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The biomethane, silage and biomass yield obtainable from three accessions of *Cynara cardunculus*

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ABSTRACT

In the light of the possible contribution of bioenergy to reducing the dependence on fossil fuel imports and to controlling greenhouse gas emission, a present priority is to develop crops which can be raised using minimal production inputs. The potential of the cardoon (*Cynara cardunculus*) has been investigated here by assessing the outcome of anaerobically digesting two cultivated forms and one wild one. Fermentation lab tests were performed on ensiled biomass, as in the real scale conditions, and they were aimed at measuring the biomethane production per unit of feedstock. The cultivated forms ('Altilis 41' and 'Bianco avorio') produced, respectively, 19.1 and 16.8 t dry matter (DM) per hectare per year, from which, respectively 4074 and 4162 Nm^3 of biomethane was generated; the wild cardoon accession was less productive both in terms of biomass accumulation (11.8 t DM per hectare per year) and estimated biomethane yield (2867 Nm^3). The yield of biomethane ranged from ~200 ('Altilis 41') to 245 ('Bianco avorio' and wild cardoon) Nm^3 per t DM, a level sufficiently high to justify considering *C. cardunculus* as a promising candidate bioenergy crop in the Mediterranean environment.

1. Introduction

The Asteraceae species *Cynara cardunculus* L. includes, in addition to the familiar edible globe artichoke (var. *scolymus*), both the cultivated (var. *altilis*) and wild (var. *sylvestris)* cardoon. The latter is thought to be the progenitor of both of the cultivated forms. It is a perennial C3 species native to the Mediterranean Basin (Sonnante et al., 2007; Mauro et al., 2009), which begins its growth cycle in the autumn and ends it in the early summer, thereby avoiding the hottest and driest time of the year (Fernandez et al., 2006; Mauro et al., 2012). The globe artichoke is cultivated for its immature inflorescence, which is consumed both as fresh and processed (Portis et al., 2005; Bianco, 2011). More than 70% of its production is generated from the Mediterranean Basin, with the remainder grown in the Americas and in China (Faostat, 2016). The cultivated cardoon is prized for its leaf petiole and leaf midrib; its area of cultivation is thought to extend over 2–3 kha, spread over Spain, Italy, France and Greece (Ierna and Mauromicale, 2010). The wild cardoon currently provides a source of vegetarian rennet used for producing some cheeses in Portugal, Spain, Morocco and Italy (Barbagallo et al., 2006). Both cardoon forms have been proposed as possible bioenergy crops (Foti et al., 1999; Fernandez et al., 2006; Grammelis et al., 2008; Gominho et al., 2011; Acquadro et al., 2013; Mauromicale et al., 2014), since they produce a large quantity of biomass even when the plants are only provided with minimal inputs, which allows the crop to be targeted to land not usually used for cropping (Mauromicale et al., 2014; Mauro et al., 2015). Energy can be produced from the biomass either directly via its combustion (Foti et al., 1999; Fernandez et al., 2006), while the oil accumulated in the achenes is suitable as a feedstock for biodiesel (Encinar et al., 2002). Alternatively, the high polysaccharide and low lignin content of cardoon biomass provides opportunities for ethanol or biomethane production via fermentation (Gominho et al., 2001; Ballesteros et al., 2008; Cotana et al., 2015; Fernandes et al., 2015); the latter is currently achieved largely from maize silage, although bread wheat and triticale biomass is also exploited on a smaller scale (Berglund and Börjesson, 2006; Dressler et al., 2012).

Under Italian law, biomethane produced from crop residues and non-food crops attracts a higher subsidy than that produced from food crops (Ministerial Decree of December 5, 2013). The use of crop residues or biomass obtained from a crop such as cardoon, which has a minimal environmental impact, improves environmental performances of energy production through anaerobic digestion (Duca et al., 2015). The suitability of *C. cardunculus* biomass for biomethane generation has thus far concentrated on the by-products of globe

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artichoke production (Ros et al., 2013; Fabbri et al., 2014; De Menna et al., 2016), on cardoon stalks (Oliveira et al., 2012) and on cardoon in mixtures with cattle manure (Kalamaras and Kotsopoulos, 2014). The objectives of the present research were to assess the performance of two cultivated and one wild cardoon entries under low/zero inputs and to evaluate the yield of biomethane obtained by the anaerobic digestion of their biomass (leaves, stalks and inflorescence) ensiled at the beginning of capitulum and seed ripening phenological stage.

