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Effect of weed control on production and quality in a plantation of *Echinacea purpurea* (L.) Moench in Galicia (NW Spain)

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Echinacea purpurea (L.) Moench. is a perennial plant used for the treatment and prevention of the common cold and flu because of its immunomodulatory and anti-inflammatory effects. At present, it is one of the most widely used medicinal herbs in the world. This study evaluated several weeding methods during three years of *E. purpurea* cultivation: mulching with black polyethylene film, corn gluten meal treatment, white clover living mulch, flame weeding, and metribuzin application. The adventitious flora was periodically inventoried, recording the coverage (%) and density (individuals/m²). Furthermore, yields of biomass were determined and concentrations of alkaloids and caffeic acid derivatives were analyzed. Plastic mulching and metribuzin controlled weeds efficiently, allowing profitable biomass production. The highest yield of aerial and underground parts was afforded by plots mulched with black polyethylene film (5800 kg DM/ha and 1032.5 kg DM/ha at year 3). The highest concentrations of alkaloids were found in plants from plots treated with metribuzin. Caffeic acid derivatives concentration in plants from plots treated with metribuzin was similar to that from plots treated with plastic mulching.

Key words: Adventitious flora, alkaloids, biomass, caffeic acid derivatives, flame weeding, live mulch, medicinal plant, metribuzin, polyethylene film.

INTRODUCTION

Coneflowers are perennial herbs of the genus *Echinacea* (Asteraceae) found naturally in North American grasslands and open woodlands (Binns et al., 2002). Three species of purple coneflower, *E. purpurea* (L.) Moench, *E. pallida* (Nutt) Nutt and *E. angustifolia* DC, are valued for their medicinal effects (Mistríková and Vaverková, 2007). All three have traditionally been used by native North Americans in remedies against ills including pain, colds, influenza, cough and fever, and even as antidotes against snakebites (Foster, 1991; Kindscher, 1999; Li, 1998). In modern studies they have been observed to possess antiviral, antibacterial, anti-inflammatory, antioxidant and immunostimulatory properties (Bodinet et al., 1993; Facino et al., 1995; Skwarek et al., 1996; Burger et al., 1997; Sloley et al., 2001),

apparently due to the combined actions of caffeoyl phenols, alkaloids and polysaccharides that are present, in different proportions, in all three species (Briskin, 2000; Speroni et al., 2002; Randolph et al., 2003; Raduner et al., 2006; Pillai et al., 2007).

For several years now the cultivation of these medicinal *Echinacea* species has been increasing in various parts of the world in order to supply a growing market. The most extensively cultivated, and the most widely used for medicinal products, is *E. purpurea* (Loaiza et al., 2004). Products based on *E. purpurea* extracts are among the world's best-selling natural medicines for the prevention of colds, influenzas and respiratory infections, and for stimulation of immunomodulation (Mahady et al., 2001; Vimalanthan et al., 2005; Hinz et al., 2007).

Though *E. purpurea* is successfully cultivated in the USA, Canada, Europe, Russia and New Zealand, relatively little has been published on the influence of plantation management practices on biomass and bioactive substance yields (Chen et al., 2008). One of the aspects of plantation

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management to which most attention has been paid is weed control, especially in newly established plantations, since coneflowers compete poorly with adventitious weeds, by which they are easily displaced (Li, 1998; Kristiansen et al., 2008).

Farmers frequently use herbicides to control adventitious flora in echinacea plantations because of their ease of application and efficiency. Nevertheless, some studies prove that in echinacea plantations treated with chemical herbicides significantly high levels of these chemicals are found in the echinacea plants (Kucharski, 2000). To avoid this potential problem the ecological culture of echinacea is recommended, by using other methods for weed control, as hand weeding, tillage or mulch (Kristiansen et al., 2007).

In Galicia, NW Spain, the company Milhulloa cultivates and processes medicinal plants, included *Echinacea purpurea* (L.). Control of weeds is for this company the most important problem to be solved, especially because of the prevailing humid warm climate of the region, which favours weed growing.

In this study we investigated the influence of various methods of weed control (black polyethylene mulch, corn gluten meal, *Trifolium repens* cover, flaming, and metribuzin treatment) on the biomass yield and alkamide and phenolic acids contents of a NW Spanish *E. purpurea* plantation during three successive years.

MATERIALS AND METHODS

Experiments were carried out 470 m above sea level in Palas de Rei (Lugo, NW Spain) (7° 56' W, 42° 53' N), an area with an annual mean temperature of 11.9 °C and mean annual precipitation of 1305 mm. The main chemical characteristics of the soil of the experimental site, a silt loam, are listed in Table 1.

At the beginning of April 2008, an area of 250 m² was ploughed and fertilized with 1 t/ha of Biof[®], an organic fertilizer composed of granulated dehydrated chicken manure that is 4 % N, 2.2 % K₂O and 2.6 % P₂O₅. The dose was calculated taking into account the initial characteristics of the soil, and the recommendations of other authors based on field experiments (Galambosi and Valo, 1995; Shalaby et al., 1997). Eighteen 1.6 × 5.0 m experimental plots separated from each other by 0.5 meter-width paths were defined, and the paths were covered with 50-µm-thick black polyethylene film. Three plots were assigned to each of five weed control treatments (mulching with 50 µm black polyethylene film, a live mulch of white clover, corn gluten meal treatment, flame weeding, and metribuzin treatment) and to the statistical control treatment (no weed control) in a completely randomized design.

