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Cultivating for the Industry: Cropping Experiences with *Hypericum perforatum* L. in a Mediterranean Environment

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Abstract: *Hypericum perforatum* is an intensively studied medicinal plant, and much experimental activity has been addressed to evaluate its bio-agronomical and phytochemical features as far. In most cases, plant material used for experimental purposes is obtained from wild populations or, alternatively, from individuals grown in vases and/or pots. When *Hypericum* is addressed to industrial purposes, the most convenient option for achieving satisfactory amounts of plant biomass is field cultivation. Pot cultivation and open field condition, however, are likely to induce different responses on plant's metabolism, and the obtained yield and composition are not necessarily the same. To compare these management techniques, a 4-year cultivation trial (2013–2016) was performed, using three *Hypericum* biotypes obtained from different areas in Italy: PFR-TN, from Trento province, Trentino; PFR-SI, from Siena, Tuscany; PFR-AG, from Agrigento province, Sicily. Both managements gave scarce biomass and flower yields at the first year, whereas higher yields were measured at the second year (in open field), and at the third year (in pots). Plant ageing induced significant differences in phytochemical composition, and the total amount of phenolic substances was much higher in 2015 than in 2014. A different performance of genotypes was observed; the local genotype was generally more suitable for field cultivation, whereas the two non-native biotypes performed better in pots. Phytochemical profile of in-pots plants was not always reflecting the actual situation of open field. Consequently, when cultivation is intended for industrial purposes, accurate quality checks of the harvested material are advised.

Keywords: St. John's wort; *Hypericum perforatum*; secondary metabolites; cropping technique

Citation: Lazzara, S.; Carrubba, A.; Napoli, E. Cultivating for the Industry: Cropping Experiences with *Hypericum perforatum* L. in a Mediterranean Environment. *Agriculture* **2021**, *11*, 446. https://doi.org/10.3390/agriculture11050446

Academic Editor: Silvia Tavarini

Received: 17 April 2021
Accepted: 11 May 2021
Published: 15 May 2021

1. Introduction

Hypericum perforatum L. (fam. *Hypericaceae*) is one of the most famous and widespread medicinal plants in the world. Due to its many pharmaceutical activities, ranging from antioxidant [1], anti-inflammatory [2], antiviral [3], antimicrobial [4], and antiproliferative [5], this species is traditionally used throughout the world for a number of internal and external applications. According to the European Pharmacopoeia, *Hypericum* drug (Hyperici herba) consists of the plant's dried aerial part and flowering tops, collected at flowering time [6,7]. Within continental Europe and the whole Mediterranean area, the *Hypericum* oleolite (Hyperici oleum) represents a very popular remedy against minor wounds and skin conditions, burns, and sunburst [8,9]. This extract is obtained by macerating the flowers in vegetable oil (mainly sunflower or olive oil), with a Drug Extract Ratio (DER) varying from 1:4 to 1:20 according to the given traditional recipe [6,7,10].

The interest towards the plant sharply rose in the early 1980s, when specific antidepressant and anxiolytic properties were discovered [11,12]. For a long time, hypericin was thought to be the main responsible for *Hypericum* antidepressant activity [13], and

Hypericum-based products started to be valued according to their content in hypericins. Later on, research enlightened the role played by hyperforin first [14], and then by other plant constituents, including many phenolic compounds. So far, a general consensus has been reached on the shared synergic pharmacological importance of the many constituents of *Hypericum* extract, that therefore should be properly considered a phytocomplex [15–17].

Despite its high commercial importance, however, the availability of *H. perforatum* raw material is presently rather limited. In Europe, the major production areas are located in Germany, Italy, and Romania [18], but collection from wild populations still forms a large part of the total *Hypericum* supply.

It appears that cultivating *Hypericum* for industrial purposes, i.e., aimed at achieving high and stable amounts of the desired active metabolites, could be a great resource for farmers. Nevertheless, the definition of a comprehensive set of information about the field management techniques still requires a great research effort, as many factors are known to deeply affect the yield and proportion of active compounds in *Hypericum* [19]. The available literature shows that scarce research indeed is addressed to the evaluation of the bio-agronomical and phytochemical response of *H. perforatum* to open field conditions. As a matter of fact, the majority of available papers are based on plant samples collected from the wild, or, when plants are cultivated, on individuals grown in constrained conditions, mostly in vases and pots. Although these kinds of experiments have many advantages—first of all reproducibility, some differences between pots cultivation and open field condition are likely to occur [20], especially when physiological, chemical, or yield response are evaluated on individually-grown plants. This issue can have striking consequences especially in Mediterranean environments, where the high variability of climatic and environmental conditions is expected to play an additional and important role on cultivated plants' metabolism. Hence, there is room for a straightforward research, aimed at comparing the phytochemical and biomass response of *H. perforatum* in pots and open field conditions. In this work we analyze the results of a cultivation trial, performed throughout the whole crop duration (2013–2016), of three *H. perforatum* biotypes, obtained from different Italian geographical areas, with contrasting cultivation methods.

2. Materials and Methods

2.1. Plants Management and Data Collection

Mature seeds of *H. perforatum* were retrieved in spring-summer 2012 from three different geographical areas of Italy: mount Bondone (TN-Trento province, Trentino), Massa Marittima (SI-near Siena, Tuscany), and San Biagio Platani (AG-Agrigento province, Sicily), representative of Northern, Central, and Southern Italy, respectively.

