

Biomass and biomethane yield of cultivated and wild cardoon grown with zero input in the Mediterranean environment

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Abstract

The cardoon, both the cultivated (*Cynara cardunculus* var. *altilis*) and the wild one (*C. cardunculus* var. *sylvestris*), are suitable for energy production as they produce large amounts of biomass and minimize the demand for inputs. Furthermore, they are native to the Mediterranean basin and therefore well adapted to its environment, of which they make the most of its resources, escaping its harshness. The aim of the study is to provide a contribution of new knowledge on the production of biomethane in the Mediterranean environment starting from cardoon plants, which have not been supplied with fertilization, irrigation, weed control, or pest control. In the present work, two genotypes of cardoon have been compared. On the one hand 'Altilis 41', a cultivated cardoon genotype selected for its ability to produce biomass, on the other hand 'Sylvestris Marsala', a wild cardoon ecotype. The plants were 5 years old and were grown in a randomized block design. The plants' regrowth was naturally allowed by rains at the end of September. Their epigeal biomass was collected at the flowering phenological stage, then ground and finally placed in silobags to simulate 3-month ensiling. 'Altilis 41' produced 19.8 t of dry biomass (DM) per hectare, while 'Sylvestris Marsala' produced under 9.5 t DM ha⁻¹. Biomethane yield, obtained from the ensiled biomass subjected to anaerobic digestion for 27 days, was 178 Nm³ for 'Sylvestris Marsala' and 167 Nm³ for 'Altilis 41'. However, the latter was more productive on a per hectare basis, due to its higher productivity in terms of biomass compared to 'Sylvestris Marsala' (3177 vs 1512 Nm³ ha⁻¹).

Keywords: cultivated cardoon, wild cardoon, biomass, biomethane, biogas, silage

INTRODUCTION

The cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC.) was bred since ancient times to produce leaves to eat (Pesce and Mauromicale, 2019), while the use of wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori] as vegetable rennet is a very ancient tradition, but still alive, especially in the Iberian Peninsula (Francisco-José et al., 2010). Alongside these traditional uses, there are alternative uses that emerged in recent decades (Gominho et al. 2018), such as the production of molecules with various industrial, pharmaceutical and cosmetic applications (Pandino et al., 2015; Scavo et al., 2018, 2019, 2020), the extraction of oil from achenes (improperly called "seeds") for human consumption, paper pulp production, fodder for livestock, ornamentals (Lanteri et al., 2012) and biomass for energy purposes (Pesce et al., 2017; Portis et al., 2018). The latter use is among the most promising in the Mediterranean environment for at least three reasons: 1) because the cardoon is native to the Mediterranean basin and therefore well adapted to its soil and climatic conditions, 2) because it can produce a large amount of biomass (especially the cultivated one) even in the face of low inputs, and 3) because it is a perennial species and can guarantee prolonged production over the years (especially the wild one), even on marginal lands. A substantial literature already testifies to the energetic attitude of *C. cardunculus*, in particular of the cultivated

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cardoon. Its ability to produce biomass was evaluated both under optimal input (Ierna et al., 2012) and low input conditions (Mauromicale et al., 2014). The possibility of using the oil extracted from the seeds to produce biodiesel after transesterification was even studied (Curt et al., 2002). Furthermore, the species *C. cardunculus*, including crop residues of the globe artichoke (var. *scolymus*), has been evaluated for bioethanol production (Pesce et al., 2020, 2022; Tavares et al., 2022). Its suitability to produce biogas and bioethanol was also evaluated. As in Pesce et al. (2017), this work aims to investigate the potential of cardoon silage, both cultivated and wild, to produce biomethane through anaerobic fermentation. However, this time the biomass was produced from 5-year-old plants to which no inputs were provided, neither in terms of fertilization, nor in terms of irrigation, or weed control. Ultimately, the goal of the work is the evaluation of the productivity in terms of biomethane per hectare, both cultivated and wild, in old age, also considering the missing plants that have occurred over the years.