The plots with polyethylene mulch were completely covered with this material. The live mulch was established by sowing white clover (*Trifolium repens* L.)

cv. Huia at a rate of 38 kg/ha. For the gluten meal treatment, corn gluten (3.3 t/ha) was spread homogeneously on the plot surface. The herbicide treatment (metribuzin; Sencor 70 WG ®, Bayer) was applied at the beginning of May under dry sunny weather conditions, by spraying at a rate of 0.75 kg/ha. Finally, flame weeding was carried out twice, a few days before planting (9th May) and one month later, using a Dessicagaz[®] (model 1B) burner.

Two-month-old homogeneous *E. purpurea* seedlings germinated in a seedbed under greenhouse conditions were planted in mid May with a distance of 30 cm between successive plants in rows 50 cm apart. After 1 year, during flowering (early September to early October), their aerial parts were harvested and, immediately after collection, leaves, flowers and stems were separated and their fresh and dry weights were determined. This was repeated in the second and third years of cultivation, when the harvest also included roots, which were washed, cut in pieces, dried, and weighed (the roots of half the plants of each plot were collected in year 2, and those of the other half in year 3). To avoid the thermal degradation of active principles, drying was in all cases carried out in an oven at 35°C.

The adventitious flora in each plot was periodically inventoried, recording the coverage (%), density (individuals/m²) and total biomass of each species (only data for the last inventories of the first and second years, taken just before harvest, are presented here). Species were identified using *Flora Europea* (Tutin et al., 1964-1980) and *Flora Ibérica* (Castroviejo et al., 1986-2013). In the case of stoloniferous and rhizomatous species, every fragment composed of roots, stem and leaves was considered as one individual.

The levels of the major *Echinacea* alkamides (dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides) and of the caffeic acid derivatives caftaric acid and cichoric acid were determined in representative samples from polyethylene- and metribuzin-treated plots. One-gram powdered samples were extracted three times with 25 ml of ethanol, sonicating each suspension for 5 min, and the pooled extracts were analysed by HPLC as follows.

HPLC Analysis

HPLC was performed using 75×46 mm (L×ID) Superspher[®] 100 RP-18 cartridges (particle size 3 µm; from BDH, Toronto, Canada). For alkamides, the chromatographic conditions were adapted from Bauer and Reminger (1989) to take into account the shorter column used in our work: gradient elution with acetonitrile/water at a flow rate of 1.0 ml/min was performed using a linear rise from 40% to 80% acetonitrile over 15 min, followed by a linear fall to 40% acetonitrile in 1 min, and 6 min at 40%; cut in peaks were

Table 1. Chemical analysis of the soil of the experimental plot.

pH H ₂ O	pH KCl	C (%)	OM (%)	N (%)	C/N	P (mg/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	K (cmol/kg)	Al (cmol/kg)	% sat Al
5.70	5.30	3.88	6.71	0.244	37.5	6.37	5.34	0.69	0.78	0.29	0.38	5.03

Table 2. Yields of aerial parts (flowers, stems and leaves) of *Echinacea purpurea* (kg of dry matter (DM) per ha) after 1 year of cultivation in control plots and in plots given different weed control treatments. Values with different letters in the same column differ significantly ($P < 0.05$).

Weed treatment	control	Yield (kgDM/ha) Aerial parts	Flowers	Stem	Leaves
Control		24.8 a	0 a	5.8 a	19.0 a
Metribuzin		378.5 b	7.6 b	95.5 b	275.4 b
Flame weeding		96.4 c	0 a	30.3 a	66.1 a
Gluten meal		86.6 c	0 a	27.0 a	60.2 a
Live mulch		86.6 c	0 a	27.7 a	58.9 a
Polyethylene		1543.1 d	87.1 c	359.5 c	1096.5 c

monitored simultaneously at 210 and 260 nm, and the UV spectrum of each peak between 200 and 400 nm was recorded on-line. For phenolics, gradient elution at a flow rate of 1.5 ml/min was carried out using 50 mM sodium dihydrogen phosphate brought to pH 2.80 with phosphoric acid (solvent A) and 1% 0.1 M phosphoric acid in acetonitrile (Solvent B): a linear gradient from 5% to 25% B over 7 min was followed by 2 min at 25%, a fall to 5% B in 1 min, and a 5 min equilibration with 5% B; detection was carried out at 320 nm (Bergeron et al., 2000).

After verification of normality using Kolmogorov-Smirnov tests, data were analysed by one-way ANOVA, followed when appropriate by *post hoc* tests Duncan's test for homoscedastic data, the Games-Howell test for heteroscedastic data). Statistical analyses were performed with SPSS 14.0 software.

RESULTS AND DISCUSSION

Yields of Aerial and Underground Parts

The highest yield of aerial parts at first harvest (1 year after plantation) was afforded by plots mulched with black polyethylene film, which produced 1543.1 kgDM/ha (Table 2). This exceeds the first-year yield obtained in the USA by Coltrain (2001), 1120 kg/ha. The untreated control plots were the least productive (24.8 kgDM/ha). No significant differences were found among the 86.6-

96.4 kgDM/ha yields of plots treated with white clover cover, gluten meal or flame weeding, all of which were higher than that of the control plots, lower than that of the metribuzin plots (378.5 kgDM/ha), and much lower than that of the plots with polyethylene mulch. The only coneflowers to flower were those of polyethylene- or metribuzin-treated plots.

