Project acronym:	**EuroBioRef**
Project Title:	EUROpean multilevel integrated BIOREFinery design for sustainable biomass processing
Instrument:	Large Scale Collaborative Project
Thematic Priority:	FP7-ENERGY.2009.3.3.1
Grant Agreement:	241718
Start Date of Project:	01/03/10
Duration:	48 Months

SP2 – STUDIES ON BIOMASS FEEDSTOCK AND OPTIMISATION FOR THE SELECTED VALUE CHAIN
WP2.1 – BIOMASS FEEDSTOCK OPTIONS

Deliverable report

Due Date of Deliverable: M24 - 29/02/2012
Actual Submission Date: M25 - 14/03/2012

Deliverable Identification	
Deliverable Number:	D2.1.5
Deliverable Title:	Laboratory analyses and database with physical and chemical properties for a variety of feedstocks
Responsible Beneficiary:	CRES
Contributing Beneficiaries:	SOABE, CRES, UWM
To be Submitted to the EC:	No

History			
Version	**Author**	**Modification**	**Date**
V1	Myrsini Christou	1st Draft	10/03/2012

Approval				
	Name	**Organization**	**Date**	**Visa**
Deliverable Responsible	Myrsini CHRISTOU	CRES	10/03/2012	OK
Work Package Leader	Myrsini CHRISTOU	CRES	10/03/2012	OK
Sub-Project Leader	Myrsini CHRISTOU	CRES	10/03/2012	OK
Coordinator	Franck DUMEIGNIL	CNRS-UCCS	14/03/2012	OK

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Initial key performance indicator:
Successful fields and crop strategies, corresponding to the achievements of deliverable D2.1.3

Executive summary

Description of the deliverable objective and content

This deliverable aims at reporting the physical and chemical properties of the lignocellulosic crops studied in this project. Along with these data, information on the field trials will also be provided.

Brief description of the state of the art

Lignocellulosic energy crops lately received a lot of interest. The recent "food *vs.* fuel" debates, in which the biofuels have become a scapegoat to blame for the recent price increases in the agricultural commodities urge for a careful selection of biomass crops. The introduction of energy crops into current agricultural systems remains very uncertain under existing financial and legislative frameworks. Among the most promising identified options so far are perennial crops. Such crops have low input requirements over 10-25 years of productive life coupled with high yielding potential. Such crops offer not only an important energy resource but can also positively contribute to biodiversity, soil protection, landscape improvement *etc*. Perennial crops have low input requirements making maintenance costs low.

Such crops have permanent rooting / rhizome systems that reduce risk of erosion, which can be an important benefit in some regions. Grass species are relatively easy harvested and collected – though systems need improvement. Short rotation coppice provides near-permanent vegetative cover, with only short periods every three years or so when the crop has been cut to ground level. With unintensive chemical inputs and its tall and varied architecture, coppice can provide important habitat for flora and fauna. There have been several sizeable pan-Europe initiatives for perennial rhizomatous grass species (JOUB-0070, AIR 2 CT92 0294, FAIR CT 96-1707, AIR 3 CT94 2465, FAIR3 CT96 2028, FAIR CT97 3701, ENK6 CT2001 00524). However, perennial energy crop research is relatively young and knowledge is severely limited because of the fact that the majority of trials have been discontinued when funding has ended. Thus, there is little information on crop development from ~ 4 years onwards to the end of crops life-span at 15-25 years. This is particularly the case for perennial rhizomatous grasses; fortunately, there are longer trials of coppice in the UK and Sweden. This causes important uncertainties: it is not known if yields improve with successive rotations, whether crops physical character becomes more difficult for harvesting etc. In the
Table 1 below, information on the R&D status of the selected energy crops in a European level is tabulated. Drawing conclusions is also complex because experimental designs and agronomic practices were not uniform among different EU-funded research projects, partly due to researchers' different aims and methods, but also due to the fact that agronomic research is by necessity species and site specific.

Table 1 - Cultivation status of the selected energy crop species in Europe.

Energy Crop	Yields (t/yr/ha)	Total Cultivated Area (ha)	Country	Type of cultivation	Uses
Miscanthus*	6-29	350	DK, DE, UK, IE, GR, IT, ES, NL, AT, FR, CH, TR, PT	Experimental, pilot large fields	Energy
Giant reed	3-37	15	UK, DE, GR, IT, ES, FR	Experimental, pilot large fields	Energy
Switchgrass	5-23	13	NL, GR, IT, ES, FR	Experimental, pilot large fields	Energy
Reed canary grass*	5-14	6,250	SW, FI, UK, IE, DE, DK	Experimental, demonstration	Energy
Willow*	5-20	18,000	SE, UK, FI, DK, IE, IT, NL, BE, DE, AT	Experimental, demonstration, pilot,large scale	Energy
Eucalypt*	8-20	500,500	PT, ES, FR, IT, GR	Experimental, demonstration,	Pulp, energy

				pilot,large scale	

*: data from the European Energy Crops Overview (EECO) Project, Abbreviations: AT Austria, CH Switzerland, E Belgium, ES Spain, DK Denmark, FI Finland, FR France, DE Germany, GR Greece, IE Ireland, IT Italy, NL Netherlands, PT Portugal, SE Sweden, TR Turkey and UK United Kingdom.

Deviation from objectives and corrective actions

The deliverable is prepared according to schedule and is following the work being done in D2.1.2 and D2.1.4

Innovation brought and technological progress

The progress beyond the state-of-the-art refers to the use of existing small scale fields with a variety of feedstock (giant reed, switchgrass, cardoon, miscanthus, SRC) to report on yielding performances and physical and chemical characteristics over a period of 7-10 years that will be studied in the project using also pre-existing knowledge. From the proposed crops, switchgrass, miscanthus and willow have been successfully grown in a wide range of EU environments and agricultural zones, whereas cardoon, giant reed and black locust are more adapted in the Mediterranean agricultural zone.

Analysis of the results

The compilation of information regarding the physical and chemical properties of the lignocellulosic feedstocks will serve as a guide towards using the most appropriate crops in the several biorefinery streams.

Impact of the results

The results obtained by the field trials will be used as a basis for refining the general strategies/approach for the project and define biomass feedstocks matching to several biorefinery options.

Related IPR

Publishable information

The report on the physical and chemical properties of the crops tested will be uploaded on the project website and will be publicly available.

Conclusion

This deliverable presents the main results of the lignocellulosic crops and will be updated annually.

Physical characteristics for a variety of feedstocks

1. Perennial crop field trials in Greece: Cardoon, giant reed, Miscanthus, switchgrass

1.1. Methods and materials

Fields of the perennial herbaceous crops, cardoon, giant reed and switchgrass have been harvested in February 2011; Miscanthus field was established in March 2011. The same split plot experimental design that is designed for giant reed and switchgrass is also followed. The three irrigation levels are: I0: no irrigated, I1: 50% of ET, I2: 100% of ETo and the three nitrogen fertilisation rates are: N0: 0 kg N/ha, N1: 40 kg N/h, N2: 120 kg N/ha.

