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Nachwachsende Rohstoffe

Phenotypic analysis of different *Silphium perfoliatum* L. accessions and evaluation as a renewable resource for material use

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Martin Georg Höller

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Referent:	Prof. Dr. Ralf Pude
Korreferent:	Prof. Dr. Wolfgang Büscher

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Abstract

Cup plant (*Silphium perfoliatum* L.) is a promising alternative to silage maize as an energy crop for biogas production and a potential raw material for the industry. This non-food perennial from the US possesses a highly ecological value due to its long blooming period, ability to grow in low-input agriculture and positive influence on soil structure. This thesis analyzes five cup plant populations for their phenotype as a starting point for further breeding programs (I). Additionally, the biomass was analyzed for the use as a renewable raw material for the paper and building industry (II, III).

So far, there have been almost no breeding attempts for cup plant. Most of the experiments showing its high biomass yield were carried out in Europe with European plant material of unclear ancestry. The comprehensive assessment of five such populations for their biomass and phenotype parameters revealed phenotypic variations indicating the possibility of improving these traits through selection and breeding. Higher biomass yield is likely to be achieved by breeding for secondary traits such as plant height, shoot diameter and shoot number. Genetic structure and phylogeny analyses revealed that all the plants belong to the same gene pool and share a common ancestry. Four out of five populations demonstrate a low genetic differentiation, whereas the fifth population represents a clear example of population stratification. To start a successful domestication and breeding of this new high-yielding perennial crop, a broader base of genetic diversity needs to be ensured and complemented by innovative breeding strategies, driven by molecular genetics and genomic approaches.

While the demand for paper and packaging material is increasing, industry and consumers are searching for more sustainable raw materials aside from wood. This thesis evaluates three non-wood perennials to find alternative raw materials for the paper and pulp industry. Biomass of cup plant, *Sida hermaphrodita* (L.) Rusby and meadow hay were analyzed for their fibre morphology and used as paper feedstock. After undergoing three different mechanical grinding methods, the plant material was screened, pulped in NaOH and beaten in a PFI mill. Birch fibre has been chosen as a short-fibre control and blend base. Hand-sheets with different pulp blends of birch and one of the three raw materials were made, and paper properties were measured. For the biomass of cup plant, Virginia mallow and meadow hay, fibre lengths of 0.9, 1.3, and 0.5 mm were measured. Therefore, all perennial plant materials have fibre lengths comparable to hardwoods. Meadow hay blends containing 50% and 75% birch pulp,

generated higher paper strength when compared to the pure birch paper. The paper strength of cup plant and Virginia mallow blends is comparable to the strength of the birch control.

Biomass for non-food applications is considered as a substitute for petroleum-based materials such as expanded polystyrene (EPS). This thesis analyzes the physical properties of an EPS containing commercial bonded leveling compound (BLC), which was substituted with cup plant biomass. The measured pore size of the parenchyma cells is comparable to the size of the EPS pores. The natural variation in parenchyma content of several European cup plant accessions is promising, regarding the future development of cultivars with suitable biomass traits for material use. Two binders with different proportions of cup plant and EPS were used to produce samples of BLC for thermal conductivity and compression strength tests. The compression strength of 0.92 N mm^{-2} and thermal conductivity of 84 mW m^{-1} K^{-1} were analyzed and deemed comparable to the commercial BLC. The thermal conductivity within the tested borders appears nearly independent of the biomass content. The mechanical strength and thermal conductivity are strongly influenced by the water absorption of the biomass and their influence on the water/cement ratio.

Zusammenfassung

Die Durchwachsene Silphie (*Silphium perfoliatum* L.) ist eine vielversprechende Alternative zu Silomais als Energiepflanze für die Biogaserzeugung und ein möglicher nachwachsender Rohstoff für die Industrie. Aufgrund ihrer langen Blütezeit, ihres geringen Nährstoffbedarfs und ihres positiven Einflusses auf die Bodenstruktur besitzt die mehrjährige Staude aus den USA einen hohen ökologischen Wert. Als Ausgangspunkt für eine züchterische Bearbeitung der Pflanze werden in dieser Dissertation fünf Herkünfte auf ihren Phänotyp hin untersucht (I). Zusätzlich wird die Kultur als nachwachsender Rohstoff für die Papier- und Bauindustrie analysiert (II, III).

S. perfoliatum wurde bislang kaum züchterisch bearbeitet. In Europa wurden bisher Feldversuche mit nur wenigen europäischen Herkünften unklarer Abstammung durchgeführt. Eine umfassende Bewertung fünf solcher Herkünfte hinsichtlich ihres Biomasseertrages und pflanzenbiologischer Unterschiede ergab phänotypische Variationen, was auf die Möglichkeit hinweist, diese Merkmale durch Selektion und Züchtung weiter zu verbessern. Ein höherer Biomasseertrag ließe sich wahrscheinlich durch Züchtung auf sekundäre Merkmale wie Pflanzenhöhe, Triebdurchmesser und Triebanzahl erzielen. Genetische Struktur- und Phylogenie-Analysen ergaben, dass alle Pflanzen zum selben Genpool gehören und eine gemeinsame Abstammung haben. Vier der fünf Populationen weisen eine geringe genetische Differenzierung auf, während die fünfte ein deutliches Beispiel für eine Populationsstratifizierung darstellt. Um eine erfolgreiche Domestizierung und Züchtung dieser neuen mehrjährigen Pflanze zu erreichen, muss eine breitere Basis genetischer Vielfalt sichergestellt und durch innovative Züchtungsstrategien auf Grundlage der Molekulargenetik und moderner genomischer Ansätze ergänzt werden.

Während die Nachfrage nach Papier und Verpackungsmaterial steigt, ist die Industrie auf der Suche nach nachhaltigeren Rohstoffquellen im Vergleich zu Holz. Drei mehrjährige, nachwachsende Rohstoffe wurden untersucht, um ihre Nutzung als alternative Rohstoffe für die Papier- und Zellstoffindustrie zu prüfen. Biomasse von *Silphium perfoliatum* L., *Sida hermaphrodita* (L.) Rusby und Wiesenheu wurde auf ihre Fasermorphologie und ihre Verwendung als Papierrohstoff untersucht. Nach drei verschiedenen mechanischen Zerkleinerungsverfahren wurde das Pflanzenmaterial gesiebt, in NaOH aufgeschlossen und in einer PFI-Mühle gemahlen. Birkenzellstoff diente sowohl als Kontrolle und Mischungsgrundlage. Es wurden Laborblätter mit verschiedenen Anteilen aus Birkenzellstoff und einem der drei Rohstoffe hergestellt und die Papiereigenschaften ermittelt. Für *S. perfoliatum*, *S. hermaphrodita* und Wiesenheu

wurden Faserlängen von 0,9, 1,3 und 0,5 mm gemessen. Somit haben sie vergleichbare Faserlängen wie Harthölzer. Laborblätter aus Wiesenheu und einem Birkenzellstoffanteil von 50% und 75% ergaben eine höhere Papierfestigkeit als die reinen Laborblätter aus Birkenzellstoff. Die Papierfestigkeit der Mischungen aus *S. perfoliatum* und *S. hermaphrodita* ist vergleichbar mit der Festigkeit der Laborblätter aus 100% Birkenzellstoff.

Nachwachsende Rohstoffe könnten in Zukunft immer häufiger als Erdölersatz herangezogen werden, etwa als Ersatz für expandiertes Polystyrol (EPS). In dieser Arbeit werden die physikalischen Eigenschaften einer gebundenen Schüttdämmung analysiert, deren EPS-Leichtzuschlag anteilig durch einen Partikelmix aus Cortex und Parenchym der Durchwachsenen Silphie ersetzt wurde. Die gemessenen Porengrößen des Parenchyms sind vergleichbar mit der Größe der EPS-Poren. Die natürlichen Unterschiede im Parenchymgehalt verschiedener europäischer Silphie-Herkünfte sind vielversprechend für die Entwicklung von Sorten mit geeigneten Eigenschaften für die Bauindustrie. Zwei Bindemittel mit unterschiedlichen Anteilen an Silphie-Biomasse und EPS wurden zur Herstellung von Probenkörpern für Wärmeleitfähigkeits- und Druckfestigkeitstests verwendet. Die Druckfestigkeit von 0,92 N mm^{-2} und eine Wärmeleitfähigkeit von 84 mW m^{-1} K^{-1} wurden gemessen und sind mit einer kommerziellen Schüttdämmung vergleichbar. Die Wärmeleitfähigkeit innerhalb der untersuchten Grenzwerte scheint nahezu unabhängig vom Biomasseanteil im Leichtzuschlag zu sein. Die mechanische Festigkeit und die Wärmeleitfähigkeit werden von der Wasseraufnahme der Biomasse stark beeinflusst.

Contents

List of Publications

The present work is a compilation of three peer-reviewed journal articles, together covering the results obtained within this dissertation:

1) Christian Wever, Martin Höller, Lukas Becker, Andrea Biertümpfel, Johannes Köhler, Delphine van Inghelandt, Peter Westhoff, Ralf Pude, Elena Pestsova. Towards high-biomass yielding bioenergy crop *Silphium perfoliatum* L.: phenotypic and genotypic evaluation of five cultivated populations. Biomass and Bioenergy, 124 (2019).

   DOI: https://doi.org/10.1016/j.biombioe.2019.03.016
   The published article is reproduced in Chapter 2 of the dissertation.

2) Martin Höller, Anne Lunze, Christian Wever, Alexander L. Deutschle, Alexander Stücker, Niklas Frase, Elena Pestsova, Antje C. Spiess, Peter Westhofff, Ralf Pude. Meadow hay, *Sida hermaphrodita* (L.) Rusby and *Silphium perfoliatum* L. as potential non-wood raw materials for the pulp and paper industry. Industrial Crops & Products 167 (2021).

   DOI: https://doi.org/10.1016/j.indcrop.2021.113548
   The accepted manuscript of this article is included in Chapter 3 of the dissertation.

3) Lüders Moll, Martin Höller, Charlotte Hubert, Christoph A. C. Korte, Georg Völkering, Christian Wever and Ralf Pude. Cup Plant (*Silphium perfoliatum* L.) Biomass as Substitute for Expanded Polystyrene in Bonded Leveling Compounds. Agronomy 12 (2022).

   DOI: https://doi.org/10.3390/agronomy12010178
   The published article is reproduced in Chapter 4 of the dissertation.

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List of Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
AEM	Adjusted entry means
ANOVA	Analysis of variance
BLAST	Basic Local Alignment Search Tool
BLC	Bonded leveling compound
CKA	Campus Klein-Altendorf of the University of Bonn
C_3 plant	Plant that utilizes the C_3 carbon fixation pathway
CO_2	Carbon dioxide
CP	Cup plant
DM	Dry mass/matter
DMY	Dry tons per hectare
DNA	Deoxyribonucleic acid
dt	Decitonne
EJ	Exajoule, 1 EJ=10^{18} J
EOD	Earth overshoot day
EPS	Expanded polystyrene
EU	European Union
E-value	Number of expected hits of similar quality that could be found just by chance
F	Inbreeding coefficient
FeMax	Length according to Feret
FeMin	Width according to Feret
FM	Fresh mass
F_{ST}	Fixation index
Gb	Gigabase
GBS	Genotyping-by-sequencing
GWAS	Genome-wide association study
ha	Hectare
H_E	Expected heterozygosities

H_O	Observed heterozygosities
HSD	Honestly significant difference
IBN	Inflorescence branch number
IN	Number of internodes
K	Kelvin
kV	Kilovolt
kWh	Kilowatt per hour
l/w	Length-to-width ratio
M	Million
m^3	Cubic meter
Mb	Megabase
Mg	Megagram, 1,000 kg
MHY	Methane hectare yield
mW	Milliwatt
n	Sample size
N	Newton
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
Nl	Standard liter
nm	Nanometre, as 1×10^{-9} m
Nm	Newton-meter
oDM	Organic dry matter
p	P-value
Pa	Pascal, 10^{-5} bar
PAUP	Phylogenetic Analysis Using Parsimony
PCA	Principal Component Analysis
PH	Plant height
pH	Potential of hydrogen
Q	Aggregate size as cumulative share
r	Correlation coefficient
RFN	Ray florets number
rpm	Revolutions per minute

SD	Shoot diameter
SEM	Scanning electron microscope
SMY	Specific methane yield
SN	Shoot number per plant
SNP	Single-nucleotide polymorphism
SPHT	Sphericity index
t	Metric ton
tGBS	Tunable genotyping-by-sequencing
TLLLR, TLL	Thüringer Landesamt für Landwirtschaft und Ländlichen Raum
VCF	Variant call format
vol%	Percentage by volume
w/c	Water cement ratio
w/l	Width to length ratio
wt%	Percentage by weight
Xarea	Radius of an equivalent circle
ε	30 L/g*cm
π	Nucleotide diversities
°C	Degree Celsius
µm	Micrometer, 1×10^{-6} m
Y_{ijr}	Phenotypic observation of the i^{th} accession of the r^{th} replication in the j^{th} year
μ	General mean
A_i	Effect of the i^{th} accession
Y_j	Effect of the j^{th} year
R_r	Effect of the r^{th} replication
$(A.\ Y)_{ij}$	Interaction between the i^{th} accession and the j^{th} year
e_{ijr}, e_{ij}, e_{ig}	Residual
Y_{ig}	Phenotypic observation of the g^{th} genotype nested in the i^{th} accession

In 1971, for the first time, humanity consumed more resources than the ecosystems could regenerate. From this year on, humans have increasingly deviated from living in accordance with nature`s natural cycle. Over the years, the so-called “Earth Overshoot Day” has been reached at incrementally earlier times of the year. In 2022, Earth Overshoot Day was July 28th, meaning that humans lived the remaining 156 days of the year at the expense of nature. 1.7 planets would be needed in order to generate all required resources and stop the exploitation of Earth (EOD 2022).

1 Introduction

The climate crisis is man-made. This is the conclusion of 97% of all climate researchers (Anderegg et al. 2010). Researchers even doubt whether we are still living in the Holocene, the Earth age that began about 12,000 years ago after the end of the last cold period, or already in the Anthropocene a geological epoch that is significantly influenced by us humans (Greek: *ánthropos*). The main reason for this is our use of fossil resources i.e., fossil carbon. Carbon is the scaffold structure of DNA, proteins and cells and therefore one of the most frequent elements on earth; it is essential for all organisms. At the same time, carbon is one of the most important energy sources and raw materials. The problem is that the use of fossil carbon, adds CO_2 to the atmosphere, which promotes the greenhouse effect, and with that, global warming. Human civilization is built upon fossil carbon, in the form of oil, natural gas and coal. The industrialization of the last 200 years would not have been possible without it. In 2020, our world economy still depended up to 80% on fossil carbon (EESI 2021). Thus, we have to shift our carbon consumption from fossil to renewable resources in an effort to maintain global warming at 1.5 °C.

Biomass can play a crucial role as alternative to fossil resources, since it is the only sustainable carbon source (GBEP 2007). Like fossil carbon, it can be applied both for energy production and raw material use. However, the agricultural production of biomass also contributes to climate change as well, because it also uses fossil resources. As Crews points out, “humans have figured out how to address virtually every ecological limiting factor to crop growth – nutrients, water, insect herbivory, weed competition, disease – with fossil fuels” (2018). It is not unusual in today's industrialized cropping systems to use four times the amount of fossil fuel calories to grow food, than the food itself contains (Pimentel and Pimentel 2007). To correct this imbalance, agriculture has to change by producing food and biomass in a more resource efficient way. This transformation could even result in agriculture no longer exacerbating the climate crisis, but instead helping to solve it by capturing carbon from the atmosphere into biomass and providing it to the economy (Abl 2021). This new biomass-based economy is called bioeconomy. Nonetheless, this transition from fossil to renewable biomass requires adequate substitutes. Since oil and coal are nothing but biomass which captured CO_2 during growth thousands of years ago, biomass will become a key substitute. Using special ingredients (oil) and structures (fibre, parenchyma) of the plants directly would be advantageous, since they would not have to first be broken down into their carbon building blocks (as is usually the case for crude oil) and then be reassembled into a product. When compared to annual biomass crops, perennial biomass crops could be even more sustainable. Due to their perennial life cycle, they are often more yield and cost efficient and able to deliver more ecological benefits than annual crops. These are important qualities in the era of phosphate depletion and high CO_2 emissions of nitrogen fertilizers (Ruf and Emmerling 2021). However, in Germany in 2020 most of the renewable biomass produced was annual and roughly 90% of it was only used for energy purposes (FNR 2022c). An energetic use of the biomass requires high volumes of biomass and produces only low-value outputs. The opposite is the case for a material use that should be preferred for sustainability reasons. A cascade utilization could be a solution by using the plant for low volume, high-value products at first, and for low-value uses at the end of its lifespan (Kraska et al. 2015).

In recent years, the perennial *Silphium perfoliatum* L. (cup plant) has gained increasing interest by scientists and farmers, due to its variety of ecological and economic advantages of the plant (Peni et al. 2020). When compared to silage maize cup plant has a similar harvest and methane yield, and is used as forage. Next to its methane yield, cup plant produces ingredients which could be interesting for the pharmaceutical and chemical industries (Lunze et al. 2021). Furthermore, the seeds could potentially be used for oil production, much like what has been investigated for *Silphium integrifolium Michx.* (Reinert et al. 2019), and as a fibre or parenchyma resource for a material use (Chapter 3 and 4). As a result, *Silphium* could become a future third-generation biomass crop. Third-generation biomass crops should be able to produce both food (either staple foods or high-quality animal feed) and industrial raw materials (fuels, fibres, etc.). For instance, a perennial crop may be harvested late in the season for edible grain, or earlier in the season for biogas production/fodder, or during the winter as a raw material (Wever et al. 2020). This thesis examines the field traits of five *Silphium perfoliatum L.* accessions cultivated in Europe and the use of *S. perfoliatum* as raw material for paper and building materials.

1.1 Research questions

The foundation of this thesis is the phenotyping and material use of the perennial *Silphium perfoliatum* L. five commonly cultivated European *S. perfoliatum* accessions were analyzed (East Germany, Russia, Ukraine, Northern Europe and USA; accession names do not have a secure origin reference) to see if they differ in pheno- and genotype and if they are a suitable starting point for further breeding (Chapter 2). Moreover, *S. perfoliatum* was analyzed as a raw material for the pulp and paper industry after a mechanical and chemical treatment (Chapter 3), and after a mechanical treatment as a substitute for polystyrene in building material (Chapter 4). This thesis will give a brief introduction into renewable resources, as well as the botany of *Silphium perfoliatum* L. and two other perennial biomass plants (*Sida hermaphrodita* L. and meadow hay). These perennials were studied and compared in the paper experiment. Subsequently, the thesis is divided into three experimental publications (Chapter 2, 3, 4) where the following research questions were addressed:

I. Are the five *S. perfoliatum* L. accessions a resilient starting point for breeding programs?

II. Is *S. perfoliatum* L. a possible raw material for the pulp and paper industry?

III. Is the biomass of *S. perfoliatum* L. suitable for replacing EPS in a bonded leveling compound?

In answering these experimental questions, further questions could be posed and also answered. It was possible to analyze the genetic diversity of five S. perfoliatum accessions and their population structure. Furthermore, yield data for winter harvesting could be collected. In the case of the material use, the fiber lengths of *S. perfoliatum*, *Sida hermaphrodita* and meadow hay were determined and resulting paper properties such as tensile strength, bulk and yellowness were measured. In addition to specific building material measurements like the thermal conductivity or the compression strength, the parenchyma amount of the different accessions and the pore size of *S. perfoliatum* parenchyma were determined and the water immersion of the grounded biomass was measured.

The final discussion and conclusion will combine the major findings of the three research papers and point out future breeding and research directions.

1.2 Annual and perennial plants

Perennial plants dominate almost all terrestrial or land-based ecosystems because, once established as seedlings, perennials have an inherent advantage over annual plants at the start of the growing season (Raunkiaer 1977). Annual plants are vulnerable to being out-competed for sunlight or soil resources, such as water, by established perennial plants that emerge quickly from dormancy at the end of winter, because they do not have to restart their growth cycle from a relatively small seed every year (Pimm 1983). However, annuals last a few years as the dominant vegetation type in ecosystems subjected to frequent, severe disturbance, such as annual flooding or long droughts (Olson D. and Cox R. 2017). Since these disturbances are rare in agricultural ecosystems, the benefits of annuals, such as short juvenile phase, rapid transition to flowering, and prolonged seed dormancy, are less important for commercial cultivation (Bergonzi and Albani 2011; Venable 2007).

Cultivating annuals normally depends on frequent and intense tillage. Soil erosion, soil carbon loss, and nutrient runoff into ground as well as surface waters are all linked to frequent tillage. Moreover, those fields lack vegetation cover for long periods of time (Power 2010). Alternatively, perennials provide year-round soil coverage, as well as about twice the carbon inputs (50–67%) from roots and decaying plant material, when compared to that of annuals (15–30%) (Crews and Rumsey 2017; Goudriaan et al. 2001; Roy and Saugier 2008). Perennials grains are expected to have lower water productivity than annuals, because they transpire over a longer growing season and allocate less of their total photosynthate to seed, they require more water per unit of food produced (Vico and Brunsell 2018).

Agricultural systems that resemble natural ecosystems are expected to reduce industrial agriculture's greenhouse gas emissions since the reduced need for tilling, minimizes the need for heavy machinery and fuel. A further reduction would result by replacing energy-intensive inputs like synthetic nitrogen fertilizers with biological nitrogen fixation (Jensen et al. 2012; Glover et al. 2010). Perennial crops may also provide significant adaptation benefits in the face of a more volatile and extreme climate. Climate change will bring new pests and diseases, as well as short-term floodings and heat waves, all of which will have an impact on yields and profits (IPCC 2014). Some climate change staple crops may increase in yield due to an increase in temperature and atmospheric CO_2, but major ones including wheat, maize and rice will suffer under climate change (Rosenzweig et al. 2014; Liu et al. 2016; Challinor et al. 2014).

Progress toward diverse perennial agricultural systems has the potential to transform agricultural practices from one that produces an unsustainable set of ecosystem disservices, to one that adds many of the essential services provided by natural ecosystems (Crews et al. 2018).

1.3 Biomass crops as renewable resource

Society is confronted with the need to stop climate change while also dealing with twin crises like continual biodiversity loss, land system change or altered nitrogen and phosphate flows (Rockström et al. 2009). The use of renewable, instead of fossil resources, is part of the solution to these problems (UBA 2013).

Renewable resources can be divided into renewable energy (solar, wind, geothermy) and renewable raw materials. This biomass can be used for energy (maize, rapeseed) or material (wood, rice straw) purposes. In principle, biomass is captured sun energy through photosynthesis. The plants utilize sun energy, water and atmospheric CO_2 to produce carbon compounds like sugars, starch and cellulose. The difference between fossil and renewable resources lies in their storage format. Fossil resources, like coal or oil, are former biomass and organisms grown in earlier geological eras and stored under oxygen deprivation in deep soil layers. Their usage adds CO_2 to the atmosphere, which has been captured for millions of years. While the use of renewable raw materials in the form of currently grown biomass also adds CO_2 to the atmosphere, their use is actually carbon neutral. The amount of CO_2 produced during its use is equal to the amount that the plant removed from the atmosphere during its growth (IEA Bioenergy 2022).

Biomass from agriculture and forestry can be used as a raw material or as an energy source. The energetic use is often less efficient when compared to the use of wind or solar energy (UBA 2018). In 2020, Germany produced biomass crops on 2,577,000 ha. Only 9% of this area was used for producing renewable raw material, while the majority of the area was used for energetic purposes (FNR 2020). However, the share of the renewable raw materials is increasing due to the decrease of fossil resources and climate change adaptation (Türk 2014). To meet the biomass demand of the EU, an increase of 40–70% is needed by 2050 (Fig. 1).

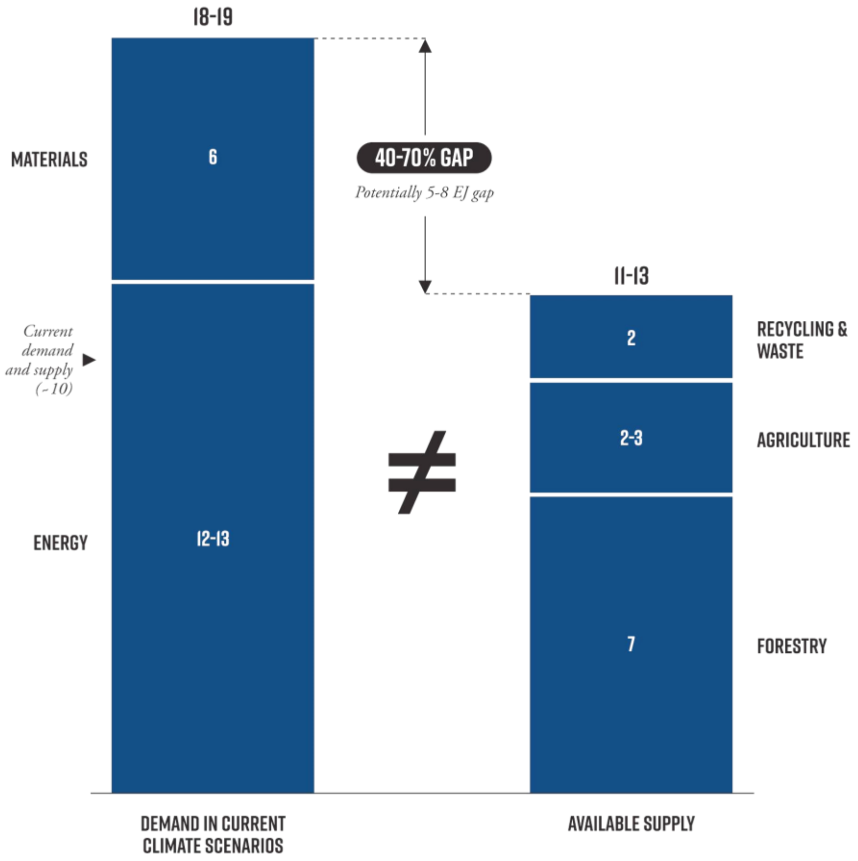

Fig. 1: 2050 biomass supply and demand for materials and energy in the EU. Primary energy equivalents in EJ per year. From Material Economics Sverige AB (2021).

Another possibility would be to substitute biomass for energy production with solar or wind energy, as much as possible. In Europe, this would make available 30–40 million ha of land that would otherwise be needed for growing bioenergy crops. Furthermore, it contributes to the agenda of restoring biodiversity in European natural systems (Material Economics 2021). Halving meat and dairy consumption in the EU would save another 9.2 million ha of grassland and 14.5 million ha of arable land (Westhoek et al. 2014). In addition, cascade utilization could be a solution for a sustainable biomass use (Fig. 2). At first, the biomass is used as a raw material for the production of high-value products like pharmaceuticals, chemicals, food, paper or building material, and after its lifespan, for heat production (Kraska et al. 2015).

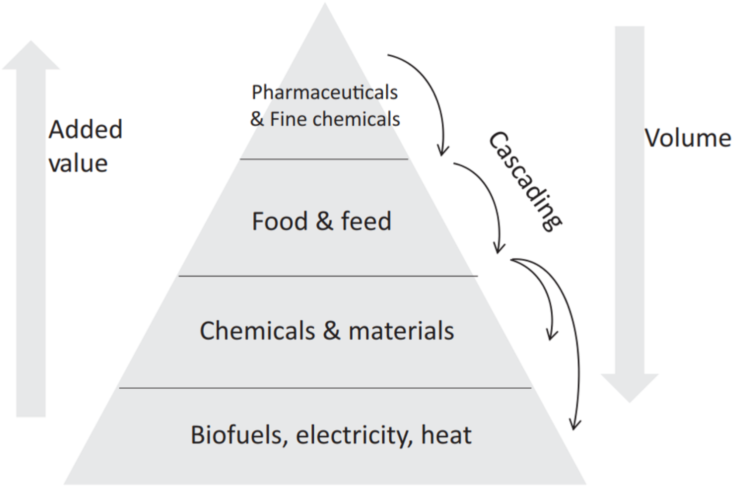

Fig. 2: Biomass value pyramid in the bioeconomy. From Eickhout (2012) adapted by Choi et al. (2019a).

As a result, policymakers, scientists, and industry should shift their focus away from seeing biomass as bulk contributions to aggregate energy targets and toward areas where the unique properties of biomass make the greatest contribution to a net-zero economy (Material Economics 2021).

1.3.1 Energetic use

Desired characteristics, local climate, and soil conditions must be considered when choosing crops for energy production. Ideally, the crops would have high dry matter yields, high calorific values, low energy and low cost inputs, minimal fertilization, and low nutrient requirements (McKendry 2002). Because perennial crops do not require annual replanting or soil tillage, the input-output balance could be greatly improved (Crews et al. 2018). Glover et al. showed that a harvested perennial grassland required 11.75 times less energy to produce a similar output when compared to nearby annual cropping systems (2010). In Europe, cup plant is mainly used as a biogas substrate. It produces a methane yield of 2333–7675 m^3 ha^{-1} depending on the analytical procedure, year, location and used *Silphium* accession (Vetter et al. 2010; Peni et al. 2020) averaging 72 m^3 methane $[t\ FM]^{-1}$ or 35.100 kWh ha^{-1} (FNR 2021b). Chapter 1.4.1 analyzes this topic in more detail. The subsequent conversion of biogas into methane gas allows it to be used as a transportation fuel of comparable quality to natural gas (Koniuszy et al. 2020). Since 2018, cup plant is compatible with the recent European law on advanced biofuels (European Commission 2018b).

1.3.2 Material use (direct, indirect)

Choi et al. show that renewable raw materials are needed for fuels, lubricants, adhesives, polymers, construction materials, fibers, and other raw materials (2019a). By definition, a renewable raw material is a material of plant, or microbial biomass, which is based on the photosynthetic primary production and is used by humans outside the food and feed category, and instead for material or energy production (Dahlson-Rutherford 2012). The agricultural production of renewable raw materials has two main aims: to reduce the use of finite fossil carbon and to decrease the CO_2 footprint of products and their production processes. Nonetheless, it is common sense that agriculture has to first serve human nutritional needs; plants should only be used for other purposes if the nutrition is ensured.

Renewable raw material can be used directly and indirectly. In the indirect use, the biomass is harvested and brought to a biorefinery. The biorefinery converts the biomass into platform chemicals (Takkellapati et al. 2018). Those chemicals can either be used as a substitute for current fuels and chemicals or they can be synthesized into novel building blocks for new applications. This method has the advantage to feed these platform chemicals into common refineries and produce products similar to those made with crude oil platform chemicals. The disadvantage to this process is that a lot of energy is required for the pre-crushing and chemical conversion in the biorefinery (Cherubini and Ulgiati 2010).

The direct method is often less energy intensive because the material requires less processing. This is leading to an advantage and disadvantage at the same time. A universal application like the use as a platform chemical is not possible. But, instead of breaking the plant tissue into platform chemicals and rebuilding it up again, the natural structures of the plant material can be used directly. For example, the direct use of a tree entails the utilization of the wood for furniture, since the wood only needs minor processing (sawing, leveling, etc.). An indirect use of a tree would be the utilization of residual phenolic oligomers as printing ink. This usage requires intensive processing of the raw material. The wood has to be converted into xylochemicals with a subsequent catalysis (Liao et al. 2020). *S. perfoliatum,* can be used indirectly after an intensive pretreatment, enzymatic hydrolysis and organosolv or fermentation in a biorefinery to produce bio-based platform chemicals (Lunze et al. 2021), or the biomass can be used directly after a less intensive pretreatment for paper production (Chapter 3) or building materials (Chapter 4).

The shoots, harvested in winter, consist primarily of cortex and parenchyma. Their chemical analysis showed a holocellulose content of ~ 28%, which was the starting point for the paper experiments. The optical and gravimetric analysis revealed similar pore sizes and weight between expanded polystyrene and the parenchyma, putting the focus on the use of *S. perfoliatum* as an insulation material.

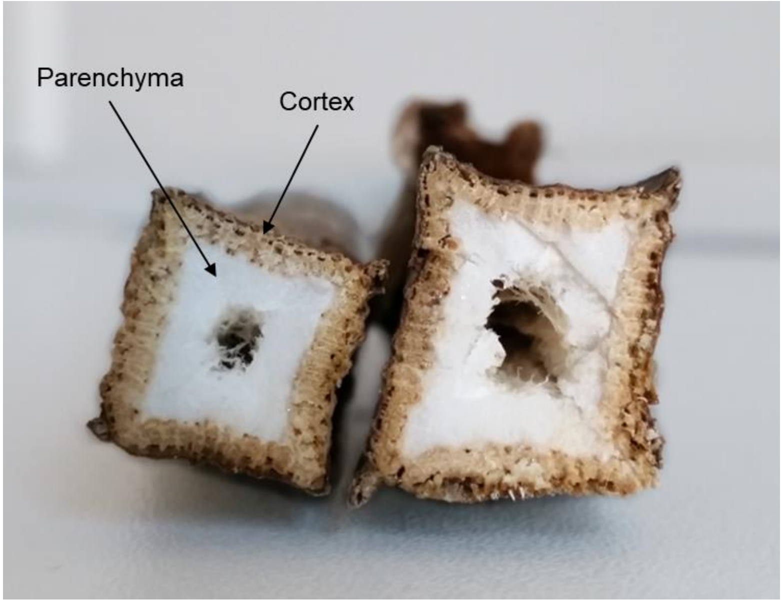

Fig. 3: *Silphium perfoliatum* L. quadrangular stalks after harvest in November with inner parenchyma surrounded by cortex.

According to Wever et al., biomass crops of the third generation should be able to produce both food and raw materials. Farmers would be able to respond to global markets and protect global food security with such flexibility. At the same time, third-generation biomass crops must improve agricultural sustainability and biodiversity. To achieve such goals, it needs de novo domestication of wild plants, improvement of orphan crops or forages and genetic adaptation to field conditions (2020; Gasparini et al. 2021). *S. perfoliatum* could become a feasible candidate as a biomass crop of the third generation. When harvested in the summer, *Silphium* can be used as biorefinery feedstock (Lunze et al. 2021), biogas substrate (Siwek et al. 2019; Cossel et al. 2020) or cattle feed (Neumerkel and Märtin 1982). When harvested in the autumn or winter, the seeds could potentially be used to produce oil (Reinert et al. 2019) and the stalks could be used as raw material for paper and building materials (Fig. 4).

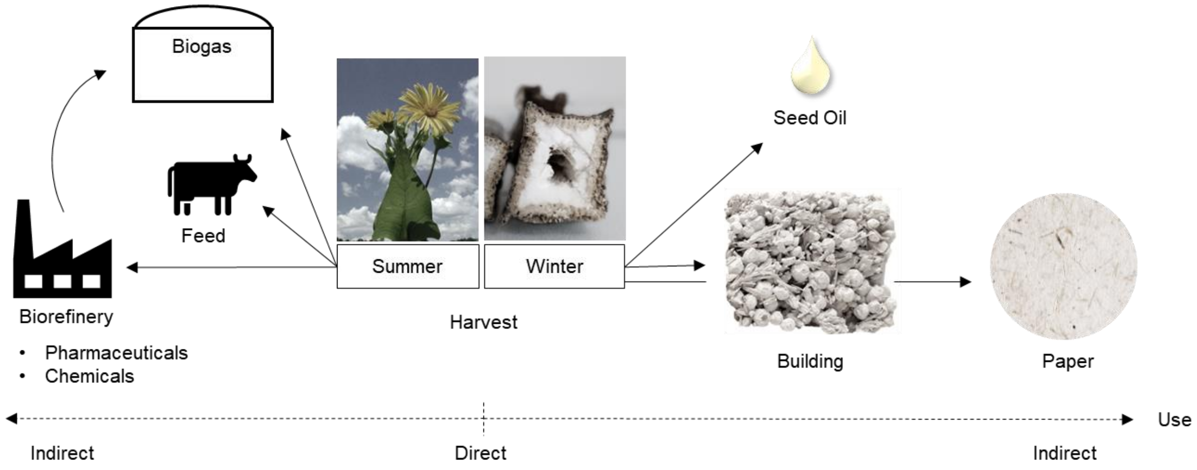

Fig. 4: Imaginable direct and indirect use of *S. perfoliatum* L. after summer and winter harvest.

1.4 Review of the three analyzed plant resources *Silphium perfoliatum* L., *Sida hermaphrodita* (L.) Rusby and meadow hay

1.4.1 *Silphium perfoliatum* L.

1.4.1.1 Origin and Botany

Silphium perfoliatum L. is a perennial from the *Asteraceae* family, of the same tribe as the sun flower (*Heliantheae*) within the genus *Silphium* (Clevinger and Panero 2000). *S. perfoliatum* is native to the central and eastern United States and the south of Canada. It was imported to Europe in the 18th century. North American ecologists and conservationists recognized it in the 1930s and 1940s for its ability to withstand a variety of stressors (Leopold 1968; Weaver et al. 1935).

In the first year of planting or sowing, the yellow flowering C_3 plant forms around 12–14 leaves in a rosette and a strong horizontal rootstock with numerous side roots (Neumerkel 1978; Stanford 1990). In the second year, it forms 3–15 hairy quadrangular stalks with a height of 2–3.5 m. The cultivation period is 15–20 years. The stalks per plant increase to up to 20–80 stalks as the plant ages. On the nodes, the leaves are arranged in pairs, forming cups that are perforated by the stalk that is why it is also known as cup plant (Gansberger et al. 2015; Neumerkel and Märtin 1982; Hayek 1918). The leaves are triangular to oval in shape with serrated edges and reach a maximum length of 40 cm and width of 25 cm (Wrobel 2013). The flowering phase lasts from July to September, due to the constant formation of new flower heads. Each head blooms for approximately 10–12 days. At least 8–10 flower heads, composed of male tubular flowers and female (Assefa et al. 2015b) ligulate ray flowers, develop per inflorescence branch (Neumerkel and Märtin 1982), with each measuring 4–8 cm in diameter. *S. perfoliatum* is a cross-pollinating or self-pollinating plant (Neumerkel 1978; Vacek and Repka 1992). It creates an attractive agricultural landscape in contrast to non-flowering crops (Feldwisch 2011; Franzaring et al. 2013; Sokolov and Gritsak 1972). After flowering, *S. perfoliatum* develops around 18–30 fruits per flower head (Neumerkel and Märtin 1982; Niqueux 1981). The fruits are flat achenes, green-brown in color (Wrobel 2013; Kowalski and Wiercinski 2004; Assefa et al. 2015b), measuring approximately 9–15 mm in length, 6–9 mm in width, and no more than 1 mm in thickness (Niqueux 1981). The rhizome of *S. perfoliatum* produces cluster roots. Individual plant side shoots develop a large number of well-branched adventitious roots, which can quickly fill the available soil volume. During the growing season, the shoot/root ratio shifts in favor of aboveground biomass (Schorpp et al. 2016).

1.4.1.2 Cultivation

This crop is well adapted to the diverse climates of Europe (Neumerkel and Märtin 1982). The optimal temperature for growth is around 20 °C. Full sun promotes optimal growth (Stanford 1990). Additionally, *S. perfoliatum* can withstand temperatures as low as -25 °C (Phillips et al. 1991). Its water requirements are already met with 400–500 mm of rain year^{-1} and between 200–250 mm during the vegetation period (Grebe et al. 2012).