At harvest time in the second and third years of cultivation, the aerial parts of coneflowers in plots treated with gluten meal, flame weeding or live mulch consisted only of the basal rosette, while in control plots coneflowers were absent, having been totally displaced by weeds. Consequently, aerial parts were only harvested in plots treated with polyethylene or metribuzin, the yields in both years being almost five times higher in the former, a significant difference (Table 3). In both cases, the second- and third-year yields were very similar, and 3-4 times higher than the first-year yield. These results are in consonance with yields obtained in experiments carried out in Italy and Germany (Aiello et al., 2002; Bomme, 2000).

Roots were harvested in all plots in years 2 and 3, highest yields being obtained from polyethylene- and metribuzin-treated plots (Table 4). These yields are lower than the 1.8-3.0 t/ha/year reported from Germany, New Zealand or the USA (Loaiza et al., 2005). Though it is recognized that coneflowers are poor-soil plants, no definitive information on their nutrient requirements are available (Berti, et al., 2002). Nevertheless, soil pH appears to be a key factor in obtaining commercially viable

Table 3. Yields of aerial parts (flowers, stems and leaves) of *Echinacea purpurea* (kg of dry matter (DM) per ha) after 2 and 3 years' cultivation in plots treated with metribuzin or polyethylene mulch. Values with different letters in the same column differ significantly ($P < 0.05$).

Weed control treatment	Year	Yield (kgDM/ha)			
		Aerial parts	Flowers	Stem	Leaves
Metribuzin	2	1145.6 a	152.9 a	476.4 a	516.2 a
	3	1223.8 a	163.8 a	510.1 a	550.0 a
Polyethylene	2	5673.6 b	858.6 b	2225.0 b	2592.0 b
	3	5800.0 b	900.2 b	2789.0 b	2110.8 b

Table 4. Yields of *Echinacea purpurea* roots (kg of dry matter (DM) per ha) after 2 and 3 years' cultivation in control plots and in plots given different weed control treatments. Values with different letters in the same column differ significantly ($P < 0.05$).

Weed control treatment	Year 2		Year 3	
	Mean	SD	Mean	SD
Control	0 a		0 a	
Metribuzin	382.08 c	16.02	565.05 c	52.79
Flame weeding	106.00 b	5.96	94.97 b	7.88
Gluten meal	105.03 b	8.17	92.55 b	8.27
Live mulch	55.13 b	3.78	54.85 b	3.46
Polyethylene	1080.70 d	247.49	1032.50 d	192.50

Data are present as mean and standard deviation

yields (Loaiza et al., 2002). The values at which coneflower production has been reported to be most profitable, pH 7.0-7.5 (Douglas and Parmenter, (2001) are considerably higher than that of our experimental plots, pH 5.7).

Weed Control

The above yield results faithfully reflected the efficacy of the various methods of weed control, the most effective methods being polyethylene mulching and metribuzin treatment (Table 5, bottom panel). White clover cover also significantly reduced weed coverage compared to the 100% coverage of control plots, but only by about 50%. Flame weeding and gluten meal treatment were quite ineffective.

In the control plots coneflower was unable to withstand competition from weeds and was displaced, confirming that the initial stages of plantation establishment are critical (Galambosi, 2004).

A total of 34 weed taxa were identified; those for which more than 10 individuals/m² were recorded are listed in Table 5 (top panel). In control plots, the highest densities were those of *Agrostis stolonifera* L., *Holcus lanatus* L. and *Fallopia convolvulus* (L.) A. Love. Gluten meal and flame weeding were not only inefficient but even encouraged the proliferation of some species, such as *Achillea millefolium* L., *Agrostis stolonifera* and *Holcus*

lanatus. White clover cover effectively suppressed some species (*Fallopia convolvulus*, *Corrigiola telephiifolia* Pourret), but not others (*Achillea millefolium*, *Agrostis stolonifera*, *Holcus lanatus*). Polyethylene mulch totally prevented the establishment of weeds, while metribuzin was effective against broad-leaved species but not grasses.

The efficacy of polyethylene mulching for weed control is well documented for many crops (Perry and Sanders, 1986; Bonanno and Lamont, 1987), including medicinal and aromatic plants (Ricotta and Masiunas, 1991; Hoeberechts et al., 2004). Mulches contribute to weed management in organic crops by reducing weed seed germination, blocking weed growth, and favoring the crop by conserving soil moisture and sometimes by moderating soil temperature. Opaque synthetic mulches like black plastic provide an effective barrier to most weeds and are amenable to mechanized application (Tadasle and Mohler, 2000). The high cost of laying down the polyethylene film can be counterbalanced by its durability, since polyethylene can be kept on the ground during the 3-4 year duration of echinacea cultivation (Galambosi and Szebeni-Galambosi, 1992), though the cost of removing the film prior to establishment of a new plantation must also be taken into account.

