Management and drought effects on growth and herbage yield of Tall Wheatgrass (*Agropyron elongatum*) for biogas production in Central Europe

Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Agrarwissenschaften
der Georg-August-Universität Göttingen

vorgelegt von
Michael Dickeduisberg
geboren in Soest

Göttingen, Dezember 2017

D 7

1. Referentin/Referent: Prof. Dr. Johannes Isselstein

2. Korreferentin/Korreferent: Prof. Dr. Harald Laser

Tag der mündlichen Prüfung: 12.02.2018

Contents

List of figures

List of tables

List of abbreviations

DM	Dry matter
DMY	Dry matter yield
dpi	Dots per inch
GSI	Germination Speed Index
HBT	Hohenheim Biogas Yield Test
MHY	Methane hectare yield
Nm^3	Standard cubic meter
oDM	Organic dry matter
PEG	Polyethylene Glycol
SMY	Specific methane yield
TSW	Thousand seed weight
WUE_{agr}	Agronomic water use efficiency
WUE_{int}	Intrinsic water use efficiency

1. General introduction


Figure 1: Tall wheatgrass has a height of almost 2 m at first harvest.

Over the course of the last few decades, arable farming has increasingly been confronted with new challenges, such as a growing world population, increasingly scarce fossil resources and climate change. These interdependent demands have changed and will continue to change the nature of agricultural production.

In 2009, the European Parliament passed a directive to promote energy from renewable sources. By 2020, at least 20% of energy consumption and 10% of fuels must be derived from renewable sources (European Parliament, 2009). Moreover, the German Renewable Energy Sources Act mandates that 40–45% of electricity consumption must come from renewable sources by 2025 (EEG, 2017). The volatility of wind and solar power precludes their use as a constant source of electricity for households and industrial plants. Therefore, balancing these energy sources with biogas will become crucial for stability of electrical grids. Agricultural crops have become very popular as substrata in biogas plants. By the end of 2014, the registered 7,944 plants had an installed electrical capacity of 4,100 MW (FNR, 2016). Common crops, such as maize, had become the predominant and best economic substrata. More than 45% of total substrata in German biogas plants, i.e. energy crops and slurry, consist of maize (Dahlhoff, 2013). The consequently increasing share of maize in crop rotations (cp. BMEL, 2013; BMELV, 2001) has led to phytosanitary, economic and publicity problems in the form of increasingly frequent maize diseases, reduced soil fertility and increased erosion. In addition, the lack of economic diversity has resulted in an increased dependency on maize yields and loss of public support (Liu et al., 2006; Schittenhelm et al., 2011). This lack of biological diversity is reflected in the narrower range of insect species and decreased population of certain insects that are in the focus of public complaints (Herbes et al., 2014). One possible solution to this may rest in partially substituting maize with other crops that promote greater ecological biodiversity, while simultaneously satisfying the economic and agricultural demands of biogas production. Attracting and protecting wildlife is usually associated with low maintenance crop management (i.e. no pesticide applications) and low tillage intensity.

Besides the national problem of the high proportion of maize in crop rotations, there is the already well-discussed and globally recognised problem of climate change with different regional characteristics. Different scenarios predict a warming trend and alteration in the distribution of annual precipitation. In Germany, a long-term annual warming of +1.6°C up to +3.8°C, and precipitation shifting from the summer months to autumn and winter is predicted (Zebisch et al., 2005). Other reports specifically forecast an increasing frequency of drought, even in regions of Great Britain and the centre and north of Europe (Parry et al., 2007). On the one hand, drying soils present a challenge in terms of crop seeding and establishment; on the other hand, the soil of harvested fields is vulnerable to erosion and nitrate leaching by high amounts of precipitation if left fallow (Sutton et al., 2008). For both these reasons (i.e. high share of maize in crop rotation and climate change), new drought-tolerant plants that are capable of being transformed or used as energy providers must be identified, tested and adapted to central European environmental conditions.

Permanent crops, such as giant knotweed (*Fallopia sachalinensis* var. Igniscum), cup plant (*Silphium perfoliatum*) or tall wheatgrass (*Agropyron elongatum*) are promising candidates. The intense blooming of giant knotweed and cup plant attracts bloom visitors. The dense stands are retreats for wildlife and enhance the occurrence of ground beetles (Platen et al., 2017). As permanent crops have a useful lifetime of several years, the risk of insufficient field emergence by seeding in dry soil can be avoided, and erosion and nitrate leaching are controlled by intense rooting (Dinnes et al., 2002). Nevertheless, while the positive ecological effects are obvious, economic revenue from these is often low. Mast et al. (2014) found the specific methane yield of giant knotweed, and indeed the relevant methane hectare yield, were less than 50% of the methane yield of the reference maize. From an economic point of view, the low methane hectare yield and high costs for plant establishment by using cuttings disqualifies giant knotweed as a serious alternative crop. For cup plant, the specific methane yield (approximately 75% of the reference maize) and methane hectare yield were significantly higher than for giant knotweed (Mast et al., 2014); however, farmers are cautious about the high cost and expected useful life of 15 years for cup plant (Dickeduisberg and Köhler, 2016), which restricts farmers options when it comes to short-term reactions in cultivation planning in response to changes in agricultural policy.

Tall wheatgrass (*Agropyron elongatum*) may present a viable option as a new biogas crop. Early research reveals a high biogas potential in the range of 0.311–0.376 Nm^3 kg oDM^{-1} (Mast et al., 2014). Hence, specific gas yields are on the same level as maize with approximately 0.350 Nm^3 kg oDM^{-1} (Amon et al., 2007; Heiermann et al., 2009). High specific gas yields and comparative dry matter biomass yields of 17.6–19.3 t DM ha^{-1} (Geißendörfer, 2012) produce a per hectare methane yield on a par with the reference yield of maize. Although this methane yield is auspicious, successful crops must be economically advantageous and easily implemented in farming systems. These requirements, however, have not been intensely evaluated for tall wheatgrass in Germany. Tall wheatgrass is established by seed and harvested using a common harvester, with cultivation being similar to other grassland species (Csete et al., 2011). Therefore, no special equipment is necessary for tall wheatgrass, which translates into significant benefits in terms of implementing tall wheatgrass in farming systems. Tall wheatgrass is a typical grassland species native to nearby Hungary in southeastern Europe, as well as to western Asia (USDA, 2014), and it has been planted all over the world over the past 100 years (Liu and Wang, 2011; Weintraub, 1953). As the robust stem and rough texture of the hairy leaves are less palatable for cattle than other forage grasses with a high yield potential, such as *Lolium perenne*, it is not a common roughage in diary production. On other continents, it is commonly employed for hay and pasture (Scheinost et al., 2008) in regions with alkaline soils and the absence of water (Moore et al., 2006). Therefore, tall wheatgrass is considered a drought-tolerant crop (Heinz, 2015), and might provide stable yields in regions with periods of drought that might otherwise produce only low yields of maize and other forages as a result of the increasing scarcity of water in spring and summer (Tröster, 2015; Zebisch et al., 2005).

In light of these characteristics, there is an opportunity to substitute a proportion of maize with tall wheatgrass without the need for additional public funding. Thus, many of the issues surrounding increasing use of maize for biogas feedstock can be addressed. Western corn root worm, a pest with a rising impact on intensive maize production (Baufeld and Enzian, 2005), can be readily reduced by installing crop rotations or alternative crops (Kiss et al., 2005). Moreover, as tall wheatgrass is a perennial crop, positive effects, such as minimising nutrient leaching (Dinnes et al., 2002), reducing soil erosion (Pimentel et al., 1987) and increasing the content of humus (and carbon) in the soil (Freibauer et al., 2004), contribute to complying with ecological requirements of biogas production and improving the image of renewable resources, thus leading to greater public support.

Although the features of tall wheatgrass presented here should foster cultivation of this new energy crop, only a small number of pioneers grow it in their fields. Many questions dealing with specific expertise for successful planting have yet to be developed. Moreover, few experimental results have been available for growth under Central European conditions. The first reports have revealed small field emergence and low germination rates. Furthermore, the application of mineral fertilizer and digestate need to be optimized for increasing yields and decreasing costs as well as cutting heights and frequencies.

1.1. Working hypothesis

The aim of the present study is to better understand tall wheatgrass sward establishment for sustainable biogas production under the conditions of predicted climate change in Germany. The results of this study should allow for the optimisation of tall wheatgrass production. Unfortunately, specific cultivars for biogas production in central Europe are not available. International plant breeding programmes are focused on different aspects of tall wheatgrass, such as soil and climate conditions, and the utilisation of tall wheatgrass across various continents. As such, different cultivars, harvested all over the world, were taken into account and tested for adaption in the German agricultural system.

A series of experiments were conducted, ranging from germination to drought resistance and cutting management. Two questions were addressed throughout these experiments:

- What is the general suitability of tall wheatgrass for becoming an alternative to maize in biogas production, and how does it perform?
- Does the provenance of tall wheatgrass seeds influence the performance of the grass under Central European conditions?

More detailed questions were the focus of the following individual experiments:

a) Germination

   Based on the reported problems with field emergence and low germination, identified through our own first field trial in 2011, the germination strategy under Central European conditions was analysed in a climate chamber experiment (see chapter 2). Pre-treatment effects and speed of germination were quantified in darkness and daytime illumination with varying temperatures typical for seeding in spring or autumn. A variety of moisture contents simulated moderate to intense drought periods after seeding, the intention being to overcome some of the problems during earlier experiences:

   - What are the reasons for low germination of tall wheatgrass?
   - How can the germination be increased?

b) Drought resistance

   In addition to the germination test, a pot experiment was conducted (see chapter 3) to focus on aboveground and below ground biomass production according to levels of water availability. There were even a number of reports concerning tall wheatgrass growth during drought stress, although most of these occurred in the initial weeks after emergence or in saline soil. Therefore, while tall wheatgrass was considered to be drought-tolerant (Heinz, 2015), there were no data available for the plants reaction to longer periods of drought, e.g. reduced tiller elongation or leaf area, and regrowth during periods of adequate water availability, as is the predicted climate change scenario of Central Europe. Furthermore, no data were available for the reaction of biomass production to the periods of drought and a subsequent period of resilience in an annual two-cut defoliation system, which is common for biogas production. Therefore, the following questions were addressed:

- Is tall wheatgrass a suitable energy crop capable of providing stable yields under the influence of increasing drought stress brought about by climate change?
- How does tall wheatgrass react during long periods of water scarcity, and do drought periods affect regrowth during the subsequent period of resilience?

c) Cutting management

Whereas the climate chamber and container experiment prioritised reactions because of and in adaptation to drought stress, chapter 4 considers approaches for the optimisation of sward and cutting management. Until this study, a stubble height of 15 cm was advised for German farming in accordance with preliminary studies and experiments from other continents (Scheinost et al., 2008; USDA, 2014). However, in many parts of South America and Australia, tall wheatgrass is considered a drought and saline prairie grass for intensive to extensive grazing or hay production. In North America, it is also cultivated via a one-cut system for the purpose of bio-refining (Zheng et al., 2007). Consequently, the demands of regrowth and quality vary intensely between utilisations of biomass. Hence, the third experiment sought to determine

- optimum cutting frequencies and dates for sustainable biogas production in Central Europe, and
- the optimum stubble height for biogas production.

Whether tall wheatgrass cultivation will be extended in Germany in the future will depend on the economic revenue and political regulations on crop production, such as efforts to limit maize cultivation or the promotion of crops with ecological benefits by political directives. The intention of this study is to adapt tall wheatgrass production to the Central European environment for the purposes of bioenergy production. The results of this study have practical implications in terms of providing agricultural guidance for reducing uncertainty in the cultivation of tall wheatgrass. Consequently, the findings of this study support substituting a share of maize in order to mitigate some of the consequences of climate change. Further research dealing with the protection of ground water by reducing nitrate leaching and studies on the ecological impact of fauna should be conducted.

1.2. References

Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., Gruber, L., 2007. Biogas production from maize and dairy cattle manure – Influence of biomass composition on the methane yield. Agriculture, Ecosystems and Environment 118, 173-182.

Baufeld, P., Enzian, S., 2005. Western corn root worm (*Diabrotica virgifera virgifera*), its potential spread and economic and ecological consequences in Germany. In: Alford, D.V., Backhaus, G.F., Plant protection and plant health in Europe – Introduction and spread of invasive species. Humboldt University, Berlin, pp. 149-154.

BMEL, 2013. Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten der Bundesrepublik Deutschland (Statistical yearbook on food, agriculture and forestry of the Federal Republic of Germany), 57. edition. Federal Ministry of Food, Agriculture and Consumer Protection, Landwirtschaftsverlag GmbH, Münster-Hiltrup, Germany.

BMELV, 2001. Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten der Bundesrepublik Deutschland (Statistical yearbook on food, agriculture and forestry of the Federal Republic of Germany), 45. Edition. Federal Ministry of Food, Agriculture and Consumer Protection, Landwirtschaftsverlag GmbH, Münster-Hiltrup, Germany.

Csete, S., Stranczinger, S., Szalontai, B., Farkas, A., Pal, R.W., Salamon-Albert, E., Kocsis, M., Tovari, P., Vojtela, T., Dezsö, J., Walcz, I., Janowszky, Z., Janowszky, J., Borhidi, A., 2011. Tall wheatgrass cultivar Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) as a potential energy crop for semi-arid lands of eastern Europe. In: Nayreripour, M., Kheshti, M. (editors), Sustainable growth and application in renewable energy source. InTech, Rijeka, Croatia, pp. 269–294.

Dahlhoff, A., 2013. Auswertung der Biogasanlagen-Betreiberdatenbank der Landwirtschaftskammer Nordrhein-Westfalen (Evaluation of biogas plant survey of the chamber of agriculture in North Rhine-Westphalia). Center of renewable resources of the chamber of agriculture for North Rhine-Westphalia, Bad Sassendorf, Germany.

Dickeduisberg, M., Köhler, J., 2016. Mit Mais Erträge schon im ersten Jahr (Harvest in the first crop year with maize. dlz-agrarmagazin, 12/2016, pp. 60-64.

Dinnes, D.L., Douglas, L.K., Jaynes, D.B., Kaspar T.C., Hatfield, J.L., Colvin, T.S., Cambardella, C.A., 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained midwestern soils. Agronomy Journal 94, 153-171.

EEG, 2017. Renewable Energy Sources Act from 21.07.2014 – latest version 17.07.2017. German Parliament, Berlin, Germany. Available at https://www.clearingstelle-eeg.de/files/node/8/EEG_2017_Englische_Version.pdf (accessed 28 November 2017).

European Parliament, 2009. Directive 2009/28/EC of the European Parliament and Council of 23 April 2009 on the promotion of the use on energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. European Parliament, Brussels, Belgium. Available at http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32009L0028 (accessed 19 June 2014).

FNR, 2016. Bioenergy in Germany – Facts and figures, edition 2016. Agency for Renewable Resources Germany, Guelzow-Pruezen, Germany.

Freibauer, A., Rounsevell, M.D.A., Smith, P., Verhagen, J., 2004. Carbon sequestration in the agricultural soils of Europe. Geoderma 122, 1-23.

Geißendörfer, H., 2012. Neues Energiepflanzenprojekt gestartet (New energy crop project started). In: Biogas Journal, Sonderheft 2. Edition, pp. 30-31.

Heiermann, M., Idler, C., Herrmann, C., Scholz, V., 2009. Estimating the impact of plant species and ensiling on substrata quality and biogas. Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Potsdam, Germany.

Heinz, M., 2015. Riesenweizengras - eine Energiepflanze mit Zukunft (Tall wheatgrass – a potential energy crop). Biogas Journal 2/2015, 48-50.

Herbes, C., Jirka, E., Braun, J.P., Pukall, K., 2014. Der gesellschaftliche Diskurs um den Maisdeckel vor und nach der Novelle des Erneuerbaren-Energien-Gesetzes (EEG) 2012 (The social discourse on the maize cap before and after the 2012 amendment of the German renewable energies act (EEG)). GAiA 23, 100-108. doi: 10.14512/gaia.23.2.7

Kiss, J., Komáromi, J., Bayar, K., Edwards, C.R., Hatala-Zsellér, I., 2005. Western corn root worm (*Diabrotica virgifera virgifera* LeConte) and the crop rotation systems in Europe. In: Vidal, S., Kuhlmann, U., Edwards, C.R., Western corn root worm – Ecology and management. CABI Publishing, Oxfordshire, England, pp. 189-220.

Liu, X., Herbert, S.J., Hashemi, A.M., Zhang, X., Ding, G., 2006. Effects of agricultural management on soil organic matter and carbon transformation – a review. Plant Soil and Environment 52, 531–543.

Liu, Z.W., Wang, R.C., 2011. Alkar – tall wheatgrass. United States Department of Agriculture – Natural Resources Conservation Center, Pullmann, USA.

Mast, B., Lemmer, A., Oechsner, H., Reinhardt-Hanisch, A., Claupein, W., Graeff-Hönninger, S., 2014. Methane yield potential of novel perennial biogas crops influenced by harvest date. Industrial Crops Products 58, 194-203.

Moore, G., Sanford, P., Wiley, T., 2006. Perennial pastures for western Australia. Department of Agriculture and Food Western Australia, Perth, Australia.

Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E., 2007. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, England.

Pimentel, D., Allen, J, Beers, A., Guinand, L., Linder, R., McLaughlin, P., Meer, B., Musonda, D., Perdue, D., Poisson, S., Siebert, S., Stoner, K., Salazar, R., Hawkins, A., 1987. World agriculture and soil erosion – Erosions threatens world food production. BioScience 37, 277-283.

Platen, R., Konrad, J., Glemnitz, M., 2017. Novel energy crops: an opportunity to enhance the biodiversity of arthropod assemblages in biomass feedstock cultures? International Journal of Biodiversity Science, Ecosystem Science and Management 13, 162-171.

Scheinost, P., Tilley, D., Ogle, D., Stannard, M., 2008. Tall wheatgrass – Plant guide. United States Department of Agriculture – Natural Resources Conversation Service, Pullman, USA.

Schittenhelm, S., Reus, D., Kruse, S., Hufnagel, J., 2011. Assessment of productivity and profitability of sole and double-cropping for agricultural biomass production. Journal of Cultivated Plants 63, 387-395.

Sutton, W.R., Block, R.I., Srivastava, J., 2008. Adaption to Climate Change in Europe and Central Asia Agriculture. In: Fey, M., Managing Uncertainty: Adapting to Climate Change in ECA Countries, World Bank. Available at http://siteresources.worldbank.org/ECAEXT/Resources/258598-1243892418318/ECA_ClimateChangeAdaptation_AgricultureRepor.pdf (accessed 07 December 2017).

Tröster, M., 2015. Trockenheit setzt Mais zu. Bietigheimer Zeitung online. Available at http://www.swp.de/bietigheim/lokales/landkreis_ludwigsburg/Trockenheit-setzt-Mais-zu;art1188795,3365644 (accessed 17 December 2015).

USDA, 2014. United States Department of Agriculture – Agricultura Research Service – Germplasm Resources Information Network. Germplasm Resources Laboratory, Beltsville, USA. Available at http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?314153 (accessed 21 July 2014).

Weintraub, F.C., 1953. Grasses introduced into the United States; United States Department of Agriculture, Handbook 58. United States Government Print Office, Washington, USA.

Zebisch, M., Grothmann, T., Schröter, D., Hasse, C., Fritsch, U., Cramer, W., 2005. Climate change in Germany – Vulnerability and adaption strategies of climate-sensitive sectors – Summary. Potsdam Institute for Climate Impact Research, Published by Federal Environmental Agency of Germany, Dessau, (Germany).

Zheng, Y., Zhongli, P., Zhang, R., Labavitch, J., Wang, D., Teter, S., Jenkins, B.M., 2007. Evaluation of different biomass materials as feedstock for fermentable sugar production. Applied Biochemistry and Biotechnology 137, 423-435.

2. Light/darkness germination as a function of drought, hydropriming, prechilling and nitrate of four provenances of tall wheatgrass (*Agropyron elongatum spp.*) in different temperatures

Figure 2: Germination of tall wheatgrass seeds.

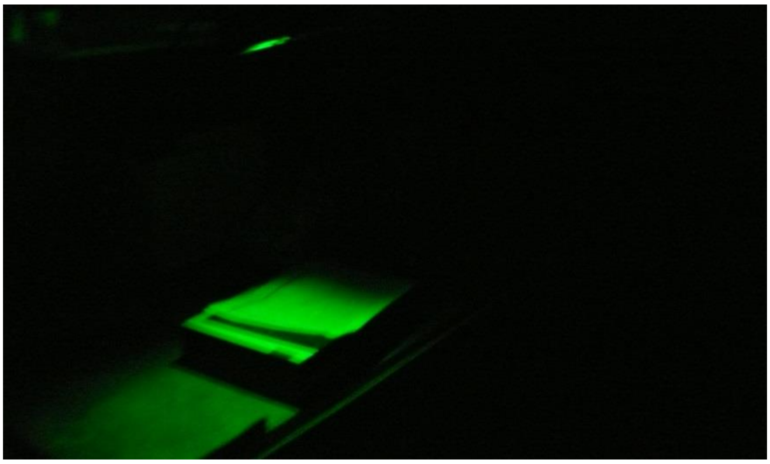

Figure 3: Counting tall wheatgrass under green safety light.

Abstract

Agropyron elongatum is a new energy crop in Europe and considered to be drought tolerant. Therefore, it appears adapted to the predicted climate change. However, in some regions, field emergence is insufficient and may be strongly affected by insufficient germination. This study examined the influence of environmental conditions and priming treatments on germination of *A. elongatum* provenances bred in various regions. Seeds of four provenances were hydroprimed, not primed, or prechilled, before starting tests at 10°C, 20°C, or alternating temperatures under either light or darkness. In addition, drought stresses of -0.1 and -1 MPa were induced and compared to 0 MPa and KNO_3^- solution treatments. Germination Speed Index (GSI) and percentage germination were measured until day 22 as well as induced dormancy in a following test. GSI was most affected by water availability and especially severe drought of -1 MPa inhibited germination. Priming and higher temperature induced fast germination, and interactions were significant. Percentage of germination under intense drought stress could be enhanced by alternating temperature and complete darkness from 9% to 51%. Provenances differed in percentage germination and GSI, but factor interactions were not significant. In conclusion, farmers should adapt cultivation to water availability by varying seeding depth and irrigation that needs to be in the focus of further investigations.

2.1. Introduction

Arable farming is currently facing new challenges: Climate change will influence growing conditions, and biomass production for bioenergy has increased as part of the prevailing energy transition of the European Union (von Gehren et al., 2016). In Europe, biogas production is an important bioenergy production pathway. Hence, the cultivation of maize, the most important biogas crop, has increased (Kiesel and Lewandowski, 2017). The high acreage of maize has led to phytosanitory problems, public concerns, biodiversity decrease (Herbes et al., 2014; Schittenhelm et al., 2011) and economic dependency of farmers. Alternative crops for biogas production are therefore needed in order to mitigate these problems. Initial research has indicated that tall wheatgrass (*Agropyron elongatum* (Host) P. Beauv.) has a chance to substitute maize, as it can reach equivalent specific methane yields (Amon et al., 2007; Heiermann et al., 2009; Mast et al., 2014) and biomass yields (Heinz, 2015).

The increasing role of arable farming in the current energy transition takes place against the background of global climate change. Reports forecast increasing frequency of droughts (Parry et al., 2007) and a resultant 25% reduction of biomass yields by 2080 (Cline, 2008).

Raising the share of drought-tolerant crops on arable land, such as *A. elongatum* (Moore et al., 2006), is an agricultural response to climate change threats and a safety factor for stable yields in the future. Unfortunately, research (Scheinost et al., 2008) and farmers report irregular field emergence of tall wheatgrass in Europe. That might be the result of a low germination rate due to suboptimal field conditions like soil compaction (Batey, 2009; Larsen and Isely, 1967), specific environmental conditions that inhibit germination (Hartmann et al., 2010) or a small specific range of environmental interactions for high germination. Especially intense drought stress can completely inhibit germination of tall wheatgrass (Pouzesh et al., 2012). This can cause serious problems when establishing tall wheatgrass in Europe, where frequent drought periods in spring or autumn are predicted under climate change (EEA, 2016). Even though tall wheatgrass is supposed to be drought-tolerant (Moore et al., 2006), the period of germination seems to be a critical phase.

Low germination because of drought stress can be reinforced or attenuated by temperature. The more water that is available, the larger is the range of temperatures that is favorable for germination (Knipe, 1973). Alternating temperature can especially influence drought effects on germination in many species (Knipe, 1973). For example, the low germination rate under drought observed in western wheatgrass (*Agropyron smithii*), which is a close relative to *A. elongatum*, is increased by an alternating temperature of approximately 15°C / 25°C and was independent from the illumination treatment. In Central Europe, it is suggested to sow *A. elongatum* in early spring, when temperatures are low, to enable a long growing season for establishing *A. elongatum* in the field, or after the harvest of a preceding crop in the summer, when temperatures are higher. Nevertheless both strategies have issues with low germination. A number of other factors may influence germination, like light or darkness. Both presence and/or absence of light, as influenced by seeding depth, have been shown to improve germination under unfavorable conditions for *Agropyron smithii* (Toole 1976), *Cynosurus cristatus*, *Poa annua* and *Poa trivialis* (Williams, 1983).

Also, varieties have adapted their germination capability to the prevailing environmental conditions of their origin. For example, populations of *Agropyron spicatum* differ significantly in germination when they are grown outside of optimum temperatures. Young et al. (1981) found that 54% of cultivar ‘P-737’, but only 12% of ‘P-739’, germinated at 2°C. Likewise, the environmental conditions during seed development and maturing influence the germination capability of the seeds (Bewley and Black, 1994; Brown, 1995). Li and Liu (2016) reported that certain responses of many plants to specific environmental conditions, e.g. to drought stress, persist in seeds' memory and contribute to quick adaption to environmental conditions in the plant's next generation. These effects of genetic adaption and seed development that effect germination can be condensed to the factor of seed provenance. Cultivation of energy crops for the purpose of biogas production typically increases the soil nitrate content due to the return of nutrients to the field in the form of biogas digestate. Many grassland species, like perennial ryegrass (*Lolium perenne*), browntop (*Agrostis capillaries*) or Kentucky bluegrass (*Poa pratensis*), increase germination in the presence of KNO_3^- or after priming with KNO_3^- (Lush and Birkenhead, 1987). The interaction of drought, temperature, provenance and illumination might lead to heterogeneous germination and hence, high variance in field emergence of *A. elongatum* under Central European environmental conditions.

Farmers request adequate field establishment for new crops before replacing maize. As a consequence, knowledge is needed to adapt farming methods like time of seeding to the specific conditions, i.e. temperature and rainfall, of central Europe to ensure well-established plants.

Priming is an additional technique for mitigating risks and problems in field establishment. It can improve germination time, synchronize germination (Bewley and Black, 1994; Brocklehurst and Dearman, 2008) and reduce induced dormancy (CFIA, 2012; Schopfer and Brennicke, 2010). Hydropriming, osmopriming and prechilling are common techniques for priming. Preliminary research by Pouzesh et al. (2012) reported better germination of tall wheatgrass after hydropriming (73%) than after osmopriming (63%). Especially at severe drought stress, hydropriming enhanced germination by 25% when compared to the unprimed control (Pouzesh et al., 2012). Prechilling is a further promising priming technique (Schopfer and Brennicke, 2010) that increases germination in many species (Lonati et al., 2009) and is recommended for *A. elongatum* testing (ISTA, 2012). However, there are studies with conflicting results that show more complete germination without prechilling than a 5-day prechilling at 5°C (Thornton, 1966).

Determining the interactive effects of temperature, drought and illumination on germination of tall wheatgrass provenances bred in different environments is necessary in order to understand what conditions may be unfavorable for germination in Central Europe. To enhance field establishment, the best suited priming technique for improving germination under unfavorable conditions needs to be identified. To this day, there have any studies on germination of tall wheatgrass in Central Europe been published.

Therefore a germination experiment was performed with Petri dishes to test the hypotheses that:

1) Germination under drought stress is limited by too low temperatures at the common sowing dates in Central Europe;
2) seed provenance and illumination have an influence on germination;
3) priming increases germination under drought in Central European conditions; and
4) potassium-nitrate improves germination.