2. Materials and methods

2.1. Local climate and soil

The experiment was spread over three seasons, namely 2010–11 (S_1), 2011–12 (S_2) and 2012–13 (S_3). The experimental site is located on the Catania plain in Sicily (37°26′N, 14°56′E), where the soil is classified as a Typic Xerofluvent (Soil Survey Staff, 1999) with a clay loam texture. At the start of the experiments, the soil characteristics were as follows: sand (40%), silt (25%), clay (35%), limestone (2%), pH (7.9), organic matter (1.6%), total nitrogen (1.3 g kg^{-1}), assimilable P_2O_5 (50 mg kg^{-1}), exchangeable K_2O (425 mg kg^{-1}), electrical conductivity (0.41 dS m^{-1}). The local climate is characterized by mild, wet winters and hot, dry summers. The ombrothermic diagram (Fig. 1) shows the rainy and the dry periods, the latter are illustrated by dotted areas. According to Bagnouls and Gaussen (1957) classification, the local climate is thermomediterranean, with 5–6 months dry, from April to September. The mean seasonal rainfall over the period 1971–2000 was 446 mm, over 75% of which fell during the period October to March (CNMCA, 2009); the hottest month has been August (26.6 °C) and the coldest January (10.4 °C). An aridity index, calculated from the ratio between the annual rainfall and the annual reference evapotranspiration (UNESCO, 1979), was derived using the FAO Penman-Monteith method (Allen et al., 1998).

2.2. Meteorological conditions during the trial period

Precipitation during the first two seasons (S_1 and S_2) was higher than during the third season (S_3); in both S_1 and S_2, it was above the long-term mean (Fig. 1, Table 1). The aridity index also underlined that S_3 was the driest of the three seasons, including a dry month during the normally rainy autumn/winter period (Fig. 1). When all three seasons were taken together, February proved to be both the rainiest (132 mm) and the coldest (mean minimum/maximum air temperatures of 3.5 °C/14.9 °C) month. During the rainy period, October was the warmest (14.1 °C/24.6 °C) month. The hottest months were July and August (mean maximum air temperature of 33.6 °C) (Table 2).

2.3. Experimental design and crop management

The experiment was set out in randomized blocks with four replications, each of which comprised 1000 plants. The three entries comprised two cultivated cardoon lines, namely the University of Catania selection 'Altilis 41' (A41) and the cultivar 'Bianco avorio' (BA), while the wild cardoon population 'Sylvestris Marsala' (SM) was obtained from a native stand in Marsala (Western Sicily). Prior to planting, the field was ploughed to a depth of ~30 cm, harrowed and a fertilizer dressing of 100 kg per hectare N (as urea), 100 kg per hectare P_2O_5 (as double perphosphate) and 80 kg per hectare K_2O (as potassium sulphate) was given. Seedlings were transplanted in early November at the stage when they had formed the fourth true leaf (about 40 days after germination) at a rate of 0.71 plants per m^2, using an inter- and intra-row spacing of, respectively, 1.4 and 1.0 m. In S_2 and S_3, regrowth after the period of summer dormancy commenced in early September, when the first rains arrived. Irrigation was given only during S_1, in the form of a 20 mm equivalent in both April and May. Seedling establishment and early growth was supported by hand weeding. No pesticide or fungicide interventions were required.

2.4. Measurement of biomass and silage outcomes

In each of the three seasons, plants from the central area (measuring 14.1 m^2) of each plot were cut ~5 cm above ground level at phenological growth stage 8: capitulum and seed ripening (late June), when < 20% of the heads were completely yellow (code 83 according to the BBCH scale by Archontoulis et al., 2010). The number of plants in the sample was recorded, and both the total harvested biomass and the