S. perfoliatum is sawn since Schäfer et al. established a precision seeding method, prior to this, it was mostly established by the planting of plantlets (2016). As a perennial, *S. perfoliatum* utilizes nutrients more efficiently when compared to many annual plants, due to its more developed and deeper root system (McKendry 2002; Schoo et al. 2017), which also enables it to utilize deep soil water (Schoo 2013). The leaf axils, also known as cups, store water such as rain and condensate (Hayek 1918). This adaptation characterizes *Silphium* as a drought-tolerant crop. However, the water is not absorbed directly from the cups. It is rather assumed that the evaporation of this water reduces the temperature in the stock, making high temperatures more tolerable (Schoo 2013; Stanford 1990; Grebe et al. 2012). Cup plant is especially tolerant to dry periods from April to June. Pan et al. observed that *S. perfoliatum* outperforms maize in terms of water use efficiency during dry vegetation periods, but maize outperforms *S. perfoliatum* during years with normal or above-average rainfall. Additionally, Pan demonstrates that *Silphium* is capable of producing significantly more biomass than maize under arid conditions. However, in normal or wet years, maize produced comparatively slightly more biomass (2011). Only in Troxler and Daccord's (1982) experiments was a certain level of sensitivity to water scarcity observed.

S. perfoliatum is harvested from late August to September for biogas or fodder purposes and has a biomass yield comparable to maize of 1.6–32 t ha^{-1} DM and a water content of 73–75% depending on the growing conditions (Boe et al. 2019a; Peni et al. 2020). For our purposes, the crop was harvested during the winter with yields between 7–14 t ha^{-1} DM and a water content between 16–67%, depending on the accession (Fig. 5b). A late harvest, after relocations of the nutrients from the green biomass into the rhizome, could increase the cultivation period; an important topic for further research.

The economic significance of this bioenergy and forage crop in Germany has grown in recent years. The total acreage increased twelve-fold from 800 ha in 2016 to 10,000 ha in 2021 (FNR 2021a). 118

studies on the cultivation of cup plant and its multi-purpose usability have been published in Europe and worldwide between 2000 and 2020 (Peni et al. 2020).

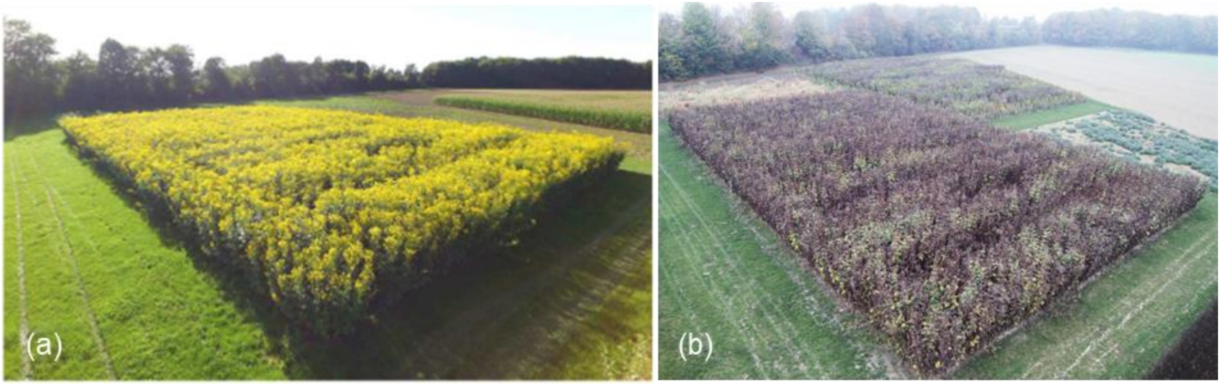

Fig. 5: *Silphium perfoliatum* L. field trial of the five accessions (East Germany, Russia, Ukraine, Northern Europe, USA) in August (a) and October (b) at the Campus Klein-Altendorf of the University of Bonn.

Fertile, humic soils, adequate moisture, and low-lying land such as woodland and along watercourses, are the characteristics of the best growing areas for the crop, whereas Hydromorphic soils are unsuitable (Sokolov and Gritsak 1972; Benke et al. 2012). *S. perfoliatum* can be used as a pioneer crop for reclaiming recultivation of degraded land (Zhang et al. 2010a; Wrobel 2013). In Southern China, *Silphium* species have been proposed for bioremediation of soils contaminated with crude oil or copper-mine tailings, as well as revegetation of eroded, acidic landscapes (Zhang et al. 2011; Zhang et al. 2006; Ouyang K. et al. 2007). These findings point out the widespread adaptation and tolerance to abiotic stressors of the crop (van Tassel et al. 2017). Due to the year-round and multi-year ground coverage, the risk of nutrient leaching, soil erosion and washout is reduced when compared to annual energy crops (Benke et al. 2012; Franzaring et al. 2013), soil formation is facilitated, and carbon sequestration occurs.

The amount of fertilizer required is determined by the soil's nutrient supply and the plant’s expected nutrient uptake. *S. perfoliatum* has a high capacity for nutrient retention and nutrient acquisition (Neumerkel 1978; Niqueux 1981). Pichard found that the first 100 kg ha^{-1} (based on nitrogen weight) had a significant effect on the yield, but the yield-increasing effect diminished after that (Daniel and Rompf 1994; Neumerkel and Märtin 1982; Pichard 2012). Due to the toxicity effect of excessive nitrogen addition, the yield is reduced and the risk of lodging increases (Pichard 2012; Benke et al. 2012). Fertilizers, both mineral and organic, are suitable. Conrad et al. (2009) fertilized their crops using mineral fertilizer and liquid digestate from a biogas plant. The highest yield was obtained by combining digestate and mineral fertilizers. They estimate that the crop requires 10 kg ha^{-1} of nitrogen to produce one ton of dry matter. Fertilization with phosphorus, magnesium, and potassium may be necessary, depending on the soil's nutrient supply. Phosphorus is removed at a rate of approximately 30 kg ha^{-1}, magnesium is removed at a rate of 60 kg ha^{-1}, and potassium is removed at a rate of approximately 250 kg ha^{-1}. Due to the low soil cover in the first year, the use of a machine hoe is inevitable (Conrad et al. 2009). Good soil shading from the second year onwards makes herbicide and machine hoe use obsolete. Its moderate fertilizer requirements, especially if harvested in winter in its brown state, makes cup plant suitable for less fertile soils, minimizing the food vs. fuel debate (Gamborg et al. 2012; Ruf and Emmerling 2021).

Fungi can cause yield-reducing damage. Wet summers can promote *Sclerotinia spp*. infection of the stems (Troxler J. and Daccord R. 1982; Stolzenburg and Monkos; Niqueux 1981). The production of permanent fruiting bodies of the fungus can be prevented by an early harvest, thereby limiting the spread of the fungus. Other fungi that contribute to biomass and seed yield losses include *Fusarium spp.*, *Alternaria spp.*, and *Botrytis spp.* (Benke et al. 2012; Grebe et al. 2012).

Despite the fact that Leopold (1968) observed that cattle preferentially grazed the foliage and that the seeds tasted like sunflower seeds, it was Soviet scientists who first attempted to exploit the genus as a forage crop in 1957 (Stanford 1990).

Fig. 6: *Silphium perfoliatum*: Different accessions in July (a), habitus (b), cup-shaped leaf axil (c), regrowth from stump 8 weeks after harvest (d).

1.4.1.3 Environmental benefits and genetics

Since cup plant is often mentioned as a maize substitute, Schorpp et al. compared their multiple ecosystems services. With nine earthworm species, cup plant fields hosted almost twice as many species when compared to maize fields (5 species). Within the earthworm communities of cup plant fields, the frequency of anectic species (*Lumbricus terrestris* and *Aporrectodea longa*) increases with increasing age of the cup plant fields (2016). As these species burrow their worm holes to great depths, water infiltration and soil gas exchange improve (Edwards and Shipitalo 1998). In addition, anectic earthworms produce humus with incorporated litter at the soil surface, which are biologically highly active “hotspots” (Schrader and Seibel 2001). Epigaic species were detected (*Lumbricus castaneus* and *Lumbricus rubellus*), which were completely absent in maize plots. However, their occurrence was restricted to later crop years (5—9 years). Cup plant fields proved to be refugia for species-rich earthworm communities that are rarely found in arable crop fields (Schorpp et al. 2016). The absence of annual tilling, has a beneficial effect on soil structure because it reduces soil erosion and promotes biodiversity (Ruf et al. 2018; Schorpp and Schrader 2016).

While pollen is pushed out of the tubular flowers, nectar is produced within the tubular flowers. Thus, for hoverfly species with a saprophagous larval feeding type, the cup plant may be of particular value as a late nectar and pollen source. For mostly short-trunked zoophagous species, access to nectar may be more difficult. The pollen and nectar supply are used by honeybees and some species of bumblebees and hoverflies with saprophagous larval feeding type. Honey bees produce new worker bees at the end of the year, which overwinter together with their queen. For the rearing of these so-called winter bees, the bee colony needs pollen, which the cup plant offers at this time. Bumblebees also use the resource offer, because at the end of the colony cycle, they produce sexual bees, which fly out to mate with each other. Some saprophagous hoverfly species exhibit a late period of activity and require energy for their long migratory flights. The late flowering of cup plant is also attractive to other wild pollinators, and provides food resources in months with scarce nectar and pollen sources (Schorpp et al. 2016). In comparison to the harvest in September for energy purposes (during flowering) the winter harvest for material use would extend the flowering period. *S. perfoliatum* was recently included in the European Commission's greening support program for farmers, indicating its high ecological value (European Commission 2018a).

In the experiments, (pulp and paper, bonded levelling compound) a mixture of two *Silphium perfoliatum* accessions was used. This mixture is mainly used in German agriculture and is distributed by the company N.L. Chrestensen Erfurter Samen- und Pflanzenzucht GmbH. It contains an open pollinated mixture of the accessions Russia and Ukraine (provided by the Thüringer Landesamt für Landwirtschaft und Ländlichen Raum, TLLLR).

1.4.2 *Sida hermaphrodita* (L.) Rusby

1.4.2.1 *Origin and Botany*

Sida hermaphrodita (L.) Rusby is a perennial from the *Malvaceae* family. The plant is native to the north-east of the United States and is also known by the names Virginia mallow and Virginia fanpetals (USDA 2021). The C_3 plant is a frost resistant shrub. In the first year, it forms mainly a deep root system, and from the second year on it forms 20–40 stems per plant which grow up to 3 m high. The upright stems, which can reach a thickness of 3 cm, are densely branched and lignify as they mature. The hand-sized leaves are light green, maple-shaped, and alternately arranged along the round stem. The leaf edge is incised and sawed (Franzaring et al. 2014). *S. hermaphrodita* produces terminal white flowers that are dispersed. The flowers range in size from 1–2 cm in diameter. The lobes of the calyx are short and broad. The petals of the long ovate flowers are tapered (Cumplido-Marin et al. 2020). The flowering season is from June to October (SidaTim 2022).

Fig. 7: *Sida hermaphrodita* in July (a) and March during harvest (b) and regrowth (c) at the Campus Klein-Altendorf of the University of Bonn.

1.4.2.2 *Cultivation and utilization*

Sowing or planting begins in the middle of May. Drill sowing is significantly less expensive than planting. However, the seed has an extremely low germination rate. While planting is more expensive, it ensures a secure plant establishment. Above-ground growth is insufficient to meet utilization requirements in the first year. From the second year of standing, a harvestable yield can be expected, and the full yield potential is reached in the third year. *Sida* has minimal site requirements; it is also suitable for marginal land (Nabel et al. 2018). However, moist, dense soils with an adequate supply of water and locations with a sunny, warm climate are ideal. On light and sandy soils, lower yields must be expected. Acidification of the soil is detrimental to the plant. In Germany, *Sida* is primarily grown on trial plots. The total area under cultivation is unknown. Depending on the soil, temperature, rainfall and number of harvests per year, yields of 2.5–24.3 t ha^{-1} DM can be harvested without fertilization (Hartmann 2018; Bury et al. 2021).

In Germany, nitrogen fertilization in the first year after seeding is no longer permitted. After the second year, *Sida* requires 100 kg N ha^{-1} to produce up to 40 t ha^{-1} FM (28% DM). Additionally, a nitrogen withdrawal of 0.25 kg N per dt ha^{-1} FM (80% DM) is anticipated for thermal use. In the spring, when new growth begins, organic fertilization with liquid manure or fermentation residues is possible (StMELF 2022).

Herb control in the first year is important. From the second year on, no additional measures are typically required in sufficiently dense stands. Infestation with *Sclerotinia* may occur depending on the previous crop and weather conditions, with the latter causing no yield losses thus far. Canola, should be avoided as a preceding crop. In the event of a severe *Sclerotinia* infestation, the crop should be harvested immediately to prevent the fungus from establishing permanent bodies, which would otherwise result in disease infestation in subsequent years (TFZ Bayern 2022).

For biogas use, *S. hermaphrodita* is cut and ensiled. It can be harvested in green stage 1–2 times per year between June and July and again in October. Harvesting requires a corn chopper with a row-independent cutting unit. The first cut yields approximately 28% DM and accounts for the majority of the annual yield. However, attaining the 28% DM level is frequently critical. According to previous research, *Sida* produces about 15–20% less methane than corn. The optimal harvest time for maximum methane yield per hectare is still being investigated (Cumplido-Marin et al. 2020). Solid fuel (pellets), biogas, or methanol can be obtained from the plant for energy production. For the use as solid fuel, *Sida* is harvested in the spring (March to April), after the leaves have fallen and the stems are sufficiently dry (>85% DM). The calorific value and aerosol forming content are comparable to that of wood chips. The ash melting point is higher when compared to other herbaceous biomass. The plant can be harvested for 15–20 years (FNR 2022a).

1.4.2.3 Environmental benefits

Feledyn-Szewczyk et al. investigated weed density and species associated with arable crops, in contrast to energy crops (2019). They analyzed an increase of 11% in perennial species (including *S. hermaphrodita*). Due to the long flowering season of *S. hermaphrodita*, pollinators receive an extended food source. *S. hermaphrodita* blooms from early summer to the first frost in autumn (Spooner et al. 1985; Kurucz et al. 2018) and can produce between 110 and 315 kg of honey per hectare (Borkowska H. and Styk B. 2006). *Sida* has the potential to reduce soil erosion and pesticide use in comparison to bioenergy crops such as maize (Cumplido-Marin et al. 2020). Due to the perennial nature of the crop, very little soil disturbance occurs after the first year of establishment, and field operations are limited to fertilization and harvest (Schorpp et al. 2016; Haag et al. 2015; Gansberger et al. 2015; Ruidisch et al. 2015). *S. hermaphrodita* is preferred in areas prone to nitrogen leaching. This is due to the crop’s capacity to absorb nitrogen, as well as its low fertilizer and pesticide requirements (Borkowska et al. 2009; Pichard 2012). Flood plains are one of Sida’s natural habitats (Spooner et al. 1985), making it an ideal candidate for flood mitigation strategies. Stolarski et al. discovered that *S. hermaphrodita* tolerates flooding better than ten other energy crops, e.g. *Silphium perfoliatum*, *Miscanthus x giganteus* and *Fallopia japonica* (2014). Additionally, the benefits of perennial crops for earthworms can improve soil aeration and water infiltration (Schorpp et al. 2016; Schorpp and Schrader 2016). Ruf et al. investigated the organic carbon, microbial biomass, and aggregate stability of three different land use systems, ranking permanent grassland first, perennial energy crops (including *S. hermaphrodita*) second, and annual energy crops third. They discovered that until the tenth year, the soil’s organic carbon content increased steadily with the plantation's age (Cumplido-Marin et al. 2020; Ruf et al. 2018).

1.4.3 Meadow hay

1.4.3.1 Botanical characteristics

In Chapter 3, the hay used was harvested on extensive permanent grassland. It consists mainly of the C_3 grasses *Lolium perenne* L., *Alopecurus pratensis* L., *Phleum pratense* L., *Poa trivialis* L. and *Dactylis glomerata* L. Those perennial species belong to the family of the sweet grasses (*Poaceae*). The grasses are typical for central European grasslands, (Britannica 2022; Opitz von Boberfeld 1994) but can be found on many continents. Grasses are herbaceous monocotyledonous plants with small flowers and long, narrow leaves (Britannica 2022). It is harvested green, as silage, or hay if it is used as fodder. Grassland vegetation types are as diverse as their yield capacity and suitability for use, depending on their location, climate conditions, utilization intensity and harvest time. Grassland also has a socioeconomic functions, such as its contribution to a cultural landscape in many regions (JKI 2022).

Fig. 8: Gras on a field (a) and as hay 5 days after harvest (b) at the Hecknaaferhof in Much (Germany).

1.4.3.2 Cultivation

Native grasslands are often relatively drought tolerant but differences between the species are significant (Craine et al. 2013). Intensively used grassland is harvested three to five times a year, depending on the location, yielding between 6–14 t ha^{-1} DM. In comparison to intensive grassland, which contains 10–15 distinct plant species, extensive grassland contains a much greater diversity of species. Once or twice a year, the extensive grassland is mowed or grazed. The harvested, biomass is primarily used in the production of hay. Yields of 2.3–15.5 t ha^{-1} DM are possible. Germany has approximately 4.7 million hectares of permanent grassland and approximately 600,000 hectares of arable fodder cultivation; under 5% of this is used for energy (mainly biogas substrate). Fertilization of extensive grassland (e.g., liming, potassium) is applied as needed to maintain the soil's nutrient content (FNR 2022b).

1.4.3.3 Environmental benefits

Grassland decreases the impact of floodings. Meadows and pastures, with their continuous plant cover, deeply rooted soil, and higher humus content, can store significantly more water than arable land. Grassland also provides erosion protection, particularly on steep slopes and in floodplains of river valleys. Meadows and pastures are also critical for the purification of our drinking water. The dense root network and the dense plant cover filter surface water. In comparison to arable land, there are few problems associated with increased agricultural nutrient inputs. Grassland that has been extensively managed acts as a buffer between adjacent water bodies and biotopes (Velthof et al. 2014). By absorbing carbon dioxide, grassland benefits the climate. Not only the plants, but especially the humus, stores large amounts of carbon (Ruf et al. 2018). Carbon sequestered in the soil is nearly twice as large as carbon sequestered in the atmosphere and three times as large as carbon sequestered in vegetation (Terrer et al. 2021). As a result, meadows and pastures act as carbon sinks, storing 0–8 t C ha^{-1} yr^{-1} (Jones and Donnelly 2004). Furthermore, grassland has a 3–5 times higher earthworm density when compared to arable land (Burmeister and Walter 2016).

2 Phenotypic and genotypic evaluation

This work has been published as:

Christian Wever, Martin Höller, Lukas Becker, Andrea Biertümpfel, Johannes Köhler, Delphine van Inghelandt, Peter Westhoff, Ralf Pude, Elena Pestsova (2019). **Towards high-biomass yielding bioenergy crop *Silphium perfoliatum* L.: phenotypic and genotypic evaluation of five cultivated populations.** Biomass and Bioenergy 124.

The manuscript has not been modified from the published version.

2.1 Introduction

To reduce our dependency on finite, high-carbon fossil fuels we need to develop and exploit sustainable sources of energy. Bioenergy from plants could make a contribution to the alleviation of global problems in climate change and energy security if high yields can be achieved (Karp and Shield 2008; Allwright and Taylor 2016). Since the beginning of the new millennium the use of biogas as a renewable energy source continues to gain significance. Germany is Europe’s biggest biogas producer and market leader in biogas technology (Bauböck et al. 2014; GBA 2018). The predominant energy crop for biogas production in Germany is silage maize and the acreage of this crop has increased dramatically in recent decades reaching 0.91 million ha in 2017 (FNR 2020). This trend resulted in extended monocultures in combination with an increased susceptibility to crop diseases and pests as well as a reduced biodiversity of insect and animal populations (Schwabe 2010; Deuker et al. 2012).

Cup plant (*Silphium perfoliatum* L.), a perennial from the *Asteraceae*, represents a promising energy crop for biogas production (reviewed in Gansberger et al. 2015). In addition to a biomass yield comparable with that of maize (Mast et al. 2014; Haag et al. 2015; Biertümpfel 2012), this non-food plant possesses a high ecological value due to a very long flowering period, which lasts from the beginning of July till the end of September, and provides a valuable source of nectar and pollen for honey bees and other insects (Schorpp et al. 2016). As a perennial the cup plant has a superior nutrient use efficiency due to the better developed and deeper root system (McLaughlin and Walsh 1998; Schoo et al. 2017). Because this plant does not require annual tilling, it has a positive influence on soil structure, reduces soil erosion and promotes biodiversity (Schorpp et al. 2016; Ruf et al. 2018; Schorpp and Schrader 2016). Recently, *S. perfoliatum* was taken in the European Commission greening support program for farmers, thus recognizing its high ecological benefits (EU Regulation 2393). However, this new emerging crop has not been well adapted to the modern agricultural management and still possesses features of a wild plant, such as uneven seed maturation, seed dormancy and the dispersal of matured seeds. The main drawback of *Silphium* cultivation represents labor- and cost-intensive field establishment due to the uneven germination and slow development during the first year (Gansberger et al. 2015).

Cup plant is native to North America and was brought to Europe in the 18th century as a decorative plant (Stanford 1990). First attempts to domesticate and breed cup plant as a forage and silage crop were made in the USSR in the late fifties (Sokolov and Gritsak 1972). Later on, a breeding program for cup plant was also initiated in East Germany (Neumerkel and Märtin 1982). After the German reunification, breeding activities in Germany had been stopped, however the cup plant was not completely forgotten: in the first decade of this millennium, it was rediscovered as a bioenergy crop. Nowadays, the economic importance of this bioenergy and forage crop in Germany has increased and there is a continuous growth of a total acreage with almost 2000 ha in 2017 (FNR 2020).

Several studies about cultivation of cup plant as a biogas substrate in other European countries have been reported (Šiaudinis et al. 2015; Slepetys et al. 2012). Remarkably all field experiments showing high biomass yield of cup plant were conducted by using only a few cultivated populations. It is not known whether these populations belong to the same gene pool or represent unrelated populations either because of different geographic origin or as a result of the previous breeding activities. Starting in 2004, the Thuringian State Institute for Agriculture (Thüringer Landesanstalt für Landwirtschaft, hereafter TLL) in Germany had collected seeds of *S. perfoliatum* from eleven different geographic origins and evaluated field performance of the corresponding populations (hereafter accessions) (Biertümpfel 2012; Biertümpfel and Conrad 2013). Based on morphology, biomass yield and biogas productivity, five accessions have been selected from this collection to be analysed in the current study. These accessions originate from East Germany, Russia, Ukraine, Northern Europe and USA. Accessions from Russia and Ukraine are of particular interest because they have been used in the breeding program of N. L. Chrestensen Erfurter Samen- und Pflanzenzucht GmbH (Erfurt, Germany) for seed production. Until recently this company was the only supplier of *Silphium* seeds and plantlets in Germany.

S. perfoliatum is a diploid species with seven chromosome pairs (Settle 1967) and a large genome of about 8 Gb (Bai et al. 2012). Until recently, the only study implying DNA sequence data of cup plant was the phylogenetic analysis of the genus and subtribe (Clevinger and Panero 2000). For bioenergy crops that have hardly been selected and bred in the past there is considerable potential to increase biomass yield in a sustainable way by better utilization of genetic resources and breeding. New DNA technologies including next generation sequencing, high-throughput genotyping and molecular breeding provide an opportunity to rapidly harness this potential and deliver higher, more sustainable yields with a high value to society (Allwright and Taylor 2016). Genotyping-by-sequencing (GBS) technology has emerged as an useful and robust approach to study genotypes in species with large genomes by reducing the genome complexity using restriction enzymes (Elshire et al. 2011; Scheben et al. 2017). The tunable GBS (tGBS®) developed by Data2Bio® provides the ability to adjust the number of targeted sites based on research goals and permits accurate genotyping of both heterozygous individuals and polyploid species by increasing average read-depth per site and reducing missing data (Ott et al. 2017). This technology is known to be effective in species without sequenced reference genomes, thus, it has been considered as the most appropriate for the analysis of the large cup plant genome.

Most studies dealing with phenotyping and yield assessment of cup plant are referring to the local, regional or geographic origin of plant material used (Haag et al. 2015; Franzaring et al. 2015; Franzaring et al. 2014; Biertümpfel and Conrad 2013). Absence of information on genetic background of plants restricts comparability of the data limiting them to the used cultivars under specific environmental conditions. Molecular genetic analysis of the cultivated cup plant populations should remove these limitations, make different studies comparable and answer the question whether the genetic diversity presented within the cultivated gene pool is broad enough for successful breeding. The main goal of this study was the evaluation of phenotypic and genetic attributes of five selected populations of cup plant in order to clarify relatedness between them and to prove the existence of regional cultivars. In the phenotypic screen, biomass and biogas yield as well as the related compositional and phenotypic parameters were characterized. In the genetic screen, homogeneity, genetic diversity and population structure of the accessions were assessed. In addition, these screens allowed the identification of useful target traits for breeding and selection of the best individuals for future crop improvement. Detected phenotype-genotype associations have a promising potential to speed up the breeding process of cup plant.

2.2 Material and Methods

2.2.1 Plant material

In this study accession is defined as seeds obtained from specific geographic origin of cup plant cultivation. Genotype is referred to an individual cup plant grown from single seed (also referred as “mother plant”) and to the genetically identical cloned plants propagated from the mother plant vegetatively. Five accessions collected by TLL were analyzed. The original ancestries of these accessions are unknown except for the fact that they must have American ancestors. The seeds of the accessions “USA” and “Russia” were obtained from local seed companies in the USA (2004) and Russia (2007), respectively. The accession “East Germany”, also known as “Benko”, derived from breeding activities in the East Germany before 1990. The seeds of the accession “Northern Europe”, also known as “Horn”, were gathered in Scandinavia in 2008. The seeds of the accession “Ukraine” were provided by an agricultural adviser cooperating with plant breeders in Kiev, Ukraine in 2009.

2.2.2 Field trials

In previous experiments the TLL has compared different cultivation practices for *S. perfoliatum*, optimized plant density and fertilizer application, and determined the best harvesting time to reach a higher biogas yield (Biertümpfel and Conrad 2013). In the current study, these optimizations were applied to the agricultural management of cup plant. Field trials for evaluation of biomass and biogas yield were conducted by TLL in Dornburg near Jena, Germany (Table A 1). Seeds of each accession were sown in December 2012 in seed trays and cultivated outdoor during 3 months for stratification. In March 2013 the seed trays were placed in a greenhouse at a temperature of 12 °C. Germination took place after 7 to 10 days. Seedlings at the 3- to 4-leaf stage were planted on May 7, 2013 and the plants were harvested on August 28, 2014, August 24, 2015 and August 31, 2016 to estimate parameters related to biogas production. The annual mean temperatures and the annual precipitations were 10.2, 10.0, 9.6 °C and 618, 488, 535 mm in 2014, 2015 and 2016, respectively. The experimental field setting was a randomized block design with four repetitions. The N fertilizer was applied taking into account the measured soil mineralization to the final rate of 100 kg ha^{-1} in 2013 and to the rate of 150 kg ha^{-1} in the next years.

The second experimental site established in 2016 is located at Field Lab Campus Klein-Altendorf in Rheinbach near Bonn, Germany (Table A 1). Annual mean temperature and rainfall were 10.3 °C and 613 mm in 2016 and 10.7 °C and 670 mm in 2017. Plants for the field trial were obtained from rhizome cuttings of 12 mother plants per accession grown in Dornburg. Each rhizome was divided into 6–25 pieces to produce on average 13 genetically identical individual plants or clones. The rhizome cuttings were pre-cultivated in the greenhouse for 10 weeks at 20–30 °C. The plantlets were planted by hand on the 11 May 2016. The trial has a randomized block design with three replications. The plants were irrigated (22 mm) after planting and 3 days later (22 mm) to safeguard plant establishment. In April 2016 100 kg/ha N fertiliser was applied. In April 2017 27 kg/ha N, 9 kg/ha P and 70 kg/ha K were applied. In the first year of cultivation, weeds were controlled manually and mechanically.

Table A 1: Geographic and meteorological conditions of the experimental sites including the plot design.

Site	**Coordinates**	**Altitude [m]**	**Soil**	**Long-term mean annual air temperature [°C]**	**Long-term mean annual precipitation [mm]**	**Plot size [m^2]**	**Plant distance [m]**	**Row distance [m]**	**Plant density [plant m^{-2}]**
Dornburg	51°00' N 11°39' E	260	Orthic Luvisol	8.9	605	13.5	0.5	0.5	4
Rheinbach	50°36’ N 6°59’ E	190	Haplic Luvisol	9.5	606	22.5	0.65	0.75	2.1

2.2.3 Estimation of biomass yield and compositional parameters

Each plot in Dornburg was harvested separately and the biomass was weighted. For the estimation of the dry biomass weight, 500 g of the freshly harvested biomass was dried at 105 °C till the achievement of constant weight. Dry tons per hectare (DMY) were extrapolated based on the dry weight of the samples for each accession. To obtain compositional data including acid detergent fibre (ADF), acid detergent lignin (ADL) and ash content the biomass samples were first dried at 30 °C and analysed using Fibertec instrument according to the protocol 6.5.2 and 6.5.3 (VDLUFA 2007). Ash content was quantified gravimetrically after incineration in an electric muffle furnace according to Annex M “Determination of crude ash” of European standard analytical procedure (EU Regulation 152). Specific methane yield (SMY) was determined once per accession per season, for this purpose the biomass of the four replications was combined to obtain representative samples. SMY was measured using the Hohenheim Biogas Yield Test according to the protocol 4.1.1 (VDLUFA 2011).

Harvest of field trial in Rheinbach took place on December 6, 2017. Twelve random plants per accession (four plants per plot) were harvested and weighted separately. The biomass was chopped and 500 g per sample was dried at 105 °C until constant weight was reached. Dry tons per hectare (DMY) were extrapolated based on the average dry weight of the samples for each accession.

2.2.4 Phenotypic traits

Phenotypic traits were evaluated at field trial at Campus Klein-Altendorf on two different dates in 2017. Twelve genotypes per accession and three cloned plants per genotype were measured, in total 180 plants. On July, 11 shoot number per plant (**SN1**) were counted, and three well-developed shoots per plant were measured for their height (**PH1**), shoot diameter **(SD1)**, number of internodes (**IN1**) and inflorescence branch number (**IBN1**). Moreover, number of ray florets (**RFN1**) was determined. On October, 19 additive measurements of plant height **(PH2)** and shoot diameter **(SD2)** were done. Plant height was measured up to the last node carrying vegetative leaves (hypsophylls). **SD1** was measured at 10 cm above ground whereas **SD2** was measured at 130 cm above ground. The parameter **IBN1** was used as proxy for flowering time, it varied from 0 (vegetative phase) to 6 inflorescence branches (shoots carrying more than 15 composite flower heads). Flower head of *Silphium* is represented by female ray florets surrounding the disk of tubular male florets. The trait **RFN1** can be easily counted and corresponds to a maximum number of seeds, which a flower head is able to set.

2.2.5 DNA extraction, tGBS analysis and SNP calling

DNA was isolated from young leaves of cup plants using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Fifty-five DNA samples (5 accessions x 11 genotypes) were isolated from the plants regrown from the rhizome cuttings. The additional 41 DNA samples were isolated from the cup plant seedlings of different origins (Supplementary Table S1).

The tunable genotyping-by-sequencing (tGBS®) including SNP calling was performed commercially by the company Data2Bio® (IA, USA) for the 96 cup plant genotypes. The number of selective bases was adjusted to 3 to concentrate sequencing reads on fewer sites (Ott et al. 2017). The samples were sequenced using six runs on an Ion Proton Instrument, generating 569.8 M raw reads (Li and Chou 2004). Clustering and multiple sequence alignments building were performed first for each sample and then across all samples using a versatile open source tool VSEARCH (Rognes et al. 2016) and a modified version of multiple sequence alignment tool POA (Lee et al. 2002). The assembled tGBS reads as a surrogate for a reference genome consisted of 1,197,534 contigs with a total length of 121.7 Mb. Reads were aligned against this reference using the program GSNAP (Wu and Nacu 2010). After SNP calling, the SNP set was filtered according to the following criteria: minimum calling rate ≥ 80% (at least 80% of samples having ≥ 5 reads at a particular locus); allele number = 2 (only biallelic SNPs were considered); minor allele frequency ≥ 3% and ≥ 1 homozygous sample with minor allele. This leads to a reduced dataset of 28,969 SNPs, which are present in at least 76 individuals. A fraction of the overall missing data points consisted 12.4%. This SNP set was stored in a variant call format (VCF), and the program VCFtools, as well as custom Python scripts, were used for further filtering and analysing the data (Danecek et al. 2011).

2.2.6 Analysis of genetic diversity and population structure

Observed heterozygosities (H_O) for each SNP were estimated by counting the proportion of observed heterozygous genotypes in a particular population. Expected heterozygosities (H_E) for each SNP were calculated with the assumption of a Hardy-Weinberg Equilibrium. H_E and H_O were averaged across all loci. Inbreeding coefficients were calculated according to the equation: $F = (H_E – H_O) /H_E$.

Nucleotide diversities (π) representing average numbers of nucleotide differences per site among individuals of each accession were calculated over the extracted and concatenated SNP dataset using the R packages “ape” and “pegas” (Paradis et al. 2004; Paradis 2010). Population fixation statistics (F_{ST}) were calculated with VCFtools according to formula described by Weir (1984). Estimations between accessions or between each single accession and the total population were performed using VCFtools commands embedded into custom Python script.

2.2.7 Principal Component Analysis and construction of phylogenetic tree

Principal Component Analysis (PCA) was performed for the set of 96 cup plant genotypes and total dataset of 28,969 SNPs using R package *SNPRelate* (Zheng et al. 2012). Results were visualized using the matplotlib graphic package (Hunter 2007). To reduce complexity, phylogenetic relationships were evaluated only for the five accessions of primary interest, represented by equal number of 11 individuals, using the dataset of 26,536 SNP sites. SVDQuartets (Singular Value Decomposition Scores for Species Quartets) appropriate for concatenated SNP datasets (Chifman and Kubatko 2014) was used to infer relationships among quartets of taxa under the coalescent model. This program was implemented in the test version of PAUP* (Phylogenetic Analysis Using Parsimony 2002). All possible quartets for each site in the alignment were estimated and combined to the species tree with the Quartet FM method (Reaz et al. 2014) implemented in PAUP*. The species level phylogeny was estimated with all possible quartets, and 100 bootstrap replicates were performed to access the reliability of the tree topology. The resulting bootstrap consensus tree was visualized and edited with the software FigTree (Rambaut 2012).

2.2.8 Statistical analysis and analysis of phenotype-genotype associations

All phenotypical analyses were made using the software R (R 2018). Two statistical models were applied for evaluation of the phenotypic data measured in Dornburg. To calculate the adjusted entry means (AEM) of DMY, ASH, ADF and ADL, the following model was fitted:

$$\mathrm{Y_{ijr}} = \mu + \mathrm{A_i} + \mathrm{Y_j} + \mathrm{R_r} + (\mathrm{A.Y})_{\mathrm{ij}} + \mathrm{e_{ijr}} \ (1),$$

where $\mathrm{Y_{ijr}}$ was the phenotypic observation of the $\mathrm{i^{th}}$accession of the $\mathrm{r^{th}}$replication in the $\mathrm{j^{th}}$year, μ was the general mean, $\mathrm{A_i}$ the effect of the $\mathrm{i^{th}}$accession, $\mathrm{Y_j}$ the effect of the $\mathrm{j^{th}}$year, $\mathrm{R_r}$ the effect of the $\mathrm{r^{th}}$replication, $(\mathrm{A.Y})_{\mathrm{ij}}$ was the interaction between the $\mathrm{i^{th}}$accession and the $\mathrm{j^{th}}$year, and $\mathrm{e_{ijr}}$ the residual. The accession effect $\mathrm{A_i}$ was of primary interest in this analysis and was, thus, considered as a fixed effect. All other effects were taken as fixed, as the number of levels of each effect was low.

For SMY and MHY, which only had measurements for three years but no replications in the separate years, the following model was used:

$$\mathrm{Y_{ij}} = \mu + \mathrm{A_i} + \mathrm{Y_j} + \mathrm{e_{ij}} \ (2).$$

Analysis of the morphological data measured in Rheinbach was performed as follows. To calculate the AEM at an accession level, the genotypes were considered as replicate for the accessions. Therefore, the arithmetic means over each plant and shoot were calculated for each genotype and for the following traits SN1, PH1, IN1, SD1, PH2, SD2, IBN1, and RFN1 the following model was fitted:

$$\mathrm{Y_{ig}} = \mu + \mathrm{A_i} + \mathrm{e_{ig}} \ (3),$$

where Y_{ig} was the phenotypic observation of the g^{th}genotype nested in the i^{th}accession. μ was the general mean, A_i the effect of the i^{th}accession and $e_{ig}$the residual. The accession effect A_i was of primary interest in this analysis and was, thus, considered as a fixed effect. The residuals were normal distributed, and the plot of the residuals against fitted values showed no violation of the assumption of constant variance. An analysis of variance (ANOVA) was performed for each trait based on the corresponding model to assess the significance of the effects and a Tukey test for multiple comparisons was performed to represent the significant difference between accessions. As there were no randomized replicate at the plant level, no AEM were calculated at the genotype level. Therefore, the arithmetic means at the genotype level were used to perform the association mapping. Phenotype-genotype associations were calculated using mixed linear model approach adapted for genome-wide association studies implemented in TASSEL v. 5.0 (Bradbury et al. 2007; Zhang et al. 2010b). Input file included averaged phenotypic data for 55 genotypes and the genotypic data for 26,536 SNPs. Matrix of five main principal components was generated to account for population structure. To correct for kinship structure a kinship matrix was calculated based on genotype data using the default parameters. Permutation test with 1000 repetitions was done to determine significance of associations.

2.3 Results

2.3.1 Estimation of biomass yield, methane yield and compositional parameters

Plant performance and biomass characterization were evaluated starting in the second year of growth over a three-year period (2014–2016) in Dornburg (Thuringia, Germany). The variation in average dry matter yield (DMY) were found to be significant, with the highest value of 17.2 t ha^{-1} observed for the accession “Northern Europe” and the lowest of 13.6 t ha^{-1} detected for the accession “Ukraine” (Table A 2, Supplementary Table S2).

Contents of acid detergent lignin (ADL), acid detergent fibre (ADF), representing a cumulative measurement of lignin and cellulose, and ash might have an impact on anaerobic digestion. The measured ADL and ADF contents of the cup plant accessions varied around 6% and 40%, respectively, with the highest values detected for the accession “Russia” (6.6%, and 42.8%) and the lowest for “Ukraine” (5.8% and 38.7%). There were minor differences among the accessions in their ash content that is in accordance with the previous finding that the main contributing factor to the variation in this trait was the field location (Biertümpfel and Conrad 2013). Specific methane yield (SMY) varied from 259 Nl [kg oDM]$^{-1}$ for the accession “Russia” to 273 Nl [kg oDM]$^{-1}$ for the accession “Ukraine”. To compare the methane yields on a hectare basis, MHY were calculated. Highest MHY was achieved for the accession “Northern Europe” with the remaining accessions ranging as follows: “East Germany”, “Russia”, “USA” and “Ukraine” (Table A 2).

Field trial established in 2016 in Rheinbach (NRW, Germany) was mostly used for phenotypic and genetic screen of five accessions. The plant material was intended to be used as renewable raw material for building industry, which demands maximally dried biomass with a low proportion of leaf material. Harvest of these plants took place in December 2017; therefore, the measured biomass yields were not directly comparable with the data gained at the end of summer time in Dornburg. Ranking of the accessions was as follows: “Russia” (14.28 t ha^{-1}), “USA” (9.57 t ha^{-1}), “East Germany” (8.94 t ha^{-1}), “Ukraine” (8.92 t ha^{-1}) and “Northern Europe” (8.36 t ha^{-1}).

Table A 2: Adjusted entry means for five accessions and general means for six biomass traits over three years (2014-2016). The letters represent significance groups (α=0.05) calculated the trial-based significant difference for Tukey multiple comparison (Honestly significant difference: HSD).

