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A Mediterranean cropping system for bioenergetics purposes:

Cynara cardunculus var. *altilis*

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Alla Vita e alla Scienza

...e alla seconda possibilità

che queste mi hanno dato.

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PREFACE

In recent years, scientists from different disciplines have been focusing on improving the production of energy from sustainable sources. The general objective is to meet current people's needs, without compromising the energy requirements of future generations.

Increasing concerns about fossil energy and its several negative impacts on the environment have accelerated the development of bioenergy at a global level (Jonson et al., 2007).

The technologies used to produce sustainable energy deal with renewable sources of energy, i.e. energy obtained from the use of biomass.

The global environmental benefits related to the use of biomass as a fossil fuel are well known, however, it's the production of biomass itself that it's able to reduce the greenhouse gases emission (Börjesson et al., 1999).

This thesis fits into this scientific scenario, and in this respect, the *Cynara cardunculus* var. *altilis* has been considered one of the most promising energy crops.

The decision to experiment in Sardinia with the thistle (*Cynara cardunculus* var. *altilis*) is justified mainly by two important factors.

First, the cardoon is a typical crop of the Mediterranean climate characterized by high production of herbaceous biomass, even if grown in a reduced inputs management.

Second, its great adaptation to Mediterranean weather allows a good production of two materials: lignocellulosic biomass and oil seeds.

After the final draft of the "Manifesto of Green Chemistry", in Sardinia, aggregations of middle and large companies want to invest on the production of bioenergy.

In this context, the cultivated cardoon is the crop chosen to provide the oil for the extraction of the polymer that produces bio plastics.

It is widely recognized that an appropriate interaction between industry and agriculture is required, in order to obtain well-structured supply chains.

This "coordination between sectors" produces real benefits when positively interacting with the local territory through models of sustainable agriculture (low input, preservation of soil fertility and biodiversity, and enhancement of marginal lands that are not suitable for food production).

The main objective of the research conducted in this thesis was to support the strategic decisions regarding the introduction of the thistle cultivated for energy production into the Mediterranean farming system.

To this purpose, the crop system has been analyzed in its complexity.

The specific objectives of the present research work were: (i) evaluate the effect of input management on yield and energy balance of a five-years standing cardoon in a Mediterranean environment (Chapter 1); (ii) evaluate the most important differences between three genotypes of *Cynara cardunculus* var. *altilis* cultivated in a Mediterranean environment; (iii) perform their growth analysis by using the indices of physiological and productive efficiency; (iv) evaluate their growth by timing of phenological events and by dynamic of biomass accumulation.

The thesis is composed of two chapters and each one describes a research work.

ABSTRACT

One of the main objectives of modern Governments is to create a global policy aimed at ensuring the availability of diversified, reliable and affordable energy resources.

In order to support the diversification of primary energy sources, *Cynara cardunculus* var. *Altilis* L. was been considered one of most promising renewable energy crop as a source of lignocellulosic biomass and oil from seeds.

However, a cultivation for bioenergy purpose is sustainable in a low-medium input context in term of irrigation, fertilization, and weed control.

To support the strategic decisions regarding the introduction of the thistle cultivated for energy production into the Mediterranean environment, the crop system was analyzed in its complexity.

A first experiment was carried out to determine the long-term effects of management intensities and of years on yield.

In the second experiment, the growth analysis of the three genotypes cardoon was investigated during 2 years.

The result showed that a reduction in management intensity can increase the energy efficiency of the low farming systems even if with evaluable yield losses within years.

Further results indicate substantial differences between genotypes, and it suggest a possibility of choose different genotypes with different productive attitudes: aboveground biomass or seeds.

These results need to be verified in a larger scale than this experimental plot.

CHAPTER 1

Effect of input management on yield and energy balance of a cardoon five-years standing in a Mediterranean environment

1.1 INTRODUCTION

Biomass is currently seen as a potentially major part of carbon mitigation strategies in the U.S. and EU. In the EU, the Renewable Energy Directive (Directive 2009/28/EC) calls for 20% of total energy to be sourced from renewables by 2020, and biomass is a major component of this plan, both in the heat and power sector and as transportation fuels. In addition, the Fuel Quality Directive (Directive 2009/30/EC) mandates a 6% greenhouse gas (GHG) reduction in transport fuels by 2020, further incentivizing biofuels. At present, the growing energy demand, is mainly satisfied by cultivated crops raising the food vs. fuel dilemma. Instead, biomass from perennial grasses crops grown in abandoned and/or marginal lands (land that is not well-suited to food production) with less negative impacts (Searle and Malins, 2014; Bouriazos et al., 2014) is object of many recommendations for political support strategies (EEA, 2011).

Perennials have strong potential as dedicated energy crops due to high conversion of sunlight into biomass, efficient water and N use, and ability to achieve high biomass yields with limited inputs (Pedroso et al., 2014; Mauromicale et al., 2014). Once established, perennial crop fields can be harvested for at least five years, reducing the energy input and costs required for annual replanting. In addition, perennial crops reduce soil erosion and nutrient leaching (Smith et al., 2013; Blanco-Canqui, 2010; Deligios et al., 2014), increasing soil organic matter content (Mishra et al., 2013), and providing habitat for wildlife (Pedroli et al., 2013).

Cardoon (*Cynara cardunculus* var. *altilis* L.), has been identified as one of the most promising energy perennial crops expanding in semi-arid Mediterranean regions. Desirable traits include its high biomass yield potential, reduced water demand, and perennial growth habit (Vasilakoglou and Dhima, 2014). Indeed, the very deep root system permits to explore the soil in depth and to extract nutrients and water accumulated along the soil profile (Christodoulou et al., 2014; Fernández et al., 2006).

Recently, some researchers highlighted the effect of low inputs of irrigation and weed control on cardoon potential productivity of biomass and oil (Vasilakoglou and Dhima, 2014). Apart from water supply that has a clear positive effect, fertilizer N is considered one of the main energy input and source of greenhouse gases emissions during perennial grasses

cultivation for biofuel purposes (Angelini et al., 2009; Mantineo et al., 2009). Furthermore, the response of cardoon to N fertilizer is not clear and results are conflicting due to variations in soil, crop management, and age of stand (Mauromicale et al., 2014; Ierna et al., 2012). Some studies have reported limited to no yield response to N fertilization (Ierna et al., 2012; Mantineo et al., 2009) while others reported significant yield responses (Archontoulis 2011; Fernández et al., 2006; Fernández et al., 2002).

According to Nassi o di Nasso et al. (2010a), among criteria accounting for the selection of suitable energy crops, energy balance is a very important tool to evaluate the energy sustainability of crop systems. There have been a number of energy budget studies on energy crops. Some researchers have reported on the energy balance of cardoon and have shown a positive response. Output/input energy ratios varied from 17.4 to 30.9 depending on growing conditions, inputs and assumed boundaries to the system (Angelini et al., 2009; Mantineo et al., 2009). As found in other research on perennial energy crops, management intensity, such as fertilization, plant density and tillage, affects energy efficiency (Larsen et al., 2014; Boléo 2011). The efficiency of energy use can be increased by reducing inputs such as fertilizer, or by increasing outputs such as crop yields (Swanton et al., 1996). In some cases, a reduction in energy inputs entails a proportional reduction in crop yield. In such cases energy efficiency is not significantly affected (Risoud, 2000; Bailey et al., 2003).

To evaluate, management intensities and crop age on cardoon yield (biomass and seed), thermochemical traits, and energy balance, we conducted experiments at a Sardinian location (Southern Italy) during the 2007 – 2012 period. Our hypothesis was that a reduced N input on cardoon cropped in Mediterranean rainfed condition would provide an increased energy efficiency of the cropping system an high-quality biomass for energetic purpose.

1.2 MATERIALS AND METHODS

1.2.1. Research site

The trial was carried out at the ‘Mauro Deidda’ Experimental Farm (Lat. 41° N, Long. 9° E, altitude 81 m) of the University of Sassari, Sardinia, Southern Italy, from 2007/08 to 2011/12 growing seasons. The climate of the study area is attenuated thermo-mediterranean, (UNESCO-FAO, 1963) with a four month drought period in summer coinciding with the highest temperatures. The soil at the experimental site is classified as a Lithic and Typic Xerorthents (USDA, 1999). Physical and chemical characteristics of the soil at different depths at the beginning of the experiment (November 2007) are presented in Table 1. Agriculture in the study region is generally rainfed, cereal based and extensively managed, with low crop yields due to the low and especially fluctuating rainfall.

Table 1: Initial soil physical and chemical characteristics (year 2007) of the experimental field.

Soil parameter	Depth (cm)			
	0-30	30-60	60-90	90-120
Sand (%)	60.5	64	69	67
Silt (%)	16.5	14.5	11	13
Clay(%)	23	21.5	20	20
pH	8.34	8.35	8.35	8.39
Total N (‰)	1.15	1.15	1.05	1.05
Soil organic matter (%)	1.97	1.57	1.41	1.26

1.2.2. Treatments and experimental design

Experiment was arranged in a randomized complete block design with two crop management intensities, replicated four times. As one of our aims was to perform an energy balance of a cardoon cropping system, large stands were required to implement the effective mechanization of all operations (i.e. site preparation, planting, fertilizing and harvesting). Therefore, each plot size was 625 m² (75 m x 8.3 m). Treatments consisted of two levels of management intensity including a conventional (CONV), and a low input system (LI). Cardoon cv. 'Bianco Avorio' was sown on 7 November 2007, 1 m between rows and 0.5 m apart within rows to achieve (Raccuia and Melilli, 2007), finally, a density of approximately 20000 plants ha⁻¹. During seedbed preparation all plots were fertilized with 80 kg ha⁻¹ of N and 100 kg ha⁻¹ of P₂O₅ using urea and triple superphosphate, respectively. At the end of each growth cycle (end of August), the plant canopy naturally dries and the fruits ripen, the aerial biomass was cut down so that a new growth cycle followed. To take into account soil P₂O₅ depletion due to crop residues removal, a restore fertilization was planned each year by applying 65 kg ha⁻¹ P₂O₅ at re-growth (BBCH00; Archontoulis et al., 2010) of the crop. Table 2 shows all the fieldwork undertaken, and amounts of fertilizers, used in each treatment. In CONV, chemical fertilizers were applied in the same quantity at re-growth of the crop and as a top-dressing at the floral elongation stage (BBCH55) in urea form (46 N) at an average total rate of 100 kg N ha⁻¹.

In the planting year, Linuron applied pre-emergence, was also used for weed control. Low input management was fertilized only at the re-growth stage at a rate of 50 kg N ha⁻¹. Soil nitrogen supply over years was designed as the minimum nutrients doses but sufficient to prevent any deficiencies. Plants were grown without supplemental irrigation in all the growing seasons.

Table 2: Summary of the operations performed for each treatment.

Field operation	CONV		LI	
	1 st year	Following years	1 st year	Following years
Machinery (number of operation)				
Mouldboard plough	1	-	1	-
Rotary harrow	1	-	1	-
Centrifugal fertilizer spreader	2	2	1	1
Crop sprayer	1	-	-	-
Conventional sower	1	-	1	-
Roller packer	1	-	1	-
Gyro rake	1	1	1	1
Baler	1	1	1	1
Harvester	1	1	1	1
Fuel consumption (kg ha ⁻¹)	132	128	62	62
Lubricant consumption (kg ha ⁻¹)	0.31	0.30	0.06	0.06
Fertilizer (kg)				
Triple Superphosphate (46% P ₂ O ₅)	100	65	100	65
Urea (46% N)	130	100	80	50
Seeds (kg)	4	-	4	-
Herbicide (L)				
Linuron	1.5	-	-	-

1.2.3. Data collection and measurements

Rainfall, daily maximum, minimum air temperature were recorded near the experimental field (Fig. 1) at an agro-meteorological station of the Sardinia Regional Environmental Protection Agency (ARPAS).

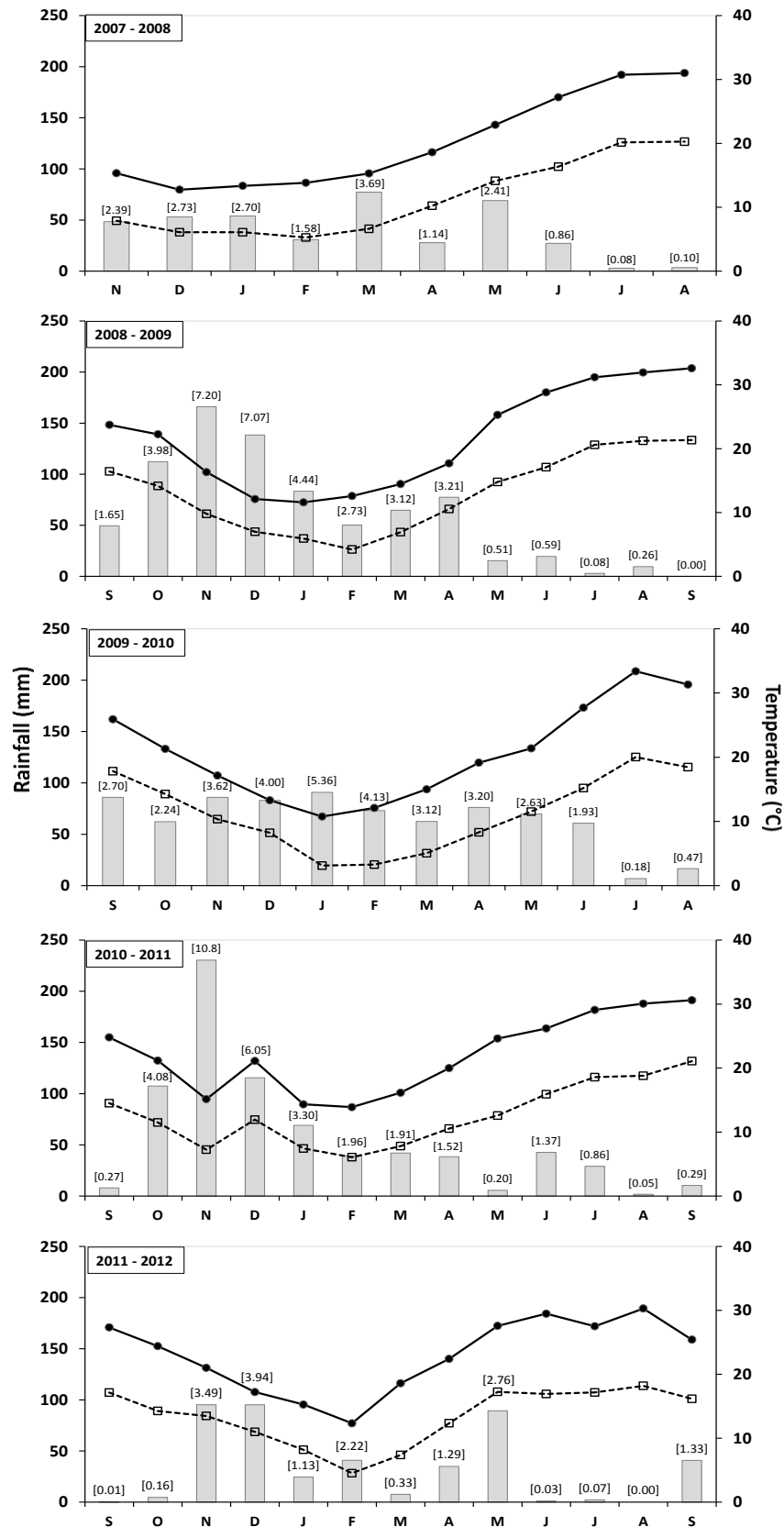


Figure 1: Climate conditions and Aridity Index (square brackets) at the field experimental station (Ottava, 41°N; 9°E).

As the experimental area is located in a Mediterranean environment, the temporary situations of below-average precipitation and of above-average temperature can occur, resulting in drought stress that is particularly harmful during the crop vegetation period.

The combined effect of temperature and precipitation can provide information on the drought level at a given site (Vogt and Somma, 2000). In this study, we applied the monthly aridity index (AI) to further understand such temporary variations during growing season.

According to De Martonne (1926), AI in a monthly basis was calculated as

$$AI = Pi/Ti + 10$$

Where:

AI = aridity index;

Pi = monthly precipitation amount;

Ti = monthly mean air temperature.

At each plot, four permanent areas (10 m² each) were monitored until the end of the 2011 - 2012 growing season to determine plants survival.

All permanent areas were monitored twice per growing season. In the first census at the beginning of the plant cycle, the number of plants that re-grew was recorded. In the second census at the end of the season, the number of plants that had reached reproduction was recorded. Survival fraction was then estimated as the ratio between the second and first census.

Following each growing season, at harvest, cardoon dry biomass, as well as height, stalk and head number plant⁻¹ were determined each year by hand-harvesting twenty cardoon plants for each plot (Aug. 28, 2008; Sept. 02, 2009; Aug. 31, 2010; Sept. 05, 2011 and Sept. 06, 2012). The border plants in the outermost row were not included in the harvested area.

Plants were, then, separated into leaf, stalk, and head. All plant samples were dried at 60 °C until constant weight and weighed. All the heads of each plant sample were threshed to separate seeds whose were then weighed. The harvest index (HI) was calculated as the ratio of seed weight to total aboveground biomass per ha at maturity. The dry vegetative samples (except seeds) were first ground in a cutting mill (Fritsch, Germany) to a fine powder (particle size ≤ 0.25 mm) and stored in sample black boxes at room temperature. After harvest, cardoon biomass residues were cut and removed.

Dry matter yield can be considered as a useful proxy to assess environmental use efficiency indicators such as water and nitrogen use efficiency (WUE and NUE, respectively) are (OECD, 2001).

The seasonal water use efficiency (WUE, $\text{g DM l}^{-1} \text{H}_2\text{O}$) was calculated as the ratios of seed yield and biomass to crop water use (mm) where, according to Mantineo et al. (2009) and Cosentino et al. (2007), the latter means water supplied by precipitation (mm).

The dry matter N concentration was measured using 1 g samples of each plant component and treatment using the Kjeldahl method after 96% H_2SO_4 hot digestion (AOAC, 1990). The nitrogen use efficiency (NUE, g g^{-1}) was calculated as the ratio between dry matter yield and dry matter N concentration (Capecchi et al., 2013; Beale and Long, 1997).

1.2.4. Biomass analysis

Biomass samples collected over treatments and years were sent to ‘Laboratorio di Ricerca e Analisi’ (LARIAN, Pomezia, Italy) for determination of their:

- higher calorific value;
- proximate and ultimate analysis;
- ash content;
- mineral composition.

The analysis of the samples was in duplicated according to the Technical Specification UNI CEN/TS (2005) and to the American Society for Testing and Materials standardized procedures (ASTM Standard, 2004).

Specifically, moisture was obtained by gravimetric analysis drying in an oven at 105 °C for 24 h. The UNI CEN/TS 14918 and UNI CEN/TS 15104:2005 procedures were used to determine higher calorific value and ultimate analysis, respectively. The ASTM D5142-02 thermogravimetric analysis (TGA) was followed for the determination of fixed carbon and volatile matter. Afterwards, the ash content was determined according to the ISO 1171 by ashing at 550 °C in a muffle furnace for 4 h. The main ash components expressed as oxides were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES).

1.2.5. Energy balance

According to Hülshbergen et al. (2001) a process analysis was performed to trace all the energy inputs into the biomass energy production system based on physical material flows.

To allow general conclusions about direct and indirect energy input in the investigated management intensities, all field operations were carried out with typical farm machinery.

Energy expended off-farm to deliver the production outside the field, for storage, processing and sale biomass were not calculated (Angelini et al., 2009).

Solar radiation and human labor were not considered (Hülshbergen et al., 2001). As reported by Rathke and Diepenbrock (2005), energy associated with human labor accounts for less than 0.2% of total energy input for most modern cropping systems, and was therefore neglected. Since the solar radiation exceeds the input of fossil energy by three orders of magnitude, its inclusion in the energy balance would have masked the variation in the input of fossil energy related to different treatments (Rathke and Diepenbrock, 2005).

Energy input includes direct input of diesel fuel and electricity and indirect input of fossil energy (production of fertilizer, pesticides and machines). The physical quantities of inputs used were converted to energy values using appropriate energy coefficients (Table 3).

Fuel and lubricant oil consumption for different operations were taken from Cocco et al. (2014) and converted to an energetic value using a conversion factor of 39.6 MJ kg⁻¹ and 40.1 MJ kg⁻¹, respectively (Hülshbergen et al., 2001; IPCC, 2006). Energy inputs for machinery were determined by estimating energy consumption for the fabrication and the repair, and by calculating the annual per hectare machinery cost (108 MJ ha⁻¹; Hülshbergen et al., 2001).

According to Angelini et al. (2009) we assumed that machinery and tools were used on 200 ha, with a lifespan of 10 years. Energy input was 78.2 MJ kg⁻¹ to produce 1 kg of N mineral fertilizer (Alhajj Ali et al., 2013). The energy cost attributed to the herbicide was obtained by multiplying the amount of active ingredient used by the energy equivalent given by Clements et al. (1995).

The energy output used in this study is based on total aboveground biomass (seed and biomass). Energy output was calculated by multiplying biomass and seed yields per the calorific value of the plant material. Calorific values per Mg of dry biomass of 16.6 GJ and 17.1 GJ were assumed for CONV and LI, respectively. As regard seed yield, a calorific value per Mg of 21.8 GJ was considered (Fernández et al., 2006). The energetic efficiency, as energy output/input ratio of the system, and net energy yield (GJ ha⁻¹), as difference between total energy output

and total energy input, were derived from the fossil energy input, the seed and biomass yields and the energy output.

Table 3: Energy equivalents for inputs in crop production (according to different authors)

Item	Energy equivalent	Units	References
Fuel			
Diesel	39.6	MJ L ⁻¹	Hülsbergen et al., 2001
Lubricant	40.2	MJ L ⁻¹	IPCC, 2006
Seeds	5.5	MJ kg ⁻¹	Angelini et al., 2009
Mineral fertilizer			
N (Urea)	70.8	MJ kg ⁻¹	Alhajj Ali et al., 2013
P ₂ O ₅ (Superphosphate triple)	15.8	MJ kg ⁻¹	Reineke et al., 2013
Pesticides			
Herbicide (Linuron)	290	MJ kg ⁻¹ a.i.	Clements et al., 2005
Machines^a	108	MJ kg ⁻¹	Hülsbergen et al., 2001

^a Refers to energy associated with production and maintenance over the item's useful life

1.2.6. Statistical analysis

The statistical analysis was focused on the relating factors of the randomized block design with repeated measures over years. All measured parameters were subjected to analyses of variance using the SAS procedure GLM (SAS Release 9.0, SAS Institute Inc., Cary, NC, USA). Year (time) and treatment were considered as fixed factor, block as random factor. GLM modeling provides a flexible framework for randomized experiments with repeated measurements and is useful for designs where the repeated factors have few levels, and where each level is observed for all or many of the experimental units. Means were statistically separated on the basis of Least Significant Difference (LSD) test, when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability level.