1.2. Results

The height of plants at the final harvest of 2011 reached 5m. A compilation of results since 2002, when the plantation was established is depicted in Erreur ! Source du renvoi introuvable.. It can be noticed that after the 5th growing period plant height tends to stabilise. The non-irrigation treatment resulted in significantly lower plants, while nitrogen fertilisation did not affected plant height. That indicates that indeed perennial grasses are low input biomass feedstocks.

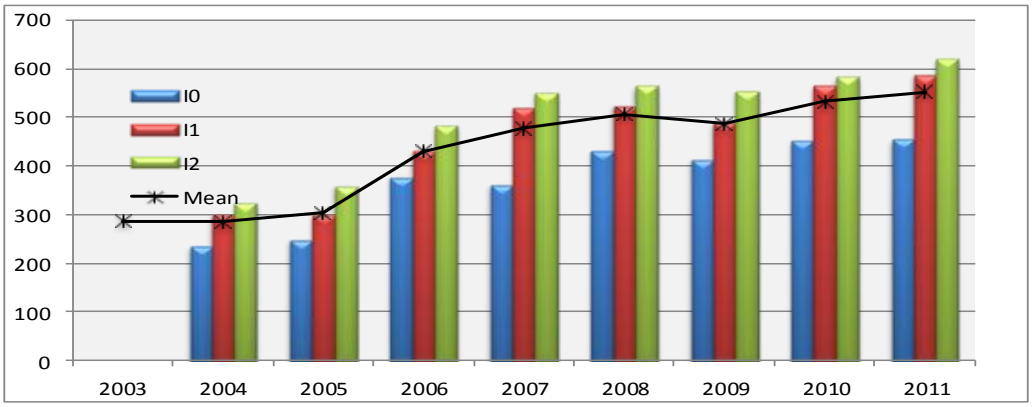

Figure 1 - Plant height (in cm) of giant reed for 9 subsequent growing periods (2003-2011) for 3 irrigation rates.

Dry matter yields in 2011 final harvest ranged from 7 – 16 t/ha, with moisture content at 54% (**Figure 2 and Figure 3**). Giant reed tends to achieve mature yield from the 4th growing season. All irrigation rates had a significant effect on yields, whereas only the lack of nitrogen fertilisation rate resulted in significantly lower yields.

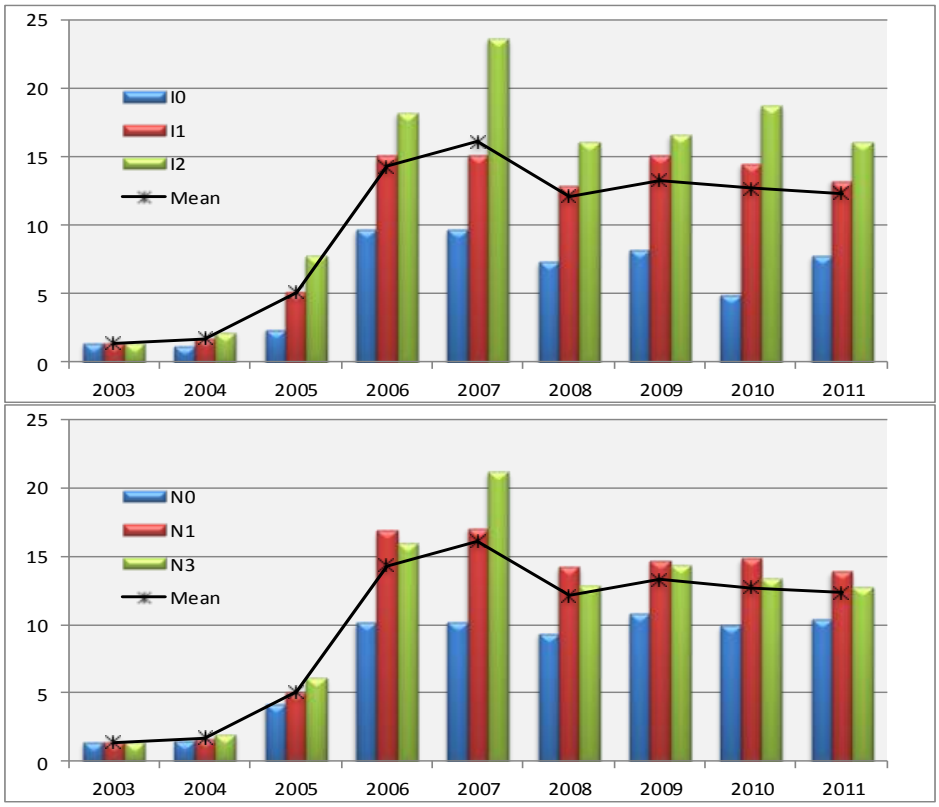

Figure 2 – Dry matter yields (in t/ha) of giant reed for 9 subsequent growing periods (2003-2011) for 3 irrigation rates and 3 nitrogen fertilisation rates

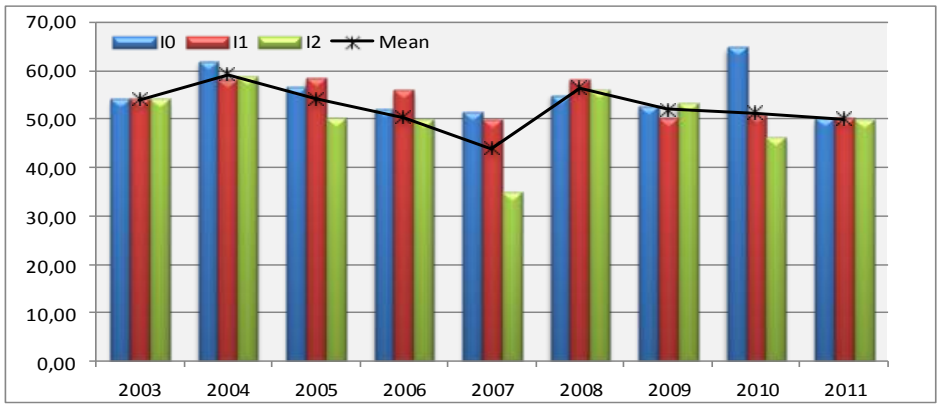

Figure 3 – Moisture content (%) of giant reed for 9 subsequent growing periods (2003-2011) for 3 irrigation rates.

Likewise, a compilation of results since 2002 are made for switchgrass (**Figure 4, Figure 5 and Figure 6**). It can be noticed that after the 3rd growing period plant height tends to stabilise. The non-irrigation and non-fertilisation treatments resulted in significantly lower yielding plants, while the two irrigation rates did not affect significantly the productivity of the plants. Dry matter yields in 2010 final harvest ranged from 7 – 26 t/ha, with moisture content at 20%.