Metribuzin treatment was quite efficient the first year, but its effect diminished with time, allowing the re-establishment of grasses and perennial asteraceae. In Polish trials of chemical herbicides for use in this kind of

Table 5. Average density (individuals/m²) of the most frequent weed species in years 1 and 2 (top panel), and total coverage of soil by weeds (bottom panel), in control plots and plots given different weed control treatments. Within rows, values with different letters differ significantly ($P < 0.05$).

Average density (individuals/ m ²)						
Species	Treatments					
	Control	Gluten	Flame	Clover	Metribuzin	Polyethylene
<i>Achillea millefolium</i> L.	21 b	33 b	42 b	24 b	3 a	0 a
<i>Agrostis stolonifera</i> L.	106 c	121 c	221 d	94 c	27 b	0 a
<i>Corrigiola telephiifolia</i> Pourret	24 b	24 b	6 a	12 ab	3 a	0 a
<i>Fallopia convolvulus</i> (L.) A. Love	42 b	18 b	9 a	2 a	0 a	0 a
<i>Holcus lanatus</i> L.	81 c	221 d	315 e	163 cd	27 b	0 a
<i>Hypochaeris radicata</i> L.	9 a	15 b	24 b	6 a	0 a	0 a
<i>Leontodon taraxacoides</i> (Vill.) Mérat	6 a	3 a	18 b	10 ab	0 a	0 a
<i>Plantago lanceolata</i> L.	9 a	10 ab	3 a	6 a	0 a	0 a
<i>Raphanus raphanistrum</i> L.	9 a	12 ab	15 b	15 b	6 a	0 a
<i>Rumex acetosa</i> L.	12 ab	9 a	12 ab	15 b	3 a	0 a
<i>Spergula arvensis</i> L.	9 a	12 ab	12 ab	12 ab	3 a	0 a
Soil coverage (%)						
Year	Treatments					
	Control	Gluten	Flame	Clover	Metribuzin	Polyethylene
1	100 d	80 d	80 d	50 c	15 b	0 a
2	100	100	100	50	50	0

plantation the most efficient chemicals were prometryn, promyzamide (to control broad-leaved species) and fluazifop-p-butyl (to control grasses) (Kordana et al., 1996; Kucharski, 1997) the herbicide levels found in the crops themselves were admissible (Kucharski, 2000).

The main advantage of flame weeding is the ability to weed more rapidly than hand-weeding. In addition, is performed standing up, which is easier on the body, cutting down on the amount of time needed bent over hoeing or hand-weeding (Cohen, 2006). In our study, flame weeding was clearly ineffective, and even favoured invasion by heat-resistant plants (*Achillea millefolium*, *Holcus lanatus*, *Hypochaeris radicata* L.). Furthermore, flame weeding is expensive, requiring costly dispensers and significant labour and energy (Nystrom and Svensson, 1987; Nemming, 1994; Cohen, 2006).

Gluten meal is a byproduct of corn (*Zea mays* L.) wet milling that contains several dipeptides that suppress seedling germination and root growth (Christians, 1993; Unruh et al., 1997). It has been patented as a natural pre-emergent herbicide, and approved for use in certified organic production systems. Gluten meal applied before weed germination reduces weed density but it is applied

shortly after germination, it acts as a nitrogen source, promoting the development of both crop and weeds (Christians, 1993).

In this study corn gluten meal had no weed control effect; on the contrary, it encouraged the development of weeds and their subsequent competition with the crop. Studies in Kentucky concluded that gluten meal has a low herbicidal effect at recommended rates (Bomford et al., 2006).

Live white clover mulch is both an inexpensive weed control method and also improves soil fertility (Hargrove and Frye, 1987). Legume cover crops provide a substantial amount of biological fixed N to the primary crop and possess a strong ability to absorb low available nutrients in the soil profile and can help in increasing concentration of plant nutrients in the surface layers of soil (Fageria et al., 2005). Its efficacy in weed control lies in its quickly covering bare soil (Romero et al., 2001), and is diminished if perennials with vigorous root systems are present. On the other hand, clover also competes with the crop, which in our case may explain the low yields and poor root development of echinacea in our white clover plots: the average DM weight of roots per plant

Table 6. Total alkamide concentration (mg/g DM) in flower heads and roots of echinacea plants from plots treated with metribuzin (Met) or polyethylene mulch (PEt), after 2 and 3 years' cultivation. Within columns, values with different letters differ significantly ($P < 0.05$).

Year	Treatment	Roots		Flower heads	
		Mean	SD	Mean	SD
2	Met	0.587 b	0.148	0.16a	0.03
	Pet	0.146 a	0.293	0.17a	0.03
3	Met	0.664 b	0.057	0.16a	0.02
	Pet	0.474 a	0.085	0.18a	0.04

Data are present as mean and standard deviation

Table 7. Concentrations of caftaric acid (Caft) and cichoric acid (Cich) (mg/g DM) in leaves, flowers, stems and roots of echinacea plants from plots treated with metribuzin (Met) or polyethylene mulch (PEt), after 1, 2 or 3 years' cultivation.