Since *Hypericum* seeds are usually considered "recalcitrant" to germinate [21], prior to sowing, seeds were submitted to a 1-week vernalization period at T = 4 °C [22]. In the second week of August 2012, seeds were sown in 104-holes expanded polystyrene trays and, after germination, plantlets were transferred to larger (about 5 cm diameter) plastic pots filled with a 1:1 sand-perlite mixed substrate for root establishment [23]. Three months after sowing (November 2012), one half of the obtained fully established plants (70 individuals per each biotype, i.e., a total of 210 plants) was transferred into larger pots (18 cm diameter), filled with a growth substrate composed of a mixture of peat, sand, and vermiculite (60%, 30%, and 10% in weight, respectively) and positioned into the facilities of CREA-DC in Bagheria (PA, Sicily, 38°5′ N,13°31′ E, 25 m a.s.l.). The remaining plants (70 individuals per each biotype) were transplanted in open field within the experimental farm "Sparacia" (Cammarata, AG, Sicily, 37°38′ N–13°46′ E; 415 m s.l.m.). The soil (Table S1) was a vertic-xerofluvent [24], characterized by a definite clayey texture, and scarcely endowed with nitrogen and organic matter. Climatic pattern recorded in Sparacia throughout the whole trial period is reported in Figure S1. Three experimental plots were set, one per each biotype, sized 10.80 m^2 (3.6 by 3.0 m); plants were arranged in rows 50 cm apart, at a distance of 40 cm one another (plant population: 5 plants m^{-2}). During the four years' timeframe and in both management systems, the crop was monitored until harvest

time, keeping note of plants' phytosanitary state and general development conditions. No intervention against pests was needed, except for weed control. That was carried out manually once a year, in springtime, before the emission of flower buds. Fertilization consisted in a light N supply in organic form. A commercial pelletized organic fertilizer, containing 5% organic N, 37% total organic carbon, and 74% organic matter, was used; fertilizer supply, corresponding to 50 kg ha^{-1} N, was distributed only once in 2012, before transplant. In all cultivation years and in both experimental conditions, plants were watered throughout spring and summer, from the transplant (in the years after the first, from the restarting of vegetation) to full flowering time (i.e., harvest time). In doing this, the amount of administered water was managed in order to achieve and maintain field capacity and, therefore, to entirely satisfy crop requirements.

From 2013 to 2016 at flowering time (between late May and early June in field, and in mid-June in pots), all plants were cut at ground level, in order to allow a quick regrowth in the following year. Samples of five plants were randomly taken per treatment, and data on weight, height, and number of stems per plant were collected. Stems were considered flowering when containing at least one fully developed flower; hence, stems of all plants were sorted by flowering and vegetative stems (without flowers). Flowers were further picked up and weighed. All separate plant fractions were open-air dried in the dark for one week, and weighed again, in order to obtain the yields of herbal product.

2.2. Phytochemical Analyses

Analyses were conducted on samples of dried flowers obtained from all experimental sets in 2014 and 2015, except for 2016, when only samples from pot-managed plants were analyzed. In all treatments and years, the flowered tops (15–20 cm) of full-flowering individuals were collected, and after cutting, plant samples were stored in paper bags and dried at 20–25 °C in the dark for further analyses. The dried flowers collected from the different experimental conditions were finely crushed and aliquots (1 g) of powder were extracted with 20 mL of ethanol for 72 h under gently continuous stirring, avoiding light exposure due to the photo sensibility of some of the metabolites of interest. The resulting deep red colored suspensions were filtered on PTFE 0.45 filters (PALL Corporation), put into 2 mL amber vials, and sent to analytical determinations.

Determinations involved some of the most relevant phenolic compounds, belonging to the chemical families of naphthodianthrones (hypericins: hypericin, pseudohypericin, protohypericin, and protopseudohypericin); phloroglucinols (hyperforin and adhyperforin); cinnamic acids and derivatives (3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, *p*-coumaroilquinic acid, and *p*-coumaric acid); flavonols (quercetin, quercitrin, rutin, hyperoside, isoquercitrin, myricitrin, and myricetin derivative); dimers (biapigenin and amentoflavone); flavan-3-ols (catechin). Quantitative analyses were carried out following the procedure already described in previous works [25]. Briefly, polyphenol quantitative analysis was carried out on a Ultimate3000 instrument equipped with a binary high-pressure pump, a photodiode array detector (Thermo Scientific, Milan, Italy). The data were processed through the Chromeleon Chromatography Information Management System v. 6.80 (Thermo Fisher Scientific Inc., Waltham, MA, USA). All chromatographic runs were performed using a reverse-phase column (Gemini C_{18}, 250 × 4.6 mm, 5 µm, Phenomenex, Italy). Chromatographic runs were carried out with a gradient of 5%–90% Buffer B (2.5% formic acid in acetonitrile) in Buffer A (2.5% formic acid in water) over 50 min after which the system was maintained for 7 min at 100% Buffer B, with a constant solvent flow of 1 mL/min. Quantifications were carried out building calibration curves using the corresponding reference substance, if applicable, or a similar molecule with analogue chromophore.

The quantitative analysis of naphthodianthrones and acylphloroglucinols was carried out on a Hitachi Chromaster instrument, equipped with a binary high-pressure pump and a photodiode array detector. Data were processed through Agilent OpenLab CDS version A.04.05 (Agilent, Santa Clara, CA, USA). Chromatographic runs were performed using the same column as the polyphenols, and were carried out with the following gradient

of Buffer B (acetonitrile) in Buffer A (ammonium acetate 20 mM in water): 0 min: 50% B; 25 min: 50% B; 35 min: 10% B; 45 min: 90% B; 50 min: 50% B. The solvent flow rate was 1 mL/min. Quantifications were carried out building calibration curves using the corresponding reference substance, if applicable, or a similar molecule with analogue chromophore. All analyses were carried out in triplicate.

2.3. Statistical Treatment of Data

Statistical analyses were performed by means of the statistical package Minitab® version 17.1.0 (Minitab Inc., State College, PA, USA, 2013). The GLM (General Linear Model) procedure was used, setting as dependent variables the data measured in all experiments, whereas the independent variables were "year," "management", and "*Hypericum* biotype," respectively. Data obtained in all trial years were preliminary submitted to the Levene's test for variance homogeneity, assessing a substantial equality of variances for some variables (number of stems per plant—including stems with flowers, stems without flowers, and total stems number—and fresh and dry weight of stems), and a significant non-homogeneity for plant height, fresh and dry weight of flowers per plant, and fresh and dry weight of total plant biomass. Hence, only in the former group of variables a complete ANOVA on pooled data was run, setting the "year" as a random factor, and "management" and "*Hypericum* biotype" as fixed factors. For the latter group of variables, separate ANOVA procedures were otherwise carried out throughout each experimental year. Chemical data were submitted to a Principal Component Analysis (PCA) by means of the PAST statistical package version 3.26b [26,27]. Values of hypericin, pseudohypericin, and hyperforin were submitted to a one-way ANOVA; because of the unbalanced structure of data, the analysis was run separately for each treatment year. The differences among means were appreciated through Tukey's post-hoc comparison test.