1.3 RESULTS

1.3.1 Environmental conditions

Growing season precipitation from 2007 to 2012 (Fig. 1) was slightly higher than the long-term average (468.9 mm), because of very high precipitation in 2008-2009 (790 mm, 68.5% above average) and 2009-2010 (773, 64.9% above average), and very low precipitation in 2007-2008 (394 mm, 15.9% below average). During the establishment year of 2007-2008, precipitation was lower than the long-term 30-year average for all months from November through April, except for March. Consistently with the total annual rainfall recorded, over the period of the experiment the aridity index varied, from year to year, between 2.80 (2010-2011) and 1.29 (2011-2012) (Fig. 1). Mean minimum air temperature in mid-winter was 6.2 °C, and mean maximum temperature in mid-summer was 29.6 °C. Temperatures over 38 °C were recorded in the summers of 2009 and 2010, and a minimum temperature of -2 °C was recorded in the winter of 2010.

1.3.2 Biometric traits, biomass yield, and partitioning

Although not statistical differences were detected, one year after planting, CONV plots had a greater rate of survival than LI plots (Fig. 2).

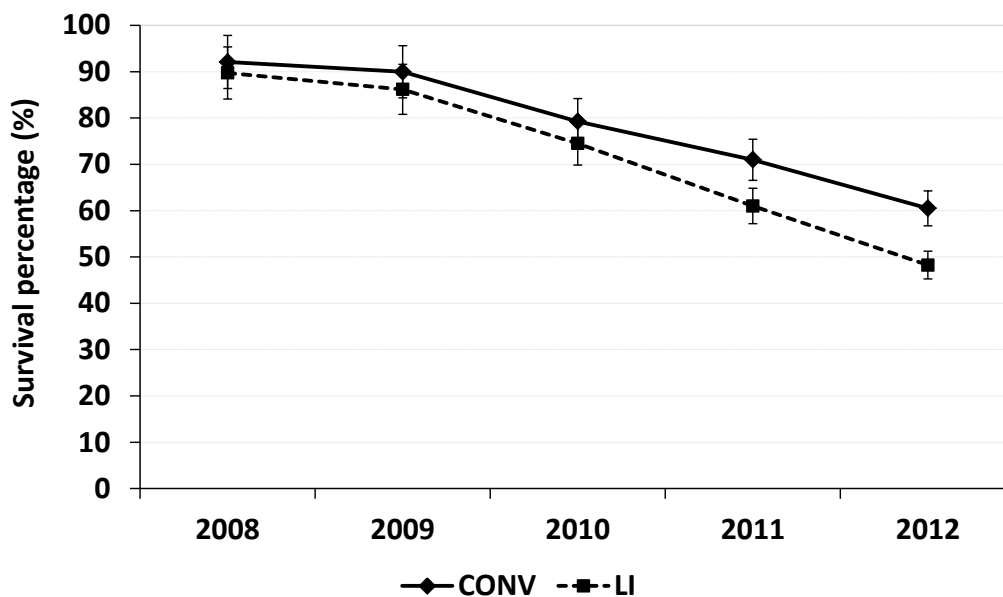


Figure 2: Plant survival percentage at each year in CONV and LI managements. Vertical bars show standard errors.

The higher survival in CONV plots may indicate better establishment and a higher chance of survivorship in subsequent years. For following years, differences between treatments were significant ($p < .0001$) being CONV plants greater than those in LI plots.

From established seedlings to a following 5-year period, our findings showed within age of stand a clear positive influence between plant survival percentage and management intensity.

This pattern was consistent with those reported by Mauromicale et al. (2014), thus demonstrate that better growing conditions ensure higher survival, and that this advantage can be maintained up at least for 5-years.

Across both treatments, all considered biometric parameters were significant higher for CONV compared to LI (Tables 4).

Table 4: Biometric parameters of cardoon as affected by treatments and year of cultivation.

Factor	Height (cm)	Leaves (no. plant⁻¹)	Stalks (no. plant⁻¹)	Heads (no. plant⁻¹)
<i>Treatment</i>				
CONV	186.4 a	44 a	12 a	8 a
LI	161.3 b	32 b	8 b	6 b
<i>Years</i>				
2008	133.3 d	31 c	6 d	6 b
2009	224.8 a	42 b	10 bc	10 a
2010	176.4 b	56 a	9 c	9 a
2011	156.7 c	30 c	14 a	6 b
2012	178.2 b	31 c	12 b	4 b
<i>P value</i>				
Treatment	0.0005	<.0001	0.0006	0.0059
Years	<.0001	<.0001	0.0001	0.0009
Treatment x Years	0.907	0.1926	0.2376	0.7609

Mean values followed by a different letter within a column and within a factor are significantly different ($P < 0.05$)

Biometric traits were, also, affected by year of cultivation. According to its perenniality, cardoon plant height changed with age and was lower at the first year and higher in the following year. Probably, rainfall distribution throughout 2010–2011 has adversely affected plant height in cardoon. Indeed, at the fifth year of cultivation, cardoon plants were significantly taller if compared to the previous year, with growth exceeding about 22 cm. The number of leaves during 2011 and 2012 was the same as that in the first year. As explained further below, stalks number increased significantly from the first to late years of cultivation. A significant increase in heads number was only found in the second and third year; in the other 3 years, heads number remained at similar levels. No significant interactions between treatment, and years occurred (Table 4).

Aboveground biomass yield of cardoon was affected by year of cultivation and treatments. The response to year of cultivation was not treatment dependent, as both treatment showed a similar pattern of changes in yield with increasing age. There was an initial period of yield increase that reached a maximum, and the greater increase in CONV during this period is evident in Fig. 3.

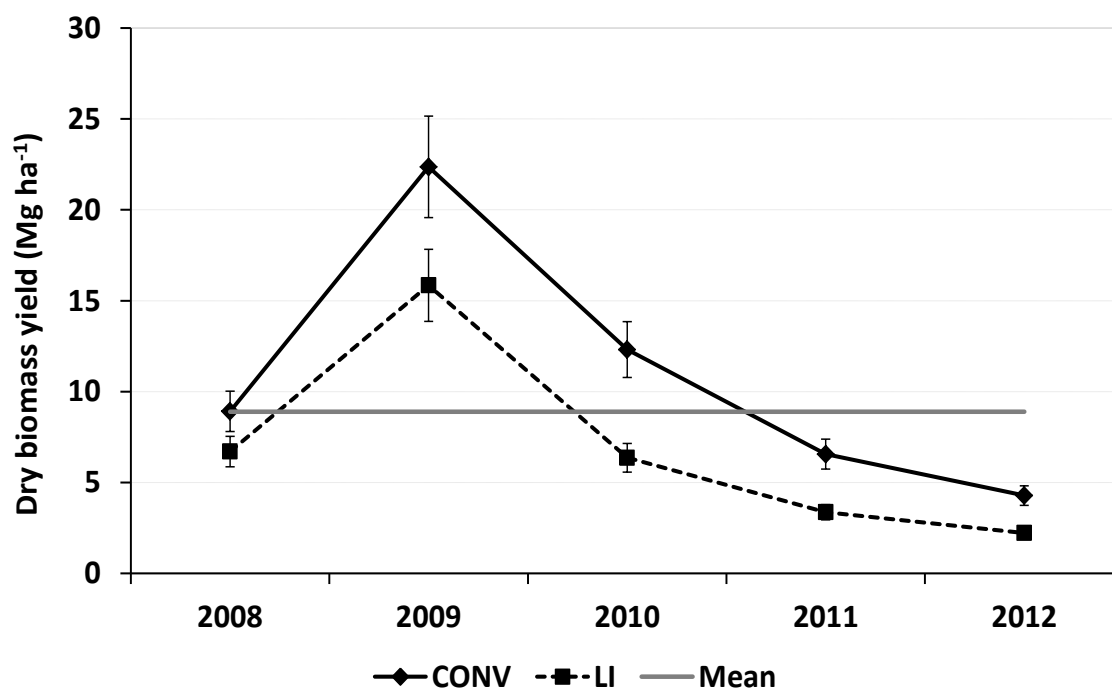


Figure 3: Dry yield (\pm standard error) in the five growing seasons for cardoon. CONV: conventional management; LI: low input management.

Following this peak of yield, yield declined and leveled in both treatments. Averaging 12.3 (CONV) – 6.4 Mg ha⁻¹ (LI) in 2010, yields clearly declined in 2011 and beyond.

This decrease in yield that we observed is earlier than expected if considering the 5 – 6 year pluriannual yields reported by Angelini et al. (2009) and Mauromicale et al. (2014) on long term trials carried out in Central and South Italy respectively.

On the contrary, a marked decline of the biomass yield from the third year onwards was observed by Foti et al. (1999), Raccuia and Melilli (2007) and Mantineo et al. (2009) in Mediterranean environment.

Possible causes of the biomass yield decline over time are the reduction of the plant density and the progressive soil susceptibility to compaction, crusting, and surface sealing resulting from a systematic removal of crop residues and cited as a possible cause of successive annual declines in biomass yield (Blanco-Canqui, 2010).

As regard biomass partitioning among plant components, no significant difference in stalks, leaves, and heads fractions were found between CONV and LI (Fig. 4).

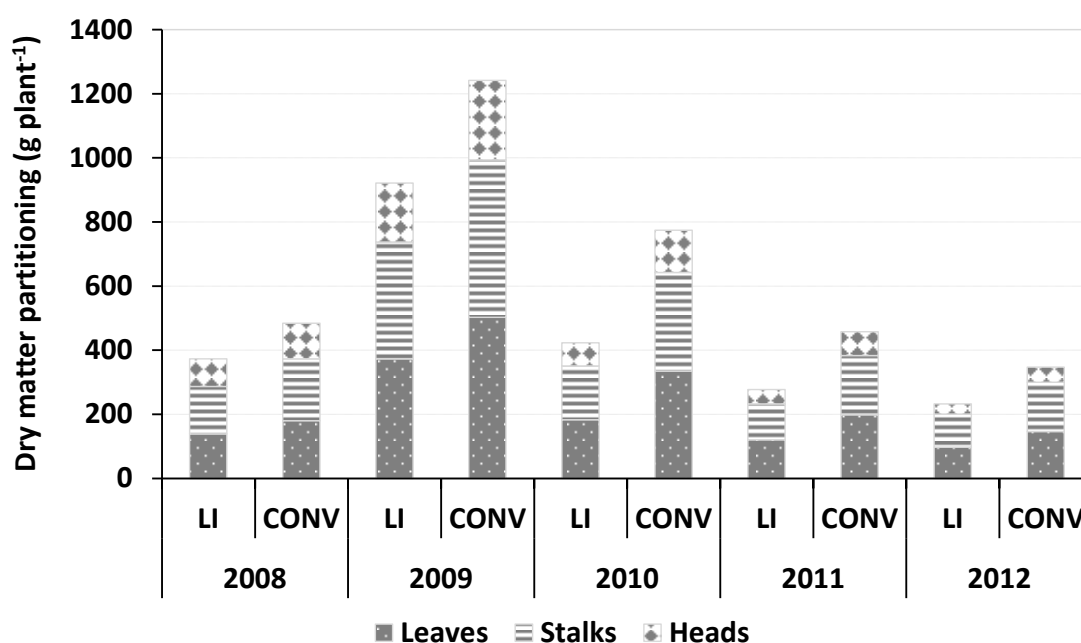


Figure 4: Dry matter partitioning in the five growing seasons for cardoon. CONV: conventional management; LI: low input management.

As expected, leaves and stalks increased from 2008 to 2011, and heads decrease was also observed over the same period, thus determining a change in biomass partitioning from early to late years (Fig. 4). In particular, the proportion of leaves and stalks biomass on total aboveground biomass was lower in 2008 (37.2% and 39.8%, respectively) and increased

constantly in the following years. The increase in the proportion of leaves and stalks over time is in line with previous findings (Ledda et al., 2013; Ierna et al., 2012) and it can be explained by change in the overall plant habit (Mauromicale et al., 2014).

In terms of efficiency of water used, CONV performed better than LI (Table 5). In the two management treatments, cardoon showed similar or even higher WUE if compared to other studies, such as irrigated cardoon (0.1 – 4.0 g DM L⁻¹), irrigated *Miscanthus x giganteus* (0.3 – 5.1 g DM L⁻¹), and *Arundo donax* (0.9 – 7.6 g DM L⁻¹) (Mantineo et al., 2009). Averaged across years, values ranged between 3.2 g DM L⁻¹ (2008) and 7.6 g DM L⁻¹ (2009), whilst in the last 2 years the values were rather low due to the reduced total yield.

Table 5: Water Use Efficiency (WUE) and Nitrogen Use Efficiency (NUE) in cardoon.

Factor	WUE (g L ⁻¹)	NUE (g g ⁻¹)
<i>Years x Treatment</i>		
2008		
CONV	3.6 a	76.9 a
LI	2.7 b	59.2 b
LSD _{0.05}	7.3	6.8
2009		
CONV	8.9 a	146.3 b
LI	6.3 a	197.1 a
LSD _{0.05}	N.S.	3.1
2010		
CONV	4.9 a	67.1 b
LI	2.5 b	122.9 a
LSD _{0.05}	3.7	2.1
2011		
CONV	2.6 a	44.0 b
LI	1.4 b	72.7 a
LSD _{0.05}	3.0	169.6
2012		
CONV	1.7 a	36.9 b
LI	0.9 b	55.1 a
LSD _{0.05}	74.5	19.4

Mean interaction values followed by a different letter within a column and within a factor are significantly different (P < 0.05).

Low input treatment was more efficient in the use of N fertilizer than CONV (Table 5), indicating that the maximum NUE was reached at 50 kg N ha⁻¹ yr⁻¹. The maximum NUE at lower N fertilization rate observed in our study is an additional advantage over other perennial species, because it indicates low N fertilization requirements to achieve maximum return of biomass per unit of N applied.

1.3.3 Seed yield and Harvest Index

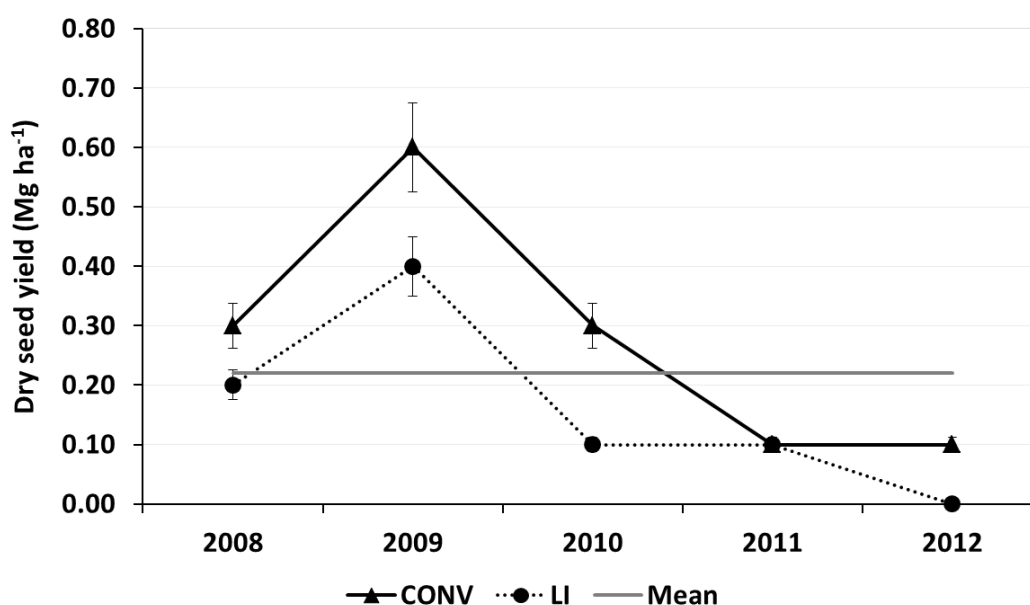


Figure 5: Dry seed yield (\pm standard error) in the five growing seasons for cardoon. CONV: conventional management; LI: low input management.

Averaged across treatments and years, cardoon harvested yields peaked in 2009 (Fig. 5, Ttable 6), which was the first full production year after the year of establishment and then declined overtime.

Table 6: Seeds yield and Harvest Index in cardoon as affected by treatments and year of cultivation.

Factor	Seeds yield (Mg ha⁻¹)	Harvest Index
<i>Treatment</i>		
CONV	0.27 a	0.0250
LI	0.18 b	0.0245
<i>Years</i>		
2008	0.24 b	0.031 a
2009	0.51 a	0.028 b
2010	0.21 b	0.023 c
2011	0.11 c	0.023 c
2012	0.06 c	0.019 c
<i>P value</i>		
Treatment	0.0015	0.2418
Years	<.0001	<.0001
Treatment x Years	0.3920	0.6478

Mean values followed by a different letter within a column and within a factor are significantly different ($P < 0.05$).

The highest yields achieved by CONV and LI in our experiment were 0.6 and 0.4 Mg ha⁻¹ yr⁻¹, respectively. However, higher yields have been reported by several authors in Mediterranean environment, although in most of case at plot scale experiments and supplying an aid irrigation to promote seeds development, or by applying high N rate, or under weed-free conditions (see table 7a,b for details).

Table 7a: Seed yields of *C. cardunculus* L. *atilis* DC. reported by several authors in Mediterranean environment

References	Site	Cycle length (years)	Genotype	Plot size (m ² or no. plants)	Water supply	Nitrogen supply (kg ha ⁻¹)	P ₂ O ₅ supply (kg ha ⁻¹)	Weeds control	Crop pests and diseases	Seeds yield (Mg ha ⁻¹)
Foti et al., 1999	Sicily, Italy	1992-93 1993-94 1994-95	Bianco Avorio	20 plants	1992-93: artichoke usual practice; 1993-94: 50 mm at May 1994-95: 50 mm at May	1992-93: artichoke usual practice; 1993-94: 50 1994-95: 50	1992-93: artichoke usual practice; 1993-94: none 1994-95: none	1992-93: artichoke usual practice; 1994-95: by hand	1992-93: artichoke usual practice; 1993-94: not declared 1994-95: not declared	1.1-2.5
Piscioneri et al., 2000	Basilicata, Italy	1993-94 1994-95 1995-96	Bianco Avorio	40 m ²	No water supply	Each year: 200	Each year: 100	2 nd year: manually removed before canopy covering the soil	Not declared	- not harvested (1 st year) - 0.5 (2 nd year) - 0.8 (3 rd year)
Raccuia and Mellili, 2007	Sicily, Italy	1998-99 2000-01 2001-02	Bianco Avorio	56 plants	1 st year: 50 mm at planting Each following year: 50 mm at May	Each year: 50	None	Each year: by hand	Not declared	- 1.2 (1 st year) - 0.9 (2 nd year) - 0.3 (3 rd year)
Ierna e Mauromicale, 2010	Sicily, Italy	1998-99 1999-00 2000-01	Gigante di Romagna	30 plants	1 st year: 30 mm +30 mm before and after transplanting, + 50 mm at April 3 rd year: 50 mm at April	Each year: 80	None	Two manual control per year	Not declared	1.4-1.7
Gominho et al., 2011	Beja, Portugal	2004-2005 2005-2006	Not declared	Total field area: 77.4 ha Sampling plot area: 7.5 m ²	Rainfed	1 st year: 50	1 st year: 90	None	Not declared	2 nd year: 0.09-1.6

Table 7a: Seed yields of *C. cardunculus* L. *atilis* DC. reported by several authors in Mediterranean environment

References	Site	Cycle length (years)	Genotype	Plot size (m ² or no. plants)	Water supply	Nitrogen supply (kg ha ⁻¹)	P2O5 supply (kg ha ⁻¹)	Weeds control	Crop pests and diseases	Seeds yield (Mg ha ⁻¹)
Ierna et al., 2012	Sicily, Italy	2000-01 2001-02 2002-03	Bianco Avorio	Sub-sub-plot: 60 plants	1 st year: 30 + 40 mm before and after transplanting, + 50 mm in spring Each following year: 50 mm in spring	Three levels: 50, 100, 200	Three levels: 50, 150, 300	1 st year: Oxyfluorfen (0.5 kg ha ⁻¹)	Not declared	- 0.8 (50 U N ha ⁻¹)* - 1.0 (100 U N ha ⁻¹)* - 1.1 (200 U N ha ⁻¹)* * Values averaged over years
Mauromicale et al., 2014	Sicily, Italy	2005-06 2006-07 2007-08 2008-09 2009-10 2010-11 2011-12	Bianco Avorio	16 m ²	1 st year: 30 m ³ ha ⁻¹ after transplanting	1 st year: 60 2 nd year: 60	1 st year: 100	1 st year: by hand after transplanting	Pest and disease control never necessary	0.55 Mean value, 7 growing seasons
Vasilakoglou and Dhima, 2014	Northern (Exp 1) And Central Greece (Exp 2)	Exp 1: 2007-08 2008-09 2009-10 2010-11 Exp 2: 2009-10 2010-11 2011-12	Bianco Avorio	Main plot: 91 m ² Sub-plot: 35 m ²	Two levels: 90 mm season ⁻¹ ; No irrigation	1 st year: 50 Following years: none	1 st year: 25 Following years: - none	Two levels: weed-free by hand; weedy	1 st year: Chlorpyrifos (2 kg ha ⁻¹)	Exp1: - 1.1 (No irrigation)* - 1.6 (Irrigated)* Exp2: - 1.9 (No irrigation)* - 2.6 (Irrigated)* * Values averaged over years
Ierna et al., 2012	Sicily, Italy	2000-01 2001-02 2002-03	Bianco Avorio	Sub-sub-plot: 60 plants	1 st year: 30 + 40 mm before and after transplanting, + 50 mm in spring Each following year: 50 mm in spring	Three levels: 50, 100, 200	Three levels: 50, 150, 300	1 st year: Oxyfluorfen (0.5 kg ha ⁻¹)	Not declared	- 0.8 (50 U N ha ⁻¹)* - 1.0 (100 U N ha ⁻¹)* - 1.1 (200 U N ha ⁻¹)* * Values averaged over years
Gominho et al., 2014	Ferraria, Portugal	2004-2005 2005-2006	Not declared	Total field area: 8.1 ha Sampling plot area: 7.5 m ²	Rainfed	1 st year: 50	1 st year: 90	None	Not declared	2 nd year: 0.45-1.6

Our results are consistent with ones reported by Mauromicale et al. (2014) in a long term experiment with low input management and by Gominho et al. (2011, 2014) who worked at large field scale.