2.2. Material and methods

A germination test was conducted in Petri dishes with the factors of provenance, pretreatment, temperature, illumination and medium. The factor medium included two drought levels: a control and a KNO_3^- treatment (Table 1).

2.2.1. Experimental factors and factor levels

Seeds of four provenances of *A. elongatum* were contributed by seed-breeding companies. They were harvested across four continents (Table 2), 6-12 months before the test started, and were stored in dry paper bags in the dark at 10°C and 50% air humidity, after they arrived.

For the hydropriming treatment, seeds were soaked in water for 12 hours at 25°C. Afterwards, soaked seeds were dried at an average temperature of 25°C (Tavili et al., 2009) in a cabinet dryer with medium airflow for 30 hours, after which no further reduction of seed weight occurred. To protect seeds from light, the complete procedure was performed under a green safety light. The International Seed Testing Association rules (ISTA, 2012) were followed for the prechilling treatment.

Three temperature regimes were tested consecutively in the same climate cabinet, each for 22 days. First, an alternating temperature of 10°C and 20°C, within a day and night cycle, was set. This treatment started with 20°C for 13 hours, cooled down to 10°C within 3 hours, and was held at 10°C for 5 hours before heating up to 20°C within 3 hours. In the second temperature regime, a temperature of 10°C was held constant. For the last temperature regime, a temperature of 20°C was maintained in the climate cabinet. Seeds were counted in another climate chamber at the same temperature as the temperature treatment.

Illumination was varied by keeping half of the Petri dishes in light-proofed boxes; the other half were kept in translucent boxes that permitted exposure to an artificial day and night cycle (16 hours of light and 8 hours of darkness per day). The temperature settings in the climate cabinet were synchronized with the artificial day and night cycle. The most intense wavelengths of the fluorescent tube were 400–450 nm and 530–640 nm.

Germination was tested under three different drought treatments: control (distilled water), slight drought (-0.1 MPa) and severe drought (-1.0 MPa) (ISTA, 2012) and were additionally placed in a 0.2% potassium nitrate (KNO_3^-) solution (ISTA, 2012). Seeds in the two drought treatments were immersed in PEG 6000 (Michel and Kaufmann, 1973). As temperature influences osmotic potentials at given PEG concentrations, different amounts of PEG were dispensed in distilled water at the various test temperatures, following the calculation of Michel and Kaufmann (1973). For the alternating temperature treatment, PEG concentration was calculated for a mean temperature of 15°C. To prevent dishes from dehydration, the covers and bottoms were sealed with Parafilm™. Because of gas exchange from regularly opening and counting seeds, water loss was compensated for by adding distilled water (Ma, 2010). Overcompensating and unintentionally reducing concentrations of PEG and KNO_3 were avoided by weighing the Petri dishes and keeping the weight constant.

Table 1: Factors and levels of germination test.

Factor	**Levels**
Provenance of seeds	Australia, Argentina, Hungary, USA
Pretreatment	No pretreatment, hydropriming, prechilling
Illumination	16 hours light, complete dark
Temperature	Constant 10°C, alternating 10/20°C, constant 20°C
Medium	Control (0 MPa), slight drought (-0.1 MPa), severe drought (-1 MPa) KNO_3^-

Table 2: Thousand-seed weight (TSW) of *Agropyron elongatum* provenances coated (with husks) and uncoated (separated from husks). Seeds were harvested in 2012.

	Provenance			
	Argentina	Australia	Hungary	United States of America (USA)
TSW coated seeds (g)	7.88	7.46	6.60	5.25
TSW uncoated seeds (g)	4.47	5.27	5.02	4.08
TSW ratio of uncoated:coated seeds	0.73	0.71	0.76	0.78

2.2.2. General conditions of germination testing

Twenty seeds were placed on 80 mm diameter filter paper disks moistened with a 2 ml solution in sterilized plastic Petri dishes. Dishes were placed in transparent boxes or lightproof wooden boxes in a climate cabinet (Rumed, Laatzen, Germany). Seeds were monitored daily for 10 days and every second day thereafter. To control for the effect of illumination, the number of germinated seeds in the dark treatment was counted in green safety light (Opitz von Boberfeld et al., 2001) with a wavelength between 500 and 600 nm. They were defined as germinated when the radicle was visible (Butler et al., 2014; Ma, 2010) and were then removed from the Petri dishes. After 22 days, the test was terminated. To assess induced dormancy by environmental conditions, non-germinated seeds were cleaned of PEG and KNO_3^- by washing in distilled water and placed on new wet filter paper. They were kept in a 24 hour illuminated room at approximately 22°C for another 28 days, as advised by CFIA (2012), after which the number of germinated seeds was counted.

2.2.3. Measurements

Response variables were cumulated germination in 22 days (G_{22d}), maximum germination (G_{max}), which was determined for evaluating the conditions that cause secondary dormancy (CFIA, 2012), and Germination Speed Index (GSI). G_{max} was the proportion of seeds that germinated over the total 50-day period, including both the first 22 days and the 28-day period assessing secondary dormancy.

GSI is a time-weighted cumulative germination index that was calculated based on Maguire's (1962) formula:

GSI = $[\frac{N1}{d1} + \frac{N2}{d2} + \cdots + \frac{Nn}{dn}]$ with

N = Number of seeds germinated on day 1, 2, …, n

d = days after start; beginning at first day after start (1)

Quotients of GSI can range between 0, when there was no visible germination over 22 days, and 20 for complete germination on the first day.

2.2.4. Data analysis

This experiment employed a fully factorial block design with four replicates and five factors (Table 1). Replicates were tested in separate boxes, and light and dark treatments within a replicate were tested in separate boxes. Boxes in the climate cabinet and Petri dishes within those boxes were rotated following each counting.

For comparable data analysis, the cumulated percentage germination after the 22ndday (G_{22d}) and maximum germination (G_{max}) were expressed in relative values. To meet assumptions of normality and homogeneity of variances, G_{22d} and G_{max} were transformed by the arcsine of the square root prior to analysis (Gomez and Gomez, 1994). Before transformation, data of 0% and 100% were replaced by $1/(4n)$ and $100 - [1/(4n))]$ (where *n* represents the number of tested seeds; Knödler, 2001), respectively. The absolute value of GSI was log-transformed after adding one to each value.

Statistical analysis was conducted using the statistical software R, version 3.1.0 (R Core Team, 2015) and the packages “nlme” (Pinheiro et al., 2016) and “lsmeans” (Lenth, 2015). Linear mixed effects models for G_{22d}, G_{max} and GSI contained the fixed effects of provenance, illumination, temperature and medium (drought level and KNO_3^-). For the analysis of the pretreatment effect, the fixed factor pretreatment was included. Taking the experimental design of a split plot with different boxes into account, the models contained random effects of the main plot “repetition” and the sub-plot “illumination”. For significant effects post-hoc comparison of means were performed using Tukey tests. A significance level of $\alpha = 0.01$ was chosen throughout.

2.3. Results

2.3.1. Provenance

Effects of temperature, illumination, water availability and provenance on germination of *A. elongatum* were analyzed. Interactive effects between provenance and the other factors were absent for G_{22d} and GSI (Table 3). G_{22d} differed significantly between the provenances of the USA (65.7%), on the one hand, and those of Australia (75.7%), Argentina (73.3%) and Hungary (74.5%) on the other hand (Table 4). In contrast, the GSI of the US provenance (3.94) was significantly ($p < 0.01$) lower than the Argentinian (4.47) and Australian (4.67) provenance but was on the same level as the Hungarian (4.09) provenance. The effect of provenance on G_{max} was similar to its effect on G_{22d} and stronger than the temperature effect. Significant interactive effects of the provenance were only found with the temperature. US provenance had a lower G_{max} in the constant temperature of 10°C (80.8%) than in alternating temperatures of 10°C/20°C (92.2%) and the Argentinian provenance had a higher G_{max} in alternating temperatures (96.7%) than in constant 20°C (90.0%) temperature.

2.3.2. Environmental effects

Drought was the experimental factor with the strongest effect on G_{22d}, GSI and G_{max} (Table 3). In medium x temperature x illumination interaction, intense drought stress strongly decreased G_{22d} compared to the control (on average, 15.9% compared to 91.6%), whereas slight drought (90.8%) and the KNO_3^- treatment (90.9%) did not differ significantly from the control. Temperature and illumination only affected G_{22d} under intense drought stress. Darkness in combination with alternating temperature increased G_{22d} significantly, to a value of 50.9%, compared to the darkness treatment in 10°C and 20°C. The same was true for GSI, which was significantly reduced under intense drought stress (mean GSI = 0.23) in all temperature x illumination interactions, an effect that was mitigated by a combination of alternating temperatures and darkness. Even the GSI was significant for the interaction of temperature, illumination and drought effect, the illumination had no significant effect on GSI at a specific drought treatment in a specific temperature regimen. The GSI was significantly lower under 10°C than under 20°C or alternating temperature in light and in darkness in the slight drought treatment, the potassium nitrate treatment and the control treatment.

Table 3: Results of linear mixed effects models testing the effects of *Agropyron elongatum* provenances (P), temperature (T), illumination (I), drought/potassium nitrate (D), and their interactions on percentage germination on the 22nd day (G_{22d}), Germination Speed Index (GSI) and maximum percentage germination until day 50 (G_{max}) of *A. elongatum*.

Effect	G_{22d}		Germination Speed Index (GSI)		G_{max}	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
P	**20.98**	**< 0.0001**	**19.19**	**< 0.0001**	**25.25**	**< 0.0001**
T	**17.98**	**< 0.0001**	**459.95**	**< 0.0001**	**13.30**	**< 0.0001**
I	3.54	0.1566	0.01	0.9237	0.02	0.9051
D	**705.54**	**< 0.0001**	**3285.77**	**< 0.0001**	3.30	0.0210
P x T	1.15	0.3348	2.02	0.0633	**3.20**	**0.0047**
P x I	1.56	0.1996	1.15	0.3308	1.59	0.1926
P x D	0.94	0.4899	1.35	0.2096	1.60	0.1154
T x I	**8.69**	**0.0002**	1.77	0.1717	0.19	0.8250
T x D	**5.12**	**0.0001**	**43.20**	**< 0.0001**	2.61	0.0177
I x D	**9.42**	**< 0.0001**	**14.60**	**< 0.0001**	0.62	0.6043
P x T x I	0.13	0.9927	0.82	0.5522	0.70	0.6500
P x T x D	1.25	0.2189	1.10	0.3539	1.01	0.4480
P x I x D	1.25	0.2648	1.11	0.3562	0.71	0.6978
T x I x D	**8.04**	**< 0.0001**	**12.09**	**< 0.0001**	0.44	0.8520
P x T x I x D	0.56	0.9245	0.67	0.8418	0.62	0.8864

Table 4: Effect of *Agropyron elongatum* provenances on percentage germination at 22nd day (G_{22d}), Germination Speed Index (GSI) and maximum percentage germination (G_{max}), the latter in interaction with temperature. Letters indicate significant differences of means within target variable ($p < 0.01$).

	Provenance							
	Argentina		Australia		Hungary		United States of America (USA)	
G_{22d}	73.3	a	75.7	a	74.5	a	65.7	b
GSI (Germinating Speed Index)	4.47	ab	4.67	a	4.09	bc	3.94	c
Maximum percentage germination								
10°C	90.5	abcd	94.4	ab	95.2	ab	80.8	d
10°C/20°C	96.7	a	95.8	ab	95.0	ab	92.2	abc
20°C	90.0	bcd	94.1	ab	92.8	ab	84.7	cd

Table 5: Percentage germination of *Agropyron elongatum* at 22nd day (G_{22d}) of three-way interaction temperature, illumination and drought/potassium nitrate (KNO_3^-) across four provenances. Small letters indicate significant differences for $p < 0.01$.

	Temperature						
	Constant 10°C		Alternating 10°C / 20°C		Constant 20°C		Means of medium
	Illumination		Illumination		Illumination		
	Dark	Light	Dark	Light	Dark	Light	
Medium: Drought/KNO_3^-:							
KNO_3^-	91.6 a	90.0 abc	92.2 a	92.2 abc	90.9 a	88.8 abc	90.9
Control (0 MPa)	85.3 ad	90.0 abc	95.6 a	95.3 ab	91.6 a	91.9 ab	91.6
Slight (-0.1 MPa)	89.4 a	90.6 abc	93.4 a	92.8 ab	86.9 a	91.6 abc	90.8
Intense (-1 MPa)	16.3 cf	6.3 ef	50.9 be	9.4 ef	3.8 f	9.1 def	15.9
Means of temperature x illumination	70.6	69.2	83.0	72.4	68.3	70.3	
Means of temperature	69.9		77.7		69.3		

Table 6: Germination Speed Index (GSI) of *Agroypron elongatum* seeds of three-way interaction temperature, illumination and drought/potassium nitrate (KNO_3^-), according to ANOVA (Table 3). Small letters indicate significant differences for $p < 0.01$.

Temperature	Constant 10 °C		Alternating 10 °C / 20 °C		Constant 20 °C		
	Illumination		Illumination		Illumination		Means of medium
	Dark	Light	Dark	Light	Dark	Light	
Medium: Drought/KNO_3^-:							
KNO_3^-	3.17 fe	3.36 cdf	5.79 bd	6.46 abe	6.77 abcd	6.65 abe	5.64
Control (0 MPa)	3.27 fe	3.58 cdf	6.33 abcd	7.33 abe	7.69 ac	7.29 abe	5.91
Slight (-0.1 MPa)	3.21 fe	3.32 cdf	5.86 bd	6.46 abe	6.77 abcd	6.65 abe	5.38
Intense (-1 MPa)	0.17 h	0.06 hg	0.87 g	0.11 hg	0.05 h	0.13 hg	0.23
Means of temperature x illumination	2.45	2.58	4.71	5.15	5.44	5.41	
Means of temperature	2.52		4.93		5.43		

2.3.3. Strategies of improvement

The pretreatment showed significant interactions with temperature and medium for G_{22d} as well as for GSI (Table 7). It was obvious that hydropriming had neither a positive nor a negative influence on G_{22d} or GSI when compared to the control. Prechilling on its own did not increase G_{22d} but increased GSI in the control, KNO_3^- and slight drought treatments, whereas no positive effect was observed under intense drought stress. In particular, decreased temperatures caused an increase of GSI by treatment prechilling. The GSI was lowest at 10°C (Table 8), but was more than doubled (factor 2.5) by prechilling, whereas, the factor was 1.7 for 20°C. For G_{max}, interactions were not significant, but hydropriming resulted in a slightly lower G_{max} (89.5%) than the control treatment (91.8%).

Table 7: Results of analysis of variance (ANOVA) testing the effect of pretreatment (PT) on *Agropyron elongatum* provenances (P), temperature (T), illumination (I), drought/potassium nitrate (D), and their interactions on percentage germination on 22nd day (G_{22d}), Germination Speed Index (GSI) and maximum percentage germination until day 50 (G_{max}). Only results for interactions including the factor pretreatment are shown.

Effect	G_{22d}		Germination Speed Index (GSI)		G_{max}	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
PT	**11.887**	< **0.0001**	**1076.062**	< **0.0001**	**12.67**	< **0.0001**
P x PT	**4.732**	< **0.0001**	**18.022**	< **0.0001**	2.39	0.0271
T x PT	0.969	0.4234	**33.465**	< **0.0001**	1.38	0.2403
I x PT	1.490	0.2259	**7.247**	**0.0008**	0.33	0.7161
D x PT	1.021	0.4100	**143.500**	< **0.0001**	1.62	0.1388
P x T x PT	0.611	0.8338	0.861	0.5875	1.21	0.2733
P x I x PT	1.026	0.4068	1.338	0.2373	0.56	0.7661
T x I x PT	2.783	0.0257	1.591	0.1745	0.45	0.7718
P x D x PT	0.527	0.9465	1.599	0.0539	0.89	0.5904
T x D x PT	**3.361**	< **0.0001**	**4.718**	< **0.0001**	1.00	0.4429
I x D x PT	1.215	0.2959	0.866	0.5193	0.38	0.8942
P x T x I x PT	0.677	0.7750	1.066	0.3857	0.80	0.6495
P x T x D x PT	0.606	0.9682	0.872	0.6854	0.77	0.8336
P x I x D x PT	0.971	0.4919	0.774	0.7328	0.46	0.9740
T x I x D x PT	1.871	0.0344	1.978	0.0234	1.44	0.1435
P x T x I x D x PT	1.253	0.1483	1.042	0.4034	0.65	0.9476

Table 8: Percentage germination at 22nd day (G_{22d}) and Germination Speed Index (GSI) of *Agropyron elongatum* seeds at various temperature and drought after pretreatment. Small letters indicate significant differences for $p < 0.01$ within a column grouped by medium (highlighted by indices). Potassium nitrate = KNO_3^-.

		G_{22d}			GSI		
		Temperature			Temperature		
Medium: Drought/ KNO_3^-	Pretreatment	Constant 10°C	Alternating 10°C / 20 °C	Constant 20°C	Constant 10°C	Alternating 10°C / 20 °C	Constant 20°C
KNO_3^-	No	90.8 a_1	92.2 a_5	89.8 a_9	3.26 b_{13}	6.24 b_{17}	7.43 b_{21}
	Hydropriming	88.8 a_1	88.6 a_5	88.1 a_9	3.67 b_{13}	6.16 b_{17}	7.28 b_{21}
	Prechilling	89.4 a_1	92.3 a_5	88.3 a_9	8.25 a_{13}	10.23 a_{17}	12.81 a_{21}
Control (0 MPa)	No	87.7 a_2	95.5 a_6	91.7 a_{10}	3.42 b_{14}	6.83 b_{18}	7.49 b_{22}
	Hydropriming	87.0 a_2	91.7 a_6	89.5 a_{10}	3.79 b_{14}	6.66 b_{18}	7.61 b_{22}
	Prechilling	90.0 a_2	90.6 a_6	90.0 a_{10}	9.48 a_{14}	10.64 a_{18}	14.14 a_{22}
Slight (-0.1 MPa)	No	90.0 a_3	93.1 a_7	89.2 a_{11}	3.26 b_{15}	6.16 b_{19}	6.71 b_{23}
	Hydropriming	86.7 a_3	90.8 a_7	88.8 a_{11}	3.74 b_{15}	6.09 b_{19}	6.55 b_{23}
	Prechilling	87.2 a_3	92.8 a_7	85.6 a_{11}	7.68 a_{15}	10.22 a_{19}	11.94 a_{23}
Intense (-1 MPa)	No	11.3 ab_4	30.2 a_8	4.4 ab_{12}	0.12 a_{16}	0.49 a_{20}	0.09 a_{24}
	Hydropriming	11.4 a_4	24.2 ab_8	4.2 b_{12}	0.12 a_{16}	0.37 ab_{20}	0.06 a_{24}
	Prechilling	4.7 b_4	16.4 b_8	10.6 a_{12}	0.05 a_{16}	0.24 b_{20}	0.16 a_{24}

2.3. Discussion

In recent years, there was the problem of low germination and field emergence of the new energy crop *A. elongatum* in Central European farming. To determine the reason for low germination and to find a solution for improved germination, a germination test with Petri dishes was conducted. Its focus was on the seed provenance, the Central European environmental conditions for germination, i.e. temperature, drought and availability of KNO_3^-, illumination, and improvement by pretreatments. While intense drought stress diminished germination, alternating temperatures improved germination significantly under intense drought stress in the darkness treatments. The amount of induced dormancy was small in all treatments. Prechilling increased the GSI with the highest impact in cold temperatures. The parameter GSI was chosen because of low germination under intense drought stress. Other more common indices for the germination speed, like the t_{50}, are more difficult to interpret with a wide range of germination as seen in this study (Ranal and de Santa, 2006; Thomson and El-Kassaby, 1993).

2.3.1. Provenance

Except for germination under the influence of intense drought stress, the G_{22d} of *A. elongatum* was on a high level (91%) across all provenances and comparable to more than 90% germination of *A. elongatum*, as observed by Moradi et al. (2012). Compared to these results, percentage of germination for *Agropyron smithii*, evaluated by Knipe (1973), did not exceed 80%. In another study, germination capacity of *A. elongatum* could reach 100% (Moradi et al., 2012). The wide range of germination capacity is caused by pre-harvest (Bewley and Black, 1994; Knödler, 2001) and storage conditions (Brown, 1995), seed position in the inflorescence (Gonzalez-Rabanal et al. 1994; Gutterman, 2000) or genetic variations (Andrés and Guillen, 2003), such as in different cultivars. In the present study, these unknown effects on germination were condensed into the factor provenance.

The provenances consistently varied in G_{22d} and GSI across all factor combinations. Hence, the environmental effects affected all provenances in the same way. Furthermore, G_{max} was determined after an additional test period revealed an analogous pattern. The US provenance differed from the other provenances by having the lowest G_{22d}, GSI and G_{max}. It also had the lowest thousand seed weight (TSW = 5.25 g). Usually, there is a strong correlation between germination and seed weight. Higher TSW values are associated with higher germination rates (Larsen and Andreasen, 2004). This might generally explain the difference in total germination and GSI as seen for the Australian (TSW = 7.46 g, GSI = 4.7) and Argentinian (TSW = 7.84 g, GSI = 4.5) provenances, but in spite of the high TSW of the Hungarian provenance (6.60 g), its GSI (4.1) was low compared to that of the Argentinian provenance. Occasionally, smaller seeds have higher germination in dry environments based on their greater access to water that results from their higher surface-to-volume ratios than those of larger seeds (Wulff, 1995). As the provenance to drought interaction was not significant in this study, the lower TSW of the US provenance did not result in higher germination under intense drought stress. In general, the provenances reacted the same way to varying environmental conditions, indicated by no significant interaction with other factors for G_{22d} and GSI. Hence, there was no different strategy of germination but merely an effect of environmental conditions. Therefore, the Australian provenance had the highest G_{22d} and GSI, whereas the US provenance showed the lowest G_{22d} and GSI in all treatments. Compared with other grassland species, faster and higher rates of germination were observed by Rouhi et al. (2011a) for *A. elongatum* than for *Festuca arundinacea*, *Festuca ovina*, and *Bromus inermis*.

The absence of strong effects from temperature or drought on G_{max} suggests a lack of induced dormancy. Reduced or absent dormancy is a typical characteristic of bred crop species, like *Triticum aestivum* or *Hordeum vulgare* (Zöll and Soppe, 2011). While *A. elongatum* has not been bred as intensively as wheat or barley, it has still lost many characteristics of wild grasses from artificial selection pressure and is consequently well adapted to farming.

2.3.2. Environmental effects

Intense drought stress reduced G_{22d} significantly compared to the control, slight drought and KNO_3^- treatments as a result of reduced seed metabolic activity under low water availability (Dutt and Sharma, 1982). Within the intense drought stress treatment, G_{22d} was exceptionally high under alternating temperature treatment in darkness. Dark conditions occur when seeds are buried in the soil where they are better protected from drying out than germinating on the soil surface with exposure to intense solar radiation. As generally found in most of the grass species, light is required by small seeds (Taylorson, 1987) with low thousand seed weight, which contain small amounts of reserve materials (Wang et al., 2008) to ensure germination close to the soil surface, as found in most of the grass species. In contrast, seeds with higher thousand seed weight can germinate under dark conditions, when seeds are covered with soil. The absence of light protects the seedling better from drying out than germination on the soil surface with exposure to intense solar radiation. With respect to the tall wheatgrass seeds, the soil conditions and the equipment Csete et al. (2011) and Scheinost et al. (2008) found that seeding deeper than 2.5 cm reduces field emergence significantly.

Nevertheless, why the positive effect of darkness on germination was just found under intense drought stress at alternating temperatures could not be fully explained. Beside advantages in G_{22d} by alternating temperature, alternating and constant temperature at 20°C enhanced GSI through a quick emergence. The positive effect of alternating temperatures on germination might be promoted by calculating the PEG concentrations in the alternating 10°C and 20°C temperature treatment for a mean temperature of 15°C and elicited osmotic pressures between -1.07 MPa at 10°C and -0.93 MPa at 20°C (cp. Michel and Kaufmann, 1973) for intense drought stress. For 13 hours each day, the temperature was maintained at 20°C. Hence, lower osmotic pressures during that period might have reduced the drought stress and increased germination when compared to 10°C and 20°C, which is comparable to the results of Pouzesh et al. (2012) for different osmotic potentials. Nevertheless, Knipe (1973) found the germination rate of western wheatgrass in alternating temperature was a function of the maximum temperature when the difference between daily minimum and maximum temperature was on a constant level in all treatments. As this study examined only one alternating temperature treatment, further studies should be conducted to find the best suited level of alternating temperatures and thus give advice regarding the optimal month for seeding tall wheatgrass under Central European conditions. Based on the results of this study, it is advisable to sow *A. elongatum* in 2–2.5 cm seedbeds in the late spring or early summer, when cool nights at 10°C and sunny days with 20°C alternate. Furthermore, seeding in this period reduces the risk of being affected by periodic summer drought and enhances field establishment. The subsequent fast field emergence after seeding is crucial for weed management and supports a good sward establishment (Soltani et al., 2001).

No positive effect was determined in G_{22d} and GSI by putting seeds in 0.2% KNO_3^- solution. Consequently, higher nitrogen availability due to mineralization in spring (Bhatti and Cresser, 2015) should not enhance germination of tall wheatgrass and does not influence the choice of seeding period.

2.3.3. Pretreatment

None of the pretreatments affected G_{22d} in *A. elongatum*. For primed *Festuca arundinacea*, Rouhi et al. (2011b) estimated increased germination from 58% to 77%. As expected, however, prechilling increased germination speed (ISTA, 2012; Schopfer, 1989). During the process of priming the physiological conditions of the embryo alter, and enzymes get activated and increase the production of soluble nutrients. Thus the system is prepared for prompt germination (Kattimani et al., 1999). But hydropriming neither increased G_{22d} nor GSI. As in the current study, Rouhi et al. (2011b) found no effect of hydropriming on final germination in the absence of drought stress or, at the least, weak drought stress. However, in contrast to this study, they found hydropriming increased germination rates under strong drought stress at -1.2 MPa. Pouzesh et al. (2012) concluded an increase of germination from 55.6% to 73.2% when seeds were hydroprimed due to faster water uptake and earlier initiation of metabolism processes. At -0.9 MPa, they also found hydroprimed seeds had higher germination (35% vs. 26%), whereas at -1.2 MPa, no germination was identified. The results of Pouzesh et al. (2012) are also in agreement with Moradi et al. (2012), who presented positive effects of hydropriming on germination, which also depended on the duration of hydropriming. Although the duration of hydropriming in this study followed Moradi et al. (2012) and the choice for hydropriming (instead of osmopriming) was based on the work of Pouzesh et al. (2012), differences in germination were not detected between hydropriming and no pretreatment. Moradi et al. (2012) found a significant effect of the duration of hydropriming on germination. Hydropriming seeds of *A. elongatum* for more than 12 to 24 hours did not differ in germination compared to untreated seeds. Extended hydropriming for 36 hours even reduced germination (Moradi et al., 2012). Similar results were reported by Dastanpoor et al. (2013) for other species. In contrast, Tavili et al. (2009) found no impact with duration of hydropriming on germination. However, these studies suggest that hydropriming for 12 hours in the current study was not carried out for too long. Prechilling enhanced GSI and, therefore, appears to be the most appropriate method of seed priming. Even though G_{22d} could be increased by prechilling, rapid germination after seeding can improve field emergence. Further studies are necessary to demonstrate a positive influence of prechilling in field experiments.

2.4. Conclusions

This study found germination capacity of *A. elongatum* at a high level, which contrasts with reports of Central European farmers. Therefore, germination capacity is not a general obstacle for agricultural cultivation of tall wheatgrass. Differences in germination speed (GSI) and G_{22d} of provenances did not interact with the other tested factors. Induced dormancy did not complicate the germination of tall wheatgrass. Seeding in dry conditions causes heterogeneous and slow germination. Prechilling accelerated initial germination in an adequate water supply but did not increase overall germination. Germination speed was considerably lower at temperatures of 10°C. However, total germination did not differ between 10°C and 20°C. It appears that a farmer’s choice of provenance is less important for germination than the environmental conditions during for growth. Seeding at the end of spring or early summer, when temperatures shift between 20°C in the day and 10°C at night, along with a seeding depth of 2–2.5 cm that leads to darkness, can even improve germination if expected precipitation does not occur. The results presented here may provide evidence that helps to increase cultivation of *A. elongatum* as a climate-change adapted crop for biogas production.