3. Results and Discussion

3.1. Plant Growth and Yield

In the first cultivation year, the height of plants was not different between the two managements (Table 1; Figure 1), whereas remarkable differences among biotypes emerged, with the highest mean value (67.8 cm) in the AG biotype and rather small values (about 30 cm) in the other two biotypes. In the second year, higher values were observed but the trend was similar: biotype from AG gained the maximum height value of the whole experiment (85.0 cm) and, in general, plants grown in pots reached higher values than those in open field. In the last two trial years, plant height seemed to stabilize on rather constant values; however, plants managed in pots, although not statistically significant, expressed an overall decrease in mean height.

The total number of stems per plant (vegetative + flowered) (Table 2) was significantly influenced by the different management systems in all years and in each different biotype (YxM and MxB interactions significant at $p \leq 0.05$). This result, combined with the observation of the mean YxMxB interactions (Figure 2) allows to drive some general consideration about the plants' response throughout the different years and conditions.

Table 1. Results of ANOVA (*F* values) for the height of plants at flowering time of 3 *H. perforatum* biotypes cultivated from 2014 to 2016 in open field and in pots.

Source of Variability	DF	2013	2014	2015	2016
Management (M)	1	<1 n.s.	33.67 ***	2.14 n.s.	3.52 n.s.
Biotype (B)	2	111.94 ***	162.66 ***	1.71 n.s.	<1 n.s.
M × B	2	<1 n.s.	<1 n.s.	<1 n.s.	1.03 n.s.
Error	24				
Total	29				

***: significant at $p \leq 0.001$; n.s.: not significant.

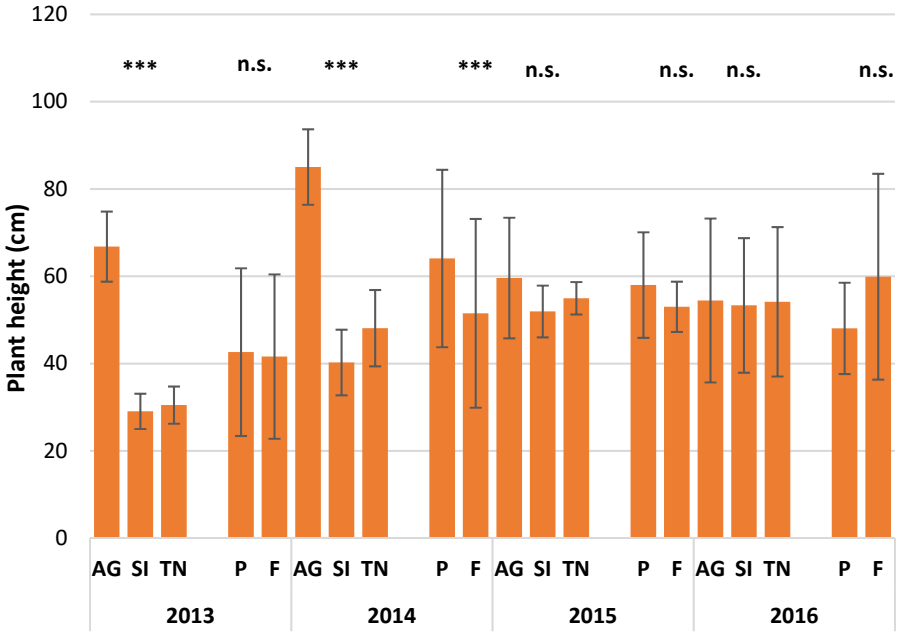

Figure 1. Height (cm) at flowering time of 3 *H. perforatum* biotypes cultivated from 2014 to 2016 with two management systems. Mean values by biotype (AG—Agrigento, SI—Siena, and TN—Trento) and plant management (P—pots and F—open field) across cultivation years. Error bars indicate standard deviation. Symbols above each group refer to the significance at ANOVA (***: $p \leq 0.001$; n.s.: not significant).

Table 2. Results of ANOVA (*F* values) for the number and the fresh (FW) and dry (DW) weight of stems per plant in 3 *H. perforatum* biotypes cultivated from 2014 to 2016 with two management systems.

Source of Variability	DF	Number of Stems (no.)			Weight of Stems (g)	
		with Flowers	Vegetative	Total	FW	DW
Year (Y)	3	<1 n.s.	2.65 n.s.	<1 n.s.	7.69 n.s.	4.15 n.s.
Management (M)	1	1.10	<1 n.s.	<1 n.s.	16.36 *	5.00 n.s.
Biotype (B)	2	<1 n.s.	15.73 **	<1 n.s.	2.37 n.s.	5.94 *
Y × M	3	3.27 n.s.	1.7 n.s.	4.87 *	<1 n.s.	<1 n.s.
Y × B	6	2.52 n.s.	1.53 n.s.	4.1 n.s.	1.31 n.s.	1.38 n.s.
M × B	2	10.16 *	1.4 n.s.	8.22 *	3.34 n.s.	4.81 n.s.
Y × M × B	6	3.28 ***	1.29 n.s.	1.67 n.s.	7.27 ***	5.40 ***
Error	96					
Total	119					

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$; n.s.: not significant.

Although ANOVA did not highlight significant differences on the YxMxB interactions, it is worth noting that pots in 2015 allowed both the maximum (27.5 stems/plant, biotype TN) and the minimum (7.2 stems/plant, biotype SI) of the whole experiment. Nonetheless, on average, the highest number of stems per plant was reached under field conditions in 2014 and 2016, whereas the lowest could be observed in pots in 2014. In the ANOVA table of the weight of stems per plant (Table 2) a significant three-factor interaction (YxMxB) shows up, underlining the outstanding differences in the behavior of the three biotypes as a consequence of the tested experimental factors. In all four trial years, total aerial plant biomass (stems + flowers), either fresh or air-dried, varied significantly according to all experimental factors, and in all cases but 2016, the MxB interaction was also significant (Table 3).