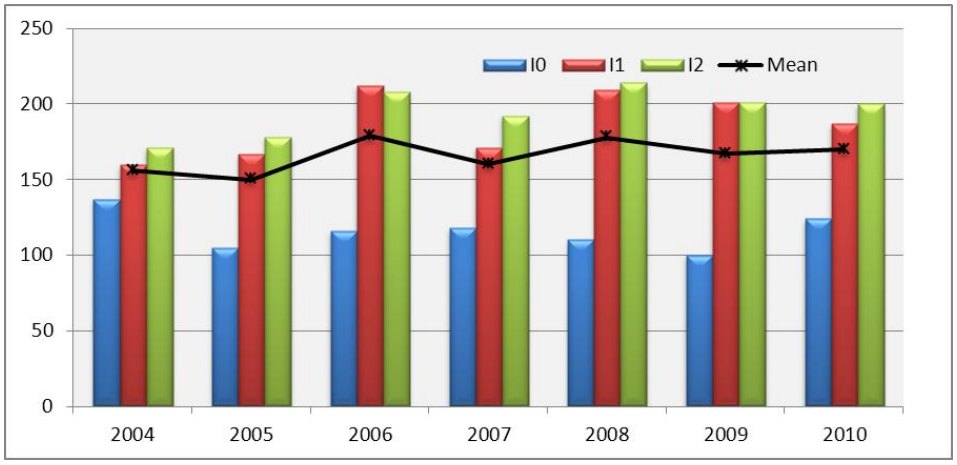

Figure 4 - Plant height (in cm) of switchgrass for 7 subsequent growing periods (2004-2010) for 3 irrigation rates.

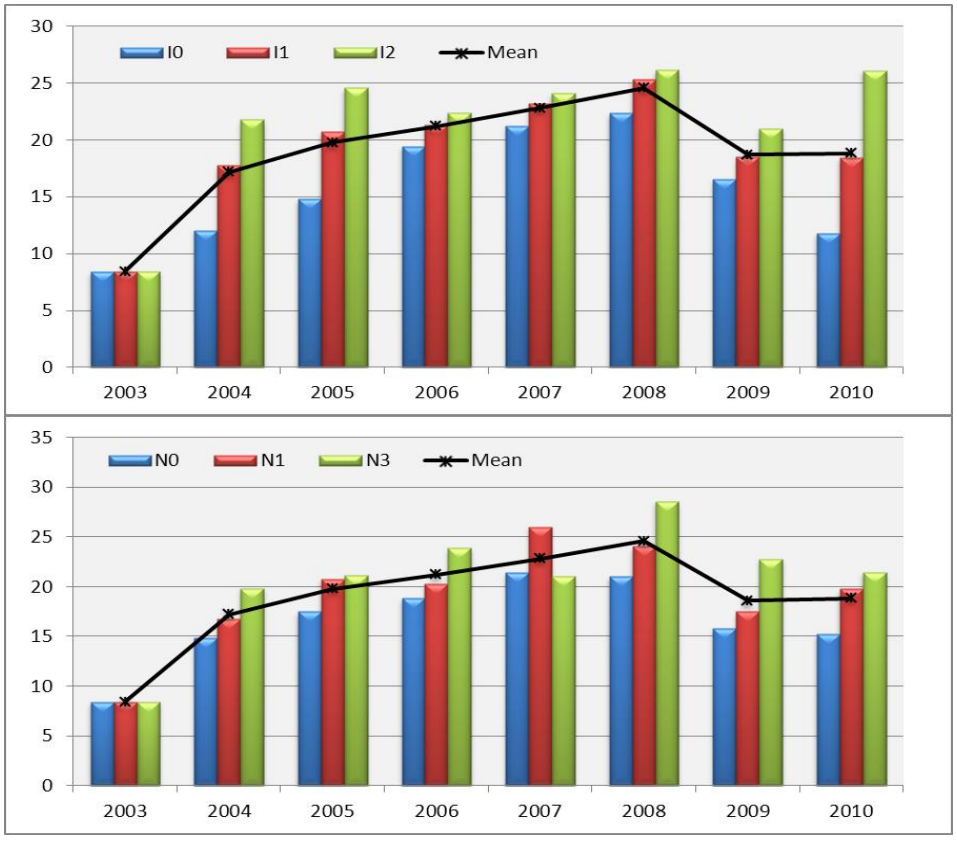

Figure 5 - Dry matter yields (in t/ha) of switchgrass for 8 subsequent growing periods (2003-2010) for 3 irrigation rates and 3 nitrogen fertilisation rates.

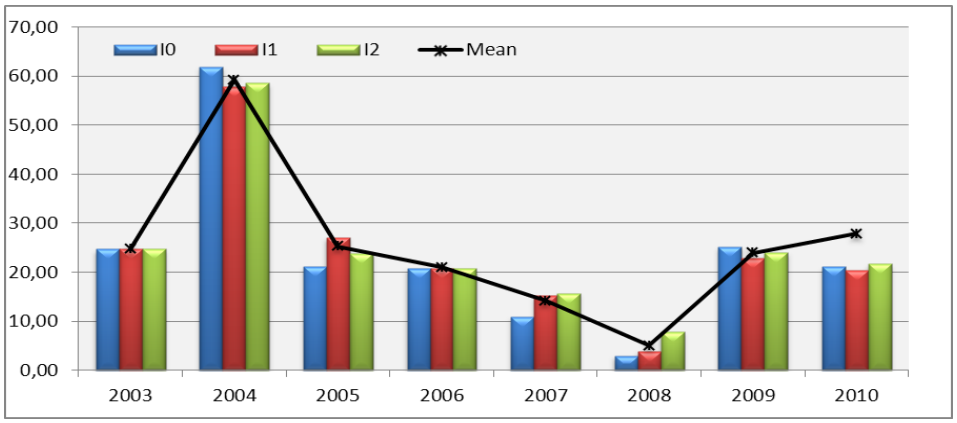

Figure 6 – Moisture content (%) of switchgrass for 8 subsequent growing periods (2003-2010) for 3 irrigation rates.

Cardoon yields ranged from 1.8 to 6 t/ha in the 2010 growing period (**Figure 7 and Figure 8**). The plant is not irrigated. Data are still being analysed and will be reported in the following reporting period.

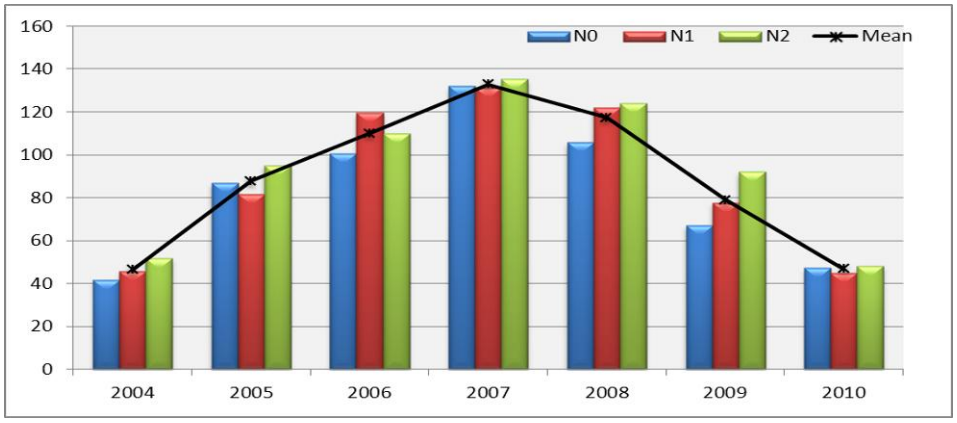

Figure 7 – Plant height (in cm) of cardoon for 7 subsequent growing periods (2004-2010) for 3 nitrogen fertilisation rates.