2.5. Acknowledgements

The authors thank Prof. Schweizer and Dr. Ahrens from the Fraunhofer Application Center for Inorganic Phosphors in Soest, Germany, who thoroughly tested the wavelengths of the light in the climate cabinet and the green safety light four counting the seeds of the darkness treatments.

2.6. References

Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., Gruber, L., 2007. Biogas production from maize and dairy cattle manure – Influence of biomass composition on the methane yield. Agriculture, Ecosystems and Environment 118, 173–182.

Andrés, A., Guillen, R., 2003. Genetic variability within two adapted population of tall wheatgrass (Thinopyrum ponticum) in Argentina. International Grassland Congress. Available at http://www.internationalgrasslands.org/files/igc/publications/2001/id1203.pdf (accessed 17 July 2014).

Batey, T., 2009.. Soil compaction and soil management – a review. Soil Use and Management 25, 335-345. doi: 10.1111/j.1475-2743.2009.00236.x

Bewley, J.D., Black, M., 1994. Seeds – Physiology of development and germination. 2nd ed. Plenum Publishing Corporation, New York, USA.

Bhatti, A., Cresser, M.S., 2015. Does low N-mineralisation rate in soils under grass help limit nitrate leaching in summer? Chemistry and Ecology 31, 334-344. doi:10.1080/02757540.2015.1008465

Brocklehurst, P.A., Dearman, J., 2008. Interaction between seed priming treatments and nine seed lots of carrot, celery and onion II. Annals of Applied Biology 102, 583–593.

Brown, R., 1995. The water relations of range plants: adaptations to water deficits. In: Bedunah, D.J., Sosebee, R.E. (Eds.), Wildland plants: Physiological ecology and development morphology. Society for Range Management, Denver, USA, pp. 291-413.

Butler, T.J., Celen, A.E., Webb, S.L., Krstic, D., Interrante, S.M., 2014. Temperature affects the germination of forage legume seeds. Crop Science 54, 2846–2853. doi:10.2135/cropsci2014.01.0063

CFIA, 2012. Canadian methods and procedures for testing seed - 2012 version. Canadian Food Inspection Agency, Saskatoon, Canada.

Cline W.R., 2008. Global warming and agriculture. Finance and Development 3, 23-27.

Csete, S., Stranczinger, S., Szalontai, B., Farkas, A., Pal, R.W., Salamon-Albert, E., Kocsis, M., Tovari, P., Vojtela, T., Dezsö, J., Walcz, I., Janowszky, Z., Janowszky, J., Borhidi, A., 2011. Tall wheatgrass cultivar Szarvasi-1 (Elymus elongatus subsp. ponticus cv. Szarvasi-1) as a potential energy crop for semi-arid lands of eastern Europe. In: Nayreripour, M., Kheshti, M. (Eds.), Sustainable growth and application in renewable energy source. InTech, Rijeka, Croatia, pp. 269-294.

Dastanpoor, N., Fahimi, H., Shariati, M., Davazdahemami, S., Hashemi, S.M.M., 2013. Effects of hydropriming on seed germination and seedling growth in sage (Salvia officinalis L.). African Journal of Biotechnology 12, 1223–1228. doi:10.5897/AJB12.1941

Dutt, N.R.G., Sharma, P.K., 1982. Screening chickpea cultivars with polyethylene glycol for drought tolerance during germination. International Chickpea Newslatter 7, 11.

EEA, 2016. Climate change impacts and adaption. European Environment Agency, Copenhagen, Denmark. Available at http://www.eea.europa.eu/soer-2015/europe/climate-change-impacts-and-adaptation (accessed 1 April 2017).

Gomez, K.A., Gomez, A.A., 1994. Statistical procedures for agricultural research. John Wiley & Sons, New York, USA.

González-Ranabal, F., Casal, M. and Trabaud, L., 1994. Effects of high temperatures, ash and seed position in the inflorescence on the germination of three Spanish grasses. Journal of Vegetation Science 5, 289-294.

Gutterman, Y., 2000. Maternal effects of seeds during development. In: Fenner, M. (Ed.), Seeds: The ecology of regeneration in plant communities, 2nd edition. CABI Publishing, Wallingford, UK, pp. 59-84.

Hartmann, H.T., Kester, D.E., Davies, F.T., Geneve, R.L., 2010. Plant Propagation: Principles and Practices. Prentice Hall, New Jersey, USA.

Heiermann, M., Idler, C., Herrmann, C., Scholz, V., 2009. Ermittlung des Einflusses der Pflanzenart und der Silierung auf Substratqualität und Biogasausbeute in Labor und in der Praxis (Estimating the impact of plant species and ensiling on substrata quality and biogas). Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Potsdam, Germany.

Heinz, M., 2015. Riesenweizengras − eine Energiepflanze mit Zukunft (Tall wheatgrass − a potential energy crop). Biogas Journal 2, 48–50.

Herbes, C., Jirka, E., Braun, J.P., Pukall, K., 2014. Der gesellschaftliche Diskurs um den Maisdeckel vor und nach der Novelle des Erneuerbaren-Energien-Gesetzes (EEG) 2012 (The social discourse on the maize cap before and after the 2012 amendment oft he German renewable energies act (EEG)). GAiA 23, 100-108. doi: 10.14512/gaia.23.2.7

ISTA, 2012. International rules for seed testing - Seed science and technology. International Seed Testing Association, Zürich, Switzerland.

Kattimani, K.N., Reddy, Y.N., Rao, B.R., 1999. Effect of pre-sowing seed treatment on germination, seedling emergence, seedling vigour and root yield of Ashwagandha. Seed Science and Technology 27, 483-488.

Kiesel, A., Lewandowski, I., 2017. Miscanthus as biogas substrate – cutting tolerance and potential for anaerobic digestion. Global Change Biology Bioenergy 9, 153-167.

Knipe, O.D., 1973. Western wheatgrass germination as related to temperature, light, and moisture stress. Journal of Range Management 26, 68-69.

Knödler, C., 2001. Keimungsverhalten verbreiteter Festuca-, Brometea- und Molinietalia-Arten (Germination of common Festuca-, Brometea- and Molinietalia-species). Ph.D.-thesis, Justus-Liebig-University, Giessen, Germany.

Larsen, A., Isely, D., 1967. Relation between germination, vigor, and field emergence in alfalfa seed. Proceedings of the Association of Official Seed Analysts 57, 60-66.

Larsen, S.U., Andreasen, C., 2004. Light and heavy turfgrass seeds differ in germination percentage and mean germination thermal time. Crop Science 44, 1710–1720. doi:10.2135/cropsci2004.1710

Lenth, R., 2015. Lsmeans: Least-Squares Means. R package version 2.20-2.

Li, X., Liu, F., 2016. Drought stress memory and drought stress tolerance in plants: biochemical and molecular basis. In: Mohammad Anwar, H., Wani, S.H., Bhattacharjee, S., Burritt, D.J., Tran, L.S.P. (Eds.), Drought stress tolerance in plants, volume 1. Springer International Publishing, Switzerland, pp. 17-44.

Lonati, M., Moot, D.J., Aceto, P., Cavallero, A., Lucas, R.J., 2009. Thermal time requirements for germination, emergence and seedling development of adventive legume and grass species. New Zealand Journal of Agricultural Research 52, 17-29. doi:10.1080/00288230909510485

Lush, W.M., Birkenhead, J.A., 1987. Establishment of turf using advanced 'pregerminated' seeds. Australian Journal of Experimental Agriculture 27, 323-337.

Ma, H., Liang, Z., Wu, H., Huang, L., Wang, Z., 2010. Role of endogenous hormones, glumes, endosperm and temperature on germination of Leymus chinense (Poaceae) seeds during development. Journal of Plant Ecology 3, 269–277. doi:10.1093/jpe/rtp035

Maguire, J.D., 1962. Speed of germination – aid in selection and evaluation for seedling emergence and vigour. Crop Science 2, 176–177.

Mast, B., Lemmer, A., Oechsner, H., Reinhardt-Hanisch, A., Claupein, W., Graeff, S., 2014. Methane yield potential of novel perennial biogas crops influenced by harvest date. Industrial Crops and Products 58, 194–203.

Michel, B.E., Kaufmann, M.R., 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiology 51, 914–916.

Moore, G., Sanford, P., Wiley, T., 2006. Perennial pastures for western Australia. Department of Agriculture and Food Western Australia, Perth, Australia.

Moradi, A., Zadeh, F.S., Afshari, R.T., Amiri, R.M., 2012. The effects of priming and drought stress treatments on some physiological characteristics of tall wheat grass (Agropyron elongatum) seeds. International Journal of Agricultural Crop Science 4, 596–603.

Opitz von Boberfeld, W., Knödler, C., Ziron, C., 2001. Keimungsstrategien von Arten verschiedener Grünland-Pflanzengesellschaften (Germination strategies of species of different grassland communities). German Journal of Agronomy 5, 87–95.

Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E., 2007. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. Nlme: Linear and nonlinear mixed effects models. R package version 3.1, 128.

Pouzesh, H., Abbasi, M., Enayati, A., Hedayati, A., 2012. Investigation the effect of hydropriming and osmopriming treatments on seeds germination of tall wheatgrass (Agropyron elongatum) under drought stress. Annals of Biological Research 3, 4874–4879.

Ranal, M.A., de Santa, D.G., 2006. How and why to measure the germination process? Brazilian Journal of Botany 29, 1–11.

R Core Team, 2015. R: A Language and Environment of Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rouhi, H.R., Aboutalebian, M.A., Sharif-Zadeh, F., 2011a. Effects of hydro- and osmopriming on drought stress tolerance during germination in four grass species. International Journal of AgriScience 2, 107–114.

Rouhi, H.R., Aboutalebian, M.A. and Sharif-Zadeh, F., 2011b. Seed priming improves the germination traits of tall fescue (Festuca arundinacea). Notulae Scientia Biologicae, 3, 57–63.

Scheinost, P., Tilley, D., Ogle, D., Stannard, M., 2008. Tall wheatgrass – Plant guide. United States Department of Agriculture – Natural Resources Conversation Service, Pullman, USA.

Schittenhelm, S., Reus, D., Kruse, S., Hufnagel, J., 2011. Assessment of productivity and profitability of sole and double-cropping for agricultural biomass production. Journal of Cultivated Plants 63, 387-395.

Schopfer, P., 1989. Experimentelle Pflanzenphysiologie – Einführung in die Anwendungen (Experimental Plant Physiology – Introduction and Application), 2nd edition. Springer, Berlin / Heidelberg, Germany.

Schopfer, P. and Brennicke, A., 2010. Pflanzenphysiologie (Plant Physiology), 7th edition. Spektrum Akademischer Verlag, Heidelberg, Germany.

Soltani, A., Zeinila, E., Galeshi, S., Latifi, N., 2001. Genetic variation for and interrelationships among seed vigor traits in wheat from the Caspian Sea coast of Iran. Seed Science Technology 29, 653-662.

Taylorson, R.B., 1987. Environmental and chemical manipulation of weed seed dormancy. Review of Weed Science 3, 135–154.

Tavili, A., Zare, S., Enayati, A., 2009. Hydropriming, ascorbic and salicylic acid influence on germination of Agropyron elongatum Host. seeds under salt stress. Research Journal of Seed Science 2, 16–22.

Thomson, A.J., El-Kassaby, Y.A., 1993. Interpretation of seed-germination parameters. New Forests 7, 123–132.

Thornton, M.L., 1966. Seed dormancy in tall wheatgrass. In: Proceedings of the Association of Official Seed Analysts, vol. 56. Association of Official Seed Analysts, USA, pp. 116-119.

Toole, V.K., 1976. Light and temperature control of germination in Agropyron smithii seeds. Plant and Cell Physiology 17, 1263-1272. doi: 10.1093/oxfordjournals.pcp.a075390

von Gehren, P., Gansberger, M., Mayr, J., Liebhard, P., 2016. The effect of sowing date and seed pretreatments on establishment of the energy plant Silphium perfoliatum by sowing. Seed Science and Technology 44, 1-10.

Wang, L., Huang, Z., Baskin, C.C., Baskin, J.M., Dong, M., 2008. Germination of dimorphic seeds of the desert annual halophyte Suaeda aralocaspica (Chenopodiaceae) a C4 plant without Kranz anatomy. Annals of Botany 102, 757–769.

Williams, E.D., 1983. Effects of temperature, light, nitrate and prechilling on seed germination of grassland plants. Annals of Applied Biology 103, 161-172. doi: 10.1111/j.1744-7348.1983.tb02752.x

Wulff, R.D., 1995. Environmental maternal effects on seed quality and germination. In: Kigel,J., Galili, G. (Eds.), Seed development and germination. Marcel Dekker Inc., New York, USA, pp. 491-507.

Young, J.A., Eckert, R.E., Evans, R.A., 1981. Temperature profiles for germination of blue-bunch and beardless wheatgrasses. Journal of Range Management 34, 84-89.

Zöll, C., Soppe, W.J.J., 2011. Der Schlaf und das Erwachen von Pflanzensamen (The sleeping and waking of plant seeds). In: Jahrbuch des Instituts. Max Planck Institute for Plant Breeding Research, Cologne, Germany. Available at https://www.mpg.de/1169860/Dormanz_Samenkeimung (accessed 6 April 2017).

3. Response of tall wheatgrass (*Agropyron elongatum spp.*) to water stress compared to tall fescue (*Festuca arundinacea*)

Figure 4: Containers with tall wheatgrass and tall fescue at harvest after the period of drought. From the left to the right: Three pots with 85% of field capacity (FC) with the grasses tall fescue, tall wheatgrass of Australian provenance and tall wheatgrass of Hungarian provenance; followed by three pots with 50% FC with grasses in the same order and three pots with 31% of FC.

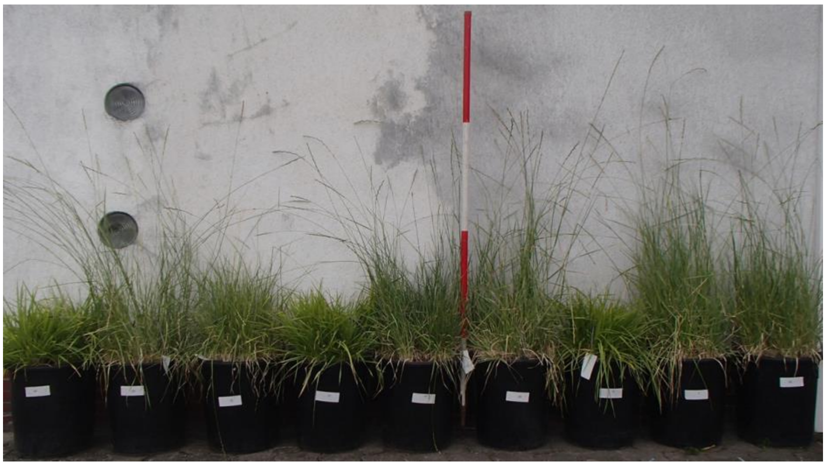

Figure 5: Containers with tall wheatgrass and tall fescue at harvest after the post-drought period. Containers are in the same order as in the figure above.

Abstract

Ecological problems, due to the increasing share of maize for German biogas facilities and the consequent economic dependence on drought-sensitive maize yields, together with extended summer droughts resulting from climate change, are challenges for Central European biogas production. *Agropyron elongatum* is suggested as an alternative crop, providing high methane yields and being potentially suitable for semi-arid regions, although little is known about its drought resistance and resilience in temperate climate.

To address this, the performance of two *A. elongatum* cultivars was compared to that of *Festuca arundinacea* grown in 30 dm^3 containers in an outdoor-climate greenhouse. For 63 days, the soil volumetric water content was maintained at 18% (severe drought), 26% (moderate drought) and 35% (control). Plants were harvested afterwards, and again after a 97 days post-drought period with a soil water content of 35%. The following characteristics were determined: dry matter yield (DMY), leaf area, agronomic water use efficiency (WUE_{agr}), intrinsic water use efficiency via carbon isotope composition, tiller production, stem:leaf ratio, and root biomass at the final harvest.

In the drought period, *F. arundinacea* was more sensitive to severe drought, reducing DMY by 53%, compared to a maximum DMY reduction of 37% for tall wheatgrass. In the post-drought period, previously drought-stressed *A. elongatum* reached higher DMY than the control. The resulting aggregated DMY over both periods was higher than that of the drought-stressed *F. arundinacea*. Morphological adaptations, in response to drought, contributed to increased WUE_{agr} in *A. elongatum* during both the drought and post-drought periods. Consequently, *A. elongatum* offset biomass losses during the severe drought treatment with higher yields in the post-drought period.

The results show the agricultural potential of more stable DMY by *A. elongatum* compared to *F. arundinacea* in the face of climate change-related drought, and should be taken into focus by further researches.

3.1. Introduction

In Germany, maize is the most important crop-derived feedstock for almost 8,000 biogas plants, with an input proportion of 73% fresh matter (FNR, 2017). The increasing share of maize in German crop rotations, as a result of biogas production (Dahlhoff, 2013), has led to phytosanitary and ecological problems (Creutzig et al., 2015; Herbes et al., 2014; Sauerbrei et al., 2014; Schittenhelm et al., 2011) and low economic diversity, causing, in turn, a dependency on maize yields. In addition to the problems of high acreage of maize cultivation, the impact of climate change on the distribution of annual precipitation in Central Europe includes a predicted shift in precipitation from the summer months to the autumn and winter months, while maintaining consistent annual precipitation (EEA, 2016; Zebisch et al., 2005). Limited water availability during the growing season will particularly decrease the dry matter yield (DMY) of shallow-rooting summer annuals, such as maize (Schittenhelm and Schroetter, 2013).

As the cultivation of energy crops is a major contributor to the greenhouse gas emissions of biogas production (Lijó et al., 2014; Pacetti et al., 2015), a high resource use efficiency in the crop production – i.e. high energy output:input ratios obtained by high DMY and low input of agrochemicals – is required (Kiesel and Lewandowski, 2017). In addition, high and stable yields are a prerequisite for profitable bioenergy production, and to control the costs of producing the biogas plants. Therefore, under the forecasted climate change, drought-tolerant energy plants need to be identified and adapted to the impending environmental conditions so as to mitigate the negative impacts of summer drought induced by climate change. New cropping systems should be established to benefit from increasing winter and autumn precipitation through a longer growing season than annual crops, and regrowth after the summer drought (Hickman et al., 2010). Consequently, developing and enhancing adaption of cropping systems and crops to climate change requirements must focus on the drought period and the following period of regeneration.

Perennial crops have been suggested as an alternative to maize. Compared to maize, they offer advantages with regard to the risks of nitrate leaching (Randall et al., 1997) and soil erosion (Pimentel et al., 1987). After a drought period, they have the potential to regenerate as soon as water availability increases, and the resulting regrowth can compensate for yield losses during the preceding drought period. A rapid vegetative growth after the onset of autumn rains, and in late spring, through an increased exploitation of residual moisture supports yield formation (Pecetti et al., 2011). In contrast to maize, the multiple harvests per year of perennial crops allow for a cutting management adapted to the expected precipitation. Perennial grassland species, such as perennial ryegrass (*Lolium perenne*) or tall fescue (*Festuca arundinacea*), could therefore be considered as promising alternative crops in light of the expected future climate in Central Europe. Temperate perennial grasses differ in degrees of drought tolerance, however. Perennial ryegrass is known to be susceptible to drought stress (Hoekstra et al., 2014), while tall fescue is considered to be more drought-tolerant (Perlikowski et al., 2013; Virkajärvi et al., 2012) to periodic summer drought in Europe, Australia and North of America. Problems with using the more drought-tolerant tall fescue include the relatively high costs and the greenhouse gas emissions caused by the relatively high number of cuttings per year that are recommended in order to obtain a high yield and energy content. Therefore, an alternative could be tall wheatgrass (*Agropyron elongatum*), which has already been shown to have high biogas yields (Mast et al., 2014) from a cost-efficient two-cutting system (Dickeduisberg et al., 2017), and is considered to be drought-tolerant (Moore et al., 2006). Hence, tall wheatgrass could have positive effects on Central European biogas production, especially in a changing climate. Tall fescue is gaining increasing attention in forage production in Central Europe, and has been suggested as an alternative to ryegrass swards. Tall wheatgrass – so far – does not play any significant role in herbage production in Central Europe.

The drought resistance and resilience of tall wheatgrass has not been studied in any depth. Some research has examined the response of tall wheatgrass to drought stress in the initial weeks after emergence in semi-arid regions (Bahrani et al., 2010; Sadeghi and Halagh, 2007), or has focused on drought resistance in saline soils (Roundy, 1985). For example, Bahrani et al. (2010) exposed a range of forage species to drought, and obtained the highest DMY under these conditions from tall wheatgrass; however, as the drought stress treatment was applied to two-week-old seedlings and lasted only for 26 days, the results are of limited significance for the longer drought periods that are likely to occur in the future climate.

In general, the effects of drought on the growth and yield formation of temperate grasses has been studied to some extent. Typical responses are a decreasing leaf area, leaf mass, tiller number (López et al., 2013), and a reduced stomatal conductance (Ludlow and Muchow, 1990). Species-specific adjustments to drought events result in different levels of aboveground herbage yield reduction. For tall wheatgrass, little is known about growth responses to drought. Better insight into the drought-resistance mechanisms of tall wheatgrass, and their consequences for DMY, is required.

As has recently been shown for grassland and forage crops, a full account of the consequences of drought on the DMY of multiple harvest crops should address the immediate effect of drought on herbage growth, but should also include the indirect effects on the following regrowth, when water is no longer limited (Carlsson et al., 2017; Hofer et al., 2016). The direct response of the crop to drought is often known as resistance, while responses in the following regrowth are named resilience (Hofer et al., 2016; Pimm, 1984). Therefore, a study of the drought effects on the DMY of tall wheatgrass should not only consider the yield losses occurring during a drought period because an increased post-drought growth might compensate for such earlier yield losses. This would contribute to the mitigation of drought-induced yield losses, when considering the total DMY of a full year, and ensure calculable DMY for biogas production (Zwicke et al., 2013). To assess the drought resistance and resilience of tall wheatgrass, it is necessary to compare it to a reference crop. A container experiment was conducted to evaluate the drought resistance and ensuing resilience on DMY, and the corresponding morphological adaption mechanisms of two tall wheatgrass provenances, compared to tall fescue. A recommended two-cut system was employed, wherein the first regrowth was exposed to drought, while the second regrowth was well supplied with water, in order to assess the resilience of the swards.

The following hypotheses were tested:

1. Tall wheatgrass provenances are both more resistant against severe drought and more resilient during a post-drought period than tall fescue;
2. the differences in drought tolerance are mediated by the intensity of morphological and physiological adaptation in the two species;
3. tall wheatgrass cultivars are able to compensate drought-related DMY losses during the post drought period and thus achieving comparable aggregated DMY under drought and control treatments over the full growing season (two harvests).

3.2. Material and methods

A container experiment was carried out in an outdoor-climate greenhouse near Soest, Germany. Treatments were replicated in four blocks in a factorial arrangement, with tall wheatgrass (*Agropyron elongatum* (Host) P. Beauv.) of Australian provenance (tall wheatgrass AP) and Hungarian provenance (tall wheatgrass HP), and the reference tall fescue (*Festuca arundinacea* Schreb.; cultivar Hykor), and three drought levels (Table 9). The drought period was set up in spring 2014, immediately after a spring cut had been taken, and seven months after the experiment had been sown. The drought stress treatment ended with a regular cut, and was followed by a post-drought period, where the soil moisture content of all containers was maintained at the level of the control treatment.

Table 9: Soil water content in percent (%) of field capacity, and soil water tension at three levels of drought stress in the drought and post-drought periods. Minimum and maximum water tensions, which were observed immediately before watering, are reported. Containers were irrigated up to the target level of percent of field capacity.

	Drought period			Post-drought period		
	Target value		Minimum and maximum soil water tension between watering [bar]	Target value		Minimum and maximum soil water tension between watering [bar]
Drought level	% of field capacity	Soil water tension [bar]		% of field capacity	Soil water tension [bar]	
Control	90	~ 0.13	0.13 – 0.32	90	~ 0.13	0.13 – 0.32
Moderate	67	~ 1.00	1.00 – 1.58	90	~ 0.13	0.13 – 0.32
Severe	46	~ 3.16	3.16 – 6.31	90	~ 0.13	0.13 – 0.45

3.2.1. Location and soil

The greenhouse prevented the containers from being exposed to natural precipitation. Data on global daily radiation, and mean temperatures of the drought and post-drought periods, are presented in Table 10.

The bottoms of the 48 containers, holding 30 dm³ (0.33 m diameter, 0.57 m height), were filled with two litres of crushed rock to protect the plants from the harmful effects of waterlogging. A water- and air-permeable mulch fleece (Windhager, Austria) separated the soil and drainage layers. The rest of the container was filled with approximately 40 kg of dry soil, with a bulk density of 1.2 Mg m^{-3}. Following the soil classification system from Ad-hoc-AG Boden (2005), the soil was a medium clayey silt (16.5% clay, 80.2% silt, 3.2% sand). Before filling the containers, the soil pH (6.5) was measured in $CaCl_2$ (VDLUFA, 1991a). Plant available soil phosphorus (183 mg kg^{-1} soil) and potassium (140 mg kg^{-1} soil) were obtained, using the common calcium-ammonium-lactate (CAL) extraction method (VDLUFA, 1991b). As the phosphorus content was considered to be high, no phosphorus fertilizer was applied. Potassium was added to the soil before the grasses were sown at a rate of 52 mg K kg^{-1} soil. Nitrogen was applied as ammonium sulphate saltpetre at a rate equivalent to 240 kg N ha^{-1} at the beginning of the drought period. The field capacity for determining drought levels was assessed according to Eckelmann et al. (2005). The values were validated by assessing the field capacity from the weight of fully water-saturated and covered containers 72 hours after the watering.

Table 10: Average air temperature and photosynthetic active radiation (PAR) at the experimental site (air temperature was measured each hour 1 m above soil surface; PAR was measured at a weather station in 13 km linear distance).

	Average air temperature [°C]	Deviation from long-term average temperature [°C]	PAR [kWh m^{-2}]	Deviation from long-term average PAR [kWh m^{-2}]
March	7.2	+1.4	36	+3
April	9.7	+0.2	42	-19
May	12.7	-0.8	55	-7
June	15.0	-1.4	60	-4
July	18.7	-0.1	60	-1
August	15.5	-2.9	51	-1

3.2.2. Sward establishment

The seeds were harvested in 2013 by breeding companies. As it is common practice to sow grassland after the harvest of a pre-crop, tall wheatgrass and tall fescue were sown at the beginning of autumn (on 30 August 2013), at a depth of 2 cm (Csete et al., 2011). A template was employed to ensure homogeneous spacing. The number of plants per container was reduced two weeks after emergence to 30 individuals per container. The plants were regularly watered until the end of the growing season. Two weeks after the onset of plant growth in spring, the watering regime was gradually adapted, over a period of two weeks, to achieve different levels of drought. The drought experiment started after a cut at a stubble height of 10 cm on 23 March 2014. During the following drought period, the watering regime was held constant until the first harvest.

3.2.3. Drought stress treatments

The level of severe drought was aimed at maintaining a soil-water content close to the permanent wilting point, but not below. In contrast, the control treatment was targeted at excluding any water limitation. As growing plants increase a container's weight, and influence water-content calculations, the fresh weight was estimated twice during the drought period by destructive harvests of additional containers. Evaporation was controlled by covering the soil with 2 cm of expanded clay that was distributed on the surface after establishment. Pre-tests demonstrated that such a cover decreased evaporation losses down to 44%, compared to bare soil. Adjacent non-planted containers were covered with expanded clay to estimate evaporation losses during the experiment. Evaporation was further decreased by watering the containers at dusk, at low temperature and radiation.

The containers were weighed and watered daily to maintain the target soil moisture content and water uptake that was adjusted by evaporation, to calculate the net water uptake. To ensure a homogeneous distribution of irrigated water in the soil, a special technique was employed. The containers were watered manually with a 100 ml syringe, modified with a 6-mm-diameter injection needle. The top of the aluminium needle was tapered to enable penetration without plugging the needle. Each day, between one and three injections, depending on the amount of water added, were randomly administered in the container. The injection depth varied between 8 cm above the mulch fleece and 5 cm below the soil surface, by chance. Watering was performed carefully to avoid leaching from the bottom of the containers. Nevertheless, the containers were placed on boxes to collect runoff water.