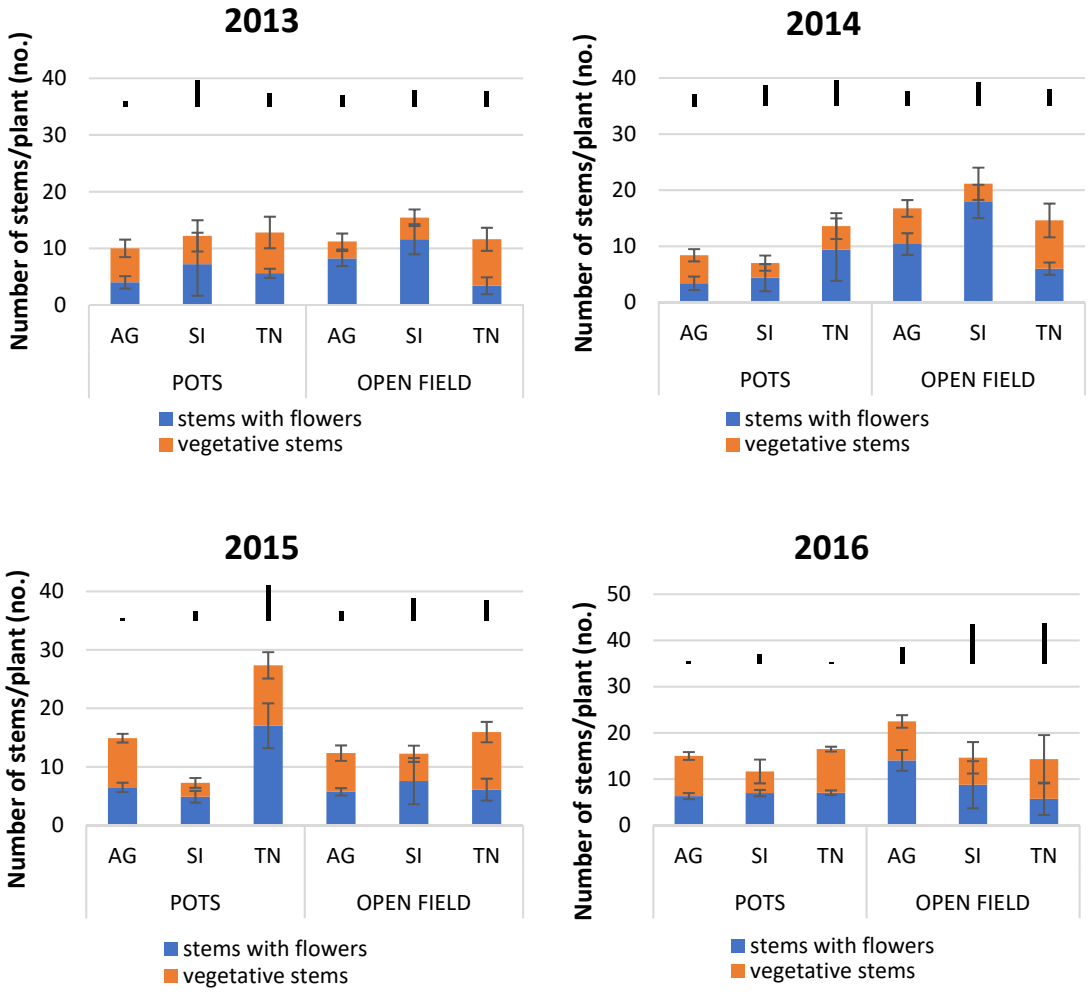

Figure 2. Average number per plant of stems with flowers (blue bars) and vegetative stems (orange) in 3 *H. perforatum* biotypes cultivated from 2014 to 2016 in pots and in open field. Error bars represent the standard deviations of each mean. Vertical bars indicate the standard deviations of the total number of stems (flowered + vegetative).

Table 3. Results of ANOVA (*F* values) for the fresh (FW) and dry (DW) weight of aerial plant biomass from 2014 to 2016 according to crop management (M) and biotype (B).

Source of Variability	DF	2013		2014		2015		2016	
		FW	DW	FW	DW	FW	DW	FW	DW
Management (M)	1	8.18 **	14.77 ***	1.96 n.s.	4.66 *	19.91 ***	5.94 *	11.12 **	6.58 *
Biotype (B)	2	52.00 ***	59.67 ***	4.84 *	10.65 ***	38.04 ***	28.79 ***	2.01 n.s.	2.31 n.s.
M × B	2	9.03 ***	12.97 ***	13.11 ***	8.05 **	13.25 ***	8.73 ***	<1 n.s.	<1 n.s.
Error	24								
Total	29								

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$; n.s.: not significant.

These results indicate that the effect of management methods varied in years and, within each year, among biotypes tested. Together with the lower height values (Figure 1), lower number of stems (Figure 2), and weight of plant biomass (Figure 3), accounted for an overall lower plant size in the first year. However, this general trend was differently pronounced according to the biotype and the cultivation management. In terms of plant biomass, the most productive year for plants grown in open field was the second (2014), whereas, an increased plant biomass was observed in the third trial year (2015) in pot

cultivation. Higher herbage yields of *Hypericum* in the second year after sowing have been already reported by other authors [28]; however, various response patterns have been recognized between different *H. perforatum* genotypes [29], and this inherent variability may explain the different outcome obtained when the same genotype is cultivated with contrasting cultivation managements.