The relatively low seed yields achieved in our study by cardoon are likely due to the impact of factors that are important when considering large scale cultivation of this crop nearby globe artichoke cropping areas, as pests and diseases that we observed in our pluriannual experiment. Although, pests control, aid irrigation or increasing and splitting the N fertilization rates may result in higher yields, the costs and energy input associated with this management needs to be carefully taken into account.

As regard Harvest Index, no significant difference resulted between CONV and LI. Averaged across years, HI varied from 0.02 to 0.03 (Table 6), values markedly lower if compared to the range 0.104 – 0.196 reported by Archontoulis et al. (2010). Anyway, these results highlight that cardoon cannot be considered an actual oleaginous crop and its seed production is only a secondary product compared to its potential biomass production.

1.3.4 Proximate and ultimate analyses

As regards biomass chemical characteristics, ANOVA analysis outlined no significant effect of the years on biomass. Therefore data were bulked and reported in Tables 8 and 9 differentiating on the basis of treatments and biomass repartition among plant components.

The proximate and ultimate analyses, as well as the calorific values of the studied samples, are presented in Table 8.

Table 8: The mean least significant differences test (LSD) at 5% significant level for proximate and ultimate analysis of cardoon averaged over years

	Moisture (%)	Volatile matter (%)	Fixed C (%)	Calorific value (MJ kg⁻¹)	C (% d.w.)	H (% d.w.)	N (% d.w.)	S (% d.w.)	O (% d.w.)	Cl (% d.w.)
<i>Treatment</i>										
CONV	9.78	90.8	7.8	16.6	42.4	6.5	2.1	<0.3	48.8	16.6
LI	9.76	90.0	7.7	17.1	42.4	6.3	2.1	<0.3	49.1	16.9
LSD _{0.05}	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
<i>Plant component</i>										
Leaves	9.80	86.0	5.8	16.7	41.3	5.9	2.4	<0.3	50.3	14.1
Stalks	10.6	92.3	7.8	15.8	41.1	6.4	1.5	<0.3	50.8	18.1
Heads	8.85	93.5	9.7	18.1	44.9	6.8	2.4	<0.3	45.8	10.1
LSD _{0.05}	1.3	1.3	8.3	12.9	2.3	0.6	0.3	N.S.	3.3	2.6

For both ultimate and proximate analyses, no significant differences were observed between treatments, although LI had slightly more ash and lower C than CONV (Table 8).

Moisture is an important parameter in biomass because heating value decreases with moisture content and decreases combustion temperatures affecting efficiency of thermal processes and producing maintenance difficulties. Cardoon moisture content of samples varied from 8.9% to 11% as can be seen in Table 8. As regard single plant organs, differences concerned calorific values that was almost 13% higher in heads (Table 8). Calorific values varied from 18.1 to 15.8 MJ kg⁻¹ respectively, mainly due to moisture differences explained. Cardoon volatile matter varied from 86% to 93.5%. These results are higher or similar than volatile matter value (74.9%) reported by Nassi o di Nasso et al. (2010b) in other types of biomass such as giant reed or in *Miscanthus* (67.7%), *salix* (70.5%), and wheat straw (68.1%) reported by Butler et al. (2013).

In our experiment Cl content ranged from 0.00% (heads) to 0.01% in (stalks). Chlorine levels were slightly (- 3%) lower than those found in a test in central Italy (Angelini et al., 2009), which could have resulted from differences in analysis method, soil Cl concentrations, or environmental variables. Despite our initial hypothesis that different N fertilization levels played a key role in the biomass chemical traits, the results demonstrated that N management intensities did not significantly affect ultimate analysis in term of N concentrations. Heads and leaves N concentrations recorded in our study exceeded the guiding value for unproblematic thermal utilization (Oberberger, 1998). Nitrogen content is an important parameter as several investigations showed that one of the main environmental impacts of solid biofuels combustion is caused by NO_x emissions (Nussbaumer, 2002). The NO_x emissions thus increase with increasing fuel N content (Oberberger et al., 1995; Leckner and Karlsson, 1993).

However, as shown in Table 8, stalks ultimate analysis (41.1% C, 6.4% H, 1.5% N and 50.8% O on average) are comparable to other perennial crops values such as arundo, kenaf, and reed canary grass (48.7% C, 6.1% H, 0.6% N and 44.5% O; 48.4% C, 6.0% H, 1.0% N and 44.5% O; 49.4% C, 6.3% H, 1.5% N and 42.7% O), and to straws of crops such as rape, barley and corn (49.4% C, 6.1% H, 1.2% N and 43.2% O) according to the data reported in literature (Vassilev et al., 2010).

Regarding to sulfur parameter, the S contained in solid biofuels forms gaseous SO₂, sometimes SO₃, alkali, alkali-sulphates. Sulfur content in all cardoon samples was lower than 0.003%. No significant differences were found in relation to ash content between the two compared treatments. Inorganic content can be high due to the nature of the biofuel or contamination with other products such as soil, sand or stones.

Table 9: The mean least significant differences test (LSD) at 5% significant level for ash content and composition (% d.w.) averaged over years

		Ash composition (% d.w.)					
		Ash (% d.w.)	K₂O	Na₂O	CaO	Fe₂O₃	Al₂O₃
<i>Treatment</i>							
CONV	15.2	15.0	12.0	27.0	0.0027	0.0003	
LI	15.5	15.7	11.2	29.3	0.0031	0.2950	
LSD _{0.05}	N.S	N.S	N.S	N.S	N.S	N.S	
<i>Plant component</i>							
Leaves	20.2	10.5	19.0	52.8	0.0067	1.1528	
Stalks	11.9	12.8	14.3	19.5	0.0008	0.1590	
Heads	9.2	22.8	1.5	12.3	0.0012	0.5220	
LSD _{0.05}	2.5	5.6	2.7	7.8	0.00004	0.1	

Total ash content of biomass, averaged across treatments, was over 15% (Table 9), a relatively high value when compared to other types of biomass (e.g. 4.3 mass % and 2.7 mass % for grass and wood biomass, respectively) (Vassilev et al., 2010). These ash results are consistent with those reported previously for cardoon (Encinar et al., 2000, 2002), although differences in ash content were present among plant components (Table 9). Leaves had the highest ash levels, about 50% higher than the other organs (Table 9). Analysis of major oxides indicated significant differences among plant components (Table 9). Heads and leaves had the highest K₂O and Na₂O, respectively. Stalks had a slight advantage over heads and leaves, in this regard, showing the lowest Fe₂O₃ and Na₂O. This variation could have a significant impact on fuel quality. High concentration in K₂O and CaO can result in ashes with low melting temperatures, leading to the tendency of slag formation, fouling and corrosion (Dahl and Obernberger, 2004).

1.3.5 Energy balance

As regards the establishment year (2007), our results showed that the total energy input per area unit in CONV was 16.1% greater than observed in LI. The total energy requirement was caused mainly by machinery and fertilization, which requested about 28.0 GJ ha⁻¹ in CONV and about 19.6 GJ ha⁻¹ in LI (Table 10).

Table 10: Energy input (MJ ha⁻¹) for cardoon biomass production from the crop establishment (2007) to the 5th year of growth cycle.

Field operation	1 st year		Following years	
	CONV (MJ ha ⁻¹)	LI (MJ ha ⁻¹)	CONV (MJ ha ⁻¹)	LI (MJ ha ⁻¹)
Tillage (ploughing + harrowing + rolling)	2295	2295	-	-
Fertilization				
Spreading	324.4	324.4	324.4	324.4
Triple Superphosphate	3429	3429	2229	2229
Urea	21974	13529	16969	8446
Weeding				
Crop spraying	166.3	-	-	-
Herbicide (Linuron)	435.0	-	-	-
Sowing				
Conventional sowing	515.2	515.2	-	-
Seeds	22.0	22.0	-	-
Harvesting	3201	3201	3201	3201
<i>Total</i>	<i>32362</i>	<i>23385</i>	<i>22723</i>	<i>14254</i>

Fertilisation was responsible, on average, for 79.5% of energy input in CONV, while it represented 74.2% in LI. Mechanization required less energy than fertilisation and mainly concerned soil tillage and crop harvest. In first year, tillage total energy requirements ranging from 7.1% to 9.8% for CONV and LI treatments, respectively (Table 10). With respect to harvest, energy requirement accounts for 9.9% in CONV and 13.7% in LI. Because of the greater inputs of N fertilizers and herbicide, the energy input was clearly higher in the case of CONV than for the other LI.

Considering the following years (2009-2012) of the cardoon five-year- cycle, according to Angelini et al. (2009); the energy input decreased because the planting operation costs were not more included. As cardoon is a perennial crop, tillage, sowing and weeding (CONV treatment) were applied only for the planting year. In the following years, the major difference in total energy requirements between CONV and LI was mainly due to the reduction of machinery (Table 10). Fertilization, and in particular fertilizers, were the main energy input, accounting for 85.9% in CONV and 77.5% in LI. This was followed by harvest, representing about 14.1–22.5% in the two compared treatments. Table 11 summarizes energy balance of the entire growing cycle.

Table 11. Mean values of global energy balance for conventional (CONV) and low input (LI) treatments from the crop establishment (2007) to the 5th year of growth cycle.

Growing seasons	Yield (Mg ha ⁻¹)				Energy inputs (GJ ha ⁻¹)		Energy output (GJ ha ⁻¹)				Total Energy output ^{a+b} (GJ ha ⁻¹)		Net energy yield ^c (GJ ha ⁻¹)		Energy efficiency ^d			
	Biomass		Seed		CONV	LI	Biomass ^a		Seed ^b		CONV	LI	CONV	LI	CONV	LI	CONV	LI
	CONV	LI	CONV	LI			CONV	LI	CONV	LI								
2008	8.6	6.5	0.3	0.2	32.4	23.4	142.8	111.2	6.5	4.4	149.3	115.5	116.9	92.1	4.6	4.9		
2009	21.8	15.4	0.6	0.4	22.7	14.3	361.9	263.3	13.1	8.7	375.0	272.1	352.3	257.8	16.5	19.0		
2010	12.0	6.3	0.3	0.1	22.7	14.3	199.2	107.7	6.5	2.2	205.7	109.9	183.0	95.6	9.1	7.7		
2011	6.5	3.3	0.1	0.1	22.7	14.3	107.9	56.4	2.2	2.2	110.1	58.6	87.4	44.3	4.8	4.1		
2012	4.2	2.2	0.1	0.0	22.7	14.3	69.7	37.6	2.2	0.0	71.9	37.6	49.2	23.3	3.2	2.6		

^c Calculated as output–input

^d Calculated as output/input

Both treatments showed a positive energy balance (Table 11). Averaged over years (Table 11), the mean energy output was slightly higher for CONV (182.4 GJ ha⁻¹ year⁻¹) than for either LI treatment (118.7 GJ ha⁻¹ year⁻¹), as result of the lower LI dry yield (about 57.8 % lower than in CONV). For both treatments, the lowest energy outputs occurred in 2011/12, ranging from 110.1 GJ ha⁻¹ year⁻¹ for CONV to 37.6 GJ ha⁻¹ year⁻¹ for LI, a result of the naturally senescence of the crop. Averaged across years, energy efficiency showed significant differences between treatments with higher values in LI than in CONV (+ 1.3%), and particularly in 2008/09 growing season (19.2 vs 16.5 for LI and CONV, respectively) (Table 11). The net energy yield was significantly higher in CONV (157.8 GJ ha⁻¹) than in LI treatment (102.6 GJ ha⁻¹) (Table 11). The value of this variable ranged from 352.3 GJ ha⁻¹ for CONV to 23.4 GJ ha⁻¹ for LI (Table 11).

1.4 CONCLUSIONS

Our results in rainfed conditions on large scale plots showed that a reduction in management intensity can increase the energy efficiency of the low farming systems even if with evaluable yield losses within years. Overall effects of N fertilization on dry matter yield were rather modest in the study, allowing for a significant input reduction, also supported by energy balance results.

Within the timeframe of this study, in both treatment dry matter yield was not maintained, but rather declined progressively. The findings clearly call for research to discover how the higher earlier yields might be maintained into the longer term. This in turn will allow improved management to avoid the clear yield decline observed here to provide reliable feedstock supply to meet bioenergy demand.

We found that cardoon input management with particular regard to N fertilization does not influence biomass mineral composition, as no significant difference was detected between two analyzed managements, making LI treatment more suitable for bioenergy use (more energy yield per nutrient removal).

More research is needed to investigate whether different crop managements can influence the sustainability of the bio-energy chain from an environmental point of view. In addition, the energy balance also needs to be extended to the fuel-processing phase. This would help to

establish whether the production of fuel is truly sustainable and to standardize conventions for estimating the energy budgets that define the system boundaries and the equivalent coefficients.

CHAPTER 2

Productive characterization and growth analysis in three different cardoon genotypes

2.1 Introduction

One of the main objectives of modern Governments is to create a global policy aimed at ensuring the availability of diversified, reliable and affordable energy resources.

Generally speaking, most strategic policies are aimed at reducing communities' energy dependence, and the risks connected to it. Therefore, there is a strong need to support the diversification of primary energy sources, of production technologies and energy distribution, and to encourage a more widespread use of alternative sources to hydrocarbons (e.g. renewable, nuclear, alternative fuels).

An alternative source of energy is the biomass.

In addition, the large utilization of biomass for energy can provide a basis for rural development and employment in developing countries, involving significant environmental benefits.

In fact, if the crop is managed in a sustainable way, the CO₂ released during combustion is balanced by the CO₂ extracted from the atmosphere during the photosynthesis. In synthesis, the production and the use of biomass do not create accumulation of carbon dioxide (CO₂) in the atmosphere. (Hall et al., 1993).

The biomass can be obtained from residues or from specifically dedicated crops.

A number of old and recent studies have identified in *Cynara cardunculus* var. *Altilis* L. one of most promising renewable energy crop.

This crop belongs to the *Asteraceae* family together with globe artichoke (*Cynara cardunculus* var. *scolymus* L.) and their common ancestor wild cardoon (*Cynara cardunculus* var. *silvestris* Lam.).

The Cardoon is native of the Mediterranean basin and the area cultivated with this crop has never been large. Its commercial relevance has been reached in Italy, Spain and southern France, where it is used for the preparation of traditional dishes (Portis et al., 2005).

The thistle has been used since ancient times as human food for its wide fleshy and succulent petioles, and for milk clotting.

The interest as bioenergy crop in a Mediterranean environment was due to various plant characteristics, as its low water requirement, as a consequence of its good adaptation to the native environment (Ledda et al. 2013)

It was been considered for its cellulose production and other industrial uses as liquid biofuel (bioethanol from fibers, and biodiesel from the grain), paper pulp and biogas (Ballesteros et al., 2008; Fernández et al., 2006; Gominho et al., 2011; Grammelis et al., 2008; Raccuia and Melilli, 2007; Oliveira et al., 2012).

Essentially, cardoon is a source of lignocellulosic biomass and oil from seeds.

The potentialities of *C. cardunculus* as an oil crop in a perennial cultivation system are confirmed in terms of seed oil content and fatty acid composition. These parameters are not different from those measured on other crops grown for the same purpose.

Thus, the utilization of *C. cardunculus* would have also the advantage to use the aerial biomass for energy production (Curt et al., 2002).

Its good adaptation to Mediterranean climates and its high potential yields suggest that *C. cardunculus* can be grown as a perennial crop with an annual harvest of the aboveground biomass in areas with a dry Mediterranean climate.

The great adaptation to semi-arid Mediterranean climate derives from the way the culture escapes the summer drought: the plant dries, the roots stay quiescent and they awaken with first rains.

The interest in *Cynara* as bioenergy crop in the Mediterranean environment, it's also justified by other plant characteristics.

In fact, a cultivation for bioenergy purpose is sustainable in a low-medium input context.

Low input management concerns mainly irrigation, fertilization, and weed control.

In this contest, cultivated cardoons are highly competitive energy crops: they show high biomass and energy yield, particularly evident at low and medium fertilization and it's a perennial crop highly competitive to weeds and with minimal needs of nitrogen and other nutrients (Grammelis et al. 2008). Vasilakoglou and Dhima, in their recently conducted study (2014), confirmed a good production of the Cardoon in stress water conditions and weed competition.

This is very important in order to meet the needs of bioenergy farmings and also to encourage the farmers to change their old cultivation with a new energy crop. These ones require a greater profit obtainable by a low input cultivation system (Ierna et al., 2012).

To support the strategic decisions regarding the introduction of the thistle cultivated for energy production into the Mediterranean farming system, the crop system must be analyzed in its complexity.

However, studies concerning temperature and photoperiod effects on the development rate of *Cynara* are lacking in literature (Archontoulis et al., 2010), and research investigation is required to inquire into different genotypes of the crops for potential biomass and grain yield, in order to identify suitable agronomic methods for optimizing qualitative crops performances (Ledda et al., 2013).

For these purposes, in this research has been carried out the analysis of plant growth and development.

Therefore, the phenology and the dynamics of the aboveground biomass production of the three genotypes cardoon were investigated during 2 years.

In plant growth analysis, data are usually obtained from successive destructive harvests performed within the plant growth cycle, from which the growth rates are calculated.

In the present work, we used two growth rates principally. These are LAD (leaf Area Duration), RGR (Relative Growth Rate) , NAR or ULR (Net Assimilation Rate or Unit Leaf Area).

Two main approaches exist for estimating growth rates:

- the classical approach: mean values of growth rates are calculated using data of two consecutive harvests;
- the functional approach, where mathematical functions are fitted throughout the growth data over time.

The functional approach provides, in the polynomial curves, instantaneous values of growth rates.

In our case, we exploited the opportunity provided by Porter in its publication of 1989, where it propose a synthesis of the two previous approaches.

To recap the main aims of the study were as follows:

- to analyse the dynamics of the above-ground biomass;
- to analyse the dynamics of biomass allocation in the productivity components;
- to analyse the timing and duration of the phenological stages;
- to evaluate the aboveground biomass and grain yield;

All evaluations were made for the three genotypes.

2.2 MATERIALS AND METHODS

2.2.1 Experimental site

From 2012 to 2014, a field experiment has been conducted in North-West of Sardinia (Italy) in Ottava, near Sassari, at the Experimental Farm of the University of Sassari (41°46'N, 8°29'E, 81 m a.s.l.).

According to the USDA Soil Taxonomy, most of the soils at the site are classified as Lithic Xerorthents (Madrau et al., 2006). In this classification system, these soils belong to the “Entisols” order, “Orthents” suborder, “Xerorthents” great group, “lithic” subgroup and in general are underdeveloped young soils, with a xeric moisture regime that has a lithic contact within 50 cm of the mineral soil surface.

Regarding the meteorological trend in the area, as show in Figure 6, the climate at the site is typically Mediterranean with 54 years long-term average annual rainfall (1958-2012) of 554 mm, occurring mostly in October, November and December. Rainfall during summer does not exceed 30 mm with a lowest value in July. The mean annual temperature ranges from 9.9 °C (January and February) to 23.7°C (August).

Minimum temperature values below 0°C are not common, the mean of the annual average minimum and maximum temperature are about 11.4°C and 20.75°C respectively.

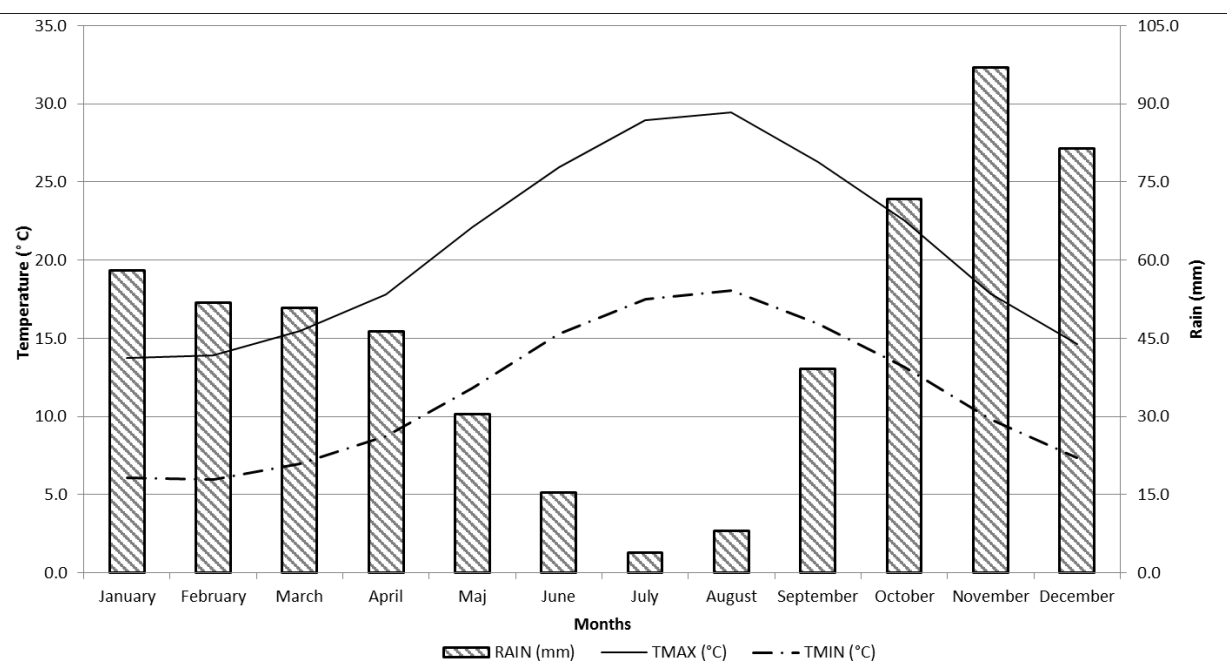


Figure 6: Meteorological trends for the 1958-2012 period at the experimental site: monthly maximum (TMAX °C) and minimum (TMIN °C) mean temperature and rainfall (bar chart).

2.2.2 Crop management and experimental design

In 2010 and 2011, the experimental field in Ottava was uncultivated and it was previously grown with durum wheat (*Triticum durum* L.).