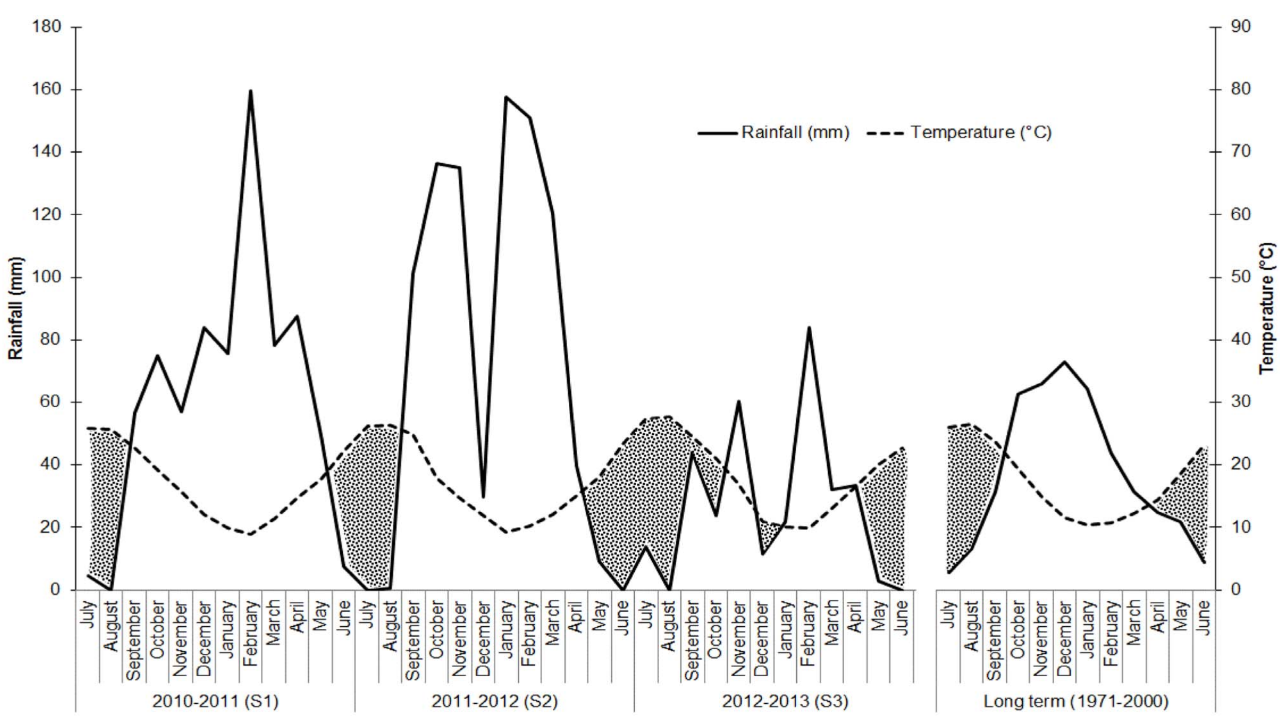

Fig. 1. Ombrothermic diagram of 2010–2013 mean monthly temperature and total monthly precipitation compared to the long term average (1971–2000).

Table 1
Comparison of monthly and seasonal rainfall (mm) between S_1, S_2, S_3 and long term period. The symbols + and − indicate values higher or lower than the long term value within each column.

	Month												Seasonal total rainfall		Seasonal aridity index
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun			
S_1 (2010–11)	5 −	0 −	57 +	75 +	57 −	84 +	76 +	160 +	78 +	88 +	49 +	8 −	**736**	+	**0.49**
S_2 (2011–12)	0 −	0 −	101 +	136 +	135 +	30 −	158 +	151 +	121 +	40 +	9 −	0 −	**881**	+	**0.55**
S_3 (2012–13)	14 +	0 −	44 +	24 −	60 −	12 −	22 −	84 +	32 =	34 +	3 −	0 −	**328**	−	**0.20**
Long term (1971–2000)	6	13	31	63	66	73	64	44	32	25	22	9	**447**		

Table 2
Monthly averages of maximum and minimum temperatures (°C) during the three seasons of experiment.

		S_1		S_2		S_3		Mean S_1–S_3	
		max	min	max	min	max	min	max	min
	July	31.6	19.6	33.5	19.0	35.6	18.9	33.6	19.2
	August	31.6	19.7	33.6	19.5	35.7	19.4	33.6	19.5
	September	27.8	17.6	31.4	18.4	31.9	17.2	30.4	17.7
Rainy period	October	23.7	14.7	23.1	12.8	27.1	14.7	24.6	14.1
	November	21.1	10.5	19.3	10.2	22.3	10.8	20.9	10.5
	December	17.4	6.8	17.2	6.9	16.6	5.2	17.1	6.3
	January	15.9	4.3	14.7	4.1	15.7	4.4	15.4	4.3
	February	14.4	3.6	14.5	3.3	15.8	3.7	14.9	3.5
	March	17.0	6.2	19.0	5.8	18.5	7.7	18.2	6.6
	April	21.1	8.9	21.7	8.5	23.0	10.0	21.9	9.1
	May	23.6	12.0	25.9	10.1	27.0	13.0	25.5	11.7
	June	29.1	16.1	32.2	14.7	30.6	15.2	30.6	15.3