MATERIALS AND METHODS

Site and soil

The field trial was carried out at the Experimental farm of the University of Catania (37°25'N, 15°03'E, 10 m a.s.l.), sited in the Catania plain, Sicily (Southern Italy), in Typic Xerofluvent with clay texture. The local climate is characterized by mild, wet winters and hot, dry summers. According to Bagnouls and Gausson classification, the local climate is thermomediterranean. The Peguy diagrams (Figure 1) show the rainy and the dry months of both the long period and the 2014-2015 period. The polygon depicting the long-term trend (Figure 1A) is elongated in the direction of the x-axis and represents a climatic condition characterized by high annual temperature ranges, with six temperate months (from November to April), two warm and humid months (September to October), and four arid months (May to August). The mean annual rainfall over the period 2002-2022 was 637 mm, of which more than 75% fell during the period October to March (SIAS, 2023); the hottest month was August (26.8°C) and the coldest one January (10.7°C).

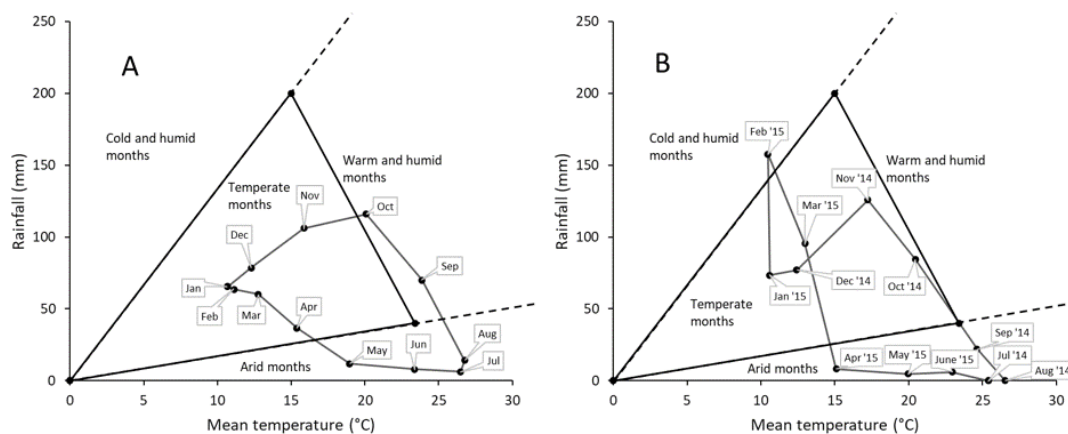


Figure 1. Peguy diagrams of mean monthly temperature and monthly precipitation in the period of the trial (A) compared to the long-term average (2002-2022) (B) in the site of the trial.

Meteorological conditions during the trial period

Precipitation during the trial was similar to the long-term mean (655 vs. 637 mm) (SIAS, 2023). The polygon that depicts the temperature and rainfall for the period 2014-2015 (Figure 1B) is instead very irregular, with six arid months and one particularly rainy month (February 2015), with 158 mm of rainfall. February was also the coldest month, with 10.5°C

mean air temperature. The hottest month was August, with 26.6°C.