After 63 days of the drought period with induced water stress, the biomass was cut by 5 cm height. Thereafter, a soil water content of 90 % of field capacity was established in all containers within three days, and maintained for the 97 days of the post-drought period, after which the biomass was harvested again.

3.2.4. Measurements

Immediately before each harvest, the number of all visible tillers and elongated tillers that were longer than 45 cm were counted in the containers. In both harvests, the grasses were cut to a height of 5 cm above the soil surface. Samples of the harvested biomass were dried at 105°C to constant weight to establish dry matter content and calculate DMY.

A double-sampling method was utilised to determine the leaf area per container. The leaf area was measured using a flatbed scanner at 600 dpi and applying the Simple Pic Compare V1.1 software. A random sample of leaves was collected at harvest from a tiller subsample that was taken from the centre of each container to fill a 297 mm x 420 mm sheet for scanning. The fresh weight of the leaves, as well as the function of leaf-area-to-fresh-weight, were used to estimate total leaf area. The number of leaves per container was extrapolated by the counted number of tillers per container, and the number of green leaves of the tiller subsamples. Total root biomass per container was quantified after the harvest following the post-drought period. The roots were carefully separated from the soil under running water, using sieves of various mesh sizes. The smallest sieve had a mesh size of 0.065 mm².

Samples for determining the intrinsic WUE (WUE_{int}) via the carbon isotope composition were collected from eight plants per container on the day of harvest. The youngest leaves were pooled and ground into a homogeneous powder with a ball mill. Subsamples of 0.5 to 1.0 mg were analysed for $\delta^{13}C$ through a Delta V Advantage (Thermo Electron, Bremen, Germany) Analysis System. To distinguish variations in WUE_{int} on the basis of $\delta^{13}C$, the approaches of Seibt et al. (2008) and Saurer and Siegwolf (2007) were applied. The agronomic WUE (WUE_{agr}) was estimated from the ratio of aboveground biomass to net water uptake, i.e. transpired water without evaporation losses.

3.2.5. Statistical analyses

The containers were arranged according to a randomized block design, with four replications (blocks). Containers within the blocks were repositioned every second day to mitigate environmental effects stemming from container positioning. The blocks themselves were rotated at seven-day intervals.

Statistical analyses were carried out with the R software (R Core Team, 2015) and the packages ‘nlme’ (Pinheiro et al., 2016) and ‘lsmeans’ (Lenth, 2015). Linear mixed-effects models were fitted with the fixed effects of provenance (including the reference tall fescue) and drought level, as well as their factorial interaction. In the analysis of elongated tillers and stem:leaf ratios of the second harvest, the reference was excluded from the model, since none of the plants had formed any elongated tillers. Assumptions of normality and homoscedasticity were visually assessed. Where these conditions were not fulfilled, appropriate variance function structures were defined. One extreme value each for leaf area, WUE_{int}, stem:leaf ratio from the first harvest and stem:leaf ratio from the second harvest were omitted from the analysis. For significant effects, a post-hoc comparison of means was performed with Tukey’s test. A significance level of $\alpha = 0.05$ was chosen throughout. Pearson’s correlation test was employed to determine correlations parameters.

3.3. Results

3.3.1. Drought period

In the control treatment, tall fescue produced greater DMY than tall wheatgrass AP. In severe drought stress, both provenances of tall wheatgrass showed higher DMY than tall fescue (Table 11). There was a 53% decrease from the control to severe drought stress for tall fescue, 37% for tall wheatgrass HP, and 29% for tall wheatgrass AP.

WUE_{int} and WUE_{agr} increased with drought across all grass species and provenances. Tall fescue achieved higher WUE_{int} in the control treatment than both tall wheatgrass provenances, and higher WUE_{int} in severe drought than tall wheatgrass AP. Both tall wheatgrass provenances had higher WUE_{agr} than tall fescue in the severe drought treatment. The net water uptake was significantly affected by the drought treatment, while the provenance/species x drought level interaction was not significant.

The number of leaves per container differed significantly between the species, but was the same at all drought levels. Both grasses responded similarly to drought with a reduced total leaf area (Table 11). Hence, the lamina size, as well as the leaf dry mass, was reduced. The total tiller number, and the number of elongated tillers, differed between species. A significant relationship between the number of elongated tillers and the DMY was found, with r-values of 0.82, 0.72 and 0.78 for tall wheatgrass AP, tall wheatgrass HP and tall fescue, respectively. The stem:leaf ratio (dry matter) was significantly different between tall wheatgrass and tall fescue. When exposed to drought, tall fescue decreased the stem:leaf ratio, while this was not the case for the tall wheatgrass provenances. Apparently, tall fescue showed a stronger decline in stem mass when grown under drought, compared to the leaf mass. In contrast, tall wheatgrass and, in particular, the HP provenance, responded with a relative increase in stem compared to leaf mass.

Table 11: Dry matter yield (DMY), water use efficiency (WUE), water uptake and morphological traits of tall fescue and two provenances of tall wheatgrass after a two-months drought period with three drought levels. Different letters within a column indicate significant differences between mean values (Tukey $p < 0.05$).differences between mean values (Tukey $p < 0.05$).

		DMY [g container^{-1}]	WUE_{agr} [g DM dm^{-3}]	Intrinsic WUE [μmol_{CO2} mol_{H2O}^{-1}]	Net water uptake [dm³ container^{-1}]	Tillers [number container^{-1}]	Elongated tillers (>45 cm) [number container^{-1}]	Leaves [number container^{-1}]	Leaf area [m² container^{-1}]	Dry matter stem:leaf ratio
Provenance:										
Tall wheatgrass Australian provenance (AP)		164.0	5.24	35.13	33.35	242 b	132 a	602 b	13.80	1.90
Tall wheatgrass Hungarian provenance (HP)		181.0	5.86	37.75	32.59	214 b	112 b	548 b	11.92	2.82
Tall fescue		174.8	4.75	50.01	38.14	310 a	74 c	832 a	14.41	1.84
p-value		0.1331	**<0.0001**	**<0.0001**	0.5221	**<0.0001**	**<0.0001**	**<0.0001**	**0.0038**	**<0.0001**
Drought level:										
Control		212.2	4.71	30.32	45.41 a	272	117 a	687	18.61	2.07
Moderate		181.3	4.70	34.57	38.88 a	246	111 a	673	13.57	2.24
Severe		126.4	6.43	61.98	19.80 b	248	90 b	623	8.08	2.29
p-value		**< 0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1031	**0.0003**	0.2604	**<0.0001**	0.1523
Provenance x drought level:										
Tall wheatgrass AP	Control	179.4 bc	4.45 de	21.56 g	40.38	244	139	597	17.32 abc	1.84 c
	Moderate	182.8 bc	4.54 cde	24.47 f	40.40	248	142	660	15.14 bc	1.96 c
	Severe	129.8 de	6.72 a	56.69 b	19.28	234	114	550	8.93 de	1.91 bc
Tall wheatgrass HP	Control	214.5 ab	5.12 bcd	28.01 e	42.19	248	121	663	16.83 a	2.52 b
	Moderate	193.0 b	5.30 bc	31.80 d	36.60	195	116	513	12.62 cd	2.59 ab
	Severe	135.6 cde	7.16 a	69.10 a	18.97	199	100	468	6.48 e	3.35 a
Tall fescue	Control	242.6 a	4.56 cde	41.40 c	53.66	324	90	801	21.67 a	1.86 c
	Moderate	168.0 bcd	4.26 e	44.92 c	39.63	295	75	845	12.72 cd	2.17 bc
	Severe	113.9 e	5.43 b	63.72 a	21.14	310	57	851	8.84 de	1.38 d
p-value		**0.0036**	**0.0031**	**<0.0001**	0.3142	0.4189	0.6667	0.0887	**0.0326**	**0.0001**

3.3.2. Resilience in the post-drought period

The resilience of the grass swards was assessed through the growth in the post-drought period, during which the water availability was high. In general, regrowth of the tall wheatgrass provenances was greater than that of tall fescue. The formerly severely drought-stressed treatments outperformed the control treatments significantly in tall wheatgrass AP, by 61%, whereas increases in DMY were not significant in tall wheatgrass HP (17%) and tall fescue (15%). Tall wheatgrass HP did not exhibit any significant DMY differences, with respect to the previous drought levels (Table 12). The DMY of the previously moderate and severe drought treatments of both tall wheatgrass provenances was greater than in the corresponding drought treatments of tall fescue. When the DMY of the drought and post-drought period were summed up, no significant effects of the drought treatment were found in the two tall wheatgrass provenances, while the tall fescue showed a clear reduction in DMY with increasing drought.

In contrast to the DMY, the net water uptake was not significantly affected by the drought treatment x provenance/species interaction. The tall wheatgrass provenances had a higher WUE_{agr} in the previous severe drought treatment, compared to the control treatment. The WUE_{agr} of tall fescue in the post-drought period was not significantly affected by the drought treatment of the preceding period. The WUE_{int} of tall fescue was higher than that of the tall wheatgrass provenances, but there was no effect of the previous drought. The WUE_{int} of all grasses were at similar levels, as in the control treatments in the former drought period.

The higher resilience of tall wheatgrass after severe drought was obviously related to a stronger adaptation of tiller and leaf growth, compared to tall fescue. Tall wheatgrass had a lower tiller density than tall fescue, a lower number of leaves, and a lower leaf area per container. An enhanced tiller elongation, and increased stem:leaf ratio, after severe drought were observed in both tall wheatgrass provenances, while tall fescue showed no tiller elongation at all. Yet, differences between the tall wheatgrass provenances were found with respect to the amount of elongated tillers and the corresponding stem:leaf ratio. The root mass, which was determined at final harvest (post-drought period) showed no significant effects from any of the investigated factors. Across all treatments, the average root mass per container was 104 g dry matter (standard deviation = 18.7).

Table 12: Dry matter yield (DMY), water use efficiency (WUE), water uptake and morphological traits of tall fescue and two provenances of tall wheatgrass after a three-months post-drought period (with three drought levels in the former drought period). Different letters within a column indicate significant differences between mean values (Tukey $p < 0.05$). * Values of *Festuca arundinacea* were excluded from the analyses because of zero inflation.

		DMY [g container^{-1}]	WUE_{agr} [g DM dm^{-3}]	Intrinsic WUE [μmol_{CO2} mol_{H2O}^{-1}]	Net water uptake [dm³ container^{-1}]	Tillers [number container^{-1}]	Elongated tillers (>45 cm) [number container^{-1}]*	Leaves [number container^{-1}]	Leaf area [m² container^{-1}]	Dry matter stem:leaf – ratio*
Provenance:										
Tall wheatgrass Australian provenance (AP)		95.8	2.07	25.38 b	46.87	221 b	28 b	578 b	7.56 b	1.23 b
Tall wheatgrass Hungarian provenance (HP)		114.7	2.40	26.99 b	49.67	201 b	51 a	486 b	7.85 b	2.11 a
Tall fescue		68.0	1.39	44.03 a	50.32	301 a	0	774 a	13.41 a	0
p-value		**<0.0001**	**<0.0001**	**<0.0001**	0.4548	**<0.0001**	**<0.0001***	**0.0006**	**<0.0001**	**0.0003***
Previous drought level:										
Control		81.0	1.88	32.05	43.71 b	236	31 b	635	8.96 a	1.37 b
Moderate		92.5	1.72	31.86	53.92 a	256	37 b	631	9.62 a	1.32 b
Severe		105.0	2.27	32.49	49.22 ab	231	50 a	572	10.24 a	1.73 a
p-value		**0.0002**	**<0.0001**	0.9018	**0.0135**	0.4446	**0.0034***	0.5155	**0.0141**	**0.0001***
Provenance x previous drought level:										
Tall wheatgrass AP	Control	71.4 bc	1.59 d	25.08	44.99	231	15	568	5.79	1.11
	Moderate	100.7 a	1.99 bcd	25.03	50.88	232	29	573	8.31	1.10
	Severe	115.3 a	2.63 abc	26.03	44.73	199	42	594	8.58	1.47
Tall wheatgrass HP	Control	109.5 a	2.63 b	26.65	41.86	196	48	466	8.79	1.63
	Moderate	106.6 ab	1.78 cd	26.58	59.56	216	46	581	6.75	1.54
	Severe	128.1 a	2.80 a	27.75	47.58	192	59	413	8.01	2.08
Tall fescue	Control	62.2 c	1.43 d	44.41	44.29	281	0	872	12.29	0
	Moderate	70.2 c	1.38 d	43.98	51.33	320	0	739	13.80	0
	Severe	71.5 c	1.37 cd	43.71	55.34	302	0	711	14.14	0
p-value		**0.0072**	**0.0002**	0.9905	0.2175	0.6378	0.1795*	0.6562	0.5411	0.5142*

3.4. Discussion

Summer drought, as a result of climate change, reduces the DMY of annual energy crops, such as maize. In contrast, perennial energy crops can mitigate this effect by compensating for summer DMY losses over the full growing season. To assess the suitability of tall wheatgrass as a drought-tolerant energy crop, a greenhouse container experiment was conducted comparing two provenances of tall wheatgrass with tall fescue as a reference. The results confirmed the hypotheses (1 and 2) that morphological adaption mechanisms of tall wheatgrass enhance DMY under severe drought conditions, and the subsequent post-drought period, compared to tall fescue. These high levels of drought resistance and resilience led to indifferent aggregated DMY of the tall wheatgrass treatments, in contrast to tall fescue.

3.4.1. Tall wheatgrass having high drought tolerance

Aggregated over the drought and post-drought period, the DMY of tall wheatgrass did not differ between the drought and control treatments, as the plants were able to compensate for yield reductions during the drought period by increased yields in the following post-drought period. The capability of grasses to reduce biomass losses to a minimum because of severe drought, from a cumulative point of view, has also been reported by Hofer et al. (2016), who found a high resilience in grasses such as *Lolium perenne* (up to +62%). While the control treatment of tall fescue achieved the same aggregated DMY as the two drought-stressed tall wheatgrass provenances, the drought treatments led to significant DMY reductions. Higher drought resistance and higher resilience of tall wheatgrass, as well as a different seasonal distribution of biomass growth, contributed to the different performances of the two species.

3.4.2. Adaption to drought

In the control treatment, tall fescue achieved a higher DMY than the tall wheatgrass provenances. In the drought treatments, however, both absolute and relative DMY reductions were greater in tall fescue than in tall wheatgrass. As a consequence, no differences in DMY were found between the species under severe drought.

All tested grasses reduced leaf area under severe drought (tall wheatgrass AP: -48%; tall wheatgrass HP: -61%; tall fescue: -59%). As the number of leaves was not decreased, drought led to reduced leaf sizes in this experiment. This typical response of grasses, and especially of tall wheatgrass, to drought was found in a previous study by Gazanchian et al. (2007), who observed a leaf-width reduction by up to 79% as a consequence of drought. Similar results were obtained by Bahrani et al. (2010), who recorded decreases in leaf area of 43% in tall wheatgrass and 78% in tall fescue. Transpiration losses mainly occur in leaves (Bleby et al., 1997; Kowal et al., 1978), which, among other functions, cools the leaves (Hashimoto et al., 1984). Consequently, plants are able to reduce transpiration losses linearly, by reducing leaf area under drought (Blum, 2005; Kowal et al., 1978; Ritchie, 1974). This reduction in transpiration losses comes at the expense of productivity and DMY (Lazaridou et al., 2004). Conversely, the capability of increasing leaf area led to greater DMY of tall wheatgrass HP and tall fescue, with sufficient water availability (control treatment). Tall wheatgrass HP could partly compensate for leaf DMY declines by enlarging the stem:leaf ratio, whereas tall fescue decreased the stem:leaf ratio in response to drought. The lenticular transpiration losses of the stem (approximately 0.1% of the total transpiration loss) are negligible compared to the stomatal transpiration of the leaves that contributes more than 90% to the total transpiration (Kotbal et al., 2007). As such, tall wheatgrass HP reacted to drought by adapting the stem:leaf ratio, reducing transpiration losses and, consequently, increasing WUE_{agr}. This effective adaptation to drought might require a fully established sward that has reached the growth stage of stem elongation, as another study of Bahrani et al. (2010) detected decreasing WUE_{agr} in two-week-old tall wheatgrass and tall fescue that were exposed to drought for 26 days.

In contrast to the WUE_{agr} at the whole-plant level, the WUE_{int} at the leaf level hardly differed between tall wheatgrass HP and tall fescue under severe drought, while WUE_{int} in the control treatment was significantly higher for tall fescue than for the tall wheatgrass provenances. This result indicated a strong capacity of the tall wheatgrass provenances to adapt stomatal conductance to drought.

There are further strategies that can limit transpiration losses. The leaf surface of tall wheatgrass is hairy (Scheinost et al., 2008), being different from tall fescue, thus transpiration rates can be more efficiently slowed by boundary layers (Sterling, 2004), especially when DMY production is limited by water under severe drought conditions.

The observed differences between the tall wheatgrass provenances are thought to originate in unequal breeding histories, such as tall wheatgrass HP that was developed for high-volume biogas production in temperate regions, with an increased production of leaf biomass (Brand, 2015). The environmental conditions of the origin of seed ripening, such as frequent drought, play an important role in the plants reaction to drought. Certain responses of many plants to specific environmental conditions persist in seeds' memory (Li and Liu, 2016) and contribute to quick adaption to environmental conditions in the plant's next generation. Tall wheatgrass AP was a cultivar, originally bred and harvested in South Australia, for cultivation under frequent drought periods in the summer months (Bleby et al., 1997; BOM Australia, 2016; Smith and Kelman, 2000). Hence, different reactions to drought of the two provenances could be expected.

3.4.3. Increased DMY by resilience

In the post-drought period, severely drought-stressed plants outperformed plants in the control treatment by 61% for tall wheatgrass AP, by 17% for tall wheatgrass HP, and by 15% for tall fescue. Across all drought treatments, the DMY of tall fescue decreased most from the drought period to the post-period than did the tall wheatgrass provenances. This can be attributed to the total lack of tiller elongation in tall fescue during this period, since DMY in the tall wheatgrass provenances was strongly related to the number of elongated tillers ($r = 0.78$). As tall fescue needs a so-called 'double induction' of vernalisation, followed by long days, for generative stem elongation (Heide, 1994), it generally only forms short vegetative tillers after the first cut (Virkajärvi et al., 2012). Consequently, its low DMY in the post-drought period was independent of the different degrees of drought stress, and can rather be attributed to a seasonal distribution of biomass growth that differs from that of tall wheatgrass.

In contrast to tall fescue, both tall wheatgrass provenances formed elongated tillers in the post-drought period, thus gaining higher dry matter stem:leaf ratios. The number of elongated tillers was higher in tall wheatgrass HP than in AP, as was the stem:leaf ratio. Both tall wheatgrass provenances exhibited resilience by increasing tiller elongation, as well as the stem:leaf ratio, after severe drought, compared to the control treatment. Enhanced tiller elongation could be ensured by a larger reserve pool of water-soluble carbohydrates in the stubble of drought-stressed plants that could promote regrowth, once the water supply is adequate (Volaire et al., 1998). We suggest that higher nitrogen availability after severe drought can also contribute to resilience (Carlsson et al., 2017) because of diminished microbial growth, and restricted bacterial movement due to low water availability (del Pino

Machado, 2005), lead to a lower rate of mineralization and, therefore, nitrogen limitation during the drought period. Compared to tall fescue, both provenances of tall wheatgrass could produce greater DMY because they were able to produce a second crop of elongated tillers, subsequent to the first harvest. This was mainly a result of the cutting management, as other studies have indicated that two annual cuts, with an early first harvest in May, were most conductive to subsequent shoot elongation (Dickeduisberg et al., 2017; Hyder and Sneva, 1963; Laplace et al., 1997).

Higher DMY from the increased stem:leaf ratio resulted in significantly higher WUE_{agr} values for the severe drought treatments of the tall wheatgrass provenances in the post-drought period, whereas no effect of previous drought treatment on water uptake was detected. Similar observations, of increased WUE_{agr} after drought, have also been made by Kørup et al. (2018) for numerous grasses. Nevertheless, the WUE_{agr} was considerably lower in the post-drought period than in the drought period. This can at least partly be attributed to a longer harvest interval and lower DMY in the post-drought period.

Differences in WUE_{int} between species were apparent, as seen in the drought period. In both species, levels were similar to those of the control treatment during the drought period. This indicates that, after harvest and rewatering, plants no longer reacted to the previous drought by adapting stomatal conductance. Similar observations were noted by Gazanchian et al. (2007), who determined that relative water content and leaf width in tall wheatgrass under severe drought returned to well-watered levels following a 14-day rest period.

3.5. Conclusions

Tall wheatgrass, in contrast to tall fescue, could offset biomass productivity losses during droughts through its high resilience in the post-drought period. As a result, its aggregated DMY over the vegetative period was independent of the drought intensity. The high resilience of tall wheatgrass is likely mostly attributable to enhanced tiller elongation and a greater stem:leaf ratio following severe drought. There was little difference between the provenances, regarding the maximum aggregated DMY, and in terms of intensity of response to drought.

As such, tall wheatgrass achieves a more stable DMY in the vegetative period – the main objective from an agronomic point of view – than tall fescue. Therefore, tall wheatgrass can be regarded as a drought-tolerant crop that is better adapted to the increasing frequency of early summer drought, brought about by climate change, than the currently grown summer annuals. It may be considered for adoption as a new crop for biogas production in Central Europe, which is presently facing the problems of an increasing share of maize cultivation. Further research is warranted, via field experiments, to identify the most suitable provenances/cultivars of tall wheatgrass and tall wheatgrass farming systems, regarding DMY, drought tolerance and competitiveness, compared to maize, especially in regions with insecure maize yields.

3.6. Acknowledgements

The authors thank Barbara Hohlmann for support in the determination of carbon isotope composition and Balint Jakli for scientific consulting on the intrinsic WUE.

3.7. References

Ad-hoc-AG Boden, 2005. Bodenkundliche Kartieranleitung (Manual of soil mapping), 3rd edition. Schweizerbart, Hannover, Germany.

Barker, D.J., Caradus, J.R., 2001. Adaption of forage species to drought. In: Proceedings of the 18th International Grassland Congress 46. Sao Paolo, Brazil. Available at: http://www.internationalgrasslands.org/files/igc/publications/2001/tema6-1.pdf (accessed 20 February 2017).

Bahrani, J.M., Bahrami, H., Haghighi, A.A.K., 2010. Effect of water stress on ten forage grasses native or introduced to Iran. Japanese Society of Grassland Science 56, 1-5.

Bleby, T.M., Aucote, M., Kennett-Smith, A.K., Walker, G.R., Schachtman, D.P., 1997. Seasonal water use characteristics of tall wheatgrass [Agropyron elongatum (Host) Beauv.] in a saline environment. Plant Cell Environment 20, 1361-1371.

Blum, A., 2005. Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? Crop and Pasture Science 56, 1159–1168.

BOM Australia, 2016. Australian climate averages – rainfall (climatology 1961-1990). Australian Government – Bureau of Meteorology, Australia. Available at http://www.bom.gov.au/jsp/ncc/climate_averages/rainfall/index.jsp?period=an&area=oz#maps (accessed 5 December 2016).

Brand, G., 2015. CEO of BSV-Saaten. Ismaning, Germany. Personal communication on 03 December 2015.

Carlsson, M., Merten, M., Kayser, M., Isselstein, J., Wrage-Mönnig, N., 2017. Drought stress resistance and resilience of permanent grasslands are shaped by functional group composition and N fertilization. Agriculture, Ecosystems and Environment 236, 52-60. doi: 10.1016/j.agee.2016.11.009

Creutzig, F., Ravindranath, N.H., Berndes, G., Berndes, G., Bolwig, S., Bright, R., Cherubini, F., Chum, H., Corbera, E., Delucchi, M., Faaij, A., Fargione, J., Haberl, H., Heath, G., Lucon, O., Plevin, R., Popp, A., Robledo-Abad, C., Rose, S., Smith, P., Stromman, A., Suh, S., Masera, O., 2015. Bioenergy and climate change mitigation: an assessment. Global Change Biology Bioenergy 7, 916-944. doi: 10.1111/gcbb.12205

Csete, S., Stranczinger, S., Szalontai, B., Farkas, A., Pal, R.W., Salamon-Albert, E., Kocsis, M., Tovari, P., Vojtela, T., Dezsö, J., Walcz, I., Janowszky, Z., Janowszky, J., Borhidi, A., 2011. Tall wheatgrass cultivar Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) as a potential energy crop for semi-arid lands of eastern Europe. In: Nayreripour, M., Kheshti, M. (editors), Sustainable growth and application in renewable energy source. InTech, Rijeka, Croatia, pp. 269–294.

Dahlhoff, A., 2013. Auswertung der Biogasanlagen-Betreiberdatenbank der Landwirtschaftskammer Nordrhein-Westfalen (Evaluation of biogas plant survey of the chamber of agriculture in North Rhine-Westphalia). Center of renewable resources of the chamber of agriculture for North Rhine-Westphalia, Bad Sassendorf, Germany.

del Pino Machado, A.S., 2005. Estimating nitrogen mineralization potential of soils and the effect of water and temperature and crop residues on nitrogen net mineralization. Dissertation, University of Göttingen, Germany.

Dickeduisberg, M., Laser, H., Tonn, B., Isselstein, J., 2017. Tall wheatgrass (*Agropyron elongatum*) for biogas production: Crop management more important for biomass and methane yield than grass provenance. Industrial Crops and Products 97, 653-663. doi: 10.1016/j.indcrop.2016.12.055

Eckelmann, W., Sponagel, H., Grottenthaler, W., Hartmann, K.J., Hartwich, R., Janetzko, P., Joisten, H., Kühn, D., Sabel, K.J., Traidl, R., 2005. Bodenkundliche Kartieranleitung (Manual of soil mapping), 5th edition. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, Germany.

EEA, 2016. Climate change impacts and adaption. European Environment Agency, Copenhagen, Denmark. Available at http://www.eea.europa.eu/soer-2015/europe/climate-change-impacts-and-adaptation (accessed 1 April 2017).

FNR, 2017. Bioenergy in Germany – Facts and Figures 2016. Agency of Renewable Resources, Gulzow, Germany.

Gazanchian, A., Hajheidari, M., Sima, N.K., Salekdeh, G.H., 2007. Proteome response of *Elymus elongatum* to severe water stress and recovery. Journal of Experimental Botany 58, 291-300.

Hashimoto, Y., Taketoshi, I., Kramer, P.J., Naylor, A.W., Strain, B.R., 1984. Dynamic analysis of water stress of sunflower leaves by means of a thermal image processing system. Plant Physiology 76, 266-269.

Heide, O.M., 1994. Control of flowering and reproduction in temperate grasses. New Phytologist 128, 347-362.

Herbes, C., Jirka, E., Braun, J.P., Pukall, K., 2014. Der gesellschaftliche Diskurs um den Maisdeckel vor und nach der Novelle des Erneuerbaren-Energien-Gesetzes (EEG) 2012 (The social discourse on the maize cap before and after the 2012 amendment oft he German renewable energies act (EEG)). GAiA 23, 100-108. doi: 10.14512/gaia.23.2.7

Hickman, G.C., Vanloocke, A., Dohleman, F.G., Bernacchi, C.J., 2010. A comparison of canopy evapotranspiration for maize and two perennial grasses identified as potential bioenergy crops. Global Change Biology Bioenergy 2, 157-168.

Hoekstra, N.J., Finn, J.A., Hofer, D., Lüscher, A., 2014. The effect of drought and interspecific interactions on depth of water uptake in deep- and shallow-rooting grassland species as determined by $\delta^{18}O$ natural abundance. Biosciences 11, 4493-4506.

Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N., Lüscher, A., 2016. Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. Journal of Applied Ecology 53, 1023-1034.

Hyder, D.N., Sneva, F.A., 1963. Studies of Six Grasses Seeded on Sagebrush-Bunchgrass Range. Agricultural Experiment Station of Oregon State University, Corvallis, USA. Available at https://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/8783/tec_bul_71.pdf?sequence=1 (accessed 20 December 2016).