Accession	**Trait**[a]					
	DMY[a] **[t ha^{-1}]**	**ADL [%-DM]**	**ADF [%-DM]**	**Ash [%-DM]**	**SMY [Nl [kg oDM]$^{-1}$]**	**MHY [m^3 ha^{-1}]**
“USA”	15.03 ab	6.48 a	41.28 bc	9.36 a	267.6 a	4026.67 a
“East Germany”	15.62 ab	5.95 a	41.23 bc	9.29 a	262.8 a	4123.33 a
“Russia”	15.82 ab	6.56 a	42.75 c	9.19 a	258.4 a	4094.00 a
“Northern Europe”	17.21 b	6.25 a	40.84 b	9.40 a	268.8 a	4634.33 a
“Ukraine”	13.59 a	5.81 a	38.73 a	8.86 a	273.0 a	3696.67 a
General Mean	15.46	6.21	40.96	9.22	266.1	4115.00
HSD	*2.33*	*0.76*	*1.71*	*0.76*	*28.22*	*1134.11*

[a] DMY, dry matter yield; ADL, acid detergent lignin; ADF, acid detergent fibre; SMY, specific methane yield; MHY, methane hectare yield

2.3.2 Phenotypic characteristics of the accessions

Field trial in Dornburg, as well as greenhouse observations, have shown that there are phenotypic differences between the analyzed accessions. All seedlings and young plants of the accession “Ukraine” revealed purple coloration of leaves and stems, most likely because of their higher anthocyanin content. Other four accessions possess intra-accession heterogeneity in this phenotype with some plants being weak purple and others completely green. Two accessions (“Ukraine” and “Northern Europe”) showed almost no leaf and stem hairiness whereas other accessions were heterogeneous again. Fig. A 1 exhibits a representative young plant of the accession “Ukraine” and a selected plant of the accession “Russia” demonstrating the phenotype opposite to the “Ukraine”. Based on the described observations, variability among accessions in other phenotypic traits could be expected. In the current study we focused on the morphological traits related to biomass yield (PH, SN, SD, IN and IBN) and seed production (RFN).

Fig. A 1: Variations in young plant phenotype. Selected plants of two accessions are shown.

Phenotypic traits were measured in the second year of cultivation to exclude a possible effect of rhizome size on growth parameters in the year of planting. For five of six traits measured in July 2017 (PH1, SD1, IN1, IBN1 and RFN1) significant differences between accessions were detected (Table A 3 and Supplementary Table S3). Interestingly, at the time of measurements all the “USA” plants flowered already (AEM for IBN1 = 2.88) whereas many of the “East Germany” plants still stayed in the vegetative phase (AEM for IBN1 = 1.22). As far as the latter plants were still actively growing, the measurements of plant height and shoot diameters were repeated at the end of the season (PH2 and SD2). Indeed, a highest increment in PH (20.6 cm) was detected for the late flowering “East Germany” plants. In general, evaluated parameters showed high reproducibility and repeatedly measurements were highly correlated (Table A 4). The accession “Russia” revealed the tallest plants with the largest shoot diameters, IN1 and RFN1 whereas the accession “Ukraine” demonstrated the smallest plants with the lowest shoot diameters and RFN1 (Table A 3). Interestingly, the accession “Northern Europe” being most productive in Dornburg showed relatively low PH and SD but the highest SN in Rheinbach.

While genetically identical plants demonstrated a high homogeneity in their phenotypes, a high phenotypic variability was observed among different genotypes inside of accessions. Unfortunately, the applied experimental design and the absence of randomized replicates at the genotype level did not allow us the statistical comparison between the genotype and the accession effects.

Table A 3: Adjusted entry means of the morphological traits for the five accessions. The letters represent significance groups (α=0.05) calculated the trial-based significant difference for Tukey multiple comparison (Honestly significant difference: HSD).

Accession	**Trait**[a]							
	PH1[a] **[cm]**	**SD1 [mm]**	**SN1**	**IN1**	**IBN1**	**RFN1**	**PH2 [cm]**	**SD2 [mm]**
"USA"	213.31 ab	19.56 ab	11.28 a	9.20 a	2.88 c	25.12 bc	218.36 a	12.79 bc
"East Germany"	200.80 a	18.65 ab	9.86 a	10.46 b	1.22 a	21.77 ab	221.39 a	11.26 ab
"Russia"	231.44 b	20.70 b	9.92 a	10.84 b	1.58 ab	27.06 c	242.96 b	13.67 c
"Northern Europe"	199.89 a	17.89 a	12.31 a	9.90 ab	2.42 bc	19.88 a	208.00 a	10.44 a
"Ukraine"	197.75 a	17.87 a	11.47 a	9.90 ab	1.71 ab	18.57 a	203.68 a	11.74 abc
General Mean	208.64	18.93	10.97	10.06	1.96	22.48	218.87	11.98
HSD	*21.02*	*2.44*	*3.54*	*1.03*	*0.97*	*4.26*	*18.81*	*2.25*

[a] PH, plant height measured on July 11, 2017; SD1, shoot diameter at 10 cm above ground; SN1, shoot number per plant; IN1, internode number per shoot; IBN1, inflorescence branch number; RFN1, number of ray flowers per flower head; PH2, plant height measured on October 19, 2017; SD2, shoot diameter at 130 cm above ground

Table A 4: Correlation coefficients between morphological traits.

Trait[a]	**PH1**	**SD1**	**SN1**	**IN1**	**IBN1**	**RFN1**	**SD2**	**PH2**
PH1	1							
SD1	0.67**[b]	1						
SN1	ns	ns	1					
IN1	0.52**	0.37**	ns	1				
IBN1	ns	ns	ns	-0.54**	1			
RFN1	0.47**	0.42**	ns	ns	ns	1		
SD2	0.64**	0.71**	ns	0.34*	ns	0.35*	1	
PH2	0.81**	0.56**	ns	0.63**	-0.27*	0.48**	0.69**	1

[a] PH, plant height measured on July 11, 2017; SD1, shoot diameter at 10 cm above ground; SN1, shoot number per plant; IN1, internode number per shoot; IBN1, inflorescence branch number; RFN1, number of ray flowers per flower head; PH2, plant height measured on October 19, 2017; SD2, shoot diameter at 130 cm above ground

[b] (*) significant at the 0.05 level, (**) significant at the 0.01 level

2.3.3 Genetic diversity, population structure and phylogenetic inference based on tunable genotyping-by-sequencing data

To explore genetic diversity and population structure of the cultivated *S. perfoliatum* accessions*,* sequencing data were generated for 55 mother plants from Dornburg (11 plants per accession) as well as for 41 other random cup plant genotypes (Supplementary Table S1) using the tGBS® approach (Ott et al. 2017). In the absence of a reference genome, the assembly of reads was done *de novo* (Data2Bio®, USA). The total length of the aligned reads was 121.7 Mb, which corresponds to approximately 1.5% of the 8 Gb cup plant genome. The SNP calling conducted under strict conditions resulted in 28,969 high-quality biallelic polymorphisms, which are present in at least 80% of all genotyped individuals. When the subset of the 55 individuals was considered then the number of SNPs consisted of 26,536 loci.

These polymorphisms were used to infer genetic variances among cup plant individuals and to evaluate a population structure. The conducted PCA allowed a graphical visualization of genetic distances between the individuals. The first three principal components captured 8.64, 7.29 and 5.95% of the measured genetic variances, respectively (Fig. A 2, Supplementary Fig. S1).

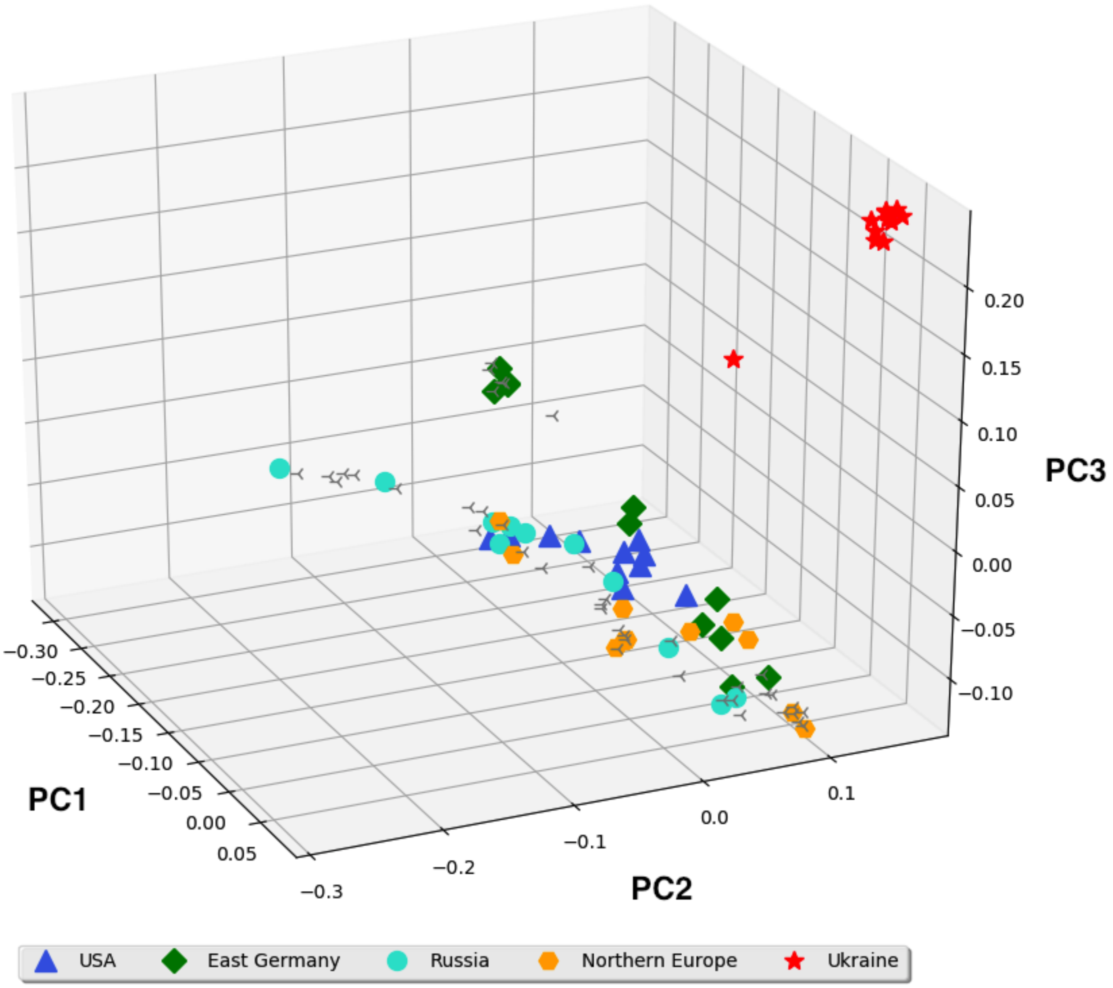

Fig. A 2: Principal component analysis (PCA) of 96 individual cup plants. Legend shows color symbols for plants belonging to five TLL accessions, the rest plants are designated as grey upsilons. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

The only accession forming separated clade was “Ukraine” with ten of the eleven analyzed individuals to be very closely genetically related (Fig. A 3, exception individual 6_2). Also, four individuals of the accession “East Germany” (3_1, 3_2, 3_4 and 3_5) formed a small separated clade relatively distant to other individuals of this accession, thus demonstrating that this accession is not homogeneous and consists of several subgroups. The accession “Russia” revealed the most dispersed distribution of its members. In general, it was not possible to resolve all but one accession into distinct clades suggesting that they belong to a common gene pool and share common alleles.

Table A 5: Nucleotide diversity (π), expected and observed heterozygosity (H), inbreeding coefficient (F) and Weir and Cockerham weighted F_{ST} in relation to the total population.

Accession	Plant No.	π	H_E	H_O	F	F_{st}
„USA“	11	0.0389	0.183	0.138	0.247	0.048
„East Germany“	11	0.0229	0.196	0.162	0.173	0.070
„Russia“	11	0.0390	0.197	0.167	0.151	0.050
„Northern Europe“	11	0.0371	0.186	0.144	0.222	0.052
„Ukraine“	11	0.0052	0.106	0.098	0.078	0.219
Average	**11**	**0.0286**	**0.174**	**0.142**	**0.174**	**0.087**

To infer phylogenetic relationships and ancestry of *Silphium* plants belonging to the five accessions a species tree was computed (Fig. A 3). It should be noted that in some cases bootstrap values of the branches could not provide high confidence about relationships between the clades or individuals. Except for two individuals of the accession “USA” (1_1 and 1_11) located in the beginning of the phylogeny, all other plants formed a single node phylogenetic tree suggesting a possible common ancestry of the analyzed individuals. All individuals of the accession “Ukraine” were clustered together, whereas the individuals of the other accessions were found in two (“East Germany”) or even more clusters (all others). The four East German individuals forming a separate clade in the PCA were placed together again with the highest bootstrap values. The phylogenetic tree confirms that these individuals have common ancestry with two other individuals of this accession (3_3 and 3_6). In turn, these six plants of the accession “East Germany” are related to three individuals of the accession “Northern Europe” and one individual of the accession “Russia”. Moreover, the obtained data suggest a recent ancestry among the rest individuals of the accession “East Germany” with the accession “Russia” as well as close relatedness of several individuals of the accession “Northern Europe” to one individual of the accession “USA”.

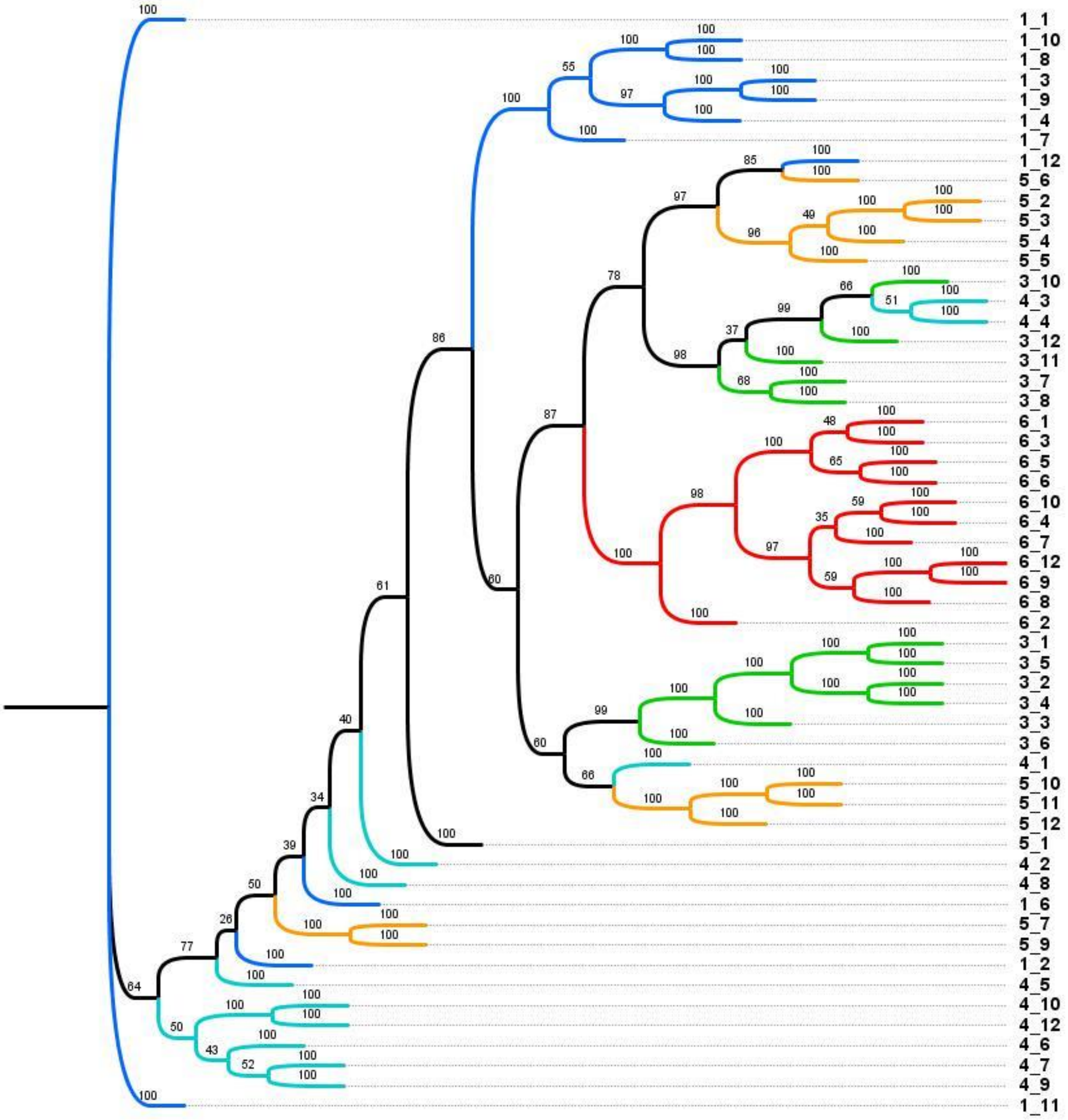

Fig. A 3: Unrooted coalescence tree of the 55 individuals of the five TLL accessions : 1 – “USA”, 3 – “East Germany”, 4 – “Russia”, 5 – “North Europe” and 6 – “Ukraine”. The node order is increasing and the branch length is proportional to the estimated values. The same color code is used as in Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Heterozygosity levels of 9.8–16.7% observed among accessions were lower than expected based on Hardy–Weinberg Equilibrium (Table A 5). This suggests that inbreeding has occurred within all of the accessions, with the accessions “USA” and “Northern Europe” showing relatively high inbreeding coefficients (F) and the accession “Ukraine” the lowest one. Nucleotide diversity (π) revealed similarly high values (0.038–0.039) for the accessions “USA”, “Russia” and “Northern Europe” and the lowest value of 0.005 for “Ukraine”. The low H_O and π values within the latter accession suggest a smaller number of polymorphic loci and increased homozygosity compared to the other accessions.

A remarkably low F detected for this accession seems to be in contradiction with these data. To clarify that, H_O and F were evaluated for each single plant of the Ukrainian as well as other accessions (data not shown). Ten of the eleven Ukrainian individuals were highly homozygous whereas one plant (6_2) revealed an increased H_O and a negative F (outbreeding), thus influencing the averaged values. Interestingly, this plant was located outside of the dense clade of individuals of the accession "Ukraine" as visualized by PCA and phylogeny (Fig. A 2 and Fig. A 3). Analysis of the individuals from other accessions showed that all accessions except "USA" were not homogeneous for H_O and F values and revealed outbreeding for some members.

Fixation index F_{ST} describes the amount of genetic differentiation due to variances in allele frequencies within one subpopulation either compared to the total population or for pairwise comparisons with other subpopulations. This parameter can vary from zero (no genetic divergence, unstructured population) to one (fixation of alternative alleles in different subpopulations). When the accessions were compared with the total population the estimated F_{ST} values varied from 0.048 for "USA" to 0.219 for "Ukraine" with an average value of 0.087 (Table A 5). This suggests that only 8.7% of total genetic diversity is accounted for by genetic differences among accessions. In pairwise comparisons the lowest value of 0.044 was shown between "Russia" and "USA" and largest value of 0.291 between "Ukraine" and "USA" (Table A 6).

Table A 6: Pairwise Weir and Cockerham weighted F_{ST} estimated between populations.

Accession	**"USA"**	**"East Germany"**	**"Russia"**	**„Northern Europe"**	**„Ukraine"**
„USA"	0				
„East Germany"	0.091	0			
„Russia"	0.044	0.080	0		
„Northern Europe"	0.062	0.086	0.066	0	
„Ukraine"	0.291	0.280	0.288	0.290	0

2.3.4 Phenotype-genotype association study

To prove whether the collected data set could be useful for discovery of significant phenotype-genotype associations, a genome-wide association study (GWAS) was performed under assumption that the identified SNPs are randomly distributed over the genome of *Silphium*. Due to the small population size (55 genotypes), only two adjacent SNPs located on the same *Silphium* sequence (Silphium_1043395) and associated with SD1 reached the significance threshold calculated based on the permutation test (8.5×10^{-7}). Supplementary-Table S4 reports 38 most significant associations ($p \leq 1 \times 10^{-4}$) detected for eight morphological traits. The BLAST alignment of 32 *Silphium* sequences harboring these 38 SNPs against the NCBI nucleotide collection identified no significant hits, whereas BLAST alignment against *Helianthus annuus* genome (sunflowergenome.org, HA412HO bronze assembly) revealed five sequences with significant hits (E-value < 10^{-30}, supplementary Table S4). Four of those have hits on several sunflower chromosomes and one (Silphium_122074 associated with SD2) shows homology to the gene Ha5_00005340 coding for tesmin/TSO1-like protein containing CXC domain.

2.4 Discussion

2.4.1 Biomass and methane yield of cup plant in comparison to maize

As reported in the literature, the biomass yield of cup plant in favorable environment can exceed 20 t ha^{-1} (Haag et al. 2015; Assefa et al. 2015a; Biertümpfel and Conrad 2013). In our study, the maximum average biomass yield of 17.2 t ha^{-1} was detected in one of the analyzed accessions, “Northern Europe”, whereas other accessions revealed a lower DMY (Table A 1). Despite of the biomass yield comparable with maize, *Silphium* is usually inferior to maize for the total biogas yield and MHY because of a lower SMY production. Indeed, the measured SMY of 259–273 Nl [kg oDM]$^{-1}$ is similar to that published in the literature (228–290 Nl [kg oDM]$^{-1}$) (Mast et al. 2014; Haag et al. 2015; Franzaring et al. 2015) and lower than SMY of maize (330-365 Nl [kg oDM]$^{-1}$) (Mast et al. 2014; Schittenhelm 2008; Grieder et al. 2012).
A reduced methane production is known to be associated with an increased lignin content of the biomass since lignin blocks access of the enzymes to cellulose (Jablonowski et al. 2017; van der Weijde et al. 2017). Negative correlations between SMY, ADL and ADF have been previously reported (Haag et al. 2015; Grieder et al. 2012; van der Weijde et al. 2017) and could also be observed in our data ($r = -0.32$ and -0.6 for ADL and ADF, respectively). Interestingly, a recent study of Damm et al. (2017a) reports a similar lignin content for maize and *Silphium* but a different lignin composition. Certainly, other cell wall key components such as cellulosic and non-cellulosic polysaccharides, their ratios and cross-linking also play an important role for the total biogas yield.

2.4.2 Genetic variation in biomass yield related traits and breeding targets for crop improvement

The presence of substantial variations in the biomass and methane yield among the accessions evidences the feasibility to improve these traits by selection and breeding. However, the observed genetic heterogeneity of the accessions, which will be discussed below, stresses the necessity of additional survey of these parameters in genetically homogeneous populations or vegetatively propagated single genotypes.

Several studies have already reported genetic variations in biomass yield and biomass-related morphological traits in cup plant (Haag et al. 2015; Franzaring et al. 2014; Assefa et al. 2015a; Albrecht and Goldstein 1997; Vetter et al. 2010). In the study of Haag *et al*. (2015) seven cup plant varieties from the TLL collection were tested for their methane formation potential whereby four of them were the same as in the current study (“Northern Europe” = *Horn*, “Russia” = *RU*, “USA” = *USA* and “Ukraine” = *U rot*). Even though only a slight variation between the chemical compositions of the varieties was observed, the highest SMY coupled with the lowest ADL and ADF was reported for the variety *U rot*, that is in agreement with our data.

In comparison to other accessions, “Ukraine” displays a more homogeneous phenotype with consistent purple coloration of young leaves and stems, and absence of leaf hairiness. Even if an exact ancestry of this accession is unknown, it can be suggested that this accession has undergone the breeding process and, most likely, has been selected for forage quality, for which low fiber content (ADF) and loss of leaf hairiness might be important. There are active breeding programs in Ukraine to enhance nutritional value of *Silphium* species as a forage crop for livestock. Since 2009 registration of three new cultivars for *S. perfoliatum* and two new cultivars for the related species *Silphium integrifolium* for forage purposes has been reported (YIECP 2018).

Differences in ranking of the accessions based on their biomass yield were observed in Dornburg and Rheinbach although this comparison should be done with caution because of the different harvest time and only one-year data from Rheinbach. Besides, the field in Rheinbach was less diverse since it had been planted with a subset of 60 vegetatively propagated mother plants from Dornburg. The observed differences in the biomass yield between locations could be a result of phenotypic plasticity of the accessions and a better adaptation to different climatic conditions. A high effect of location has been reported also in other studies (Assefa et al. 2015a; Biertümpfel and Conrad 2013; Vetter et al. 2010). Thus, Assefa et al. (2015a) observed 50% more biomass production in Arlington than in Brookings and an inconsistency in rank of families between the two locations.

Phenotyping cup plant for biomass and methane yield is costly and time consuming. Furthermore, direct selection based on these parameters might be ineffective due to high level of genotype x environment interactions. More heritable and easy to measure morphological traits are needed for effective breeding. The study of thirty-three halb-sib cup plants families derived from natural populations from Minnesota and Illinois (Assefa et al. 2015a) revealed significant positive correlations between biomass yield and four morphological traits: mass shoot^{-1}, plant height, crown area and internodes shoot^{-1}. The heritability estimates for these biomass-related morphological traits ranged from 0.52 to 0.72, suggesting that the biomass yield could be improved by selection for yield per se as well as by indirect selection for any of the correlated traits. Whereas only low to moderate variations in biomass and methane yield parameters were observed between the accessions in the current study, the differences in the related morphological traits were highly significant (Table A 3). Comparison of the DMY in Rheinbach with morphological data revealed the best correlations for PH1 (average r = 0.91) and SD (average r = 0.87), suggesting these two secondary traits as useful breeding targets for cup plant.

Flowering time has a crucial influence on plant development and, in crop species, responses to day length have been extensively manipulated, creating varieties that can grow, flower and set seeds at latitudes outside of the range occupied by the wild progenitor (Dimitrijevic and Horn 2017; Brambilla et al. 2017). Long vegetative growth duration and delayed flowering increase the biomass yield and resilience in C_4 grasses (Mullet 2017; Warnasooriya and Brutnell 2014). Recently, importance of flowering time for breeding high-biomass yielding cup plants has been also discussed (Assefa et al. 2015a). Significant differences in IBN1, the parameter related to flowering time, detected among the five cup plant accessions open the possibility to modify this trait for better adaptation to distinct environments and biomass yield improvement.

Up till now, establishment of cup plant in the field was mainly achieved by labour- and cost-intensive planting of seedlings (Gansberger et al. 2015; Biertümpfel and Conrad 2013). However, development of new sowing techniques, usage of pretreated and pelleted seeds demonstrate that direct seeding is conceivable (Gehren et al. 2016; Schäfer et al. 2017). To further improve production and commercialization of cup plant the genetic variation for seed quality, achene traits (Assefa et al. 2015b) as well as in seed set ability (RFN1) detected in our study may be useful.

2.4.3 Possible monophyletic origin and a common gene pool of the accessions

Heterozygosity is a measure of genetic variation within a population; thus, heterozygosity values can give insights into the degree of genetic uniformity and diversity. The average observed heterozygosity (H_O) levels among the analyzed *S. perfoliatum* accessions (0.142) is higher than H_O levels in cultivated sunflower crops (0.076) but lower compared to the H_O in wild sunflower individuals (0.286) (Mandel et al. 2011). This moderate heterozygosity level may represent a combined result of a population bottleneck that occurred during the plant introduction to Europe, specifics of breeding activities and geographical isolation of the analyzed *S. perfoliatum* populations. The H_O levels among accessions differ with the lowest level of 9.8% observed in the accession “Ukraine” and the highest 16.7% in the accession “Russia” (Table A 5). Furthermore, it has been found that the H_O levels vary significantly among individuals within most of the accessions. This heterogeneity of the accessions may result in a skewed estimation of population genetic parameters that stresses an importance to analyze more plants per accession in order to obtain unbiased results. Effect of sample size on a number of population genetic parameters were discussed elsewhere (Subramanian 2016).

Only 8.7% (average F_{ST} = 0.087) of the total genetic variation found in the SNP dataset of the European *S. perfoliatum* accessions is attributed to genetic differences among them (Table A 5). However, F_{ST} values differ among subpopulations and the average values cannot explain the whole magnitude of genetic variation. Whereas little differentiation compared to the total population was detected for the accessions “Russia”, “USA”, “East Germany” and “Northern Europe” (5–7%), almost 22% of genetic variation could be accounted for by differences with the “Ukraine” which demonstrated even higher F_{ST} values in the pairwise estimations (Table A 5). These evidences for population stratification together with the results of the PCA and the phylogenetic inference are consistent with the differing morphological, compositional and biomass yield parameters of the Ukrainian accession and support the idea that the accession “Ukraine” must be a result of the breeding programs in Ukraine, which have been mentioned above.

Even though the pairwise genetic differences of 4–9% (Table A 5) were detected between the remaining four accessions, the PCA and phylogenetic analysis could not discriminate them properly. In several cases, very close genetic distances were detected for the plants belonging to different accessions placing them in the same clade. Thus, different genetic origin of the seeds does not automatically mean their genetic separation suggesting either a common ancestry of the cultivated accessions or recent exchange of seeds between the origins. Limited value of the geographical origin along to the genetic structure of cultivated outcrossing plants for which the exchange of genetic material is not inhibited has also been shown in other studies (Otto et al. 2017).

The phylogenetic inference presented in Fig. A 3 suggests a monophyletic origin of all but two analyzed plants. One could speculate about recent common ancestor of the cup plants cultivated in Europe, however, the presence of nine individuals obtained from the seed company in the USA within the same node of phylogeny complicates this simple picture. Nevertheless, the single node phylogenetic tree might indicate a bottleneck of genetic diversity which had happened already at this very early stage of cup plant domestication and breeding. To investigate this, we have recently collected seeds from the wild populations throughout the range of *S. perfoliatum* distribution area in the USA (https://www.biosc.de/spread). Survey of this new collection should help us to estimate genetic variation at the level of a whole species and to find out an origin of the European cup plant. We are hoping to detect phenotypically and genetically diverse plants and use them for *de novo* domestication of *S. perfoliatum* (van Tassel et al. 2017), to ensure a broad base of genetic diversity for future breeding.

2.4.4 Opportunities and challenges in analysis of large genomes of non-model species

The conducted research has shown that tGBS® is a powerful tool for the analysis of non-model organisms with a large genome such as *S. perfoliatum* and represents one of the best approaches for such studies. It allowed the discovery of 28,969 high-quality biallelic polymorphisms, which were useful for phylogenetic inference, genetic diversity and population structure estimations. However, despite the obvious advantages of the deep sequencing tGBS® method, there are still a lot of challenges.

S. perfoliatum possesses a complex genome with many repetitive and paralogous sequences, as it has been shown for other members of the Asteraceae (Badouin et al. 2017; Reyes-Chin-Wo et al. 2017). Paralogous and repetitive sequences, which harbour recognition sites for restriction enzymes used during the tGBS®-procedure, may lead to an extensive sequencing of those regions. If these sequences contain SNPs, insertion or deletion, false assembly of contiguous sequences is often unavoidable. Furthermore, the subsequent variant calling may result in homozygous alleles to be miscalled as heterozygous or *vice versa* as well as in other ambiguities. Short length of the reads produced by the applied tGBS® technology limits the capability to resolve complex regions with repetitive or heterozygous sequences. However due to the deep coverage of isolated genome regions, stringent mapping and the downstream filtering procedure, an accurate variant calling with little false positive SNPs can get achieved.

For resolving complex genomes, further sequencing and genome assemblies should be carried out implying new sequencing strategies in long-read sequencing together with new bioinformatic solutions (Li et al. 2017). The sequencing and assembly of the recently published and high complex sunflower genome is a good example for the power of third generation sequencing technologies (Badouin et al. 2017; Renaut 2017). Nevertheless, significant efforts and high costs were necessary to construct a final reference genome assembly of the sunflower, as it mainly consists of long and highly similar repeats. The 8 Gb genome of *S. perfoliatum* is more than twice as large than the 3.6 Gb genome of *H. annuus* that will make assembly even more challenging.

The knowledge of chromosomal positions and the availability of high-quality genetic map are prerequisites for detection of genes or genetic regions influencing important morphological and physiological traits and/or biomass yield components. Due to the lack of a reference genome for *S. perfoliatum* we were not able to detect the position of the tGBS loci in the genome. Bearing in mind this limitation and assuming a random genomic distribution of the discovered polymorphic loci, we performed GWAS between the SNPs and the measured phenotypic traits. The identification of several significant associations demonstrates that the application of GWAS is feasible even in the absence of a genetic map. However, further analysis of the detected associations is hampered by the fact that the sequences harboring SNPs are very short and the probability that they are located within genes is very low. In our case only one SNP putatively associated with shoot diameter could be located within the gene coding for tesmin/TSO1-like protein. The TSO1 mutant of *Arabidopsis taliana* exhibits normal vegetative development but aberrant ovules and flowers (Hauser 1998). Participation of TSO1 protein in cytokinesis and cell expansion processes (Hauser 2000) allows a speculation on importance of these pathways in shoot diameter regulation. Definitely, all of the detected associations should be considered with caution because of a small population size in our study. Multiple experiments using large populations are necessary to provide robust and broadly relevant associations.

2.5 Conclusion

The demand for alternative bioenergy crops in Germany is increasing since greening measures and political aims envisage the establishment of more diverse agricultural landscapes with a broader range of crop species (BMELV 2012). Cup plant is a promising alternative to annual energy crops as it comes with the advantage of ecosystem services. Published studies along with our data demonstrate that this plant, being purely adapted and hardly bred in the past, is able to produce biomass yield comparable to that of maize, the predominant energy crop for biogas production in Germany. Even though cup plant is still inferior to maize regarding the biogas yield, the observed genetic variation indicates the possibility of cup plant improvement through selection and breeding.

The conducted phylogenetic and population structure analysis revealed a common origin of the analysed plants and implied a possible bottleneck of genetic diversity within the gene pool of the cultivated cup plant populations. To maintain a broad base of genetic diversity for *de novo* domestication and future breeding we are currently aiming a survey of genetically diverse germplasm from a wide geographic origin. Integration of new approaches in genotyping and sequencing technologies with new breeding strategies informed by multiple disciplines give us a unique opportunity to create a new high-yielding herbaceous perennial crop delivering many specific ecosystem services (van Tassel et al. 2017). An additional support for this long-term goal provides the attempts to improve the crop profitability by its usage as feedstock for chemical or pharma industry (Lunze et al. 2021), or as renewable raw material for production of paper and insulating materials (Höller 2017).

3 Pulp and paper

This work has been published as:

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The manuscript has not been modified from the published version.

3.1 Introduction

With increasing global wealth, paper demand will almost double by 2050 (Lamberg et al. 2012). Trees are the main paper and pulp feedstock in the world. They are a valuable resource for many industries; moreover, trees are replaced by arable land for crop production. In the future, forests will be needed to reduce global net CO_2 emissions due to their high potential for carbon sequestration (Hawes 2018). Beside wood fibres, fibres from non-wood resources are becoming interesting alternatives for paper production (Kamoga et al. 2013). Wood pulp is a premium fibre with high strength at a high cost; alternative fibres become interesting for low-cost production and new paper products with lower brightness, for example in packaging, which is the fastest growing paper sector (FAO 2015).

To tackle the global paper demand and to reduce the industry's CO_2 consumption, new sustainable fibrous plants are needed. However, no new relevant plants for the paper production have been introduced into the industry since 1802, when straw was used by M. Koops (Franklin Inst. 1835) with a global market share of about 44% (Liu et al. 2018). Perennial biomass plants have a similar lignocellulosic structure compared to trees (Saijonkari-Pahkala 2001), thereby they could become additional paper resources; their low nutrient requirements and high yields are good prerequisites (Cossel et al. 2019; Wever et al. 2019; Cumplido-Marin et al. 2020).

Liu et al. (2018) indicate that some major problems of the paper industry include the shortage of resources, and the level of technical equipment required. This study aimed to find additional non-wood perennials for the paper industry to diversify the paper resources and work with simple technical grinding equipment. Three plant species were tested, that are already cultivated for bioenergy production (Cossel et al. 2020; Tsapekos et al. 2019; Šiaudinis et al. 2015; Siwek et al. 2019), as proportional substitutes of wood fibres: cup plant (*Silphium perfoliatum* L.), Virginia mallow (*Sida hermaphrodita* (L.) Rusby), and meadow hay (mainly *Lolium perenne* L. and *Alopecurus pratensis* L.). It is possible to grow all three perennial species on marginal soils so that there are no issues in the food or fuel discussion (Wever et al. 2020; Boe et al. 2019b; Nabel et al. 2014; Meehan et al. 2017). In the short-term, meadow grass, or rather meadow hay, could be of high interest due to its considerable CO_2 sequestration of 4.4 megagram hectare^{-1} year^{-1} in soil and roots and its 3.4 bn ha permanent acreage and "billions of tons" of cuttings from non-agricultural areas, river embankments, highway verges, parks or high-nature-value grasslands that are not used properly (Tilman et al. 2006; Suttie et al. 2005). The FAO (2019) assumes that pastures and rangelands could represent a carbon sink that could be greater than forests if adequately managed. Single grass species like tall fescue (*Festuca arundinacea* Schreb.), reed canary grass (*Phalaris arundinacea* L.), switchgrass (*Panicum virgatum* L.), meadow fescue (*Festuca pratensis* Huds.) and different Ugandan grasses have been analyzed as future paper feedstocks, but these usually form only single-species stands and are not available in large-scale (Danielewicz et al. 2015; Finell and Nilsson 2004; Gorouard and Samson 2000; Kamoga et al. 2014; Saijonkari-Pahkala 2001; Thykesson et al. 1998). Paper trials with Virginia mallow have been carried out, but only for handmade paper (Mejouyo et al. 2020) or they are old, and detailed test descriptions are not available (Medvedev 1940). To our knowledge, nobody has previously tested cup plant and meadow grass, or rather meadow hay, that naturally consists of a variety of grass species.

In the long-term, cup plant and Virginia mallow could become a profound alternative to common energy and fibre plants (Wever et al. 2020). They are high yielding perennials with up to 25 t dm ha^{-1} a^{-1}, a superior nutrient use efficiency due to their extensive root system (Schoo et al. 2017; Franzaring et al. 2015), and cup plant offers a wide range of useful chemical substances for the pharmaceutical or agrochemical industry (Lunze et al. 2021). Furthermore, they provide environmental benefits like phytoremediation, pollination, soil health regulation, CO_2 sequestration, habitat connectivity, as well as an increase in biodiversity and minimization of soil erosion (Cumplido-Marin et al. 2020; Schorpp et al. 2016). Moreover, a cascade utilization according to *Miscanthus x giganteus* is conceivable (Kraska et al. 2015). Hammett et al. (2001) indicated that pulping non-wood plants is cheaper than pulping wood. They are low in lignin and, thus, do not require as many chemicals. All these traits ensure a rising scientific interest in the plants, and the area under cultivation of these wild plants increases. Their utilization as paper feedstock e.g., for sustainable packaging could add an additional usage. Thus, farmers could become fibre suppliers, which would decentralize the fibre market and ensure a second mainstay for the farmers worldwide.

This research aims to show the feasibility of all three plants as new paper feedstock. Starting with three different grinding types commonly used in agriculture: a hammer-, cutting- and edge mill, followed by soda-pulping and beating. We substituted 25% and 50% of birch fibre with the three raw materials. Virginia mallow and cup plant reached lower, and the meadow hay blends reached higher paper properties compared to birch (control).