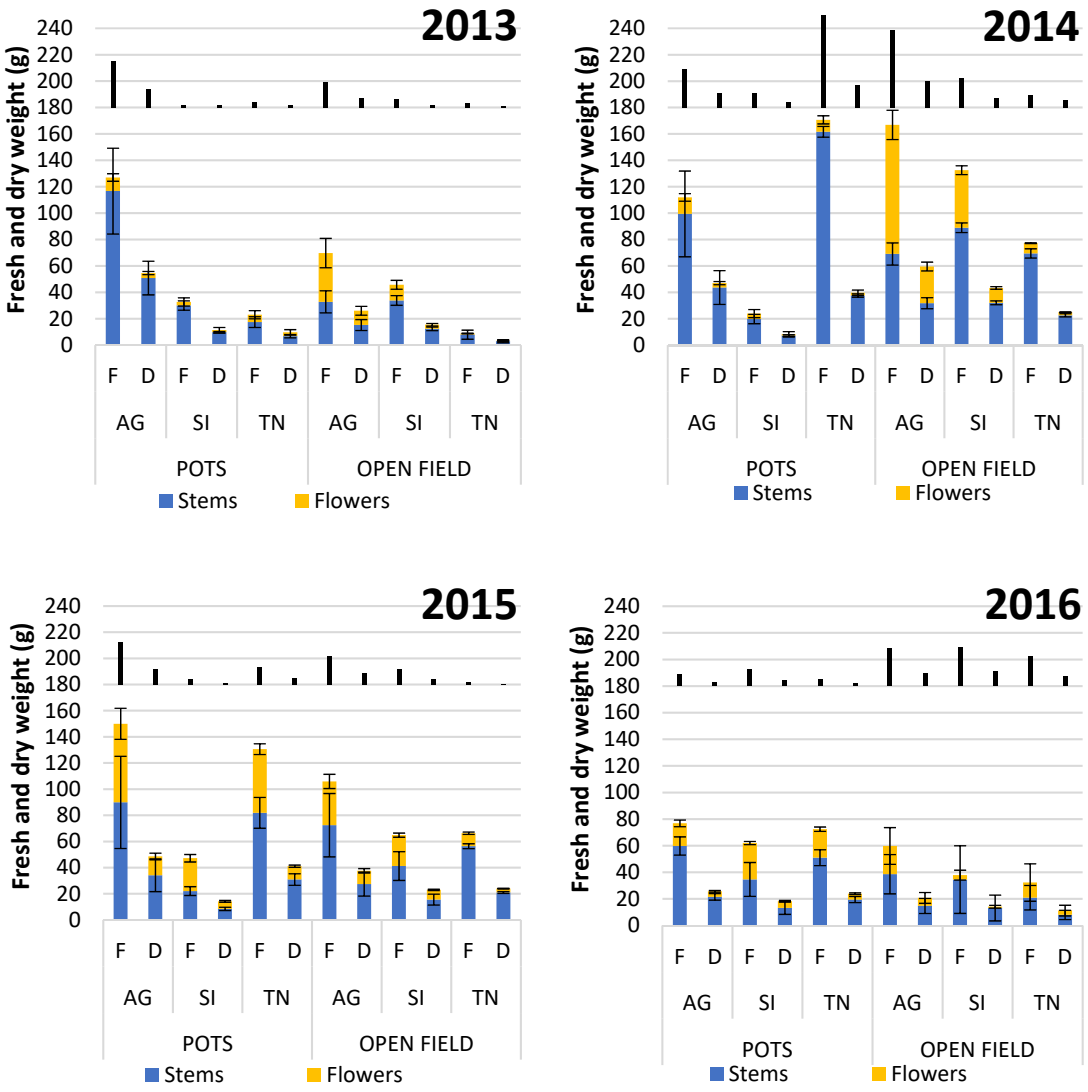

Figure 3. Fresh (F) and dry (D) weight of flowers (yellow) and stems (blue) in 3 *H. perforatum* biotypes cultivated from 2014 to 2016 in two management systems. Error bars represent the standard deviations of each mean. Vertical bars in the upper part of each graph indicate the standard deviations of the total aerial mass (flowers + stems) per plant.

All three biotypes reached the highest value of total aerial biomass in field cultivation, with the only exception of the TN biotype, which, in this respect, found the best cultivation conditions in pot. It must be observed that the TN biotype experienced the highest susceptibility to contrasting cropping conditions, showing both the highest (170.7 g/plant in pots in 2014) and the lowest (8.8 g/plant in open field in 2013) mean values of plant biomass throughout the whole experiment (Figure 3).

In the last trial year (2016), in the majority of experimental conditions—except for the SI biotype cultivated in a pot—plant biomass decreased, achieving values similar to those obtained in the first year. As expected, similarly to the whole aerial biomass, flower yields (Figure 3) were generally lower in the first year and higher in the second; in the remaining two years, flower yields decreased, until reaching values similar to those achieved in the

first year. However, relevant exceptions may be found, as assessed by the highly significant MxB interactions in all trial years (Table 4). The highest yields (97.8 g fresh flowers/plant) were obtained in the second trial year (2014) by the AG biotype grown in open field; in most cases, open field conditions allowed highest flower yields in 2014, whereas, when grown in pot, the same biotypes achieved the highest flower yields in the following year.

Table 4. *Hypericum perforatum* L. results of ANOVA (*F* values) for the fresh and dry mass of flowers according to crop management (M) and biotype (B).

Source of Variability	DF	2013		2014		2015		2016	
		FW	DW	FW	DW	FW	DW	FW	DW
Management (M)	1	24.17 ***	6.42 *	31.26 ***	29.50 ***	104.10 ***	35.69 ***	8.56 **	1.32 n.s.
Biotype (B)	2	34.54 ***	20.89 ***	13.97 ***	13.60 ***	38.74 ***	48.31 ***	<1 n.s.	1.59 n.s.
M × B	2	18.34 ***	12.93 ***	11.56 ***	10.66 ***	25.34 ***	22.30 ***	5.81 **	2.96 n.s.
Error	24								
Total	29								

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$; n.s.: not significant.

3.2. Phytochemical Composition

The majority of the components in the extracts from *H. perforatum* flowers resulted phloroglucinols (hyperforins) and flavonols (myricetin derivates, rutin, myricitrin, hyperoside, isoquercitrin, quercitrin, and quercetin), adding up from 68% to 84% of total identified phenols. This feature is typical of *H. perforatum*, and allows a rather precise separation of this species from many others, even if taxonomically close [25,30].

The PCA on chemical data, grouped by families of compounds (Table 5; Figure 4), allowed in first instance a sharp partitioning of samples among years. All samples are closely distributed near the first Principal Component (PC), showing some dispersion towards negative values above all in 2014. The first PC allows very easily to discriminate samples collected in 2014 from those of 2015 and, to a lesser extent, 2016. Total phenolics content and phloroglucinols affect the composition of the first PC, whereas flavonols affect the second PC. Hence, total phenolics content and phloroglucinols appear to be a relevant factor in discriminating among years. The retrieved amount of both groups of compounds was indeed much lower in 2014 than in 2015 (Figure 5).

Table 5. Loadings, eigenvalues, and variance (%) accounted for by the 7 components found by PCA.