Biomass evaluation and analysis

The experiment was set out in a randomized block design, with four replications per genotype, based on a net plot size of 7×5 m per replication. The two genotypes were a cultivated cardoon line, namely 'Altilis 41' (A41), specifically selected for biomass production in a breeding program of the University of Catania, and a wild cardoon genotype, namely 'Sylvestris Marsala' (SM), obtained from an ecotype found in the countryside of Marsala (western Sicily). Inter- and intra-row spacing were 1.4 and 1.0 m, respectively. Regrowth after summer dormancy started at the end of September, after the first autumn rains. No irrigation and no fertilization were provided, and no pesticide or fungicide applications were required. The aboveground biomass was harvested and ensiled on June 5th at the phenological growth stage between 6 and 7: flowering and development of capitula. Plants were harvested from the central area of each plot, by cutting them at about 5 cm above the soil level. Harvested plants were immediately weighed to determine their fresh weight. For ensilage, 9 plants for each genotype were first chopped using a garden chipper (Model BIO 80 made by Caravaggi – Brescia), then the plants were ensiled in threes in black polyethylene bags to obtain 6 bags. The latter were stored for 90 days at room temperature (20±2°C). After ensiling, the biomass was characterized 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 fiber (NDF), and acid detergent fiber (ADF). Finally, the loss of biomass due to ensiling was evaluated, by weighing the biomass before and after ensiling.

Biogas and biomethane production

Three replicated samples coming from each bag (a total of 27 samples) were prepared, for evaluating the potential of the biomass to generate biomethane via anaerobic fermentation. The silage samples 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 (Negri et al., 2014). 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 of fresh silage biomass corresponding to 37.8 g of dry silage biomass (Negri et al., 2014). The fermentation was prolonged until there was no significant difference between the samples and the blank (inoculum only). The volume of gas generated was recorded daily. The chemical composition of the gas was monitored using a Combimass GA-m portable gas analyzer (Binder, Ulm, Germany) equipped with an infrared dispersion cell to detect both biomethane and carbon dioxide.

Statistical analysis

All data were subjected to an analysis of variance 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 before the analysis of variance.

RESULTS AND DISCUSSION

Overview

The results are presented in terms of biomass and biomethane production relative to the genotype and referred to either mass or surface unit, while also considering the survival of the plants after five years of cultivation without any additional inputs.

Research data

1. Growth cycle and weather conditions.

Both cultivated and wild cardoons showed very similar growth and development behaviors. At the end of September, after the first rainfall, axillary buds of the underground

fraction of the plant naturally sprout in young shoots and the new growth cycle started. From November to February, rosettes grew both in size and number of leaves, and the canopy rapidly covered the soil surface, competing effectively against weeds. During the cold months (January and February), the plant growth rate slowed down but not stopped. In the last ten days of February, apices of the stems passed to the reproductive stage, but stem elongation started at the end of March/beginning of April, because of the warmer and longer days. Anthesis started in mid-May. Overall, the length of the cycle was approximately 250 days: from regrowth to biomass harvest done at full flowering (on June 5th, 2015.). During the growth cycle, the evapotranspiration demand of the atmosphere, expressed in terms of ET₀ (691 mm), was almost fully satisfied by the precipitations (633 mm) (Figure 2). Furthermore, Figure 2 shows that the growth cycle avoids the summer harshness of the Mediterranean climate (Figure 2). This reaffirms once again the harmony of *C. cardunculus* growth and development cycle with the Mediterranean climate.

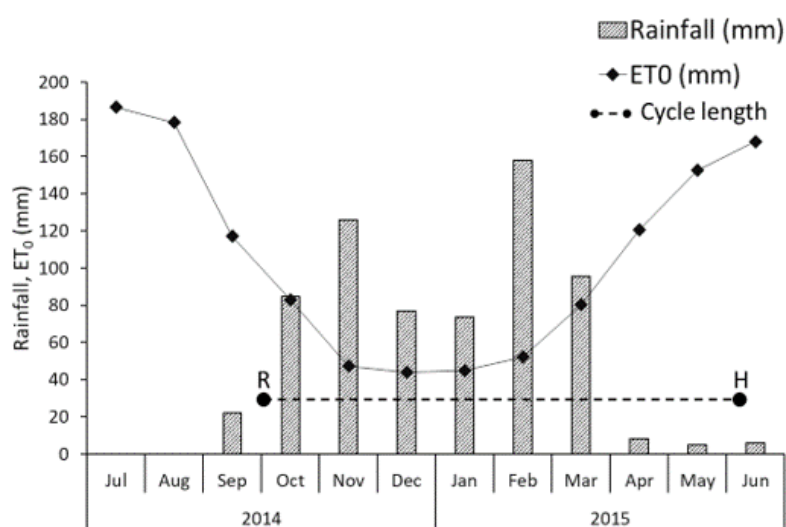


Figure 2. Comparison between precipitation and reference evapotranspiration (ET₀) at the site and in the period in which the test was carried out. R = plant regrowth; H = biomass harvest.