weight of stalk, leaf and inflorescence material separately, were weighed. The tissue moisture content was obtained by baking a ~200 g sample of each component at 105 °C until a constant weight had been reached. For ensilage, ~30 kg of fresh material was first chopped, then packed into a sealed black polythene bag. More in details, the fresh biomass was chopped using a garden chipper (Model BIO 80 made by Caravaggi – Brescia), while the size of the particle ranged between 5 and 15 mm. Three samples, of about 1000 g each, were collected from each MINISILO to be subjected to analysis. The bags were stored for 90 days at room temperature (20 ± 2 °C), then sampled for content analysis following Faithfull (2002); the following parameters were recorded: dry matter (DM), pH, dry organic matter (DOM), ash, crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), glucose, fructose, mannitol, ethanol, lactic acid, acetic acid, propionic acid and butyric acid.

2.5. Biogas and biomethane production

To assess the potential of the biomass to generate biomethane via anaerobic fermentation, three replicated samples of each of the three entries were prepared. The samples of silage were fermented without any pre-treatment and no additives were used. A set of unstirred lab scale fermenters was held at 40 °C within a water bath (for more details about the lab scale fermenters see Negri et al., 2014a). The inoculum was collected from various full-scale anaerobic digesters. In each fermenter, the inoculum/substrate ratio was 2:1 on a DOM basis: on average, each fermenter contained 1.4 kg inoculum and about 100 g fresh silage biomass corresponding to 37 g of dry silage biomass (Negri et al., 2014a). Fermentation continued until the quantity of gas produced by the inoculum + substrate was the same as by the inoculum alone. The volume of gas generated was recorded on a daily basis. The chemical composition of the gas was monitored using a Combimass GA-m portable gas analyser (Binder, Ulm, Germany) equipped with an infrared dispersion cell to detect both biomethane and carbon dioxide.

2.6. Statistical analysis

All data were subjected to an analysis of variance (Snedecor and Cochran, 1989), and the means for each trait were separated by Fisher's least significance difference test, applying a threshold of 0.05. Values recorded as percentages were subjected to angular transformation prior to the analysis of variance.

3. Results and discussion

3.1. Biomass yield

Averaged across all three seasons, the mean biomass accumulated by A41 was 19.1 t DM per hectare per year, followed by BA (16.8 t DM) and SM (11.8 t DM) (Fig. 2). This level of biomass yield agrees well with those experienced in similar experiments carried out by Gherbin et al. (2001), Angelini et al. (2009), Ierna et al. (2012) and Mauromicale et al. (2014). The performance of the cultivated cardoon entries was equal or superior to that achieved by maize, wheat, ryegrass and triticale. Maize, a crop widely grown in Italy as both a fodder and as feedstock for bioenergy via fermentation, can produce between 15.0 and 26.1 t DM of biomass per hectare (González-García et al., 2013; Negri et al., 2014a; Bacenetti et al., 2014). The earliest maize classes, grown in the second sowing (to follow grain or fodder autumn winter crops) under irrigation, produce a quantity of biomass comparable to that produced by the cardoon entries described here, while late-maturing ones are much more productive. Wheat and triticale crops typically yield, respectively, 12.3 and 14.5 t DM per hectare, while ryegrass yields in the range 8–15 t DM per hectare (Bortolazzo et al.,

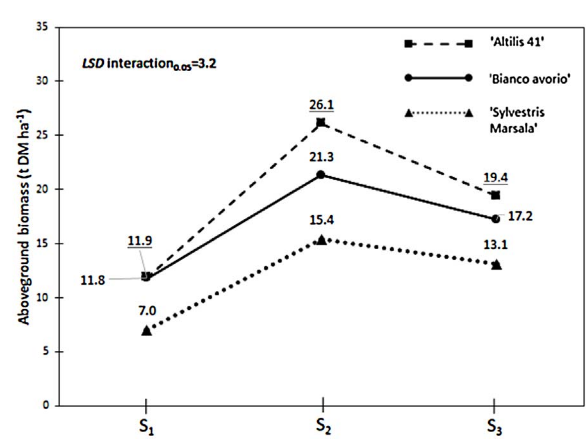

Fig. 2. Effect of interaction "Genotype x Season" on cardoon aboveground biomass yield.

