemon balm, although at higher temperatures, the damage would probably be greater [Wills 1976]. Delaying the treatment until lemon balm produces first true leaves would increase its resistance to bentazon. At the same time, however, weeds would overgrow the crop, and also become much more resistant to the herbicide. In the experiment of Krawiec et al. [2019], two-year-old lemon balm plants with a height of approximately 30 cm were completely resistant to bentazon applied at a dose of 960 g·ha^{-1}. The effectiveness of both herbicides was similar due to the very similar share of grasses and dicotyledonous weeds in the total weed infestation (Tab. 3). The results obtained in the present experiment confirmed high effectiveness of fluazifop-P-butyl in controlling dangerous weeds in the cultivation of herbal plants such as couch grass and barnyardgrass [Kordana et al. 2002] and confirmed high resistance of annual meadow grass to this preparation [Wang et al. 2014]. Among the herbicides compared in the experiment, fluazifop-P-butyl showed the slowest action – it killed the seedlings of barnyardgrass within a week, and couch grass plants – within 2–3 weeks. In the case of abundant grasses and dicotyledonous weeds growing in the field, it would be possible to use both herbicides separately. The obtained results confirm their usefulness as stated by Korbas [2020] for weed control in the first year of cultivation of lemon balm sown directly into the field.

Lemon balm seeds were sown in accordance with the sowing standards recommended in Poland [Król 2018], ensuring high plant density. The weeding methods did not affect the number of emergence, and the significant differences in both years of research (Tab. 4) could possibly be related to the quality of the seeds used in the experiment each year. Although lemon balm is a perennial that grows up to a height of 1 m [Seidler-Łożykowska et al. 2015], in the present experiment, in the first year of cultivation, at the beginning of flowering, it reached an average height of 44.3 cm in the season with more rainfall, and 32.9 cm in the drier season (Tab. 4). It is consistent with the results obtained by Dzida et al. [2015] and Gorgini Shabankareh et al. [2017]. In the experiment, lemon balm plants grown from direct sowing in high density reached greater height, and produced a greater yield of fresh herb than plants grown Dzida et al. [2015] from transplants at much lower density. The yields harvested in this experiment (968–1426 g·m^{-2}, Tab. 4) were also higher than those obtained by Politycka and Seidler-Łożykowska [2009] for lemon balm grown from transplants, and similar to the average yields obtained from transplants by Seidler-Łożykowska et al. [2015] in several places in the second and third year under conventional and organic cultivation. The drought in the second half of the growing season in 2015 limited the growth of plants, and therefore the yields of fresh herb, despite greater plant density, were significantly lower than in 2014. The weeding methods did not affect the plant height or the yield of lemon balm fresh herb.

High air temperatures at the turn of July and August favoured the production of essential oil. Its content in the tested dry lemon balm leaves was relatively high, and ranged from 1.9 to 2.1% (Tab. 4). These values were similar to those found by Politycka and Seidler-Łożykowska [2009] and Dzida et al. [2015]. They were in the upper range of the oil content determined by Klimek et al. [2000] and Patora et al.

[2003], and considerably higher than those determined by Seidler-Łożykowska et al. [2015] in lemon balm herb. The oil content depends on many factors, especially environmental conditions, cultivation method, genetic characteristics of lemon balm plants, and distillation method [Sari and Ceylan 2002, Patora et al. 2003, León-Fernández et al. 2008, Khalid et al. 2009]. A significantly beneficial effect of drought in the second half of the growing period on the content of essential oil in lemon balm leaves found in this experiment is consistent with the results obtained by Abbaszadeh et al. [2009], Radácsi et al. [2016] and Gorgini Shabankareh et al. [2017]. The weed control methods studied in the experiment had no effect on the content of essential oil in the lemon balm leaves. No information regarding this subject is still available in the literature.

The number of compounds detected in the essential oil was similar to that recognized by León-Fernández et al. [2008] in the oil produced by plants cultivated in the field, and much higher than that determined by Khalid et al. [2009] in the oil produced by plants grown in a greenhouse. The main compounds were geranial (29.59–33.69%), neral (25.78–35.20%), caryophyllene oxide (7.31–11.57%), geranyl acetate (3.08–4.77%), citronellal (2.84–4.20%), α-cadinol (1.46–4.62%), and (*E*)-caryophyllene (1.41–4.62%), and the content of monoterpene aldehydes was approximately two hundred times greater than that of monoterpene alcohols (Tab. 5). This is largely in line with results obtained by Pino et al. [1999], Klimek et al. [2000], Patora et al. [2003], León-Fernández et al. [2008] and Seidler-Łożykowska et al. [2015] under field conditions, and differs considerably from those obtained by Khalid et al. [2009] in a greenhouse. Content of 6-methyl-5-hepten-2-one (0.65–0.77%), characteristic of lemon balm oil, was approximately twice higher than the average content in lemon balm leaves studied by Klimek et al [2000], almost three times higher than the average content determined by Patora et al. [2003], and over three-five times lower than that found in tropical climate by Pino et al. [1999] and León-Fernández et al. [2008]. Content of individual components in oils obtained from plants weeded using the methods tested in the experiment was, similarly to studies by Klimek et al. [2000], Patora et al. [2003] and Seidler-Łożykowska et al. [2015], varied to some extent, but did not clearly depend on the method of weeding. According to Klimek et al. [2000], large variability in the composition of lemon balm oil is its characteristic feature.