Loadings	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Cat	0.54750	0.59633	−0.01455	0.27531	−0.03179	0.51733	1.97×10^{-14}
Phlor	0.98032	−0.19132	−0.04742	−0.00513	−0.00964	−0.00125	3.02×10^{-16}
Napht	0.69665	−0.40432	0.58406	−0.09964	−0.01061	−0.00619	1.7×10^{-15}
Cinn	−0.48407	0.56480	−0.33371	−0.16055	0.55611	−0.01671	4.41×10^{-15}
Flav	−0.11142	0.98737	−0.07198	−0.06860	−0.05277	−0.00416	9.72×10^{-16}
Dim	0.66202	0.63131	0.11850	0.38590	−0.00336	−0.01434	2.23×10^{-15}
Phen	0.98775	0.15402	0.02353	0.00109	0.00800	0.00106	-2.6×10^{-16}
Eigenvalues	1029.890	79.784	6.816	1.657	0.860	0.034	2.43×10^{-18}
% variance	92.03	7.13	0.61	0.15	0.08	0.00	2.17×10^{-19}

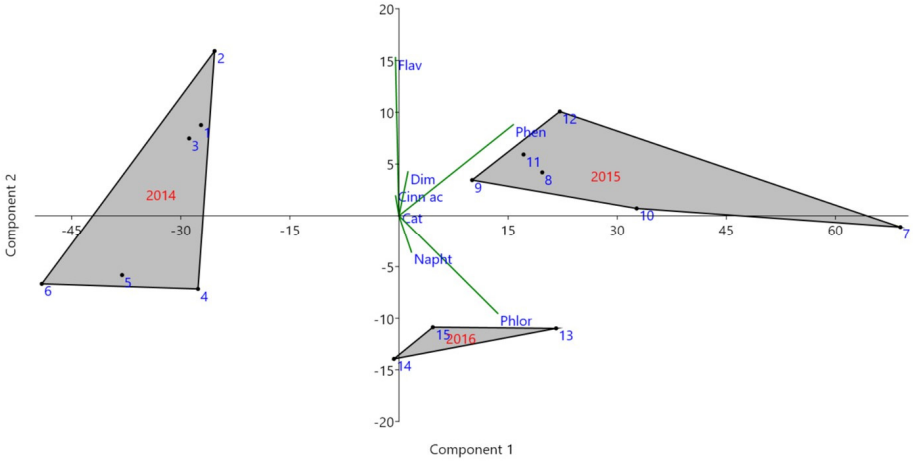

Figure 4. PCA biplot for the major groups of phenolic compounds detected in flowering tops of 3 *H. perforatum* biotypes, grown with two management systems and in three years. N = 15. Explained variance: PC1 = 92.1%; PC2 = 7.1%. Cinn—cinnamic acids; Phlor—phloroglucinols; Dim—dimers; Napht—naphthodianthrones; Cat—catechins; Flav—flavonols; Phen—phenols tot.

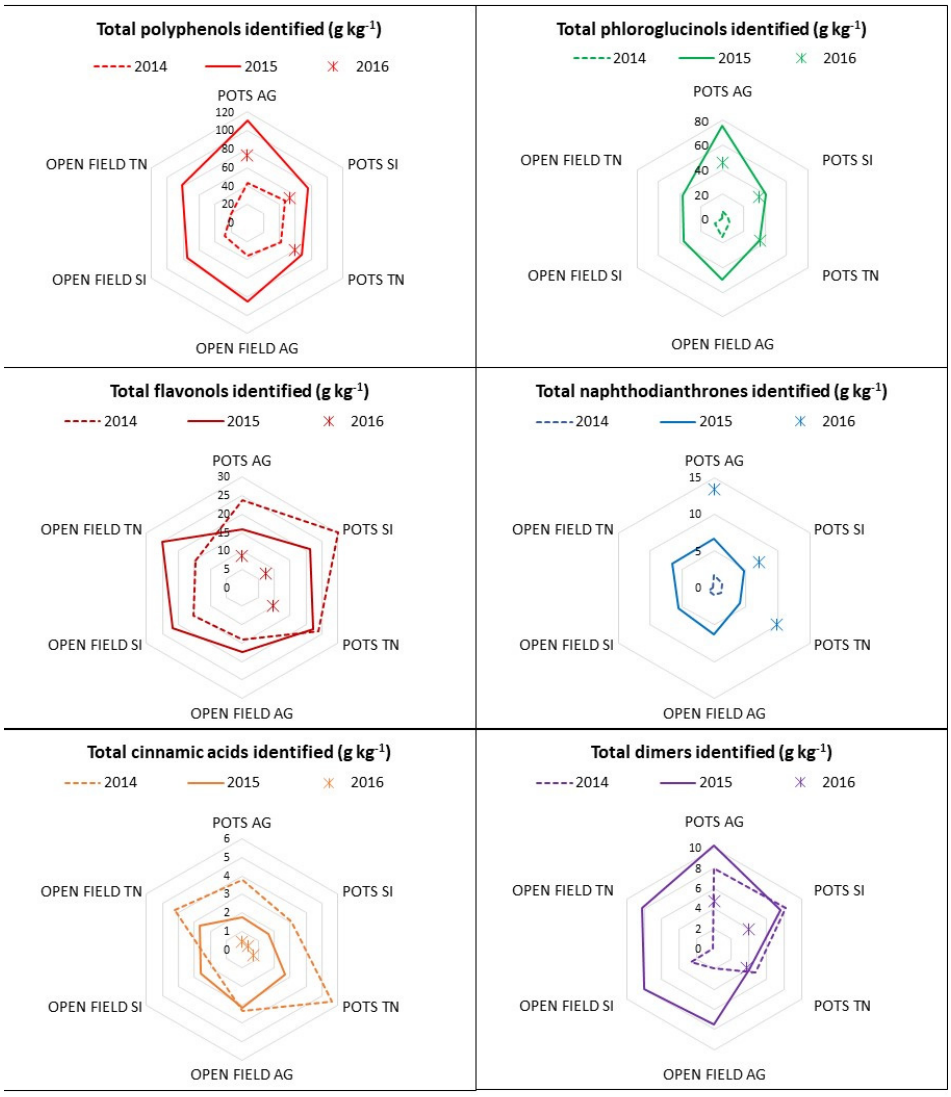

Figure 5. Radar diagrams of the detected content in metabolites, grouped by chemical family, in flowering tops of 3 biotypes of *H. perforatum*. Results from 2014, 2015, and 2016 cultivations in pots and in open field.