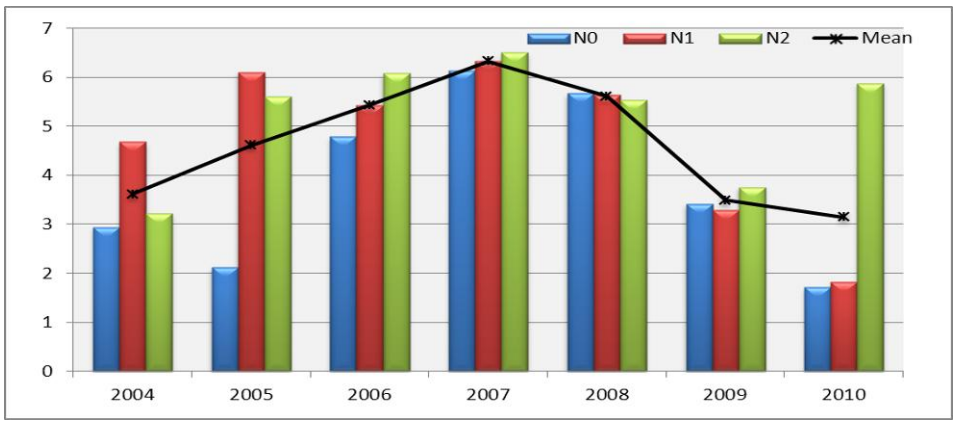

Figure 8 – Fresh matter yields (in t/ha) of cardoon for 7 subsequent growing periods (2004-2010) for 3 nitrogen fertilisation rates.

The plantations

Photo 1 – A view of the newly established Miscanthus field trials in Greece

Photo 2 – Non irrigated-fertilised giant reed plants (on the left) and highly irrigated giant reed plants (on the right) in the field trials in Greece

Photo 3 – Non irrigated-fertilised (with yellow colour) vs highly irrigated and fertilised (with green colour) switchgrass plants in the field trials in Greece

2. Willow field trials in Poland

The Warmia and Mazury Region is located in north-eastern Poland. Olsztyn is the largest city and natural centre of this region (**Photo 4**). The University of Warmia and Mazury is situated in Olsztyn and our experimental station in Łężany is located about 75 km from Olsztyn. Agricultural land at the station in Łężany covers an area of about 1400 ha. The farm is very well equipped with technical equipment. One large field with an area of about 21 ha (**Photo 5**) was designated and prepared for the purpose of carrying out the tasks of producing biomass from willow and oil plants for the needs of the EuroBioRef project.

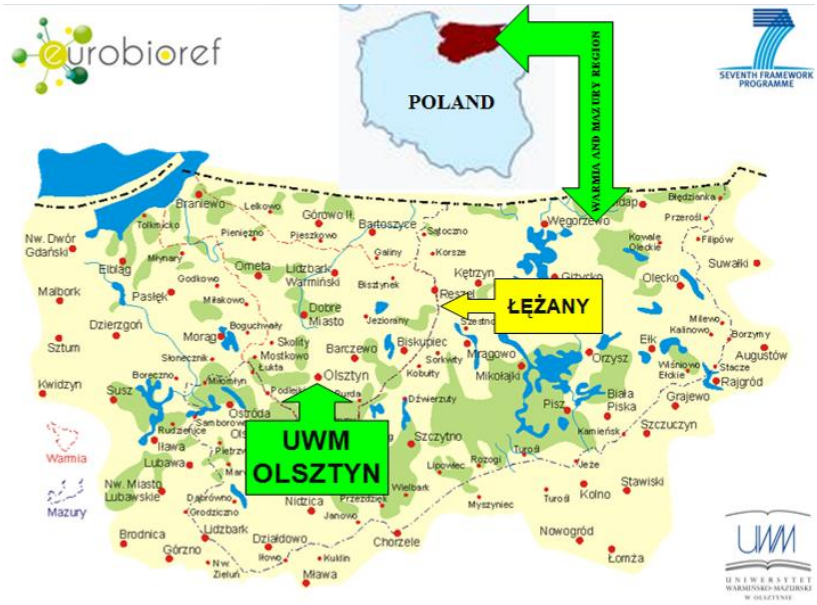

Photo 4 - Warmia and Mazury Region in Poland and the UWM in Olsztyn

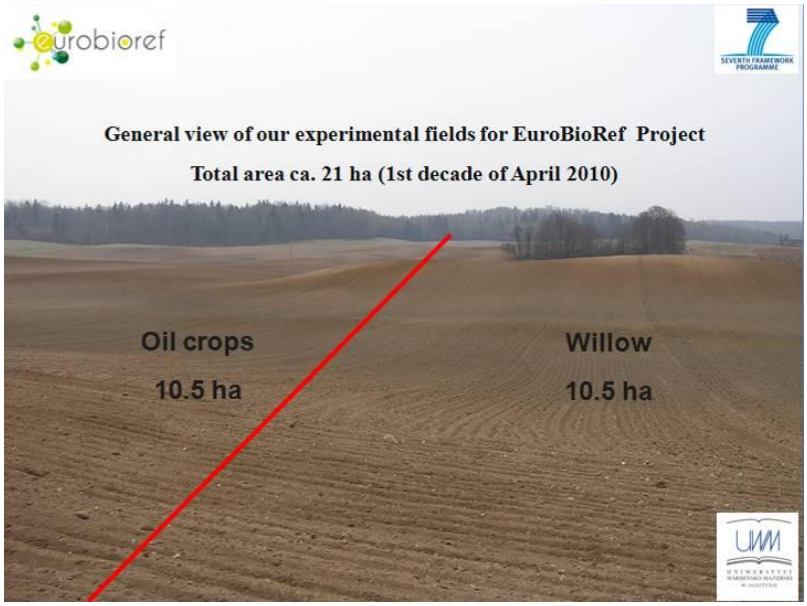

Photo 5 - The field designated for the cultivation of willow and oil plants

2.1. Methods and materials

The willow plantation was started between the 11th and 20th of April 2010 at the Educational and Research Station in Łężany belonging to the University of Warmia and Mazury in Olsztyn. The main factor in the field experiment are three varieties and four clones of willow, all of them created by the Department of Plant Breeding and Seed Production of the University of Warmia and Mazury in Olsztyn.

1. Start – variety from the species *Salix viminalis*
2. Tur – variety from the species *Salix viminalis*
3. Turbo – variety from the species *Salix viminalis*
4. UWM 006 – clone from the species *Salix viminalis*
5. UWM 035 – clone from the species *Salix pentandra*
6. UWM 043 – clone from the species *Salix viminalis*
7. UWM 155 – clone from the species *Salix dasyclados*

Plantation area amounts to 10.5 ha. In the second year of plants growth the cultivation of recurrences around the plantation was conducted. Moreover nitrogen was sown in the form of ammonium nitrate just before the start of plant vegetation at the dose of 50 kg ha^{-1}. The second part of the nitrogen dose (40 kg ha^{-1}) was applied between the 21st and 30th of May.
A spraying for monocots with Targa Super herbicide with dose of 3 l ha^{-1} was carried out. At this part of the plantation mechanical weeding (mainly for clone UWM 155) was conducted as well.