Before the planting phase, the field was ploughed with a disc plough to create good conditions for seed germination. During the seed-bed preparation by a disk harrow, the soil was fertilized with N and P₂O₅ using 200 kg ha⁻¹ of diammonium phosphate. In both years, a dose of 200 kg ha⁻¹ of urea was applied about 1 months before blooming. More specifically, the agronomic practices listed in Table 12, were applied to the experiment.

During the 2 year-experiment, the energy input for crop management was minimized, and the crop water requirements were satisfied by rain.

Table 12: main agronomic operations carried out in the experiment field.

Activities	Period (date)	Details
Land preparation	December 10 th 2012	Ploughed with disc plough (depth: 30-40 cm)
		Harrowed with disc arrow
Planting	December 11 th 2012	Manually, 3-4 seeds per hole Depth of planting: 3-4 cm
Thinning	March 21 st 2012	Manually, to get 1 plant per sqm
Fertilizer applied		
N	Sowing	36 Kg ha ⁻¹
	Vegetative stage, before elongation floral (for both years)	92 Kg ha ⁻¹
P	Sowing	92 Kg ha ⁻¹
	Vegetative stage	0 Kg ha ⁻¹
Weeding	April 2013	mechanical weeding
Pesticide applied	Stage seeds ripening (2013)	- Decis
	Before flowering (2014)	- Confidor
Harvest	September 1 st , 2013	At physiological maturity of seeds and plant senescence (BBCH90)
	September 5 th , 2014	

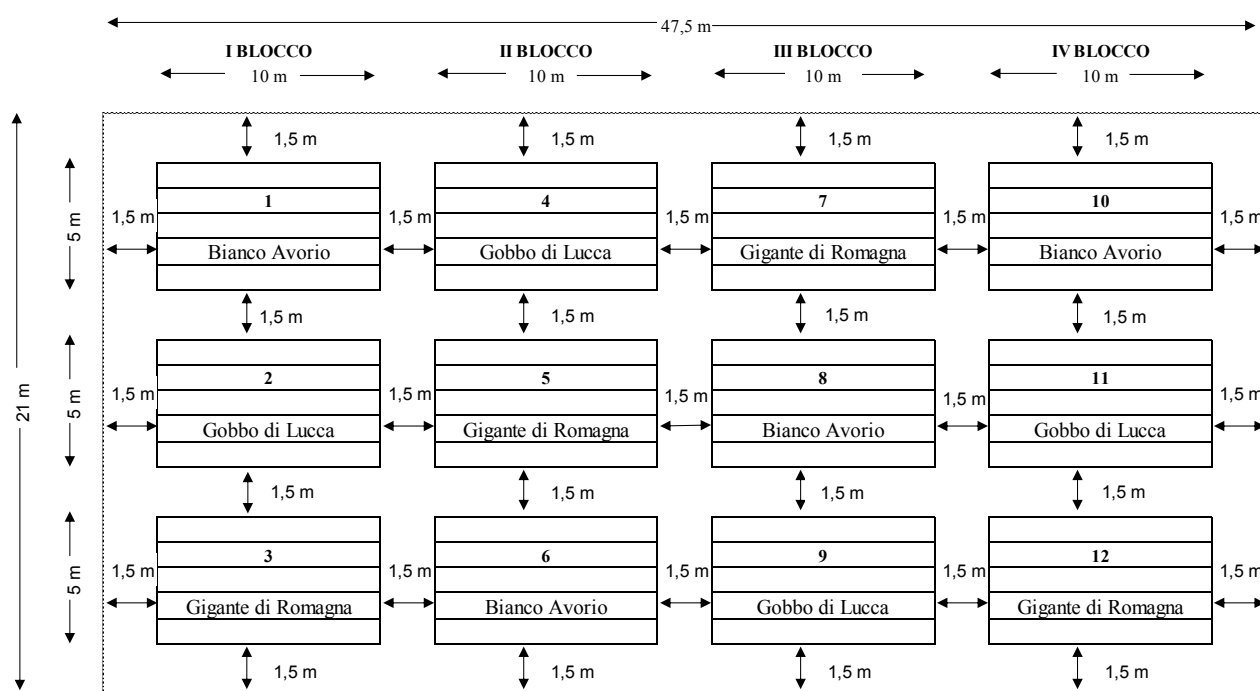
Three genotypes of cultivated cardoon (listed in Table 13) were compared four times in a complete randomized block experimental design.

Table13: Genotypes studied with related codes and botanical variety

Genotype	Code	Botanical Variety
Bianco Avorio	BA	<i>Cynara cardunculus</i> L. var. <i>altilis</i> DC
Gigante di Romagna	GR	<i>Cynara cardunculus</i> L. var. <i>altilis</i> DC
Gobbo di Lucca	GL	<i>Cynara cardunculus</i> L. var. <i>altilis</i> DC

The genotypes, studied for their high biomass production, were “Bianco avorio”, “Gigante di Romagna” e “Gobbo di Lucca”. They are commonly grown in Italy as vegetables (for food purposes), and already studied in literature for their large biomass production and seeds yield. The Figure 7 shows the map of the experimental field.

Figure 7: map of the experimental field.



The sowing was done manually using 3-4 seeds per hole, on December 11th 2012, allowing 1 m between and within rows. Each plot (5 m x 10 m) consisted of 50 plants placed in five rows with ten plants each one.

Three-month-old cardoon plants were thinned out and blanks were replaced with the same age plants.

In the first year, an inter-row weeding was conducted mechanically.

The crop was grown for two consecutive years and at the end of each annual crop cycle, at complete maturation of achenes, the aboveground biomass was cut down; the crop regrowth was naturally carried out by autumn rain.

2.2.3 Data collection and measurements

After the initial operation of soil preparation, the experimentation started with data measurement activities.

2.2.3.1 Soil and meteorological measurements

The soil characteristics were determined by sampling before planting. Chemical and physical analysis were carried out in the laboratories of the University of Sassari.

The soil characteristics were obtained by sampling each block from three increasing depths (0-15 cm; 15-30 cm; 30-45 cm) in December 2012, in the day before sowing.

All 12 samples were analyzed for main physical and chemical properties: texture, pH, organic matter, exchangeable potassium (K), and phosphorus (P), nitrate (NO₃⁻) and ammonium (NOH⁴⁺) concentration.

The weather data such as, rainfall and daily maximum and minimum air temperature was measured by an agro-meteorological station of ARPAS (the Sardinia Regional Environmental Protection Agency) placed near the field.

2.2.3.2 Plant growth and development

Data on plant phenology, growth dynamics of plants, aboveground biomass yield and its partitioning and grain yield were marked for the first two years of cycle.

a. PHENOLOGY

The experimental protocol registration of the experimental data was the same for both years.

The 10 plants on the central row of each plot were chosen as permanent samples areas for phenology measurements.

Once a week, after the emergence, systematic phenological observations were carried out in according to BBCH scale (Archontoulis et al., 2010).

For the *Cynara*, 10 (0-9) principal vegetative and reproductive growth stages and 10 (0-9) secondary growth stages were recorder.

The main growth stages recorded are listed and described in Table 14.

Table 14: Phenological growth stages of *Cynara cardunculus*, according to the BBCH scale (Archontoulis et al., 2010).

BBCH CODE	DESCRIPTION	
	I° GROWTH CYCLE	II° GROWTH CYCLE
	<i>Germination</i>	<i>Sprouting and bud development</i>
00-09	<p>First year: the development from seeds starts in stage 0 and ends when cotyledons emerge through the soil surface (50-60 °C-days using a minimum threshold temperature of 10°C);</p> <p>Second year, onwards: after crop harvesting, regrowth starts from vegetative buds that are positioned on the upper part of the root system. This stage ends when buds show green tips.</p>	
	<i>Areal leaf development from seed of the young plant</i>	<i>Leaf development from sprouting</i>
10-19	<p>First year: leaf development from seeds begins after the cotyledons are fully unfolded and ends when at least nine leaves are visible.</p> <p>Second year, onwards: leaf development from sprouting begins after the first leaf is expanded and ends when the ninth leaf is developed.</p>	
30-39	<i>Rosette growth, crop cover</i>	
	This principal stage describes the percentage of soil cover by plants	
	<i>Development of harvestable vegetative plant part</i>	
40-49	This stage reflects the increase of leaf biomass as percentage of this maximum biomass. This increase is the result of the fast leaf expansion and production of new leaves. In this stage, the crop can be harvested for forage purposes.	
	<i>Inflorescence emergence and development</i>	
50-59	This stage describe the inflorescence of the whole plant, whit particular reference to the main stem inflorescence.	
	<i>Flowering and capitulum formation</i>	
60-69	This principal stage assesses the flowering of the main inflorescence and the progressive blossoming of the heads.	
	<i>Development of capitulum</i>	
70-79	This stage refer to the development of the heads (capitulum) and achenes (fruits, seeds).	
	<i>Capitulum and seed ripening</i>	
80-89	This phase begin when the heads have reached physiological maturity and they starts to charge color to yellow and to brownish-yellow	
	<i>Senescence</i>	
90-99	This principal stage describes the gradual decrease of the plant moisture content	

For database elaboration and interpretation a methodology proposed and used in 2014 by Shiping et al. was used. This method was adapted for specific experimental conditions and for cardoon crop.

Thus, the same ten plants were followed across the first and second growing season.

For results interpretation, seven main phenological stages, evaluated as more important and representatives than others, were chosen.

The first day of each phenological event was calculated as the day of the year in which the phenological characteristics were visible for 10% of the sample, therefore in one plant per plot.

If the stage was evaluated by plant tissue colors, for each plant the date was calculated as the day of the year in which 90% of considered organs were colored.

The seven phenological stages taken into account including:

1. BBCH10: emergence of first leaf.
2. BBCH31: when the leaves cover 10% of ground, at the beginning of the rosette growth.
3. BBCH41: beginning of development of harvestable vegetative plant part, biomass storage.
4. BBCH51: beginning of stem elongation, inflorescence emergence and development stage.
5. BBCH60: onset of flowering with first flower petals visible on main stem inflorescence.
6. BBCH70-80: development of capitulum (or head) and seed ripening.
7. BBCH90: full development and early senescence.

The length of each physiological stage (vegetative and reproductive growth) was established as the average number of days between events for all plants in sample areas.

As in Shiping et al. (2014), during the observation of reproductive events the ripening seed was ignored because it was difficult to be monitored in the field.

The vegetative growing season goes from germination (first year) or bud sprouting (second and following years) to the beginning of flower stalk.

The observation of the reproductive stage included all the other developmental phenologic events but excluded the event of seed ripeness as it was too difficult to monitor in the field.

b. GROWTH DYNAMICS OF BIOMASS AND ITS COMPONENTS

Every two weeks in the first growth cycle year, and every month in the second one, 1 plant per plot was randomly chosen in the row near the centre. The border plants in the outer row were discarded from the observation.

The plants were cut at the ground level and immediately brought in the laboratory, in order to determine their fresh aboveground biomass and partitioning.

The leaves, stems and inflorescences were weighed separately to determine their fresh weight and then were dried in a heater at 60°C until constant weight, in order to determine the total dry aboveground mass and their partitioning for each organ and for each date.

The roots were not considered, as their extraction from the soil was too hard.

All measurements were done for each of the twelve plots.

The crop dynamic of development and growth was described using an efficiency index: RGR “Relative Growth Rate”.

In this case, the rates of change were calculated in terms of total dry weight increase per unit of dry weight. This is an index of overall performance.

The development dynamic was also described by "Compounded growth rates”.

In particular, the plant total growth was considered as the sum of CPR effects (Component Production Rate).

The purpose was to subdivide RGR into indices, which represent the individual performances of system components.

In this way, this work describes the components of yielded biomass, but also the role of these components on the plant growth dynamic.

Operationally, the plant’s aboveground relative growth rate (in weight) was subdivided into an expression, which includes the relative growth rates of the individual organs of the plant such as stems, leaves and heads:

$$Rw = Jl + Js + Jh;$$

Where:

Rw : is aboveground plant’s relative growth rate (in weight);

J : is the rate of dry weight production of the plant component (wl , ws , wh) expressed per unit of total dry weight (W);

Jl: CPR for leafs;
Js: CPR for stems;
Jh: CPR for heads;

$$Jl = \frac{Rl(wl)}{W} = CPR (leafs);$$

$$Js = \frac{Rs(ws)}{W} = CPR (stems);$$

$$Jh = \frac{Rh(wh)}{W} = CPR (heads);$$

Rl: is the relative growth rate of *wl* (weight of leaves);

Rs: is the relative growth rate of *ws* (weight of stems);

Rh: is the relative growth rate of *wh* (weight of heads);

W: is the total dry weight of aboveground biomass, and *wl*, *ws*, *wh* in sum equaling *W*:

$$W = wl + ws + wh;$$

So, to recap, the sum of the relative growth rates of the weight of leaf, stem and heads, equals *Rw* (Relative growth rate of total dry aboveground plant):

$$Rw = \frac{Rl(wl)}{W} + \frac{Rs(ws)}{W} + \frac{Rc(wh)}{W}$$

Hunt, 1990

To calculate the RGR and the CPR, the method proposed by Hendrik Poorter in his 1989 work was used. The author proposed a "synthesis of the classical and the functional approach" as a method of calculating the relative growth rates (RGR).

The method allows the fitting of a polynomial through the relative growth rate value. RGRs were calculated with the classical approach instead of the ln-transformed plant weights as in the functional method.

Variability caused by the size of the sample collected and by the heterogeneity of the sample in the classical approach was rejected through an additional step in the calculation.

In order to improve the classical approach, the additional step reduces the difference between the sample mean and the population mean (Poorter, 1989), which is only one of the many phases of improvement of the classical method.

Essentially, in this way the RGR was not calculated with consecutive values from adjacent harvests but by skipping one harvest each time.

The first harvest was compared with the third rather than second, the second with the fourth instead of the third etc.. The two or three values obtained for each day were averaged (Wickens and Cheeseman, 1988), obtaining only one daily representative value.

The use of this combination of classical and functional approaches in the growth analysis was aimed at minimizing their mistakes.

The classical method may result in an overestimation of the variances of the growth rates (Causton, 1994); the functional approach in the other hand has some problems related to the choice of the appropriate function (Hunt, 1982; Poorter, 1989).

All growth rates were expressed as $\text{g g}^{-1} \text{ day}^{-1}$

c. BIOMETRIC CHARACTERIZATION

At the flowering stage (65BBCH code), i.e. when for each sampled plant 50% of heads in blossom were observed, plant height, number of heads per plant and number of offshoots per plant (only second year) were measured.

d. LEAF AREA INDEX

In each plant cut at ground level for destructive analysis, the photosynthetic leaf area was measured.

A LI-COR planimeter model LI-3000 (Li-Cor, Lincoln, Nebraska, USA) determined the photosynthetic leaf area.

The Leaf Area Index (LAI) was calculated by leaf area and by the ground surface from which the samples were taken.

In order to build the LAI curve, instantaneous values calculated as the mean of the time interval were used (see the method proposed by Wickens and Cheeseman, 1988).

e. TOTAL ABOVEGROUND BIOMASS YIELD AND ITS PARTITIONING

The harvested of aboveground biomass, capitulum enclosed, was carried out on September 1st, and September 5th 2013 when the plants had completely dried up. In both years, five plants standing in the middle row of each plot were harvested and leaves, heads and stalks were separately weighed.

The moisture content of biomass components (stalks, leaves and capitulum) was measured by weighing 200 g of plant material and placing it in a thermoventilated oven at 60°C until constant weight was reached.

Biomass production per plant were expressed as g plant⁻¹ of DM (dry matter) and the biomass yield as t ha⁻¹ of DM

f. GRAIN YIELD AND ITS PARTITIONING

At harvest, the heads were separated from leaves and stalks. The heads were immediately weighed to determine fresh weight and then were dried in oven at 60°C until constant weight, in order to determine their dry matter.

In the first year, all harvested heads were manually threshed to separate fruits (achenes), then they were weighed and counted.

In the second year instead, the number of heads increased significantly and therefore only a sample of 10 heads per plant were manually threshed.

Grain production per plant were expressed as g plant⁻¹ DM and grain yield as t ha⁻¹ DM.

2.2.4 Data analysis

Data were checked for homogeneity of variance and then analyzed using ANOVA (SAS version 9.02, 1999)

The statistical analysis was focused on the relating factors of the randomized block complete design with repeated measures in both years. All measured parameters were subjected to analyses of variance using the SAS procedure GLM (SAS Release 9.0, SAS Institute Inc., Cary, NC, USA). Treatment was considered as fixed factor, block as random factor. Means were statistically separated on the basis of Least Significant Difference (LSD) test, when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability level.

2.3 RESULTS AND DISCUSSION

2.3.1 Soil and weather conditions during the two years of interest

Meteorological trends for the two years of cultivation at the experimental site are shown in the Figure 8.

On average, seasonal maximum temperatures during the first growing cycle (2012/2013) were slightly lower than those of second cycle (2013/2014), 19.8°C and 20.4°C respectively.

Similarly, the minimum mean temperature during the second cycle was higher than the one observed during the first crop cycle (11.6°C vs. 10.8°C).

On the other hand, the crop year 2013/2014 experienced more rain than the first growth cycle (568.4 and 476.2 mm respectively).

In general, for the three climatic factor considered, the second growing year is closer to the trends observed over the last 60 years. (Figure 6).

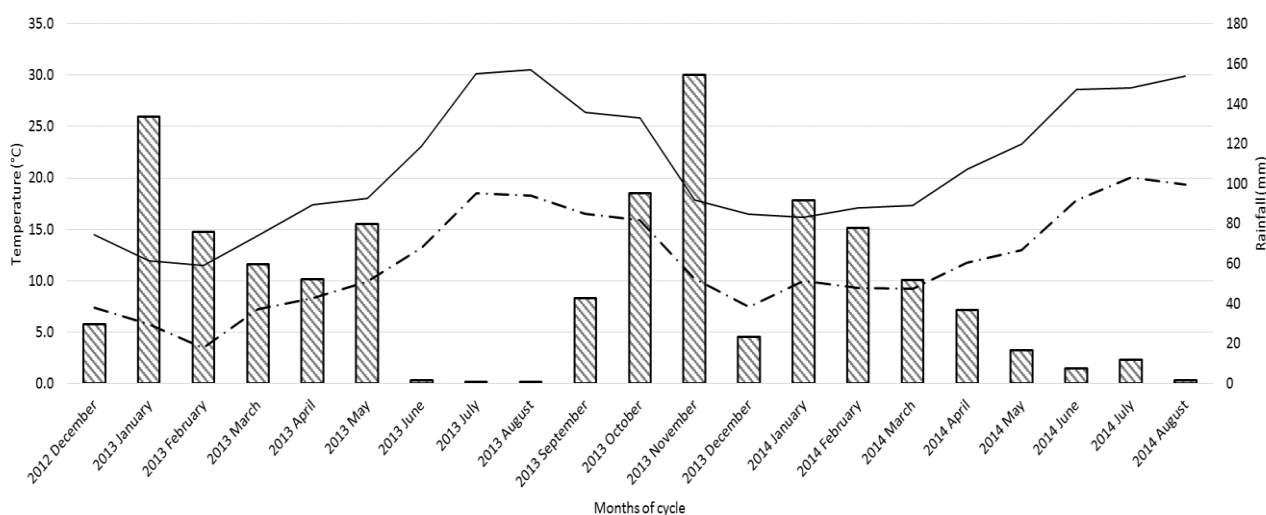


Figure 8: monthly maximum (solid line) and minimum (dashed line) mean temperatures and rainfall (bar chart) during the two years of cultivation at the experimental site.

The overall minimum temperature was reached in January of the first cycle (-2°C), and the same month was also the wettest with about 134mm of rain; the highest temperature recorded was 39.5°C in August 2013.

During the second growing season, instead, the lowest value of temperature was reached in November 2013 (2°C), the highest value in July 2014 (37.3°C) and, the highest monthly value of rainfall was recorded in January 2014 (91.6 mm) (Figure 8 and Table 15)

Table 15: Extreme values of temperature for the two growing seasons (maximum and minimum).

<i>Month</i>	<i>2012-2013</i>	<i>2013-2014</i>
Maximum air temperature (C°)		
October		31
November		25
December	18	19
January	18	19
February	16	24.1
March	17	24.1
April	27	24.9
May	23	32.5
June	30	36
July	39	37.3
August	39.5	33.2
September	32	
Minimum air temperature (°C)		
October		11.5
November		2
December	3	4
January	-2	7
February	-1	6
March	0	5.5
April	4	6.8
May	6	9.5
June	9	12
July	16	16.8
August	15.6	15.5
September	13	

The soil analyses were conducted by the Department of Agricultural Laboratories, and the physical and chemical characteristics of the soil at the beginning of the experiment (December 2012), for different depths, are presented in Table 16 and 17.

Table 16: Soil physical properties of the experimental field (Ottava, Italy, 41° N, 9° E).

	Depth (cm)		
	0-15	15-30	30-45
Physical properties			
Stones (%)	2.5	1.9	2.1
Sand (%)	63.4	64.7	63.5
Silt (%)	11.9	11.5	11.2
Clay (%)	22.2	21.9	22

Table 17: Soil chemical properties of the experimental field (Ottava, Italy, 41° N, 9° E).

Chemical properties	Depth (cm)		
	0-15	15-30	30-45
pH	8.25	8.35	8.30
Total N (‰)	1.45	1.4	1.35
Available P ₂ O ₅ , Olsen (ppm)	32.75	24.75	25.25
K ₂ O for exchange (ppm)	331.3	306.9	278.6
Total limestone (%)	4.3	6.3	4.8
Active limestone (%)	-	-	-
CSC (meq/100 g)	24.5	24.7	24.5
ECe (dS m ⁻¹)	0.19	0.15	0.17
Assimilable P (Olsen, ppm)	32.75	24.75	25.25
Exchangeable K (ppm)	331.4	307.0	278.6
Ca (ppm)	3983.9	4140.3	4218.4
Mg (ppm)	125.2	125.2	125.2
Na (ppm)	99.4	93.7	93.7
Soil organic matter (%)	2.75	2.4	2.625
Organic C (%)	1.6	1.4	1.525

As reported in the table above, the soil at the site was sandy-clay-loam with a C/N ratio of 10.9, with a pH ranging between 8.25 and 8.35, with a percentage of organic carbon varying from 1.4 to 1.6%, nitrogen and phosphorus-Olsen ranging from 1.35 to 1.45 ppm and from 24.75 to 32.75 ppm respectively. For this last element, the significantly high value - especially in the upper layer - is most likely due to some fertilizer application.