3.2 Material and methods

3.2.1 Plant material

Cup plant and Virginia mallow were grown at the Field Lab *Campus* Klein-Altendorf of the University of Bonn (Rheinbach, Germany) near Bonn using standard farming equipment. Cup plant biomass was harvested (Champion 1200, Kemper) in December 2016, 3 months later than the typical harvest date for energy purposes, since this plant was senescent and the remobilization of nutrients into the rhizome was already completed. The raw material had 46% water content, which was reduced to 15% after drying on a drying trailer, and 16% leaf content. Virginia mallow biomass was harvested nearly leafless after winter (350 MXF, Schliesing) in April 2017 according to Stolarski et al. (2014) with 13% water content and required no additional drying. The chopped raw materials of cup plant and Virginia mallow had a particle length of 2–10 cm and a width of 0.3–2 cm. The meadow hay was harvested in June 2017 on extensive permanent grassland (Much, Germany) near Bonn, dominated by *Lolium perenne* L., *Alopecurus pratensis* L., *Phleum pratense* L., *Poa trivialis* L., and *Dactylis glomerata* L. The meadow hay was cut (GMD 4010, Kuhn) and bailed (582, John Deere), after drying for three days on the field, with a water content of 10%. The meadow hay stalks were approximately 30–40 cm long. Industrial kraft pulped and bleached birch fibre (*Betula verrucosa* ROTH) served as control.

3.2.2 Grinding and sieving of the raw materials

The main process steps are grinding, sieving, soda pulping, and beating. Each of the raw materials was ground either by a hammer mill "HM" (BHS 100, Buschhoff), cutting mill "CM" (SM 300, Retsch), or edge mill "EM" (33-390, KAHL). The primary aim of the grinding was to produce particles with a maximum length of 1–6 mm comparable to long fibre pulps (Björklund et al. 2016), so that the fibres would not be too long for paper making in the case of incomplete pulping. The secondary aim was to increase the available specific surface area of the raw materials to improve the pulping (Kim et al. 2016). The chosen screen size of each mill was 6 mm round hole recommended by Gil and Arauzo (2014). To avoid blockage of the edge mill due to long grass blades, the grass was pre-cut with the hammer mill (15 mm). The three raw materials before and after grinding can be seen in Fig. B 1. After grinding, the particles were sieved with an oscillating sieving machine (Ass-100, S&F). Two sieves of 0.5 mm and 0.75 mm mesh size were used to provide a sieve fraction between 0.5–0.75 mm for the experiment. An illustration of the grinding and pulping process that was used is shown in Fig. B 2.

Fig. B 1: Blades of meadow hay (a) along with shoots of Virginia mallow (b), and cup plant (c) before and after grinding by the hammer mill (screen size 6 mm, round hole) used in this study. With a size scale of 1 cm.

3.2.3 Pulping and paper production

An externally heated beaker with 1 L of volume was used for pulping. For each digestion, 30 g of dry matter and 600 ml of NaOH solution were used, resulting in a solid to liquid ratio of 1:20. NaOH concentration was 10 weight% of the dry weight, in accordance to Marin et al. (2009) who analyzed the pulping conditions of the perennial *Miscanthus giganteus* and produced the highest amount of long and short fibres with 10.5% NaOH. For all experiments, the reaction temperature was kept at 98 ± 2 °C (RCT classic, IKA) for 3 h. The pulp was stirred continuously at 500 rpm (MINISTAR 80, IKA). The experimental conditions were similar to previously published studies for the semichemical pulping of non-wood materials (Marín et al. 2009; Guadalix et al. 1996; Danielewicz and Surma-Ślusarska 2019). After digestion, the pulp was pressed through a 600 µm sieve to remove black liquor and was washed with 2 L deionized water. Industrial kraft pulped birch fibre served as reference. According to ISO 5264-2 the pulps were beaten in a PFI mill (PFI-Mühle, FRANK-PTI). Due to preliminary tests a high beating intensity of 5000 revolutions was choosen to separate the fibre bundles of the perennial pulps and maintain the tensile strength apex of the birch fibre. Finally, the pulps were washed again on the 600 µm sieve with deionized water until they reached pH 7.

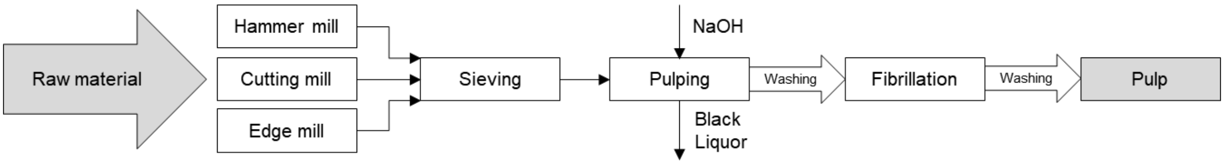


Fig. B 2: Grinding and pulping process of the raw materials : meadow hay, Virginia mallow and cup plant. Grinding with either the hammer mill, cutting mill or edge mill and subsequent sieving of the individual batches. Followed by NaOH pulping and washing before beating (PFI mill). Last washing step and resulting pulps for the paper production.

Round hand-sheets with a basis weight of 75 g m^{-2} and a diameter of 200 mm were produced using a Rapid-Köthen apparatus, according to ISO 5269-2. Paper blends using birch and one of the raw materials were produced containing 25% or 50% of the raw materials. Hand-sheets of 100% birch pulp served as control. Hand-sheets were conditioned for 24 h at 23 °C and 50% relative humidity according to ISO 187. Tensile index according to ISO 12625-4 was determined using a FRANK-PTI Universal Tester Vertical (Type: S818380002). Further tests were carried out according to the following standards: paper basis weight (DIN EN ISO 536), thickness (ISO 12625-3), bulk (inverse density) (DIN EN ISO 534), brightness (ISO 12625-7), and yellowness (DIN 6167). For each testing, 4–6 sheets were used, and 7 measurements per sheet were taken using different parts of the hand-sheets.

3.2.4 Optical and chemical characterization of the raw materials

The sieved particles were measured by dynamic image analysis (CamSizer P4, Retsch) for particle geometries shown in Table B 1. For chemical characterization, the following standards have been used: moisture (ISO 18134-3), ash 525 °C (ISO 1762), ash 900 °C (ISO 2144). We refer to the raw material before pulping as "particle" and after pulping as "fibre". Using the term "fibre" for all materials would be misleading since the "particles" before pulping do not have fibre proportions like typical paper pulps. The particle and fibre properties shown in Table B 1, Table B 2 and Fig. B 4 are the arithmetic means of 6 (particle) and 3 (fibre) samples. A fibre-water suspension was analyzed via image analysis using a Fibre Tester plus+ by L&W for fibre proportions according to ISO 16065-1. Fibres under 0.2 mm length were defined as fines, and objects were defined as particles with a length-to-width ratio under 4 : 1.

Two-step acid hydrolysis for chemical characterization of the raw material and pulp were performed in triplicates, according to NREL TP-510-42618. This method was initially developed for raw woody materials (Sluiter et al. 2011), and has been used for pulp/fibre analysis before (Gschwend et al. 2016;

Ilanidis et al. 2021; Lunze et al. 2021). The acid insoluble residue (AIR), consisting mainly of lignin, was determined gravimetrically with the analytical balance (XA105 Dual Range, Mettler Toledo). In the liquid phase, acid soluble compounds were detected using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (ICS-5000+, and a CarboPac™PA100-column, ThermoScientific), according to Anders et al. (2015). Acid soluble lignin (ASL) was detected photometrical at 320 nm (ε = 30 L/g*cm) for all materials, as described by Sluiter et al., using a Synergy Mx photometer and Gen 5 2.0 software (BioTek, USA) (Sluiter et al. 2011). The results of the characterization of the raw materials are shown in Table B 4 and Table B 5.

3.3 Results and discussion

3.3.1 Particle shapes after grinding and sieving

After grinding and sieving, the average meadow hay particles (5.2 mm) are approximately twice the length of Virginia mallow particles (2.6 mm) and more than three times longer compared to cup plant particles (1.4 mm). The particle length of meadow hay and Virginia mallow differs negligibly between the three mills, whereas cup plant shows the same particle length for all three mills (Table B 1). For Virginia mallow and cup plant, the width of the particles increases from HM over CM to EM. The EM produces the widest particles for all plants, conceivably due to the pressure applied by the rollers (Hauhouot-O'Hara et al. 1999). Those proportions result in a length-to-width ratio for meadow hay of 4.2 : 1, Virginia mallow of 3.7 : 1, and cup plant of 2 : 1. The hammer mill produces the highest length-to-width ratio for meadow hay, whereas for Virginia mallow, it is the CM and for cup plant HM and CM. The cup plant particles are more or less rectangular, the meadow hay and Virginia mallow particles are more elongated but not comparable to fibres like birch with 39 : 1 (Table B 2).

Table B 1: Particle length [mm], particle width [µm] and length-to-width ratio (l/w) of meadow hay, Virginia mallow and cup plant after grinding and sieving. Divided by the three grinding types hammer mill (HM), cutting mill (CM) and edge mill (EM). Measured by dynamic image analysis. Table shows the mean ± standard deviation.

	Meadow hay			Virginia mallow			Cup plant		
Mill	HM	CM	EM	HM	CM	EM	HM	CM	EM
Length [mm]	6.2 ± 1.5	4.5 ± 0.6	5.0 ± 1.5	2.6 ± 0.3	3.0 ± 0.5	2.4 ± 0.3	1.3 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
width [µm]	924 ± 40	1024 ± 66	1029 ± 78	684 ± 18	718 ± 24	770 ± 26	633 ± 9	683 ± 5	745 ± 15
l/w ratio	6.7 : 1	4.4 : 1	4.8 : 1	3.9 : 1	4.2 : 1	3.1 : 1	2.1 : 1	2.1 : 1	1.9 : 1

The grinding result can be explained by the original shape of the harvested raw materials. The meadow hay blades are soft, long, and thin compared to the more solid, shorter, and wider Virginia mallow and cup plant chaff, allowing them to pass the screen of the mill faster. This results in less retention time in the mill, and therefore less size reduction (Saensukjaroenphon et al. 2017; Bueno et al. 2010). The HM creates air suction, which transports the material through the mill. At the same time, the thin meadow hay particles tend to fall vertically through the sieving screens, resulting in less efficient sieving (Schmidt et al. 2003). One reason why the average width of the meadow hay particles lies over the 0.75 mm of the sieve screen, could be because the particles are softer compared to the other raw materials, causing them to deform as they pass through the sieve.

The proportions of the Virginia mallow and cup plant chaff are similar. Cup plant does not shed its leaves during winter, resulting in a high leaf content of the material (13%) and an increased number of fines (Table B 2), as well as a decreased average length of the particles.

3.3.2 Fibre characterization of the three different pulps

Fig. B 3 shows the length-weighted average fibre lengths for Virginia mallow of 1.3 mm, followed by cup plant with 0.8–1 mm and meadow hay with 0.5 mm. The measured fibre length of birch was 0.9 mm, as described by Przybysz et al. (2017). The analyzed fiber lengths of the raw materials are similar to the average of hardwood trees with 0.5–1.5 mm (Björklund et al. 2016). In comparison, cup plant ranges in the middle, meadow hay at the lower, and Virginia mallow at the upper limit of this average. Virginia mallow has a fibre length similar to Rye straw or hemp (Burley et al. 2004). The fibre lengths of cup plant is comparable to birch (Danielewicz and Surma-Ślusarska 2017). Compared to other grasses, like reed canary with 0.7–0.8 mm or tall fescue with 1.1–2.0 mm, meadow hay had shorter fibre lengths (Finell and Nilsson 2004; Kennedy et al. 1996). The different mills show no effect on the fibre lengths

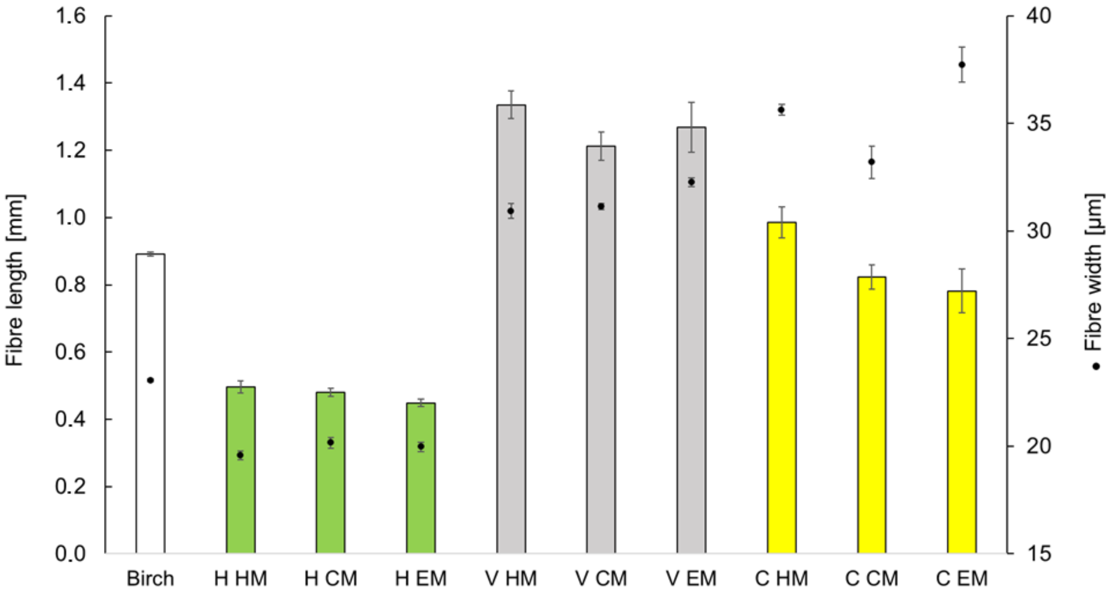


Fig. B 3: The Fibre length [mm] shown on the primary axis and fibre width [µm] shown on the secondary axis after pulping and refining of birch fibre (control), meadow hay (H), Virginia mallow (V) and cup plant (C). Subdivided by the grinding types hammer mill (HM), cutting mill (CM) and edge mill (EM). Fibre length and width are shown as length-weighted average with standard deviation. Determined by dynamic fibre analysis.

The meadow hay fibres (20 µm) have similar widths compared to birch (23.1 µm). Virginia mallow (31 µm) and cup plant fibres (35 µm) are wider. The various mills are showing small differences in fibre width for Virginia mallow and cup plant. For both raw materials, the EM produces the widest fibres, which is likely due to its compressive forces between the roller and the die (Hauhouot-O'Hara et al. 1999). The particle width of meadow hay is not affected by the use of different mills. Meadow hay and cup plant have a similar length-to-weight ratio of 24 : 1 and 25 : 1. The length-to-width ratio of Virginia mallow (40 : 1) is slightly higher compared to birch fibres (39 : 1).

Virginia mallow HM (17.0%) and CM (18.3%) have a slightly higher fines content compared to birch (15.1%). Hay follows with 24.2–27.4%. The fines content of Virginia mallow and cup plant increased from HM to CM and EM. Cup plant has the highest fines approximately as a result of its high leaf content of 16% (Wisur et al. 1993). Moreover, cup plant EM has a very high fines content of 43.4%, which might relate to the design of the edge mill. The raw material in the EM has to resist more shear force by virtue of more intensive grinding compared to HM and CM, which are cutting more than grinding (Berk 2018). Another reason for the high fines could be the beating with 5000 revolutions in the PFI mill. Further investigations should test less revolutions or even unbeaten pulps, especially for cup plant (Wisur et al. 1993), and distinguish between primary and secondary fines, to determine their origin and consider a further utilization (Gharehkhani et al. 2015; Thykesson et al. 1998). Nevertheless, an improvement of the sieving after the grinding of the raw materials, by using other sieving methods or air separation, will reduce the leaf content of the three raw materials and consequently the fines content (Finell and Nilsson 2004).

Table B 2: Fines content [%], objects per 10^5 fibres and length-to-width ratio of meadow hay, Virginia mallow and cup plant after pulping and beating. Subdivided by the grinding types hammer mill (HM), cutting mill (CM) and edge mill (EM). Measured by image analysis. Table shows the length-weighted average ± standard deviation.

	Birch	Meadow hay			Virginia mallow			Cup plant		
Mill		HM	CM	EM	HM	CM	EM	HM	CM	EM
Fines [%]	15.1 ± 0.5	24.2 ± 0.6	27.4 ± 1.0	25.2 ± 0.9	17.0 ± 0.3	18.3 ± 0.3	22.8 ± 0.4	27.8 ± 0.9	32.9 ± 3.5	43.4 ± 2.4
Objects [per 10^5 fibres]	154 ± 16	168 ± 80	242 ± 74	301 ± 122	1607 ± 144	1986 ± 199	3305 ± 101	5246 ± 736	5407 ± 109	10120 ± 797
l/w ratio	39 : 1	25 : 1	24 : 1	22 : 1	43 : 1	39 : 1	39 : 1	28 : 1	25 : 1	21 : 1

On average, meadow hay (237 per 10^5 fibres) has a similar meager objects quantity to birch (154). For Virginia mallow, the object number rises from HM (1607) to EM (3305). Cup plant EM (10120) showed the highest number of objects, followed by cup plant CM (5407) and HM (5246). The object number seems to correlate with the tensile strength. Cup plant and Virginia mallow show a high number of fibre bundles and contaminations compared to birch and meadow hay. Fig. B 4 shows dark field microscopy images of fibres and fibre bundles of all three raw materials.

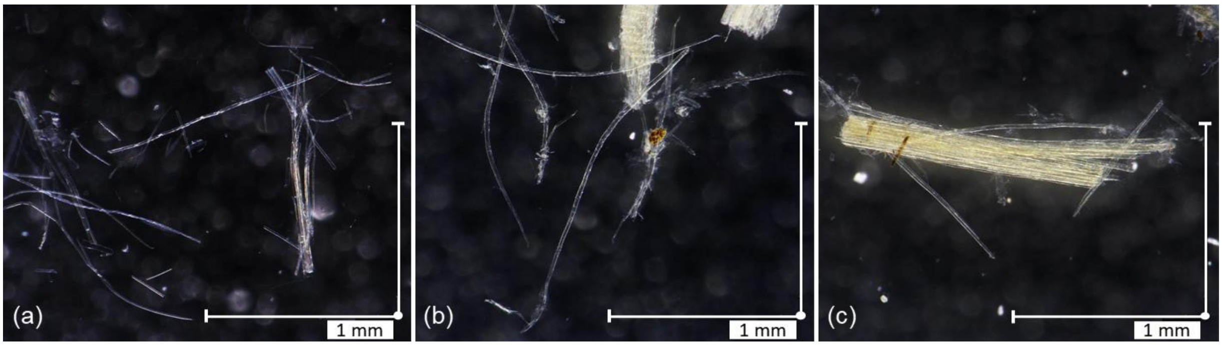


Fig. B 4: Dark field microscopy image (150x) after pulping and beating of hammer milled meadow hay (a), Virginia mallow (b) and cup plant (c). Showing fibres, fibre bundles and the differences in fibre lengths of meadow hay (0.5 mm), Virginia mallow (1.3 mm) and cup plant (0.8–1 mm). With a size scale of 1 mm.

3.3.3 Mechanical, structural and optical paper properties

Table B 3 shows the properties of the paper blends produced from birch fresh fibre with meadow hay, Virginia mallow, and cup plant. The highest tensile strength was measured for meadow hay, followed by Virginia mallow and cup plant (Table B 3, Fig. B 5). The effect of the different grinding types varies between the raw materials. For meadow hay, the cutting mill produced the highest values for tensile index (88.1 Nm g^{-1}); even higher than the birch control. No differences between the three mills could be observed for Virginia mallow, and for cup plant, the cutting mill showed the lowest tensile index.

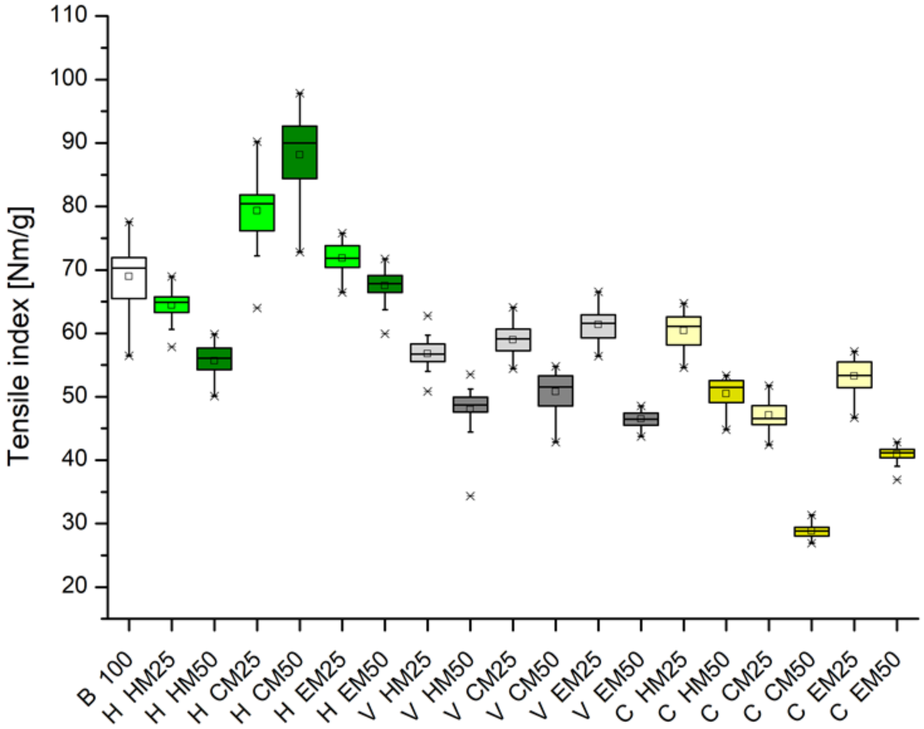


Fig. B 5: Tensile index of the produced paper sheets. The three raw materials meadow hay (H), Virginia mallow (V), and cup plant (C) differentiated by grinding type (HM, CM, EM) and blend share (25%, 50%) compared to the birch control (B 100). Boxes show median and interquartile range (25th and 75th percentile); mean (□); vertical whiskers, 1.5 interquartile range beyond the 25th and 75th percentiles; and cross, more extreme outliers.

Apart from meadow hay CM50 with the highest tensile index of all samples, the other 50% blends, of meadow hay Virginia mallow and cup plant, showed lower values compared to the 25% samples. The most significant differences in tensile index are observed for the meadow hay blends. With 64.4 and 55.7 Nm g^{-1} for the hammer mill, 71.9 and 67.5 Nm g^{-1} for the edge mill, and up to 79.3–88.1 Nm g^{-1} for the cutting mill. Przybysz et al. (2017) found similar tensile indices for hand-sheets from 100% tall fescue pulped using 26% active alkali and a sulfidity of 30%. Virginia mallow shows a consistent trend. The 25% samples show values close together with 56.8, 59, and 61.4 Nm g^{-1}. The same applies for the 50% samples with 48.0, 50.8, and 46.5 Nm g^{-1}. The cup plant measurement values differ and it is the same for meadow hay, but the mill order is reversed. The highest tensile index was reached for hammer mill 25% (60.4 Nm g^{-1}), followed by the edge mill (53.3 Nm g^{-1}) and the cutting mill (47.1 Nm g^{-1}). The meadow hay samples of CM25 and EM25 have a higher tensile index than birch (69 Nm g^{-1}). Meadow hay HM25 and EM50, as well as Virginia mallow EM25 and cup plant HM25, are laying slightly under the birch control.

Danielewicz (2015) analyzed the breaking strain for kraft pulped birch and tall fescue comparable to our raw materials. Thicker hand-sheets often showed lower tensile indices. Probably, because the bigger particles did not fit into the fibre network, resulting in less interlocking of the individual fibres (Annergren et al. 2009b). The thickest meadow hay hand-sheets were produced with the raw material of the HM, followed by EM and CM. Only for cup plant, this order is reversed.

Table B 3: Mechanical, structural and optical paper properties of hand-sheets with 25% or 50% share of meadow hay, Virginia mallow or cup plant and different grinding types: HM: Hammer mill; CM: Cutting mill; EM: Edge mill. Table shows the mean ± standard deviation.

Raw material	Raw m. share [%]	Tensile index [Nm/g]	Breaking strain [%]	Thickness [µm]	Bulk $[cm^3/g]$	Brightness [%]	Yellowness [index]
Birch	100%	69 ± 4.7	2.3 ± 0.4	142.1 ± 16.2	1.8 ± 0.2	82.9 ± 0.1	8.6 ± 0.1
Meadow hay	HM 25%	64.4 ± 2.6	1.8 ± 0.2	166.4 ± 16.1	2.2 ± 0.2	46.3 ± 0.6	31.1 ± 0.6
	HM 50%	55.7 ± 2.5	1.3 ± 0.1	176.8 ± 10.2	2.3 ± 0.1	34.1 ± 0.5	41.0 ± 0.7
	CM 25%	79.3 ± 5.1	2.4 ± 0.4	129.9 ± 1.2	1.7 ± 0.2	43.7 ± 0.3	35.2 ± 0.4
	CM 50%	88.1 ± 6.2	2.1 ± 0.3	127.2 ± 14.5	1.7 ± 0.2	31.7 ± 0.3	44.8 ± 0.3
	EM 25%	71.9 ± 2.3	2.2 ± 0.2	136.2 ± 10.4	1.8 ± 0.1	43.7 ± 9.2	33.5 ± 0.2
	EM 50%	67.5 ± 2.7	1.7 ± 0.2	136.7 ± 9.3	1.8 ± 0.1	33.7 ± 0.2	43.6 ± 0.3
Virginia mallow	HM 25%	56.8 ± 2.5	2.1 ± 0.2	152.1 ± 6.5	2.0 ± 0.1	51.3 ± 0.4	23.9 ± 0.5
	HM 50%	48 ± 3.6	1.7 ± 0.2	158.5 ± 9.4	2.1 ± 0.1	39.8 ± 0.3	32.4 ± 0.5
	CM 25%	59 ± 2.3	2.1 ± 0.2	149.0 ± 9.1	1.9 ± 0.1	48.0 ± 0.3	23.6 ± 0.4
	CM 50%	50.8 ± 2.9	1.9 ± 0.3	157.1 ± 10.8	2.1 ± 0.1	36.3 ± 0.4	32.0 ± 0.7
	EM 25%	61.4 ± 2.5	2.4 ± 0.2	157.1 ± 12.3	2.1 ± 0.2	51.4 ± 0.3	23.2 ± 0.3
	EM 50%	46.5 ± 1.3	1.7 ± 0.1	163.2 ± 6.3	2.2 ± 0.1	38.6 ± 0.2	32.7 ± 0.3
Cup plant	HM 25%	60.4 ± 2.8	1.9 ± 0.3	159.2 ± 11.8	2.1 ± 0.2	42.6 ± 0.2	26.4 ± 0.3
	HM 50%	50.5 ± 2.6	1.8 ± 0.5	174.6 ± 13.8	2.3 ± 0.2	30.9 ± 0.3	35.4 ± 0.7
	CM 25%	47.1 ± 2.3	1.7 ± 0.3	196.1 ± 11.3	2.6 ± 0.2	52.8 ± 0.6	18.8 ± 0.5
	CM 50%	28.8 ± 1.1	1.4 ± 0.1	224.6 ± 15.3	3.0 ± 0.2	36.7 ± 0.7	30.4 ± 0.9
	EM 25%	53.3 ± 2.6	2.0 ± 0.3	168.0 ± 13.9	2.2 ± 0.2	42.7 ± 0.3	25.6 ± 0.3
	EM 50%	40.9 ± 1.3	1.6 ± 0.2	186.9 ± 13.6	2.5 ± 0.2	30.9 ± 0.4	34.8 ± 0.7

Breaking strain for the birch paper was 2.3%. In comparison to 100% birch pulp, a mixture of 25% hay (CM) or Virginia mallow (EM) with 75% birch pulp rendered the same result. 50% of meadow hay, Virginia mallow, or cup plant reduced the value. For meadow hay, breaking strain drops from CM to EM and HM. For the cup plant, the highest breaking strain was attained by the HM, followed by EM and CM. For Virginia mallow, the edge mill showed the highest values (2.4%). Breaking strain has the same mill sequence as tensile index, starting with the highest elongation rates for the cutting mill followed by the edge mill and hammer mill. For cup plant, the trend is quite similar, but the HM and EM results are equal.

The control paper (birch 100%) was 142.1 µm thick. The proportionate replacement of birch pulp by the alternative pulps resulted in a higher thickness of the paper in almost every sample, due to unsolved particles and fibre bundles in the paper, as seen in Fig. B 4. Dietz et al. (2015) reported similar findings for recycling pulp with 30% unpulped meadow hay particles. For Virginia mallow and cup plant, the 50%

paper sheets are always thicker compared to the 25% sheets, which was not observed for meadow hay. The meadow hay hammer mill samples are thicker than the birch control, the cutting- and edge mill samples thinner. The different grinding types of Virginia mallow did not affect the paper thickness. The lowest thickness was observed for meadow hay processed with the cutting mill (127.2 µm, 50%). The highest increase in paper thickness was observed for cup plant processed with the cutting mill (224.6 µm, 50%). This means that the cutting mill produced on the one hand the thinnest and on the other hand the thickest paper. The thickness of the meadow hay paper seems to have an impact on the paper strength. The thinner the paper, the higher the tensile index and breaking strain. Simultaneously, the cup plant samples show the highest thickness and the lowest tensile indices. Annergren et al. indicated that finer grounded fibres improve the bonding ability of the fibres and this increases the tensile index. Nevertheless, an increased thickness can also be an advantage, especially for board and other products, which require a high bending stiffness (Annergren et al. 2009b).

Most of the samples have bulk values in the range of the 100% birch paper. The 50% samples have higher bulk values than the 25%, except for hay CM and EM, those samples have almost the same thickness between the 25% and 50% sample and therefore similar bulk values. On the other hand those four samples show the highest tensile indices due to the correlation between tensile index and bulk (Seth 1990). Hay HM shows an increased bulk and at the same time a lower object number (Table B 2) so an inefficient pulping resulting in more fibre bundles is not probable. This suggests that pulping was not influenced by the different grinding methods but the CM and EM compressed the fibres more, resulting in lower fibre widths (Fig. B 3) and therefore thinner paper with lower bulk. Cup plant has an average bulk of 2.4 cm^3 g^{-1}, which is 25% higher when compared to the birch control, so their use in the middle layer of cardboard could be of interest (Andreasson et al. 2009). The high bulk of cup plant could be an indication of an incomplete fibre separation after pulping and beating (Niskanen 2011).

The brightness of the 100% bleached birch paper was 82.9%. The highest values of the three raw materials ranged between 46.3 (meadow hay HM), 51.4 (Virginia mallow EM), and 52.8 (cup plant CM) for the 25% samples. The 50% paper blends showed lower brightness than the 25% paper blends. The lowest values of the 50% samples varied between 31.7 (meadow hay CM), 36.3 (Virginia mallow CM) and 30.9 (cup plant HM). The yellowness index shows the same but reverse results compared to brightness. The brightest samples also have the lowest yellowness index. The light microscopy images in Fig. B 6 show the color differences between the 50% hand-sheets and the 100% birch paper.

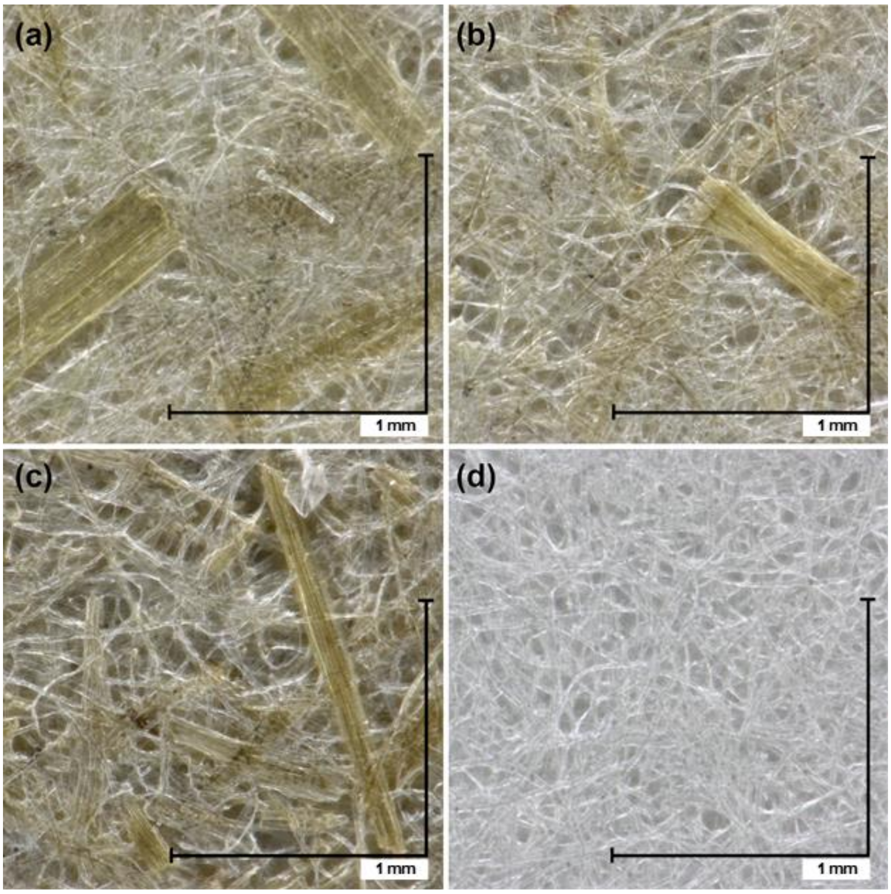


Fig. B 6: Light microscopy images (150x) of the hammer milled 50% paper blends ; Meadow hay + birch (a), Virginia mallow + birch (b), cup plant + birch (c) and 100% birch (d). With a size scale of 1 mm.

3.3.4 Chemical composition of the raw materials and fibres

The analysis of the lignocellulose and ash (particles: Table B 4, fibres: Table B 5) serves to interpret the pulping process and the paper properties of the produced hand-sheets. With birch used as a reference, values reported in Table B 4 and Table B 5 were taken from published studies. In comparison to the other raw materials (Table B 4), birch had the highest cellulose content, depending on the literature, between 41–46% that was about two-fold higher compared to the perennials. Of the perennials, meadow hay had the lowest cellulose content, with about 16%, and Virginia mallow the highest with about 24%. The hemicellulose content of birch was also higher when compared to the perennials, while the lignin content was rather evenly distributed at roughly 20%. The different grinding types had no significant effect on the analysis of the chemical composition of the raw materials before pulping.

Table B 4: Chemical composition of birch, meadow hay, Virginia mallow and cup plant before pulping. Subdivided by the grinding types hammer mill (HM), cutting mill (CM) and edge mill (EM). Table shows the mean ± standard deviation; basis for calculation, basis weight %.

	Birch	Meadow hay			Virginia mallow			Cup plant		
Mill		HM	CM	EM	HM	CM	EM	HM	CM	EM
Moisture [%]	12–15 [a]	8.4 ± 0.4	8.1 ± 0.2	8.2 ± 0.2	8.3 ± 0.3	8.1 ± 0.2	8.1 ± 0.2	9.4 ± 0.3	8.5 ± 0.3	9.1 ± 0.5
Ash at 525 °C [%]	0.6 [b]	5.9 ± 0	5.9 ± 0.1	5.7 ± 0.3	2.5 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	9.0 ± 0.1	9.8 ± 0.0	8.6 ± 0.2
Ash at 900 °C [%]	0.3 [c]	3.6 ± 0.1	3.6 ± 0.0	3.3 ± 0.9	1.8 ± 0.1	2.2 ± 0.0	2.2 ± 0.1	5.4 ± 0.5	5.9 ± 0.2	5.6 ± 0.3
Lignin [%]	19–22 [c,d]	18.5 ± 1.4	19.1 ± 0.4	19.3 ± 1.5	17.5 ± 1.1	18.7 ± 0.5	18.9 ± 0.6	21.4 ± 0.4	24.8 ± 4.5	22.3 ± 1.5
Cellulose [%]	41–46 [c,d]	16.6 ± 0.5	16.8 ± 0.7	16.1 ± 0.3	24.3 ± 2.7	23.7 ± 1.4	24.8 ± 0.4	19.3 ± 0.6	17.1 ± 0.2	17.2 ± 0.6
Hemicellulose [%]	32.4–36 [c]	12.3 ± 0.2	13.3 ± 0.4	12.2 ± 0.5	13.3 ± 1.3	12.8 ± 0.6	12.9 ± 0.2	11 ± 0.8	9.9 ± 0.4	9.8 ± 0.7

[a] Grosser and Teetz W. 1998, [b] Buzała et al. 2019, [c] Wüstenberg 2015, [d] Björklund et al. 2016.

After pulping (Table B 5), the cellulose content of the fibres increased compared to the particles before pulping. This was expected, as metabolites dissolve during the pulping process, which increases the fraction of complex carbon structures like cellulose and hemicellulose compared to the total weight (Hintz and Lawal 2018). As meadow hay was harvested in vegetative growth and not in senescence, it contained more metabolites compared to Virginia mallow and cup plant, and thus, the cellulose increase was exceptionally high, resulting in a cellulose content of 40% in the meadow hay pulp. The hemicellulose content in the pulp of the perennials increased to different extents. Again, the highest increase was shown for meadow hay pulp with a hemicellulose content of 25%, which can be explained by the harvest date. The lignin content was lowest for kraft pulped birch. The lignin content in meadow hay pulp decreased by smaller extends to 12%. This decrease is desired and expected, as alkaline pulping breaks structural linkages between lignin and complex carbon structures, and disrupts the lignin structure (Zeldin et al. 1988). For Virginia mallow and cup plant pulp, the lignin content increased, conceivably because the pulping process was too soft for both plants, resulting in the lignin not dissolving completely. The higher lignin content in the perennials compared to birch may also be the reason for the low brightness and high yellowness (Falkehag et al. 1966) (Table B 3). Thus, the higher lignin surface coverage of non-wood pulps compared to softwood pulps could be a reason to try higher NaOH concentrations (Yu et al. 2002). The ratio of lignin to cellulose is 0.3 for meadow hay, 0.69 for Virginia mallow, and 0.75 for cup plant. With lower lignin content, the cellulose fibres are less linked to lignin polymers, so they have more options for fibre-fibre bonding (Andreasson et al. 2009). Thus, the adverse properties of the higher lignin content as well as the lower cellulose content in Virginia mallow and cup plant, could provide a reason for the lower paper quality with respect to tensile index and breaking strain. Zhai and Lee indicated that the non-fibre parenchyma cells of straw have higher lignin concentration than the fibres themselves. As a result, the separation of parenchyma and cortex before pulping could reduce the lignin content of cup plant and Virginia mallow (Zhai, H., Lee, Z. 2006).

Table B 5: Chemical composition of the control fibres (birch, kraft[1] pulped) and the soda[2] pulped fibres of meadow hay, Virginia mallow and cup plant. Subdivided by the grinding types hammer mill (HM), cutting mill (CM) and edge mill (EM). Table shows the mean ± standard deviation; basis for calculation, basis weight %.