Kiesel, A., Lewandowski, I., 2017. Miscanthus as biogas substrate – cutting tolerance and potential for anaerobic digestion. Global Change Biology Bioenergy 9, 153-167. doi: 10.1111/gcbb.12330

Kørup, K., Lærke, P.E., Baadsgaard, H.. Andersen, M.N., Kristensen, K., Münnich, C., Didion, T., Jensen, E.S., Martensson, L.M., Jørgensen, U., 2018. Biomass production and water use efficiency in perennial grasses during and after drought stress. Global Change Biology Bioenergy 10, 12-27. doi: 10.1111/gcbb.12464

Kotbal, R.I., Bendre, A., Tyagi, K.N., 2007. Concepts of Biology – Class XI. Rastogi Publications, Meerut, India.

Kowal, J.M., Kassam, A.H., 1978. Agricultural Ecology of Savanna: A Study of West Africa. Oxford Univ. Press (Clarendon), London and New York.

Laplace, S., Brizuela, M.A., Cid, M.S., 1997. Management of Tall Wheatgrass based on the Leaf Appearance during spring. In: Proceedings of the 18th International Grassland Congress, Winnipeg, USA, pp. 27-28. Available at http://www.internationalgrasslands.org/files/igc/publications/1997/1-07-027.pdf (accessed 2 February 2017).

Lazaridou, M., Koutroubas, S.D., 2004. Drought effect on water use efficiency of berseem clover at various growth stages. In: Proceedings of the 4th International Crop Science Congress, Brisbane, Australia.

Lenth, R., 2015. Lsmeans: Least-Squares Means. R package version 2.20-2.

Li, X., Liu, F., 2016. Drought stress memory and drought stress tolerance in plants: biochemical and molecular basis. In: Mohammad Anwar, H., Wani, S.H., Bhattacharjee, S., Burritt, D.J., Tran, L.S.P. (Eds.), Drought stress tolerance in plants, volume 1. Springer International Publishing, Switzerland, pp. 17-44.

Lijó, L., González-García, S., Bacenetti, J., Negri, M., Fiala, M., Feijoo, G., Moreira, M.T., 2013. Environmental assessment of farm-scaled anaerobic co-digestion for bioenergy production. Waste Management 41, 50-59. doi: 10.1016/j.wasman.2015.03.043

López, I.F., Kemp, P.D., Dörner, J., Descalzi, C.A., Balocchi, O.A., Garcia, S., 2013. Competitive strategies and growth of neighbouring *Bromus valdivianus* Phil. and *Lolium perenne* L. plants under water restriction. Journal of Agronomy and Crop Science 199, 449-459.

Ludlow, M.M., Muchow, R.C., 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy 43, 107-153.

Mast, B., Lemmer, A., Oechsner, H., Reinhardt-Hanisch, A., Claupein, W., Graeff-Hönninger, S., 2014. Methane yield potential of four novel perennial biogas crops influenced by harvest date. Industrial Crops and Products 58, 194-203. doi: 10.1016/j.indcrop.2014.04.017

Moore, G., Sanford, P., Wiley, T., 2006. Perennial Pastures for Western Australia. Department of Agriculture and Food Western Australia, Perth, Australia.

Pacetti, T., Lombardi, L., Federici, G., 2015. Water–energy nexus. Journal of Cleaner Production 101, 278–291.

Pecetti, L., Annicchiarico, P., Abdelguerfi, A., Kallida, R., Mefti, M., Porqueddu, C., Simoes, N.M., Volaire, F., Lelièvre, F., 2011. Response of Mediterranean tall fescue cultivars to contrasting agricultural environments and implications for selection. Journal of Agronomy and Crop Science 197, 12-20.

Perlikowski, D., Kosmala, A., Rapacz, M., Koscielniak, J., Pawlowicz, I., Zwierzykowski, Z., 2013. Influence of short-term drought conditions and subsequent re-watering on the physiology and proteome of *Lolium multiflorum / Festuca arundinacea* in introgression forms, with contrasting levels of tolerance to long-term drought. Plant Biology 16, 385-394. doi: 10.1111/plb.12074

Pimentel, D., Allen, J, Beers, A., Guinand, L., Linder, R., McLaughlin, P., Meer, B., Musonda, D., Perdue, D., Poisson, S., Siebert, S., Stoner, K., Salazar, R., Hawkins, A., 1987. World agriculture and soil erosion – Erosions threatens world food production. BioScience 37, 277-283.

Pimm, S.L., 1984. The complexity and stability of ecosystems. Nature 307, 321–326.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. Nlme: Linear and nonlinear mixed effects models. R package version 3.1 - 128.

R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Randall, G.W., Huggins, D.R., Russelle, M.P., Fuchs, D.J., Nelson, W.W., Anderson, J.L., 1997. Nitrate losses through subsurface tile drainage in conservation reserve program, alfalfa, and row crop systems. Journal of Environmental Quality 26, 1240-1247.

Ritchie, J.T., 1974. Atmospheric and soil water influences on plant water balance. Agricultural Meteorology 14, 183-198.

Roundy, B.A., 1985. Root penetration and shoot elongation of tall wheatgrass and basin wildrye in relation to salinita. Canadian Journal of Plant Science 65, 335-343.

Saurer, M., Siegwolf, R.T.W., 2007. Human impacts on tree-ring growth reconstructed from stable isotope. In: Dawson, T.E., Siegwolf, R.T.W. (Eds.), Stable Isotopes as Indicators of Ecological Change (eds), Academic Press, pp. 49-62.

Sadeghi, S.H.R., Halagh, N.R., 2007. Comparison of wheatgrass (*A. intermedium*) and tall wheatgrass (*A. elongatum*) in transpiration viewpoint. Caspian Journal of Environmental Sciences 55, 41-44.

Sauerbrei, R., Eckschmitt, K., Woters, V., Gottschalk, T.K., 2014. Increased energy maize production reduces farmland bird diversity. Global Change Biology Bioenergy 6, 265-274.

Scheinost, P., Tilley, D., Ogle, D., Stannard, M., 2008. Tall wheatgrass – Plant guide. United States Department of Agriculture – Natural Resources Conversation Service, Pullman, USA.

Schittenhelm, S., Schroetter, S., 2013. Comparison of drought tolerance of maize, sweet sorghum, and sorghum-sudangrass hybrids. Journal of Agronomy and Crop Science 200, 46-53.

Schittenhelm, S., Reus, D., Kruse, S., Hufnagel, J., 2011. Assessment of productivity and profitability of sole and double-cropping for agricultural biomass production. Journal of Cultivated Plants 63, 387-395.

Seibt, U., Rajabi, A., Griffiths, H., 2008. Carbon isotopes and water use efficiency: sense and sensitivity. Oecologia 155, 441-454. doi: 10.1007/s00442-007-0932-7

Smith, K.F., Kelman, W.M., 2000. Register of Australian Herbage Plant Cultivars. Australian Journal of Experimental Agriculture 40, 119-120. doi: 10.1071/EA99156_CU

Sterling, T.M., 2004. Transpiration – Water Movement through Plants. New Mexico State University, USA. Available at https://www.sciencemag.org/site/feature/misc/webfeat/vis2005/show/transpiration.pdf (accessed 30 January 2017).

VDLUFA, 1991a. VDLUFA Methodenbuch Band I – Böden – Kapitel A 5.1.1 (VDLUFA Method Manual Part 1 – Soils – Chapter A 5.1.1). Verband Deutscher Landwirtschaftlicher Untersuchung- und Forschungsanstalten, Darmstadt, Germany.

VDLUFA, 1991b. VDLUFA Methodenbuch Band I – Böden – Kapitel A 6.2.1.1(VDLUFA Method Manual Part 1 – Soils – Chapter A 6.2.1.1). Verband Deutscher Landwirtschaftlicher Untersuchung- und Forschungsanstalten, Darmstadt, Germany.

Virkajärvi, P., Pakarinen, K., Hyrkäs, M., Seppänen, M., Bélanger, G., 2012. Tiller Characteristics of Timothy and Tall Fescue in Relation to Herbage Mass Accumulation. Crop Science 52, 970-980. doi: 10.2135/cropsci2011.01.0039

Volaire, F., Thomas, H., Lelievre, F., 1998. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought. New Phytologist 140, 439-449.

Zebisch, M., Grothmann, T., Schröter, D., Hasse, C., Fritsch, U., Cramer, W., 2005. Climate change in Germany – Vulnerability and adaption strategies of climate-sensitive sectors – Summary. Potsdam Institute for Climate Impact Research, Federal Environmental Agency of Germany, Dessau, Germany.

Zwicke, M., Alessio, G.A., Thiery, L., Falcimagne, R., Baumont, R., Rossignol, R., Soussana, J.F., Picon-Cochard, C., 2013. Lasting effects of climate disturbance on perennial grassland above-ground biomass production under two cutting frequencies. Global Change Biology 19, 3435–3448.

4. Tall wheatgrass (*Agropyron elongatum*) for biogas production: Crop management more important for biomass and methane yield than grass provenance

This chapter is an article published in Industrial Crops and Products.
Dickeduisberg, M., Laser, H., Tonn, B., Isselstein, J., 2017.
Industrial Crops and Products 97, 653-663.
http://dx.doi.org/10.1016/j.indcrop.2016.12.055

Figure 6: Cutting tall wheatgrass with a Hege 212 in the field experiment.

Abstract

Tall wheatgrass (*Agropyron elongatum*) has been proposed as a new energy crop of a dry matter yield (DMY) and methane hectare yield (MHY) potential similar to maize. So far, little is known about the agronomy of the grass for biogas production in temperate Europe. In a field trial the hypothesis was tested that aboveground DMY and MHY are affected by the frequency of cutting and cutting height, and that these management effects interact with the tall wheatgrass germplasm. Four wheatgrass provenances were sown in a three-factorial block design with three cutting heights at harvest (5, 10, and 15 cm above soil surface) and three levels of cutting frequency (one, two, and four cuts $year^{-1}$). Aboveground DMY (two full harvest years), crude nutrient and fibre content as well as the specific methane yield (SMY, one full harvest year) and MHY were determined.

In general, only small differences in the target variables among the different provenances were found. Likewise, a significant interaction of provenance x crop management was only found for the DMY in the second year. The cutting frequency strongly affected the DMY with a two-year average of 14.6, 18.4, and 14.9 t DM ha^{-1} and the MHY of 3505, 5705, and 5384 Nm^3 methane ha^{-1} for the one-, two-, and four-cut regime, respectively. The cutting height was less important with DMY values of 17.2, 16.3, and 14.4 t DM ha^{-1} for 5, 10, and 15 cm cutting height, respectively. SMY was well predictable from growing degree days. In conclusion for Central European conditions the performance of tall wheatgrass is suitable for biogas production and a clearly responds to cutting frequency and cutting height with highest DMY and MHY in the two–cut system and 5-10 cm cutting height. The choice of the wheatgrass germplasm was less important. It remains to be shown for how long tall wheatgrass stands will maintain their performance.

4.1. Introduction

During the last decade maize has become the most important feedstock for the increasing number of biogas plants in Germany (Dahlhoff, 2013). In several regions it is now the dominant crop in agricultural systems. As a consequence, public concerns over the sustainability of biogas production have risen because the increasing acreage of maize is linked to increasing pest pressure, high soil erosion, nutrient losses and biodiversity decrease (Herbes et al., 2014; Schittenhelm et al., 2011). The predicted climate change with a precipitation shift from summer to winter months and an increased frequency of summer droughts (Zebisch et al., 2005), further calls for alternative bioenergy crops.

Tall wheatgrass (*Agropyron elongatum* (Host) P. Beauv.) has been proposed as an alternative crop to maize. It is considered to be drought tolerant (Moore et al., 2006) and it is a representative for perennial crops with little erosion risk (Pimentel et al., 1987) and small nutrient losses (Dinnes et al., 2002). As it is a new crop to temperate Europe the pressure of pests and diseases is likely to be small. In addition, replacing maize to some extent with tall wheatgrass would contribute to the diversification of crop rotations, which is a goal of the EU Common Agricultural Policy (European Parliament, 2013). Preliminary studies in Germany have demonstrated the yield potential of tall wheatgrass with dry matter yield (DMY) comparable to maize (Heinz, 2015). Apart from DMY the specific methane yield (SMY) is important for the profitability of an energy crop. Mast et al. (2014) found that the SMY of tall wheatgrass was up to 8% higher than that of maize (0.376 vs. 0.349 Nm^3 kg oDM^{-1}). Hence cultivating tall wheatgrass potentially has many advantages and can help to reduce the ratio of maize in biogas substrates.

The introduction of tall wheatgrass into crop rotations requires a sound knowledge of the agronomy of this crop, which is not yet available for Central European conditions. In addition, the variability of tall wheatgrass germplasm with regard to the agronomy of the crop has not been investigated. Moore et al. (2006) point to the fact that there has been some genetic selection among tall wheatgrass varieties in countries where this grass has been grown before, and it is known that there is genetic variation in several plant traits (Oram, 1981). This variation refers to the time of reproductive growth and maturation, leaf characteristics, shoot morphology, tolerance of alkaline or saline soils and the level of dry matter productivity (Brand, personal communication; Hanson, 1972; Oram, 1981; Smith and Kelman, 2000; Smith et al., 1994; UPOV, 2015). Yet, to what extent this genetic variation affects the agronomy of the crop for Central European conditions is not known.

When introducing a perennial grass that is adapted to multiple cuttings per year into cropping systems some questions arise. How often should the sward be harvested and at what height the grass should be cut in order to obtain a high biomass as well as methane hectare yield (MHY), which ideally can be maintained over several years. So far, no scientific reports on harvest management of tall wheatgrass with a high MHY potential have been published. Concerning grasses in perennial grassland in general, maximum yield is obtained when the first cut is taken shortly after ear or panicle emergence and a further two or three cuts taken at intervals of approximately eight weeks (Williams, 1980). Increasing the frequency of cutting reduces yield when the stem elongation is interrupted too early, because this is the period of maximum biomass production. Decreasing cutting frequency also decreases yield because of greater portions of senescent leaves with low photosynthetic productivity. Rate and form of regrowth after cutting depend on whether or not the apical meristem is removed, the level of carbohydrates within remaining organs, photosynthetic activity of remaining green plant parts that were previously shaded, root mass and activity, and also water and nutrient availability (Kerrisk and Thomson, 1990; Pearson and Ison, 1996). In consequence, the cutting frequency affects the regrowth after the harvest and therefore the annual DMY (Undersander and Naylor, 1987). Similarly, the cutting height affects the DMY and the regrowth potential. For tall fescue (*Festuca arundinacea* Schreb.) it was shown that a reduction of stubble height from 9 cm to 5 cm resulted in a DMY increase of 9-12% (Burns et al., 2002). The frequency of cutting and the cutting height have a direct effect on carbohydrate storage, which ensures a rapid regrowth of grass. Based on experiences mainly from the USA (Scheinost et al., 2008; USDA, 2013; Wasser et al., 1986) a cutting height of 15 cm for a sustainable production of tall wheatgrass is recommended. With regard to the cutting frequency, Schrabauer et al. (2014) found higher competitiveness in a one-cut compared to a two-cut regime, the difference being dependent on the wheatgrass variety. Mast et al. (2014) found increasing SMY when the cutting interval was reduced. This is obviously due to a lower cell wall as well as fibre and lignin content compared to plants harvested in longer cutting intervals and thus having higher cell wall content (Beever et al., 2000). Thus, cutting frequency and cutting height have to be balanced carefully in order to produce high DMY at reasonable SMY and to maintain high productivity over several years.

In the present study a field experiment was conducted with two consecutive full harvest years after the sowing year to test the hypotheses that (1) aboveground DMY and MHY are affected by the frequency of cutting and the cutting height, and (2) crop management interacts with tall wheatgrass germplasm of different provenances. These have been developed under different conditions such as frequent summer droughts or alkaline soils and are thus differently adapted to such conditions.

4.2. Material and methods

4.2.1. Site description

The experimental field was located at Haus Duesse centre of agricultural research and education in Bad Sassendorf, Germany (51°38’15.3’’N, 8°11’8.0’’E), at an altitude of 69 m above sea level. It is representative of the upper Central Rhineland, lower Rhine, and southern Munsterland region with respect to the segmentation of soil-climate-regions of the Federal Republic of Germany (Graf et al., 2009; JKI, 2014). The soil has clay migration from topsoil to subsoil and is influenced by stagnant water between 40 and 80 cm depth and groundwater deeper than 80 cm depth. Furthermore, the parent material is loess (Hellmich, 2006). Following the soil classification system by USDA (1987), the top 30 cm layer represents silt loam. Prior to imposing treatments, soil pH (6.3) was measured with $CaCl_2$ (VDLUFA, 1991a). Plant available soil phosphorus (150 mg kg^{-1} soil) and potassium (84 mg kg^{-1} soil) were obtained by using the common Calcium-Ammonium-Lactate (CAL) extraction method (VDLUFA, 1991b). As the soil phosphorus content is considered to be high, no phosphorus fertilizer was applied. Potassium fertilizer was applied at an amount that replaced the potassium offtake with the harvested grass.

The sowing year was rather dry with markedly lower annual precipitation than the 25-year average (Figure 7, Table 13). Yet, germination and emergence of the grass were high and the crop was well established at the end of the sowing year. In the full harvest years the rainfall was close to the long-term average. Although the average temperatures were slightly above the long-term values, the second full harvest year was characterized by low temperatures in spring and autumn with an unusual period of snowfall in October just before the last harvest under frequent cutting.

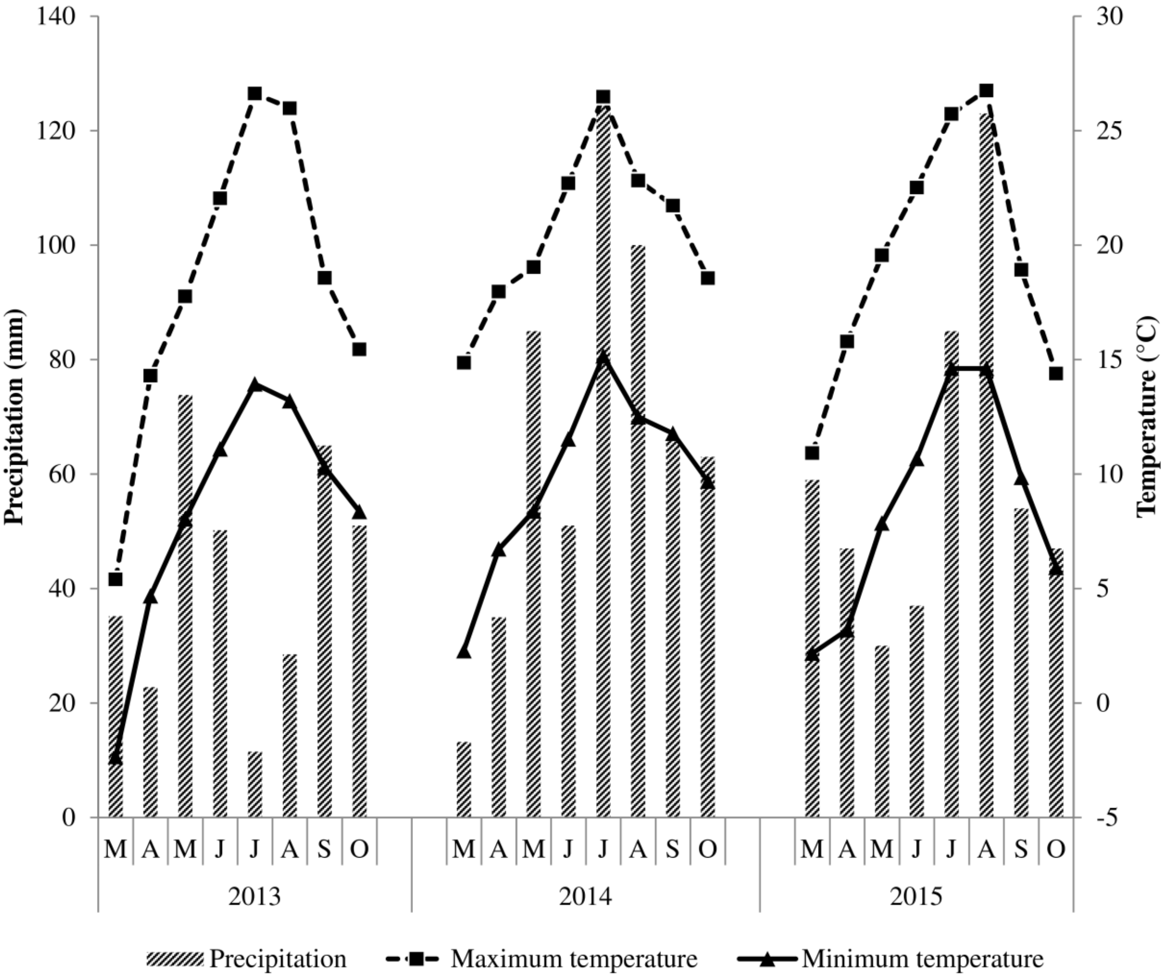

Figure 7: Monthly (March–October) precipitation and average temperature data for Haus Duesse. Air temperature was measured each hour 2 m above soil surface.

Table 13: Average air temperature and annual precipitation in the year of establishment (2013) and in the first (2014) and second (2015) full harvest year.

Year	Average air temperature (°C)	Deviation from long-term average temperature (°C)	Annual precipitation (mm)	Deviation from long-term average precipitation (mm)
2013 (year of establishment)	10.0	- 0.2	513	- 262
2014 (first year)	12.0	+ 1.8	708	- 67
2015 (second year)	11.4	+ 1.2	718	- 57

4.2.2. Treatments

The experiment was conducted with four factors (Table 14) in an orthogonal structure with three replicates. Plots (12 m X 1.5 m) were arranged in a randomized split-split-plot design with main plot "provenance", sub-plot "cutting frequency" and sub-sub-plot "cutting height".

Timing of harvest was usually scheduled according to plant growth using the BBCH scale of Meier et al. (2001) that rates crop growth with numbers from 0 to 99.

The four harvests of the frequent cutting were scheduled at intervals of 56 days, with the initial cut set at the beginning of shoot elongation (BBCH 31-33). In the two-cut system (double cutting) the first harvest was done at the phenological growth stage from mid to end of heading (BBCH 55-59). The second cut was done at a later stage (end of flowering, BBCH 69) before the vegetation period ended. Under single cutting, harvesting was planned for August (full ripening, BBCH 89). Because of lodging the crop had to be harvested earlier when plants were at a growth stage between BBCH 71 and 89.

Table 14: Factors and levels

Factor	Levels
Cutting frequency	frequent (4 cuts year^{-1}), double (2 cuts year^{-1}), single (1 cut year^{-1})
Cutting height	5 cm, 10 cm, 15 cm
Provenance of seeds	Argentina, Australia, Hungary, USA
Year of harvest	first full harvest year (2014), second full harvest year (2015)

The seeds of the different provenances were obtained from breeding companies. They were harvested across four continents (Table 14) six to 12 months before sowing. Meanwhile, seeds were stored dry in paper bags under dark conditions at 10 °C and 50% air humidity.

The Australian seeds were collected near Keith in the state of South Australia in February 2013 (Teague, personal communication). They originated from tall wheatgrass that was introduced to Australia from the USA and included in tests in Western Australia in the 1950s (Hanson, 1972; Oram, 1981; Rogers and Bailey, 1963). Thereafter, the line was developed through breeding to better cope with the Australian growing conditions (Oram, 1981). Since the 1990s a new cultivar was developed as a leafy, productive alternative to the existing cultivar (Smith and Kelman, 2000).

In Argentina, tall wheatgrass is mainly planted in waterlogged and saline soils in the Pampa region (Andrés and Guillen, 2003). It is commonly used for grazing livestock and for hay production. Due to its salt tolerance, tall wheatgrass has replaced native grasslands to some extent (Taboada et al., 1998). The provenance from the USA originated from a seed collection set up in 1934 in the former USSR (Liu and Wang, 2011). Later it was used for pasture in wet and alkaline conditions or semi-arid regions (Scheinost et al., 2008). Currently it is planted in the Northern Great Plains and Intermountain West (Liu and Wang, 2011; Wasser et al., 1986; Weintraub, 1953). The Hungarian provenance is based on a new breeding program that focuses on European bioenergy production and was launched on the German seed market in the 2010s (Brand, personal communication).

4.2.3. Crop establishing and management

Tall wheatgrass was sown on 13 May 2013 and emerged two weeks later. In the sowing year, the sward was cut twice on 12 August and 30 October 2013 at a cutting height of 10 cm. Weeds were controlled by applying an herbicide (50 g ha^{-1} Tribenuron-Methyl) on 25 July. Amount and timing of mineral fertilizer application were identical for all treatments. In the establishment year nitrogen was applied at a rate of 50 kg N ha^{-1} as calcium ammonium nitrate. In the subsequent full harvest years the level of plant available nitrogen of 280 kg N ha^{-1} was calculated for 22.9 t ha^{-1} DMY to avoid considerable limitations. At the beginning of each season the plant available soil nitrogen was determined up to 90 cm depth. Further annual mineralisation of digestate that was applied throughout the previous years was predicted by 30 kg N ha^{-1}. The residual amount of nitrogen fertilizer was split into three doses whereas potassium fertilizer was timed with the first nitrogen application (first year: 194 kg N ha^{-1} and 220 kg K_2O ha^{-1}; second year: 240 kg N ha^{-1} and 230 kg K_2O ha^{-1}).

4.2.4. Measurements and biomass sampling

The growth stage of the plants was evaluated weekly throughout the season following the extended BBCH scale of Meier et al. (2001). Prior to each harvest, weed invasion was determined for each plot by estimating weed cover using the decimal scale of Londo (1984). The growing degree days (GDD) is the sum of daily mean temperatures above a threshold temperature of 0 °C. Calculation started with the beginning of vegetative growth at GDD = 200 °C, where positive temperatures were rated half in January and at 75% in February (Ernst and Loeper, 1976). DMY was determined from harvesting a 10 m strip in the centre of each plot (15 m²) using a combine forage harvester (Hege 212, MDW Mähdrescherwerke GmbH, Singwitz, Germany). The cutting height was controlled with a tension roller, so that the target cutting height was met with a deviation not higher than 1 cm. If plants were lodging they were erected manually to achieve constant cutting heights.

4.2.5. Laboratory analysis and NIRS validation

Subsamples of each plot were dried at 105 °C to determine dry matter (DM) content. Additional subsamples were taken and dried at 60 °C for 48 h for further laboratory analysis. Samples from the first year were ground (SM 2000, Retsch GmbH, Haan, Germany) to pass a 1 mm sieve, prior to compositional analysis. Compositional analysis was done using near infrared reflectance spectroscopy (NIRS, FOSS 5000, FOSS GmbH, Hamburg, Germany). An existing calibration function developed for a wide range of chemical composition, needed especially for wide range of cell-wall materials and lignification in three cutting frequencies, of bioenergy crops (VDLUFA , 2010) was used to predict ash, crude protein (CP), crude fibre (CF), crude fat (ether extract), neutral detergent fibre on an organic matter basis (NDF), acid detergent fibre on organic matter (ADF), acid detergent lignin (ADL), and enzyme-soluble organic matter (ESOM) from the NIRS measurements.

For NIRS validation (Table 15), a subset sample (n = 36) was chosen for wet chemical analysis of ESOM (VDLUFA, 1976), Weende proximate analysis (Commission Regulation (EC) No 152/2009, 2009), and fibre content according to van Soest (Van Soest, 1991) following VDLUFA guidelines (1995a, 1995b, 1995c). These samples were selected by performing a cluster analysis with the Hartigan and Wong (1979) algorithm in the “k-means” function in the software R (R Core Team, 2015). Using ESOM as the target variable, the data were clustered into six groups. Six samples from the centre of each group were selected for validation. Due to distinct grouping of the results, correction of systematic bias was performed separately for samples from single cutting and samples from double or frequent cutting. Even though the complete validation had high coefficients of determination (R^2) for the parameters it was considered to be more appropriate for determining the best suited values for correction of systematic bias by distinguishing the cutting frequencies into two groups (one versus double/frequent cutting) with respect to clear differences in the chemical composition. Nevertheless the validation is feasible for a methodical clear bias-correction, but this specific NIRS-validation could only be applied on biomass samples of the first harvest year at this specific location and therefore not be generalised for further tall wheatgrass samples.