2.2 Characterization and evaluation of climate conditions in the second year of plant vegetation

Meteorological data of mean monthly air temperature and participation was gathered and analysed in purpose of climate conditions analysis. The mean air temperature in vegetation period in 2011 (14.4 ^{o}C) was 0.8 ^{o}C higher than the value from the multi-annual period 1998-2007 (**Table 2**). The warmest month was July (18.5 ^{o}C). the temperature in that month was analogical to temperature in the multi-annual period. In all remaining months of vegetation period of analysed year, air temperatures were higher than in multi-annual period from 0.2 in May to 1.6 ^{o}C in April and June.

The total precipitation in 2011 (447.3 mm) was practically on the same level as mean for years 1998-2007 (**Table 3**). Participation was lower from the beginning of Spring than in the multi-annual period. In March was 19.1 mm, In Aprli 33.2 mm and In May 46.8 mm. It amounted to respectively 49%, 92% and 81% value of the multi-annual period. Thus, water deficit was found on the beginning of second vegetation. In June participation were almost 2% higher than in multi-annual period, while in July amounted to 154 mm and were 2 times higher than in multi-annual period. In next month of vegetation period in 2011 were lower than in multi-annual period. As much as 84.9 mm fell in August, in September only 18.3 mm and in October 51.9 mm.

Table 2 - The mean air temperature (°C) in 2011 and from multi-annual period

Year / Month	2011	Deviation	1998-2007
January	-1.7	0.3	-2.0
February	-6.0	-4.5	-1.5
March	1.9	0.3	1.6
April	9.8	1.6	8.2
May	13.3	0.2	13.1
June	17.4	1.6	15.8
July	18.5	0.0	18.5
August	18.1	0.5	17.6
September	14.8	1.4	13.4
October	8.8	0.6	8.2
November	3.4	0.8	2.6
December		0.5	-0.5
Mean (IV-X)	14.4	0.8	13.5
Mean (I-XII)	8.9	1.0	7.9

Summing up, it can be generally stated that 2011 was warmer in regard to mean air temperatures. The participation was practically the same as for in multi-annual period. However their distribution was unfavorable, which undoubtedly limited plants growth.

Table 3 - Total precipitation (mm) in 2011 and from multi-annual period

Year / Month	2011	% from multi-annual period	1998-2007
January	32.1	74.7	43.0
February	56.5	171.2	33.0
March	19.1	49.0	39.0
April	33.2	92.2	36.0
May	46.8	80.7	58.0
June	79.5	101.9	78.0
July	154.0	200.0	77.0
August	84.9	98.7	86.0
September	18.3	34.5	53.0
October	30.6	51.9	59.0
November	9.6	18.8	51.0
December		0.0	44.0
Sum (IV-X)	447.3	100.1	447.0
Sum (I-XII)	564.6	85.9	657.0

2.3. Characterization of the dynamics of willow plant growth in the first year of vegetation

The dynamics of willow growth in the first year of vegetation are presented in **Figure 9**. Clone UWM 155 was harvested after first year of grow. Its dynamic shows growth in subsequent months only for vegetation period of 2011. The height of plants after first vegetation period is included in May 2011for all remaining varieties and clones.

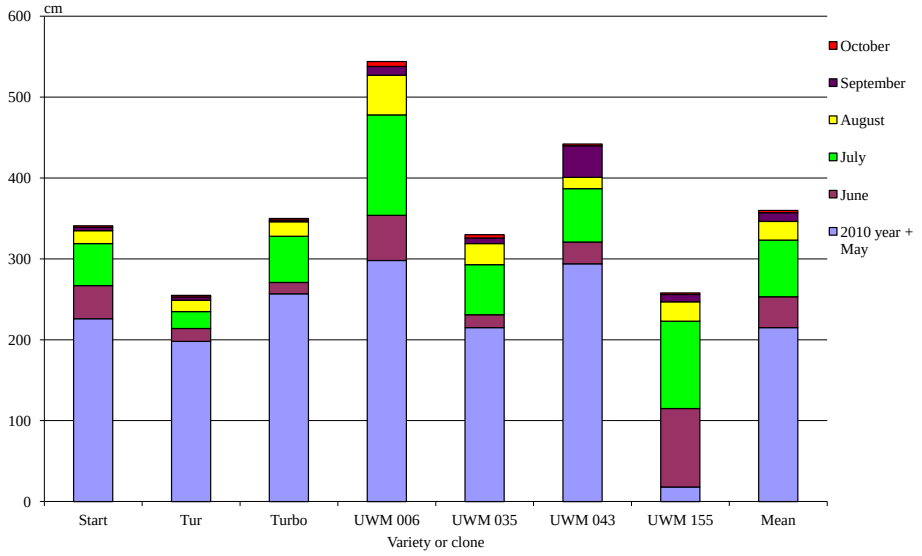

Figure 9 - Dynamics of willow plant growth in 2011

Photo 6- Willow plantation on 18.01.2011

Photo 7 - Willow plantation on 08.02.2011

2.3.4 Characterization of the plants and evaluation of the willow biomass quality after the second year of vegetation

The number of the willow plants at the end of 2010 amounted on average to 13,929 plants ha^{-1}, and the value of standard deviation amounted to over 2,7 plants ha^{-1} (**Table 4**). Among the studied varieties, by far the most UWM 043 plants survived – 15,667 plants ha^{-1}. The Start and Turbo varieties belonged to the second homogeneous group. Tur and UWM 035 were in the third group. Significantly, the fewest plants (8.000 plants ha^{-1}) survived in the case of UWM 155 from the species *Salix dasyclados*.

It was also found that willow plant losses after the first year of vegetation were high and amounted on average to 22.6% (**Table 4**). The value of that characteristic ranged from 12.96% for UWM 043 to as much as 55.56% for UWM 155. Such high willow plant losses were generally the result of low precipitation in April 2010, year of planting. The very high losses of the UWM 155 clone plants resulted from the drought and harm from the soil-applied herbicide.

Table 4 - Survival of willow plants after second vegetation period

Variety or clone	Number of plants		Losses	
	(plants ha^{-1})	stand. dev. (±)	(%)	stand. dev. (±)
Start	15500.0 a	1000.0	13.89	5.56
Tur	13777.8 b	544.3	23.46	3.02
Turbo	15555.6 a	601.8	13.58	3.34
UWM 006	15111.1 ab	480.1	16.05	2.67
UWM 035	13888.9 b	1263.6	22.84	7.02
UWM 043	15666.7 a	587.9	12.96	3.27
UWM 155	8000.0 c	654.2	55.56	3.63
Mean	13928.6	2664.0	22.62	14.80

± standard deviation
a, b, c… homogenous groups

The number of 1st order ramifications in the experiment amounted on average to 2.83 (**Table 5**). The significantly highest number of stems was found in the UWM 155 clone (6.3). Meanwhile, for four varieties and clones the number of 1st order ramifications was in the range of 1.63-3.30.