Year	Phenolic acid	Treatment	Leaves		Flowers		Stems		Roots	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	Caft	Met	0.196	0.012	0.954	0.095	0.129	0.08		
		Pet	0.141	0.025	1.096	0.019	0.265	0.06		
	Cich	Met	0.326	0.03	2.609	0.061	0.775	0.12		
		Pet	0.446	0.17	2.622	0.099	0.587	0.08		
2	Caft	Met	1.514	0.065	1.201	0.074	1.129	0.12	0.978	0.097
		Pet	1.513	0.084	1.213	0.055	1.016	0.06	0.676	0.017
	Cich	Met	2.750	0.02	2.527	0.293	0.775	0.12	1.891	0.074
		Pet	3.245	0.36	2.737	0.369	0.587	0.08	1.813	0.251
3	Caft	Met	1.670	0.069	1.517	0.066	1.006	0.10	0.766	0.02
		Pet	1.499	0.029	1.521	0.039	1.129	0.12	0.544	0.03
	Cich	Met	2.363	0.257	2.821	0.425	0.725	0.10	1.750	0.032
		Pet	2.498	0.261	2.628	0.131	0.871	0.03	1.897	0.094

Data are present as mean and standard deviation.

was about 4 g in these plots, similar to the 3.3-4.0 g in plots treated with gluten meal or by flame weeding, which had 80-100% weed coverage, and significantly less than the 18 g of plots treated with polyethylene mulch and 10 g of those treated with metribuzin.

Active principle contents (alkamides and caffeic acid derivatives)

Previous studies have shown that active principle content varies greatly among individual coneflower plants (Kreft, 2005; Qu et al., 2005). Accordingly, we only determined active principles in plots in which there were enough plants to guarantee that the value determined would with reasonable certainty be representative of the corresponding treatment. This limited the study of active principles to the plots treated with polyethylene mulch or metribuzin, which retained most of the plants initially planted.

Alkamide content was significantly higher in roots than in flower heads, and significantly higher in metribuzin-treated roots than in polyethylene-mulch-treated roots; and, regardless of treatment, root alkamide content was

significantly higher at second root harvest (year 3) than at first harvest (year 2) (Table 6).

A number of studies have shown that alkamide levels are much lower in leaves than in roots (Perry et al., 1997; El Gengaihi et al., 1998; Kim et al., 2000; Stuart and Wills, 2000). That perennial parts (roots and rhizomes) have the highest levels is interpreted as a defence against herbivores, insects and larvae (Pietta, et al., 1998; Ramírez-Chávez et al., 2004; López-Bucio et al., 2006).

The alkamide levels observed in echinacea roots in this work (Table 6) are within the range of those obtained in Taiwan (Chen et al., 2009), and clearly higher than those found in Germany (Bauer and Reminger, 1989). The difference between the root alkamide levels found in metribuzin-treated and polyethylene-mulch-treated plots is in keeping with reports that polyethylene mulching can affect the quality of various crops (Elkner and Kaniszewski, 1995; Farias-Larios and Orozco-Santos, 1997; Andrady, 2003). These observations confirm the influence of geographical, climatic and soil factors, and of management practices, on the concentration of active principles (Yu and Kaarlas, 2004).

Like those of alkamides, the concentrations of caffeic acid derivatives (caftaric and cichoric acids) varied among different parts of the plant (Table 7). This is in agreement with the results obtained by other authors (Stuart and Wills, 2003; Callan et al., 2005; Kreft, 2005). Regardless of which weed control treatment was used, cichoric acid levels varied little in flowers and stems, but in leaves were lower in the first year than in subsequent years, as were caftaric acid levels in all three tissues (especially leaves and stems). In the third year, concentrations were similar to those recorded in the second year. Unlike alkamide content, caftaric and cichoric acid contents did not differ significantly between plants from plots treated with polyethylene mulch and metribuzin. In general, the observed levels of both caftaric and cichoric acid were lower than those reported previously (Qu et al., 2005; Perry et al., 2001; Binns et al., 2002; Kreft, 2005; Pellati et al., 2005; Wu et al., 2007; Chen et al., 2009). However, these results should be considered cautiously since variability in the concentration of the bioactive compounds may be explained not only by the management conditions. It has been found that other factors such as the time of day at which samples were taken, weather conditions, the location of the plots, or the handling of the sample in the course of its chemical analysis, may cause changes in the concentrations of active ingredients (Parmenter and Littlejohn, 1997; Stuart and Wills, 2000; Dou et al., 2001; Berti et al., 2002; Perry et al., 2004). Cichoric acid, in particular, is known to be susceptible to enzymatic degradation (Bauer, 1997; Nüsslein, 2000) and sensitive to pre- and post-harvest processing (Tanko et al., 2005). Also, the echinaceas planted in this study were grown from seed, and genetic differences may therefore have influenced our results (Binns et al., 2002).

CONCLUSIONS

Throughout the growing cycle of *Echinacea purpurea* (L.) Moench. in Galicia, we have tested five control methods: adventitious flora mulching with black polyethylene, corn gluten meal treatment, white clover living mulch, flame weeding, and metribuzin application. The study revealed that only treatments with black polyethylene mulch and metribuzin are effective throughout the whole cycle. The higher yields of biomass were obtained, both for aerial and buried parts, in the plots in which the adventitious flora was monitored with black polyethylene mulch.

Harvested plants in plots treated with metribuzin offered higher performance in alkamides than those obtained in the plots of black polyethylene mulch.

The concentrations of caffeic acid derivatives were similar in the adventitious control treatments with metribuzin and black polyethylene mulch.