On the same samples of the wet chemical analysis the Hohenheim Biogas Yield Test (HBT) was performed according to VDI Guideline 4630 (VDI-Richtlinie, 2006) at the State Institute of Agricultural Engineering and Bioenergy at the University of Hohenheim in triplicates. The test was carried out as described by Hellfrich and Oechsner (2003) and Mittweg et al. (2012) under mesophilic conditions with constant temperature of 37 ± 0.5 °C. Methane production was measured over a period of 35 days by mixing 0.4 g of ground, dry biomass (0.348 – 0.376 g oDM) with a 30 ml inoculum (30 g, 49 g kg^{-1} DM, 230 g kg^{-1} oDM) in an oDM-ratio of inoculum to substrate of at least 2:1. The inoculum was standardized and well controlled, to get a high repeatability of the results (c.f. Mast et al., 2014). It was based on liquid digestate, collected from more than three different biogas plants, running under mesophilic conditions (37 – 40 °C). To guarantee a high stabilisation of the inoculum it was treated in a 400 l digester at a temperature of 37 ± 0.5 °C and fed daily with a broad spectrum of nutrients (carbohydrates, protein, fat and fibres) with a low loading rate of 0.5 kg volatile solids m^{-3} d^{-1}, to receive a broad spectrum of methanogenic microbes and a low gas production of the inoculum itself. The contents of the fermenter were revitalized every 2 months with fermentation substrate from several biogas plants (2 – 5 vol%) (Mittweg et al., 2012). For protection of the quality of the results, two well-known standard substrates were digested parallel to the samples.

To calculate SMY of individual samples, a linear regression model was fitted for the 36 samples for which HBT had been measured. The model included the NIRS estimates (ash, CP, CF, ether extract, NDF, ADF, ADL, ESOM) as independent variables (multiple $R^2 = 0.91$). This model was used to predict SMY for all samples based on the NIRS estimates of chemical composition.

4.2.6. Statistical analysis and modelling

Statistical analyses were carried out with the software R (R Core Team, 2015) and the packages “nlme” (Pinheiro et al., 2016) and “lsmeans” (Lenth, 2015). Depending on the level at which data were analysed, different types of linear mixed effects models were fitted. Models for MHY in the first full harvest year and for relative reduction of annual DMY between the first and second harvest year contained the fixed effects of provenance, cutting height and cutting frequency as well as their factorial interactions. For the analysis of annual DMY, year and its interactions with the other fixed effects were included as well. For all analyses focussing on results of single harvests, a parameter named "harvest date" was formed as unique combination of cutting frequency and number of harvest within year, which resulted in a factor with 4 + 2 + 1 = 7 levels. This factor was included as fixed effect, together with provenance and cutting height, to analyse SMY, biomass quality parameters and proportional DMY reduction of single harvests between first and second full harvest year. Taking into account the experimental layout of a split-split plot, all models contained subplot (cutting frequency) nested in main plot (provenance) as nested random effects. Where repeated measurements within one subplot were considered, i.e. the two harvest years and/or several harvests per harvest year, sub-sub plot (cutting height) was included as a further random effect, nested in subplot. This random effects structure was also used in the model assessing the relationship between SMY and GDD, where the fixed effects were GDD as both linear and quadratic term.

In all cases, model residuals were visually inspected for normality and homoscedasticity. Where these conditions were not fulfilled, the response variable was transformed, or variance function structures were defined, as was necessary. Global models containing all fixed effects described above were simplified using the package "MuMIn" (Barton, 2016). The minimum adequate model was chosen as the model with the lowest value of Akaike's Information Criterion (AIC) and was used for further analysis. Fixed effects included in this model were tested for their significance using sequential Wald tests. For significant effects, post-hoc comparison of means was performed using Tukey tests. A significance level of $\alpha = 0.05$ was chosen throughout.

Table 15: Results of NIRS validation of chemical composition parameters on a subset of 36 samples. Coefficient of determination (R^2) and standard error of prediction (SEP, % of dry matter) over all samples; standard error of prediction corrected for bias (SEP(C), % of dry matter) separately for samples from single cutting (n = 7) and samples from double and frequent cutting (multiple cutting) (n = 29). Asterisks indicate significant SEP© and Bias for $p \leq 0.05$.

	R^2	SEP	SEP(C)		Bias	
			single cutting	multiple cutting	single cutting	multiple cutting
Ash	0.90	1.45	0.66	0.84*	0.66*	1.32*
Crude protein	0.97	1.10	0.41	0.72*	-1.22*	-0.78*
Crude fibre	0.90	1.76	1.28*	1.30*	2.62*	0.46
Ether extract	0.93	0.68	0.18	0.36*	0.37*	0.65*
Neutral detergent fibre	0.66	11.66	2.16	2.44	-2.12*	12.68*
Acid detergent fibre	0.93	1.49	0.82	1.50	-1.11*	0.40
Acid detergent lignin	0.90	1.92	0.21	0.80*	-2.03*	-1.72*
Encyme-soluble organic matter	0.92	3.87	3.05*	2.17*	-0.86	-3.45*

4.3. Results

4.3.1. DMY

DMY in the first year was affected by cutting frequency and cutting height (Table 16) but was not significantly different among the different provenances (Table 17). On average over cutting heights and provenances, the maximum DMY was obtained by double cutting. However, there was an interaction between cutting frequency and cutting height. Varying the cutting height did not change the DMY under frequent cutting, but lowering the cutting height under double frequency from 15 cm to 5 cm increased the yield significantly (+17%). Under single cutting, reducing the cutting height resulted in a steady increase of the DMY with 13.4, 17.9, and 20.2 t ha^{-1} at 15, 10, and 5 cm height, respectively. Thus, the less frequent the sward was harvested, the more important was the cutting height for the DMY in the first year.

Compared to the first year, the DMY was significantly lower in the second year. The proportional yield reduction was strongest under frequent cutting whereas proportional yield reduction under double cutting was similar to single cutting. When simply comparing the means of single harvests (harvest dates) between the two years, DMY reductions were similar under all cutting intensities in the first (-28%) harvest, whereas under frequent cutting the relative yield reduction increased over the year up to -57 and -81% in the third and fourth harvest, respectively.

In the second year, reducing the cutting height did not result in higher DMY under the double cutting frequency (Table 17) as was found in the first year. Similar to the first year, the highest DMY was found by double cutting. However, in contrast to the first year, the level of DMY under frequent cutting at 15 cm (9.7 t ha^{-1}) sank to the level under single cutting at the same cutting height (8.9 t ha^{-1}).

On average over both years, double cutting tended to gain higher DMY than single or frequent cutting. Interactive effects of cutting frequency and height were clearest expressed under single cutting, where yield increased by up to 53% when cutting height was decreased.

While no significant provenance effect on DMY was found in the first year, provenances differed significantly among each other in the second year (Table 17). When considering both years, a significant interaction year x provenance on DMY was found. The Argentinean provenance showed the strongest DMY decrease (-41%) from the first to the second year compared to the other provenances, whereas Hungarian provenance had significantly higher DMY than the Argentinian and the Australian provenance over cutting height and frequency.

The seasonality of biomass production was assessed by comparing the different harvests within years (results not shown). Under double cutting the first harvest contributed 70% to the annual DMY in both years (second harvest 30%). Under frequent cutting the first harvest was more important (first year 39%; second year 48%) than the second harvest (both years 39%) in the second harvest year. The third (first year 12%; second year 9%) and the final harvest (first year 11%; second year 4%) contributed with minor relevance to the accumulated DMY.

Table 16: *F* and *p* values of the linear mixed effects models for dry matter yield (first and second harvest year) and methane hectare yield (first harvest year). Only main effects and interactions that were significant ($p < 0.05$; bold) for at least one target variable are shown.

Source	Dry matter yield		Methane hectare yield	
	F value	*p* value	*F* value	*p* value
Year	**803.1**	**< 0.0001**		
Provenance	**4.3**	**0.0154**	1.9	0.1638
Cutting height (height)	**48.3**	**< 0.0001**	**20.9**	**< 0.0001**
Cutting frequency (frequency)	**33.0**	**< 0.0001**	**109.6**	**< 0.0001**
Year x provenance	**4.7**	**0.0041**		
Year x frequency	**23.2**	**< 0.0001**		
Height x frequency	**17.8**	**0.0420**	10.1	0.1638
Year x height x frequency	**2.6**	**< 0.0001**		

4.3.2. Chemical composition

The chemical composition varied significantly between the harvest dates (Table 18), i.e. the unique combination of cutting frequencies and number of harvest within the year. Although height x harvest date interactions were significant for the parameters ash, ADF, ADL and ESOM, there were no differences between cutting heights at individual harvest dates, with the exception of ash content, which was lower for 5 cm (6.15%) than for 15 cm (7.49%) cutting height at the first harvest under double cutting (data not shown). The impact of the chemical composition on the SMY was apparent (Table 19).

Table 17: Dry matter yield (t ha^{-1}) of four provenances of tall wheatgrass under different cutting frequencies and cutting heights in the first and second full harvest year. Lower case letters indicate significant differences between means of frequency x height within each year or the relative development of dry matter yield between the harvest years; capital letters significant differences between means of provenance x year, averaged over cutting frequency and cutting height ($p < 0.05$).

Provenance/treatments	Single (1 cut a^{-1})			Double (2 cuts a^{-1})			Frequent (4 cuts a^{-1})			Means of provenance x year
	Cutting height			Cutting height			Cutting height			
	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	
first full harvest year										
Argentina	22.3	17.1	13.1	22.5	21.3	19.5	20.1	19.5	21.7	19.7 A
Australia	17.3	18.1	12.4	21.2	20.1	16.9	18.5	19.6	19.9	18.2 A
Hungary	22.3	17.9	14.1	25.8	23.2	21.9	18.5	17.9	17.8	19.9 A
USA	19.0	18.4	14.1	21.8	21.3	19.9	19.6	19.6	19.3	19.2 A
Means of frequency x height	20.2 ab	17.9 c	13.4 d	22.8 a	21.5 ab	19.5 cb	19.2 cb	19.1 cb	19.6 cb	
second full harvest year										
Argentina	13.3	11.1	7.0	15.4	13.7	12.4	10.8	10.7	10.0	11.6 C
Australia	12.2	12.3	8.8	14.5	12.7	14.7	9.7	11.2	10.0	11.8 C
Hungary	18.3	14.6	10.1	18.0	18.2	17.8	11.1	10.8	9.2	14.2 B
USA	13.7	13.3	9.5	16.0	16.3	15.2	11.5	11.2	9.5	12.9 BC
Means of frequency x height	14.4 ab	12.8 bc	8.9 d	16.0 a	15.2 ab	15.0 ab	10.8 cd	11.0 cd	9.7 d	
Relative change in means of frequency x height from first to second full harvest year	-27% cd	-28% bcd	-34% bcd	-30% d	-29% d	-22% d	-44% ab	-42% bc	-50% a	

4.3.3. SMY and MHY

Biomass conversion to methane depends on the chemical composition of the grass. The corresponding linear mixed effects model (Table 18) revealed a strong influence of harvest date ($p < 0.0001$), where different harvest dates correspond to different stages of development and variations in GDD (Table 19). Later stages of development with higher GDD (Figure 8) that were harvested under single cutting showed significantly lower SMY (0.204 Nm^3 kg DM^{-1}) than earlier development stages harvested under double cutting (0.265 Nm^3 kg DM^{-1}) or frequent cutting (0.282 Nm^3 kg DM^{-1}) with short periods between harvests and therefore lower GDD.

The cutting height had only a minor effect on SMY (Table 18). SMY increased significantly when cutting at 15 cm (0.268 Nm^3 kg DM^{-1}) rather than at 5 cm (0.264 Nm^3 kg DM^{-1}), but an increase of 1.5% was not as relevant as the potential for increasing SMY by 41.7% depending on the date of harvest (Table 19).

The corresponding MHY was calculated based on DMY and SMY at individual harvest dates (Table 20). A higher DMY at the low harvest intervals (frequent cutting treatment) could compensate for a lower SMY. This resulted to some extend in equal MHY in contrast to significantly differing DMY as seen under frequent and double cutting at 5 cm cutting height in the first year (Table 17).

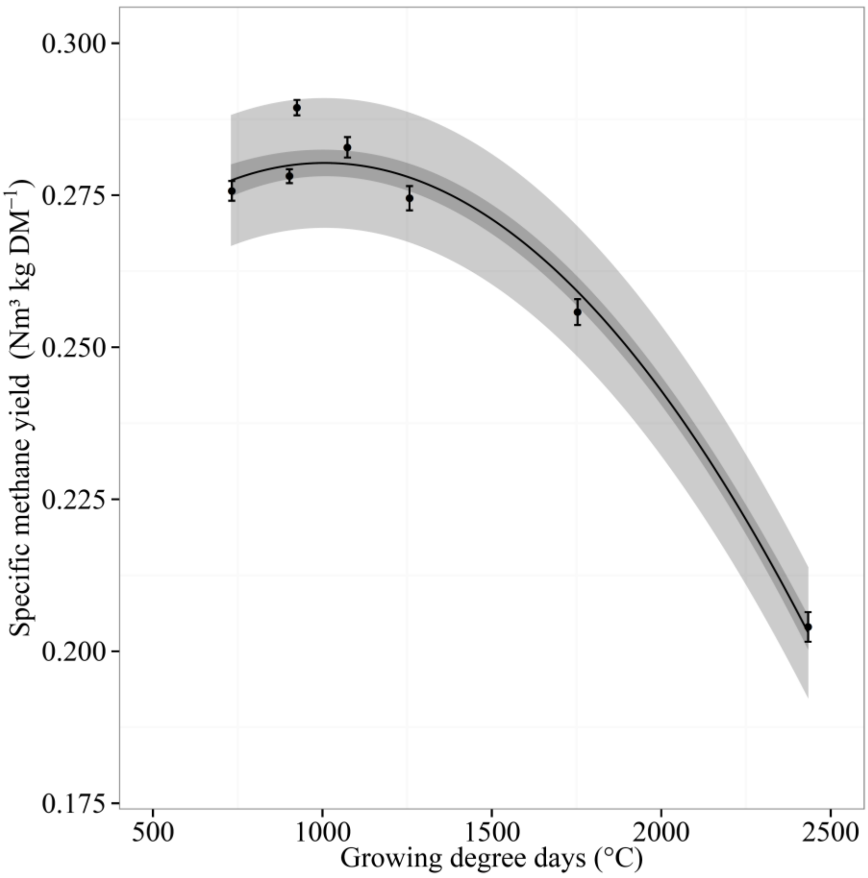

Figure 8: Relationship between growing degree days (GDD) of each growth interval and the specific methane yield (SMY) of the harvested biomass in the first full harvest year. Points represent the mean values of single harvest dates over three cutting heights and four provenances; error bars indicate the standard error of the mean. The black line indicates the response predicted by the linear mixed effects model (SMY [Nm^3 kg DM^{-1}] = $-3.79*10^{-8}$ GDD^2 $+7.63*10^{-5}$ GDD + 0.242; GDD^2: $p < 0.0001$, GDD: $p < 0.0001$; proportion of variance explained: 0.882), with confidence interval (dark grey) and prediction interval (light grey); ($\alpha = 0.05$).

Table 18: p values of the linear mixed effects models for SMY and chemical composition. Only main effects and interactions that were significant ($p < 0.05$; bold) for at least one target variable are shown (n = 252 NIRS results).

Parameter	SMY	Ash	CP	CF	Ether extract	NDF	ADF	ADL	ESOM
Provenance	0.0581	0.7023	**0.0026**	**0.0215**	**0.0439**	0.0595	0.1863	< **0.0001**	0.7404
Cutting height (height)	**0.0400**	0.1100	0.3147	0.1994	0.7342	0.4055	0.7539	0.0921	0.4826
Harvest date (date)	< **0.0001**	< **0.0001**	**0.0001**	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**
Provenance x date		0.2127	**0.0038**	**0.0009**	**0.0145**	**0.0003**	**0.0006**	< **0.0001**	**0.0001**
Height x date		**0.0011**	0.1657	0.1041	0.2028	0.0904	**0.0375**	**0.0173**	**0.0447**

Table 19: Specific methane yield and chemical composition of tall wheatgrass biomass in individual harvest dates of the first full harvest year (n = 252 NIRS results). Lower case letters indicate significant ($p < 0.05$) differences within rows. Growth stages were determined by following the guidelines of Meier et al. (2001).

	Cutting frequency						
	Single (1 cut a^{-1})	Double (2 cuts a^{-1})		Frequent (4 cuts a^{-1})			
	Cut no. 1	Cut no. 1	Cut no. 2	Cut no. 1	Cut no. 2	Cut no. 3	Cut no. 4
Growth stage	71-89	55-58	65-67	33	56-58	31-33	29
Growing degree days (°C)	2433	1257	1753	733	903	1074	925
Specific methane yield (Nm^3 kg DM^{-1})	0.204 e	0.275 c	0.256 d	0.276 c	0.278 bc	0.283 b	0.289 a
Ash (g kg^{-1})	46 e	68 d	74 c	8.7 b	75 c	91 b	109 a
Crude protein (g kg^{-1})	68 d	86 c	90 c	13.1 b	76 d	147 a	151 a
Crude fibre (g kg^{-1})	400 a	398 a	378 b	33.4 c	381 b	303 d	294 d
Ether extract (g kg^{-1})	12 e	20 c	21 c	2.8 b	18 d	30 b	34 a
Neutral detergent fibre (g kg^{-1})	687 b	740 a	742 a	63.0 c	731 a	587 d	574 d
Acid detergent fibre (g kg^{-1})	430 a	424 ab	423 ab	35.5 c	414 b	323 d	323 d
Acid detergent lignin (g kg^{-1})	67 a	43 c	51 b	2.5 d	47 c	16 e	10 f
Enzyme-soluble organic matter (g kg^{--1})	350 e	392 d	373 e	49.2 c	405 d	514 b	534 a

Table 20: Methane hectare yield (Nm³ ha^{-1}) of four provenances of tall wheatgrass under different cutting frequencies and cutting heights in the first full harvest year. Lower case letters indicate significant differences between means of frequency x height, averaged over provenance ($p < 0.05$).

Provenance/treatments	Single (1 cut a^{-1})			Double (2 cuts a^{-1})			Frequent (4 cuts a^{-1})			Means of provenance
	Cutting height			Cutting height			Cutting height			
	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	
Argentina	4604	3389	2761	5972	5796	5321	5623	5473	6097	5004
Australia	3522	3786	2337	5518	5466	4623	5197	5466	5591	4612
Hungary	4508	3769	2898	6778	6225	5796	4984	4951	4903	4979
USA	3734	3745	3007	5884	5670	5409	5482	5453	5385	4864
Means of frequency x height	4092 c	3673 c	2751 d	6038 a	5789 ab	5287 b	5321 ab	5336 ab	5494 ab	

4.3.4. Weed pressure and plant development

Weed cover was highest under frequent cutting whereas almost no weeds were present under double and single cutting at the end of both harvest years (Table 21). After the first year the invasion of weeds under frequent cutting, expressed by the cover, was lower for all cutting heights than after the subsequent year. Weed pressure increased to the last harvest in the second year and was strongest for low cutting height of 5 cm.

Table 21: Development of weed infestation in different cutting frequencies and cutting heights of tall wheatgrass, expressed as mean cover of soil by weed biomass ± standard deviation following the decimal scale of Londo (1984).

	Cutting height					
Cutting frequency	5 cm		10 cm		15 cm	
	End of first harvest year	End of second harvest year	End of first harvest year	End of second harvest year	End of first harvest year	End of second harvest year
Single cutting	0%	0%	0%	0%	0%	0%
Double cutting	0%	0%	0%	0%	0%	0%
Frequent cutting	2 ± 2%	14 ± 4.4%	0.5 ± 0.7%	6.8 ± 2.6%	0.2 ± 0.6%	3 ± 2.7%

The growth stage was a function of cutting frequency and harvest date. The single cutting had the longest period of growing and reached growth stage ripening (BBCH 81- 85) until harvest, but did not bolt afterwards (BBCH 29). Under double cutting the shoots elongated until first harvest (BBCH 55-61) as well as until the second annual harvest (BBCH 65). In contrast, the first harvest under frequent cutting was conducted at BBCH 33 (third node at least 2 cm above second node), but shoot elongation occurred by the second cut (BBCH 56 – 68). Over the summer period tall wheatgrass development was inhibited until third cutting at beginning of stem elongation (BBCH 31) and also in autumn of the second year until final harvest (BBCH 29; end of tillering). In the first year some tillers were elongated by the fourth cut (BBCH 51; beginning of heading), but most did not grow more than BBCH 29.

The plant development was fastest in the beginning of the year. The period of growth to reach BBCH 65 was shorter after first harvest under frequent cutting (60 days) in May than after the first harvest of the double cutting (95 days) in June.

4.4. Discussion

The present study investigated the agronomy of tall wheatgrass. Four provenances were grown over two full harvest years under varied cutting frequency and cutting height. The hypotheses were tested that (1) aboveground DMY and MHY are affected by cutting frequency and height, and (2) crop management interacts with tall wheatgrass germplasm of different provenances.

The best suited management strategy was different for optimum DMY and MHY, due to the potential of compensating lower DMY under frequent cutting by higher SMY. The DMY as well as the SMY were strongly affected by the cutting frequency. In general, double cutting gained the highest DMY in both years and the best MHY, this latter trait being determined only in the first year. DMY reduction from the first to the second year was significantly stronger under frequent cutting compared to double and single cutting. The effect of the cutting height was less relevant although reducing cutting height increased DMY and MHY. Differences in DMY among the provenances were small and only found in the second harvest year ($p < 0.05$) without interaction with the crop management.

4.4.1. DMY response to cutting frequency

An important finding of our experiment was the strong interaction of year x cutting frequency with a considerable yield decrease in the second year in particular with frequent cutting. In agreement with these studies, Schrabauer et al. (2014) described a decreasing DMY from the first to the second year, but also showed a contrasting trend of increasing yield at a second location. Less information is available on the interaction of tall wheatgrass crop management with soil and weather conditions. In the present investigation the environmental conditions of the first year with an early start of the season in spring and higher temperatures in autumn were more favourable than in the second year (cf. Figure 7). This has obviously contributed to increased growth and overall DMY in the first year (Fang et al., 2003; Wan et al., 2005). Lower DMY in the second year might also be related to an impaired persistence of the grass sward. In general, grassland plants are adapted to frequent cutting as they maintain leaf area and growing points below the harvested horizon of the sward and can thus recover (Virkajärvi et al., 2012). However, grassland plants are not all similarly affected. Erect growing bunchgrasses, like tall wheatgrass (Scheinost et al., 2008), secure less green leaves and tillers after cutting and thus suffer from short harvest intervals (Hodgkinson et al., 1989) with decreasing DMY and persistence. With regard to tall wheatgrass, there is no clear evidence as to how the grass responds towards cutting frequency: Malinowski et al. (2003) found a considerable decrease of the annual DMY in intensive crop utilization, whereas Moore et al. (1981) did not find a similar response. From our results we deduce that the DMY and persistence of tall wheatgrass with varying cutting frequency are depending on the climatic and growing conditions.

4.4.2. DMY response to cutting height

The DMY was significantly affected by the cutting height. On average over cutting frequency, a low cutting height resulted in generally higher yields compared to the higher cutting. This is in line with observations in tall fescue. Burns et al. (2002) in a three year experiment found a 21% higher yield of tall fescue when it was regularly cut at 5 cm instead of 9 cm. However, this relationship is not a simple one. In our experiment, the effect of cutting height was dependent on the frequency of cutting (Table 17). Under frequent cutting the cutting height showed no significant effect on the DMY whereas at single cutting lowering the cutting height from 15 to 5 cm raised the DMY by 51% (first year) and 62% (second year). At double cutting the DMY was increased by 17% when the cutting height was reduced. Other studies also point to more complex interactions of crop management with years (Malinowski et al., 2003). The complex response of DMY towards the cutting is also confirmed by experiences from the US. Based on results of various experimentations with tall wheatgrass on alkaline soils and in a semi-arid environment, Scheinost et al. (2008), USDA (2013) and Wasser et al. (1986) recommended an optimum stubble height at harvest and at the end of the growing season of approximately 15 cm. This recommendation is obviously related to the particular site conditions where the regrowth after a cut is hampered by a limited water and related nitrogen availability. Apparently, the site of the present experiment has a much higher availability of water (cf. Table 13) and soil mineral nitrogen. Hence, the regrowth after cutting is less limited. This is likely to explain the different DMY response towards the cutting height in our experiment compared to the American experience.

4.4.3. Provenance effect on DMY

Compared to the effect of cutting height and cutting frequency, the germplasm only showed a minor effect on the DMY. The Hungarian provenance was slightly higher yielding than the other provenances, which were not different among each other. This superiority was significantly stronger in the second year. The reason for the higher DMY of the Hungarian provenance is obviously related to the breeding background. This provenance has been developed fairly recently with a focus on high biomass and methane yields under central European conditions (Brand, personal communication). The breeding history of the other provenances reaches back much further and their breeding purpose was clearly a different one due to less favourable environmental conditions. Alkaline and semi-arid soils dominate the growing area of tall wheatgrass in South and North America and Australia, where tall wheatgrass was adapted to the purpose of grazing and forage production. Consequently, it needed to fulfil other requirements than for biogas production in Europe; such as drought and alkaline tolerance, adaptation to frequent cutting, and a good forage quality (Malinowski et al., 2003; Moore et al., 2006; Scheinost et al., 2008; Wasser et al., 1986; Weintraub, 1953). Similar results for double cutting were obtained by Heinz (2015) at a higher altitude (450 m) in two consecutive years with small difference in DMY between the Hungarian (mean 18.2 t ha^{-1}) and the American provenance (mean 18.0 t ha^{-1}). Lunenberg and Hartmann (2016) showed differences between DMY of two American provenances of the cultivar Jose (mean 17.6 t ha^{-1}) and Alkar (mean 15.9 t ha^{-1}) in the harvest years 2014 and 2015. They also found a yield reduction from 2014 to 2015.

4.4.4. Chemical composition, SMY and MHY

Cutting height was less important for the SMY and MHY than for the DMY. Decreasing the cutting height from 15 cm to 5 cm raised the SMY by only 1.5%. The cutting frequency, however, had a much stronger impact on SMY ranging from 0.204 Nm³ kg DM^{-1} under single cutting to 0.289 Nm³ kg DM^{-1} in the fourth cut of the frequent cutting treatment. This finding is readily explained by the increasing cell wall content, the higher lignification of the cell wall and thus lower digestibility when the grass is cut at a more mature growth stage. The importance of the stage of maturity of grasses from extensively managed grasslands as indicated by the crude fibre content for the SMY has also been shown by Prochnow et al. (2009, 2005).

The small effect of cutting height on the SMY and other biomass quality characteristics was not expected. Other researches had shown that a variety of temperate grass species responded with a deterioration of biomass quality with reduced cutting height (Cherney and Cherney, 2005; Parsons et al., 2012). This was attributed to the spatial arrangement of grass swards with younger tissue and a higher share of leaves being allocated to the upper part of the sward (Suksombat and Buakeeree, 2005; Willms and Beauchemin, 1990). Other studies showed little response of the biomass quality with varying cutting height. For Napier grass (*Pennisetum purpureum* Schumach) Tessema et al. (2010) did not find any significant effect of cutting height on most of the chemical components and of in-vitro DM digestibility, except that of ash and CP. Little effects of the cutting height may be related to a particular morphology of the grass species and the sward. A higher stem to leaf ratio can affect the concentration of fibrous constituents of the plant (Willms and Beauchemin, 1990). Hoekstra et al. (2007) associated inconsistent effects of the cutting height on *Lolium perenne* L. with soil and dead material in the harvested biomass rather than the cutting height itself. Our results showed that the harvest date as a function of cutting frequency was highly relevant for the SMY, which in turn is linked to the chemical composition (Table 19). The ADL content increases when the plant turns from the elongation to the flowering and ripening stage and the concurrent stabilisation of the stem (Chen et al., 2002). ADL levels of up to 67 g kg^{-1} were found under single cutting at a growth stage of BBCH 71-89, which confirms the findings of Salon et al. (2010). As lignin is not fermentable in biogas plants and protects other cell wall compounds against microbial degradation, its content is important for the SMY (Mittweg et al., 2012).