In 2015 and 2016, phloroglucinols (35 to 75 g kg^{-1} d.m.) shared more than 50% of total *Hypericum* phenolic content, whereas in 2014 they were less abundant (from 3% to 43%). Hyperforins in plant material mainly take two forms: hyperforin and its homologue adhyperforin. Both compounds are unstable in the presence of light, and are rapidly oxidized [31,32]. According to environmental and cropping conditions, hyperforin content in *H. perforatum* can range between 37–43 g kg^{-1} [9,25], and hyperforin high-yielding and low-yielding genotypes are often recognizable [9]. In this trial, hyperforins production (Figure 6) confirmed to be genotype-dependant, meaning the most high-yielding genotype (the AG biotype) ranked first under all experimental conditions; however, field cultivation seems to stabilize hyperforins yield of all genotypes.

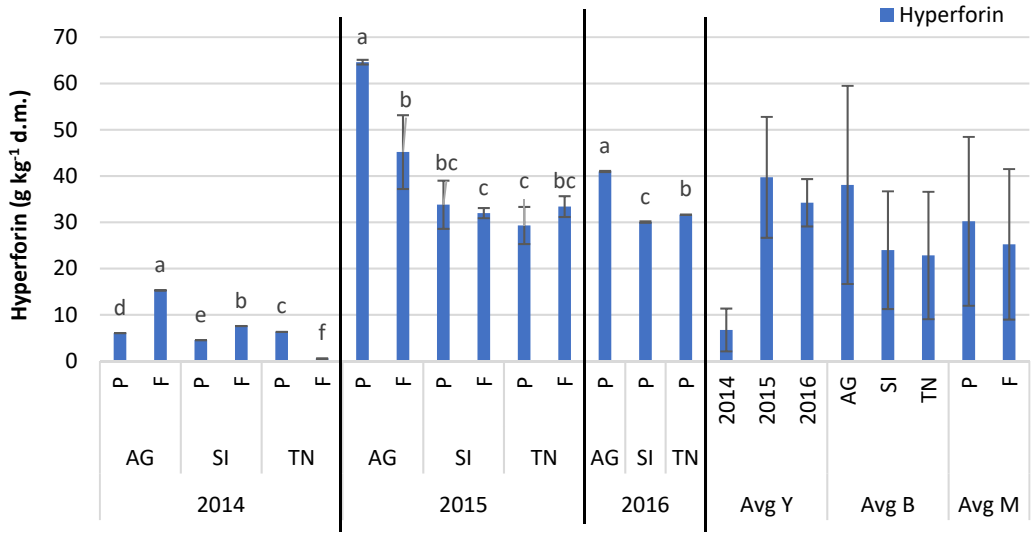

Figure 6. Mean values of hyperforin content (g kg^{-1} d.m.) in flowering tops of 3 biotypes of *H. perforatum* (AG, SI, and TN), obtained in 2014, 2015, and 2016 from cultivations in pots (P) and in open field (F). Within each year, means that do not share a letter are significantly different at $p \leq 0.05$ (Tukey's test). Avg Y—yearly averages; Avg B—averages by biotype; Avg M—averages by cultivation management.

Hypericins (naphthodianthrones) (Figure 7) significantly increased from 2014 to 2016 (on average, from 1.0 to 10.1 g kg^{-1} d.m.), while maintaining rather stable values across biotypes and cultivation management. Within this chemical group, pseudohypericin was always more abundant (from 30% higher, to more than twice) than hypericin. As previously assessed [9], pseudohypericin and hypericin amounts were always linearly associated (r = 0.82), consistent with the hypothesis that they originate from the same precursors [33]. On average, the highest hypericins content (hypericin + pseudohypericin + the precursors protohypericin and protopseudohypericin) was measured within the local biotype (AG, 6.5 g kg^{-1} d.m.), which achieved the highest hypericins yield (13.4 g kg^{-1} d.m.) in 2016 and in pots cultivation. However, although a large variability in hypericins content was found, all analyzed samples showed values higher than the threshold value of 0.8‰, i.e., the minimum amount pointed out by the European Pharmacopoeia [7].

Flavonols (Figure 5) were predominant in 2014, whereas in the two following years they ranked second, after phloroglucinols. According to the European Pharmacopoeia [7], flavonols are mainly represented by glycosides of the flavonol quercetin (hyperoside, rutin, isoquercitrin, and quercitrin), and account for 2%–4% of phytochemical components in *Hypericum* herb. Research has showed that this chemical group has a great importance in determining *Hypericum* bioactivity, most importantly with regards to its significant antioxidant and radical-scavenging activity [25,34]. Although flavonols content was slightly higher in plants from open field than in pots (about 18.7 vs. 17.1 g kg^{-1} d.m.), the widest variations in this group of compounds were undoubtedly due to the year of cultivation. In flowers harvested in 2016, they averaged less than 9 g kg^{-1} d.m, compared to the 25.9 and

19.7 g kg^{-1} d.m. obtained, in the same management conditions (pots) in 2014 and 2015, respectively. Hyperoside, rutin, isoquercetin, and quercitrin, listed in decrescent order, were the most represented flavonols in the analyzed samples.