The height of the plants at the end of the second vegetation season amounted on average to 3.6 m in the experiment, and the value of standard deviation amounted to 1.58 m (**Table 5**). The significantly highest plants were developed by the UWM 006 clone (5.44 m). The plants of clone UWM 043 were approx. 1 m shorter (4.42 m). The Turbo, Start, Tur and UWM 035 plants (3.3-3.3 m) belonged to another homogeneous group. The significantly lowest plants were given by UWM 155 and Tur. Plants of UWM 155 plants were short because regrown from stump after harvest In first year of growth. The Tur plants were eaten by wild animals in wintertime that resulted in their propagation but low increase of height.

The diameter of the main willow shoot amounted on average to 20.98 mm (**Table 5**). The plants of the UWM 006 clone were characterized by the significantly thickest shoots, on average 37.71 mm, and the value of standard deviation amounted to 2.97 mm. The second group in that characteristic included UWM 043. The UWM 155 and Tur varieties developed the significantly thinnest shoots after the second year of vegetation (12.7-13.3 mm).

Photo 8 - Willow plantation - UWM 043 clone on 30.09.11

Table 5 - Number of stems, height and shoot diameter of the willow plants after the second year of vegetation

Variety or clone	Number of stems		Height		Diameter	
	quantity	stand. dev. (±)	(m)	stand. dev. (±)	(mm)	stand. dev. (±)
Start	3.30 b	0.51	3.41 c	0.10	14.75 cd	1.55
Tur	2.17 c	0.19	2.55 d	0.28	12.71 d	2.26
Turbo	2.20 c	0.14	3.50 c	0.19	20.85 c	2.21
UWM 006	1.63 c	0.32	5.44 a	0.40	37.71 a	2.97
UWM 035	2.40 c	0.13	3.30 c	0.18	21.98 bc	3.99
UWM 043	1.81 c	0.13	4.42 b	0.47	25.50 b	2.37
UWM 155	6.33 a	0.78	2.58 d	0.15	13.33 d	1.35
Mean	2.83	1.58	3.60	1.00	20.98	8.61

± standard deviation
a, b, c… homogenous groups

Photo 9 - Willow plantation - UWM 043 clone on 01.12.11

After the second year of grow the yield of *Salix spp*. was assessed. The fresh mass of one plant amounted to 1.5 kg in average (**Table 6**). The value of this feature varies between 0.85 and 2.47 kg. respectively for clone UWM 155 and UWM 006. The yield of fresh biomass for these clones was respectively 6.85 t ha^{-1} and 37.08 t ha^{-1}. After moisture was taken into account, the yield of dry matter was assessed for plants after second year of growth, which amounted to 10.65 t ha^{-1} in average. Among all tested varieties the highest yield was given by clone UWM 006 (18.25 t ha^{-1}). Similar yield was acquired from clone UWM 043. Varieties Tur, Turbo i UWM 035 are in third homogeneous group. After recalculation of obtained results for one year of plantation cultivation it may be stated that this value were in range from 1.6 to 9.13 t d.m. ha^{-1} $year^{-1}$ respectively for clones UWM 155 and UWM 006.

Table 6 - Estimated biomass yield of *Salix* spp. after the second year of vegetation

Variety or clone	Fresh biomass one plant		Fresh biomass yield		Dry biomass yield		Dry biomass yield	
	(kg)	stand. dev. (±)	(t/ha)	stand. dev. (±)	(t/ha)	stand. dev. (±)	(t/ha/year)	stand. dev. (±)
Start	1.59 b	0.46	24.91 ab	8.18	12.21 ab	4.20	6.10 ab	2.10
Tur	1.02 c	0.25	14.04 bc	3.78	7.05 b	1.93	3.53 b	0.96
Turbo	1.42 b	0.31	21.96 b	4.67	10.44 b	2.21	5.22 b	1.10
UWM 006	2.47 a	0.57	37.08 a	7.55	18.25 a	3.35	9.13 a	1.67
UWM 035	0.91 c	0.30	12.58 bc	4.29	6.10 b	2.04	3.05 b	1.02
UWM 043	2.26 ab	0.95	35.55 a	15.60	17.31 a	7.69	8.66 a	3.84
UWM 155	0.85 c	0.20	6.85 c	2.21	3.20 c	0.98	1.60 c	0.49
Mean	1.50	0.75	21.85	12.89	10.65	6.32	5.33	3.16

± standard deviation
a, b, c… homogenous groups

Photo 10 - Willow plantation - UWM 043 clone on the left and Turbo variety on the right (01.12.11)

Photo 11 - Willow plantation - Turbo variety on the left and Start variety on the right (01.12.11)

Photo 12 - Willow plantation - Start variety on the left and UWM 006 clone on the right (01.12.11)

Photo 13- Willow plantation - UWM 006 clone on the left and Tur variety on the right (01.12.11)

Photo 14 - Willow plantation - Tur variety on the left and UWM 155 clone on the right (01.12.11)

Photo 15 - Willow plantation - UWM 155 clone on the left and UWM 035 clone on the right (01.12.11)

In the 2011 vegetation season, the willow plants generally were not settled by pathogens. Because of this, no significant negative impact of diseases or pests on the growth or development of the plants was observed. Admittedly, isolated colonies of aphids were observed during July, mainly on the plants of the UWM 006 clone. However, after about two weeks ladybirds appeared, which effectively naturally reduced their numbers and eventually the willow plants were free from aphids. The leaves of the willow plants had intense, natural, green colouring practically till the end of the vegetation season.

1. Perennial crops: Cardoon, giant reed, miscanthus, switchgrass

The summary of the results obtained from laboratory analysis conducted so far for the four studied energy crops is presented in the following **Table 7**. Also a range for short rotation coppice is presented in order to compare SRC to the four studied energy crops. As it is shown cardoon exhibited the highest ash content in comparison to the other three crops indicated that energy conversion of cardoon may face problems. Also, cardoon energy content, measured as gross calorific value, is much lower than the measured one for the three grasses (miscanthus, giant reed and switchgrass). Last, nitrogen content for cardoon is higher than the other crops which may also have negative effect due to the higher possibility for NOx formation during energy conversion.

Table 7 - Laboratory analysis results for the four studied energy crops.

		Proximate			Gross Calorific Value	Elemental		
		Volatile Matter (%)	Ash (%)	Fixed Carbon (%)	(kcal/kg)	C (%)	H (%)	N (%)
Cardoon	**mean**	**73,62**	**15,63**	**10,75**	**3923,35**	**41,76**	**5,50**	**1,40**
	min	72,82	15,05	10,40	3799,17	39,51	5,13	0,74
	max	74,33	16,79	11,23	4102,53	43,64	6,01	2,05
Miscanthus	**mean**	**81,47**	**3,05**	**15,48**	**4334,78**	**45,76**	**6,29**	**0,12**
	min	81,09	2,63	15,43	4217,61	45,47	6,26	0,10
	max	81,85	3,48	15,54	4451,95	46,05	6,32	0,14
Arundo	**mean**	**77,18**	**4,75**	**18,06**	**4267,36**	**45,07**	**5,96**	**0,64**
	min	74,81	3,75	16,21	4106,96	42,44	5,58	0,19
	max	78,70	8,85	19,07	4405,55	46,47	6,25	1,78
Switchgrass	**mean**	**78,66**	**4,93**	**16,41**	**4179,79**	**44,83**	**6,08**	**0,33**
	min	76,61	2,56	15,42	4024,00	42,90	5,86	0,11
	max	81,09	6,35	17,35	4272,42	45,91	6,47	0,85
SRC*	**min**	80.94	0.52	16.35	4596	48.18	5.71	0.15
	max	82.55	1.33	18.26	4711	50.73	5.92	0.57

* An Atlas of Thermal Data for Biomass and Other Fuels, National Renewable Energy Laboratory, U.S. Department of Energy, June 1995.