The maturity of plants has earlier been shown to serve as an indicator of the growth stage, which in turn is related to the GDD (Miller et al., 2001). GDD are known to affect the forage quality of grasses (Hill et al., 1995; Mitchell et al., 2001). Especially cell wall components increase with rising temperature (Jelmini and Nösberger, 1978). This relationship had been analysed for several grass species. For sand bluestem (*Andropogon gerardii* var. paucipilus (Nash) Fern.) and prairie sandreed (*Calamovilfa longifolia* (Hook.) Scribn) Hendrickson et al. (1997) reported high correlations up to -0.99 between GDD and in vitro dry matter digestibility and a correlation up to 0.83 between GDD and lignin, respectively. In the present investigation, GDD for the first time was used to predict SMY of tall wheatgrass (Figure 8). A fairly close relation of 0.882 was found in this experiment in the first harvest year, indicating the potential to deduce SMY values from GDD. To what extent this relation would be confirmed in different environments and would thus have the potential for a more general rapid assessment of the biogas yield of a tall wheatgrass crop remains to be proven in further studies.

The mean SMY of tall wheatgrass in the present study was slightly lower than demonstrated by Mast et al. (2014). These authors found SMY values being variable depending on the date of harvest, however, maximum values even exceeded average SMY values of silage maize (0.349 Nm^3 kg oDM^{-1}). In our study, the SMY of a maize crop that had been tested as a reference crop in the HBT (0.334 Nm^3 kg DM^{-1}) was not reached by tall wheatgrass. Yet, the SMY of tall wheatgrass from the first harvest of the double cutting treatment grown was only 12% lower compared to the maize reference. Similar values had been reported by Herrmann et al. (2016) who graded the relative SMY of tall wheatgrass as 82% of that of maize. Both studies harvested tall wheatgrass in similar growth stages that led to SMY on the same level because of the effect of the stage of maturity on SMY like shown in Table 19.

4.4.5. Weed pressure

In the current study, weeds occurred only under frequent cutting with a marked increase from the first to the second year. Under single and double cutting, weeds were not playing any role. The weed invasion might have resulted in a DMY decline from the first to the second year that was stronger for the frequent than for the other cutting treatments. As has been shown above (4.3.4.), the growth of the tall wheatgrass stands was obviously impaired by frequent cutting, allowing weeds to invade and becoming stronger competitors to the crop. A similar result was obtained by Schrabauer et al. (2014) who found that a two-cut system led to weed coverage of more than 20% in the second year whereas in the one-cut system weeds were almost completely suppressed over a period of two consecutive years. Results of Csete et al. (2011) indicate a strong linear relationship between biomass production and the logarithmic values of total weed cover. They found that a weed cover of 50% was related with low DMY of less than 10 t ha^{-1} whereas a weed cover of 5% and lower was found when the DMY was higher than 20 t ha^{-1}. The cutting height also showed to have an effect on weed occurrence. At the higher cutting (15 cm) the weeds were much less important (3%) than at the lower cutting (5 cm, 14%). Geber (2002) reported similar observations when comparing two and four annual cuts with different heights of reed canary grass (*Phalaris arundinacea* L.), with a significantly higher weed cover in the frequent cutting. It can thus be concluded that in order to control weeds and to ensure persistency of tall wheatgrass over several years, the swards should either be cut in double rather than frequent intervals. If for biomass quality reasons tall wheatgrass is cut frequently, a rather tall cutting height should be aimed at.

4.5. Conclusions

The experiment showed that the cutting management of tall wheatgrass is highly important for the performance as a crop for biogas production, whereas the genomic imprinting of grass species caused by provenance was small. The cutting frequency had a stronger effect on DMY, SMY and corresponding MHY than the cutting height. Extending the cutting interval led to increased maturation of the harvested biomass with considerable losses in SMY. The prediction of SMY using GDD had a high accuracy in this research. The cutting frequency of tall wheatgrass should, however, not solely be adapted to obtain highest SMY as with frequent cutting we found a considerable yield decline from the first to the second full harvest year and significant weed invasion. Obviously, frequently cut swards are less persistent, which is exacerbated by a low cutting height. It is therefore concluded that a double cutting frequency, i.e. two cuttings per year combined with a cutting height of 5-10 cm, has a high potential of providing sustainable dry matter and biogas yields, while the risk of weed invasion is low. Further long-term effects of severe drought, fertiliser management, or varying stubble heights between spring and autumn harvest should be examined in subsequent research.

4.6. Acknowledgements

The laboratory analysis was co-funded by the German Federal Ministry of Food and Agriculture via the Agency for Renewable Resources under grant number 22002010. The authors gratefully acknowledge Konstantin Sümmermann for thoroughly conducting the

NIRS analyses, Hans Oechsner for his technical support in biomass fermentation and Peter Tillmann for the subsequent validation of the NIRS data.

4.7. References

Andrés, A., Guillen, R., 2003. Genetic variability within two adapted population of tall wheatgrass (*Thinopyrum ponticum*) in Argentina. Available at http://www.internationalgrasslands.org/files/igc/publications/2001/id1203.pdf (accessed 17 July 2014).

Barton. K., 2016. MuMIn: Multi-Model Inference. R package version 1.15.6.

Beever, D.E., Offer, N., Gill, M., 2000. The feeding value of grass and grass products. In: Hopkins, R. (Ed.), Grass: Its production and utilization, Blackwell Scientific, Oxford, UK, pp. 140–195.

Burns, J.C., Chamblee, D.S., Giesbrecht F.G., 2002. Defoliation intensity effects on season-long dry matter distribution and nutritive value of tall fescue. Crop Science 42, 1274-1284.

Chen, L., Auh, C., Chen, F., Cheng, X.F., Aljoe, H., Dixon, R.A., Wang, Z.Y., 2002. Lignin deposition and associated changes in anatomy, enzyme activity, gene expression, and ruminal degradability in stems of tall fescue at different development stages. Journal of Agriculture and Food Chemistry 50, 5558-5565. doi: 10.1021/jf020516x

Cherney, D.J.R., Cherney, J.H., 2005. Forage yield and quality of temperate perennial grasses as influenced by stubble height. Forage and Grassinglands 3, 0-0. doi: 10.1094/FG-2005-0215-01-RS

Commission Regulation (EC) No 152/2009, 2009. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Brussels, Belgium: Commission of the European Communities.

Csete, S., Stranczinger, S., Szalontai, B., Farkas, A., Pal, R.W., Salamon-Albert, E., Kocsis, M., Tovar, P., Vojtela, T., Dezso, J., Walcz, I., Janowszky, Z., Janowszky, J., Borhidi, A., 2011. Tall wheatgrass cultivar Szarvasi-1 (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) as a potential energy crop for semi-arid lands of eastern Europe. In: Nayreripour, M., Kheshti, M., (Ed,). Sustainable growth and application in renewable energy source. InTech, Rijeka, Croatia, pp. 269-294.

Dahlhoff, A., 2013. Auswertung der Biogasanlagen-Betreiberdatenbank der Landwirtschaftskammer Nordrhein-Westfalen (Evaluation of biogas plant survey of the chamber of agriculture in North Rhine-Westphalia). Center of renewable resources of the chamber of agriculture for North Rhine-Westphalia, Bad Sassendorf, Germany.

Dinnes, D.L., Douglas, L.K., Jaynes, D.B., Kaspar, T.C., Hatfield, J.L., Colvin, T.S., Cambardella, C.A., 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained midwestern soils. Agronomy Journal 94, 153-171.

Ernst, P., Loeper, E.G., 1976. Temperaturentwicklung und Vegetationsbeginn auf dem Grünland (Temperature and beginning of vegetation growth in grasslands). Das wirtschaftseigene Futter 22, 5-11.

European Parliament, 2013. Regulation (EU) No 1307/2013 of the European Parliament and of the Council of 17 December 2013.

Fang, J., Piao, S., Field, C.B., Pan, Y., Guo, Q., Zhou, L., Peng, C., Tao, S., 2003. Increasing net primary production in China from 1982 to 1999. Frontiers in Ecology and Environment 1(6), 293-297.

Geber, U., 2002. Cutting frequency and stubble height of reed canary grass (*Phalaris arundinacea* L.): influence on quality and quantity of biomass for biogas production. Grass and Forage Science 57, 389-394.

Graf, R., Michel, V., Rossberg, D., Neukampf, R., 2009. Definition pflanzenartspezifischer Anbaugebiete für ein regionalisiertes Versuchswesen im Pflanzenbau (Definition of crop specific areas for planning of distribution of field trials). Journal of Cultivated Plants 61, 247-253.

Hanson, A.A., 1972. Grass varieties in the United States – Agricultural Handbook No. 170. Agricultural Research Service, Washington D.C., USA.

Hartigan, J.A., Wong, M.A., 1979. A K-means clustering algorithm. Applied Statistics 28, 100-108.

Heinz, M., 2015. Riesenweizengras - eine Energiepflanze mit Zukunft (Tall wheatgrass – a potential energy crop). Biogas Journal 2/2015, 48-50.

Hellfrich, D., Oechsner, H., 2003. Comparison of different laboratory techniques for the digestion of biomass. Landtechnik 58, 148-149.

Hellmich, W., 2006. Digitales Fachinformationssystem Bodenkunde – Bodenkarte zur Standorterkundung (Digital information system for soil science – soil map for site investigation). Geologischer Dienst Nordrhein-Westfalen, Krefeld, Germany.

Hendrickson, J.R., Moser, L.E., Moore, K.J., Waller, S.S., 1997. Leaf nutritive value related to tiller development in warm-season grasses. Journal of Range Management 50, 116-122.

Herbes, C., Jirka, E., Braun, J.P., Pukall, K., 2014. Der gesellschaftliche Diskurs um den Maisdeckel vor und nach der Novelle des Erneuerbaren-Energien-Gesetzes (EEG) 2012 (The social discourse on the maize cap before and after the 2012 amendment oft he German renewable energies act (EEG)). GAiA 23, 100-108. doi: 10.14512/gaia.23.2.7

Herrmann, C., Plogsties, V., Willms, M., Hengelhaupt, F., Eberl, V., Eckner, J., Strauße, C., Idler, C., Heiermann, M., 2016. Methane production potential of various crop species grown in energy crop rotations. Landtechnik 71, 194-208. doi: 10.15150/lt.2016.3142

Hill, N.S., Cabrera M.L., Agee, C.S., 1995. Morphological and climatological predictors of forage quality in tall fescue. Crop Science 35, 541–549.

Hodgkinson, K.C., Ludlow, M.M., Mott, J.J., Baruch, Z., 1989. Comparative responses of Savanna grasses Cenchrus ciliaris and Themeda triandra to defoliation. Oecologia 79, 45-52.

Hoekstra, N.J., Struik, P.C., Latinga, E.A., Schulte, R.P.O., 2007. Chemical composition of lamina and sheath of *Lolium perenne* as affected by herbage management. Wageningen Journal of Life Sciences 55, 55-73.

Jelmini, G., Nösberger, J., 1978. Effect of temperature on growth, development, nonstructural carbohydrates and nitrogen of *Festuca pratensis* HUDS, *Lolium multiflorum* LAM, *Trifolium pratense* L and *Trifolium repens* L. Journal of Agronomy and Crop Science 146, 143-153.

JKI, 2014. GeoPortal-Application for soil-climate-areas. Julius Kühn-Institut, Federal Research Center for Cultivated Plants, Quedlinburg, Germany. Available at http://geoportal.julius-kuehn.de/map?app=bodenklimaraum (accessed 18 April 2016).

Kerrisk, J.J., Thomson, N.A., 1990. Effect of intensity and frequency of defoliation on growth of ryegrass, tall fescue and phalaris. In: Proceedings of the New Zealand Grassland Association 51, pp. 135-138.

Lenth, R., 2015. Lsmeans: Least-Squares Means. R package version 2.20-2.

Liu, Z.W., Wang, R.C., 2011. Alkar – tall wheatgrass. United States Department of Agriculture – Natural Resources Conservation Center, Pullmann, USA.

Londo, G., 1984. The decimal scale for relevés of permanent quadrats. In: Knapp, R. (Ed.), Sampling methods and taxon analysis in vegetation science. *d*en Haag, Netherlands, pp. 45-49.

Lunenberg, T, Hartmann, A., 2016. Riesenweizengräser – Sortenunterschiede am Standort Straubing (Tall wheatgrass – effects of the cultivar at Straubing). TFZ-Merkblatt 16PLb004, Technologie und Förderzentrum, Straubing, Germany.

Malinowski, D.P., Hopkins, A.A., Pinchak, W.E., Sij, J.W., Ansley, R.J., 2003. Productivity and survival of defoliated wheatgrasses in the Rolling Plains of Texas. Agronomy Journal 95, 614-626.

Mast, B., Lemmer, A., Oechsner, H., Reinhardt-Hanisch, A., Claupein, W., Graeff-Hönninger, S., 2014. Methane yield potential of novel perennial biogas crops influenced by harvest date. Industrial Crops and Products 58, 194-203.

Meier, U., Bleiholder, H., Buhr, L., Feller, C., Hess, M., Klose, R., Lancashire, P.D., Stauss, R., van den Boom, T., Weber, E., Wicke, H., 2001. Growth stages of mono- and dicotyledonous plants, 2nd edition. Federal Biological Research Center for Agriculture and Forestry, Braunschweig, Germany.

Miller, P., Lanier, W., Brandt, S., 2001. Using growing degree days to predict plant stages. Montana State University. Available at http://store.msuextension.org/publications/AgandNaturalResources/MT200103AG.pdf (accessed 11 August 2016).

Mitchell, R.B., Fritz, J., Moore, K., Moser, L., Vogel, K., Redfearn, D., Wester, D., 2001. Predicting forage quality in switchgrass and big bluestem. Agronomy Journal 93, 118–124. doi: 10.2134/agronj2001.931118x

Mittweg, G., Oechsner, H., Hahn, V., Lemmer, A., Reinhardt-Hanisch, A., 2012. Repeatability of laboratory batch method to determine the specific biogas and methane yield. Engineering in Life Science 12, 270-278.

Moore, G., Sanford, P., Wiley, T., 2006. Perennial pastures for western Australia. Department of Agriculture and Food Western Australia, Perth, Australia.

Moore, J, Murphy, J.M., Rouquette, F.M., Holt, E.C., 1981. Influence of defoliation height and frequency on yield and in vitro dry-matter digestibility of Jose tall wheatgrass. In: Forage Resarch in Texas - Department Technical Report No. 81-12, Department of Soil and Crop Sciences, Pecos, USA, pp. 155-156.

Oram, R.N., 1981. Register of Australian herbage plant cultivars: a grasses, 18. wheatgrass *Agropyron elongatum* (Host.) Beauv. (Tall wheatgrass) cv. Tyrrell. Reg. No. A-18a-1. J. Australian Institute of Agricultural Science 47, 179-180.

Parsons, D., McRoberts, K.C., Cherney, J.H., Cherney, D.J.R., Bosworth, S.C., Jimenez-Serrano, F.R., 2012. Preharvest neutral detergent fiber concentration of temperate perennial grasses as influenced by stubble height. Crop Science 52, 923-931. doi: 10.2135/cropsci2011.09.0478

Pearson, C.J., Ison, R.L., 1996. Agronomy of grassland systems. Second edition. Cambridge University Press, United Kingdom.

Pimentel, D., Allen, J., Beers, A., Guinand, L., Linder, R., McLaughlin, P., Meer, B., Musonda, D., Perdue, D., Poisson, S., Siebert, S., Stoner, K., Salazar, R., Hawkins, A., 1987. World agriculture and soil erosion - Erosions threatens world food production. BioScience 37, 277-283.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. Nlme: Linear and nonlinear mixed effects models. R package version 3.1 - 128.

Prochnow, A., Heiermann, M., Drenckan, A., Schelle, H., 2005. Seasonal pattern of biomethanation of grass from landscape management. Agricultural International: The CIGR Ejournal, Manuscript EE 05 011, II, 1-17.

Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., Hobbs, P.J., 2009. Bioenergy from permanent grassland – a review: 1. Biogas. Bioresource Technology 100, 4931-4944.

R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rogers, A.L., Bailey, E.T., 1963. Salt tolerance trials with forage plants in south-western Australia. Australian Journal of Experimental Agriculture and Animal Husbandry 3, 125-130.

Salon, P.R., Mayton, H., Hansen, J., van der Grinten, M., Horvath, T., 2010. Tall wheatgrass for biofeedstock energy: Yield, seeding rate and time of harvest study. United States Department of Agriculture - Natural Resources Conversation Service, Corning, USA.

Scheinost, P., Tilley, D., Ogle, D., Stannard, M., 2008. Tall wheatgrass – Plant guide. United States Department of Agriculture – Natural Resources Conversation Service, Pullman, USA.

Schittenhelm, S., Reus, D., Kruse, S., Hufnagel, J., 2011. Assessment of productivity and profitability of sole and double-cropping for agricultural biomass production. Journal of Cultivated Plants 63, 387-395.

Schrabauer, J., Buchgraber, K., Moder, K., Liebhard, P., 2014. Potential of ten alternative grass species under different cutting regimes in Central Europe. Journal of Cultivated Plants 66, 189-202.

Smith, K.F., Kelman, W.M., 2000. Register of Australian Herbage Plant Cultivars. Australian Journal of Experimental Agriculture 40, 119-120. doi: 10.1071/EA99156_CU

Smith, K.F., Lee, C.K., Borg, P.T., Flinn, P.C., 1994. Yield, nutritive value and phenotypic variability of tall wheatgrass grown in a non-saline environment. Australian Journal of Experimental Agriculture 34, 609-614.

Suksombat, W., Buakeeree, K., 2005. Effect of cutting interval and cutting height on yield and chemical composition of hedge Lucerne (*Desmanthus virgatus*). Asian-Australian Journal of Animal Sciences 19, 31-34.

Taboada, M.A., Rubio, G., Lavado, R.S., 1998. The deterioration of tall wheatgrass pastures on saline sodic soils. Journal of Range Management 51, 241-246.

Tessema, Z.K., Mihret, J., Solomon, M., 2010. Effect of defoliation frequency and cutting height on growth, dry-matter yield and nutritive value of Napier grass (*Pennisetum pupureum* (L.) Schumach). Grass and Forage Science 65, 421-430.

Undersander, D.J., Naylor, C.H., 1987. Influence of clipping frequency on herbage yield and nutrient content of tall wheatgrass. Journal of Range Management 40, 31-35.

UPOV, 2015. Elytrigia elongata International union for the protection of new varieties of plants. Available at http://www.upov.int/edocs/mdocs/upov/en/twa_44/tg_elytr_proj_5.pdf (accessed 8 March 2016).

USDA, 1987. Soil mechanics level 1 – module 3 – USDA textural classification. Soil Conversation Service of the United States Department of Agriculture, USA. Available at https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1044818.pdf (accessed 19 December 2016).

USDA, 2013. Conservation release brochure for ‘Jose’ tall wheatgrass. Plant Materials Center of the United States Department of Agriculture, Los Lunas, USA.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 72, 3583-3597.

VDI-Richtlinie 4630, 2006. Fermentation of organic materials – Characterization, of substrate, sampling, collection of material data, fermentation tests. VDI-Gesellschaft Energietechnik, Düsseldorf, Germany.

VDLUFA, 1976. Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik, 3. Auflage (Handbook of agricultural research and analysis methods, 3rd edn.). Verband Deutscher Landwirtschaftlicher Untersuchung- und Forschungsanstalten, Meldungen, Germany.

VDLUFA, 1991a. VDLUFA Methodenbuch Band I – Böden – Kapitel A 5.1.1 (VDLUFA method manual part 1 – soils – chapter A 5.1.1). Verband Deutscher Landwirtschaftlicher Untersuchung- und Forschungsanstalten, Darmstadt, Germany.

VDLUFA, 1991b. VDLUFA Methodenbuch Band I – Böden – Kapitel A 6.2.1.1 (VDLUFA method manual part 1 – soils – chapter A 6.2.1.1). Verband Deutscher Landwirtschaftlicher Untersuchung- und Forschungsanstalten, Darmstadt, Germany.

VDLUFA, 1995a. Method 6.5.1, Determination of Amylase-treated neutral-detergent fibre (aNDF) and Amylase-treated neutral-detergent fibre exclusive of residual ash (aNDFom). In: VDLUFA-Methods Book, Part III Feed Analysis, 4th edition, 8th suppl., VDLUFA-Verlag, Darmstadt, Germany.

VDLUFA, 1995b. Method 6.5.2, Determination of acid detergent fibre (ADF). In: VDLUFA-Methods Book, Part III Feed Analysis, 4th edition, 8th suppl., VDLUFA-Verlag, Darmstadt, Germany.

VDLUFA, 1995c. Method 6.5.3, Determination of acid detergent lignin. In: VDLUFA-Methods Book, Part III Feed Analysis, 4th edition, 8th suppl., VDLUFA-Verlag, Darmstadt, Germany.

VDLUFA, 2010. Biomasse-Kalibrierung entwickelt in dem FNR-Projekt „Bewertung nachwachsender Rohstoffe zur Biogaserzeugung für die Pflanzenzüchtung (naRoBi)" Teilprojekt 4: "Entwicklung von NIRS-Kalibrierungen an getrocknetem Material und Umsetzung der NIRS-Untersuchungen unter praktischen Bedingungen", FKZ 22001906 (Biomass-calibration developed by FNR-project „Evaluation of renewable resources for biogas production in plant breeding" Part 4:"Evaluation of NIRS-calibration of dry matter and implementing in practical conditions", grant number 22001906). VDLUFA Qualitätssicherung NIRS GmbH, Kassel, Germany.

Virkajärvi, P., Saarijärvi, K., Rinne, M., Saastamoinen, M., 2012. Grass physiology and its relation to nutritive value in feeding horses. In: Saastamoinen, M., Fradinho, M.J., Santos, A.S., Miraglia, N. (Eds.), Forages and grazing in horse nutrition. Wageningen, the Netherlands, pp. 17–43.

Wan, S., Hui, D., Wallace, L., Luo, Y., 2005. Direct and indirect effect of experimental warming on ecosystem carbon processes in a tallgrass prairie. Global Biogeochemical Cycles 19, GB2014. doi: 10.1029/2004GB002315

Wasser, C.H., Dittberner, C.L., Dietz, D.R., 1986. Tall wheatgrass. Department of the Army – US Army Corps of Engineers, Vicksburg, USA.

Weintraub, F.C., 1953. Grasses introduced into the United States. In: United States Department of Agriculture - Handbook 58. United States Government Print Office, Washington, USA, p. 4.

Williams, T.E., 1980. Herbage production: grasses and leguminous forage crops. In: Holms, W. (Ed.). Grass - its production and utilization. Blackwell Scientific Publications, Oxford, London, Edinburgh, United Kingdom, pp. 25-28.

Willms, W.D., Beauchemin, K.A., 1990. Cutting frequency and cutting height effects on forage quality of rough fescue and Parry oat grass. Canadian Journal of Animal Science 71, 87-96.

Zebisch, M., Grothmann, T., Schröter, D., Hasse, C., Fritsch, U., Cramer, W., 2005. Climate change in Germany – Vulnerability and adaption strategies of climate-sensitive sectors – Summary. Federal Environmental Agency of Germany, Dessau, Germany.

5. General discussion

Figure 9: Harvest of tall wheatgrass by a maize chopper with a direct-cutting unit.

In this study, the effects of drought, brought about by climate change, on tall wheatgrass production in Central Europe were investigated. Furthermore, the common forage management strategies for tall wheatgrass production were optimized to increase the competitiveness of tall wheatgrass as an alternative bioenergy crop to maize. To this end, three experiments were conducted: a laboratory germination test, a container experiment and a field trial.

The first experiment – the germination test – was inspired by reports from farmers, and our own experiences, of the insufficient field emergence and germination of tall wheatgrass. The second experiment, performed in containers, was intended to gather more information on direct response of tall wheatgrass to drought, in terms of the moderate cutting system for biogas production in the Central European environment. The direct response of the crop to drought is often known as resistance, while responses in the following regrowth are named resilience (Hofer et al., 2016; Pimm, 1984). Up to now, detailed recommendations concerning cultivation (e.g. based on germination behavior, drought resistance etc.) have only been available from overseas and addressed the use of tall wheatgrass for forage instead of biogas in a different climate background. Hence, the third experiment was conducted in the field to adapt the cutting regime to local demands, and enhance the competitiveness of tall wheatgrass as a biogas substrate. The central questions across all the experiments were:

- What is the general suitability of tall wheatgrass for becoming an alternative to maize in biogas production, and how does it perform?
- Does the provenance of tall wheatgrass seeds influence the performance of the grass under Central European conditions?

Against the background of climate change, drought stress was the focus of the germination test and the container experiment. Tall wheatgrass is considered a drought-tolerant crop (Moore et al., 2006), and is consequently cultivated in arid and semi-arid regions of the world (Roundy, 1985; Weintraub, 1985). It can be assumed that there was selection pressure for drought resistance, besides specific challenges, such as alkaline soils in Australia, when producing seeds in arid regions. In addition, certain environmental responses in many seed crops can persist in the next sexual generation. Thereby, the specific reaction for growing under drought conditions could be memory-based (Li and Liu, 2016). Provenance-specific variations in germination and growth under drought conditions, and different performances in the cutting management of Central European tall wheatgrass, were therefore expected, and formed the focus of this research.

Farmers have reported low field emergence of tall wheatgrass and, consequently, insufficient plant density for satisfactory biomass yields. Hence, solving this problem is of fundamental importance to subsequent research on drought resistance, and here we used a germination test.

The speed of germination was most strongly affected by water availability. Intense drought conditions of -1 MPa mostly inhibited germination. Increased temperatures and priming accelerated germination speeds. As the mean germination rate from our experiment was approximately 90%, the farmers' reports could not be fully explained by our results; however, it was appropriate to suspect that the seed harvest and seed storage conditions (Bewley and Black, 1994) were responsible for the low germination in the farmers' fields. This thesis is supported by the proven low germination of a specific seed charge from one retailer that was sold to German and Austrian farmers. Unfortunately, this insight was announced after the germination test was finished. Nevertheless, field conditions and seed vigour are important for plant establishment.

As tall wheatgrass needs more time for field establishment than other grasses that are commonly cultivated in Central Europe, e.g. ryegrasses (*Lolium* spp.), the optimal conditions for tall wheatgrass germination, identified for Central Europe in this study, should be taken into account when choosing the date of sowing. In addition, dealing with competition from weeds is also important for good field establishment (Scheinost et al., 2008). The field-specific weed seed bank, and time of weed germination, can both influence tall wheatgrass field emergence (Pallutt, 2000). Furthermore, lower tillage intensity and weed regulation in the pre-crop affect competition in the subsequent crop of tall wheatgrass, with consequences on field emergence and field establishment (Schwarz and Pallutt, 2014). In this situation, application of herbicides can enhance field establishment (Scheinost et al., 2008). In conclusion, the optimal strategy for establishing tall wheatgrass is a combination of seeding conditions and weed management.

In the container experiment, drought resistance and plant reaction to drought was investigated. Two tall wheatgrass provenances were compared to the native tall fescue cultivar Hykor. Prior studies focused on short-term experiments in the initial weeks after germination (Bahrani et al., 2010; Sadeghi and Halagh, 2007), and on alkaline soil (Roundy, 1985). Alkaline soil is rare in German agricultural production, however, and the interest in Central European biogas production is predicated on resistance to drought periods and the biomass yield of a full year. With these facts in mind, our tall wheatgrass was grown on clayey silt, which is typical for agricultural production, for a full harvest year, with two cuts. The observed advantages of tall wheatgrass in intense drought, e.g. increased efficiency of water-use and high resilience, confirmed the findings of previous studies concerning high drought resistance. Compared to field experiments, the volume available for root elongation was limited by the containers. In fact, moderate drought in the period of field establishing to first harvest can promote adaption of the plant to a scarcity of water. Shoot growth is inhibited in favour of root growth, as Schopfer and Brenicke (2010) explained for *Agropyron smithii*. Deep rooting to water-bearing layers enhances the plants' access to water in periods of intense drought in the field. Hence, field studies assess the dehydration avoidance strategy of the plants, whereas the focus of this research was on the plants' adaption to tolerating dehydration (see Volaire, 2008).