	Birch [1]	Meadow hay [2]			Virginia mallow [2]			Cup plant [2]		
Mill		HM	CM	EM	HM	CM	EM	HM	CM	EM
Moisture [%]	5.6 ± 0.1	7.4 ± 0.3	7.3 ± 0.7	8.2 ± 0.6	5.2 ± 0.1	5.0 ± 0.1	5.1 ± 0.0	7.2 ± 1.4	6.5 ± 0.1	6.7 ± 2.1
Ash at 525 °C [%]	0.5 ± 0.0	2.0 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.0	1.3 ± 0.2	4.2 ± 0.3	3.3 ± 0.1	3.8 ± 0.1
Ash at 900 °C [%]	0.3 ± 0.1	1.8 ± 0.2	1.5 ± 0.5	1.6 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	2.7 ± 0.5	1.9 ± 0.0	2.3 ± 0.1
Lignin [%]	3.1–9.8 [a, b]	12.2 ± 2.1	12.6 ± 1.1	11.8 ± 1.3	25.6 ± 1.8	24.6 ± 1.2	22.8 ± 1.1	25.1 ± 1.1	23.2 ± 0.4	23.8 ± 0.4
Cellulose [%]	70–94.6 [a, b]	42.4 ± 0.2	40.3 ± 1.2	40.8 ± 1.4	35.6 ± 0.9	35 ± 1.3	35.9 ± 0.3	35.8 ± 0.4	28 ± 0.9	31.9 ± 0.8
Hemicellulose [%]	2.2–8.7 [b]	27.9 ± 0.5	22.8 ± 0.8	24.5 ± 1.1	13.6 ± 0.1	13.9 ± 0.2	14.1 ± 0.4	15.3 ± 0.3	12.8 ± 0.4	12.4 ± 0.2

[a] Borrega and Sixta 2013, [b] Buzała et al. 2019.

Kim et al. (2016) conclude that grinding increases the available specific surface area for hydrolysis, which is expected to have an effect during the pulping process. Unexpectedly, grinding had only a minor impact on the composition of meadow hay, Virginia mallow and cup plant. There are three main grinding mechanisms: cutting (shortening), shearing (external fibrillation), and compression (internal fibrillation) (Gharehkhani et al. 2015; Kim et al. 2016). As each grinding type processed the fibres in a different way, different compositions after pulping were expected. Therefore, all three grinding types were suitable for preparing the raw materials for pulping.

Direct comparison of these lignocellulose results to other studies is difficult due to different analytical methods, cultivation and genotypes used. Nevertheless, using the applied two step acid hydrolysis method, all relevant compounds for the pulp and paper process can be determined; the subsequent analytical method then enables a comparison of the raw materials used here. Furthermore, HPAEC-PAD has been used for the analysis of the biomass such as Virginia mallow and cup plant before (Lunze et al. 2021; Damm et al. 2017a; Damm et al. 2017b).

Cup plant raw material shows the highest ash content followed by meadow hay and Virginia mallow, due to its high leaf content of 16% within the harvested biomass. Furthermore, the brown outer layer of the cup plant shoot (Fig. B 1c) could have a bark-like composition and therefore an increased ash content (Björklund et al. 2016). The use of a shredder with cyclone could separate the dry and light leaves from the shoots and lead to a lower leaf content in the harvested material of the cup plant, resulting in a decrease of the ash content. The NaOH pulping resulted in a reduction of the ash content, but all pulps have significantly higher ash contents compared to the birch control. To decrease the ash content for further experiments, the grinding and sieving should be adjusted to produce fewer fines and therefore less ash (Yancey et al. 2013; Finell and Nilsson 2004; Thykesson et al. 1998).

3.4 Conclusion

This study showed that all three perennial crops meadow hay, Virginia mallow, and cup plant are possible new paper feedstocks. The paper of meadow hay and birch pulp in combination with a cutting mill pretreatment resulted in a paper strength increase of 27% compared to pure birch pulp. Virginia mallow and cup plant revealed good paper properties and the longest and widest fibres. The unbleached fibres of all three crops are yellowish, show high strength potential and are thicker compared to the birch control. Therefore, a first implication of these raw materials could be the use in one- or multilayer cardboard for sustainable packaging, where fibre strength and bulk is needed but white bleached fibres are not required.

The different mills had no impact on the fibre proportions; their impact on the paper properties is relatively low. This means that all three simple agricultural grinding types can be used sufficiently. Soda pulping worked, but an evaluation of common pulping methods should be performed to find the best method. Nevertheless, there is much potential in adjusting the grinding and sieving to produce smaller particles or even fibrous material and increase the performance of Virginia mallow and cup plant to the level of meadow hay or other hardwood pulps. Parameters like recyclability, optical properties, homogeneity of the fibres and pulp yield are crucial factors for an ecologic and economic efficiency calculation. Further trials should focus on these parameters in particular. New data like the particle and fibre proportions or chemical composition of the raw materials could be of interest for other researchers in the fields of biochemistry or material science.

It can be concluded that the analyzed raw material could be of interest for the paper and pulp industry. An alternative fibre supply besides recycling and wood fibres would support the growing fibre demand worldwide. Equally important, it will accompany a variety of major ecological benefits, like biodiversity increase and soil health regulation using these perennials. Farmers could build another mainstay and cooperate with fibre producers, which will improve the land-use efficiency, preferably of marginal soils. This would reduce the pressure on the wood market and maybe lead to an increase of forests and properly utilized pastures worldwide, resulting in a decreased CO_2 footprint of one of the most energy-intensive industries in the world.

4 bonded levelling compound

This work has been published as:

Lüders Moll, Martin Höller, Charlotte Hubert, Christoph A. C. Korte, Georg Völkering, Christian Wever and Ralf Pude (2022). **Cup Plant (*Silphium perfoliatum* L.) Biomass as Substitute for Expanded Polystyrene in Bonded Leveling Compounds.** Agronomy 12.

The manuscript has not been modified from the published version.

4.1 Introduction

Building and construction materials are responsible for 11% of global annual greenhouse gas emissions, and actually building operations are adding a further 28% (United Nations 2009). Therefore, this industrial sector becomes an integral element of battling climate change. Construction materials, for example, could be used as longterm storage for CO_2 (Churkina et al. 2020). Atmospheric carbon can be trapped using technical solutions such as carbon capture and storage (Mikunda et al. 2013), or through the photosynthesis of plants (Terrer et al. 2021; Di Vita et al. 2017). Compared to annual plants, perennial crops allow a more sustainable biomass production due to lower nutrient requirements and increased stability against volatile climatic conditions (van Tassel et al. 2010; Crews et al. 2018; Bai et al. 2012; McLaughlin and Walsh 1998; Schoo et al. 2017; Wever et al. 2019).

Cup plant (*Silphium perfoliatum* L.) is an undomesticated wild crop (van Tassel et al. 2017) with a dry matter yield of 14–25 Mg $ha^{-1}y^{-1}$ (Bury et al. 2020), if harvested in August for biogas production or of 8.4–14.3 Mg $ha^{-1}y^{-1}$ in case of harvesting in December as a raw material for building materials (Wever et al. 2019). This perennial crop can be harvested annually for a period of 15–20 years. Furthermore, cup plant presents several ecological benefits like pollen, nectar and soil health regulation resulting in an increase in biodiversity and minimization of soil erosion (Schorpp et al. 2016; Cumplido-Marin et al. 2020; Ruf and Emmerling 2021). It is also discussed as a high-yielding bioenergy plant for problematic areas such as periodically waterlogged cropland (Ruf et al. 2019). Furthermore, cup plant has been found to substitute silage maize in biomass yield and quality (Grunwald et al. 2020). Due to these factors, and their high amounts of carbohydrates and proteins, cup plant is currently being used for biogas and fodder (van Tassel et al. 2017; Siwek et al. 2019; Wever et al. 2020; Gansberger et al. 2015). Among other biomasses, cup plant is under investigation as a potential greenhouse gas remedy through the production of biofuels (Cumplido-Marin et al. 2020). However, ecological improvement necessitates a biomass feedstock that is procured in a manner that allows an overall increase in sustainability (McLaughlin and Walsh 1998; Ruf and Emmerling 2021; Lunze et al. 2021; Jain et al. 2017). To take full advantage of the ecological benefits, a late harvest with the least possible agronomical input is necessary (Ruf and Emmerling 2021). In contrast to biogas applications, late harvests take place after the end of the flowering period so the full pollen and nectar supply is available to pollinators (Schorpp et al. 2016).

Furthermore, non-wooden perennial biomass has already been examined as a replacement for woody biomass in the paper industry (Höller et al. 2021) and particleboard manufacturing (Klímek et al. 2016; Klímek et al. 2021). Plant-based materials could play another key role in CO_2 fixation and sustainable construction (Bozsaky 2019; Ljungberg 2007). Due to its positive environmental impact, biomass is suggested as a possible substitute for traditional insulation (Bozsaky 2019; Latif et al. 2015; Rojas et al. 2019). Schulte et al. shows that the perennial grass Miscanthus may compete with expanded polystyrene (EPS) for insulation applications (2021). Other applications include light concretes or foam concrete systems. Those are investigated to improve the building materials CO_2 and ecological balance by substituting scarce sand, reducing the compound weight, and improving thermal insulation properties (Ramamurthy et al. 2009). Integration of natural aggregates in lightweight concrete (Pude et al. 2004; Pude et al. 2005), concrete (Acikel 2011; Vo and Navard 2016) and foamed concrete has been studied as reinforcements (Castillo-Lara et al. 2020). A partial substitution may be a step towards the development of implemented biomass concretes.

The physical performance of this building material substituted with cup plant may suggest different selection criteria for specific applications. Due to visual similarities of the cup plant parenchyma and EPS we assume similar insulating performance for building materials. So, the goal of the study was to investigate if cup plant can be used as lightweight aggregate and partially substitute EPS in bonded leveling compound.

4.2 Materials and Methods

4.2.1 Study Design

The parenchyma contents of several European cup plant accessions were analyzed as quality traits for insulation purposes. Bonded leveling compound served as test application. Its insulation properties and moderate strengths requirements are ideal to analyze the effect of the biomass substitution. The lightweight aggregate constitutes the high-volume compound of the BLC to reduce the overall weight. It was examined if EPS can be substituted by cup plant aggregates and if adverse biomass effects can be reduced by changing the binder. We increased the biomass (0–45 vol%) and thus decreased the EPS share along with the use of two different mineral binders. The thermal conductivity was determined by guarded hot plate analysis and the strength was determined at 20% sample compression.

4.2.2 Plant Material

The cup plant biomass was grown at the Field Lab Campus Klein-Altendorf (Rheinbach, Germany). The cup plant biomass used for the BLC samples was obtained from a plot resembling the commercially available feedstock. The field trial was established by planting of plantlets in 2014 (N.L. Chrestensen Erfurter Samen und Pflanzenanzucht GmbH, Erfurt, Germany).

The plant material for the comparison of different European accessions derived from the Thüringer Landesamt für Landwirtschaft und Ländlichen Raum (TLLLR). The plants were established at the Campus Klein-Altendorf in 2016. The accessions ‘USA’, ‘Germany’, ‘Russia’, ‘Northern Europe’ and ‘Ukraine’, described by Wever et al. (Wever et al. 2019), were used for the parenchyma quality trait. The annual mean temperature was 9.4 °C with a mean precipitation of 603 mm. The growing season included 165–170 days.

4.2.3 Biomass Preparation for the Construction Material Trial

The harvest of the cup plant biomass for the BLC samples was carried out in December 2016 (Champion 1200, Maschinenfabrik Kemper GmbH & Co.KG, Breul, Germany). At this time the relocation of nutrients from the stems into the rhizomes was completed and the plants were senescent. The harvested biomass had a water content of 46% which was reduced to 15% after drying on a drying trailer. To produce a vegetal lightweight aggregate with a similar size distribution comparable EPS the biomass was ground with a hammer mill (BHS 100, Th. Buschhoff GmbH & Co., Ahlen, Germany) equipped with a 10 mm grinding screen. The sieve fraction used for the BLC was 1–6 mm, produced on an oscillating screen (ASM 100, S&F GmbH, Grünkraut, Germany). After sieving no further biomass processing was performed (Fig. C 1).

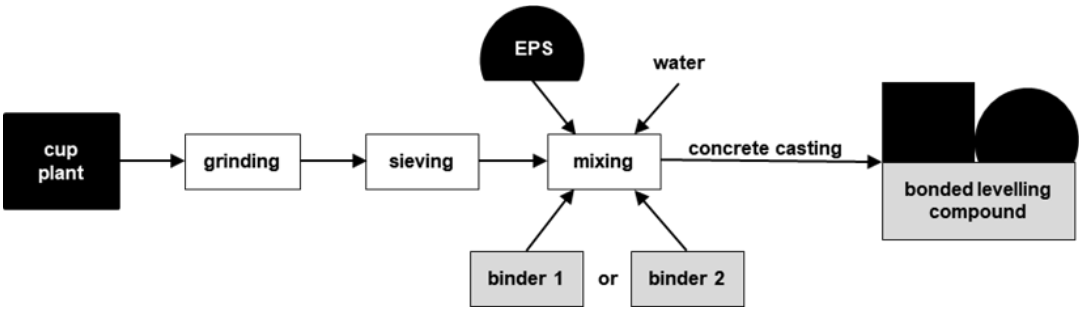


Fig. C 1: Schematic depiction of the study design. Grinding and sieving of the cup plant biomass, followed by mixing with expanded polystyrene (EPS), water, and one of the binders.

4.2.4 Parenchyma Analysis

The biomass used for the determination of the parenchyma content was harvested after senescence in November 2021. The stems were cut 10 cm above ground at the cut height of a field chopper. The inflorescence and remaining leaves were removed. The water content of the stems was 49%. The stems were dried for 24 h at 105 °C to mass stability. Three internodes at different stem heights were used as segments for the parenchyma determination. The low segment was defined as the lowest intact internode above the cut, the high segment was the highest internode, and the third segment was the respective middle internode. From each internode a central segment of 5 cm length was sawed out and the width of each specific stem segment was recorded.

The mass ratio of cortex to parenchyma was established by longitudinal sectioning of the segments and scraping the parenchyma from the cortex pieces. Afterwards both cortex and parenchyma were weighed separately (ME 54TE, Mettler Toledo, Columbus, OH, USA).

For the estimation of the parenchyma density the cortex was removed from the lower segments and the size of the parenchyma cuboids was measured. The raw density was estimated by the individual mass of the parenchyma cuboids and their truncated volume (n= 10).

4.2.5 Binder Systems

Binder 1 was extracted from a commercial bonded leveling compound (Fermacell, Bonded Leveling Compound) by sieving. Binder 2 (Otterbein, PROMPT Fix) was chosen for its high compatibility with biomass aggregates as demonstrated for hempcrete (Chaloupková et al. 2019). The binder systems cannot be compared directly, as Binder 1 is a ready-mix of a cementitious quick setting binder with EPS lightweight aggregates. In contrast, Binder 2 is a highly hydraulic lime cement powder with high early strength development.

4.2.6 Lightweight Aggregates and Concrete Specimens

The original lightweight aggregates (EPS) of the commercial bonded leveling compound (BLC) were separated by sieving over a 1 mm sieve. The mass ratio in the commercial BLC was recorded at 80% binder and 20% EPS. The compositions for the substitution of EPS by cup plant aggregates were measured volumetrically. The biomass ratio ranged between 0–45 vol%, thus the EPS partition shifted between 100–55 vol%. The final list of the lightweight compositions is shown in Table C 1.

Table C 1: Volumetric compositions and bulk densities of the lightweight aggregate mixtures containing cup plant (CP) and expanded polystyrene (EPS).

	Sample Composition [%]				
lightweight component	**CP 0**	**CP 15**	**CP 30**	**CP 45**	**CP 100** *
bulk density [g cm^{-3}]	0.045	0.05	0.06	0.07	0.10
EPS [ml]	6500	5525	4550	3575	-
cup plant [ml]	-	975	1950	2925	-

* **CP 100** was used as reference for pure biomass.

All samples were produced using the same weight ratio between binder and aggregate (8:1) according to the commercial BLC. Each sample batch had ~500–600 g (6.5 L) lightweight aggregates and 2–2.2

kg mineral binder sufficient material for 4 slabs (15 × 15 × 3 cm) and 6 prisms (4 × 4 × 16 cm). The Binder 1 samples were produced by mixing 10 wt% lightweight aggregate, containing 0–45% cup plant with 80 wt% mineral Binder 1, and water to a w/c of 0.45–0.50. The Binder 1 sample with 0% cup plant (CP 0) was the commercial product and served as control. The Binder 2 samples were produced likewise, but with Binder 2 and ascorbic acid (8 g L^{-1}) as a setting delay agent, according to the manual (Chaloupková et al. 2019), and water, to a w/c of 0.76. To measure the direct substitution potential of cup plant aggregates, the mix-water was given as determined by the product manuals, and no additional water was given to treat the water uptake of the biomass. The lightweight aggregates were mixed and then wetted with 75% of the water. Subsequently, the binder and the residual water were added and mixed for 5 min. The molds for the sample slabs and prisms were filled and compacted by manual agitation. The samples for the compression strength were produced in triplet prism molds, cured at room temperature for 7 days, and finally sawn into cubes (4 cm edge) for compression testing. The compression tests were carried out on a Hess TMN 10 (Richard Hess MBV GmbH, Sonsbeck, Germany) with compression plates at 10 mm s^{-1}. For those tests, a preload of 5 N was used and the force was recorded at 20% sample compression. The slabs for the thermal conductance were cast into slabs, cured for 7 days at room temperature, and sanded flush. The slabs were dried in an oven at 60 °C. During the testing procedure, the slabs were wrapped in cling film to eliminate humidity uptake. The thermal conductance measurements were carried out at an average of 10 °C with 15 K temperature difference using a guarded hot plate apparatus of the type Lambdameter EP500e (Lambda-Meßtechnik GmbH, Dresden, Germany).

4.2.7 Lightweight Aggregate Analysis

Random samples of the lightweight aggregate mixtures CP 0–CP 45 and the raw biomass CP 100 were homogenized on a sample divider (Retsch, PT 100, Haan, Germany). The analysis of the aggregate sizes was carried out using dynamic image analysis on a CamSizer P4 (Retsch). The shape parameters included in the analysis are Xarea (radius of an equivalent circle), FeMax (length according to Feret), and FeMin (width according to Feret). The width to length ratio (W/L) and sphericity index (SPHT) were calculated from the shape parameters (Neufert and Neff 1997). The water uptake was performed as cyclic water immersion, while the weight was recorded on the universal testing machine (TMN 10, Richard Hess MBV GmbH, Sonsbeck, Germany). After preliminary experiments, the machine was programmed to hold both extreme positions (immersed, well above water) for 30 s, to traverse the Z-axis at 10 mm s^{-1}, and to repeat the immersion process 20 times. Samples for the scanning electron microscope (SEM) were prepared from extracted cup plant parenchyma and EPS by drying in a desiccator for 24 h. The microscopes used were a Phenom ProX (Phenom, Thermo Fisher Scientific, Waltham, MA, USA) and a VHX-7000 (Keyence, Osaka, Japan). The pore size measurements were performed using ImageJ software (v 1.52. URL: https://imagej.nih.gov/ij/download.html accessed on: 01.05.2021).

4.2.8 Statistics

Data analysis was conducted in R (R Core Team, 2021) under Version 4.1.0 (18 May 2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/ (accessed on: 18.05.2021). The used packages were: (Bengtsson 2022; Graves et al. 2022; Signorell 2022; Wickham et al. 2022; Wickham et al. 2019; Lenth 2022; Gibb and Strimmer 2012). A one-way ANOVA was performed to compare the effect of biomass ratio on the compression strength and the effect of biomass ratio on the thermal conductivity. The ANOVA was followed by Tukey HSD for homogeneous groupings. The calculations were performed on 6 repetitions for the compression strength values and on 4 repetitions for the thermal conductivity while normal distribution was assumed. The correlation coefficient of thermal conductivity vs. density was determined by the Pearson method.

4.3 Results and Discussion

4.3.1 Cup Plant Parenchyma

Due to its foamlike structure, parenchyma seems to be a suitable feedstock for insulation materials. Scanning electron microscopy (SEM) of EPS and cup plant parenchyma cells showed that the macropore diameters range the same order of magnitude for both materials as can be seen in Fig. C 2. The cell size of the cup plant material (Fig. C 2a) varies between 141–217 µm and the shape is rectangular. In comparison, the EPS reveals rounded cells with size variations between 44–140 µm in Fig. C 2b. Neroth et al. assume that pores have to be closed and as small as possible for low thermal conductivity (Neroth and Vollenschaar 2011). Therefore, the pore size similarity of EPS and cup plant parenchyma suggest similar thermal conductivities.

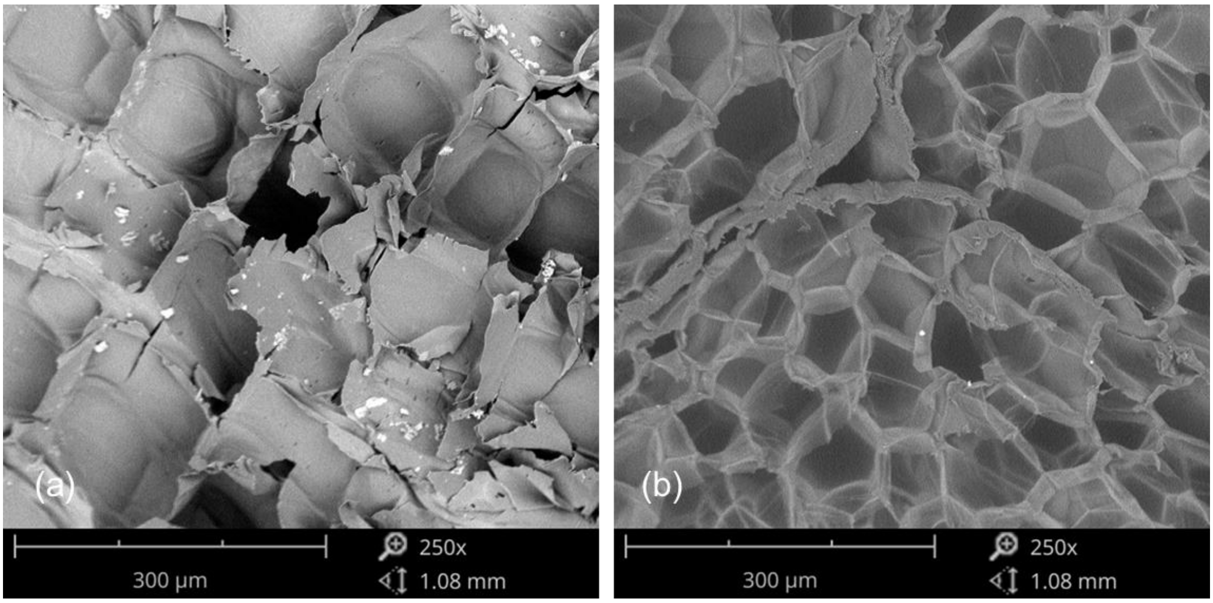


Fig. C 2: Structural similarities of (a) cup plant parenchyma, (b) EPS granules under scanning electron microscopy with 250x magnification [10 kV, 60 Pa, image scalebar = 300 µm].

The cross section of the cup plant stem shows the outer cortex as pink tissue after reaction with Wiesner stain and the inner non lignified parenchyma tissue (Fig. C 3). Cross-sections of the stem display a parenchyma area of approximately 44%. Cup plant shows a high volumetric amount of parenchyma throughout the plant stem. The estimated density of parenchyma at 0.041 mg mm^{-3} is in the order of magnitude of EPS with 0.01–0.03 mg mm^{-3}.

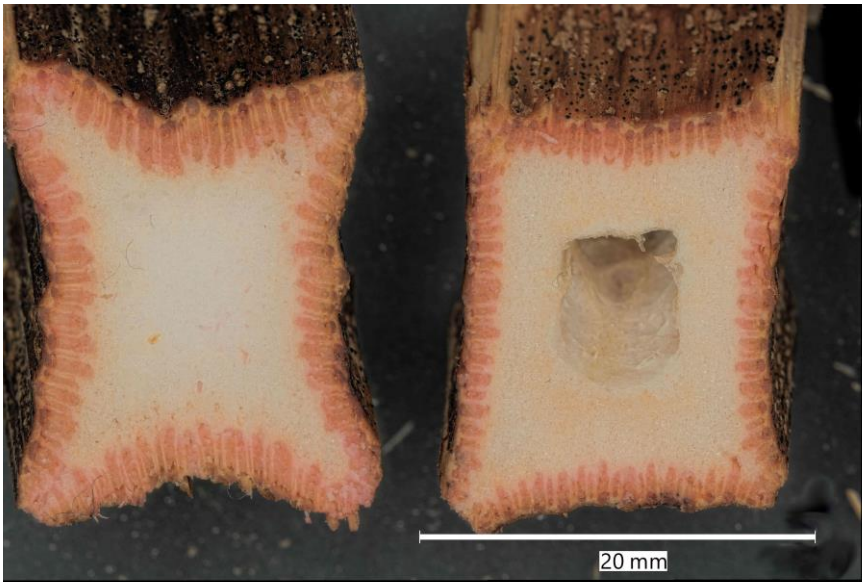


Fig. C 3: Cross-sections of commercial cup plant stems with visible parenchyma and cortex , Wiesner staining of the lignified cortex, under 20x magnification [image scalebar= 20 mm].

As all cup plants, the shoots, from the N.L. Chrestensen plots display a decreasing stem diameter at increasing stem heights. The stem diameter decreases up to 53% from the bottom to top. The absolute gravimetric amount of parenchyma decreases to 38% as shown in Table C 2. Due to the low parenchyma density an approximately tenfold amount of cortex is observed. The high standard deviation of stem width and mass is likely caused by the phenotypic variation of the N.L. Chrestensen genotypes. Due to the fact, that the commercially available seed materials (N.L. Chrestensen) is a mixture of several European accessions.

Table C 2: Biomass qualities of commercially available cup plant material (N.L. Chrestensen) at three different stem heights [n = 32].

	Diameter [mm]	Weight Cortex [g]	Weight Parenchyma [g]	Cortex to Parenchyma
high	6.8 ± 2.8	0.60 ± 0.34	0.05 ± 0.03	12
mid	10.1 ± 3.3	0.93 ± 0.49	0.09 ± 0.06	10.3
low	12.8 ± 2.8	1.63 ± 0.87	0.12 ± 0.08	13.6

The European cup plant accessions were analyzed for biomass quality in terms of parenchyma quantity. Of the five cup plant accessions displayed in Fig. C 4a ‘Russia’ displayed a significantly larger parenchyma weight. With 0.121 g the parenchyma weight of ‘Russia’ is 104% higher than the accession with the lowest parenchyma weight ‘Northern Europe’ (0.059 g). This agrees with the previous findings where ‘Russia’ showed the highest stem thickness. However, the highest annual dry matter yield is reported for ‘Northern Europe’ (Schoo et al. 2017). Due to the high phenotypic variation of 104% for parenchyma yield, this trait offers the possibility to develop adapted cultivars focusing on a material use of the cup plant biomass.

The parenchyma weight and stem diameter show a correlation coefficient of 0.777 over all height levels and all accessions. Therefore, cultivating cup plant accessions with increased shoot diameters define a new cup plant ideal type for material use. The variance of biomass quality in the European gene pool shows that various accessions could be differently suited as feedstock for materials production.

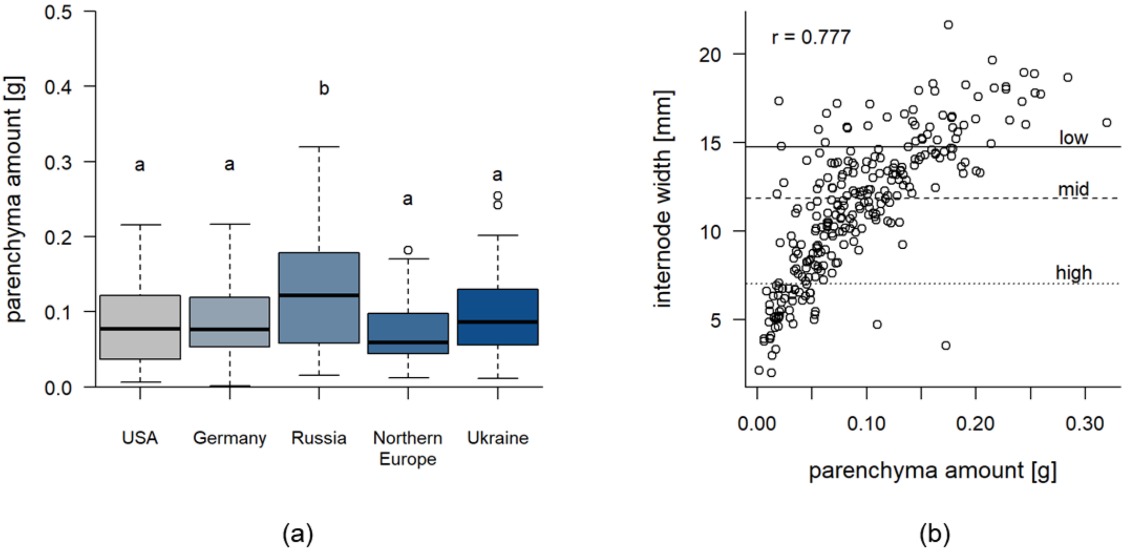


Fig. C 4: Qualities of different European cup plant accessions . The data presented were acquired by surveying internode segments of 5 cm length. Three internodes in different heights (high, mid, low) were examined per stem. In total five accession (‘USA‘, ‘Germany‘, ‘Russia‘, ‘Northern Europe‘, ‘Ukraine‘) with 18 stems each were analyzed. (a) Boxplots (n = 54) of parenchyma amounts as a function of their accession. The letters above represent homogenous groupings, calculated via ANOVA with following post hoc test (Tukey-HSD). Different letters indicate significance at $p < 0.05$. (b) Scatterplot of internode width against parenchyma amount (n = 270). The correlation coefficient of both parameters equals 0.777 over all five accessions. Horizontal lines display the arithmetic means of internode widths on different positions on the shoot (independent of accession).

4.3.2 Aggregate Analysis

To substitute cup plant in the BLC the shape parameters need to match the original EPS aggregates. The observed cup plant aggregates exist mainly as rod-like shapes of different aspect ratios. Elongated aggregates with low aspect ratios appear to be dominated by the outer section of the cortex. In contrast, the shorter aggregates contain more parenchyma. The resulting biomass is a wide spread of rod-like larger aggregates and more granulated smaller aggregates. In comparison, the EPS used in this study consists of mainly spherical aggregates.

Fig. C 5a shows the high volumetric parenchyma content in the native stem. Shredding of the plant during harvest and processing in the hammer mill causes losses, which can be observed in Fig. C 5a. The overall size of the cup plant aggregates (CP 100) obtained by milling and sieving comparable to the expanded polystyrene (CP 0) separated from the concrete mix. However, the shape of the cup plant aggregates is inhomogeneous with mainly rod-like aggregates (Fig. C 5a) in contrast to the round EPS (Fig. C 5b).

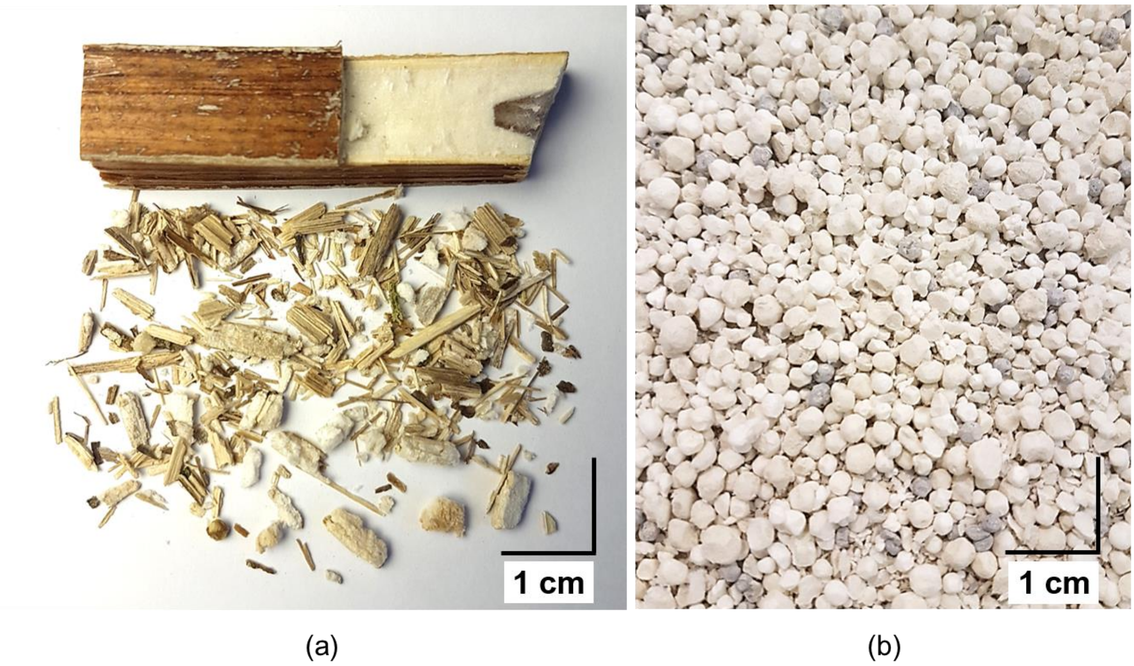


Fig. C 5: Lightweight aggregate examples (a) Cup plant stem and milled sieve fraction; (b) Expanded polystyrene aggregates (sieve fraction: 1–6 mm) [Scalebar = 1 cm].

The composition of the lightweight aggregates shows distinct differences in size (Fig. C 5 and Fig. C 6) and shape distributions for EPS (CP 0) (Fig. C 6a), the mixture of cup plant aggregates and EPS (CP 45) (Fig. C 6b), as well as pure milled cup plant (CP 100) (Fig. C 6c). The aggregate size as cumulative share (Q) of the total distribution allows to represent the influence of the amount of different aggregate sizes on the total composition of the mixtures. The shape of the EPS (CP 0) curves (Fig. C 6a) is approximately a parallel sigmoidal curve in all measured shape parameters. This indicates relatively uniform aggregate shape and aspect ratios. The distinct sigmoid shape also shows a sharp aggregate size cutoff, where 10% of the aggregates have a width or equivalent radius below 3 mm. Aggregates above 6 mm have nearly no contribution. The same behavior is observable for the aggregate length but shifted to a size interval of 4–7 mm. In the case of CP 100 (Fig. C 6c), the sigmoid for the length distribution (FeMax) displays a decreasing slope with increasing aggregate size compared to both other parameters. The size range of the pure biomass up to 3 mm width spans 85%, while it reaches 75% at a length up to 7 mm. For the mixtures of biomass and EPS (CP 45), the overall shape of all sigmoid curves shows an increased presence of small aggregates (Fig. C 6b). A shift towards smaller aggregate girths (FeMin) raises the number of aggregates below 3 mm to 25%. The length span of the aggregates is slightly increased (FeMax) from 3–8 mm.

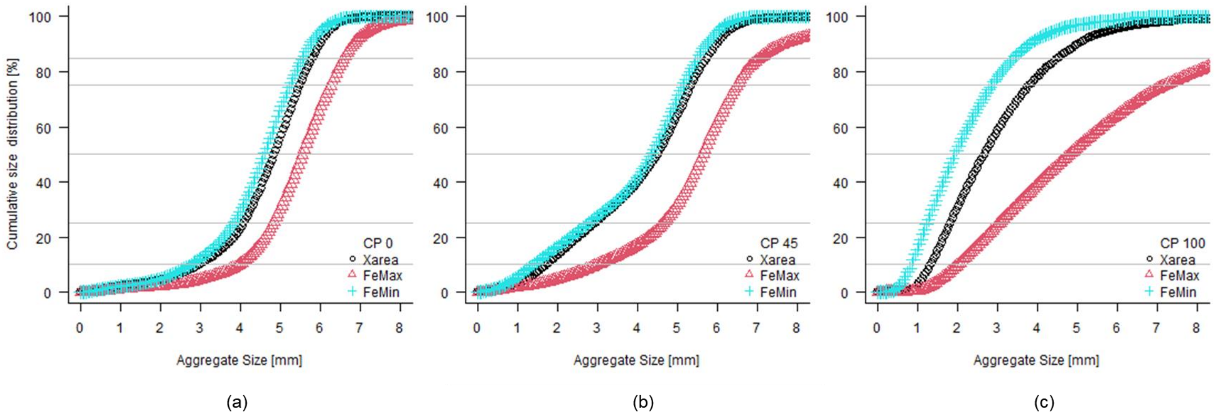


Fig. C 6: Average development of aggregate shape parameters with increasing cup plant ratio (CP) as cumulative size distribution Q. (a) CP 0: EPS extracted from the commercial mix; (b) CP 45: mix composition of 45 vol% milled cup plant aggregates and 55 vol% extracted EPS; (c) CP 100: cup plant aggregates obtained by milling and sieving. The parameters are: Feret minimal diameter (FeMin) as width; equivalent circle radius (Xarea); Feret maximal diameter (FeMax) as length [n = 4].

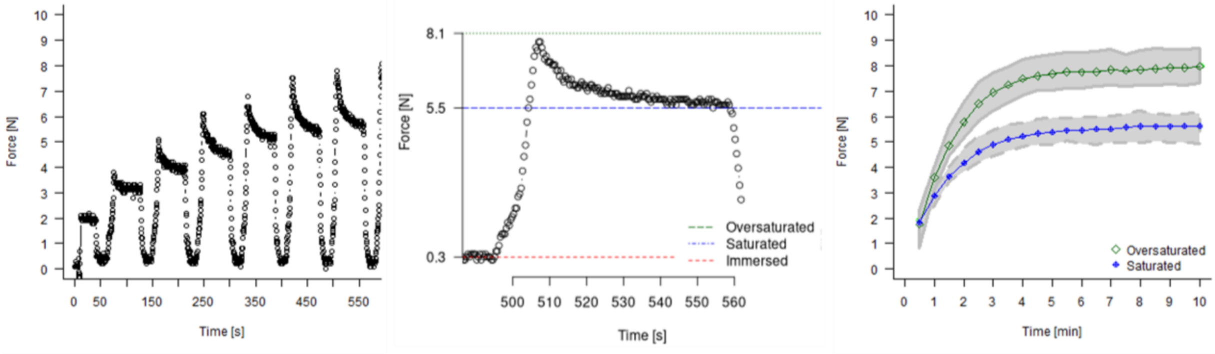
Considering the change in general shape parameters such as the width to length ratio (W/L) and sphericity (SPHT) in Table 3 at the cumulative readout Q, the influence of the biomass in the mixtures CP 15–CP 45 of up to 85% is minor for the main amounts of the aggregates. The size and shape characteristics are still dominated by the EPS for most aggregates in all lightweight aggregate mixtures used in this study. In order to substitute EPS with an alternative feedstock, this similarity of shapes is necessary to ensure similar workability as rheology and compaction are influenced by the granulometry (Van der Putten J. et al. 2016; Glas et al. 2015). Otherwise, a reconstitution of an optimized mixture by predictions methods such as the Andreasen and Andersen model may be necessary (Glas et al. 2015). Partial substitution of EPS by cup plant biomass is thus possible, as long as the found feedstock shape parameters are considered.

Table C 3: Development of aggregate size [mm], aspect ratio (W/L), and sphericity (SPHT, normalized) with increasing cumulative size distribution (Q [%]) of aggregate size (equivalent circle radius) at CP levels increasing from CP 0 (pure EPS) to CP 100 (pure cup plant) [n = 4].

	CP 0			**CP 15**			**CP 30**			**CP 45**			**CP 100**		
Q [%]	size	W/L	SPHT	size	W/L	SPHT	size	W/L	SPHT	size	W/L	SPHT	size	W/L	SPHT
10	3.0	0.64	0.74	2.2	0.45	0.58	1.9	0.37	0.51	1.7	0.34	0.50	1.3	0.34	0.51
25	4.0	0.76	0.82	3.7	0.64	0.74	3.3	0.54	0.65	2.9	0.45	0.59	1.8	0.34	0.49
50	4.8	0.81	0.86	4.7	0.76	0.82	4.6	0.73	0.79	4.5	0.70	0.76	2.7	0.37	0.48
75	5.5	0.81	0.85	5.4	0.81	0.84	5.4	0.77	0.81	5.3	0.76	0.81	3.8	0.38	0.47
85	5.8	0.83	0.85	5.7	0.80	0.84	5.7	0.80	0.82	5.7	0.75	0.79	4.6	0.38	0.43

4.3.3 Early Onset Water Absorption of Cup Plant Raw Material

The water immersion cycles of cup plant biomass can be seen in Fig. C 7. The individual measurement (Fig. C 7a) consists of 3 cycle stages where 0 N corresponds to the immersed sample, followed by the oversaturated peak, and the wet saturation plateau.