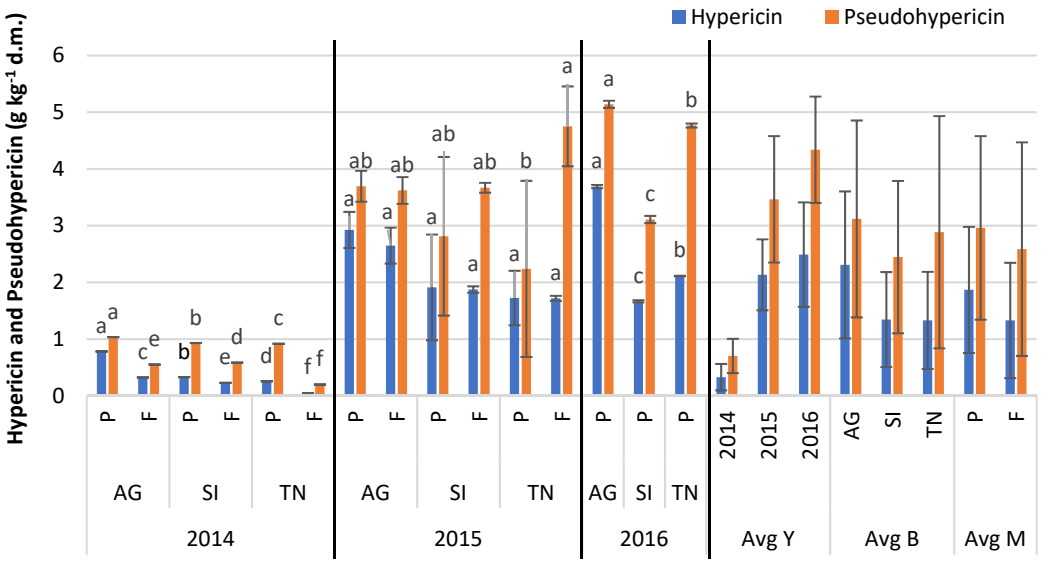

Figure 7. Mean values of hypericin and pseudohypericin content (g kg^{-1} d.m.) in flowering tops of 3 biotypes of *H. perforatum* (AG, SI, and TN), obtained in 2014, 2015, and 2016 from cultivations in pots (P) and in open field (F). Within each year and compound, means that do not share a letter are significantly different at $p \leq 0.05$ (Tukey's test). Avg Y—averages by year; Avg B—averages by biotype; Avg M—averages by cultivation management.

Cinnamic acids (3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, p-coumaroylquinic acid, and p-coumaric acid), involved in the antioxidant properties of the plant [25], showed a sharp decrease throughout experimental years (on average, 3.6, 2.4, and 0.5 g kg^{-1} d.m. in 2014, 2015, and 2016, respectively) (Figure 5). This decrease was more evident in plants cultivated in pots and less marked in plants derived from open field; in open field, two biotypes (AG and SI) exhibited amounts of cinnamic acids similar (biotype AG) and even higher (biotype SI) in 2015 with respect to 2014.

Although their mechanism of action is not perfectly elucidated yet, apigenin dimers (biapigenin and amentoflavone) are thought to be involved in *H. perforatum* pharmacological activity, being probably associated—together with phloroglucinols and naphthodianthrones—to their overall anxiolytic and antidepressant effect [35]. These compounds (Figure 5) were found in lower amounts in 2014 and 2016 (about 4.1 g kg^{-1} d.m), whereas in 2015 their total content raised to 7.6 g kg^{-1} d.m. On average, their content was higher in the AG and SI biotypes (6.7 and 6.2 g kg^{-1} d.m., respectively), and lower in the biotype from Northern Italy (TN), averaging 4.4 g kg^{-1} d.m. Biapigenin (5.8 g kg^{-1} d.m in pots and 5.2 in open field) shared the most part of this chemical group, whereas amentoflavone never surpassed the amount of 0.28 g kg^{-1} d.m.; these values agree with previous results obtained on *H. perforatum* [25].

4. Conclusions

The trial evidenced a high variability in both biomass and phytochemical response of *H. perforatum* according to the growth conditions, also including the environmental effects, enclosed in the factor "year". Some general considerations—useful both for further research and to give preliminary indications to farmers in Mediterranean environments—can be driven.

Firstly, it appeared clear that, although classed as a perennial herb [36], cultivated *Hypericum* has a limited duration. In general, this was no longer than 2–3 years—although plants grown in pots seemed more suitable for longer stands—and herbal yields tended to thereafter stabilize on lower levels.

Secondly, noticeable differences showed up between the results obtained by the two management techniques. Under field conditions, *Hypericum* allowed satisfactory yield levels, in terms of total biomass and herbal product (flowers), in the second cultivation year. Contrastingly, cultivations in pots reached their best yield performances in the following growth year, both in the local biotype and in one of the non-native ones. Some differences could also be observed in the behavior of the biotypes. In general, the locally-obtained biotype performed best in field cultivation, whereas the cultivation in pots seemed more capable of meeting the requirements of non-native biotypes.

This last outcome seems to confirm the higher suitability to field cultivation of local populations, that probably have developed in time a better fitness to local environmental conditions. Of course, further research in different environments, adopting locally-selected biotypes, is necessary to confirm this hypothesis.

All biotypes tested showed hypericins levels satisfactory for marketing. However, from the phytochemical point of view, a remarkable variability was observed. A strong variability of chemical composition due to the effect of cultivation year was expected, as it was already assessed in *Hypericum* [37], as well as in many other medicinal and aromatic plants. Therefore, the cultivation of *Hypericum* requires a properly tuned cropping technique, along with a sound choice of the genotype to be cultivated. When cultivation is addressed to the industry, the choice of the most proper genotype is necessary, but this outstanding variability requires accurate post-harvest analyses to check the qualitative features of each production prior to commercialization, in order to verify if the harvested product meets the required industrial standards.

Finally, it must be observed that pots cultivation does not reflect the performance obtainable from field cultivations, often leading to a biased response. That means, a biotype that seems to achieve exceptionally high results in pots, does not necessarily keep this exceptional performance under field conditions, and vice-versa. Hence, studies performed on *Hypericum* in pots are not able to give a definite response on plants performance in open field, making accurate post-harvest analyses necessary.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11050446/s1, Figure S1: Ten-day values of rainfall (mm) and minimum, maximum, and average temperatures (°C) recorded at Sparacia (Cammarata, AG, Sicily) from November 2012 to July 2016; Table S1: Main characteristics of the soil used for the trial at Sparacia (Cammarata, AG, Sicily).

Author Contributions: Conceptualization, S.L. and A.C.; methodology, S.L. and A.C.; validation, S.L., A.C. and E.N.; formal analysis, A.C.; investigation, S.L. and E.N.; data curation, S.L. and E.N.; writing—original draft preparation, A.C.; writing—review and editing, S.L. and E.N.; visualization, S.L., A.C. and E.N.; supervision, A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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