Even though differences between crops and between cultivars have been observed in biomass production under drought conditions, it is not known how the quality of the crop, and the specific methane yield, are affected by drought. We found tall wheatgrass to be more drought-tolerant and resilient than the native tall fescue, primarily because of greater tiller elongation and a higher stem:leaf ratio. As the forage quality of grasses decreases with the increasing proportion of the stem fraction (Barker and Caradus, 2001), our results indicate that tall wheatgrass is more likely to be a substitute for less drought-tolerant grasses for biogas production than for dairy nutrition. Prochnow et al. (2009) argued that the stage of maturity and the harvest date of tall wheatgrass are more critical to the chemical composition and quality than are drought conditions. Yet an exemplary study by Emerson et al. (2014) revealed the significant influence of intense drought on the chemical composition of mixed grasses, *Miscanthus* x *giganteus* and corn stover. So, there is still a question of whether the chemical composition of tall wheatgrass is more or less influenced by drought than other grasses, and how precisely it is affected. Nevertheless, farmers should consider growing tall wheatgrass, especially in low-precipitation areas, for gaining stable yields under the impending risk of periodic drought.

In the field experiment, the optimum cutting frequency and cutting height for biogas production in Central Europe were identified. Increasing biomass yields is crucial for greater competitiveness of tall wheatgrass versus other energy crops, such as maize. The field experiment revealed the highest biomass yield in moderate clipping intensity that consisted of two annual harvests. Reducing the stubble height from 15 cm to 5 cm increased the biomass yield by 12.5%, whereas the specific methane yield decreased by only 1.4%. Although stubble close to the ground has previously been shown to have a higher lignin content, and therefore lower digestability, than the remaining plant (Muche and Richardt, 2014; Thalmann, 2013), stubble height did not affect lignin content in our experiment. A high lignin content decreases fermentation in biogas plants (Mittweg et al., 2012). We found that the specific methane yield was more affected by the stage of maturity at harvest than by the cutting height. The results of this study are clear: moderate cutting with two harvests per year, and a low cutting height of 5-10 cm, are the preconditions for high methane yields. There are advantages for choosing a cutting height of 10 cm rather than 5 cm, however. Heinz (2015) reported that, in practical farming, cutting heights below 10 cm drastically reduced restoration and yield after four years, and consequently reduced the duration of utilisation of the tall wheatgrass sward. Hence, raising the defoliation height might tend to result in smaller annual biomass yields, but could increase economic revenue due to extended utilisation.

Casaretto and Heise (2015) gave an approximate calculation of the economics of tall wheatgrass energy crops, suggesting that they are more expensive than other crops, in terms of seeds, but that there is greater profit to made from consecutive years of harvesting and savings on seeds and tillage after establishment. Similar results were obtained by Aurbacher et al. (2017) who conducted an economic comparison between tall wheatgrass and maize over a period of three consecutive years, in a field trial close to where the field experiment of this study was carried out. The results showed that the economic revenue of tall wheatgrass was as high as maize because the biomass yields were comparable. The authors concluded that increasing the useful life of tall wheatgrass beyond three years would make its production more profitable than maize cultivation.

Contrary to our expectations, the effects of seed provenance on germination, drought resistance and biomass yield were less important than other factors, such as drought level or cutting management. The differences between the provenances were minor, especially in the germination experiment. In the container and field experiments, the focus of the Hungarian breeding program for biogas production in Central Europe was visible. Compared to the other provenances, the Hungarian provenance gained the highest biomass yields. There is potential for further improvement in tall wheatgrass production for biogas through new cultivars bred specifically for biomass production.

This study has confirmed the potential for tall wheatgrass to become a new crop for biogas production in Central Europe. By diversifying the cultivation range, it is suitable for risk mitigation in substrate production and reducing the dependency on maize. The germination and container experiments showed a high drought resistance of tall wheatgrass. Therefore, especially in suboptimal locations, with low ground water availability and increased risk of drought, it is a new crop that could help to solve rising agricultural problems, such as climate change, water scarcity (Zebisch et al., 2005) and economic pressure on bioenergy production (Purkus et al., 2015). As a matter of fact, farmers in regions with low maize yields are very interested in tall wheatgrass. Farmers with stagnant moisture in their fields, which handicaps tillage and maize cultivation in spring, could also try to seed tall wheatgrass and, thus, avoid annual problems with maize cultivation in these fields. The current investigation also showed the need to adapt crop management practices. For example, cutting height was not as important to the chemical composition of the tall wheatgrass as has been reported from other countries, although cutting to a height typical in forage production (7 cm) is not conductive to sustainable biomass production (Fisch and Buhr, 2008).

Our study did not focus on the application of pesticides. Spraying intensity, expressed via the treatment index, varies widely, with 1.2 applications in maize, 3.8 in wheat (Burth et al., 2002) and up to 32.6 per year in apple production (Roßberg and Harzer, 2015). The low spraying intensity in the present container and field experiments resulted in a treatment index of approximately 0.4 applications per year. As a consequence, the integration of a perennial tall wheatgrass crop could contribute to enhancement of weed diversity (Glemnitz and Brauckmann, 2016). Platen et al. (2017) found a higher biodiversity of carabid beetles and arachnids in tall wheatgrass than in maize. They concluded that the richly-structured tall wheatgrass plots possessed not only a higher species richness and number of species, but also a more balanced dominance structure compared to maize.

There are further questions to be answered. As permanent crops minimise nitrate leaching (Dinnes et al., 2002), the initial results of other studies have shown that tall wheatgrass can contribute to reducing nitrate loss in the winter months because of the low amounts of nitrate left after vegetation has ceased (von Buttlar, 2013). The application dates, and splitting, of the application of mineral and manure fertilization need to be improved, however.

In conclusion, tall wheatgrass is a suitable crop for biogas production in Germany. It is an alternative crop for substituting maize, which can mitigate some of the challenges of climate change. Further research should be conducted to address questions concerning fertilization and agrochemical application from an economic point of view. With regard to the future, agricultural consultants should take the results of this study, and similar reports into account when advising farmers and agricultural entrepreneurs.

5.1. References

Aurbacher, J., Kornatz, P., Müller, J., 2017. Endbericht Projekt BRAWU (Final report of the BRAWU-project). *In preparation for publication.*

Bahrani, J.M., Bahrami, H., Haghighi, A.A.K., 2010. Effect of water stress on ten forage grasses native or introduced to Iran. Japanese Society of Grassland Science. doi:10.1111/j.1744-697X.2009.00165.x

Barker, D.J., Caradus, J.R., 2001. Adaption of forage species to drought. In: Proceedings of the 18th International Grassland Congress 46, Sao Paolo, Brazil. Available at: http://www.internationalgrasslands.org/files/igc/publications/2001/tema6-1.pdf (accessed 20 February 2017).

Bewley, J.D., Black, M., 1994. Seeds – Physiology of development and germination, 2nd ed. Plenum Publishing Corporation, New York, USA.

Burth, U., Gutsche, V., Freier, B., Roßberg, D., 2002. Das notwendige Maß bei der Anwendung chemischer Pflanzenschutzmittel (The necessary application level of agrochemicals). Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 54, 297-303.

Casaretto, R., Heise, R., 2015. Hirschgras – Riesenpflanze, Riesenflopp oder Riesenchance (Tall wheatgrass – giant plant, big flop oder huge chance)? Biogas Journal 5/2015, 46-49.

Dinnes, D.L., Douglas, L.K., Jaynes, D.B., Kaspar, T.C., Hatfield, J.L., Colvin, T.S., Cambardella, C.A., 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained midwestern soils. Agronomy Journal 94, 153-171.

Emerson, R., Hoover, A., Ray, A., Lacey, J., Cortez, M., Payne, C., Karlen, D., Birrell, S., Laird, D., Kallenbach, R., Egenolf, J., Sousek, M., Voigt, T., 2014. Drought effects on composition and yield for corn stover, mixed grasses, and Miscanthus as bioenergy feedstocks. Biofuels 5, 275-291.

Fisch, R., Buhr, F., 2008. Schnitthöhe richtig gemacht (The optimal cutting height)! Dienstleistungszentrum Ländlicher Raum Eifel. Available at http://www.dlr.rlp.de/internet/global/themen.nsf/747270cf8f15f0d1c1257abb0030380e/0448a1f313f6b36ec125742600318465/$FILE/Schnitth%C3%B6he%20richtig%20gemacht.pdf (accessed at 04 January 2016).

Glemnitz, M., Brauckmann, H.J., 2016. Beitrag neuer Energiepflanzen für die Diversifizierung der Beikrautflora in Energieanbausystemen (The impact of new energy crops on weed flora diversification in energy cropping systems). 27. Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und –bekämpfung. DOI 10.5073/jka.2016.452.011

Heinz, M., 2015. Head of Department of Plant Production and Agricultural Research of Agricultural colleges Triesdorf. Triesdorf, Germany. Personal communication on 11 December 2015.

Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N., Lüscher, A., 2016. Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. Journal of Applied Ecology 53, 1023-1034.

Li, X., Liu, F., 2016. Drought stress memory and drought stress tolerance in plants: biochemical and molecular basis. In: Mohammad Anwar, H., Wani, S.H., Bhattacharjee, S., Burritt, D.J., Tran, L.S.P. (Eds.), Drought stress tolerance in plants, volume 1. Springer International Publishing, Switzerland, pp. 17-44.

Mittweg, G., Oechsner, H., Hahn, V., Lemmer, A. Reinhardt-Hanisch, A., 2012. Repeatability of laboratory batch method to determine the specific biogas and methane yield. Engineering in Life Sciences 12, 270-278.

Moore, G., Sanford, P., Wiley, T., 2006. Perennial Pastures for Western Australia. Department of Agriculture and Food Western Australia, Perth, Australia.

Muche, S., Richardt, W., 2014. Tendenzen beim Futterwert von Mais- und Grassilagen unter Berücksichtigung der in vitro verdaulichen NDF (NDFD) (Feed value of maize and grass silage under consideration of vitro digestibility NDF (NDFD)). Presentation: 7. Thüringisch-Sächsisches Kolloquium zur Fütterung, 8. October 2014. Available at http://www.tvlev.de/cms/sites/default/files/downloads/publikationen/In%20vitro%20verdauliche%20 NDF,%20Frau%20Muche.pdf (accessed at 18 December 2015).

Pallutt, B., 2000. Unkrautregulierung im ökologischen Landbau (Weed control in organic farming systems). In: Pallut, B. (Ed.), Pflanzenschutz im Ökologischen Landbau - Probleme und Lösungsansätze - Drittes Fachgespräch am 02.11.1999 in Kleinmachnow. Berichte aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft 72, Saphir Verlag, Ribbesbüttel, Germany, pp. 35-46.

Pimm, S.L., 1984. The complexity and stability of ecosystems. Nature 307, 321–326.

Platen, R., Konrad, J., Glemnitz, M., 2017. Novel energy crops: an opportunity to enhance the biodiversity of arthropod assemblages in biomass feedstock cultures? International Journal of Biodiversity Science, Ecosystem Science and Management 13, 162-171.

Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., Hobbs, P.J., 2009. Bioenergy from permanent grassland – a review: 1. Biogas. Bioresource Technology 100, 4931-4944.

Purkus, A., Gawel, E., Deissenroth, M., Nienhaus, K., Wassermann, S., 2015. Market integration of renewable energies through direct marketing – lessons learned from the German market premium scheme. Energy, Sustainability and Society 5. doi: 10.1186/s13705-015-0040-1

Roßberg, D., Harzer, U., 2015. Erhebungen zur Anwendung von Pflanzenschutzmitteln im Apfelanbau. Journal für Kulturpflanzen 67, 85-91.

Roundy, B.A., 1985. Root penetration and shoot elongation of tall wheatgrass and basin wildrye in relation to salinita. Canadian Journal of Plant Science 65, 335-343.

Sadeghi, S.H.R., Halagh, N.R., 2007. Comparison of wheatgrass (*A. intermedium*) and tall wheatgrass (*A. elongatum*) in transpiration viewpoint. Caspian Journal of Environmental Sciences 55, 41-44.

Scheinost, P., Tilley, D., Ogle, D., Stannard, M.,. 2008. Tall wheatgrass – Plant guide. United States Department of Agriculture – Natural Resources Conversation Service, Pullman, WA, USA.

Schopfer, P., Brennicke, A., 2010. Pflanzenphysiologie (Plant physiology), 7th edition. Spektrum Akademischer Verlag, Heidelberg, Germany.

Schwarz, J., Pallutt, B., 2014. Influence of tillage system on the weed infestation in a long-term field trial. Proceeding of 26th German Conference on weed Biology an Weed Control, March 11-13, 2014, Braunschweig, Germany

Thalmann, T., 2013. Im Pansen muss das Tempo stimmen (High pace in the rumen). Schweizer Bauer 6 April 2013, 23. Available at http://www.ufa.ch/files/66-22934727.pdf (accessed 18 December 2015).

Volaire, F., 2008. Plant traits and functional types to characterise drought survival of pluri-specific perennial herbaceous swards in Mediterranean areas. European Journal of Agronomy 29, 116-124.

von Buttlar, C., 2013. Untersuchung und Praxiseinführung eines grundwasserschutzorientierten Biomasseanbaus vor dem Hintergrund der Anforderungen der EG-Wasserrahmenrichtlinie (Investigation and introduction to practical use of a groundwater protecting crop production against the background of the given requirements of the EU Water Framework Directive). Bericht 2013 – Verbundvorhaben standortangepasste Anbausysteme für die Produktion von Energiepflanzen, Fachagentur Nachwachsende Rohstoffe e.V, Gülzow, Germany.

Weintraub, F.C., 1953. Grasses introduced into the United States; United States Department of Agriculture, Handbook 58. United States Government Print Office, Washington, USA.

Zebisch, M., Grothmann, T., Schröter, D., Hasse, C., Fritsch U., Cramer, W., 2005. Climate change in Germany – Vulnerability and adaption strategies of climate-sensitive sectors – Summary. Potsdam Institute for Climate Impact Research, Federal Environmental Agency of Germany, Dessau, Germany.

6. Summary

Climate change clearly influences agricultural production. Most scientific studies for climate change in Central Europe predict the scarcity of water and a changed distribution of precipitation that will lead to increased periods of drought in the coming decades. Biogas is expected to contribute to climate change. However, the yields of energy crops and other crops are predicted to be more volatile and to decrease, in general, on the regional level because of climate change.

In addition, maize has an outstanding share on substrata for biogas production. This results in the reduced diversity of crop rotations and ecological problems, due to a high share of maize cultivation for biogas production, and are in the focus of public criticism. Hence, there is a need for alternative and cost-efficient biogas feedstock. The perennial crop tall wheatgrass (*Agropyron elongatum* (Host) P. Beauv.) is already grown as a drought-tolerant forage crop on many continents, is considered to better protect soil than maize and has been shown to achieve good qualities as a biogas substrate in preliminary trials.

This study was aimed at answering questions about the drought resistance and resilience that has not been examined so far, solving germination problems observed under practical conditions, and giving expertise in optimising cutting management under Central European environmental conditions. In a series of experiments in a climate cabinet, in containers and in the field, two to four cultivars of different continental origins were compared and evaluated under various test terms.

Initially, a germination test was set up under controlled conditions in a climate cabinet. It was assumed that germination is inhibited by long durations of drought and too low temperatures. For that purpose, four provenances were submitted to three different pre-treatments (prechilling, hydropriming, nitrate-treatment), periodical illumination or complete darkness, and three temperature regimes (constant 10 °C, constant 20 °C, 10 °C/20 °C alternating temperatures) to test the effects on germination. In addition, three levels of drought stress (0 MPa, -0.1 MPa, -1 MPa) were induced. Intense drought of -1 MPa was the main effect on germination and clearly reduced germination that could only partly be mitigated by fluctuating temperatures and darkness. Approximately 90% of the seeds germinated in slight drought (-0.1 MPa) and in the control treatment (0 MPa). The effect of the provenance on germination was significant but circumstantial. The germination speed was positively influenced by priming and rising temperature.

A container experiment (30 dm³) was started to evaluate drought resistance and subsequent resilience of two cultivars of tall wheatgrass and a common cultivar of native tall fescue (*Festuca arundinacea* Schreb.). The grass species were expected to adapt differently to drought and show different reactions of biomass production in the subsequent period of resilience. Therefore, the water availability of medium clayey silt was varied in three levels of 90% field capacity (-0.013 MPa), 67% field capacity (-0.1 MPa) and 46% field capacity (-0.316 MPa) in an outdoor-climate greenhouse. All plants grew over the winter period until the first defoliation in spring. Afterwards, water availability was varied between drought treatments and was held constant for approximately two months until the first regular harvest. In the subsequent period of three months up to the final harvest, all of the plants remained at 90% field capacity. The water consumption as a function of transpiration was monitored daily. The evaporation was controlled by covering the soil. The results showed that tall wheatgrass was better adapted to intense drought than tall fescue by better exploitation of the available water. The water use efficiency of tall wheatgrass was significantly higher than that of tall fescue. Up to 7.2 g DM were produced by tall wheatgrass per litre of water, whereas tall fescue did not gain more than 5.4 g DM of over-ground biomass under severe drought. Depending on the cultivar, tall wheatgrass as well as tall fescue could use full water availability and gain high biomass yields. In contrast, tall wheatgrass achieved higher biomass yields than tall fescue in the period of resilience after severe drought.

A field trial was conducted to prove the hypotheses of higher biomass yields in Central Europe by reduced cutting height and adapted cutting-frequency compared to the commonly used harvest management on farms. Therefore, the effect of seed provenance (4 provenances), cutting frequency (1, 2, 4 cuts per year) and cutting height (5 cm, 10 cm, 15 cm) were tested. Across two harvest years, the biomass yield increased under deeper defoliation (5 – 10 cm) compared to the typically chosen cutting height (15 cm) on the farms. In contrast, the specific methane content decreased significantly by clipping closer to the ground (1.4%).Thereby, the growth stage and maturity at single harvests were more important for the specific methane content than the cutting height. Hence, the one-cut system was unsuitable for biogas fermentation. The highest methane yields were attained under two annual harvests, where dry matter yields reached up to 21.3 t DM ha^{-1}, even though specific methane content was higher under more frequent harvests. As shown by the field trial, a reduced clipping height is suitable for biogas production. Increasing biomass and methane yields improves the profitability of tall wheatgrass.

In summary, the relative drought-tolerant crop, tall wheatgrass, allows stabilisation of the biogas substrate yields in low-precipitation regions and in areas with risk of intense drought periods. Under the terms of predicted climate change in Central Europe, the relative excellence of cultivating tall wheatgrass will increase. The study has shown that optimising the cutting management increases the yield performance of tall wheatgrass and improves the competitiveness with other energy crops. Hence, a serious alternative to maize has been found that should be developed further. More insight is needed into cultivar-specific differences in germination, drought tolerance, and capability of gaining high and constant yields to determine the best-suited cultivar for different sites. Furthermore, planting the permanent crop, tall wheatgrass, is considered to mitigate nitrate leaching into the ground water, contribute to water pollution control, and upgrade agricultural biodiversity. This potential should be under consideration in further research.

7. Zusammenfassung

Die überwiegende Zahl der Prognosen für den Klimawandel in Mitteleuropa sieht für die kommenden Jahrzehnte zunehmende Dürrephasen und eine veränderte Niederschlagsverteilung voraus. Die Produktion von Biogas soll einen Beitrag zum Klimaschutz leisten, doch wird der Anbau von Energiepflanzen ebenso wie der anderer Kulturen von den Folgen des Klimawandels betroffen sein und zu regionalen Ertragsrückgängen und steigenden Ertragsunsicherheiten führen.

Derzeit hat der Mais einen überragenden Anteil an der Substratbereitstellung für die Biogaserzeugung. Die dadurch verengten Fruchtfolgen und negativen ökologischen Konsequenzen werden öffentlich kritisiert. Folglich werden alternative, kostengünstige Anbausubstrate benötigt. Das mehrjährige Riesenweizengras (*Agropyron elongatum* (Host) P. Beauv.) wird als trockentolerante Futterpflanze bereits auf vielen Kontinenten angebaut, gilt als bodenschonender im Vergleich zu Mais und erzielte in Vorversuchen gute Eigenschaften als Biogassubstrat.

Diese Studie soll bisher nicht geklärte Fragen der Trockentoleranz beantworten, zur Lösung der in der Praxis beobachteten Keimungsprobleme beitragen und Erkenntnisse darüber liefern, wie das Schnittmanagement unter mitteleuropäischen Anbaubedingungen optimiert werden kann. In einer Serie von Klimaschrank-, Gefäß- und Feldexperimenten wurden jeweils zwei bis vier Sorten unterschiedlicher kontinentaler Herkunft unter den diversen Versuchsbedingungen vergleichend geprüft.

Unter kontrollierten Bedingungen wurde im Klimaschrank ein Keimungsversuch angelegt. Es wurde angenommen, dass die Keimung durch Trockenheit und niedrige Temperaturen beeinträchtigt wird. Vier Herkünfte wurden drei verschiedenen Vorbehandlungen unterzogen (Stratifikation, Hydropriming, Nitratbehandlung) und bei periodischer Beleuchtung oder vollständiger Dunkelheit in drei Temperaturregimen (konstant 10 °C, konstant 20 °C, 10 °C/20 °C Wechseltemperatur) zur Keimung gebracht. Während der Keimungsphase wurde Trockenstress in drei Stufen (0 MPa, -0,1 MPa, -1 MPa) über das Keimungsmedium induziert. Intensiver Trockenstress von -1 MPa hatte den größten Effekt auf die Keimung und reduzierte sie deutlich, dieser Effekt wurde durch Wechseltemperaturen in Dunkelheit abgeschwächt. Bei leichtem Trockenstress (-0,1 MPa) und in der Kontrollvariante (0 MPa) lag die Keimungsrate bei ca. 90%. Die Sortenwahl hatte ebenfalls einen signifikanten Einfluss auf die Keimung, war jedoch hinsichtlich des Ausmaßes von untergeordneter Bedeutung. Durch Saatgutvorbehandlung und steigende Keimtemperatur wurde die Keimungsgeschwindigkeit positiv beeinflusst.

In einem Gefäßversuch (30 dm³ Volumen) wurde die Trockentoleranz und das anschließende Regenerationsvermögen von zwei ausgewählten Sorten Riesenweizengras mit einer in der Praxis gebräuchlichen einheimischen Sorte Rohrschwingel (*Festuca arundinacea* Schreb.) verglichen. Es wurde erwartet, dass hinsichtlich der Reaktion auf Trockenheit sowie der Ertragsbildung in der folgenden Regenerationsphase unterschiedliche Ausprägungen der getesteten Grasarten beobachtet werden können. Dazu wurde in einem Außenklimagewächshaus das Wasserangebot in tonigem Schluff in den drei Stufen 90% Feldkapazität (-0,013 MPa), 67% Feldkapazität (-0,1 MPa) und 46% Feldkapazität (-0,316 MPa) variiert. Die Pflanzen wurden über Winter angezogen und erhielten nach einem ersten Schnitt im Frühjahr eine für ca. zwei Monate unterschiedliche Wasserversorgung. Nach erneuter Ernte wurden alle Varianten bis zur abschließenden Ernte drei Monate später auf 90% Feldkapazität bewässert. Der Wasserverbrauch durch Transpiration wurde täglich ermittelt. Verluste durch Evaporation wurden mittels einer Schutzauflage unterbunden. Die Ergebnisse zeigten, dass Riesenweizengras vorhandenes Wasser besser ausnutzen konnte als Rohrschwingel und somit besser an starken Trockenstress angepasst ist. Die Wassernutzungseffizienz je Liter Wasser lag mit bis zu 7,2 g Trockenmasse (TM) über der von Rohrschwingel (5,4 g). In Abhängigkeit von der Sorte konnte Riesenweizengras eine gute Wasserverfügbarkeit ebenso wie Rohrschwingel durch hohe Biomasseerträge ausnutzen. Auf starken Trockenstress reagierte Riesenweizengras mit höherer Wassernutzungseffizienz und höheren Biomasseerträgen in der Regenerationsphase als Rohrschwingel.

In einem mehrjährigen Feldversuch wurde die Hypothese geprüft, dass durch die Reduktion der Schnitthöhe und eine angepasste Schnitthäufigkeit die Riesenweizengraserträge unter mitteleuropäischen Bedingungen gegenüber den in der Praxis üblichen bzw. empfohlenen Nutzungen gesteigert werden können. In einem dreifaktoriellen Versuchsdesign wurde der Einfluss der Faktoren Saatgutherkunft (4 Herkünfte), Schnittfrequenz (1, 2, 4 Schnitte je Jahr) und Schnitthöhe (5 cm, 10 cm, 15 cm) geprüft. Über zwei Nutzungsjahre hinweg waren bei verringerter Schnitthöhe (5 – 10 cm) die Erträge gegenüber der üblichen Schnitthöhe (15 cm) erhöht. Dem entgegen nahmen die spezifischen Gasausbeuten mit tieferen Schnitten geringfügig aber signifikant ab (1,4 %). Dabei war die Gasausbeute weniger von der Schnitthöhe als vielmehr vom Entwicklungsstadium des Erntegutes abhängig, weshalb sich insbesondere eine einschnittige Nutzung als nicht zur Verwertung in Biogasanlagen geeignet erwies. Mit maximalen Masseerträgen der zweischnittigen Nutzung von 21,3 t TM ha^{-1} und Jahr konnten trotz leicht geringerer spezifischer Gasausbeuten als bei der Vierschnittnutzung die höchsten Methanhektarerträge erzielt werden. Der Feldversuch belegt somit, dass sich die

Schnitthöhe zu Gunsten höherer Massen- und Gaserträge reduzieren lässt, was die Rentabilität des Anbaus verbessern kann.

Zusammenfassend haben die Versuche gezeigt, dass Riesenweizengras als relativ trockentolerante Kultur eine Möglichkeit bietet in niederschlagsarmen Regionen oder von periodischer Niederschlagsarmut bedrohten Anbaugebieten relativ stabile Erträge zu erzielen. Unter den Bedingungen des für Mitteleuropa prognostizierten Klimawandels dürfte die relative Vorzüglichkeit des Anbaus dieser Kultur demnach noch zunehmen. Es konnte gezeigt werden, dass durch die Optimierung des Schnittmanagements die Ertragsleistung erhöht und die Wettbewerbsfähigkeit des Anbaus gegenüber anderen Energiepflanzen verbessert werden kann. Somit liegt mit dieser Kultur eine ernsthafte Anbaualternative zu Mais vor, deren Anbau noch weiter verbessert werden kann. Sortenunterschiede in Keimung, Trockentoleranz und Ertragsfähigkeit müssen weiter ausdifferenziert werden, um standortoptimale Anbauentscheidungen treffen zu können. Perspektivisch kann die Dauerkultur Riesenweizengras dem Problem der Nitratverlagerungen ins Grundwasser entgegenwirken und somit aktiv zum Gewässerschutz beitragen sowie einen Beitrag zur Steigerung der Agrarlandbiodiversität leisten. Diese Potentiale sollten in folgenden Untersuchungen bewertet werden.

8. Acknowledgements

First of all, I would like to express my deepest sense of gratitude to my supervisor, Prof. Dr. Johannes Isselstein, for giving me the opportunity to conduct research on this interesting topic, and to write this thesis as an external PhD student. I thank him for our inspiring discussions and his immense knowledge.

Of course, I am also very grateful to my co-supervisor, Prof. Dr. Harald Laser, who offered his continuous advice, and put a great deal of effort into supporting my work with related BSc and MSc thesis of students – a huge thanks for his enormous professional input and many ideas.

I would like to express my sincerest gratitude to Dr. Bettina Tonn for supporting my statistical analyses and thoroughly reviewing my papers. I thank her for the discussions, and the proposals for improving my thesis.

My heartfelt thanks also go to the technical staff, and the student assistants, especially Konstantin. I am also grateful to my employer, the Chamber of Agriculture of North Rhine-Westphalia, which gave me the chance to pursue my PhD-“hobby” with very flexible work times.

Finally, special thanks to the numerous people and my family – especially my parents – who supported this thesis through professional discussions, work effort in conducting the experiments, proofreading and moral support. In particular, Marie and Alexander spent a lot of their free time preparing Petri dishes, watering containers and proofreading.

Last, but not least, great thanks to my wife, Svenja, who supported my research throughout, and patiently took part in my ups and downs – and especially the downs – when testing and improving the methods, analyzing the results and writing the papers.