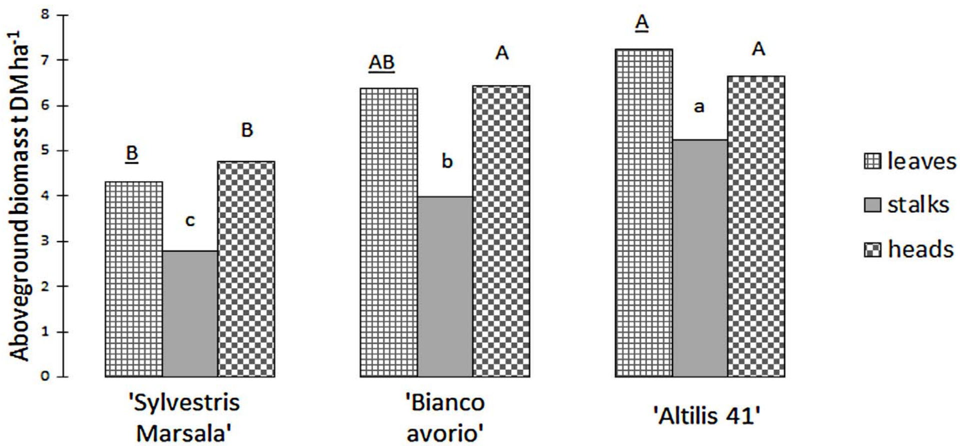

Fig. 3. Mean values over three seasons of above-ground biomass yielded by each genotype, divided in leaves, stalks and heads. Different underlined capital letters indicate significance at *LSD* test ($P \leq 0.05$) within leaves. Different small letters indicate significance at *LSD* test ($P \leq 0.05$) within stalks. Different capital letters indicate significance at *LSD* test ($P \leq 0.05$) within heads.

2009; D'Imporzano et al., 2010; Soldano et al., 2013). All three entries accumulated more biomass during S_2 than during S_1, but less during S_3 than during S_2. The extent of the extra productivity during S_2 was most marked for A41, while that of the decline during S_3 was least marked for SM (Fig. 2). The loss of productivity during the third cropping season was unexpected, since in similar experiments carried out by others (Mauro et al., 2015; Mauromicale et al., 2014; Ierna and Mauromicale, 2010), the quantity of biomass produced in the third year typically remains the same as, if not more than was produced during the second year (Angelini et al., 2009). A possible explanation for this unexpected result is that the rainfall experienced during S_3 was notably low (only 37% of what was recorded during S_2) and the aridity index was very low (0.2, compared with 0.55 during S_2) (Table 1).

When the biomass was partitioned into its various components, of the three entries, A41 produced the most leaf material (7.2 t DM per hectare per year), the most stalk material (5.3 t DM) and the most inflorescence material (6.6 t DM). The equivalent levels of performance for BA were, respectively, 6.4, 4.0 and 6.4 t DM, and those for SM only, respectively, 4.3 t DM, 2.8 t DM and 4.7 t DM (Fig. 3). Leaf material accounted for 38% of the biomass accumulated by both A41 and BA, and for 36% by SM; stalk material for, respectively, 28%, 24% and 24%; and inflorescence material for, respectively 34%, 38% and 40% (Fig. 3). Between S_1 and S_2, the amount of leaf material increased by 192%, while the increase was less marked for both the stalk (44%) and inflorescence (79%) material. In contrast, during S_3 the proportion of leaf material decreased by 47% while the production of both stalk and inflorescence remained largely unchanged (Fig. 4). During S_1, the DM was relatively equally distributed between leaf, stalk and inflorescence, while during S_2, 46% of the overall DM was composed of leaf material, and during S_3 the relative contribution of the inflorescence was prominent (43%) (Fig. 4). This plasticity reflects the ability of cardoon to adapt to its environment: during a dry season it develops proportionally more reproductive material, while during a wetter one it develops more vegetative growth.

3.2. Silage characterization and biomethane production

Table 3 displays the outcome of the ensilage experiment. The DM content of the silage ranged from 32.8% in A41/S_2 to 37.1% in BA/S_3. These values indicate that the silage was made at the right time. The pH of the silage ranged from 3.3 (A41/S_2) to 4.1 (A41/S_3), again confirming that the ensiling conditions were appropriate, and hence that there was only a modest loss of organic matter. The silage had a high ash content (10.8-13.4%) and, consequently, a low DOM content (86.6-89.2%). These values are very different from those prevailing in maize silage, which has a typical ash content of ~4% and a DOM of 96%, while both wheat and triticale silage contain ~10% ash and have a DOM of 90% (Negri et al., 2016). As a comparison, ensiled whole plant maize contains at least 30% starch, while ensiled maize ears can contain more than 60% starch (Negri et al., 2014b; Negri et al., 2016). The values of EE varied from 2.0 to 2.8%, a range which is similar to that seen in ensiled wheat, triticale and maize ears (Negri et al., 2014b; Negri et al., 2016).