(a) (b) (c)

Fig. C 7: Increasing water uptake as function over time at short wetting intervals . (a) Force against time of cyclic wetting; (b) individual force in dependence of time result of a single soaking cycle. The horizontal lines represent the three stages of soaking in water followed by dripping of adsorbed water until the wet aggregates are saturated with absorbed water; (c) force against time result as mean values of the oversaturated and saturated values. The grey area represents the value range of all samples [n = 6].

The oversaturated peak and wet saturated plateau increase asymptotically and reach a stable value within 5 min of cumulative soaking time as seen in Fig. C 7c. Pude et al. (2004) concluded that the most relevant water absorption appears in the first minutes, and pre-soaking of biomass is often performed in practical applications (Chaloupková et al. 2019; La Doudart de Grée et al. 2019; Kochova et al. 2016). Water uptake of the biomass has previously been identified as a relevant parameter (Pude et al. 2004; Pude et al. 2005; Yu et al. 2013; Boix et al. 2016; Moll et al. 2020; Doudart de la Grée, G.C.H. et al. 2014, 2014). However, water absorption is no simple linear process, as physical and chemical absorption processes are contributors as well (La Doudart de Grée et al. 2019; Brouard et al. 2018). The stable water uptake from Fig. C 7c is ≈ 200 wt% of the biomass (300%) for the saturation and ≈ 300 wt% of the biomass (400%) for the oversaturated state. The oversaturated state is of relevance for this application. Water that is superficially adsorbed may disturb the w/c ratio if it is desorbed during the mixing of the concrete. Table C 4 with its recalculated w/c values was generated under the assumption that the biomass absorbed sufficient water to reach the saturation plateau during mixing of the wet concrete. Disregarding further water competition between binder and biomass the water availability for concrete during mixing was recalculated. The w/c for Binder 1 starts at a normal level of 0.45 at CP 0 but decreases rapidly (0.36–0.20) with biomass addition. The same behavior for Binder 2 causes a transition from a high w/c (0.76) to a more usual w/c ratio (0.56) with increasing biomass content.

Table C 4: Recalculated w/c for each CP level and theoretical water demand of increasing biomass substitution levels from CP 0 (pure EPS) to CP 45 (EPS 55%, cup plant 45%).

	CP 0	**CP 15**	**CP 30**	**CP 45**
Binder 1 *w/c*	0.45	0.36	0.26	0.20
Binder 2 *w/c*	0.76	0.62	0.59	0.56
Water demand [ml]	-	200	400	600

4.3.4 Compression Strength

The compression strength did not display an increase over time in the measured intervals. This is likely caused by the quicksetting nature of both binders and the overall low strength of the BLC. The sample structure consists of the binder covered lightweight aggregates and gas cavities (Fig. C 8).

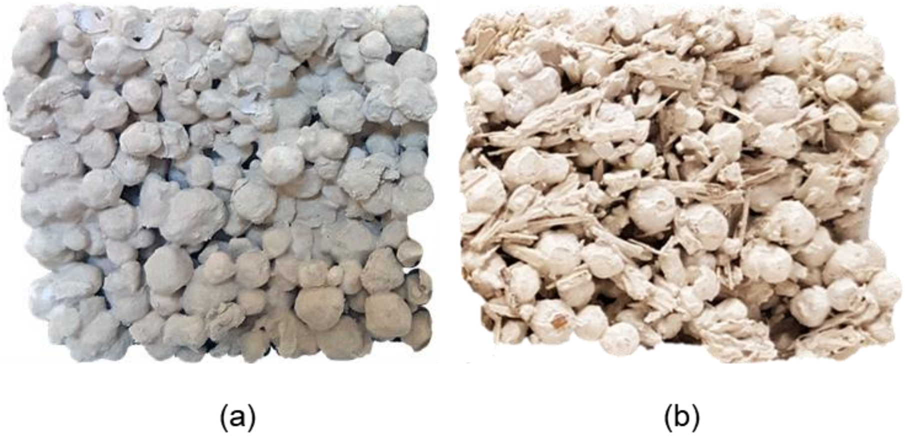
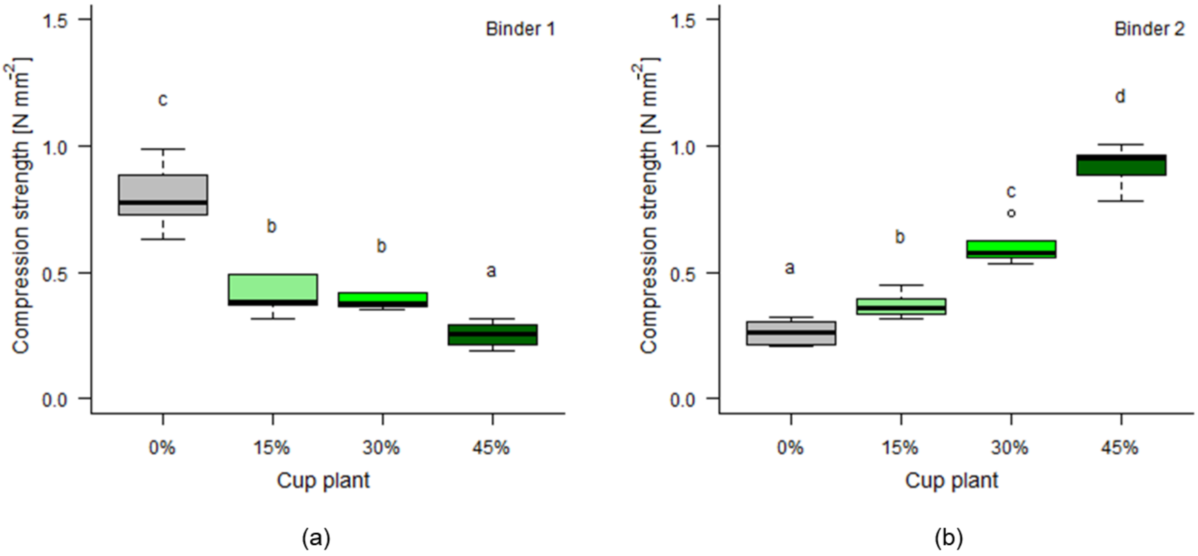


Fig. C 8: Samples of bonded leveling compound (a) CP 0 in Binder 1; (b) CP 30 in Binder 2.

According to the commercial information (JH 2022), Binder 1 is designed to solidify after 6 h and should reach the final compression strength of 0.4–0.5 N mm^{-2} after 28 days. Binder 2 on the other hand is an inherently quick setting binder and is designed to lower a final compression strength above 0.3 N mm^{-2} with biomass, according to the commercial information (Chaloupková et al. 2019). The compression strength of the control (Binder 1, CP 0) fluctuated between 0.73 and 0.86 N mm^{-2} at an average of 0.79 N mm^{-2}. With an increase of the cup plant aggregates, the compressive strength of Binder 1 diminished from an average of 0.79 N mm^{-2} (CP 0) to 0.40 N mm^{-2} (CP 15) and, respectively, further to 0.38 and 0.25 N mm^{-2} for CP 30 and CP 45 (Fig. C 9a). The compression strength is in the same order of magnitude as other biomass containing concretes such as Miscanthus concretes from Pude et al. (2004) (0.28–0.75 N mm^{-2}), waterproofed EPS based lightweight aggregate concretes (0.42–0.47 N mm^{-2}) (Li and Li 2014), Hemp lime systems from Benfratello et al. (2013). (0.09–0.46 N mm^{-2}) but considerably lower than the higher density (1160–1520 kg m^{-3}) systems with strength values ranging from 2–28 N mm^{-2} reached by Chen (2020).



(a) (b)

Fig. C 9: Compression strength values [N mm^{-2}] at 20% compression after 28 days. Samples with 0–45% cup plant mixed with Binder 1 (a) or Binder 2 (b). [The letters a–d represent homogeneous subsets of the 28 days strength averages according to Tukey HSD, n = 6].

Investigations of the interaction of concrete with biomass extractives have shown that concrete setting will be retarded by different organic species (Peschard et al. 2006; Le Ngoc Huyen et al. 2011; Diquélou et al. 2015; Doudart de la Grée, G.C.H. et al. 2015; Kochova et al. 2017; Ye et al. 2018). The effective water to binder ratio w/c is also a factor that determines the compressive strength via concrete hydration (Pude et al. 2005; Glas et al. 2015; Yu et al. 2013; Le Ngoc Huyen et al. 2011). Even though the binders are setting quickly, the retardation effect should not be dominating since the biomass and the binder systems are still in strong competition for the available water (Caprai et al. 2018). When compared to Binder 1, the mixture for Binder 2 is especially adapted for biomass aggregates by an increased water amount.

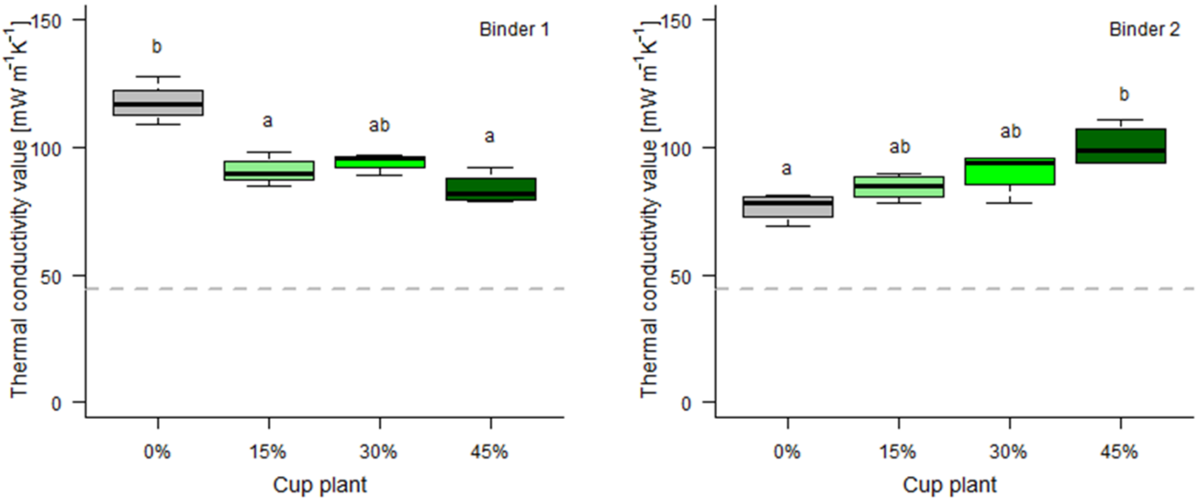
For Binder 2 the compressive strength behavior is reversed. The CP 0 mixture shows the lowest compressive strength at 0.26 N mm^{-2}. With an increased biomass ratio, the compressive strength increases from 0.37 N mm^{-2} to 0.60 N mm^{-2}, and a maximum of 0.92 N mm^{-2} (CP 45) (Fig. C 9b). The samples CP 30 and CP 45, therefore, reach the desired strength values above 0.5 N mm^{-2} of the product. The most likely reason for the strength increase is the initial water excess, causing a weakened matrix which is offset by increased water uptake by the biomass. The decrease in w/c is accompanied by a change in workability and mechanical properties as lower w/c ratios correspond to a higher cement stiffness (Tavossi et al. 1999). An increasing water uptake by the biomass may be the main explanation for both the increased compression strength at higher substitution levels in Binder 2, as well as the inverted strength behavior between Binder 1 and Binder 2. The Binder 2 sample is formed with a concrete mix that is made with an initially higher w/c ratio of 0.76 compared to w/c of 0.45 in Binder 1. The highest compression strengths of 0.79 N mm^{-2} and 0.92 N mm^{-2} are reached for CP 0 at w/c of 0.46 in Binder 1 and CP 45 at w/c at 0.76 in Binder 2. Both results correspond to the highest reached density (Table C 5) in their respective sets.

Table C 5: Density values of the compression prim samples for both binder systems at the light aggregates compositions from CP 0 (pure EPS) to CP 45 (EPS 55%, cup plant 45%).

Density [kg m^{-3}]	**CP 0**	**CP 15**	**CP 30**	**CP 45**
Binder 1	604 ± 36	457 ± 39	516 ± 22	466 ± 33
Binder 2	339 ± 37	426 ± 32	564 ± 27	701 ± 47

4.3.5 Thermal Conductivity

The thermal conductivity for Binder 1 shows two distinct groups with CP 0 around 117 mW m^{-1} K^{-1} and ≈ 82–95 mW m^{-1} K^{-1} for all other biomass samples, which is shown in Fig. C 10a. A drop in density of the same fashion can be seen in Fig. C 11, as evidenced by the values from 565 ± 36 kg m^{-3} (CP 0) to 431 ± 48 kg m^{-3} (CP 45). The reduction of the density with biomass addition in Binder 1 cannot be caused by the inherent density of the lightweight aggregates, as the cup plant biomass displays a higher bulk density than the EPS as it is referenced in Table C 1. Hence, the biomass must influence the compaction of the system indirectly.



(a)

(b)

Fig. C 10: Boxplot of the thermal conductivities [mW m^{-1} K^{-1}] of bonded leveling compound slabs produced with cup plant ratios of 0–45%. The grey lines represent reference values of the pure EPS control (45 mW m^{-1} K^{-1}) (Domínguez-Muñoz et al. 2010). (a) Thermal conductivity values of Binder 1 (b); Thermal conductivity values of Binder 2; differences in values followed by a different letter for each batch are statistically significant at $p < 0.05$ [(Tukey grouping), n = 4].

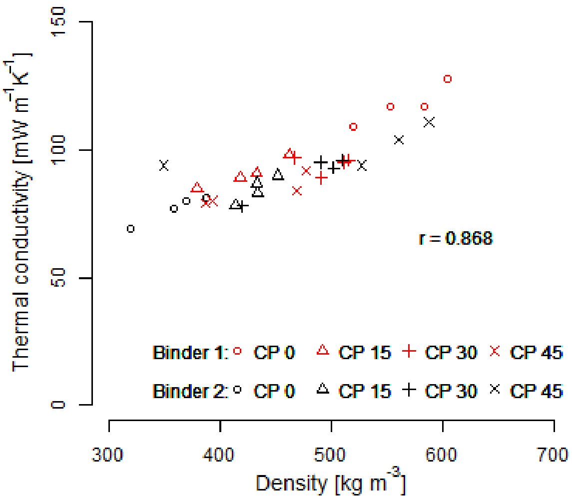


Fig. C 11: Scatterplot of sample density against thermal conductivity values for both Binders at different ratios of cup plant biomass and EPS [n = 4, r = 0.868, p = 1.245 × 10−10].

In Binder 2, the density and thermal conductivity both increase with biomass content from 78 to 99 mW m^{-1} K^{-1} and 359 ± 28 kg m^{-3} to 506 ± 107 kg m^{-3} for CP 0 and CP 45, respectively, Fig. C 10b as well as Fig. C 11. The resulting thermal conductivities for both binders are in the same order of magnitude as other insulation concretes found in literature such as hemp lime biocomposite with 83 mW m^{-1} K^{-1} at 231 kg m^{-3} (Benfratello et al. 2013), or ultra-lightweight concrete from Miscanthus fiber and expanded glass with 90 mW m^{-1} K^{-1} at 554 kg m^{-3} (Chen et al. 2020). A strong correlation (r = 0.86) between the thermal conductivity and the density can be found in Fig. C 11. These results are in accordance with the generally accepted theoretical framework as insulation materials tend to show lower thermal conductivity with lower density values (Freymuth et al. 2002).

In combination with the apparent reversed density effect due to cup plant addition, it must be concluded that compatibility is the main factor determining the thermal conductivity, as is generally the case for insulation concrete (Samson et al. 2016). However, the span of thermal conductivities for the obtained specimen is sufficient when compared with the commercial reference (120 mW m^{-1} K^{-1}), and follows the same behavior relations as foamed concretes as reported by Samson et al. (2016).

The flow behavior and packing density of concretes have been shown to depend on both the water availability and the shape characteristics of the aggregates (Van der Putten J. et al. 2016). Therefore, the difference in water availability by the addition of cup plant biomass impedes the flow behavior and the compaction. Both factors leading to an inhomogeneous density.

The thermal conductivity of the composite material is thus a complex function of the lightweight aggregates, the binder system, and the w/c. Future studies of this kind of biomass systems should entail porosity and permeability tests in relation to the parenchyma content of the concrete, as well as a quantification of rheological behavior against bioaggregate granulometry.

4.4 Conclusions

Cup plant could change the balance of CO_2 emissions for certain construction materials. The local production of the low-density cup plant biomass could be ecologically beneficial compared to the production and transport of petroleum- or mineral-based lightweight aggregates. The influence of biomass quality traits on the performance of the materials should be further researched. The phenotypic variation of parenchyma content in the European accessions offers the possibility to develop product-adapted cultivars. Longterm breeding goals for materials applications could be derived from the relation of biomass quality and materials performance. Increased stem thickness and the correlated parenchyma increase could be used as selection criteria.

To ensure a sustainable contribution of cup plant use in construction materials the following topics need to be addressed:

- Land use competition between food and biomass production;
- Life cycle analysis of cup plant as industrial raw material;
- Industrial scalability of processing and production of biobased building materials.

The results indicate that late harvested cup plant biomass could be a biobased substitute for EPS in bonded leveling compounds. The resulting compression strength of 0.92 N mm^{-2} and thermal conductivity of 99 mW m^{-1} K^{-1}, of the sample with 45% cup plant, are within the original product specification. Hence, the biobased samples allow the same applications as the reference product if the binder system is adapted. At the current state, the maximum cup plant content of ≈ 45% is limited by the w/c ratio and the rheology of the mix. The shortterm biomass water uptake of 200% and the water to cement (w/c) ratio are the determining factors of the sample density. The density governs both thermal conductivity and compression strength. To obtain a better understanding of the complex insulation system, the porosity of the resulting overall system needs to be determined. The effect of the comminution on the granulometry of the aggregates must be investigated. The resulting changes in rheology must be analyzed so that optimized mixtures can be designed.

Experimental studies for the w/c need to be performed to enhance either the thermal conductivity or the compression strength. For practical applications, solid guidelines for the handling of the biomass aggregate and the casting BLC, especially the adjustment of the water to binder ratio, must be implemented. Further studies must address fire behavior, water vapor diffusion, and alternatives to the cement binder, which continues to be the main CO_2 emitter.

5 Final Discussion

To shift from a fossil-based economy to a bioeconomy, actual crops have to be improved and crops which replace fossil resources have to be found (Schurr 2017). *Silphium perfoliatum* can play a significant role in the future bioeconomy, especially when breeding begins and it will be directed towards a material use of the plant.

The first objective of this thesis (I) was to analyze five European *S. perfoliatum* accessions for their phenotype, biomass and methane yield as a starting point for further breeding programs. Significant differences for the five traits were found: plant height, shoot diameter, internode number per shoot, inflorescence branch number and number of ray flowers per flower head. Another important plant parameter is the size of the cup plant parenchyma pores. As mentioned in Chapter 4, the pore size is essential for the thermal conductivity of insulation materials. Future investigations should analyze if there are also differences in pore quantity and pore volume between the accessions. Thus, some accessions could be favored for the use in insulation materials.

In addition to a typical harvest of *S. perfoliatum* in August with significant yield differences between the accessions (13.6–17.2 t ha^{-1}), some plots were not harvested until December. This atypical winter harvest was important for a further material use of the biomass. Only the senescent and dry stalks of *S. perfoliatum* form solid and appropriate amounts of parenchyma and the drying effort of the biomass is much lower (unpublished data). The yields reached 8.4–14.3 t ha^{-1}, which is lower when compared to Miscanthus (10–20 t ha^{-1}), but higher when compared to wheat straw (3.4–9.6 t ha^{-1}), which is also used as a raw material in the paper and building industries (Cappelletto et al. 2000; Barth et al. 2016; Espinosa et al. 2016; Dai et al. 2016).

The substantial variation in biomass and methane yields between accessions demonstrates the possibility of further improving these traits through selection and breeding. The analyses resulted in the discovery of 28,969 high-quality biallelic polymorphisms, that were useful for phylogenetic inference, genetic diversity and population structure estimations. Phenotyping cup plant for biomass and methane production is an expensive and time consuming process. Additionally, due to the high level of genotype x environment interactions, direct selection using these parameters may be ineffective. For effective breeding, more heritable and easily quantifiable morphological traits are required. The results of the phylogenetic and population structure analyses indicated that the analyzed plants shared a common ancestor and suggested a possible genetic diversity bottleneck within the gene pool of cultivated cup plant populations. Thus, there was a need to find more origins to avoid a possible bottleneck. As a result, Wever and Becker collected 42 additional origins in North America, which will be investigated at the campus in the following years (Wever and Becker 2016). This new introduced US-germplasm will maybe be a valuable cornerstone for further breeding work, aiming the de novo domestication of this wild plant. Drought resistance, nitrate scavenging, mycorrhizal associations and lodging after late harvest are all characteristics that should be monitored during domestication. Additionally, characteristics that influence the formation of soil organic matter, such as root, stem, and leaf anatomy and composition must be examined. The late harvest could possibly increase soil organic matter due to the accumulation of belowground biomass, and increase the cultivation period due to less nutrient removal.

Considering the shortage of paper resources (Berg and Lingqvist 2019), the second objective (II) was to evaluate if *S. perfoliatum*, *S. hermaphrodita* and meadow hay are possible feedstocks for the paper industry. It proved possible to produce paper from all three raw materials, so this thesis could be a step towards a diversification of the paper resources used in the industry. The pulping of the biomass using one method for all three plants, which simplifies the fibre production process, has worked. In the future, this knowledge could also allow the production of paper from a combination of all three plants, to take advantage of their different fibre properties. The successful pulping enabled the measuring of the fibre length of all raw materials. The fibre lengths are comparable to the average of hardwood trees, ranging between 0.5 and 1.5 mm (Ali et al. 2019; Muthu 2017; Björklund et al. 2016). This data is not only important for the paper industry in its effort to improve production processes; other fibre or fibre-reinforced products like textiles, biopolymers or building materials could also benefit from these findings.

Since the different mill types showed no effect on the fibre lengths, there is no need for the farmers to invest in a specific mill used only for the grinding of the paper feedstock; they can use mills they often already have. All three raw materials generated similar or higher bulk densities when compared to the control. Depending on the final product, a high bulk density can be favorable or obstructive. A higher bulk density in the packaging sector leads to a higher stiffness and volume of the cardboard, whereby raw material can be saved (Johansson 2011). On the other side, a high bulk is no option in the production of tissue or other very fine papers (Choi et al. 2019b). Further investigations would have to find alternative pulping and grinding techniques to lower the bulk.

The Meadow hay and birch blend pretreated in the cutting mill resulted in a 27% increase in tensile strength when compared to the control. Thus, birch pulp could be saved by adding meadow hay fibres; possible associated CO_2 savings should be investigated in the future.

Paper from cup plant and hay, after soda pulping, is a novelty and so far, unique. Their paper strength was comparable to that of the birch control. Virginia mallow and cup plant had the longest and widest fibres, most likely due to the thicker cell walls, but this remains to be verified (Annergren et al. 2009a). The unbleached fibres of the three plants are yellowish in color, thicker than the birch fibres and show a high strength potential. Cardboard for sustainable packaging could be a first application, where fibre strength and bulk are required but white bleached fibres are not necessary. Although bleaching of the fibers requires additional energy and is not necessary for many applications, it should still be tested to analyze its influence on the fibres. Further research and adjustments in all production steps will certainly improve the properties of these raw materials even further. For example, the separation of cortex and parenchyma, would allow more adjusted pulping methods for each plant part and thus could result in improved fibre properties.

The last objective (III) was to evaluate *S. perfoliatum* as a raw material for the building industry. Here, the focus was mainly on the parenchyma and its similarity to expanded polystyrene. The size measurement of the EPS pores and cup plant parenchyma cells revealed similar diameters of both materials. This could be a reason for the comparable insulation properties of the samples. Moreover, this finding could be of interest for other industry sectors; the parenchyma could be used as a sustainable alternative for petroleum-based packing chips or other insulation materials (Mellinger 1996). The determined water uptake of the biomass was important for the workability of the building material and a crucial factor for the water/cement ratio, and therefore the compression strength. The intentional or unintentional water uptake of the biomass will play a significant role for future building materials based on *S. perfoliatum*. Research to find a sustainable biomass coating to decrease the water uptake is of great importance, especially in cases where a water uptake is not wanted.

Regarding compression strength, some samples achieve the requirements of the commercial bonded leveling compound. A scaleup of the trials using standard processing machines like a screed pump or compulsory mixer are needed to see if the results can be transferred into practice. When combined with the apparent reversed density effect caused by the addition of cup plant, it is necessary to conclude that compatibility is the primary determinant of thermal conductivity, as is typically the case for insulation concrete (Samson et al. 2016). The thermal conductivity and compression strength of the samples are governed by the density of the raw materials. The maximum cup plant content of ≈ 45% is limited by the water/cement ratio and the rheology of the sample. The effect of comminution on the shape and granulometry of the aggregates, as well as the resulting changes in rheology, must be investigated in order to design optimized mixtures. If further investigations find a way to separate cortex and parenchyma, the sole use of parenchyma would decrease the density, decrease the thermal conductivity, and change the rheology of the building material.

An accession which loses all its leaves during ripening, or a separation of the leaves after harvest could have additional benefits. The leftover ingredients in the leafs (sugars, proteins) could cause an increase of the chemical oxygen demand of the pulp (Bantacut and Ardhiansyah 2018), while simultaneously not contributing to the strength of the paper. Their reduction would further improve the recyclability of the fibres. At the same time, remaining sugars could influence the setting process of cementitious building materials (Ahmad et al. 2020). Further research has to address the ripening of the different accessions. A low water content at winter harvest is a crucial economic factor, since energy cost for drying is likely to increase. Moreover, the topic of biomass comminution is poorly represented in literature. Most research is done in the field of rocks, minerals and plastics. However, biomass grinding and sieving are the basis for its material use. When compared to other raw materials after comminution, there is often no homogeneous size fraction. The material is divided into different size fractions and each fraction can have a different influence on the end product. However, the different size fractions could also go into different uses. Bigger particles could be of interest as animal litter and smaller ones as paper resource. Furthermore, it is important to know what influence the force that the mill exerts on the plant has on the different plant tissues. Finally, cascade utilization must be considered further. Work is already in progress on the usage of cup plant fibre after biogas utilization (Bergel 2021). Following this fibre use, the raw material could be used in the chemical industry, like unusable wood fibres, and finally used energetically (Risse et al. 2017). The results of this thesis indicate the potential and multiple applications of *Silphium perfoliatum* in the future.

6 Final Conclusion

Arable land is limited, and as a result, we have to use the available land and its biomass as efficiently and sustainably as possible. Each use must be assessed for its possible conflict with food production, resource efficiency, CO_2 savings, environmental impact and economics. Nutrient imports and exports, across agricultural cycles, must be drastically reduced. Lower production of animal products and a substitution of energetically used biomass by renewable energies frees up enough land to feed the world population and to make the industry independent of fossil resources (Willett et al. 2019; Material Economics 2021). Biomass is the main renewable carbon source; thus, its future use is important and inevitable (GBEP 2007). However, future biomass use has to change; the focus must be on direct and indirect material use. Energetic use should be favorized in special niches as part of a circular economy or following a cascade utilization. This thesis provides strong evidence that *Silphium perfoliatum* L. can play a significant role in the future bioeconomy.

So far, *Silphium perfoliatum* L. has undergone only minor breeding work so far. To begin breeding from here, with the additional origins from Wever and Becker, is a good starting point. The broad phenotypic and genetic diversity, be it biomass yield, methane content or high-quality biallelic polymorphisms, enables significant breeding opportunities. This makes it possible to adapt the plant precisely to climate change, the technological change in agriculture, and for future material uses. Possibly at the end of the breeding process, a hybrid variety will be developed whose seeds can be used for oil production, while its cortex can be used for paper production and its parenchyma for the production of insulating materials. As Wever et al. state, with the advent of new genomic-possibilities, the time has come for plant breeders to catalyze a long-term transformation of biomass agriculture (2020). The production of paper from the three different biomasses with good paper properties was feasible. The further development of the fibres with experts from the paper industry may lead to a diversification of the fiber supply. This could help meet the world's growing fibre demand and reduce dependencies in the supply of paper raw materials. This could result in less volatile prices and thus financial relief for consumers. More importantly, the use of wood could be reduced, resulting in an increase of forests and an increase in the amount of CO_2 that could be captured. More wood could go into the construction industry, a sector that stores the bound CO_2 for decades and thus contributes more to the decarbonizing of the atmosphere than when it is used in paper and pulp. Additionally, the use of these perennials will result in a variety of significant ecological benefits, such as increased biodiversity and soil health. Furthermore, the production of the raw materials is more decentralized, since arable land is more evenly distributed in Europe than forests for pulp production. This could reduce transport distances and increase regional value creation. Substantial energy savings must also be made in the building sector in order to protect the climate. The use of efficient heating technology is essential here. However, this can only be accomplished if the building is well insulated. Hence, more insulation of new and existing buildings will be needed in the future. Sustainable insulation materials are therefore required. The results of the *S. perfoliatum* insulation material are promising and should be more improved upon. Moreover, the production of a bonded leveling compound from 100% *S. perfoliatum* would not require any fossil oil for the production of the polystyrene. At the same time, the building material is a long-term storage of atmospheric CO_2. This building material could serve the environment twice, by saving fossil oil and by storing atmospheric CO_2. CO_2 certificates for the cultivation of perennials such as *S. perfoliatum* would be conceivable. Finally, the cultivation and utilization of perennial raw materials result in an increased humus content of pastures and cropland globally, while lowering the CO_2 footprint of two of the world's most energy-intensive industries.

The starting point for most of the findings mentioned above is agriculture, or more precisely, the farmers. They are the ones who grow the plants and make them available for the industry. So, each farmer can become a raw material producer for the industry with low investment, since the analyzed mills are commonly used in agriculture. This second mainstay gives them financial flexibility and security, which they lack today when deciding to grow perennials. The networking of agriculture and industry could also lead to a narrowing of the gap between society and agriculture.

In conclusion, the breeding potential of *Silphium perfoliatum* is high, its material use promising and its energetic use common practice. In an era of altered phosphate and nitrogen flows, and biodiversity loss, its low fertilizer requirements, humus accumulation and diverse biodiversity benefits will continue to pay off. All these points, whether in the field of breeding or the material use, lead to the conclusion that agriculture is most likely facing a 4th revolution by becoming the center of the new bioeconomy. This will allow agriculture to drastically reduce its contribution to the climate crisis, and make a substantial contribution to environmental protection and a net-negative carbon future.

Appendix

Chapter 2

Supplementary Table S1. List of 96 *S. perfoliatum* individuals analyzed using tGBS approach. The first 55 plants belonging to five accessions from TLL* were characterized in detail in the current study.

Accession name (if exists) and plant / seed provider	**Place**	**Country**	**Plant number**	**Numeric designation of accession**	**Numeric designation of individuals**
„USA“ (TLL)	**Dornburg**	**USA / Germany**	**11**	**1**	**1_1 etc.**
„East Germany“ (TLL)	**Dornburg**	**Germany**	**11**	**3**	**3_1 etc.**
„Russia“ (TLL)	**Dornburg**	**Russia / Germany**	**11**	**4**	**4_1 etc.**
„North Europa“ (TLL)	**Dornburg**	**North Europe / Germany**	**11**	**5**	**5_1 etc.**
„Ukraine“ (TLL)	**Dornburg**	**Ukraine / Germany**	**11**	**6**	**6_1 etc.**
Botanical Garden, Loci-Schmidt-Garten	Hamburg	Germany	6	7	7_1 etc.
Botanical Garden, HHU	Düsseldorf	Germany	6	11	11_1 etc.
Schau- und Sichtungsgarten Hermannshof e.V. (C. Schmidt)	Weinheim	Germany	6	13	13_1 etc.
SILP1, IPK Genebank	Gatersleben	Germany	5	8	8_1 etc.
SILP2, IPK Genebank	Gatersleben	Germany	4	9	9_1 etc.
„Wisconsin x Arkansas“ (K. Albrecht)	Jülich / Bayreuth	USA / Germany	8	10	10_1 etc.
Ontario Native Scape	Ontario	Canada	2	12	12_1 etc.
Botanical Garden, Basel	Pennsylvania	USA / Switzerland	3	14	14_1 etc.
„ssp. *connatum*“ (I. Kaczmarek)	Hamburg	USA / Germany	1	2	2_1
Total	-	-	96	-	-

* - Thuringian State Institute for Agriculture (Thüringer Landesanstalt für Landwirtschaft)

Supplementary Table S2. Analysis of variance (ANOVA) for six biomass traits over three years period (2014-2016) at Dornburg.

	Accession	**Year**	**Repetition**	**Accession:Year**	**Residuals**
DF	**4**	**2**	**3**	**8**	**42**
DMY_Sum_squares	82.93	22.16	46.59	54.27	171.37
DMY_Mean_squares	20.73	11.08	15.53	6.78	4.08
DMY_P_value	1.97E-03**	7.78E-02.	1.68E-02*	1.36E-01	
Ash_Sum_squares	2.26	2.48	4.91	5.57	18.40
Ash_Mean_squares	0.56	1.24	1.64	0.70	0.44
Ash_P_value	2.90E-01	7.06E-02.	1.81E-02*	1.57E-01	
ADF_Sum_squares	100.59	118.09	4.78	15.56	92.53
ADF_Mean_squares	25.15	59.05	1.59	1.94	2.20
ADF_P_value	2.32E-06***	3.15E-08***	5.43E-01	5.39E-01	
ADL_Sum_squares	5.12	14.93	0.60	2.29	18.39
ADL_Mean_squares	1.28	7.47	0.20	0.29	0.44
ADL_P_value	3.21E-02*	3.80E-06***	7.12E-01	7.29E-01	
DF	**4**	**2**			**8**
SMY_Sum_squares	361.73	1961.73			882.27
SMY_Mean_squares	90.43	980.87			110.28
SMY_P_value	5.47E-01	9.26E-03**			
MHY_Sum_squares	1359069.33	1262808.40			1424990.27
MHY_Mean_squares	339767.33	631404.20			178123.78
MHY_P_value	2.03E-01	7.90E-02.			

Signif. Codes: *** p < 0.001. **p < 0.01. * p < 0.05. . p < 0.

DMY, dry matter yield; ADL, acid detergent lignin; ADF, acid detergent fibre; SMY, specific methane yield; MHY, methane hectare yield

Supplementary Table S3. Analysis of variance (ANOVA) for eight morphological traits analyzed in 2017 at Rheinbach.

	Accession	**Residuals**
DF	**4**	**55**
SN1_Sum_squares	53.64	518.52
SN1_Mean_squares	13.41	9.43
SN1_P_value	2.39E-01	
PH1_Sum_squares	9582.09	18327.41
PH1_Mean_squares	2395.52	333.23
PH1_P_value	9.91E-05***	
IN1_Sum_squares	18.71	44.07
IN1_Mean_squares	4.68	0.80
IN1_P_value	5.46E-04***	
SD1_Sum_squares	69.97	248.01
SD1_Mean_squares	17.49	4.51
SD1_P_value	7.59E-03**	
PH2_Sum_squares	11231.08	14678.16
PH2_Mean_squares	2807.77	266.88
PH2_P_value	2.11E-06***	
SD2_Sum_squares	77.60	209.82
SD2_Mean_squares	19.40	3.81
SD2_P_value	1.47E-03**	
IBN1_Sum_squares	21.68***	38.75
IBN1_Mean_squares	5.42	0.70
IBN1_P_value	5.36E-05	
DF	4	32
RFN1_Sum_suares	431.73	257.67

*** p < 0.001. **p < 0.01. * p < 0.05. . p < 0.1

PH, plant height measured on July 11, 2017; SD1, shoot diameter at 10 cm above ground; SN1, shoot number per plant; IN1, internode number per shoot; IBN1, inflorescence branch number; RFN1, number of ray flowers per flower head; PH2, plant height measured on October 19, 2017; SD2, shoot diameter at 130 cm above ground.

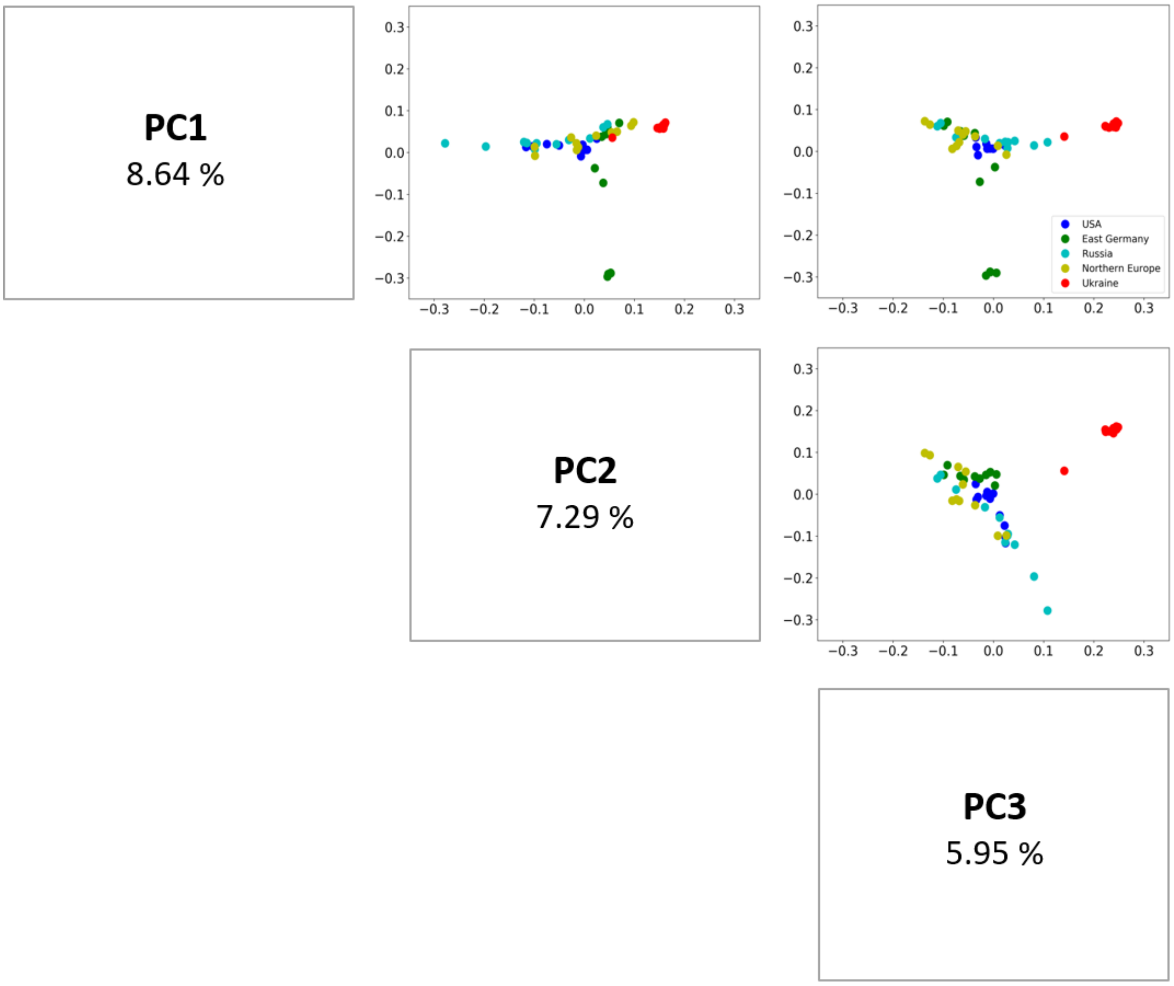
Supplementary Table S4. SNPs associated with cup plant morphological traits identified by the software TASSEL.