2. Plants survival and aboveground dry biomass yield.

At the end of the fifth year of cultivation, the survival rate of SM plants was 96.1%, while that of A41 plants was 89.3% (Table 1). These are very high percentages but in line with the data of Mauromicale et al. (2014). The harvested biomass of A41 was estimated at 19.8 t DM ha⁻¹, while that produced by SM was estimated at 9.5 t DM ha⁻¹. Interestingly, the biomass DM content was significantly higher in A41 (50.2%) than in SM (41.3%) (Table 1). These values of biomass yield are close to those found in similar trials carried out by Ierna et al. (2012), Ierna and Mauromicale (2010), Mauromicale et al. (2014), Mauro et al. (2015), and Pesce et al. (2017). 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 A41 (González-García et al., 2013; Negri et al., 2014). Wheat and triticale crops typically yield, respectively, 12.3 and 14.5 t DM ha⁻¹, while ryegrass yields in the range of 8-15 t DM ha⁻¹ (Negri et al., 2014).

3. Silage characterization and biomethane production.

The loss of dry biomass due to ensiling was evaluated at -10.4% for SM and at -4.2% for A41. This difference can be explained by the moisture content of SM biomass, significantly

higher than that of A41. The characterization of the ensiled biomass confirmed the highest DM content of A41; however, the latter has a lower content of NDF and ADF (Table 2). The silages had a very high ash content (17.1% for SM and 15.2% for A41) and, consequently, a low DOM content (82.9 and 84.8% respectively) (Table 2).

Table 1. Fifth-year plant survival, DM content and biomass yield of the two genotypes.

Variable	Genotype	
	SM	A41
Plants survival (%)	96.1 a	89.3 b
DM content (%)	41.3 b	50.2 a
Biomass yield (t DM ha ⁻¹)	9.5 b	19.8 a

DM=dry matter.

Table 2. Characterization of the silages coming from the cultivated and wild cardoon genotypes.

Variable	Genotype	
	SM	A41
DM (%)	37.0 b	48.1 a
DOM (%)	82.9 b	84.8 a
Ash ^a (%)	17.1 a	15.2 b
CP ^a (%)	14.0 a	14.4 a
EE ^a (%)	1.5 a	1.4 a
NDF ^a (%)	39.1 a	35.8 b
ADF ^a (%)	26.9 a	24.9 b
C/N (%)	21.5 a	21.4 a

DM = dry matter; DOM = dry organic matter; CP = crude protein; EE = ether extract.

NDF = neutral detergent fiber; ADF = acid detergent fiber.

^aas a fraction of dry matter.

Different small letters within each variable indicate significance at LSD test ($P \leq 0.05$).

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). The specific production of biomethane is illustrated in Figure 3, where the cumulative curves of both genotypes, along the 27 days of fermentation, are shown. The fermentation of A41 silage produced less biomethane than that produced by SM silage (167.5 ± 11.6 vs. 177.7 ± 9.0 Nm³ t⁻¹ DM) (Figure 3). The experimental data fit well with quadratic curves (Figure 3). The lower biomethane yield of A41 silage could reflect its chemical composition, more precisely the lower content in NDF and ADF (Table 2). These specific biomethane production values are decidedly lower than those found by Pesce et al. (2017) on the same genotypes. One possible explanation is that the biomass used in this trial came from plants harvested at a phenological stage in which the seeds (rich in fat) were not yet formed and therefore missed their contribution to biomethane production. However, this variability requires further testing and investigation.