The evolution of biomethane over the 27 days fermentation of

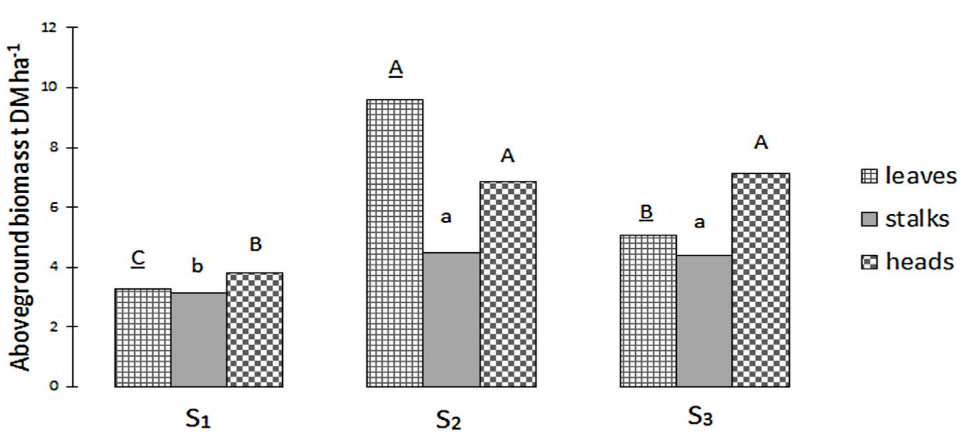

Fig. 4. Mean values of above-ground biomass of the three genotypes yielded in each season, divided in leaves, stalks and heads. Different underlined capital letters indicate significance at *LSD* test ($P \leq 0.05$) within leaves. Different small letters indicate significance at *LSD* test ($P \leq 0.05$) within stalks. Different capital letters indicate significance at *LSD* test ($P \leq 0.05$) within heads.

Table 3
Results of laboratory analysis for the silage of different cardoon genotypes in Season 2 and Season 3. Different small letters within each variable indicate significance at *LSD* test ($P \leq 0.05$) in S_2. Different capital letters within each variable indicate significance at *LSD* test ($P \leq 0.05$) in S_3.

		S_2		S_3		
		'Bianco avorio'	'Altilis 41'	'Sylvestris Marsala'	'Bianco avorio'	'Altilis 41'
DM	%	35.4 a	32.8 b	33.0 AB	37.1 A	34.6 B
pH	–	3.7 a	3.3 a	3.9 A	3.8 A	4.1 A
DOM	%	88.0 a	88.1 a	86.6 A	89.2 A	88.6 A
Ash[a]	%	12.0 a	11.9 a	13.4 A	10.8 A	11.4 A
CP[a]	%	12.8 a	14.2 a	13.9 A	13.4 A	14.6 A
EE[a]	%	2.8 a	2.5 a	2.3 A	2.4 A	2.0 A
NDF[a]	%	44.7 a	48.0 a	47.4 A	47.4 A	45.9 A
ADF[a]	%	33.2 a	28.1 b	33.3 A	32.4 A	37.4 A
Glucose[a]	%	0.3 a	0.3 a	0.2 A	0.3 A	0.2 A
Fructose[a]	%	0.5 a	0.5 a	0.3 B	0.5 A	0.6 A
Mannitol[a]	%	2.0 a	1.7 a	1.4 A	1.6 A	1.9 A
Ethanol[a]	%	0.5 a	0.5 a	0.5 A	0.4 A	0.4 A
Lactic acid[a]	%	0.9 a	1.3 a	1.4 A	0.8 A	1.3 A
Acetic acid[a]	%	1.4 a	1.4 a	1.0 B	1.9 A	1.9 A
Propionic acid[a]	%	0.2 a	0.3 a	0.2 A	0.5 A	0.4 A
Butyric acid[a]	%	0.3 a	0.2 a	0.2 A	0.1 A	0.2 A
C/N	–	25.0 a	22.5 a	22.6 A	24.1 A	22.0 A

DM = dry matter; DOM = dry organic matter; CP = crude protein; EE = ether extract. NDF = neutral detergent fibre; ADF = acid detergent fibre.
[a] as fraction of dry matter.