2.3.2 Timing and duration of phenological phases

1 year of cycle (2012-2013):

The sowing was done on December 11th, 2012.

The germination of the seed ends when the cotyledons emerge through the soil surface and this process depends on temperature, soil moisture, sowing depth and sowing period (Archontoulis et al. 2010).

In December 2012 - January 2013, the minimum and maximum average temperatures in the experimental site were about 7° and 15°C respectively, the rainfall was about 27 mm.

In our conditions of temperature and moisture, this process lasted about 30 days for both thistle types analyzed.

This trend disagrees with the work presented by Archontoulis et al. (2010), which state that this phase is about 1–2 weeks for a spring sowing, and 1–2 months for a late autumn sowing or around 50–60° C-days (using a minimum threshold temperature of 10° C).

In the present study, the crop showed lower thermal requirements or, the minimum temperature threshold defined by these authors in this work was too high to calculate the GGD (Growing Degree Days).

For the crop in these specific conditions (and this minimum threshold temperature) only about 30°C- days were needed to end the germination of the seed.

Using instead a base temperature of 7.5 (Archontoulis et al., 2011; Viridis et al., 2009), the analyzed crops show a requirement of about 120 °C-days to complete the germination in this specific experimental site.

This first phenological stage did not show different timing between genotypes.

With regard to the second period, after *Cynara*'s germination from the seed, the starting dates of almost all phenological events were affected by the genotype type factor (Figure 9).

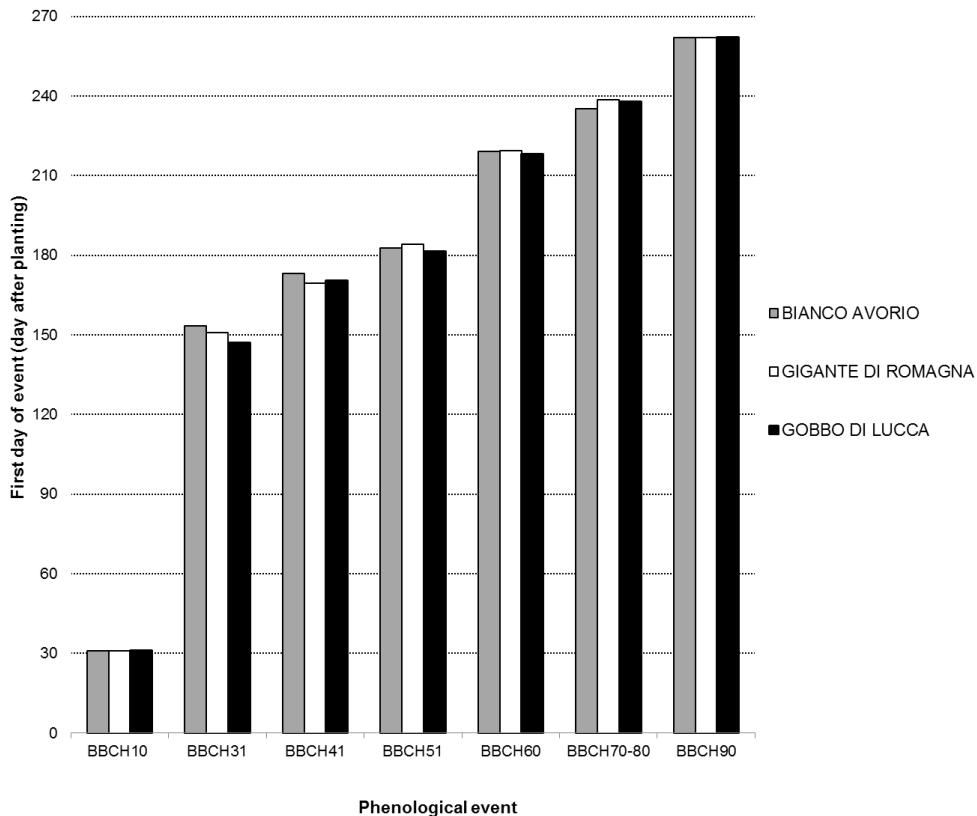


Figure 9: Timing of phenological phases (first growth year) for tree genotypes of *Cynara cardunculus* var. *altilis* (“Bianco Avorio”, “Gigante di Romagna” e “Gobbo di Lucca”): BBCH10, emergence of first leaf; BBCH31, beginning of the rosette growth (more than nine leaves); BBCH41, beginning biomass storage; BBCH51, beginning of stem elongation and inflorescence emergence; BBCH60, onset of flowering on main stem inflorescence; BBCH70-80, development of capitulum and seed ripening; BBCH90, full development and early senescence.

“Gobbo di Lucca” genotype was earlier than the others for BBCH31 and BBCH51 stages ($p < 0.0001$ and $p < 0.001$ respectively).

The genotype “Bianco Avorio” was earlier than the others only for the stage “development of capitulum and seed ripening” (BBCH70-80), ($p < 0.01$). The third genotype “Gigante di Romagna” never showed a highest precocity, except in the BBCH41 stage, where it was as advanced as the “Gobbo di Lucca” genotype ($p < 0.01$).

Regarding the first and last BBCH stage considered, these ones showed a very homogeneous timing among analyzed genotypes.

Figure 10 shows the duration of the vegetative growth for the three thistle genotypes.

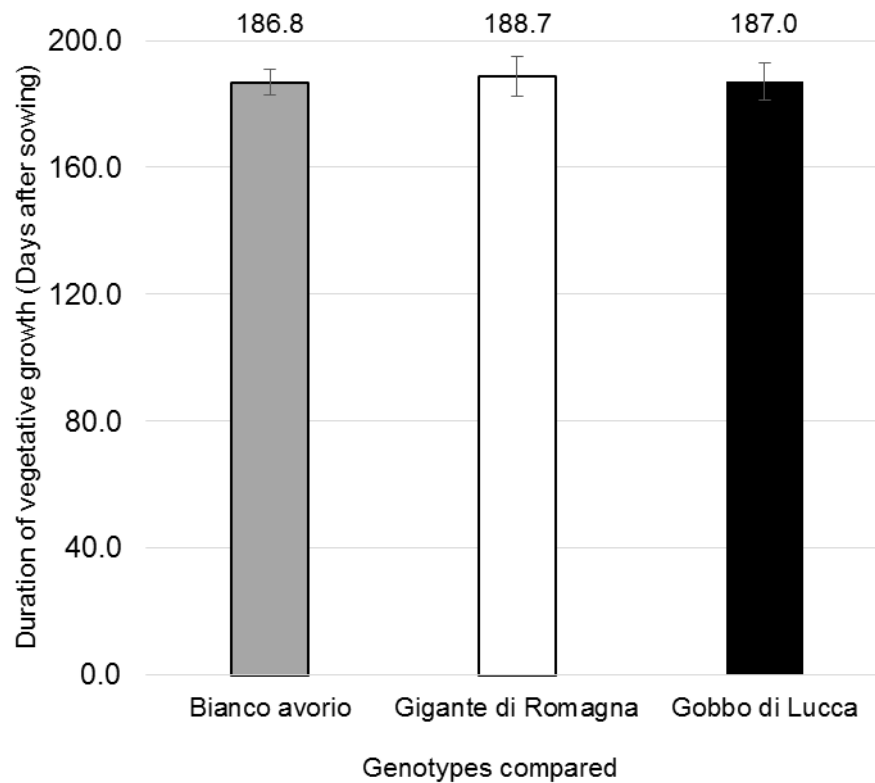


Figure 10: duration of vegetative growth stage in the first year of cycle (2012-2013).

Three principal phenological stages represented the vegetative growth in *Cynara*. These correspond to leaf development (BBCH10), rosette growth (BBCH30), development and storage of harvestable vegetative plant parts (BBCH40). At the end of this period, starts the reproductive development, until the physiological maturation.

As illustrated in figure 10, the vegetative development in the first year of cycle lasted 185.5 days on average.

This duration was not conditioned by genotype factor, in fact no significant differences in terms of days were observed ($p < 0.05$).

To analyze the length of the cycle, in relation to degree days needed to grow from stage BBCH 10 to BBCH 49, the present study affirms that the plants accumulated approximately 1100 °C days using a base temperature of 7,5°C.

This result is very different from the findings in Archontoulis et al. (2010 and 2011), where 650 °C days were counted to complete the vegetative growing (also excluding the germination from seeds).

The reproductive development includes the inflorescence emergence and its development (BBCH50), flowering (BBCH60), development of heads and achenes (BBCH70), seeds and inflorescence ripening (BBCH80), to end with the plant senescence. The duration of the reproductive growth during the first year of the experiment is represented in Figure 11.

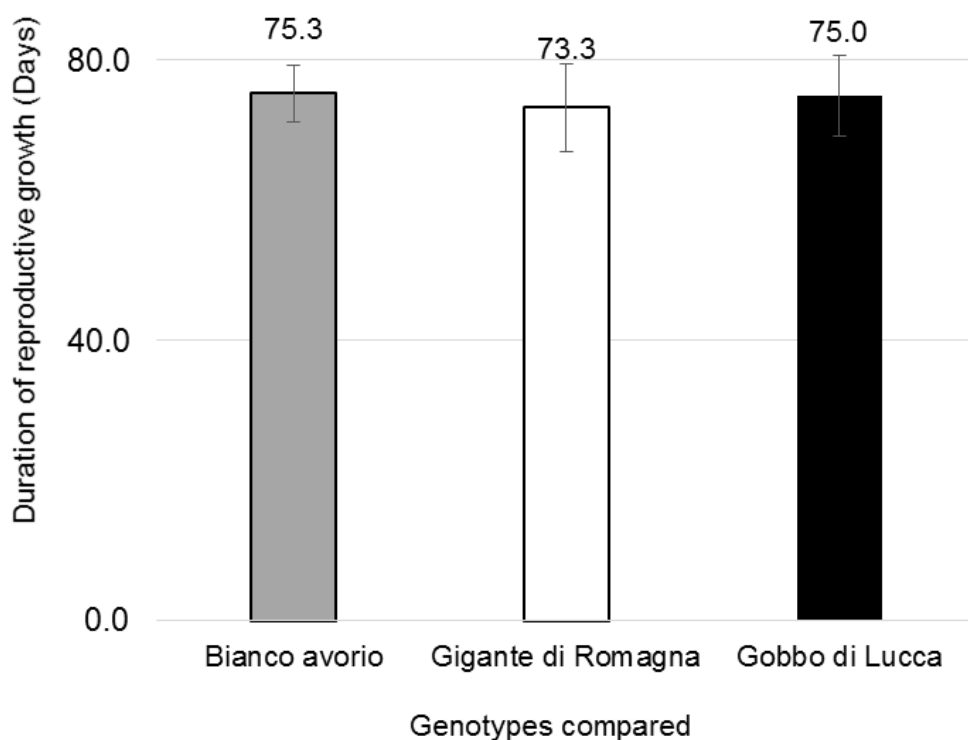


Figure 11: duration of the reproductive growth stage in the first year of cycle (2012-2013)

The duration of this long stage was calculated as the average of total days from the first day of BBCH50 stage and the harvest date (September 5, 2013). The latter should be considered the physiological maturity of the crop.

Also for the reproductive duration, there were no significant differences between studied genotypes.

To analyze the length of the cycle, in relation to degree days needed to grow from stage BBCH50 to BBCH97, the present study affirms that the plants accumulated approximately 850 °C days.

In addition, this latter value is different from of the one available in the literature: 1600°C days (Archontoulis et al. 2010, 2011).

II year of cycle (2013-2014)

The second growing season started after crop harvesting.

After the first rainfalls, regrowth starts from several vegetative buds positioned on the upper part of the root system.

The primary data, analyzed as described in Materials and Methods section, did not show any differences in relation to the first day of the phenological event.

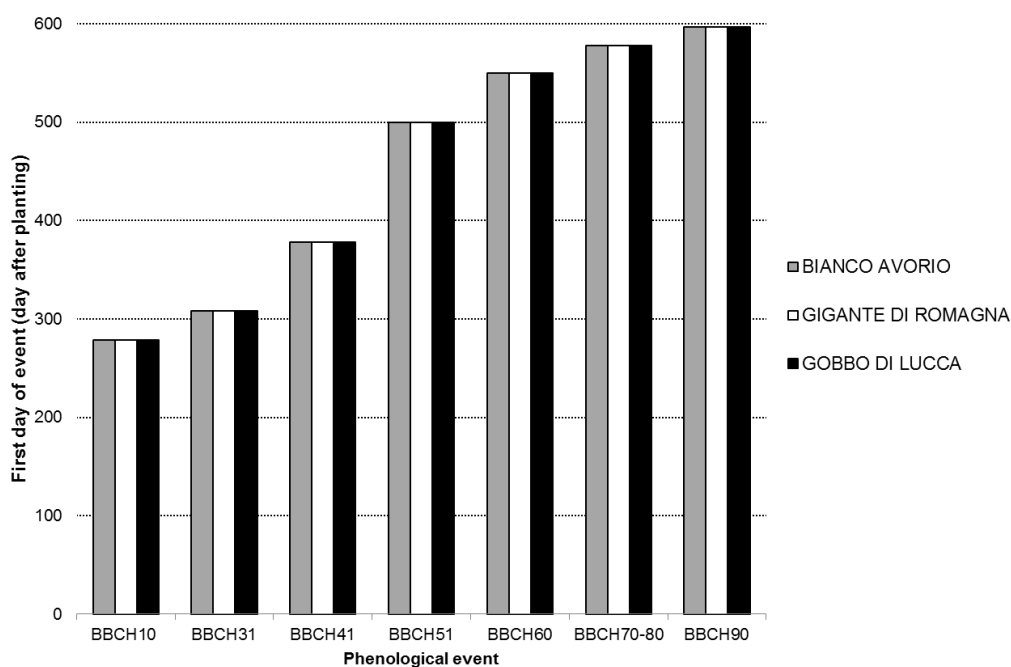


Figure 12: Timing of phenological phases (second year growth) for tree genotypes of *Cynara cardunculus* var. *altilis* (“Bianco Avorio”, “Gigante di Romagna” e “Gobbo di Lucca”): BBCH10, first leaf spread or separated; BBCH31, beginning of the rosette growth (more than nine leaves); BBCH41, beginning biomass storage; BBCH51, beginning of stem elongation and inflorescence emergence; BBCH60, onset of flowering on main stem inflorescence; BBCH70-80, development of capitulum and seed ripening; BBCH90, full development and early senescence.

As represented in Figure 12, all three genotypes observed in this research started the seven phenological phases considered at the same time.

We can observe that after an establishment year (implant), the culture becomes highly phenological homogeneous.

In the second year, the plants were composed by more offshoots, which significantly increased the number of samples on which to make observations. This fact helps the smoothing by increasing the probability of observing the same feature in more individuals.

Regarding the duration of the vegetative growth stage in the second year cycle, “Gigante di Romagna” showed a higher number of days than the other two ($p < 0.01$).

The little difference, but statistically significant, is shown in Figure 13

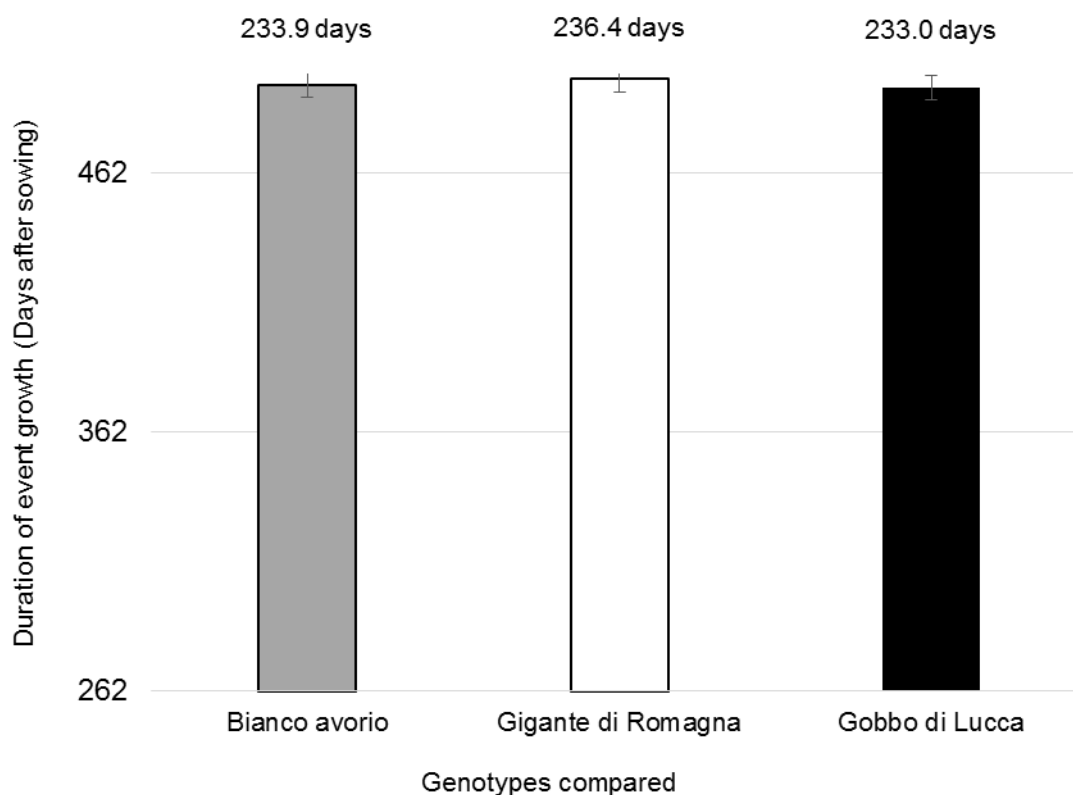


Figure 13: Duration of the vegetative growth stage in the second year of cycle (2013-2014). The count starts at the harvest day of the previous crop year (262 days after sowing). In the graph, in the Y-axis, the number of days refers to the entire duration of the experimental crop (two cycles). The numbers above the bars chart are relative only to the second crop growth.

During the second year of implant, the plants accumulated about 1700 °C days to complete the vegetative development. This count starts from the harvest in September 2013 until the beginning of BBCH51 stage.

In addition, if the count starts after the first rain (after one month) when the buds showed green tips, GDD (Growing Degree Days) is different from the values published in literature: 1400°C days instead of 650°C days established by Archontoulis et al., (2011).

As can be easily deduced from the previous results about the vegetative duration, also the graph in Figure 14 confirms that given the same total days, the genotype “Gigante di Romagna” presents the shortest reproductive phase ($p < 0.01$).

The duration of 95 days or 1300°C-days affirmed by Archontoulis et al., (2010, 2011) seems once again underestimated, compared to the observed values in our conditions.

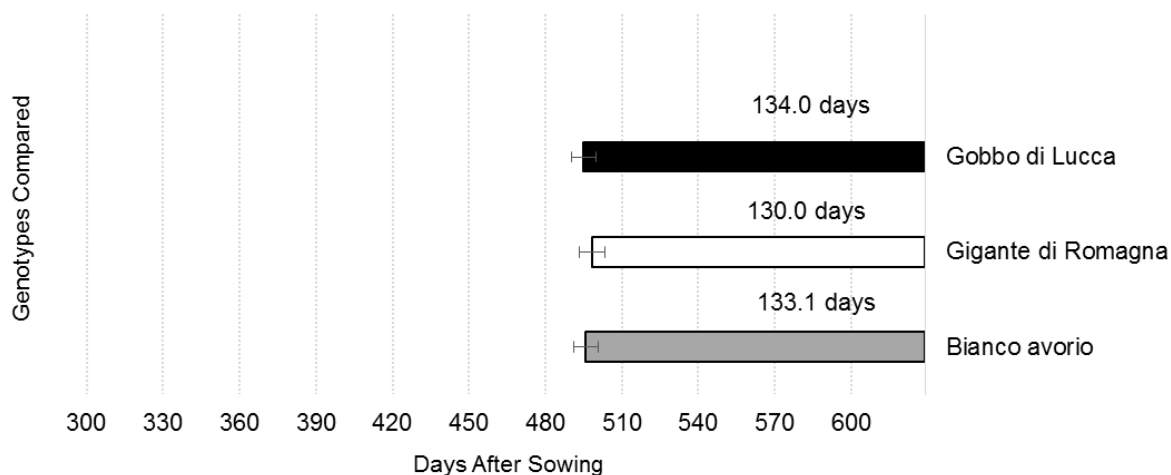


Figure 14: Duration of the reproductive growth stage in the second year of cycle (2013-2014). The graph shows the average duration of the reproductive phase compared to the total days of analysis. Total days were expressed as days after sowing (x-axis). Day number 629, is the harvest day (the same for all three genotypes)

Regarding the first and the second year of life cycle, the phenology of *Cynara cardunculus* var. *altilis* showed great variability.

The first year confirmed the characteristics of the stabilization period typical of the perennial crops.

Consequently, the phenological differences observed derived in a greater way from age of crop and "cultivation year" factor, that from the genotype type in comparison.

The differences identified with the data available in the literature, confirm that *Cynara* is a rather complex crop.

In the work cited above, there is a separation of phenological stages in time, closely linked to the seasons' change; in fact, for *Cynara*, the degree-days were further separated for the vegetative (autumn to spring) and the reproductive growth (spring to summer).

This structure is more suitable for the second year, in which, for example, flowering occurred in spring and seed ripening in summer.

This has not been observed in the first year, where the heads formation and flowering were both observed during the summer.

The GGD accounted for reproductive stages, but could have also been affected by the harvest timeliness.

However, we can confirm a substantial difference with the literature data above mentioned.

2.3.3 Biometric characterization

The following results on biometric traits were elaborated with data recorded in the flowering stage for both seasons of crop (Table 18).

Not all considered characteristics were affected by genotype.

Regarding the plants height, it changed with age and was lower in the first year and higher in the following year. This fact is in accordance with the usual trend of perennial plants.

Table 18: Biometric parameters recorded at the flowering phenological stage of cardoon (BBCH60) as affected by genotype.

<i>Factor of variation: Genotype</i>		<i>Height (cm)</i>	<i>Offshoots (no. plant⁻¹)</i>	<i>Heads (no. plant⁻¹)</i>
I YEAR	BIANCO AVORIO	87.1 a	1	8.0 a
	GIGANTE DI ROMAGNA	80.1 b	1	6.6 b
	GOBBO DI LUCCA	69.7 b	1	7.1 b
Mean I year		78.97		7.23
II YEAR	BIANCO AVORIO	246.3 a	2.7	18.3 b
	GIGANTE DI ROMAGNA	226.3 b	2.8	19.8 a
	GOBBO DI LUCCA	223.8 b	2.7	19.5 ab
Mean II year		232.13	2.7	19.20

Mean values followed by a different letter are significantly different ($P < 0.05$).