Considering the performance in terms of biomethane per hectare, A41 is the most productive genotype (3177 vs. 1512 Nm³ ha⁻¹) thanks to its greater capacity to accumulate biomass in comparison to SM (Figure 4).

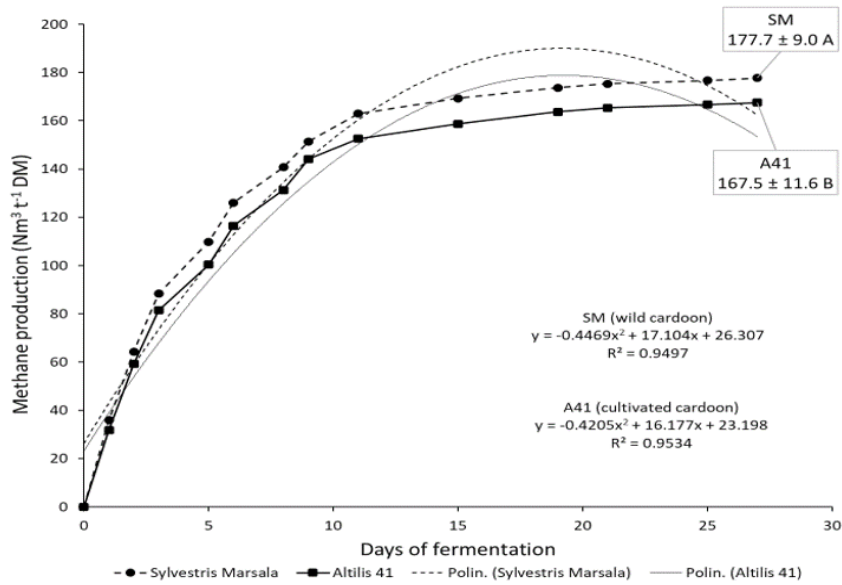


Figure 3. Cumulative curves of biomethane produced along the fermentation period by the silage of the two cardoon genotypes. Data reported in the rectangles are means after 27 days of fermentation \pm standard deviation. Different letters indicate significance at LSD test ($P \leq 0.05$).

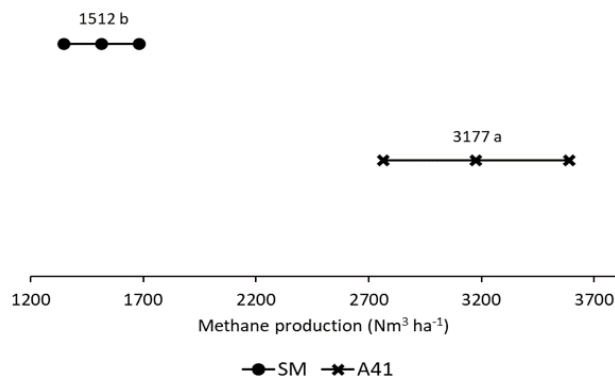


Figure 4. Confidence intervals for the mean estimated biomethane yield per hectare of each genotype. Different letters within each season indicate significance at the LSD test ($P \leq 0.05$).

CONCLUSIONS

Cardoon proved once again that it is an energy plant suitable for the Mediterranean climate, as it avoids summer aridity and takes advantage of the autumn-winter rains. The data of the present work confirm that the cultivated cardoon produces a large quantity of biomass (~ 20 t DM ha^{-1}) even from 5-year-old plants and above all without being supplied with inputs. The volume of biomethane achieved per ton of dry biomass found in this study was ~ 178 Nm^3 for wild cardoon and ~ 167 Nm^3 for the cultivated form. Although wild cardoon showed a higher specific production of biomethane than cultivated cardoon, the latter is the most productive in terms of biomethane per unit area (3177 vs. 1512 Nm^3 ha^{-1}).

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