In medicinal plant production, the use of effective non-chemical weed control methods is especially important,

since it avoids the presence of residual herbicide in commercial products.

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REFERENCES

- Aiello N, Scartezzini F, Vender C, Albasini A (2002). Influence of the crops' duration and harvest time on yield and quality of different purple coneflower species (*Echinacea angustifolia* DC. var. *angustifolia*, *E. pallida* (Nutt.) Nutt and *E. purpurea* (L.) Moench. ISAFSA Comunicazioni di Ricerca. 1: 15–27.
- Andrady AL (2003). Polyethylenes and the environment. Wiley and Sons, New York.
- Bauer R, Reminger P (1989). TLC and HPLC analysis of alkamides in *Echinacea* drugs. *Planta Med.* 55: 367–371.
- Bauer R (1997). Standardisierung von *Echinacea purpurea*-Preßsaft auf Cichoriensäure und Alkamide. *Z. Phytother.* 18: 270–276.
- Bergeron C, Livesey JF, Awang DVC, Arnason JT, Rana J, Baum BR, Letchamo W (2000). A quantitative method for identity and quality assurance of *Echinacea* products on the North American market. *Phytochem. Anal.* 11: 207–215.
- Berti M, Wilkens R, Fisher S, Hevia F (2002). Effect on harvest season, nitrogen, phosphorous and potassium on root yield, echinacoside and alkamides in *Echinacea angustifolia*. *Acta Hort.* 576: 303–310.
- Binns SE, Livesey JF, Arnason JT, Baum BR (2002). Phytochemical variation in *Echinacea* from roots and flowerheads of wild and cultivated populations. *J. Agr. Food Chem.* 50: 3573–3678.
- Bodinet C, Willigmann I, Beuscher N (1993). Host-resistance increasing activity of root extracts from *Echinacea* species. *Planta Med.* 59: 672–673.
- Bomme U (2000). Produktionstechnologie von Sonnenhut *Echinacea purpurea* (L.) Moench, *E. pallida* (Nutt.) Nutt. und *E. angustifolia* DC. Bavarian State Research Centre for Agronomy, Freising.
- Bonnano AR, Lamont WJr (1987). Effect of polyethylene mulch, irrigation method and row covers on soil and air temperature and yield of muskmelon. *J. Am. Soc. Hortic. Sci.* 112(5): 735–738.
- Bomford M, Silvernail A, Peterson A, Detenber S (2006). Corns gluten meal as organic herbicide: A worthwhile investment?. Kentucky Academy of Science Meeting, Agricultural Science Section, Morehead, KY.