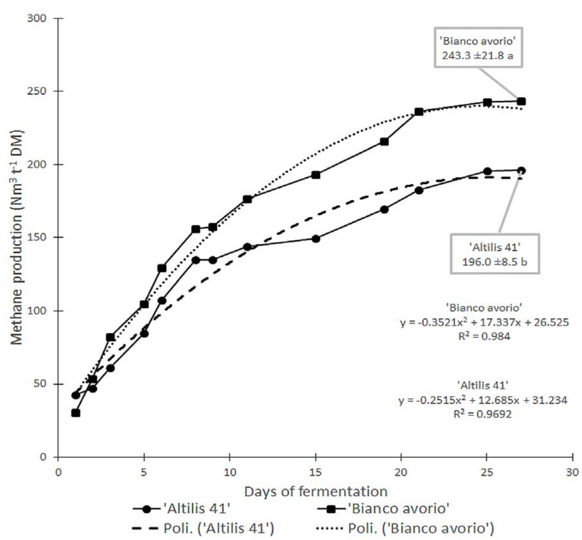

Fig. 5. Cumulative curves of biomethane production along the fermentation period of silage of two cultivated cardoon genotypes in S_2. Data reported in the rectangles are means (N = 6) after 27 days of fermentation ± standard deviation. Different letters indicate significance at *LSD* test ($P \leq 0.05$).

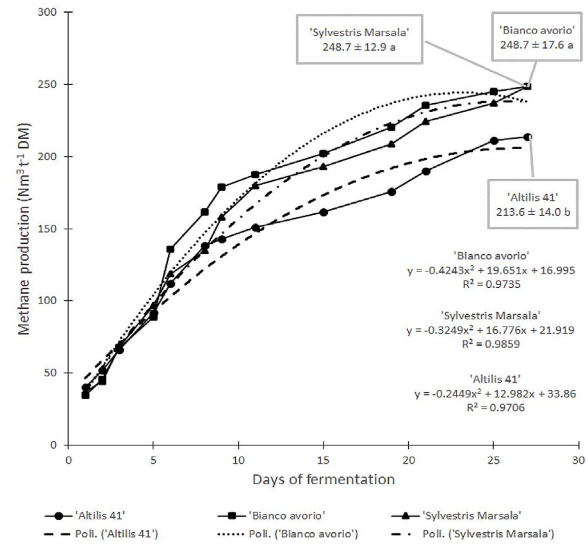

Fig. 6. Cumulative curves of biomethane production along the fermentation period of silage of three cardoon genotypes in S_3. Data reported in the rectangles are means (N = 6) after 27 days of fermentation ± standard deviation. Different letters indicate significance at *LSD* test ($P \leq 0.05$).

biomass harvested at the ends of S_2 and S_3 is illustrated in, respectively, Figs. 5 and 6. The curves are both well modelled by a quadratic relationship. The fermentation of A41/S_2 silage produced less biomethane than that produced by BA/S_2 silage (196.0 ± 8.5 *vs* 243.3 ± 21.8 $Nm^3\ t^{-1}$ DM), while the performance of SM/S_3 and BA/S_3 materials was effectively identical (248.7 ± 12.9 *vs* 248.7 ± 17.6 $Nm^3\ t^{-1}$ DM of silage), but superior to that of A41/S_3 (213.6 ± 14.0 $Nm^3\ t^{-1}$ DM). The poorer biomethane yield of A41 biomass could reflect the chemical constitution of the biomass, and specifically its higher proportion of stalks, which could be partially lignified, and the lower proportion of inflorescence material, where, moreover, the achenes are found. In the calculation of biogas production per hectare, ensiling losses were taken into account. However, such losses were not measured, because of the small scale of ensiling in the present trial, but assumed on the basis of experimental experiences on different fodders on a larger scale (Muck and Holmes, 2000; Muck et al., 2003; Muck and Holmes, 2006; Bacenetti and Fusi, 2015). The biomass losses due to ensiling was evaluated in 12% DM. When calculated on a per hectare basis (Fig. 7), thanks to its higher per unit area biomass production, the biomethane yield of A41 (4501 Nm^3 per hectare during S_2, 3647 during S_3) was not significantly different from those of BA in either S_2 (4560) or S_3 (3765); SM was by far the least productive entry. Previous studies, always carried out at experimental level, highlighted that maize silage, which tends to contain plenty of starch, achieves a biomethane production ranging from 275 to 476 $Nm^3\ t^{-1}$ DM (Amon et al., 2007; Bauer et al., 2010; Negri et al., 2014a). The productivity of the cardoon samples was therefore comparable to the lower end of what is achievable by ensiling maize, but was broadly similar to the levels reported for ryegrass, triticale and wheat. Their silages can generate between 241 and 262 $Nm^3\ t^{-1}$ DM biomethane (Murphy et al., 2011; Negri et al., 2014a; Vítěz et al., 2015), and – like cardoon – the starter DM has a low (or zero) starch content, and is dominated by cellulose. Notably, the biomethane yields achieved from cardoon are in line with those produced from globe artichoke by-products, as detailed by De Menna et al. (2016). On a per hectare basis, maize silage generates from 5300 to 9000 Nm^3 biomethane (depending on the crop's maturity time and the growing environment) (Kalač, 2011; Bacenetti et al., 2014), while the quantity of biomethane derivable from temperate cereals (wheat or triticale) varies from 2500 to 3500 Nm^3 and that from ryegrass between 2000 and 3000 Nm^3 (Prochnow et al., 2009). Contrasting BA and A41 (SM was not considered as its biomethane productivity was measured just in S_3), the analysis of variance indicated a strong entry effect ($P < 0.001$) in terms of the biomethane yield per ton of DM. Thus, while BA produced on average 246 $Nm^3\ t^{-1}$ DM per year, the equivalent yield for A41 was only 205 $Nm^3\ t^{-1}$ DM. Season significantly ($P < 0.001$) affected biomethane yield per hectare, which amounted, on average, to 4530 Nm^3 in S_2 and to 3706 Nm^3 in S_3.