In both seasons, the plants height was affected by genotype. Always, the "Bianco Avorio" was the highest compared to the other two.

These results are in disagreement with those published by Piscioneri et al., (2000) about the genotypes "Bianco Avorio" and "Gigante di Romagna" in Matera, which indicated a height slightly lower for both crops: 63 cm and 73 cm respectively for the first year and 195 cm and 210 cm respectively, for the second season growth.

The observed values are different, although the crop management and climatic trend were very similar.

Indeed, in Matera as well, the sowing was done with a density of 1 plant/m, over the winter and without water supply during the whole experimental period. Only the fertilizer applications were more abundant than in the presented study, but it seems that this practice did not condition the plants' size.

The number of flower heads per cardoon plants was affected by the genotype factor in both years. Obviously, the values were much higher during the second cycle.

In the first year, “Bianco Avorio” produced a higher number of flower heads (at flowering time) as opposite to the second season in which it produced fewer.

2.3.4 *Leaf area index*

LAI is an index of the crop leafiness. It is obtained as a derived quantity of the sequential measurements on green leaf area. D.A. Watson devised it in 1947, to reported the average number of complete layers of leaf material displayed by the crop. The index is calculated as a simple ratio, which allows an instantaneous evaluation without recurring to fitted growth curves (Hunt, 1990).

First year cycle (2012/2013):

The curves obtained elaborating the primary data recorded in the first year of cultivation and for all the thistle genotypes studied, are reported in Figure 15, 16 and 17

In particular, Leaf Area Index (LAI), or total photosynthetic leaf area per crop, is plotted against the time. This way, the area below the curves represents the crop's 'whole opportunity for assimilation' (Watson, 1947), and it is called "Leaf Area Duration" (LDA), as it was constructed through a series of leaf area measurements. The "LDA" considers not only the leaf area quantity, but also how long it persists.

LAD is therefore an index expressed per crop and per season.

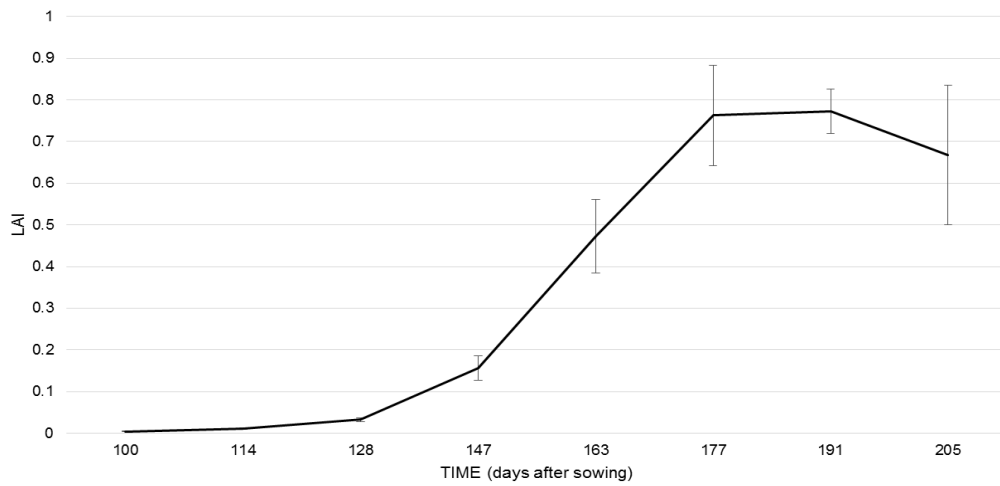


Figure 15: LAD (Leaf Area Duration) in “Bianco Avorio” genotype, first crop year (2012/2013).

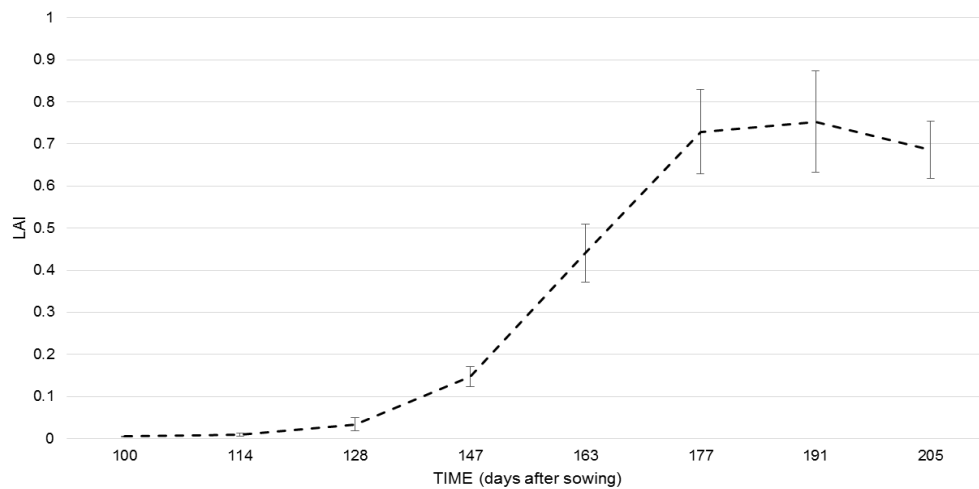


Figure 16: LAD (Leaf Area Duration) in “Gigante di Romagna” genotype, first crop year (2012/2013).

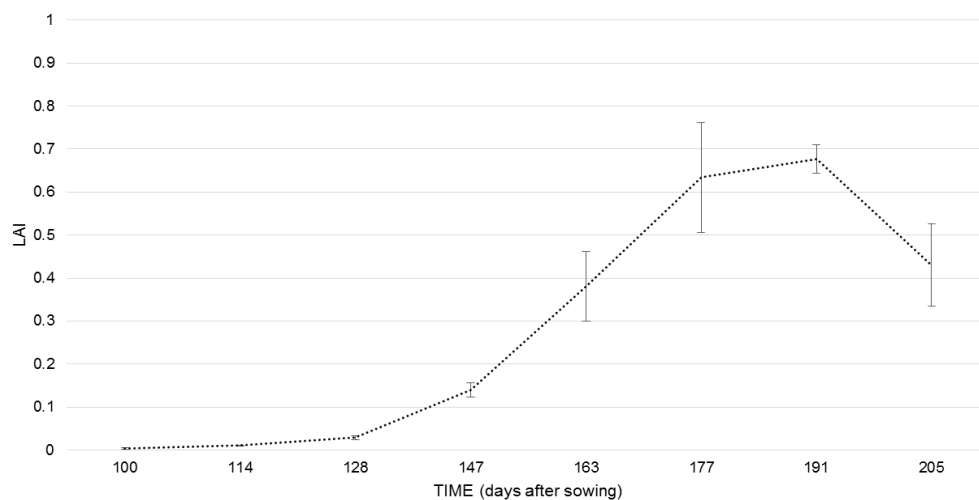


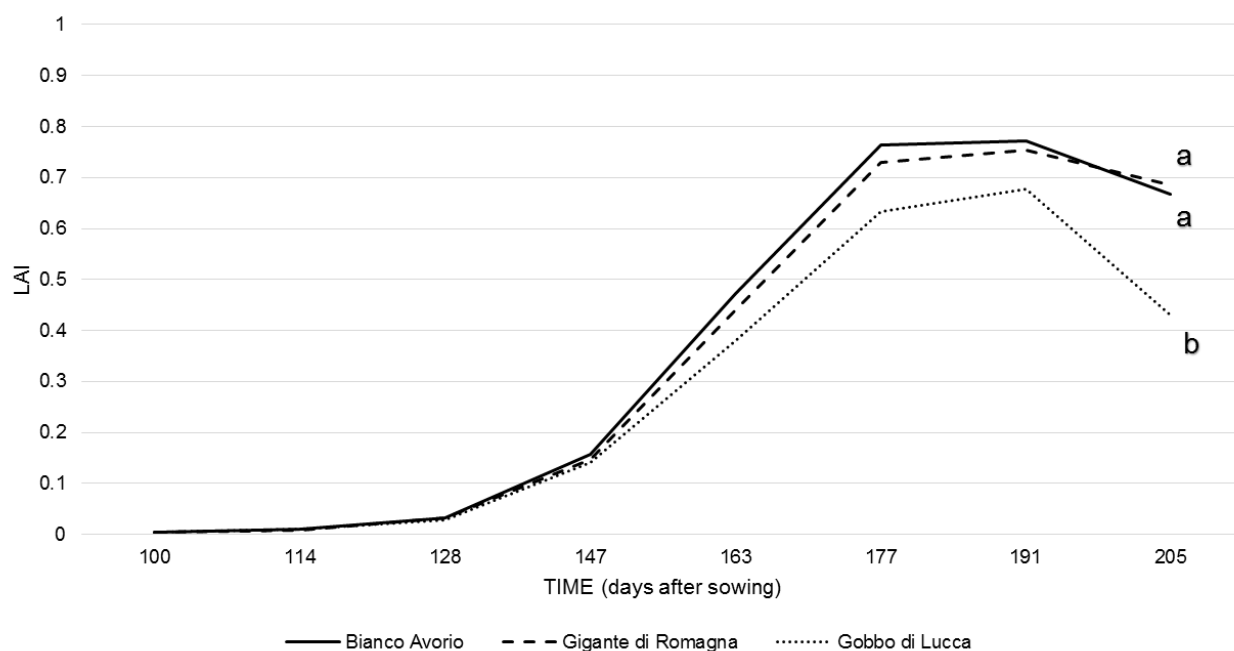
Figure 17: LAD (Leaf Area Duration) in “Gobbo di Lucca” genotype, first crop year (2012/2013).

In all graphs above, it can be observed the same trend of LAI over time.

The smallest values were recorded at emergence stage, about one month after sowing (in January), and the biggest dimensions in the June period, when it was observed the transition from vegetative development to reproductive stage (around the BBCH50 stage).

After these relative high values of the Leaf Area Index, the decreasing trend derived from the progressive senescence of leaves. The duration of this process is in accordance with the timing described by Archotouilus et al. (2010). They affirmed that the plant senescence usually takes place from mid-July and continues during August. This fact results in a steep negative slope of the curve, after the maximum value.

The Figure 18 immediately shows the comparison of the total photosynthetic leaf area by Genotypes of cardoon analyzed in this work.



Different letters indicate mean values significantly different ($P < 0.05$)

Figure 18: LAD (Leaf Area Duration) for three genotype of cardoon, first crop year (2012/2013).

The three curves show the same trend over time. This fact indicates same timing of leaf area development for the three genotypes considered in this research.

In addition, these results show also some differences on the value of LAI achieved in the last measurements.

In particular, the different genotypes do not exhibit significantly different maximum values.

The significant difference between mean values ($P < 0.05$) was observed last day only.

Therefore, when the values of the leaf area were already in decline, the "Gobbo di Lucca" genotype decreased fastest than the other two.

In terms of LAD, the photosynthetic leaf area of the genotypes "Bianco avorio" and "Gigante di Romagna" showed a greater durability, and also persistence in the field and a greater opportunity for assimilation. This fact can be deduced from the greater area under the corresponding curves.

Second year cycle (2013/2014)

The curves obtained elaborating the primary data recorded in the second year of crop are represented in Figure 19, 20 and 21.

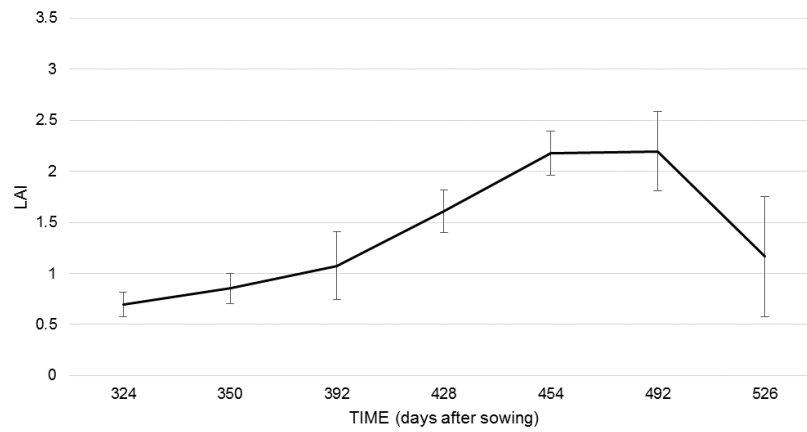


Figure 19: LAD (Leaf Area Duration) in “Bianco Avorio” genotype, second crop year (2013/2014).

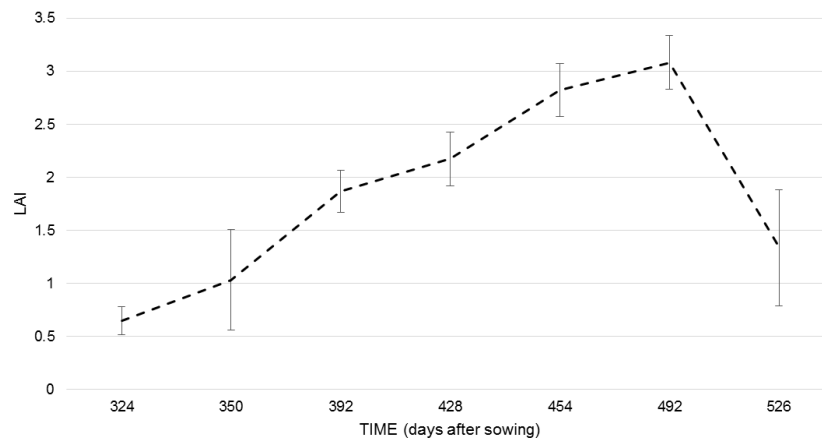


Figure 20: LAD (Leaf Area Duration) in “Gigante di Romagna” genotype, second crop year (2013/2014).

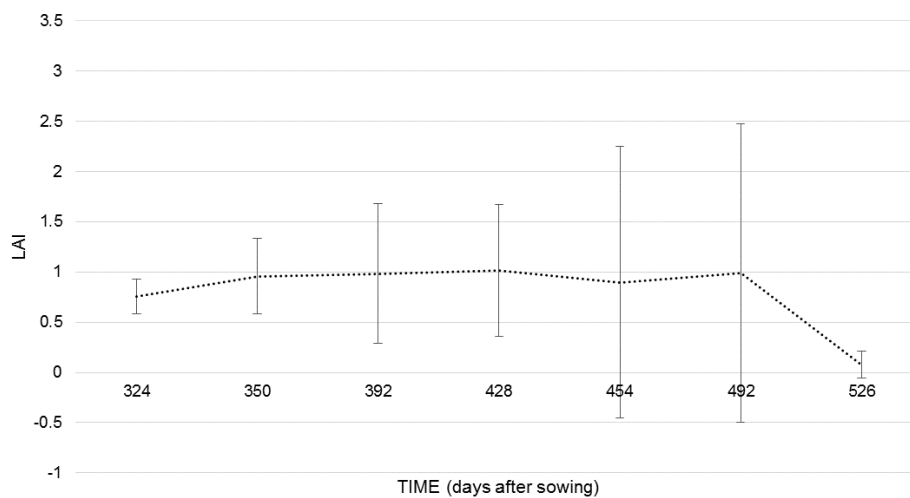


Figure 21: LAD (Leaf Area Duration) in “Gobbo di Lucca” genotype, second crop year (2013/2014).

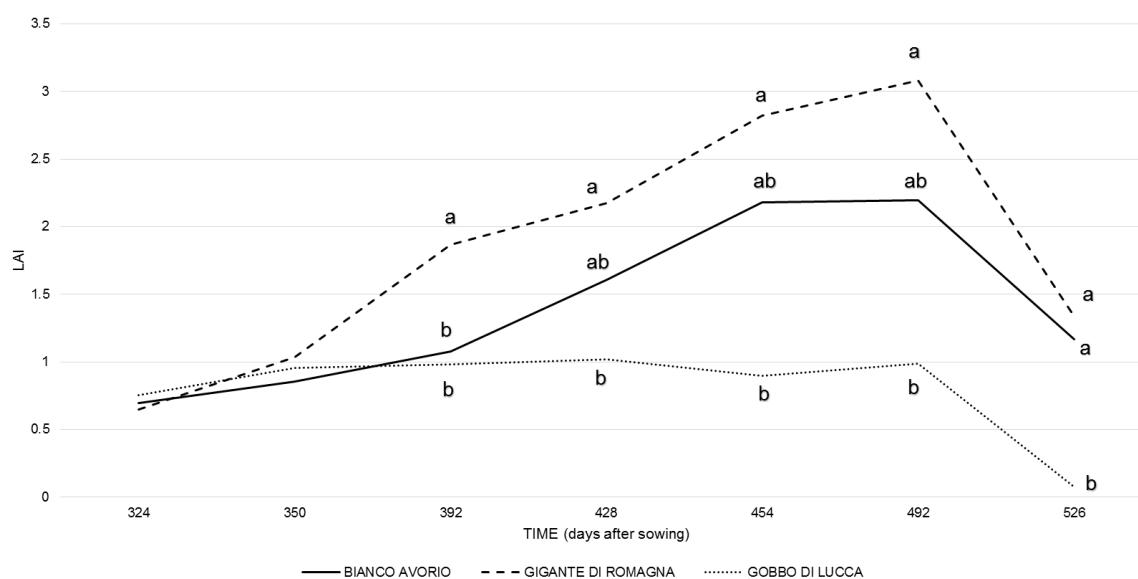
Also during the second growing season, all studied genotypes showed similar LAI trend. The highest value was recorded in the time interval around phenological stage BBCH50.

With the progress of the reproductive season, the leaf area value strongly decreased.

In this second experimental season, this event was observed in the month of April, in the middle of spring.

This temporal result is in disagreement with Archotouilus et al. (2010), as in our case, the senescence starts much earlier than summer.

The following Figure 22 highlights the differences between studied genotypes.



Different letters indicate mean values significantly different ($P < 0.05$)

Figure 22: LAD (Leaf Area Duration) in three genotypes of cardoon, second crop year (2013/2014).

The general trend was the same as during the first cycle, but during the second season the differences in leaves' area size were more evident.

These differences were observed from the third measurement onwards.

In all dates "Gigante di Romagna" proved to make a greater leaf area, in some cases double and triple than "Gobbo di Lucca".

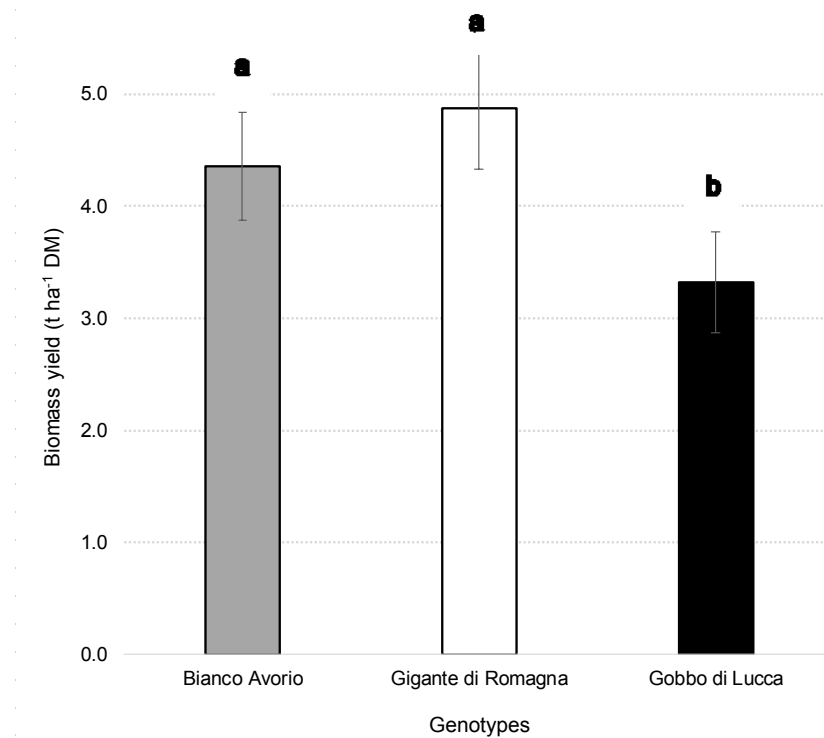
For instance, in the same date (492 days after sowing), all genotypes showed the maximum value of LAI, but for "Gobbo di Lucca" this value was three times smaller than the highest.

On the other hand, the cultivated cardoon genotype "Bianco Avorio", maintained an intermediate behavior during the whole second season.

2.3.5 Total aboveground biomass yield and its partitioning

First year cycle (2012/2013)

The following histogram (Figure 23) exhibits the total aboveground biomass produced by the cardoon genotypes in the first year of cultivation at the end of the first growth cycle.



Different letters indicate mean values significantly different ($P < 0.05$)

Figure 23: Total aboveground biomass yield. Crop season considered 2012/2013.

The different *Cynara cardunculus* genotypes, in the first year of growth, were not established yet and they showed poor growth in term of aboveground biomass stored.

The biomass yield was evaluated through the data of the latest harvest, and its values ranged from 3 to over 4 t ha⁻¹.

The production of "Gobbo di Lucca" genotype, was significantly lower than the others two.

The present production is in disagreement with Piscioneri et al., (2000).

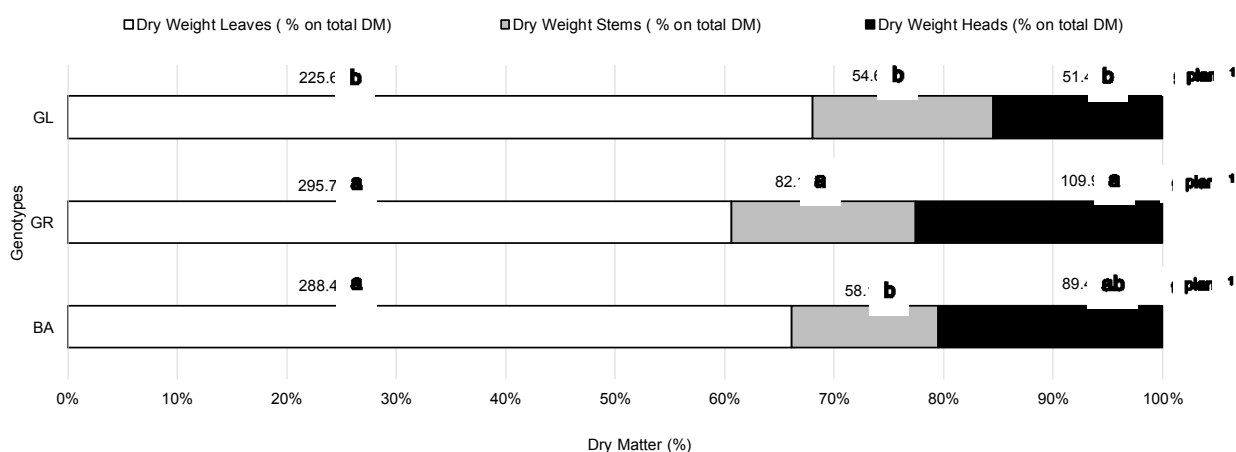
According to these authors, the total dry biomass in Bianco Avorio and Gigante di Romagna genotypes was around 1 t ha⁻¹ DM. This value was obtained in a very similar crop management and same sowing period.