- Briskin DP (2000). Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiol.* 124: 507–514.
- Burger RA, Torres AR, Warren RP, Caldwell VD, Hughes BG (1997). *Echinacea*-induced cytokine production by human macrophages. *Int. J. Immunopharmacol.* 19(7): 371-379.
- Callan NW, Yokelson T, Wall-Maclane S, Westcott MP, Miller JB, Ponder G (2005). Seasonal trends and plant density effect on cichoric acid in *Echinacea purpurea* (L) Moench. *J. Herb. Spices Med. Plants.* 11(3): 35-46.
- Castroviejo S, Lainz J, López-González J (1986-2013). *Flora Ibérica. Plantas vasculares de la Península Ibérica e Islas Baleares.* Real Jardín Botánico. Madrid.
- Chen CL, Zhang SC, Sung JM (2008). The biomass and caffeoyl phenols productions. *J. Agr. Food Chem.* 50: 3673-3687.
- Chen LC, Zhang SC, Sung JM (2009). Caffeoyl phenols and alkamides of cultivated *Echinacea purpurea* and *Echinacea atrorubens* var *paradoxa*. *Pharm. Biol.* 47(9): 835-840.
- Christians NE (1993). The use of corn gluten meal as a natural pre-emergence weed control in turf. IN: R. N. Carrow, N. E Christians, and R. C. Shearman, eds. *Int. Turfgrass Soc. Res. J.* 7: 284-290. Overland Park, KS.
- Cohen B (2006). Flame-Weeding: A Hot Alternative to Herbicides. *J. Pestic. Reform.* 26(4): 6-7.
- Coltrain D (2001). *Economic Issues with Echinacea.* Kansas State University. Agricultural Experimental Station and Cooperative Extension Service. MF-2532.
- Dou D, Cui S, Cao Y, Yan Y, Fei S (2001). Assaying of cichoric acid in introducing plant *Echinacea purpurea*. *Zhong Cao Yao.* 32: 987–988.
- El-Gengaihi SE, Shalaby AS, Agina AE, Hendawy SF (1998). Alkamides of *Echinacea purpurea* L. as influenced by plant ontogeny and fertilization. *J. Herbs Spices Med. Plants.* 5: 35–41.
- Elkner K, Kaniszewski S (1995). Effect of drip irrigation and mulching on quality of tomato fruits. *Acta Hort.* 379: 175-180.
- Facino RM, Carini M, Aldini G, Saibene L, Pietta P, Mauri P (1995). Echinacoside and caffeoyl conjugates protect collagen from *Echinacea* extracts in the prevention of skin photodamage. *Planta Med.* 61: 510-514.
- Fageria NK, Baligar VC, Bailey BA (2005). Role of cover crops in improving soil and row crop productivity. *Commun. Soil Sci. Plan.* 36: 2733-2757.
- Farias-Larios J, Orozco-Santos M (1997). Effect of polyethylene mulch colour on aphid populations, soil temperature, fruit quality, and yield of watermelon under tropical conditions. *New Zeal. J. Crop Hort. Sci.* 25(4): 369-374.
- Foster S (1991). *Echinacea Nature's Immune Enhancer.* Inner Traditions/ Bear and Co. Healing Arts Press.
- Galambosi B, Szebeni-Galambosi Z (1992). The use of black polyethylene mulch and ridges in the production of herbicide free plants. *Acta Hort.* 306: 353-356.
- Galambosi B, Valo R (1995). Kokemuksia punahatun viljelystä, Puutarha. 98(10): 560–561.
- Galambosi B (2004). Cultivation in Europe. In: *Echinacea: The genus Echinacea.* S.C. Miller and H. Yu, editors. CRC Press, Boca Raton, Fla.
- Frye W (1987). The need for legume cover crops in conservation tillage production. In: *The role of legumes in Conservation Tillage Systems.* JF Power, editor. Soil Conservation Society of America, Ankeny, Iowa (USA). 1-5.
- Hinz B, Woelkart K, Bauer R (2007). Alkamydes from *Echinacea* inhibit cyclooxygenase-2 activity in human neuroglioma cells. *Biochem. Biophys. Res. Commun.* 48: 1466-1472.
- Hoeberechts J, Nicola S, Fontana E (2004). Growth of lavender and rosemary in response to different mulchs. *Acta Hort.* 629: 245-251.
- Kim HO, Durance TD, Scaman CH, Kitts DD (2000). Retention of caffeic acid derivatives in dried *Echinacea purpurea*. *J. Agric. Food Chem.* 48: 4182-4186.
- Kindscher K (1999). The uses of *Echinacea angustifolia* and other *Echinacea* species by Native Americans in the Great Plains. In: *Proceedings 1999 AHPA International Echinacea Symposium.* American Herbal Products Association, Kansas City, MO.
- Kordana S, Kucharski W A, Nowak D, Załęcki R (1996). Badania uprawowe jeżówki purpurowej (*Echinacea purpurea* (L.) Moench. *Herba Pol.* 2: 108–113.
- Kreft S (2005). Cichoric acid content and biomass production of *Echinacea purpurea* plants cultivate in Slovenia. *Pharm. Biol.* 43: 662-665.
- Kristiansen P, Sindel BM, Jessop RS (2008). Weed management in organic *Echinacea* (*Echinacea purpurea*) and lettuce (*Lactuca sativa*) production. *Renew. Agr. Food Syst.* 23(2):120-135.
- Kucharski WA (1997). Anbautechnologie und Pflanzenschutz von *Echinacea purpurea* (L.) Moench. *Drogenreport.* 10(16): 33–36.
- Kucharski WA (2000). Herbizidversuche mit Arzneipflanzen in Polen, *Drogenreport.* 13 (24): 41–44.
- Li TSC (1998). *Echinacea: Cultivation and medicinal value.* Horttechnology. 8 (2): 122-129.
- Loaiza J, Valverde R, Cartin V, Gómez L (2004). Producción de *Echinacea purpurea* en tres localidades de Costa Rica. *Agron. Costarricense.* 29(3): 59-66.
- López-Bucio J, Acevedo-Hernández G, Ramírez-Chávez E, Molina-Torres J, Herrera-Estrella L (2006). Novel signals for plant development. *Curr. Opin. Plant Biol.* 9: 523-529.
- Mahady GB, Qato DM, Gyllenhaal C, Chadwick GB, Fong HHS (2001). *Echinacea: Recommendations for its use in prophylaxis and treatment of respiratory tract infections.* *Nutr. Clinic. Care.* 4: 199-208.
- Mistríková I, Vaverková S (2007). Morphology anatomy