In the experiment, content of rosmarinic acid in dry lemon balm leaves (2.15–2.36%, Tab. 4) was lower than that found by Lamaison et al. [1991], Tóth et al. [2003], and Shekarchi et al. [2012] in countries south of Poland, but much higher than that determined by Benedec et al. [2015], and similar to that stated by Lamaison et al. [1990] in lemon balm inflorescences. An increasing tendency of rosmarinic acid content was observed in the drier year 2015, which is consistent with the results of research by Radácsi et al. [2016]. The data cited above show that the content of this compound depends on the region of origin of lemon balm, development phase and part of the plant, as well as on the method of its determination. The experiment showed no effect of the weed control methods on the content of rosmarinic acid in lemon balm leaves.

Content of nitrogen (2.18–2.55%), phosphorus (0.30–0.41%), and potassium (2.94–3.22) in dry lemon balm herb determined in this experiment (Tab. 6) was similar to that reported by Rumińska [1983], and differed to various degrees from the content stated by several other authors. Thus, nitrogen content was within the range determined by Dzida et al. [2015], and was many times higher than that in lemon balm flowers and leaves given by Özcan [2004]. The content of phosphorus was twice as high as that stated by Dzida et al. [2015], and more than one and half times smaller than that determined by Özcan et al. [2008]. Potassium content was one and a half times higher than that determined by Özcan et al. [2008], and almost twice as high as that given by Dzida et al. [2015]. Calcium content (1.02–1.60%) was similar to that reported by Özcan et al. [2008], and one and half times higher than that reported by Dzida et al. [2015]. Moreover, calcium content was very similar to that found by Raczuk et al. [2008] in leaves, as well as that found by Özcan [2004] in lemon balm leaves and flowers. Magnesium content (0.30–0.32%) was the same as reported by Özcan et al. [2008], and was in the upper range of content determined by Dzida et al. [2015]. On the other hand, magnesium content found in the experiment was almost twice lower than its content in lemon balm leaves and flowers as determined by Özcan [2004], and almost three times lower than the content in lemon balm leaves stated by Raczuk et al. [2008]. Higher nitrogen content in the herb harvested in the year with a higher rainfall is consistent with the results obtained by Dzida et al. [2015]. However, unlike in those studies, content of phosphorus, potassium, and calcium was higher in the year with less rainfall, and magnesium content was similar in both years of the study. The results of research conducted by Brzozowska et al. [2018] show that the impact of weather conditions on the content of individual macroelements is varied, and not only the total sum of precipitation is important, but also their distribution during growing season, as well as air temperature. Like results obtained by the authors cited above, the results of this experiment evidence that lemon balm herb contains significant amounts of macroelements important for human health. According to Özcan [2004], the differences in their content might result from the growth conditions, genetic factors, geographical variations, and analytical procedures. The studied weed control methods had no effect on the content of nitrogen, phosphorus, potassium, calcium, or magnesium in lemon balm raw material.

CONCLUSIONS

1. Lemon balm cultivated in central-eastern Poland from direct sowing into the field was characterized by a long, four-week emergence, and very slow initial growth, making it highly sensitive to weed competition.

2. The investigated weed control methods proved useful in the first year of cultivation lemon balm from direct sowing onto the field. Methods with total action (flaming 90 kg propane·ha^{-1}, glufosinate-ammonium 600 g·ha^{-1}) were more effective than those with selective herbicides (bentazon 960 g·ha^{-1}, fluazifop-P-butyl 150 g·ha^{-1}). In practice, however, the effectiveness of either method will largely depend on the weed flora present in the field. Of the assessed methods, only flame weeding can be used in organic lemon balm cultivation.

3. By the time of harvest, lemon balm plants averaged 32.9–44.3 cm in height, and produced an average fresh herb yield of 968–1426 g·m^{-2} with 0.19–0.21% of essential oil content in dry leaves. In the year with less rainfall, the plants were lower, and produced a lower yield of fresh herb, while content of oil in the leaves was higher. These features were independent of weed control methods.

4. A total of 70 compounds were detected in lemon balm essential oil, of which 63 were identified. The main components of the oil were geraniol (29.59–33.69%), neral (25.78–35.20%), caryophyllene oxide (7.31–11.57%), geranyl acetate (3.08–4.77%), and citronellal (2.84–4.20%). Content of individual components in oils obtained from plants weeded with different methods varied to some extent, but did not depend on the method of weeding.

5. Dry lemon balm leaves contained significant amounts of rosmarinic acid (2.26%) and macroelements (N – 2.42%; P – 0.35%; K – 3.11%; Ca – 1.28%; Mg – 0.31%). Their content was independent of the method of weeding. Lemon balm could be a good source of these components in human nutrition.

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