Among the three grasses, in general small differences were detected. Laboratory analyses indicated that miscanthus was the most promising energy crop since it had low ash content, high calorific value, high carbon and hydrogen content and low nitrogen content.

When comparing the four energy crops to SRC, it is obvious that most of the energy characteristics of the three energy grasses are within the ranges for SRC indicating their high value as energy crops. SRC is better than grasses if ash and carbon content is considered.

Data are still being analysed thus this deliverable will be annually updated.

2. Willow

Biomass samples from the studied species collected for laboratory analyses, in the form of shoots cut with secateurs from their whole length, were packed into plastic bags and transported to the laboratory of the Department of Plant Breeding and Seed Production of the UWM in Olsztyn. The samples were then broken up with secateurs into the form of chips. The biomass moisture content was then determined in a laboratory, with the drying and weighing method. The biomass was dried at the temperature of 105 ^{o}C until a constant mass was obtained. Next, the individual biomass samples were broken up in an IKA KMF 10 basic analytic mill using a 0.25 mm mesh sieve. The ash content was determined in the prepared analytical samples at a temperature of 550 °C in an ELTRA TGA-THERMOSTEP automatic thermogravimetric analyser. In addition, the higher heating value of dry biomass was determined in an IKA C 2000 calorimeter using the dynamic method. The lower heating value of the fresh biomass was calculated on the basis of the higher heating value and moisture content of the biomass. The carbon, hydrogen and sulphur content were also identified, by means of an ELTRA CHS 500 automatic analyser. The nitrogen content was determined with the Kjeldahl method, using a K-435 digestion unit and a B-324 BUCHI distiller and the chlorine content using the Eschka mixture. All the analyses were performed in three repetitions. Chemical properties were analyzed according to Polish Norm - 92/P-50092: cellulose content by the Seifert method, lignin content by the Tappi method, holocellulose with sodium chlorite, pentosan content by the Tollens method, the content of substances soluble in alkalis (1% aqueous solution of NaOH), which provides information about the amount of hemicelluloses in wood, the content of cold-water-soluble substances, the content of hot-water-soluble substances, the content of substances soluble in ethanol.

The results of the tests were analysed statistically using STATISTICA PL software. The mean arithmetic values of the examined characteristics were calculated. Homogeneous groups for the examined characteristics were determined by means of an SNK (Student-Newman-Keuls) multiple test with the significance level set at p = 0.05.

The moisture content of the willow biomass after the second year of cultivation amounted on average to 51.4% (**Table 8**). Among analysed genotypes significantly the lowest moisture content was found in biomass of Tur variety (49.84%), while significantly the highest in biomass of clone *Salix dasyclados* UWM 155 (53.08%). Ash analysis of *Salix* spp. biomass showed it low content, 1.49% d.m. in average (tab. 6). It was found that clone UWM 006 has significantly the lowest ash content (1.3% d.m.). The value of this feature was significantly the highest in biomass of clone UWM 035 (1.64% d.m.). Remaining genotypes are assigned to one homogenous group according to ash content.

Table 8 - Moisture content and ash content in willow biomass after second vegetation period

Variety or clone	Moisture content		Ash content	
	(%)	stand. dev. (±)	(% d.m.)	stand. dev. (±)
Start	51.19 b	0.83	1.47 b	0.03
Tur	49.84 c	0.35	1.46 b	0.05
Turbo	52.45 ab	0.42	1.53 b	0.05
UWM 006	50.61 bc	1.15	1.30 c	0.01
UWM 035	51.42 b	0.99	1.64 a	0.08
UWM 043	51.36 b	0.28	1.48 b	0.03
UWM 155	53.08 a	0.76	1.52 b	0.01
Mean	51.42	1.22	1.49	0.10

± standard deviation
a. b. c… homogenous groups

Higher heating value of *Salix spp.* amounted to 19.76 MJ kg^{-1} d.m. in average (**Table 9**). The standard deviation of this feature amounted to 0.12 MJ kg^{-1} d.m. Biomass of Tur variety has significantly the highest value of this feature (19.93 MJ kg^{-1} d.m.), the lowest was found in Start variety. Higher heating

value was positively correlated with carbon, hydrogen, sulphur and nitrogen content, while negatively correlated with moisture and ash content (**Table 13**). After including of moisture content, lower heating value of biomass was calculated. Its value amounted to 8.35 MJ kg^{-1}. The highest value of this feature (8.78 MJ kg^{-1}) was found in biomass of Tur variety. Biomass of UWM 006 variety were in the second homogenous group. Its lower heating value amounted to 8.55 MJ kg^{-1}. Significantly the lowest value of this feature was analysed in clone UWM 155.

Table 9 - Higher heating value and lower heating value in willow biomass after second vegetation period

Variety or clone	Higher heating value		Lower heating value	
	(MJ kg^{-1} d.m.)	stand. dev. (±)	(MJ kg^{-1})	stand. dev. (±)
Start	19.57 e	0.00	8.30 b	0.18
Tur	19.93 a	0.03	8.78 a	0.06
Turbo	19.66 d	0.01	8.07 bc	0.10
UWM 006	19.82 b	0.03	8.55 ab	0.26
UWM 035	19.84 b	0.03	8.38 b	0.23
UWM 043	19.81 b	0.02	8.38 b	0.06
UWM 155	19.71 c	0.04	7.95 c	0.17
Mean	19.76	0.12	8.35	0.30

± standard deviation
a. b. c… homogenous groups

Carbon content was determined in biomass of analysed genotypes, which amounted to 50.67% d.m (**Table 10**). Significantly the highest carbon content was found in Tur variety and clone UWM 035. Hydrogen mean content in biomass of analysed species amounted to 6.05%, while significantly the highest was determined in biomass of clone UWM 043. The average nitrogen content of willow biomass amounted to 0.62% d.m. Significantly the highest value of this feature was marked in Turbo variety, the lowest in Tur, UWM 006, UWM 035. Willow biomass contained little amount of sulphur, 0.031% d.m. in average. Chlorine mean content amounted to 0.007% d.m.

Table 10 - Elemental analysis of the willow biomass after the second year of vegetation

Variety or clone	C		H		N		S		Cl	
	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)
Start	50.49 c	0.22	6.03 b	0.02	0.52 e	0.0	0.031 b	0.001	0.005 d	0.000
Tur	51.16 a	0.30	6.03 b	0.03	0.60 d	0.0	0.028 c	0.001	0.007 b	0.000
Turbo	50.33 c	0.01	5.97 c	0.04	0.71 a	0.0	0.032 b	0.000	0.009 a	0.001
UWM 006	50.77 b	0.10	6.09 ab	0.02	0.61 d	0.0	0.023 d	0.001	0.007 b	0.000
UWM 035	51.19 a	0.14	6.05 b	0.05	0.60 d	0.0	0.031 b	0.000	0.006 c	0.000
UWM 043	50.39 c	0.08	6.13 a	0.03	0.68 b	0.0	0.038 a	0.002	0.009 a	0.000
UWM 155	50.36 c	0.26	6.05 b	0.06	0.64 c	0.0	0.038 a	0.001	0.007 b	0.000
Mean	50.67	0.39	6.05	0.06	0.62	0.1	0.031	0.005	0.007	0.002

± standard deviation
a. b. c… homogenous groups

Mean content of substances soluble in cold water amounted to 8.48% d.m (Table 11), while mean value for substances soluble in hot water were higher and amounted to 10.38% d.m. Mean content of substances soluble in ethanol ranged between 7.26-11.14% d.m. Reaction amounted to 5.90 in average.