4. Conclusions

The present set of data has confirmed the cardoon (and particularly its cultivated form) as a genuine candidate as a renewable source of energy, both when considered from the point of view of its biomass yield and biomethane potential of its silage through anaerobic digestion. The volume of biomethane produced per ton of DM was compar-

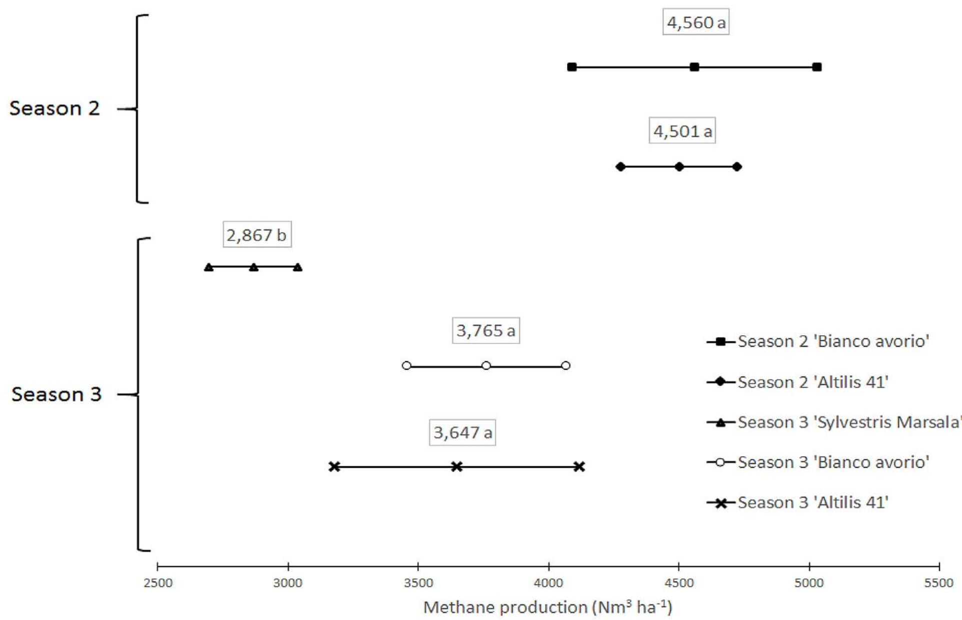

Fig. 7. 95% confidence intervals divided by seasons for the mean estimated biomethane yield per hectare of each genotype. Different letters within each season indicate significance at *LSD* test ($P \leq 0.05$).

able to those produced by ensiling ryegrass, triticale or wheat. When considered on a per hectare basis, cardoon becomes even more attractive given the large level of biomass produced per unit hectare of plants. The levels achieved by the cultivated types averaged ~4500 Nm^3 per hectare during S_2 and ~3700 during S_3, making them competitive with maize grown under an intermediate input intensity regime. What is particularly significant was that the cardoons were grown under a zero/minimal input regime with respect to fertilizer, irrigation, weed and pest control. To fully exploit the potential of cardoons as a bioenergy crop, focus will be needed to determine their optimal harvesting time in terms of DM and the sugar content of the material; the latter trait in particular has a large influence over biomethane yield through its positive effect on the biomass' digestibility.

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