Trait	Marker	Chr	Pos	df	F	p	add_effect	add_F	add_p	dom_effect	dom_F	dom_p	errordf	MarkerR2	Genetic Var	Residual Var	-2LnLikelihood	NCBI BLASTN	NCBI BLASTX_hit	NCBI BLA	BLASTN HA41	BLASTN HA412HO_E-value
SD1	Silphium_1043395-133	SILPHIUM_1043395	133	2	45,09311	7,20E-11	-2,87E+00	6,81199	0,01278	-6,51E-01	0,33573	0,56564	40	0,63633	6,75803	0,06772	165,69122	no hits	no hits	-	-	-
SD1	Silphium_1043395-143	SILPHIUM_1043395	143	2	41,54182	1,73E-10	2,86066	7,13734	0,01087	-6,26E-01	0,32457	0,57206	41	0,61932	6,3867	0,07639	167,73306	no hits	no hits	-	-	-
SN1	Silphium_1019323-20	SILPHIUM_1019323	20	2	20,3397	1,55E-06	-2,23E+00	5,69599	0,02271	5,0929	39,99183	3,28E-07	35	0,42857	29,55454	1,0895	144,33283	no hits	no hits	-	-	-
SN1	Silphium_531473-19	SILPHIUM_531473	19	1	30,82026	1,75E-06	NaN	NaN	NaN	NaN	NaN	NaN	43	0,39438	1,98335	4,29675	185,81409	no hits	N66 matrix prot	2E-06	between Ha1	7.25×10^{-05}
SN1	Silphium_463461-119	SILPHIUM_463461	119	2	18,72206	2,41E-06	0,44766	0,3503	0,55754	-3,52E+00	21,6433	4,11E-05	38	0,46426	25,52714	1,32108	156,37299	no hits	no hits	-	-	-
SN1	Silphium_788395-89	SILPHIUM_788395	89	1	28,20151	3,86E-06	NaN	NaN	NaN	NaN	NaN	NaN	43	0,37459	1,09272	4,63303	186,56057	no hits	no hits	-	-	-
SN1	Silphium_515977-130	SILPHIUM_515977	130	1	27,59689	6,92E-06	NaN	NaN	NaN	NaN	NaN	NaN	37	0,33294	5,38E-05	5,37497	162,70678	no hits	no hits	-	-	-
SN1	Silphium_863504-118	SILPHIUM_863504	118	2	15,36985	1,38E-05	-4,39E-01	0,57816	0,45185	6,74372	29,4198	3,78E-06	38	0,42039	8,44879	2,09608	154,06902	no hits	no hits	-	-	-
IBN1	Silphium_513182-47	SILPHIUM_513182	47	2	14,24931	1,89E-05	1,5293	28,49669	3,53E-06	-5,49E-01	3,50599	0,06812	43	0,33435	1,5786	0,33056	91,04607	no hits	no hits	-	-	-
IBN1	Silphium_987112-119	SILPHIUM_987112	119	2	14,76744	1,93E-05	0,90396	24,48786	1,65E-05	0,66378	4,02712	0,05212	38	0,32905	5,18E-06	0,51847	81,71107	no hits	no hits	-	-	-
SN1	Silphium_1124957-37	SILPHIUM_1124957	37	2	14,19933	1,95E-05	-2,98E+00	14,66703	4,21E-04	1,16461	1,98779	0,16593	43	0,38464	6,10135	3,76385	189,9352	no hits	no hits	-	-	-
SN1	Silphium_1124957-136	SILPHIUM_1124957	136	2	14,19933	1,95E-05	2,98293	14,66703	4,21E-04	1,16461	1,98779	0,16593	43	0,38464	6,10135	3,76385	189,9352	no hits	no hits	-	-	-
PH2	Silphium_289785-105	SILPHIUM_289785	105	2	14,38647	2,09E-05	32,28661	28,69491	4,04E-06	-1,45E+01	5,22575	0,02777	40	0,36544	225,04688	145,93669	317,09241	no hits	**putative revers**	**2E-23**	**many chrom**	$\mathbf{4.94 \times 10^{-35}}$
SN1	Silphium_921869-71	SILPHIUM_921869	71	2	13,86599	3,43E-05	5,39821	23,3894	2,48E-05	-9,17E-01	0,52513	0,47334	37	0,4137	24,72829	2,39738	165,69536	no hits	no hits	-	-	-
SN1	Silphium_761162-31	SILPHIUM_761162	31	2	13,85223	3,45E-05	-3,68E+00	26,58729	9,31E-06	-1,14E+00	1,8536	0,18183	37	0,38372	22,39274	2,26602	164,49766	no hits	no hits	-	-	-
IBN1	Silphium_570147-69	SILPHIUM_570147	69	2	13,50494	3,71E-05	0,81834	23,29041	2,28E-05	-2,07E-01	0,66332	0,42047	39	0,35147	0,15104	0,52381	85,54196	no hits	no hits	-	-	-
SN1	Silphium_984750-176	SILPHIUM_984750	176	2	13,48997	3,98E-05	-4,07E+00	16,94936	2,06E-04	1,36807	0,53487	0,46918	38	0,4059	13,16657	2,68338	165,58221	no hits	no hits	-	-	-
SN1	Silphium_1075847-81	SILPHIUM_1075847	81	1	20,37359	4,13E-05	NaN	NaN	NaN	NaN	NaN	NaN	49	0,29152	12,64234	3,45298	220,80388	no hits	no hits	-	-	-
IN1	Silphium_417033-109	SILPHIUM_417033	109	2	12,78415	5,10E-05	-9,14E-01	23,89472	1,69E-05	-2,26E-01	0,77386	0,38428	41	0,28576	0,00254	0,52009	87,40631	no hits	uncharacterized	1E-05	-	-
PH2	Silphium_288016-43	SILPHIUM_288016	43	2	12,95032	5,14E-05	20,01327	19,23675	8,84E-05	35,52088	14,68589	4,63E-04	39	0,26963	14,31596	176,99436	305,39203	no hits	uncharacterized	1E-05	-	-
SD2	Silphium_122074-158	SILPHIUM_122074	158	2	13,15813	5,48E-05	2,14074	13,83988	6,96E-04	-6,39E-01	0,59971	0,44389	36	0,31793	0,35694	2,40884	131,84797	no hits	**protein tesmin,**	**9E-11**	**Ha5_000053**	$\mathbf{5.97 \times 10^{-34}}$
IBN1	Silphium_207617-108	SILPHIUM_207617	108	1	19,66134	6,09E-05	NaN	NaN	NaN	NaN	NaN	NaN	45	0,24708	1,7288	0,35726	97,40735	no hits	**uncharacterize**	**1E-22**	**many chrom**	$\mathbf{1.84 \times 10^{-40}}$
IN1	Silphium_417033-65	SILPHIUM_417033	65	2	12,41137	6,10E-05	0,89593	20,1966	5,61E-05	-3,69E-01	1,50287	0,22723	42	0,28991	5,86E-06	0,58628	95,47291	no hits	uncharacterized	1E-05	-	-
RFN1	Silphium_942393-72	SILPHIUM_942393	72	2	14,91223	6,18E-05	0,34108	0,23747	0,63046	5,28807	29,671	1,35E-05	25	0,51449	81,79996	3,77804	108,88764	no hits	no hits	-	-	-
IBN1	Silphium_554308-55	SILPHIUM_554308	55	2	12,25814	6,41E-05	1,49129	19,25201	7,56E-05	-2,98E-01	0,23287	0,63191	43	0,30444	2,38746	0,33919	95,04746	no hits	no hits	-	-	-
PH1	Silphium_51173-26	SILPHIUM_51173	26	2	12,17922	6,44E-05	-2,47E+01	23,82279	1,49E-05	15,896	5,14339	0,02842	44	0,32124	235,99189	0,00236	346,27081	no hits	no hits	-	-	-
SN1	Silphium_1041243-124	SILPHIUM_1041243	124	2	12,30203	6,85E-05	0,04457	0,00251	0,9603	8,12626	19,95929	6,34E-05	41	0,37027	5,93796	4,37025	185,11101	no hits	no hits	-	-	-
IBN1	Silphium_1174705-5	SILPHIUM_1174705	5	2	13,00094	6,86E-05	0,22272	1,63667	0,20971	1,95568	25,92741	1,41E-05	34	0,34735	3,09348	0,26746	76,10605	no hits	no hits	-	-	-
IBN1	Silphium_808587-83	SILPHIUM_808587	83	1	19,74688	7,11E-05	NaN	NaN	NaN	NaN	NaN	NaN	40	0,28269	1,06705	0,3644	85,7241	no hits	**uncharacterize**	**8E-21**	**many chrom**	$\mathbf{2.83 \times 10^{-51}}$
SN1	Silphium_94402-129	SILPHIUM_94402	129	2	12,21367	7,24E-05	-1,44E+00	2,15545	0,14988	9,11396	23,6815	1,81E-05	41	0,36955	6,74539	4,03759	183,44749	no hits	no hits	-	-	-
IBN1	Silphium_679751-88	SILPHIUM_679751	88	2	12,05602	7,28E-05	1,16621	11,68496	0,00141	-2,11E+00	24,10287	1,43E-05	43	0,30401	1,94861	0,36287	96,09637	no hits	hypothetical pro	3E-09	-	-
SN1	Silphium_966382-14	SILPHIUM_966382	14	2	12,12716	7,29E-05	3,0658	20,41072	5,21E-05	-2,94E-01	0,03815	0,84611	42	0,34573	17,10466	2,44009	183,96741	no hits	no hits	-	-	-
SD1	Silphium_779511-34	SILPHIUM_779511	34	2	12,34896	7,79E-05	0,66189	7,87787	0,00794	-1,52E+00	13,42322	7,74E-04	38	0,36866	5,70837	0,11879	154,02748	no hits	no hits	-	-	-
SD1	Silphium_779511-84	SILPHIUM_779511	84	2	12,34896	7,79E-05	-6,62E-01	7,87787	0,00794	-1,52E+00	13,42322	7,74E-04	38	0,36866	5,70837	0,11879	154,02748	no hits	no hits	-	-	-
IN1	Silphium_417033-96	SILPHIUM_417033	96	2	12,04772	8,03E-05	-8,81E-01	22,54718	2,63E-05	-2,35E-01	0,77473	0,38402	41	0,28074	0,02259	0,52441	88,05721	no hits	uncharacterized	1E-05	-	-
IBN1	Silphium_669513-41	SILPHIUM_669513	41	2	11,56903	9,18E-05	1,35987	19,35805	6,79E-05	0,18274	0,14371	0,70644	45	0,27141	2,72812	0,32903	99,98784	no hits	**uncharacterize**	**5E-22**	**many chrom**	$\mathbf{6.04 \times 10^{-40}}$
SN1	Silphium_921869-49	SILPHIUM_921869	49	2	11,96654	9,32E-05	-5,45E+00	23,47801	2,15E-05	-1,99E+00	3,12395	0,08518	39	0,35648	23,45158	2,18774	173,32723	no hits	no hits	-	-	
IBN1	Silphium_918427-113	SILPHIUM_918427	113	2	11,79218	9,42E-05	-6,55E-01	5,31165	0,02645	-1,46E+00	23,27909	2,07E-05	41	0,31561	1,02227	0,35653	83,63148	no hits	no hits	-	-	

Sequences harboring several significant SNPs are designated in color

PH, plant height measured on July 11, 2017; SD1, shoot diameter at 10 cm above ground; SN1, shoot number per plant; IN1, internode number per shoot; IBN1, inflorescence branch number; RFN1, number of ray flowers per flower head; PH2, plant height measured on October 19, 2017; SD2, shoot diameter at 130 cm above ground

Supplementary Figure S1. Principal component analysis (PCA) performed based on a dataset of 28,969 SNPs. Only plants belonging to the five TLL accessions are shown, the same color code as in the Fig. A 2 is used. Percentages of variance explained by first three components are indicated.



Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.biombioe.2019.03.016.

Bibliography

Abl (2021): Der kritische Agrarbericht 2021. Schwerpunkt: Welt im Fieber - Klima & Wandel. Konstanz/Hamm: Arbeitsgemeinschaft bäuerliche Landwirtschaft e.V., Bauernblatt Verlag.

Acikel, H. (2011): The use of *miscanthus* (*Giganteus*) as a plant fiber in concrete production. In *Scientific Research and Essays*. DOI: 10.5897/SRE10.1139.

Ahmad, S.; Lawan, A.; Al-Osta, M. (2020): Effect of sugar dosage on setting time, microstructure and strength of Type I and Type V Portland cements. In *Case Studies in Construction Materials* 13, e00364. DOI: 10.1016/j.cscm.2020.e00364.

Albrecht, K.A.; Goldstein, W. (1997): Silphium perfoliatum: A North American Prairie Plant with Potential as a Forage Crop. In *The XVIII International Grassland Congress*, pp. 167–168.

Ali, H.M.; Ariffin, H.; Sapuan, S.M. (2019): Lignocellulose for future bioeconomy. Amsterdam: Elsevier. Available online at https://www.sciencedirect.com/science/book/9780128163542.

Allwright, M.R.; Taylor, G. (2016): Molecular Breeding for Improved Second Generation Bioenergy Crops. In *Trends in Plant Science* 21 (1), pp. 43–54. DOI: 10.1016/j.tplants.2015.10.002.

Anderegg, W.R.L.; Prall, J.W.; Harold, J.; Schneider, S.H. (2010): Expert credibility in climate change. In *Proceedings of the National Academy of Sciences of the United States of America* 107 (27), pp. 12107–12109. DOI: 10.1073/pnas.1003187107.

Anders, N.; Humann, H.; Langhans, B.; Spieß, A.C. (2015): Simultaneous determination of acid-soluble biomass-derived compounds using high performance anion exchange chromatography coupled with pulsed amperometric detection. In *Analytical Methods* 7 (18), pp. 7866–7873. DOI: 10.1039/c5ay01371b.

Andreasson, B.; Bristow, A.; Fellers, C.; Nygards, C.; Östlund, S.; Söremark, C. et al. (Eds.) (2009): Paper products physics and technology. Berlin: De Gruyter (Pulp and paper chemistry and technology, 4).

Annergren, G.; Backlund, B.; Brännvall, E.; Engstrand, P.; Gellerstedt, G.; Germgard, U. (2009a): Pulp and Paper Chemistry and Technology. Pulping Chemistry and Technology. Berlin/Boston, Ann Arbor, Michigan: De Gruyter (2).

Annergren, G.; Engström, G.; Hagen, N.; Lindström, T.; Norman, B.; Salmen, L. et al. (2009b): Pulp and Paper Chemistry and Technology. Paper Chemistry and Technology. Berlin/Boston: De Gruyter (3).

Assefa, T.; Wu, J.; Albrecht, K.A.; Johnson, P.J.; Boe, A. (2015a): Genetic Variation for Biomass and Related Morphological Traits in Cup Plant (Silphium perfoliatum L.). In *American Journal of Plant Sciences* 06 (08), pp. 1098–1108. DOI: 10.4236/ajps.2015.68114.

Assefa, T.; Wu, J.; Boe, A. (2015b): Genetic Variation for Achene Traits in Cup Plant (Silphium perfoliatum L.). In *Open Journal of Genetics* 05 (02), pp. 71–82. DOI: 10.4236/ojgen.2015.52006.

Badouin, H.; Gouzy, J.; Grassa, C.J.; Murat, F.; Staton, S.E.; Cottret, L. et al. (2017): The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution. In *Nature* 546 (7656), pp. 148–152. DOI: 10.1038/nature22380.

Bai, C.; Alverson, W.S.; Follansbee, A.; Waller, D.M. (2012): New reports of nuclear DNA content for 407 vascular plant taxa from the United States. In *Annals of Botany* 110 (8), pp. 1623–1629. DOI: 10.1093/aob/mcs222.

Bantacut, T.; Ardhiansyah, E.L. (2018): Chemical Oxygen Demand Balance and Energy Recovery in Wastewater Treatment Plant of Pulp and Paper Industry (Corrugated Board). 8th ed. (18).

Barth, S.; Murphy-Bokern, D.; Kalinina, O.; Taylor, G.; Jones, M. (Eds.) (2016): Perennial Biomass Crops for a Resource-Constrained World. Cham: Springer International Publishing.

Bauböck, R.; Karpenstein-Machan, M.; Kappas, M. (2014): Computing the biomass potentials for maize and two alternative energy crops, triticale and cup plant (Silphium perfoliatum L.), with the crop model BioSTAR in the region of Hannover (Germany). In *Environmental Sciences Europe* 26 (1), p. 19. DOI: 10.1186/s12302-014-0019-0.

Benfratello, S.; Capitano, C.; Peri, G.; Rizzo, G.; Scaccianoce, G.; Sorrentino, G. (2013): Thermal and structural properties of a hemp–lime biocomposite. In *Construction and Building Materials* 48, pp. 745–754. DOI: 10.1016/j.conbuildmat.2013.07.096.

Bengtsson, H. (2022): Functions that Apply to Rows and Columns of Matrices (and to Vectors) [R package matrixStats version 0.62.0]: Comprehensive R Archive Network (CRAN). Available online at https://cran.r-project.org/web/packages/matrixStats/index.html, checked on 8/13/2022.

Benke, M.; Formowitz, B.; Glauert, T.; Heiermann, M.; Idler, C. (2012): Energiepflanzen für Biogasanlagen: Fachagentur Nachwachsende Rohstoffe e.V. (553). Available online at https://bit.ly/3ISVwX6, checked on 8/13/2022.

Berg, P.; Lingqvist, O. (2019): Pulp, paper, and packaging in the next decade: Transformational change. In *McKinsey & Company*, 8/7/2019. Available online at https://www.mckinsey.com/industries/paper-forest-products-and-packaging/our-insights/pulp-paper-and-packaging-in-the-next-decade-transformational-change, checked on 8/13/2022.

Bergel, S. (2021): Ökobilanz von Silphie-Fasern - Fraunhofer UMSICHT. Fraunhofer-Institut für Umwelt-,Sicherheits- und Energietechnik UMSICHT. Available online at https://www.umsicht.fraunhofer.de/de/presse-medien/pressemitteilungen/2021/oekobilanz-silphiefasern.html, updated on 8/12/2022, checked on 8/12/2022.

Bergonzi, S.; Albani, M.C. (2011): Reproductive competence from an annual and a perennial perspective. In *Journal of Experimental Botany* 62 (13), pp. 4415–4422. DOI: 10.1093/jxb/err192.

Berk, Z. (2018): Size reduction. In *Food Process Engineering and Technology.*, pp. 165–191. DOI: 10.1016/B978-0-12-812018-7.00006-3.

Biertümpfel, A. (2012): Korbblütler könnte Mais verdrängen. Sonderheft Energiepflanzen. In *Biogas Journal*, p. 38. Available online at https://www.biogas.org/edcom/webfvb.nsf/id/de_bj-energiepflanzen-2012, checked on 8/13/2022.

Biertümpfel, A.; Conrad, M. (2013): Abschlussbericht zum Verbundvorhaben: Erhöhung des Leistungspotenzials und der Konkurrenzfähigkeit der Durchwachsenen Silphie als Energiepflanze durch Züchtung und Optimierung des Anbauverfahrens. Teilvorhaben 2. Optimierung des Anbauverfahrens und Bereitstellung von Selektionsmaterial. Available online at www.fnr-server.de/ftp/pdf/berichte/22012809.pdf, checked on 8/13/2022.

Björklund, M.J.; Brannvall, E.; Daniel, G.; Gellerstedt, G.; Henriksson, G.; Lennholm, H. et al. (2016): Wood chemistry and wood biotechnology. Berlin: De Gruyter (Pulp and Paper Chemistry and Technology). Available online at https://books.google.de/books?id=95QX7M_Uc5sC.

BMELV (2012): National Programme for the Conservation and Sustainable Use of Plant Genetic Resources of Agricultural and Horticultural Crops. In *Federal Ministry of Food, Agriculture and Consumer Protection*. Available online at https://www.genres.de/fileadmin/SITE_MASTER/content/Publikationen/Plant_Genetic_Resources_NationF_en.pdf, checked on 8/13/2022.

Boe, A.; Albrecht, K.A.; Johnson, P.J.; Wu, J. (2019a): Biomass Production of Cup Plant (Silphium perfoliatum L.) in Response to Variation in Plant Population Density in the North Central USA. In *American Journal of Plant Sciences* 10 (06), pp. 904–910. DOI: 10.4236/ajps.2019.106065.

Boe, A.; Albrecht, K.A.; Johnson, P.J.; Wu, J. (2019b): Biomass Production of Monocultures and Mixtures of Cup Plant and Native Grasses on Prime and Marginal Cropland. In *American Journal of Plant Sciences* 10 (06), pp. 911–924. DOI: 10.4236/ajps.2019.106066.

Boix, E.; Georgi, F.; Navard, P. (2016): Influence of alkali and Si-based treatments on the physical and chemical characteristics of miscanthus stem fragments. In *Industrial Crops and Products* 91, pp. 6–14. DOI: 10.1016/j.indcrop.2016.06.030.

Borkowska, H.; Molas, R.; Kupczyk, A. (2009): Virginia fanpetals (Sida hermaphrodita Rusby) cultivated on light soil; height of yield and biomass productivity. In *Polish J. Environ. Stud.* 18, pp. 563–568.

Borkowska H.; Styk B. (2006): Ślazowiec pensylwański (Sida hermphrodita rusby) uprawa i wykorzystanie (The Virginia mallow (Sida hermphrodita rusby): Cultivation and utilization): University of Life Sciences, Lublin.

Borrega, M.; Sixta, H. (2013): Purification of cellulosic pulp by hot water extraction. In *Cellulose* 20 (6), pp. 2803–2812. DOI: 10.1007/s10570-013-0086-1.

Bozsaky, D. (2019): Nature-Based Thermal Insulation Materials From Renewable Resources – A State-Of-The-Art Review. In *Slovak Journal of Civil Engineering* 27 (1), pp. 52–59. DOI: 10.2478/sjce-2019-0008.

Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. (2007): Tassel: software for association mapping of complex traits in diverse samples. In *Bioinformatics* 23 (19), pp. 2633–2635. DOI: 10.1093/bioinformatics/btm308.

Brambilla, V.; Gomez-Ariza, J.; Cerise, M.; Fornara, F. (2017): The Importance of Being on Time: Regulatory Networks Controlling Photoperiodic Flowering in Cereals. In *Frontiers in Plant Science* 8, p. 665. DOI: 10.3389/fpls.2017.00665.

Britannica (2022): grass | Definition, Families, & Facts. Encyclopædia Britannica. Available online at https://www.britannica.com/plant/grass, updated on 2/17/2022, checked on 8/13/2022.

Brouard, Y.; Belayachi, N.; Hoxha, D.; Ranganathan, N.; Méo, S. (2018): Mechanical and hygrothermal behavior of clay – Sunflower (Helianthus annuus) and rape straw (Brassica napus) plaster bio-composites for building insulation. In *Construction and Building Materials* 161, pp. 196–207. DOI: 10.1016/j.conbuildmat.2017.11.140.

Bueno, M.; Shi, F.; Kojovic, T.; Powell, M. (2010): Investigation on multicomponent semi-autogenous grinding. In *XXV International Mineral Processing Congress 2010, IMPC 2010* 1.

Burley, J.; Youngquist, J.; Evans, J. (2004): Encyclopedia of forest sciences. 1st ed. Oxford: Elsevier. Available online at https://books.google.de/books?id=O4eMWqdluM8C.

Burmeister, J.; Walter, R. (2016): Untersuchungen zur ökologischen Wirkung der Durchwachsenen Silphie aus Bayern. Themenheft Durchwachsene Silphie. Journal für Kulturpflanzen (10.5073/jfk.2016.12). Available online at https://ojs.openagrar.de/index.php/Kulturpflanzen journal/article/view/13223, checked on 8/13/2022.

Bury, M.; Możdżer, E.; Kitczak, T.; Siwek, H.; Włodarczyk, M. (2020): Yields, Calorific Value and Chemical Properties of Cup Plant Silphium perfoliatum L. Biomass, Depending on the Method of Establishing the Plantation. In *Agronomy* 10 (6), p. 851. DOI: 10.3390/agronomy10060851.

Bury, M.; Rusinowski, S.; Sitko, K.; Krzyżak, J.; Kitczak, T.; Możdżer, E. et al. (2021): Physiological status and biomass yield of Sida hermaphrodita (L.) Rusby cultivated on two distinct marginal lands in Southern and Northern Poland. In *Industrial Crops and Products* 167, p. 113502. DOI: 10.1016/j.indcrop.2021.113502.

Buzała, K.; Kalinowska, H.; Małachowska, E.; Boruszewski, P.; Krajewski, K.; Przybysz, P. (2019): The Effect of Lignin Content in Birch and Beech Kraft Cellulosic Pulps on Simple Sugar Yields from the Enzymatic Hydrolysis of Cellulose. In *Energies* 12 (15), p. 2952. DOI: 10.3390/en12152952.

Cappelletto, P.; Mongardini, F.; Barberi, B.; Sannibale, M.; Brizzi, M.; Pignatelli, V. (2000): Papermaking pulps from the fibrous fraction of Miscanthus x Giganteus. In *Industrial Crops and Products* 11 (2-3), pp. 205–210. DOI: 10.1016/S0926-6690(99)00051-5.

Caprai, V.; Gauvin, F.; Schollbach, K.; Brouwers, H.J.H. (2018): Influence of the spruce strands hygroscopic behaviour on the performances of wood-cement composites. In *Construction and Building Materials* 166, pp. 522–530. DOI: 10.1016/j.conbuildmat.2018.01.162.

Castillo-Lara, J.F.; Flores-Johnson, E.A.; Valadez-Gonzalez, A.; Herrera-Franco, P.J.; Carrillo, J.G.; Gonzalez-Chi, P.I.; Li, Q.M. (2020): Mechanical Properties of Natural Fiber Reinforced Foamed Concrete. In *Materials (Basel, Switzerland)* 13 (14), p. 3060. DOI: 10.3390/ma13143060.

Challinor, A.J.; Watson, J.; Lobell, D.B.; Howden, S.M.; Smith, D.R.; Chhetri, N. (2014): A meta-analysis of crop yield under climate change and adaptation. In *Nature Climate Change* 4 (4), pp. 287–291. DOI: 10.1038/nclimate2153.

Chaloupková, V.; Ivanova, T.; Krepl, V. (2019): Particle size and shape characterization of feedstock material for biofuel production. 766.2Kb. DOI: 10.15159/AR.19.152.

Chen, Y.X.; Wu, F.; Yu, Q.; Brouwers, H.J.H. (2020): Bio-based ultra-lightweight concrete applying miscanthus fibers: Acoustic absorption and thermal insulation. In *Cement and Concrete Composites* 114, p. 103829. DOI: 10.1016/j.cemconcomp.2020.103829.

Cherubini, F.; Ulgiati, S. (2010): Crop residues as raw materials for biorefinery systems – A LCA case study. In *Applied Energy* 87 (1), pp. 47–57. DOI: 10.1016/j.apenergy.2009.08.024.

Chifman, J.; Kubatko, L. (2014): Quartet inference from SNP data under the coalescent model. In *Bioinformatics* 30 (23), pp. 3317–3324. DOI: 10.1093/bioinformatics/btu530.

Choi, H.S.; Grethe, H.; Entenmann, S.K.; Wiesmeth, M.; Blesl, M.; Wagner, M. (2019a): Potential trade-offs of employing perennial biomass crops for the bioeconomy in the EU by 2050: Impacts on agricultural markets in the EU and the world. In *GCB Bioenergy* 11 (3), pp. 483–504. DOI: 10.1111/gcbb.12596.

Choi, J.H.; Lee, D.; O'Connor, L.; Chalup, S.; Welsh, J.S.; Dowling, J.; Greer, P.B. (2019b): Bulk Anatomical Density Based Dose Calculation for Patient-Specific Quality Assurance of MRI-Only Prostate Radiotherapy. In *Frontiers in Oncology* 9, p. 997. DOI: 10.3389/fonc.2019.00997.

Churkina, G.; Organschi, A.; Reyer, C.P.O.; Ruff, A.; Vinke, K.; Liu, Z. et al. (2020): Buildings as a global carbon sink. In *Nature Sustainability* 3 (4), pp. 269–276. DOI: 10.1038/s41893-019-0462-4.

Clevinger, J.A.; Panero, J.L. (2000): Phylogenetic analysis of Silphium and subtribe Engelmanniinae (Asteraceae: Heliantheae) based on ITS and ETS sequence data. In *American Journal of Botany* 87 (4), pp. 565–572. DOI: 10.2307/2656600.

Conrad, M.; Biertümpfel, A.; Vetter, A. (2009): Durchwachsene Silphie (Silphium perfoliatum L.) – von der Futterpflanze zum Koferment F.N.R. Gülzower Fachgespräche. In *Gülzower Fachgespräche*, pp. 281–289.

Cossel, M. von; Amarysti, C.; Wilhelm, H.; Priya, N.; Winkler, B.; Hoerner, L. (2020): The replacement of maize (Zea mays L.) by cup plant (Silphium perfoliatum L.) as biogas substrate and its implications for the energy and material flows of a large biogas plant. In *Biofuels, Bioproducts and Biorefining* 14 (2), pp. 152–179. DOI: 10.1002/bbb.2084.

Cossel, M. von; Lewandowski, I.; Elbersen, B.; Staritsky, I.; van Eupen, M.; Iqbal, Y. et al. (2019): Marginal Agricultural Land Low-Input Systems for Biomass Production. In *Energies* 12 (16), p. 3123. DOI: 10.3390/en12163123.

Craine, J.M.; Ocheltree, T.W.; Nippert, J.B.; Towne, E.G.; Skibbe, A.M.; Kembel, S.W.; Fargione, J.E. (2013): Global diversity of drought tolerance and grassland climate-change resilience. In *Nature Climate Change* 3 (1), pp. 63–67. DOI: 10.1038/nclimate1634.

Crews, T.E.; Carton, W.; Olsson, L. (2018): Is the future of agriculture perennial? Imperatives and opportunities to reinvent agriculture by shifting from annual monocultures to perennial polycultures. In *Global Sustainability* 1. DOI: 10.1017/sus.2018.11.

Crews, T.E.; Rumsey, B. (2017): What Agriculture Can Learn from Native Ecosystems in Building Soil Organic Matter: A Review. In *Sustainability* 9 (4), p. 578. DOI: 10.3390/su9040578.

Cumplido-Marin, L.; Graves, A.R.; Burgess, P.J.; Morhart, C.; Paris, P.; Jablonowski, N.D. et al. (2020): Two Novel Energy Crops: Sida hermaphrodita (L.) Rusby and Silphium perfoliatum L.—State of Knowledge. In *Agronomy* 10 (7), p. 928. DOI: 10.3390/agronomy10070928.

Dahlson-Rutherford, C. (2012): Renewable Raw Materials in the Industrial Chemical Industry. In *ESSAI: Vol. 10, Article 15.* Available online at http://dc.cod.edu/essai/vol10/iss1/15, checked on 8/13/2022.

Dai, J.; Bean, B.; Brown, B.; Bruening, W.; Edwards, J.; Flowers, M. et al. (2016): Harvest index and straw yield of five classes of wheat. In *Biomass and Bioenergy* 85, pp. 223–227. DOI: 10.1016/j.biombioe.2015.12.023.

Damm, T.; Grande, P.M.; Jablonowski, N.D.; Thiele, B.; Disko, U.; Mann, U. et al. (2017a): OrganoCat pretreatment of perennial plants: Synergies between a biogenic fractionation and valuable feedstocks. In *Bioresource Technology* 244 (Pt 1), pp. 889–896. DOI: 10.1016/j.biortech.2017.08.027.

Damm, T.; Pattathil, S.; Günl, M.; Jablonowski, N.D.; O'Neill, M.; Grün, K.S. et al. (2017b): Insights into cell wall structure of Sida hermaphrodita and its influence on recalcitrance. In *Carbohydrate Polymers* 168, pp. 94–102. DOI: 10.1016/j.carbpol.2017.03.062.

Danecek, P.; Auton, A.; Abecasis, G.; Albers, C.A.; Banks, E.; DePristo, M.A. et al. (2011): The variant call format and VCFtools. In *Bioinformatics* 27 (15), pp. 2156–2158. DOI: 10.1093/bio.

Daniel, P.; Rompf, R. (1994): Possibilities and limits in the utilization of Silphium perfoliatum as a fodder plant, renewable raw material and a landscape conservation plant. In *Agribiological research* 47, p. 345.

Danielewicz, D.; Surma-Ślusarska, B. (2017): Properties and fibre characterisation of bleached hemp, birch and pine pulps: a comparison. In *Cellulose* 24 (11), pp. 5173–5186. DOI: 10.1007/s10570-017-1476-6.

Danielewicz, D.; Surma-Ślusarska, B. (2019): Miscanthus × giganteus stalks as a potential non-wood raw material for the pulp and paper industry. Influence of pulping and beating conditions on the fibre and paper properties. In *Industrial Crops and Products* 141, p. 111744. DOI: 10.1016/j.indcrop.2019.111744.

Danielewicz, D.; Surma-Ślusarska, B.; Żurek, G.; Martyniak, D.; Kmiotek, M.; Dybka, K. (2015): Selected Grass Plants as Biomass Fuels and Raw Materials for Papermaking, Part II. Pulp and Paper Properties. In *BioResources* 10 (4). DOI: 10.15376/biores.10.4.8552-8564.

Deuker, A.; Stinner, W.; Rensberg, N.; Wagner, L.; Hummer, H.E. (2012): Regional risks for biogas production in Germany by maize pest Diabrotica v. virgifera? In *Journal of Agricultural Science and Technology* 2, pp. 749–767.

Di Vita, G.; Pilato, M.; Pecorino, B.; Brun, F.; D'Amico, M. (2017): A Review of the Role of Vegetal Ecosystems in CO2 Capture. In *Sustainability* 9 (10), p. 1840. DOI: 10.3390/su9101840.

Dietz, W.; Cruse, F.; Höller, M.; Szafera, S. (2015): Entwicklung eines Verfahrens zur Gewinnung von Gras als Rohstoff und Verarbeitung für die Herstellung von Papierprodukten unter besonderer Berücksichtigung des Aufbaus einer nachhaltigen Wertschöpfungskette. Az: 30990/01. In *Abschlussbericht Deutsche Bundesstiftung Umwelt (DBU)*. Available online at https://www.dbu.de/708ibook81883_38339_2486.html, checked on 7/23/2022.

Dimitrijevic, A.; Horn, R. (2017): Sunflower Hybrid Breeding: From Markers to Genomic Selection. In *Frontiers in Plant Science* 8, p. 2238. DOI: 10.3389/fpls.2017.02238.

Diquélou, Y.; Gourlay, E.; Arnaud, L.; Kurek, B. (2015): Impact of hemp shiv on cement setting and hardening: Influence of the extracted components from the aggregates and study of the interfaces with the inorganic matrix. In *Cement and Concrete Composites* 55, pp. 112–121. DOI: 10.1016/j.cemconcomp.2014.09.004.

Domínguez-Muñoz, F.; Anderson, B.; Cejudo-López, J.M.; Carrillo-Andrés, A. (2010): Uncertainty in the thermal conductivity of insulation materials. In *Energy and Buildings* 42 (11), pp. 2159–2168. DOI: 10.1016/j.enbuild.2010.07.006.

Doudart de la Grée, G.C.H.; Yu, Q.L.; Brouwers, H.J.H. (2014): Wood-wool cement board : potential and challenges, pp. 279–282. Available online at https://pure.tue.nl/ws/files/3841378/449247297565249.pdf, checked on 8/13/2022.

Doudart de la Grée, G.C.H.; Yu, Q.L.; Brouwers, H.J.H. (2015): The effect of glucose on the hydration kinetics of ordinary portland cement. Clermont-Ferrand, France. In *First International Conference on Bio-based Building Materials*.

Edwards, W.M.; Shipitalo, M.J. (1998): Consequences of earthworms in agricultural soils: aggregation and porosity.: St. Lucie Press.

EESI (2021): Fossil Fuels. Environmental and Energy Study Institute. Available online at https://www.eesi.org/topics/fossil-fuels/description, updated on 4/8/2022, checked on 8/13/2022.

Eickhout, B.; Gjaltema, J.; Jong, F. de (2012): A strategy for a bio-based economy. In *Green European Foundation* (9). Available online at https://gef.eu/wp-content/uploads/2017/01/A_strategy_for_a_bio-based_economy.pdf.

Elshire, R.J.; Glaubitz, J.C.; Sun, Q.; Poland, J.A.; Kawamoto, K.; Buckler, E.S.; Mitchell, S.E. (2011): A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. In *PLoS ONE* 6 (5), e19379. DOI: 10.1371/journal.pone.0019379.

EOD (2022): Earth Overshoot Day 2022. Earth Overshoot Day. Available online at https://www.overshootday.org/about-earth-overshoot-day/, updated on 4/6/2022, checked on 8/13/2022.

Espinosa, E.; Tarrés, Q.; Delgado-Aguilar, M.; González, I.; Mutjé, P.; Rodríguez, A. (2016): Suitability of wheat straw semichemical pulp for the fabrication of lignocellulosic nanofibres and their application to papermaking slurries. In *Cellulose* 23 (1), pp. 837–852. DOI: 10.1007/s10570-015-0807-8.

EU Regulation 152 (2009): Regulation (EU) 152/2009 of the European Parliament and of the Council of the European Union on 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. In *Offi. J. Eur. Union*.

EU Regulation 2393 (2017): Regulation (EU) 2017/2393 of the European Parliament and of the Council of 13 December 2017 amending Regulations (EU) No 1305/2013 on support for rural development by the European Agricultural Fund for Rural Development (EAFRD). In *Off. J. Eur. Union*.

European Commission (2018a): Commission delegated regulation - amending delegated regulation (EU) no 639/2014 as regards certain provisions on the greening practices established by regulation (EU) no 1307/2013 of the European Parliament and of the council. L 293, revised 61. Available online at https://eur-lex.europa.eu/legal-content/ES/TXT/?uri=OJ%3AL%3A2018%3A293%3ATOC.

European Commission (2018b): Directive (EU) 2018/2001 of the European Parliament and of the Council of 11 of December 2018 on the promotion of the use of energy from renewable sources. Available online at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.328.01.0082.01.ENG.

Falkehag, S.I.; Martin, J.; Adler, E. (1966): Chromophores in Kraft Lignin. In *Advances in Chemistry* 59 (59), pp. 75–89. DOI: 10.1021/ba-1966-0059.ch007.

FAO (2015): Pulp and paper capacities 2014-2019. Food and Agriculture Organization of the United Nations. Available online at https://www.fao.org/documents/card/en/c/5f6de718-9db4-43a4-84fc-228ac1c36ade/, checked on 8/13/2022.

FAO (2019): Fighting climate change with grasslands. Food and Agriculture Organization of the United Nations. Available online at http://www.fao.org/news/story/en/item/38916/icode/, checked on 8/13/2022.

Feldwisch, N. (2011): Umweltgerechter Anbau von Energiepflanzen (43). Available online at https://slub.qucosa.de/id/qucosa:1806, checked on 8/13/2022.

Feledyn-Szewczyk, B.; Matyka, M.; Staniak, M. (2019): Comparison of the Effect of Perennial Energy Crops and Agricultural Crops on Weed Flora Diversity. In *Agronomy* 9 (11), p. 695. DOI: 10.3390/agronomy9110695.

Finell, M.; Nilsson, C. (2004): Kraft and soda-AQ pulping of dry fractionated reed canary grass. In *Industrial Crops and Products* 19 (2), pp. 155–165. DOI: 10.1016/j.indcrop.2003.09.002.