- of *Echinacea purpurea*, *E. angustifolia*, *E. pallida* and *Parthenium intergrifolium*. Biol. Brat. 62: 2-5.
- Nemming A (1994). Cost of flame weeding. Acta Hort. 372: 205-212.
- Nüsslein B, Kurzmann M, Bauer R, Kreis W (2000). Enzymatic degradation of cichoric acid in *Echinacea purpurea* preparation. J. Nat. Prod. 63: 1615-1618.
- Nystrom P, Svensson SE (1987). Thermal weed control on hard surfaces. Experimental work 1987. In: Report 123. Swedish University of Agricultural Sciences, Department of Agricultural Engineering, Alnarp, Sweden.
- Parmenter GA, Littlejohn RP (1997). Planting density effects on root yield of purple coneflower (*Echinacea purpurea* (L.) Moench. N. Z. J. Crop Hort. Sci. 24: 169-175.
- Pellati F, Benvenuti S, Melegari M, Lasseigne T (2005). Variability in the composition of anti-oxidant compounds in *Echinacea* species by HPLC. Phytochem. Anal. 16(2): 77-85.
- Perry KB, Sanders DC (1986). Tomato yield as influenced by plant protection system. Hort Science. 21: 238-239.
- Perry NB, Burgess EJ, Glennie VL (2001). *Echinacea* standardization: analytical methods for phenolic compounds and typical levels in medicinal species. J. Agr. Food. Chem. 49: 1702-1706.
- Perry NB, Klink JWV, Burgess EJ, Parmenter GA (1997). Alkamide levels in *Echinacea purpurea*: a rapid analytical method revealing differences among roots, rhizomes, stems, leaves and flowers. Planta Med. 63:58-62.
- Perry NB, Wills RBH, Stuart DL (2004). Factors affecting *Echinacea* quality: Agronomy and processing, In: *Echinacea: The genus Echinacea*. S.C. Miller and H. Yu, editors. CRC Press, Boca Raton, Fla. pp. 111-126.
- Pietta P, Simonetti P, Mauri P (1998). Antioxidant Activity of Selected Medicinal Plants J. Agr. Food Chem. 46(11): 4487-4490.
- Pillai S, Pillat C, Mitscher L, Cooper R (2007). Use of quantitative flow cytometry to measure ex vivo immunostimulant activity of *Echinacea*: The case for polysaccharides. J. Altern. Complem. Med. 13: 625-634.
- Qu L, Chen Y, Wang X, Sealzo R (2005). Patterns of variation in alkamides and cichoric acid in roots and above ground parts of *Echinacea purpurea* (L.) Moench. Hort Science. 40: 1239-1242.
- Raduner S, Majewska A, Chen J-Z, Xie X-Q, Hamon J, Faller B, Altman K, Gertsch J (2006). Alkamides from *Echinacea* are a new class of cannabinomimetics cannabinoid type 2 receptor dependant and independent immunomodulatory effects. J. Biol. Chem. 281: 14192-14206.
- Ramírez-Chávez E, López-Bucio J, Herrera-Estrella L, Molina-Torres J (2004). Alkamides isolated from plants promote growth and alter root development in Arabidopsis. Plant Physiol. 134: 1058-1068.
- Randolph RK, Gellenbeck K, Stonebrook K, Brovelli E, Qian Y, Bankaitis-Davis D, Cheronis J (2003). Regulation of human immune gene expression as influenced by a commercial blended *Echinacea* product: Preliminar studies. Exp. Biol. Med. 228: 1051-1056.
- Ricotta JA, Massiusnas JB (1991). The effects of black polyethylene mulch and weed control strategies on herb yield. Hort Science. 21: 238-239.
- Romero R, Rigueiro A, Fernández JL (2001). Non chemical control of weeds and raspberry sucres in a raspberry plantation in Galicia. In: Proceedings I World Congress on Conservation Agriculture. Garcia-Torres L, Benites J, Martín-Vilela A, editors. 457-462.
- Shalaby AS, El-Gengaighi E, Agina E, El-Khayat AS, Hendawy SF (1997). Growth and yield of *Echinacea purpurea* L. as influenced by planting density and fertilization. J. Herbs Spices Med. Plants. 5(1): 69-76.
- Skawarek T, Tynecka Z, Glowniak K, Lutostanska E (1996). *Echinacea* I. Inducer of interferons. Herba Pol. 42: 110-117.
- Sloley BD, Urchuk LJ, Tywin C, Coutts RT, Pang PK, Shan JJ (2001). Comparison of chemical components and antioxidants capacity of different *Echinacea* species. J. Pharmacol. Pharmacother. 53: 849-57.
- Speroni E, Govoni P, Guizzardi S, Renulli C, Guerra MC (2002). Anti-inflammatory and cicatrizing activity of *Echinacea pallida* Nutt. Root extract. J. Ethnopharmacol. 79: 265-272.
- Stuart DL, Wills RBH (2000). Alkylamide and cichoric acid levels in *Echinacea purpurea* tissues during plant growth. J. Herbs Species Med. Plants. 7: 91-102.
- Stuart DL, Wills RBH (2003). Effect of drying temperature on alkamyde and cichoric acid concentrations of *Echinacea purpurea*. J. Agr. Food Chem. 51: 1608-1610.
- Tanko H, Carrier DJ, Duan L, Clausen Ed (2005). Pre- and post-harvest processing of medicinal plants. Plant Genetic Resources. 3(2): 304-313.
- Teasdale JR, CL Mohler (2000). The quantitative relationship between weed emergence and the physical properties of mulches. Weed Sci. 48: 385-392.
- Tutin TG, Heywood VH, Burges NA (1964-1980). Flora Europea. Cambridge University Press, Cambridge (UK).
- Unruh J, Christians N, Horner H (1997). Herbicidal effects of the dipeptide alaninyl-alanine on perennial ryegrass (*Lolium perenne* L.) seedling. Crop Sci. 37: 208-212.
- Vimalanthan S, Kang L, Amiguet VT, Liversey J, Arnason T, Hudson J (2005). *Echinacea purpurea* aerial parts contain multiple antiviral compounds. Pharm. Biol. 43: 740-745.
- Wu C-H, Murthy HN, Hahn E-J, Paek K-Y (2007). Large-scale cultivation of adventitious roots of *Echinacea purpurea* in airlif bioreactors for the production of cichoric acid, chlorogenic acid and caftaric acid. Biotechnol. Lett. 29: 1179-1189.
- Yu H-c, Kaarlas M (2004). Popularity, diversity, and quality of *Echinacea*. In: *Echinacea: The genus*

Echinacea. Miller SC, Yu Hc, editors. CRC Press, Boca Raton, Florida, USA. 127 – 150.