Table 11 - The content of substances soluble in ethanol, cold and hot water in willow biomass after second year of vegetation

Variety or clone	Substances soluble in cold water		Substances soluble in hot water		Substances soluble in ethanol		pH	
	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)
Start	7,17 d	0,07	8,98 e	0,04	7,26 e	0,19	6,00 a	0,08
Tur	8,81 ab	0,10	11,13 ab	0,15	8,75 c	0,04	5,79 b	0,09
Turbo	8,18 c	0,21	9,41 d	0,15	7,73 d	0,24	5,90 a	0,04
UWM 006	8,51 b	0,10	10,43 c	0,29	7,83 d	0,12	5,94 a	0,12
UWM 035	8,98 a	0,14	10,87 b	0,10	11,14 a	0,07	5,76 b	0,03
UWM 043	8,74 ab	0,23	10,50 c	0,07	9,86 b	0,04	5,94 a	0,08
UWM 155	8,95 a	0,30	11,33 a	0,33	9,71 b	0,18	5,95 a	0,02
Mean	8,48	0,63	10,38	0,84	8,90	1,33	5,90	0,11

± standard deviation
a. b. c… homogenous groups

Significantly the highest average content of cellulose (43.29% d.m.) was found in biomass of clone UWM 043 (**Table 12**). Next group with lower average content of cellulose includes the UWM 006 and UWM 155 clones, and third one with the lowest content of the polysaccharide – remaining varieties. The highest holocellulose content was found in biomass of clones UWM 043 and UWM 155. Its average content was equal to 70.37% d.m. Significantly the highest value was found in biomass of UWM 035 (72.85% d.m.) and the lowest in Start variety – 68.68% d.m. Lignin content, in the experiment, amounted to 25.21% d.m. in average. Significantly the highest value was determined in biomass of Turbo variety, while the lowest in clones UWM 006 and UWM 155. The content of pentosans was the lowest in clone UWM 035.

Table 12 - Chemical composition of willow biomass after second year of vegetation

Variety or clone	Hemielluloses		Cellulose		Holocellulose		Lignin		Pentosans	
	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)	(% d.m.)	stand. dev. (±)
Start	31,48 d	0,08	40,30 c	0,29	68,68 e	0,19	25,52 b	0,35	21,25 a	0,20
Tur	32,22 c	0,12	40,57 c	0,55	69,97 d	0,22	25,64 b	0,14	20,07 b	0,19
Turbo	32,33 c	0,01	40,62 c	0,43	67,78 f	0,24	26,73 a	0,26	21,69 a	0,35
UWM 006	32,79 bc	0,58	41,81 b	0,12	71,61 b	0,46	23,96 d	0,30	21,20 a	0,16
UWM 035	33,09 b	0,50	40,52 c	0,22	72,85 a	0,25	25,88 b	0,13	19,44 c	0,42
UWM 043	34,62 a	0,13	43,29 a	0,69	70,67 c	0,73	24,69 c	0,02	19,85 bc	0,25
UWM 155	34,43 a	0,47	42,20 b	0,15	71,05 bc	0,89	24,08 d	0,13	19,92 bc	0,31
Mean	32,99	1,14	41,33	1,12	70,37	1,69	25,21	0,97	20,49	0,85

± standard deviation
a. b. c… homogenous groups

Table 13 - Coefficient of *r* – Pearson correlation for the characteristics examined

Item	Moisture content	Ash content	Higher heating value	Lower heating value	C	H	S	N	Cl	Substances soluble in cold water	Substances soluble in hot water	Substances soluble in ethanol	Hemicelluloses	Cellulose	Holocellulose	Lignin	Pentosans	pH
Moisture content	1,00																	
Ash content	0,27	1,00																
Higher heating value	-0,49	-0,07	1,00															
Lower heating value	-0,99	-0,25	0,63	1,00														
C	-0,50	0,06	0,71	0,58	1,00													
H	-0,18	-0,16	0,39	0,23	0,19	1,00												
S	0,55	0,55	-0,31	-0,55	-0,50	0,13	1,00											
N	0,39	0,12	0,16	-0,32	-0,38	-0,04	0,34	1,00										
Cl	0,18	-0,01	0,23	-0,11	-0,35	0,15	0,34	0,87	1,00									
Substances soluble in cold water	0,08	0,19	0,73	0,07	0,33	0,32	0,14	0,49	0,36	1,00								
Substances soluble in hot water	-0,08	0,13	0,73	0,21	0,39	0,35	0,11	0,19	0,13	0,86	1,00							
Substances soluble in ethanol	0,14	0,58	0,49	-0,03	0,35	0,34	0,44	0,21	0,06	0,75	0,70	1,00						
Hemielluloses	0,36	0,11	0,21	-0,29	-0,30	0,44	0,58	0,55	0,44	0,62	0,57	0,63	1,00					
Cellulose	0,17	-0,24	0,16	-0,13	-0,40	0,58	0,43	0,45	0,47	0,43	0,35	0,30	0,79	1,00				
Holocellulose	-0,11	0,08	0,56	0,21	0,51	0,57	-0,08	-0,12	-0,24	0,66	0,66	0,73	0,44	0,27	1,00			
Lignin	-0,04	0,52	-0,14	0,01	0,17	-0,56	-0,00	0,08	0,10	-0,27	-0,43	-0,10	-0,53	-0,65	-0,50	1,00		
Pentosans	-0,00	-0,43	-0,52	-0,10	-0,33	-0,34	-0,38	-0,02	-0,01	-0,67	-0,76	-0,87	-0,57	-0,29	-0,66	0,24	1,00	
pH	0,22	-0,34	-0,62	-0,32	-0,66	0,02	0,18	-0,06	-0,09	-0,47	-0,41	-0,44	0,10	0,29	-0,26	-0,36	0,43	1,00

Red color denotes correlation coefficients significant at a level of $p \leq 0.05$ at $n = 21$

ANNEX II – Milestone achievement

This deliverable proves the achievement/partial achievement of the milestone M2.2, planned at M20. It will be updated annually, following the updates of Deliverable 2.1.5 and the outcomes of SP2 work.

1. Evaluation of lignocellulosic crops matching the understudy biorefinery concept

Samples from all perennial grasses and willow are being characterized. Some data are presented in D2.1.5 and the rest will be available in the second part of M25. The milestone and the relevant Deliverable 2.1.5 will be immediately updated. The data will be communicated to the other SPs of the project, in order to evaluate the appropriateness of the lignocellulosic crops for the various biorefinery streams.