We can easily see in the graph in Figure 23, the differences in the dry weight of total biomass observed in the present work.

Under the conditions of the work of those authors, the total dry biomass is underestimated compared to the one observed in our conditions.

Conversely, under the conditions of the work of Raccuia and Melilli (2007), the total dry biomass is very overestimated compared to the one observed in our conditions (10,7 t ha⁻¹ in BA genotype).

The Figure 24 shows the total biomass yield and its partition, with relative percentage, in the main plant parts (stems, heads and leaves) at the end of the first experimental year.



Different letters indicate mean values significantly different ($P < 0.05$)

Figure 24: Total aboveground biomass and its partition in leaves, heads and stems. The values are expressed in percent (bar chart) and in grams of dry matter per plant (above the bars). Crop season considered 2012/2013.

In this Figure, in general we can notice that the maximum dry biomass percentage is positioned in the leaves, followed by heads and stems.

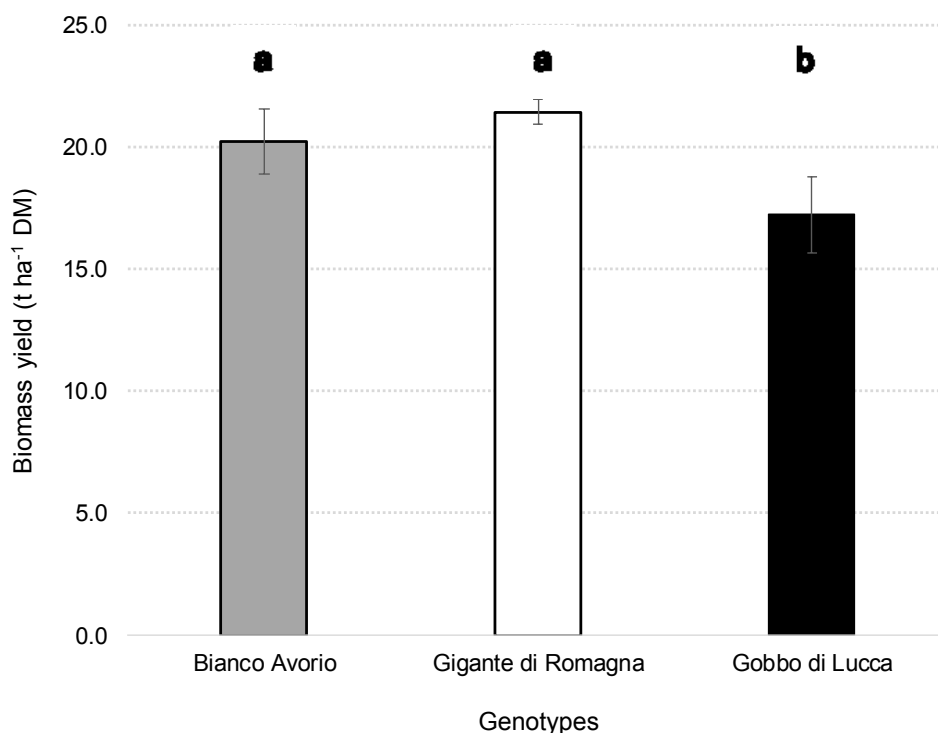
Among the three genotypes, the most productive “Gigante di Romagna” showed a greater partition of biomass over the heads and the stems. These ones typically have a higher dry weight than the leaves: this higher partition percentage favored the largest total weight.

Second year cycle (2013/2014)

In general, the good dry biomass yields obtained during the second year ranged from 18 to 23 t ha⁻¹ of dry matter. (Figure 25)

The productions obtained from the different genotypes were significantly different:

“Gigante di Romagna” followed by “Bianco Avorio” (but without statistical differences) were the most productive genotypes with regard to their total dry biomass production.

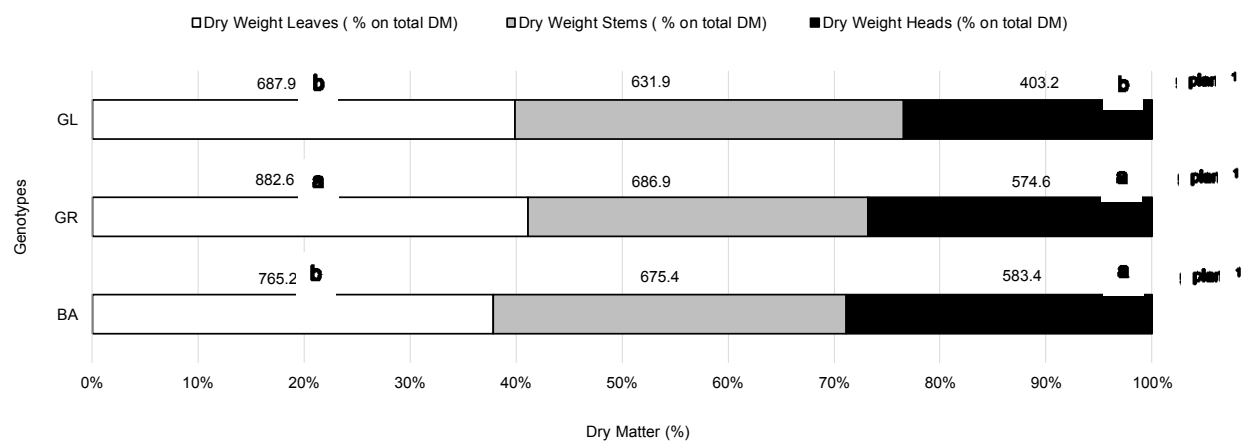


Different letters indicate mean values significantly different ($P < 0.05$)

Figure 25: Total aboveground biomass yield. Crop season considered 2013/2014.

In the contrast to the values of the first year, under the conditions of the work of Raccuia and Melilli (2007), the total dry biomass is underestimated, compared to the one observed in our conditions (18,7 t ha⁻¹ in BA genotype).

The biomass yield and its subdivision, with relative percentage, in three plant parts (stems, heads and leaves) at the end of the experimental period are reported in Figure 26.



Different letters indicate mean values significantly different ($P < 0.05$)

Figure 26: Total aboveground biomass and its partition in leaves, heads and stems. The values are expressed in percent (bar chart) and in grams of dry matter per plant (above the bars). Crop season considered 2013/2014.

In this figure, in general, one can note that the maximum dry biomass percentage is in the stems, followed by heads and leaves (very different situation from the first year).

It is important to notice the distribution of biomass yield components in the genotype more productive "GR": the percentage of leaves was higher than the other two, while maintaining a high weight of heads statistically similar to that of "BA".

This result is related to those obtained on the distribution of LAI (Figure 22). Indeed, "GR" showed in the second year the largest leaf area.

The partition towards the leaves did not penalize the weight percentage of the heads, actually, it was definitely physiologically facilitated (as confirmed later in the analysis of the seed productivity).

The present subdivision is in disagreement with Raccuia and Melilli, (2007).

According to these authors, in a total of about 2.400 g plant of dry aboveground biomass, 38% were heads and 62% were stalks and leaves. In the present study, a typical plant at its second year represents the authors' exercise.

We can easily see in the graph in Figure 26 the differences in the dry weight distribution, observed in the present work.

Under the experimental conditions of the work of those authors, the percentage by weight of the heads is overestimated compared to the one observed in our conditions.

Perhaps, the different techniques of crop management caused these variations.

One among many, the use of one irrigation per year at flowering stage, which in the second year was summed up to a rainfall higher than ours in the same growth season.

It can be hypothesized that the water availability helped the allocation to the heads.

The results published by Ierna et. al., (2012) allow us the comparison with mean values evaluated over three cultivation years in Catania.

Regarding the BA genotype, these authors evaluated the partition percentages as 44%, 29% and 27% for leaves, stalks and heads respectively.

For the GL genotype, instead, the values were 41%, 33%, and 26%.

Considering our average values for the two years, for both genotypes, the mean results are in line with our findings.

In general, it can be concluded that the genotypes “Gigante di Romagna” and “Bianco Avorio” appear to be the most productive for all the two years in regard to their total biomass production.

In the second year, the three genotypes were established and all showed good biomass production.

2.3.6 Grain yield and its components

The following Tables (19 and 20) list the total grain produced by the three cardoon genotypes in the first and second year of cultivation. The tables also describe the yield components.

Table 19: Grain yield (t ha^{-1}) and its components in different genotypes of *Cynara cardunculus*. Crop season 2012/2013.

<i>First Year Crop</i>	Cardoon Genotype		
	BA	GR	GL
Total Heads (N° plant ⁻¹)	10.2	8.3	8.3
Fertile Flower Heads (N° plant ⁻¹)	3.8 a	4.1 a	2.4 b
Dry Weight Fertile Heads (g head ⁻¹ DM)	30.6	27.6	25.8
Grains Weight (g head ⁻¹ DM)	5.60 a	3.78 b	2.18 c
Grain Yield (t ha ⁻¹ DM)	0.20 a	0.16 a	0.037 b
Number of seeds (N° heads ⁻¹)	216.9 a	136.2 b	113.6 b
1000 Seeds Weight (g)	29.3 a	28.5 a	25 b
Harvest Index	0.05	0.04	0.01

Different letters indicate mean values significantly different ($P < 0.05$)

The results show a very low grain yield, in line with all the components. Therefore, also these data - as those relative to the biomass yield - confirmed that the different *Cynara cardunculus* genotypes, in the first year of growth, were not established yet and they showed poor growth in term of reproductive components.

Among the three, in all variables, the BA genotype - and in some cases GR - showed higher values than GL genotype. In particular, BA was significantly higher than the other two for dry weight of achenes and the number of achenes per fertile head. On the contrary, BA was not different from GR for the number of fertile heads per plant, for the grain yield per hectare and for the 1000 seeds weight.

In all measured variables, the GL genotype was the less productive than the others.

The total grain yield value ranged from 0.16 t ha⁻¹ to 0.037 t ha⁻¹ for BA and GL respectively.

The very small Harvest Index is the further confirmation of a low ability to produce a good grain yield.

Table 20: Grain yield ($t\ ha^{-1}$) and its components in different genotypes of *Cynara cardunculus*. Crop season 2013/2014.

<i>Second Year Crop</i>	Cardoon Genotype		
	BA	GR	GL
Total Heads (N° plant ⁻¹)	25.1	24.6	24.0
Fertile Flower Heads (N° plant ⁻¹)	19.6	21.5	21.5
Dry Weight Fertile Heads (g head ⁻¹ DM)	25.8 b	34.9 a	24.4 b
Grains Weight (g head ⁻¹ DM)	3.83 b	6.27 a	3.27 b
Grain Yield (t ha ⁻¹ DM)	0.75 b	1.28 a	0.65 b
Number of seeds (N° heads ⁻¹)	129.8 b	190.4 a	123.7 b
1000 Seeds Weight (g)	34.2 a	36.8 a	28.1 b
Harvest Index	0.32	0.63	0.35

Different letters indicate mean values significantly different ($P < 0.05$)

By the analysis of the two previous tables, we can easily identify a greater production in the second year than in the first one. Indeed, all the analyzed variables showed a good increase over the first year.

In the second cycle, the cardoon genotype GR was the most productive with regard to all the analyzed factors. The others two showed a similar and less productive behavior.

The total grain yield value ranged from $1.28\ t\ ha^{-1}$ to $0.65\ t\ ha^{-1}$ for GR and GL respectively. The Harvest Index of the second year showed the improved ability to produce grain yield. This event is much more marked for GR in particular: HI 0.63.

The Table 21 shows some results observed in literature, with the aim to simplify the general discussion and the comparison of the results.

Table 21: some values reported in literature of *C. cardunculus* var. *altilis* DC. reported by several authors in similar environments to the trial site.

Genotype	Grain Yield (t ha ⁻¹ DM)	1000-seed weight (g)	Total Heads (n.plant ⁻¹)	Dry Weight Heads (g head ⁻¹ DM)	Year	References	Location Trial	Special features
BA	1.23	41.0 g			I	Raccuia and Melilli., 2007	Sicily, Catania	Transplant late August (20.000 plant ha ⁻¹)
	0.90	36.5 g			II			Low input
	2.5	26 g			I			Transplant in July 11.900 plants ha ⁻¹
	2.2	25 g			II			
			38.2	455	II			
GL	2.8	34 g			II	Foti et al., 1999	Sicily, Catania	Irrigation; fertilization, Weed and pest control.
			34.5	474	II			High input
GR	139 (g plant ⁻¹)	34 g			I	Ierna et al., 2010	Sicily, Catania	Transplant in Autumn 12.500 plants ha ⁻¹
	124 (g plant ⁻¹)	31 g			II			2 irrigations in April
								Low input

With the analysis of the table above, one can easily see that all values found in literature were higher than ours.

Regarding the three different genotype, it seems that all studies overestimated all features.

This fact, for Foti et al. (1999), was deductible by experimental conditions with very high input management (as for the artichoke), but the results were in strong contrast with the other two experiments.

Raccuia and Melilli (2007), and Ierna et al. (2012) experimented in fact with a low profile management input and their result still show a very high performance. The higher sowing density does not balance this difference, in fact does not explain the higher number and weight of the heads per plant. Relatively to this last feature, Foti et al. (1999) on high management input, found weights per head 10 times greater than in this work.

2.3.7 Evaluation of the crop efficiency

This section presents the results concerning the analysis of physiological and productive efficiency, for the three thistle genotypes.

The crop efficiency was evaluated using growth rates: initially the total dry weight was analyzed (RGR, Relative Growth Rate), as sum of its components dry weight (CPR, Component Production Rate), then, the total growth curves for all genotypes were derived;

- RGR and CPR (Relative Growth Rates and their Components Production Rates)

Using a methodology proposed by Poorter in 1989, it was possible to delineate the curves that best fit the RGRs trend in the first and in the second growing season.

As explained in the previous section, the total dry weight is the sum of the weights from all the different aboveground biomass components: leaf, heads and stems.

On the same way, the total Relative Growth Rate for each genotype or plant in general, is the sum of production rate components, expressed per unit of total dry weight.

In the following Figures (27-29), we can see that these rates sum up to a curve that describes the relative growth rate in dry weight of the whole plant.

First year cycle (2012/2013)

Figure 27: Contribution of CPRs to RGR. The curves for production rate component in the different organs of BA genotype: leaves (red), heads (blue), stems (green). First crop year (2012/2013)

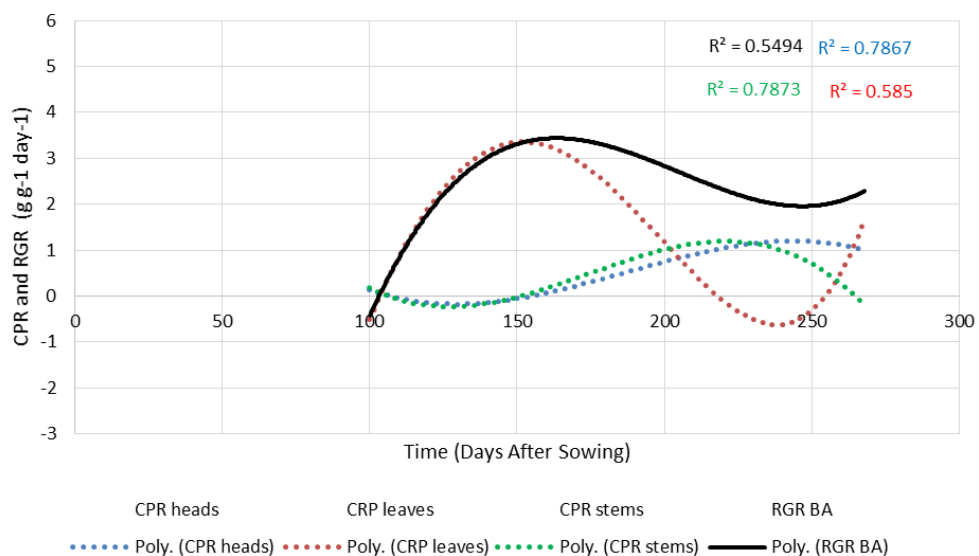


Figure 28: Contribution of CPRs to RGR. The curves for production rate component in the different organs of GR genotype: leaves (red), heads (blue), stems (green). First crop year (2012/2013)

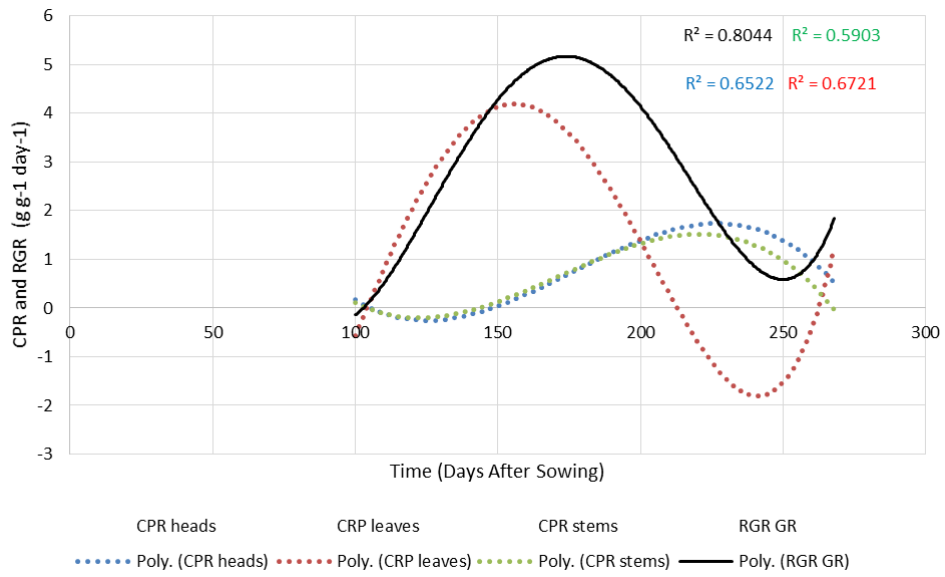
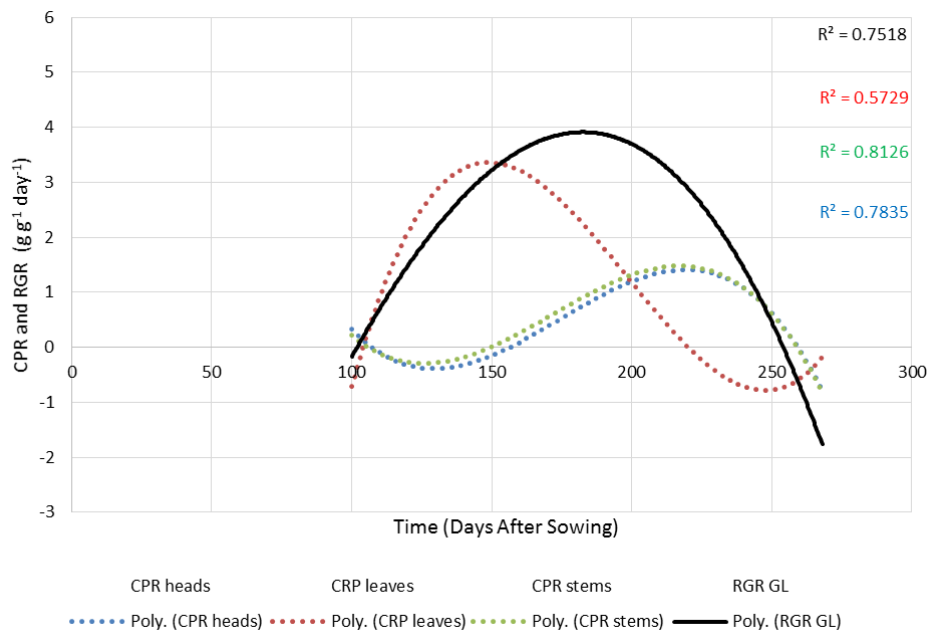


Figure 29: Contribution of CPRs to RGR. The curves for production rate component in the different organs of GL genotype: leaves (red), heads (blue), stems (green). First crop year (2012/2013)



The total RGR (black line) represents the sum of the effects of the different biomass components.

In the previous graphs, we can easily see, that the contribution of the different components varies over time, and therefore in the different physiological stages.

During early vegetative growth, the total RGR was mostly dominated by the leaf production rate.

Later, at the beginning of the early reproductive stage, "CPR leaves" exceeds the tip and begins to drop. From this level, the growth rate depends on stems and heads production rate, in a very similar way for the two components and in all three genotypes.

It can be observed the little difference in the final trend of RGR for the GL genotype. At the end of the season we do not observe the new ascent as in the other two treatments.

Probably, the stems and heads CPRs are not enough to balance the decrease of leaf CPR.

To confirm this, the Gobbo di Lucca genotype was the less productive, in all the analyses.

This fact has determined a decrease in trend of the total biomass dynamic (see Figure 32).

The instantaneous values of the different rates illustrated in the figures above permitted the representation of the biomass dynamic over the first year of cultivation (Figure 30-32).

The graphs below represent the biomass dynamics relative to the total aboveground dry weight and the dry weight of the components.

Figure 30: Biomass dynamics and its components in BA genotype. First year of cycle (2012/2013)

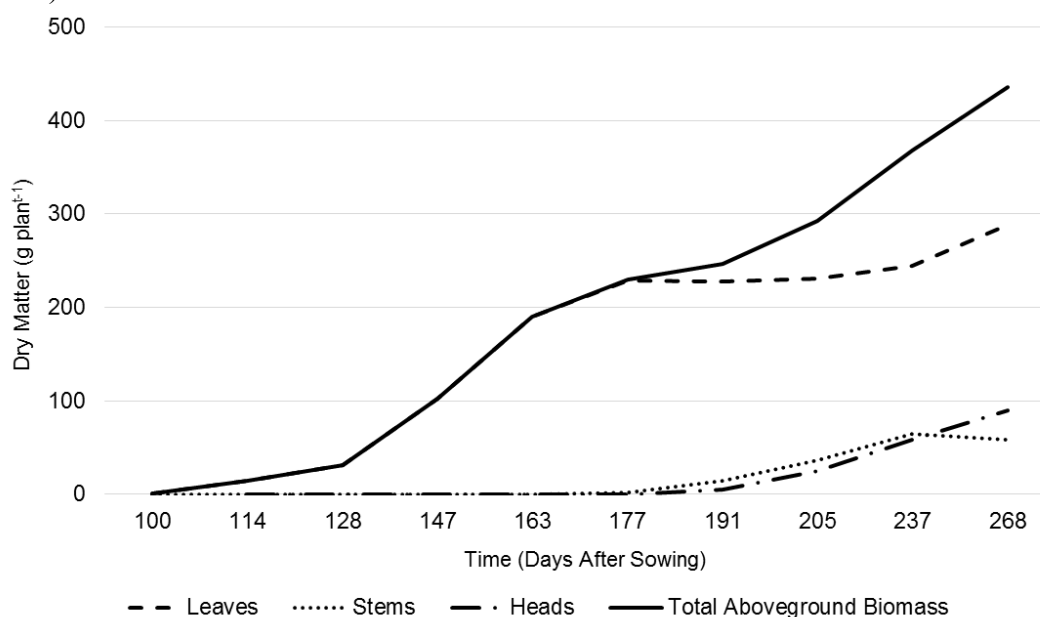


Figure 31: Biomass dynamics and its components in GR genotype. First year of cycle (2012/2013)

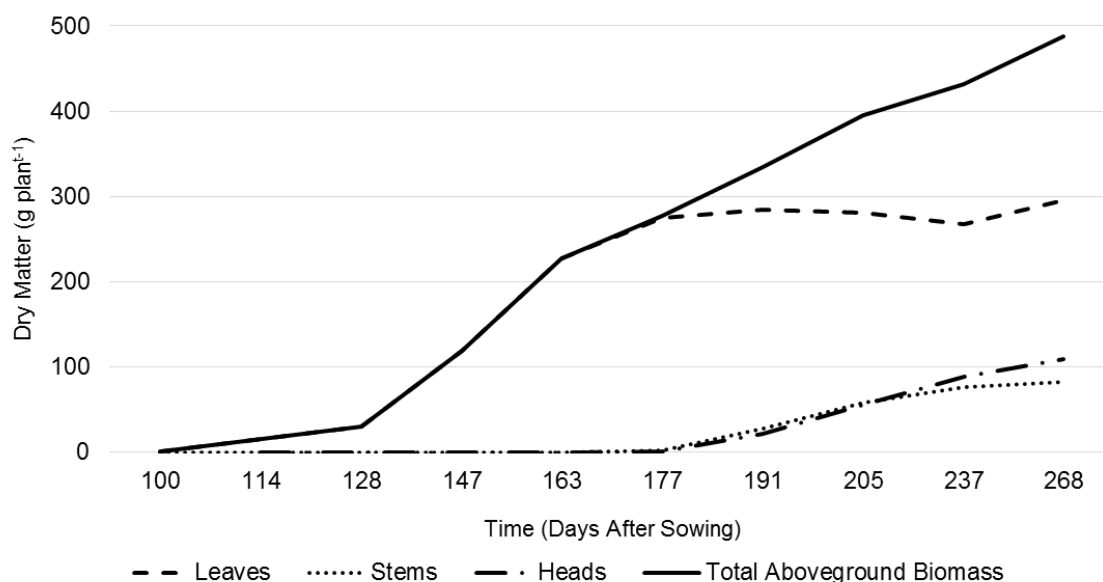
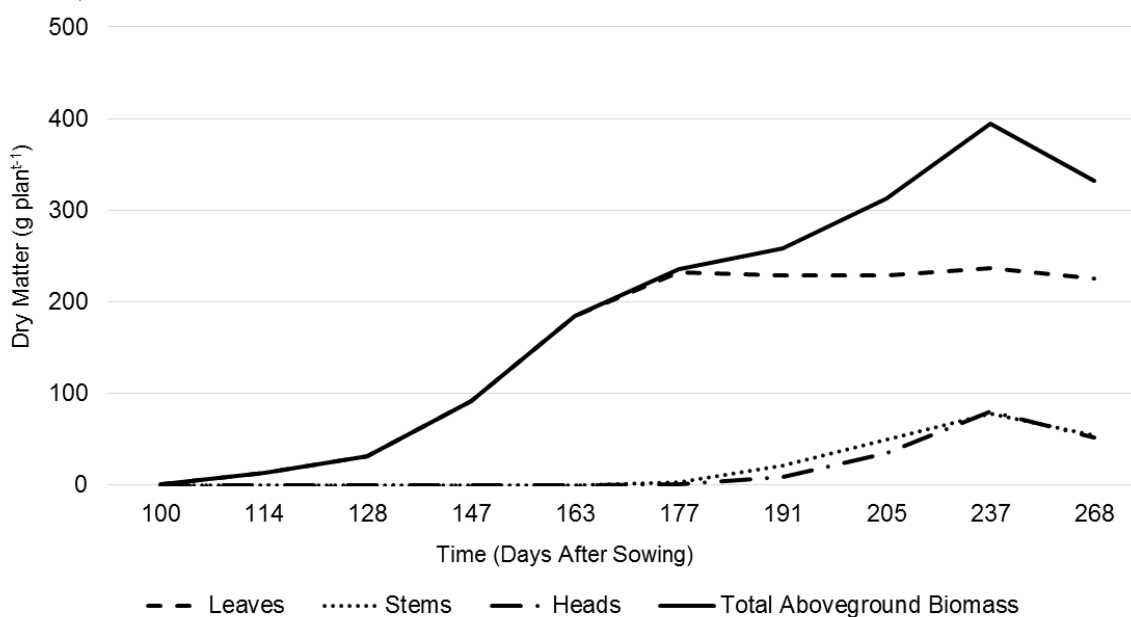


Figure 32: Biomass dynamics and its components in GL genotype. First year of cycle (2012/2013)



In general, all the genotypes did not show significant differences in trend, except for the Gobbo di Lucca, which showed a final decrease in term of biomass cumulated per day. This result could be caused by a stronger decrease in the stems and heads production rate, compared to the other two (BA and GR) in which the values did not reach such low levels.

In the last three figures above, we can clearly see the different role of the components in the storage of biomass.

Second year cycle (2013/2014)

Following the same procedure used for the first year, the second year curves are plotted below (Figures 33-35).

Figure 33: Contribution of CPRs to RGR. The curves for production rate component in the different organs of BA genotype: leaves (blue), heads (green), and stems (red). Second crop year (2013/2014)

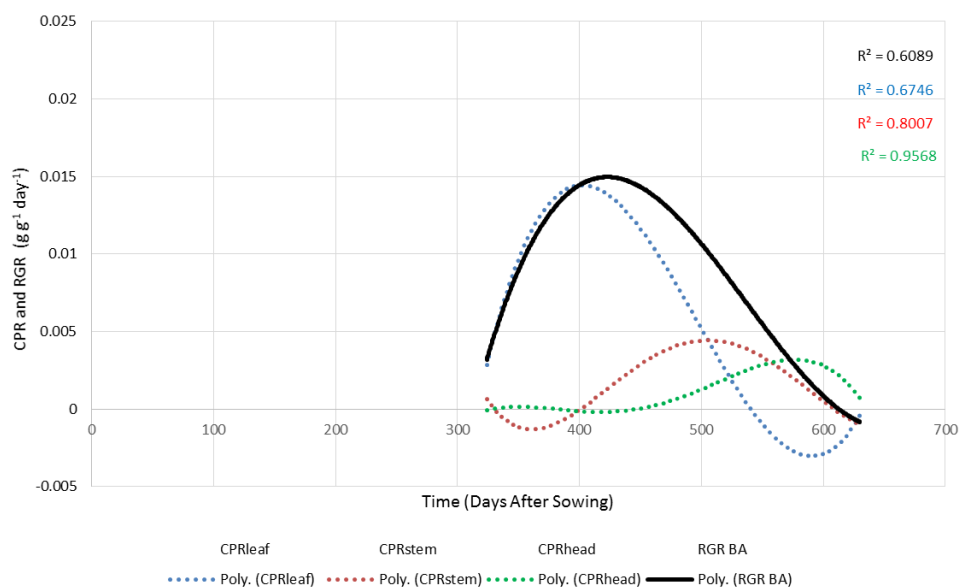


Figure 34: Contribution of CPRs to RGR. The curves for production rate component in the different organs of GR genotype: leaves (blue), heads (green), and stems (red). Second crop year (2013/2014)

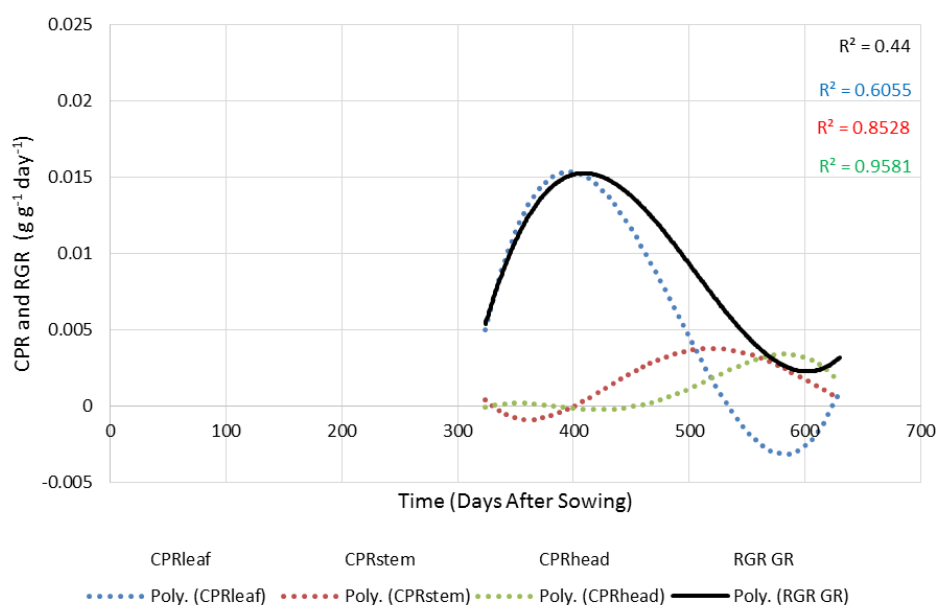
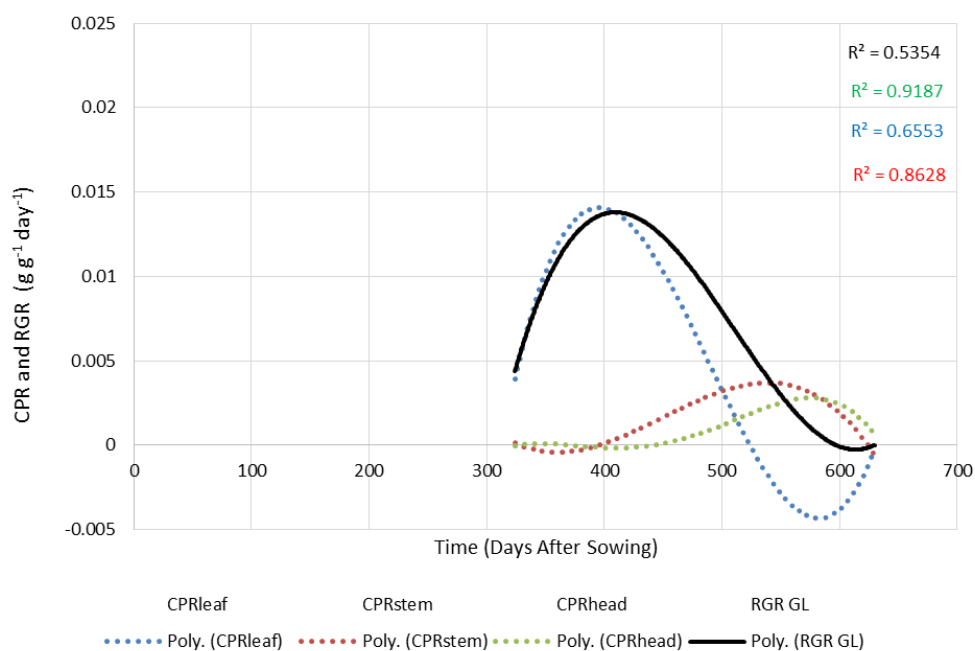


Figure 35 Contribution of CPRs to RGR. The curves for production rate component in the different organs of GL genotype: leaves (blue), heads (green), and stems (red). Second crop year (2013/2014)



In the second year, RGRs trend was very similar for all genotypes and for all production components.

The main difference is observed in comparison to the first year RGRs value size (grams of biomass produced by gram of the whole plant per day).

The second year values are particularly low as the total dry weight (denominator) was much higher than during the first year.

This is the peculiarity of using a "relative" vs. "absolute" growth rate.

Indeed, using the "Absolute Growth Rate", two performances may look similar as it is the most elementary index of plant growth: a rate of change in size, an increment of weight per time unit. Instead, the Relative Growth Rate takes into account this original difference in size.

Looking at final value of production, if two performances appeared identical, knowing their initial dry weights can observe the difference in term of "crop efficiency". By using the "Relative Growth Rates", for instance, it is easy to identify the higher performance of the species that doubled their dry weight, compared to those that increased their weight by only a tenth.

The efficiency of the plants in the first year was higher than in the second, even considering a low biomass yield.

For example: the crops of the first year ranged from 100 g to 200 g in 15 days, in the second for the same range of weight (100-200g) the crop needed about 50 days. It is easy, to see the higher efficiency of the first year.

In the following Figures (36-38) we can see the biomass dynamic accumulation and its high values. The higher yields are also justified by a much longer cycle duration.

Figure 36: Biomass dynamics and its components in BA genotype. Second year of cycle (2013/2014)

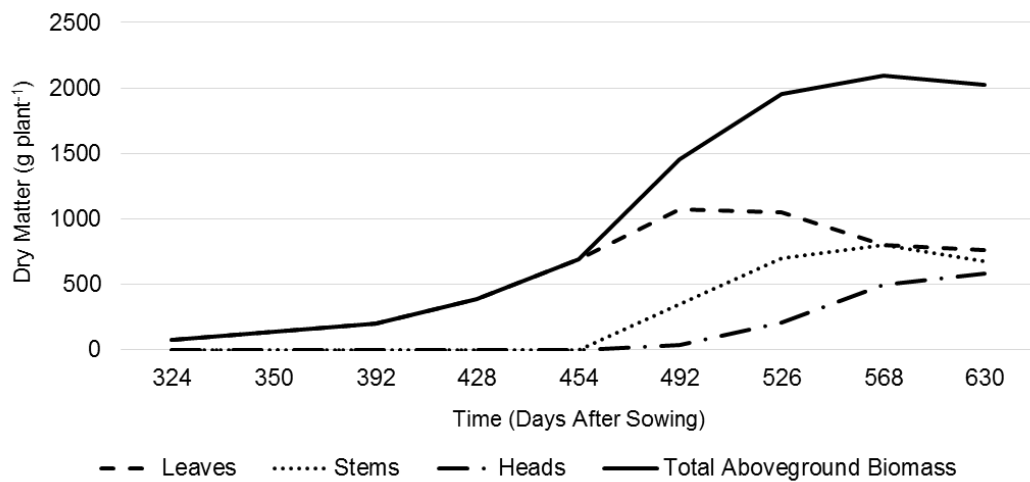


Figure 37: Biomass dynamics and its components in GR genotype. Second year of cycle (2013/2014)

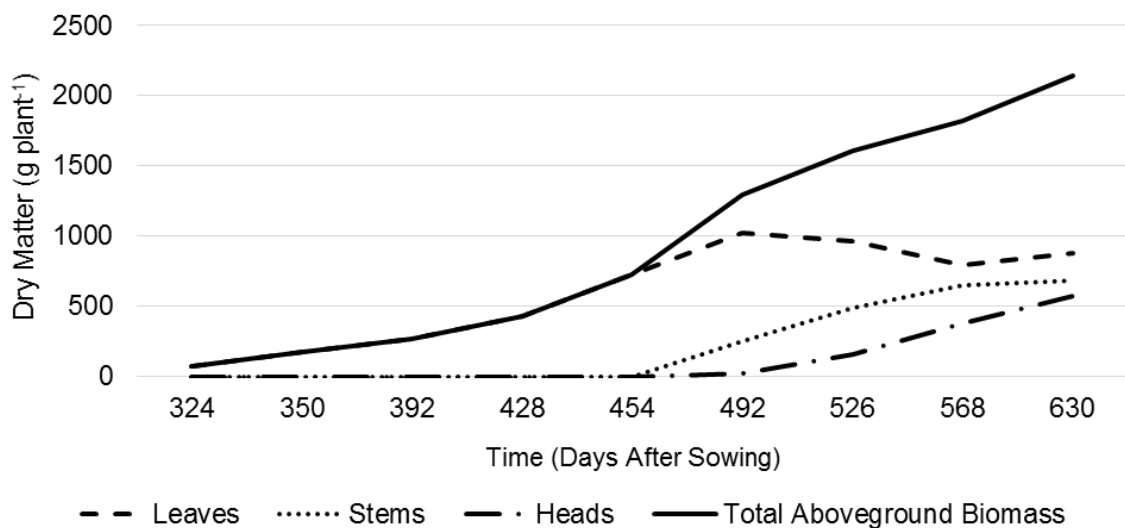
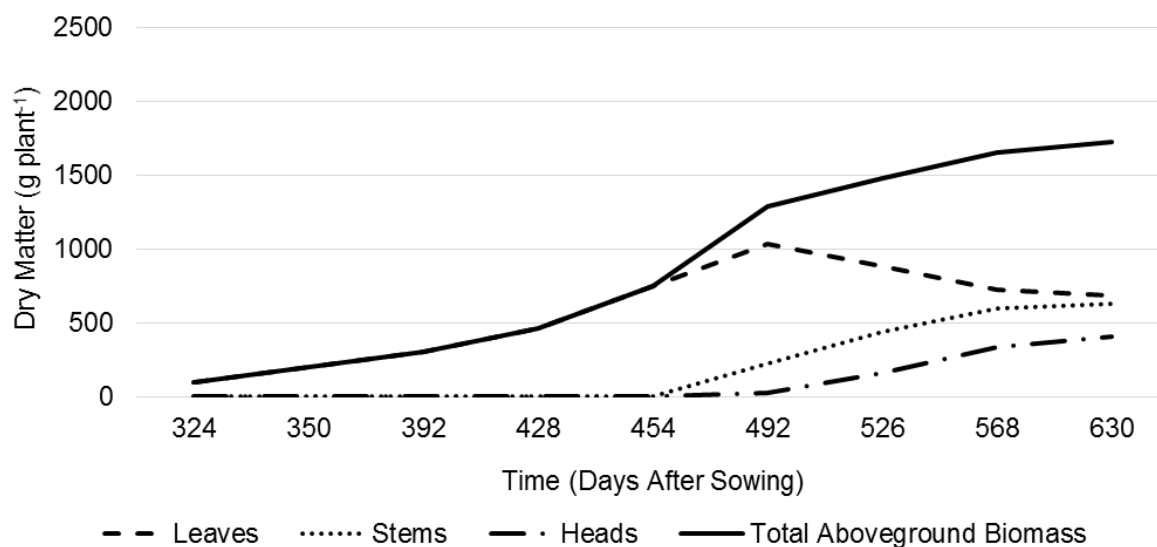


Figure 38: Biomass dynamics and its components in GL genotype. Second year of cycle (2013/2014)



In conclusion, the analysis of the crop in general, showed higher RGR in the first year, but the short duration of this growth season and the features of establishment year did not allow a higher biomass yield.

We often observed that in the biomass dynamic accumulation graphs, around the end of the cycle in both years, a decrease of biomass (total and per components).

This event is in agreement with Ierna et al., (2012). According to the authors, “the shift in harvest time from flowering to achenes ripening decreased the aboveground biomass yield (grain excepted) on average, from 21.1 to 19.0 t h but without significant effects on energy yield, as a consequence of the energy obtained from grains”.

On the same context, the decreases observed in some instances of this work, most likely suggest different optimal harvests for the three genotypes. Therefore, the conclusion is that the optimal harvest time varies with the crop purpose (biomass or seeds).

2.4 CONCLUSIONS

The scientific community, at global and local level, has been shown much interest in the cropping system "thistle" for bioenergetics purposes. For this reason, in the present research, it was been studied in deep.

Our results agree with others obtained in northern Sardinia (Ledda et al., 2013), and confirm good biomass yield of cardoon after the first year, even if slightly different from that reported for similar Mediterranean environments.

The grain yield was always lower than the published values for similar environments and managements, and it was very small quantities.

In the second year, the crop showed grains yield values still very low, except the genotype "Gigante di Romagna", which with a HI of 0.6 showed more than one ton per hectare of seed produced.

In this contest, further research is needed to assess the extent of the loss in seed yield caused by the infestation of "Larinus cynarae" and others insects, which has not been eradicated with the two pesticide distributions.

The GR genotype, in general reasoning, it showed a greater productivity than others two, and in the second year showed a substantially higher LAI.

This fact suggests the possibility to choose from different genotypes with different productive attitudes: aboveground biomass or seeds and crop residues.

These results need to be verified in a larger scale than this experimental plot. Although it was difficult to separate the phenological phases, because many growth stages are overlapping, the BBCH scale was a useful and valuable tool for analysis.

The results obtained are quite promising, because these aboveground biomass yields have been obtained without any water supply and in a hot and dry weather conditions.

C. cardunculus var. *altilis* confirmed their competitive behavior as energy crops, with a good adaptation in the characteristics of Mediterranean farming systems.

This work highlights the importance of reasoning not only on total yield, but also in term of crop efficiency.

This is especially true when we refer to bioenergy systems, because with equal yields, the crop efficiency can make the difference in term of sustainability, both economic and environmental.

In conclusion, it is highlighted the need of research with the aim to define the optimal harvest time and further investigation on physiological mechanisms related to the resource use efficiency, such water (WUE) and nitrogen (NUE) radiation (RUE).

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