FNR (2020): Sektorstudie zum Aufkommen und zur stofflichen Verwendung von Ölen und Fetten in Deutschland (2011-2016). In *Fachagentur Nachwachsende Rohstoffe e.V.* Available online at http://www.fnr-server.de/ftp/pdf/berichte/22004416.pdf, checked on 8/13/2022.

FNR (2021a): Pflanzen: Anbauzahlen. Fachagentur Nachwachsende Rohstoffe e.V. Available online at https://pflanzen.fnr.de/anbauzahlen, updated on 9/18/2021, checked on 8/13/2022.

FNR (2021b): Durchwachsene Silphie. Fachagentur Nachwachsende Rohstoffe e.V. Available online at https://pflanzen.fnr.de/energiepflanzen/pflanzen/durchwachsene-silphie, updated on 9/7/2021, checked on 8/13/2022.

FNR (2022a): Sida. Sida hermaphrodita. Fachagentur Nachwachsende Rohstoffe e. V. Available online at https://pflanzen.fnr.de/energiepflanzen/pflanzen/sida, updated on 2/10/2022, checked on 8/13/2022.

FNR (2022b): FNR - Pflanzen: Grünland. Fachagentur Nachwachsende Rohstoffe e.V. Available online at https://pflanzen.fnr.de/energiepflanzen/pflanzen/gruenland, updated on 2/17/2022, checked on 8/13/2022.

FNR (2022c): Anbau und Verwendung nachwachsender Rohtoffe in Deutschland. In *Fachagentur Nachwachsende Rohstoffe e.V.* Available online at https://www.fnr.de/index.php?id=11150&fkz=22004416, checked on 8/13/2022.

Franklin Inst. (1835): Straw paper. In *Journal of the Franklin Institute* 19 (4), p. 292. DOI: 10.1016/S0016-0032(35)91752-5.

Franzaring, J.; Holz, I.; Kauf, Z.; Fangmeier, A. (2015): Responses of the novel bioenergy plant species Sida hermaphrodita (L.) Rusby and Silphium perfoliatum L. to CO2 fertilization at different temperatures and water supply. In *Biomass and Bioenergy* 81, pp. 574–583. DOI: 10.1016/j.biombioe.2015.07.031.

Franzaring, J.; Holz, I.; Müller, M.; Kauf, Z.; Fangmeier, A. (2013): Reaktionen der Energiepflanzen Sida und Silphie auferhöhte Temperaturen, reduzierte Niederschläge und den CO2-Düngeeffekt. Projekt: FKZ 22400511. Available online at https://www.fnr.de/index.php?id=11150&fkz=22400511, checked on 8/13/2022.

Franzaring, J.; Schmid, I.; Bäuerle, L.; Gensheimer, G.; Fangmeier, A. (2014): Investigations on plant functional traits, epidermal structures and the ecophysiology of the novel bioenergy species Sida hermaphrodita Rusby and Silphium perfoliatum L. In *Journal of Applied Botany and Food Quality*. DOI: 10.5073/JABFQ.2014.087.006.

Freymuth, H.; Jenisch, R.; Klopfer, H.; Petzold, K.; Stohrer, M.; Fischer, H.-M.; Richter, E. (2002): Lehrbuch der Bauphysik. Schall - Wärme - Feuchte - Licht - Brand - Klima. 5., überarb. Auflage 2002. Wiesbaden: Vieweg+Teubner Verlag; Imprint.

Gamborg, C.; Millar, K.; Shortall, O.; Sandøe, P. (2012): Bioenergy and Land Use: Framing the Ethical Debate. In *Journal of Agricultural and Environmental Ethics* 25 (6), pp. 909–925. DOI: 10.1007/s10806-011-9351-1.

Gansberger, M.; Montgomery, L.F.; Liebhard, P. (2015): Botanical characteristics, crop management and potential of Silphium perfoliatum L. as a renewable resource for biogas production: A review. In *Industrial Crops and Products* 63, pp. 362–372. DOI: 10.1016/j.indcrop.2014.09.047.

Gasparini, K.; Moreira, J.D.R.; Peres, L.E.P.; Zsögön, A. (2021): De novo domestication of wild species to create crops with increased resilience and nutritional value. In *Current opinion in plant biology* 60, p. 102006. DOI: 10.1016/j.pbi.2021.102006.

GBA (2018): German Biogas Association. German Biogas Association (Fachverband Biogas e.V.). Available online at http://www.biogas.org/, checked on 8/13/2022.

GBEP (2007): A review of the current state of bioenergy development in G8 + 5 countries, GBEP Secretariat, Food and Agriculture Organization of the United Nations (FAO). In *Global Bioenergy Partnership*. Available online at fao.org/docrep/fao/010/a1348e/a1348e00.pdf.

Gehren, P. von; 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. In *Seed Science and Technology* 44 (2), pp. 310–319. DOI: 10.15258/sst.2016.44.2.04.

Gharehkhani, S.; Sadeghinezhad, E.; Kazi, S.N.; Yarmand, H.; Badarudin, A.; Safaei, M.R.; Zubir, M.N.M. (2015): Basic effects of pulp refining on fiber properties - a review. In *Carbohydrate Polymers* 115, pp. 785–803. DOI: 10.1016/j.carbpol.2014.08.047.

Gibb, S.; Strimmer, K. (2012): MALDIquant: a versatile R package for the analysis of mass spectrometry data. In *Bioinformatics* 28 (17), pp. 2270–2271. DOI: 10.1093/bioinformatics/bts447.

Gil, M.; Arauzo, I. (2014): Hammer mill operating and biomass physical conditions effects on particle size distribution of solid pulverized biofuels. In *Fuel Processing Technology* 127, pp. 80–87. DOI: 10.1016/j.fuproc.2014.06.016.

Glas, D.J.; Yu, Q.L.; Spiesz, P.R.; Brouwers, H.J.H. (2015): Structural lightweight aggregates concrete. In. 19. Internationale Baustofftagung "ibausil", 16.-18. September 2015, Weimar, Deutschland: F.A. Finger-Institut für Baustoffkunde, pp. 1375–1382. Available online at https://pure.tue.nl/ws/files/3849194/1454149677895.pdf, checked on 8/13/2022.

Glover, J.D.; Culman, S.W.; DuPont, S.T.; Broussard, W.; Young, L.; Mangan, M.E. et al. (2010): Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability (137). In *Agriculture, Ecosystems & Environment* (1-2), pp. 3–12.

Gorouard, P.; Samson, R. (2000): Potential role of perennial grasses in the pulp and paper industry. In *Pupl and Paper Canada*. Available online at https://www.researchgate.net/publication/289089257, checked on 8/13/2022.

Goudriaan, J.; Groot, J.R.; Uithol, P.W. (2001): Productivity of Agro-ecosystems. In *Physiological Ecology*, pp. 301–313. DOI: 10.1016/B978-012505290-0/50014-4.

Graves, S.; Piepho, H.-P.; Selzer, L. (2022): multcompView: Visualizations of Paired Comparisons. Comprehensive R Archive Network (CRAN). Available online at https://cran.r-project.org/web/packages/multcompView/index.html, updated on 6/20/2022, checked on 8/13/2022.

Grebe, S.; Beleu, T.; Döhler, H.; Eckel, H.; Frisch, J.; Fröba, N. et al. (2012): Energiepflanzen. Daten für die Planung des Energiepflanzenanbaus. 2nd ed. Available online at https://www.ktbl.de/shop/produktkatalog/19508, checked on 8/13/2022.

Grieder, C.; Mittweg, G.; Dhillon, B.S.; Montes, J.M.; Orsini, E.; Melchinger, A.E. (2012): Kinetics of methane fermentation yield in biogas reactors: Genetic variation and association with chemical composition in maize. In *Biomass and Bioenergy* 37, pp. 132–141. DOI: 10.1016/j.biombioe.2011.12.020.

Grosser, D.; Teetz W. (1998): Informationsdienst Holz. Birke. 18. Available online at https://informationsdienst-holz.de/publikationen, checked on 8/13/2022.

Grunwald, D.; Panten, K.; Schwarz, A.; Bischoff, W.-A.; Schittenhelm, S. (2020): Comparison of maize, permanent cup plant and a perennial grass mixture with regard to soil and water protection. In *GCB Bioenergy* 12 (9), pp. 694–705. DOI: 10.1111/gcbb.12719.

Gschwend, F.J.V.; Brandt, A.; Chambon, C.L.; Tu, W.-C.; Weigand, L.; Hallett, J.P. (2016): Pretreatment of Lignocellulosic Biomass with Low-cost Ionic Liquids. In *Journal of visualized experiments : JoVE* (114). DOI: 10.3791/54246.

Guadalix, M.E.; Almendros, G.; Martínez, A.T.; Camarero, S.; Barrasa, J.M.; Pelayo, M. (1996): Comparative analysis of wheat straw paperboards prepared after biomechanical and semichemical pulping. In *Bioresource Technology* 57 (3), pp. 217–227. DOI: 10.1016/S0960-8524(96)00014-4.

Haag, N.L.; Nägele, H.-J.; Reiss, K.; Biertümpfel, A.; Oechsner, H. (2015): Methane formation potential of cup plant (Silphium perfoliatum). In *Biomass and Bioenergy* 75, pp. 126–133. DOI: 10.1016/j.biombioe.2015.02.012.

Hammett, A.L.; Youngs, R.L.; Sun, X.; Chandra, M. (2001): Non-Wood Fiber as an Alternative to Wood Fiber in Chinas Pulp and Paper Industry. In *Holzforschung* 55 (2), pp. 219–224. DOI: 10.1515/HF.2001.036.

Hartmann, A. (2018): Mehrjährige Ertragsergebnisse aus Feldversuchen zur Durchwachsenen Silphie. Technologie- und Förderzentrum im Kompetenzzentrum für Nachwachsende Rohstoffe.

Hauhouot-O'Hara, M.; Solie, J.B.; Whitney, R.W.; Peeper, T.F.; Brusewitz, G.H. (1999): Effect of hammer mill and roller mill variables on cheat (Bromus secalinus L.) seed germination. In *Applied Engineering in Agriculture* 15 (2), pp. 139–145. DOI: 10.13031/2013.5757.

Hauser, B.A. (1998): Arabidopsis TSO1 regulates directional processes in cells during floral organogenesis. In *Genetics* 150, p. 411. DOI: 10.1093/genetics/150.1.411.

Hauser, B.A. (2000): TSO1 is a novel protein that modulates cytokinesis and cell expansion in Arabidopsis. In *Development* 127, p. 2219. DOI: 10.1242/dev.127.10.2219.

Hawes, M. (2018): Planting carbon storage. In *Nature Climate Change* 8 (7), pp. 556–558. DOI: 10.1038/s41558-018-0214-x.

Hayek (1918): Illustrierte Flora von Mittel-Europa: mit besonderer Berücksichtigung von Oesterreich, Deutschland und der Schweiz. Dicotyledones, pp. 495–496.

Hintz, H.L.; Lawal, S.A. (2018): Paper: Pulping and Bleaching. Paper: Pulping and Bleaching. Reference Module in Materials Science and Materials Engineering. DOI: 10.1016/B978-0-12-803581-8.11233-0.

Höller, M. (2017): Silphium perfoliatum and Sida hermaphrodita as raw material for the paper and building industry. Bioeconomy Science Center Symposium, Poster.

Höller, M.; Lunze, A.; Wever, C.; Deutschle, A.L.; Stücker, A.; Frase, N. et al. (2021): Meadow hay, Sida hermaphrodita (L.) Rusby and Silphium perfoliatum L. as potential non-wood raw materials for the pulp and paper industry. In *Industrial Crops and Products* 167, p. 113548. DOI: 10.1016/j.indcrop.2021.113548.

Hunter, J.D. (2007): Matplotlib: A 2D Graphics Environment. In *Computing in Science & Engineering* 9 (3), pp. 90–95. DOI: 10.1109/MCSE.2007.55.

IEA Bioenergy (2022): Carbon neutrality | Bioenergy. International Energy Agency. Available online at https://www.ieabioenergy.com/iea-publications/faq/woodybiomass/carbon-neutrality/, updated on 7/7/2022, checked on 8/13/2022.

Ilanidis, D.; Stagge, S.; Jönsson, L.J.; Martín, C. (2021): Effects of operational conditions on auto-catalyzed and sulfuric-acid-catalyzed hydrothermal pretreatment of sugarcane bagasse at different severity factor. In *Industrial Crops and Products* 159, p. 113077. DOI: 10.1016/j.indcrop.2020.113077.

IPCC (2014): Climate Change 2014. Impacts, Adaptation and Vulnerability. Part A: Global and Sectoral Aspects. 1. publ. With assistance of David Dokken. Intergovernmental Panel on Climate Change. New York.

Jablonowski, N.D.; Kollmann, T.; Nabel, M.; Damm, T.; Klose, H.; Müller, M. et al. (2017): Valorization of Sida (Sida hermaphrodita) biomass for multiple energy purposes. In *GCB Bioenergy* 9 (1), pp. 202–214. DOI: 10.1111/gcbb.12346.

Jain, K.K.; Kumar, S.; Deswal, D.; Kuhad, R.C. (2017): Improved Production of Thermostable Cellulase from Thermoascus aurantiacus RCKK by Fermentation Bioprocessing and Its Application in the Hydrolysis of Office Waste Paper, Algal Pulp, and Biologically Treated Wheat Straw. In *Applied Biochemistry and Biotechnology* 181 (2), pp. 784–800. DOI: 10.1007/s12010-016-2249-7.

Jensen, E.S.; Peoples, M.B.; Boddey, R.M.; Gresshoff, P.M.; Hauggaard-Nielsen, H.; J.R. Alves, B.; Morrison, M.J. (2012): Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. In *Agronomy for Sustainable Development* 32 (2), pp. 329–364. DOI: 10.1007/s13593-011-0056-7.

JH (2022): Datasheet: Fermacell Gebundene Schüttung T. James Hardie Europe GmbH. Available online at https://jameshardieeurope.my.salesforce.com/sfc/p /#200000000AOl/a/0J000000 cQCx/ Tv9_RSrXx0XB3LvTmXs_T1gH4O_3A3ThimkaxqQAAt0, updated on 6/20/2022, checked on 8/13/2022.

JKI (2022): Managed grassland. Julius Kühn-Institut - Federal Research Centre for Cultivated Plants. Available online at https://www.julius-kuehn.de/en/pb/fields-of-activity/managed-grassland/, updated on 2/13/2022, checked on 8/13/2022.

Johansson, A. (2011): Correlations between fibre properties and paper properties. Pulp Technology. In *KTH Vetenskap Och Konst*.

Jones, M.B.; Donnelly, A. (2004): Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO2. In *New Phytologist* 164 (3), pp. 423–439. DOI: 10.1111/j.1469-8137.2004.01201.x.

Kamoga, O.L.M.; Byaruhanga, J.K.; Kirabira, J.B. (2013): A Review on Pulp Manufacture from Non Wood Plant Materials. In *International Journal of Chemical Engineering and Applications*, pp. 144–148. DOI: 10.7763/IJCEA.2013.V4.281.

Kamoga, O.L.M.; Kirabira, J.B.; Byaruhanga, J.K.; Godiyal, R.D.; Anupam, K. (2014): Characterisation and Evaluation of pulp and paper from selected Ugandan grasses for paper industry Cellulose Chemistry and Technology (2), 275–284. http://hdl.handle.net/20.500.12283/103. Available online at http://hdl.handle.net/20.500.12283/103.

Karp, A.; Shield, I. (2008): Bioenergy from plants and the sustainable yield challenge. In *New Phytologist* 179 (1), pp. 15–32. DOI: 10.1111/j.1469-8137.2008.02432.x.

Kennedy, J.F.; Phillips, G.O.; Williams, P.A. (1996): The chemistry and processing of wood and plant fibrous material. Cambridge, England: Woodhead Publishing Limited. Available online at https://books.google.de/books?id=uq-jAgAAQBAJ.

Kim, S.M.; Dien, B.S.; Singh, V. (2016): Promise of combined hydrothermal/chemical and mechanical refining for pretreatment of woody and herbaceous biomass. In *Biotechnology for biofuels* 9, p. 97. DOI: 10.1186/s13068-016-0505-2.

Klímek, P.; Meinlschmidt, P.; Wimmer, R.; Plinke, B.; Schirp, A. (2016): Using sunflower (Helianthus annuus L.), topinambour (Helianthus tuberosus L.) and cup-plant (Silphium perfoliatum L.) stalks as alternative raw materials for particleboards. In *Industrial Crops and Products* 92, pp. 157–164. DOI: 10.1016/j.indcrop.2016.08.004.

Klímek, P.; Wimmer, R.; Meinlschmidt, P. (2021): TOF-SIMS Molecular Imaging and Properties of pMDI-Bonded Particleboards Made from Cup-Plant and Wood. In *Applied Sciences* 11 (4), p. 1604. DOI: 10.3390/app11041604.

Kochova, K.; Schollbach, K.; Brouwers, H.J.H. (2016): Use of alternative organic fibres in cement composites, 11/21/2016. Available online at https://pure.tue.nl/ws/files/43446922/Use_of_alternative_organic_fibres_in_cement_composites.pdf.

Kochova, K.; Schollbach, K.; Gauvin, F.; Brouwers, H.J.H. (2017): Effect of saccharides on the hydration of ordinary Portland cement. In *Construction and Building Materials* 150, pp. 268–275. DOI: 10.1016/j.conbuildmat.2017.05.149.

Koniuszy, A.; Hawrot-Paw, M.; Podsiadło, C.; Sędłak, P.; Możdżer, E. (2020): Gasification of Cup Plant (Silphium perfoliatum L.) Biomass–Energy Recovery and Environmental Impacts. In *Energies* 13 (18), p. 4960. DOI: 10.3390/en13184960.

Kowalski, R.; Wiercinski, J. (2004): Evaluation of chemical composition of some Silphium L. species seeds as alternative foodstuff raw materials. In *Pol. J. Food Nutr. Sci.* 13/54, p. 349.

Kraska, T.; Winzer, F.; Witzel, C.P.; Finger, R.; Pude, R. (2015): Cascade Utilization of Miscanthus. Mitteilungsblatt über Biomasse für Energie und Industrie in einer nachhaltigen Wirtschaft. In *Biobased Future* (4), p. 13.

Kurucz, E.; Fári, M.G.; Antal, G.; Gabnai, Z.; Popp, J.; Bai, A. (2018): Opportunities for the production and economics of Virginia fanpetals (Sida hermaphrodita). In *Renewable & Sustainable Energy Reviews* 90, pp. 824–834. DOI: 10.1016/j.rser.2018.04.007.

La Doudart de Grée, G.; Caprai, V.; van Dam, J.; van As, H.; Brouwers, H.J.H.; Yu, Q.L. (2019): Ionic interaction and liquid absorption by wood in lignocellulose inorganic mineral binder composites. In *Journal of Cleaner Production* 206, pp. 808–818. DOI: 10.1016/j.jclepro.2018.09.220.

Lamberg, J.-A.; Ojala, J.; Peltoniemi, M.; Särkkä, T. (2012): The Evolution of Global Paper Industry 1800–2050. A Comparative Analysis. Dordrecht: Springer Netherlands (World Forests, 17).

Latif, E.; Ciupala, M.A.; Tucker, S.; Wijeyesekera, D.C.; Newport, D.J. (2015): Hygrothermal performance of wood-hemp insulation in timber frame wall panels with and without a vapour barrier. In *Building and Environment* 92, pp. 122–134. DOI: 10.1016/j.buildenv.2015.04.025.

Le Ngoc Huyen, T.; Queneudec T'kint, M.; Remond, C.; Chabbert, B.; Dheilly, R.-M. (2011): Saccharification of Miscanthus x giganteus, incorporation of lignocellulosic by-product in cementitious matrix. In *Comptes rendus biologies* 334 (11), 837.e1-837.e11. DOI: 10.1016/j.crvi.2011.07.008.

Lee, C.; Grasso, C.; Sharlow, M.F. (2002): Multiple sequence alignment using partial order graphs. In *Bioinformatics* 18 (3), pp. 452–464. DOI: 10.1093/bioinformatics/18.3.452.

Lenth (2022): Estimated Marginal Means, aka Least-Squares Means [R package emmeans version 1.7.4-1]: Comprehensive R Archive Network (CRAN). Available online at https://cran.r-project.org/web/packages/emmeans/index.html, checked on 8/13/2022.

Leopold, A. (1968): A Sand County almanac. 2nd ed. Oxford, UK.

Li, C.; Lin, F.; An, D.; Wang, W.; Huang, R. (2017): Genome Sequencing and Assembly by Long Reads in Plants. In *Genes* 9 (1), p. 6. DOI: 10.3390/genes9010006.

Li, J.; Li, G.Z. (2014): Study on the Waterproofing Properties of Cement-Based Composite Thermal Insulation Materials. In *Applied Mechanics and Materials* 711, pp. 166–169. DOI: 10.4028/www.scientific.net/AMM.711.166.

Li, S.; Chou, H.-H. (2004): LUCY2: an interactive DNA sequence quality trimming and vector removal tool. In *Bioinformatics* 20 (16), pp. 2865–2866. DOI: 10.1093/bioinformatics/bth302.

Liao, Y.; Koelewijn, S.-F.; van den Bossche, G.; van Aelst, J.; van den Bosch, S.; Renders, T. et al. (2020): A sustainable wood biorefinery for low-carbon footprint chemicals production. In *Science* 367 (6484), pp. 1385–1390. DOI: 10.1126/science.aau1567.

Liu, B.; Asseng, S.; Müller, C.; Ewert, F.; Elliott, J.; Lobell, D.B. et al. (2016): Similar estimates of temperature impacts on global wheat yield by three independent methods. In *Nature Climate Change* 6 (12), pp. 1130–1136. DOI: 10.1038/nclimate3115.

Liu, Z.; Wang, H.; Hui, L. (2018): Pulping and Papermaking of Non-Wood Fibers. In *IntechOpen*. DOI: 10.5772/intechopen.79017.

Ljungberg, L.Y. (2007): Materials selection and design for development of sustainable products. In *Materials & Design* 28 (2), pp. 466–479. DOI: 10.1016/j.matdes.2005.09.006.

Lunze, A.; Heyman, B.; Chammakhi, Y.; Eichhorn, M.; Büchs, J.; Anders, N.; Spiess, A.C. (2021): Investigation of Silphium perfoliatum as Feedstock for a Liquid Hot Water–Based Biorefinery Process Towards 2,3-Butanediol. In *BioEnergy Research* 14 (3), pp. 799–814. DOI: 10.1007/s12155-020-10194-9.

Mandel, J.R.; Dechaine, J.M.; Marek, L.F.; Burke, J.M. (2011): Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, Helianthus annuus L. In *Theoretical and Applied Genetics* 123 (5), pp. 693–704. DOI: 10.1007/s00122-011-1619-3.

Marín, F.; Sánchez, J.L.; Arauzo, J.; Fuertes, R.; Gonzalo, A. (2009): Semichemical pulping of Miscanthus giganteus. Effect of pulping conditions on some pulp and paper properties. In *Bioresource Technology* 100 (17), pp. 3933–3940. DOI: 10.1016/j.biortech.2009.03.011.

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. In *Industrial Crops and Products* 58, pp. 194–203. DOI: 10.1016/j.indcrop.2014.04.017.

Material Economics (2021): EU Biomass Use In A Net-Zero Economy - A Course Correction for EU Biomass. Available online at https://materialeconomics.com/latest-updates/eu-biomass-use, checked on 8/13/2022.

McKendry, P. (2002): Energy production from biomass (part 1): overview of biomass. In *Bioresource Technology* 83 (1), pp. 37–46. DOI: 10.1016/S0960-8524(01)00118-3.

McLaughlin, S.; Walsh, M. (1998): Evaluating environmental consequences of producing herbaceous crops for bioenergy. In *Biomass and Bioenergy* 14 (4), pp. 317–324. DOI: 10.1016/S0961-9534(97)10066-6.

Medvedev, P.F. (1940): New fibrous crops in the USSR. Sel´khozgiz.

Meehan, P.; Burke, B.; Doyle, D.; Barth, S.; Finnan, J. (2017): Exploring the potential of grass feedstock from marginal land in Ireland: Does marginal mean lower yield? In *Biomass and Bioenergy* 107, pp. 361–369. DOI: 10.1016/j.biombioe.2017.10.014.

Mejouyo, P.W.H.; Nkemaja, E.D.; Beching, O.R.; Tagne, N.R.S.; Kana'a, T.; Njeugna, E. (2020): Physical and Tensile Properties of Handmade Sida rhombifolia Paper. In *International journal of biomaterials* 2020, p. 3967641. DOI: 10.1155/2020/3967641.

Mellinger, T. (1996): Packaging chips from maize starch - New procedure for using maize as renewable resource. In *Landwirtschaftliches Wochenblatt. Organ des Landesbauernverbandes in Baden Wuerttemberg. Ausg. WWL*. Available online at https://agris.fao.org/agris-search/search.do?recordID=DE19960169173, checked on 8/13/2022.

Mikunda, T.; Santos S.; Helseth J.; St. Leger H. (2013): CO_2 Capture and Storage (CCS) in energy-intensive industries. An indispensable route to an EU low-carbon economy. European Technology Platform for Zero Emission Fossil Fuel Power Plants.

Moll, L.; Wever, C.; Völkering, G.; Pude, R. (2020): Increase of Miscanthus Cultivation with New Roles in Materials Production—A Review. In *Agronomy* 10 (2), p. 308. DOI: 10.3390/agronomy10020308.

Mullet, J.E. (2017): High-biomass C4 grasses-Filling the yield gap. In *Plant science : an international journal of experimental plant biology* 261, pp. 10–17. DOI: 10.1016/j.plantsci.2017.05.003.

Muthu, S. S. (Ed.) (2017): Sustainable fibres and textiles. Textile Institute. Duxford, Cambridge, MA, Kidlington: Elsevier Science. Available online at https://ebookcentral.proquest.com/lib/kxp/detail.action?docID=4867951, checked on 8/13/2022.

Nabel, M.; Barbosa, D.B.; Horsch, D.; Jablonowski, N.D. (2014): Energy Crop (Sida Hermaphrodita) Fertilization Using Digestate under Marginal Soil Conditions: A Dose-response Experiment. In *Energy Procedia* 59, pp. 127–133. DOI: 10.1016/j.egypro.2014.10.358.

Nabel, M.; Schrey, S.D.; Temperton, V.M.; Harrison, L.; Jablonowski, N.D. (2018): Legume Intercropping With the Bioenergy Crop Sida hermaphrodita on Marginal Soil. In *Frontiers in Plant Science* 9, p. 905. DOI: 10.3389/fpls.2018.00905.

Neroth, G.; Vollenschaar, D. (Eds.) (2011): Wendehorst Baustoffkunde. Wiesbaden: Vieweg+Teubner.

Neufert, P.; Neff, L. (1997): Ökologisches Bauen. In Peter Neufert, Ludwig Neff (Eds.): Gekonnt Planen Richtig Bauen. Wiesbaden: Vieweg+Teubner Verlag, pp. 25–27. Available online at https://

www.zkw-otterbein.de/images/Otterbein/Produktbroschueren_1/PROMPT_Fix-Zement/PROMPT_Oekologisches_Bauen_de.pdf, checked on 8/13/2022.

Neumerkel, W. (1978): Silphium perfoliatum L. – eine Nutzpflanze? Wissenschaftliche Zeitschrift. In *Math. Nat. Reihe* 27, p. 31.

Neumerkel, W.; Märtin, B. (1982): Silphium (Silphium perfoliatum L.) – a new feed plant. In *Arch. Agron. Soil Sci.* 26, p. 261. Available online at https://agris.fao.org/agris-search/search.do?recordID=DD19830851295, checked on 8/13/2022.

Niqueux, M. (1981): A new forage plant: Silphium perfoliatum L. In *Fourrages* 87, p. 119. Available online at https://agris.fao.org/agris-search/search.do?recordID=XE8232584, checked on 8/13/2022.

Niskanen, K. (2011): Mechanics of Paper Products. 1. Aufl.: Walter de Gruyter GmbH Co.KG.

Olson D.; Cox R. (2017): California Central Valley Grasslands. Edited by World Wildlife Fund. Available online at https://www.worldwildlife.org/ecoregions/na0801, updated on 5/6/2020, checked on 8/13/2022.

Opitz von Boberfeld, W. (1994): Grünlandlehre. Biologische und ökologische Grundlagen ; 28 Tabellen. Stuttgart: Ulmer (Uni-Taschenbücher Agrarwissenschaften, 1770).

Ott, A.; Liu, S.; Schnable, J.C.; Yeh, C.-T.; Wang, K.-S.; Schnable, P.S. (2017): tGBS® genotyping-by-sequencing enables reliable genotyping of heterozygous loci. In *Nucleic Acids Research* 45 (21), e178. DOI: 10.1093/nar/gkx853.

Otto, L.-G.; Mondal, P.; Brassac, J.; Preiss, S.; Degenhardt, J.; He, S. et al. (2017): Use of genotyping-by-sequencing to determine the genetic structure in the medicinal plant chamomile, and to identify flowering time and alpha-bisabolol associated SNP-loci by genome-wide association mapping. In *BMC Genomics* 18 (1), p. 599. DOI: 10.1186/s12864-017-3991-0.

Ouyang K.; Wang K.; Li D.; Li Z; Liu B (2007): Establishment of sown pastures in the hilly red soil region of the subtropics in southern China. In *Tropical Grasslands* (41), pp. 92–99.

Pan, G. (2011): Water use patterns of forage cultivars in the North China Plain. In *International Journal of Plant Production* 5, p. 181.

Paradis, E. (2010): pegas: an R package for population genetics with an integrated-modular approach. In *Bioinformatics* 26 (3), pp. 419–420. DOI: 10.1093/bioinformatics/btp696.

Paradis, E.; Claude, J.; Strimmer, K. (2004): APE: Analyses of Phylogenetics and Evolution in R language. In *Bioinformatics* 20 (2), pp. 289–290. DOI: 10.1093/bioinformatics/btg412.

Peni, D.; Stolarski, M.J.; Bordiean, A.; Krzyżaniak, M.; Dębowski, M. (2020): Silphium perfoliatum—A Herbaceous Crop with Increased Interest in Recent Years for Multi-Purpose Use. In *Agriculture* 10 (12), p. 640. DOI: 10.3390/agriculture10120640.

Peschard, A.; Govin, A.; Pourchez, J.; Fredon, E.; Bertrand, L.; Maximilien, S.; Guilhot, B. (2006): Effect of polysaccharides on the hydration of cement suspension. In *Journal of the European Ceramic Society* 26 (8), pp. 1439–1445. DOI: 10.1016/j.jeurceramsoc.2005.02.005.

Phillips, R.; Rix, M.; Barnes, P.; Compton, J.; Rix, A.; Bryan, J.; Stokoe, G. (1991): Perennials. Early Perennials. 1st ed. London: Pan Books (The Pan Garden Plants Series).

Phylogenetic Analysis Using Parsimony (2002): PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods). With assistance of Swofford. Version 4.0b10.

Pichard, G. (2012): Management, production, and nutritional characteristics of cup-plant (Silphium perfoliatum) in temperate climates of southern Chile. In *Ciencia e investigación agraria* 39 (1), pp. 61–77. DOI: 10.4067/S0718-16202012000100005.

Pimentel, D.; Pimentel, M.H. (2007): Food, Energy, and Society: CRC Press. Available online at https://www.taylorfrancis.com/books/mono/10.1201/9781420046687/food-energy-society-david-pimentel-ph-marcia-pimentel, checked on 8/13/2022.

Pimm, S.L. (1983): Resource competition and community structure. Monogr. Pop. Biol. 17. Princeton University Press, Princeton, N.J. 296 p. In *Limnology and Oceanography* 28 (5), pp. 1043–1045. DOI: 10.4319/lo.1983.28.5.1043.

Power, A.G. (2010): Ecosystem services and agriculture: tradeoffs and synergies. In *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 365 (1554), pp. 2959–2971. DOI: 10.1098/rstb.2010.0143.

Przybysz, K.; Małachowska, E.; Martyniak, D.; Boruszewski, P.; Iłowska, J.; Kalinowska, H.; Przybysz, P. (2017): Yield of Pulp, Dimensional Properties of Fibers, and Properties of Paper Produced from Fast Growing Trees and Grasses (13). In *Bioresources* (1), pp. 1372–1387.

Pude, R.; Banaszuk, P.; Trettin, R.; Noga, G. (2005): Suitability of Phragmites for leightweight concrete. In *Journal of Applied Botany and Food Quality* 79, pp. 141–146.

Pude, R.; Treseler, C.H.; Noga, G. (2004): Morphological, Chemical and Technical Parameters of *Miscanthus* Genotypes. In *Journal of Applied Botany* 78, pp. 58–63.

R (2018): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Available online at https://www.r-project.org/, updated on 6/20/2022, checked on 8/13/2022.

Ramamurthy, K.; Kunhanandan Nambiar, E.K.; Indu Siva Ranjani, G. (2009): A classification of studies on properties of foam concrete. In *Cement and Concrete Composites* 31 (6), pp. 388–396. DOI: 10.1016/j.cemconcomp.2009.04.006.

Rambaut, A. (2012): FigTree V. 1.4.0. Available online at http://tree.bio.ed.ac.uk/software/figtree/, checked on 8/13/2022.

Raunkiaer, C. (1977): The life forms of plants and statistical plant geography. Being the collected papers of C. Raunkiaer: Arno P.

Reaz, R.; Bayzid, M.S.; Rahman, M.S. (2014): Accurate phylogenetic tree reconstruction from quartets: a heuristic approach. In *PLoS ONE* 9 (8), e104008. DOI: 10.1371/journal.pone.0104008.

Reinert, S.; van Tassel, D.L.; Schlautman, B.; Kane, N.C.; Hulke, B.S. (2019): Assessment of the biogeographical variation of seed size and seed oil traits in wild Silphium integrifolium Michx. genotypes. In *Plant Genetic Resources: Characterization and Utilization* 17 (5), pp. 427–436. DOI: 10.1017/S1479262119000248.

Renaut, S. (2017): Genome sequencing: Illuminating the sunflower genome. In *Nature Plants* 3 (7), p. 17099. DOI: 10.1038/nplants.2017.99.

Reyes-Chin-Wo, S.; Wang, Z.; Yang, X.; Kozik, A.; Arikit, S.; Song, C. et al. (2017): Genome assembly with in vitro proximity ligation data and whole-genome triplication in lettuce. In *Nature Communications* 8 (1), p. 14953. DOI: 10.1038/ncomms14953.

Risse, M.; Weber-Blaschke, G.; Richter, K. (2017): Resource efficiency of multifunctional wood cascade chains using LCA and exergy analysis, exemplified by a case study for Germany. In *Resources, Conservation and Recycling* 126, pp. 141–152. DOI: 10.1016/j.resconrec.2017.07.045.

Rockström, J.; Steffen, W.; Noone, K.; Persson, A.; Chapin, F.S.; Lambin, E.F. et al. (2009): A safe operating space for humanity. In *Nature* 461 (7263), pp. 472–475. DOI: 10.1038/461472a.

Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. (2016): VSEARCH: a versatile open source tool for metagenomics. In *PeerJ* 4, e2584. DOI: 10.7717/peerj.2584.

Rojas, C.; Cea, M.; Iriarte, A.; Valdés, G.; Navia, R.; Cárdenas-R, J.P. (2019): Thermal insulation materials based on agricultural residual wheat straw and corn husk biomass, for application in sustainable buildings. In *Sustainable Materials and Technologies* 20. DOI: 10.1016/j.susmat.2019.e00102.

Rosenzweig, C.; Elliott, J.; Deryng, D.; Ruane, A.C.; Müller, C.; Arneth, A. et al. (2014): Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. In *Proceedings of the National Academy of Sciences of the United States of America* 111 (9), pp. 3268–3273. DOI: 10.1073/pnas.1222463110.

Roy, J.; Saugier, B. (Eds.) (2008): Terrestrial global productivity. San Diego, CA, United States: Academic Press (Physiological ecology).

Ruf, T.; Audu, V.; Holzhauser, K.; Emmerling, C. (2019): Bioenergy from Periodically Waterlogged Cropland in Europe: A First Assessment of the Potential of Five Perennial Energy Crops to Provide Biomass and Their Interactions with Soil. In *Agronomy* 9 (7), p. 374. DOI: 10.3390/agronomy9070374.

Ruf, T.; Emmerling, C. (2021): Different life-form strategies of perennial energy crops and related nutrient exports require a differentiating view specifically concerning a sustainable cultivation on marginal land. In *GCB Bioenergy* 13 (6), pp. 893–904. DOI: 10.1111/gcbb.12830.

Ruf, T.; Makselon, J.; Udelhoven, T.; Emmerling, C. (2018): Soil quality indicator response to land-use change from annual to perennial bioenergy cropping systems in Germany. In *GCB Bioenergy* 10 (7), pp. 444–459. DOI: 10.1111/gcbb.12513.

Ruidisch, M.; Nguyen, T.T.; Li, Y.L.; Geyer, R.; Tenhunen, J. (2015): Estimation of annual spatial variations in forest production and crop yields at landscape scale in temperate climate regions. In *Ecological Research* 30 (2), pp. 279–292. DOI: 10.1007/s11284-014-1208-4.

Saensukjaroenphon, M.; Evans, C.E.; Sheldon, K.H.; Jones, C.K.; Paulk, C.B.; Stark, C.R. (2017): The Effect of Hammermill Screen Hole Diameter and Hammer Tip Speed on Particle Size and Flowability of Ground Corn. In *Kansas Agricultural Experiment Station Research Reports* 3 (7). DOI: 10.4148/2378-5977.7505.

Saijonkari-Pahkala, K. (2001): Non-wood plants as raw material for pulp and paper. DOI: 10.23986/afsci.5707. Dissertation. Faculty of Agriculture and Forestry, University of Helsinki.

Samson, G.; Phelipot-Mardelé, A.; Lanos, C. (2016): Thermal and mechanical properties of gypsum–cement foam concrete: effects of surfactant. In *European Journal of Environmental and Civil Engineering*, pp. 1–20. DOI: 10.1080/19648189.2016.1177601.

Schäfer, A.; Damerow, L.; Lammers, P.S. (2016): Durchwachsene Silphie: Bestandesetablierung mittels Aussaat. 367-371 Seiten / Journal für Kulturpflanzen, Bd. 68 Nr. 12 (2016): Themenheft Durchwachsene Silphie. Journal für Kulturpflanzen (Journal für Kulturpflanzen, 10.5073/jfk.2016.12). Available online at https://ojs.openagrar.de/index.php/ Kulturpflanzen journal/article/view/13216, checked on 8/13/2022.

Schäfer, A.; Damerow, L.; Schulze Lammers, P. (2017): Determination of the seed geometry of cup plant as requirement for precision seeding. In *Landtechnik* 72, p. 122. DOI: 10.15150/lt.2017.3159.

Scheben, A.; Batley, J.; Edwards, D. (2017): Genotyping-by-sequencing approaches to characterize crop genomes: choosing the right tool for the right application. In *Plant Biotechnology Journal* 15 (2), pp. 149–161. DOI: 10.1111/pbi.12645.

Schittenhelm, S. (2008): Chemical composition and methane yield of maize hybrids with contrasting maturity. In *European Journal of Agronomy* 29 (2-3), pp. 72–79. DOI: 10.1016/j.eja.2008.04.001.

Schmidt, P.; Körber, R.; Coppers, M. (2003): Sieben und Siebmaschinen. Grundlagen und Anwendung. Weinheim. DOI: 10.1002/3527609032: Wiley. Available online at http://onlinelibrary.wiley.com/ book/10.1002/3527609032.

Schoo, B. (2013): Vergleichende Untersuchung von Wurzelmerkmalen bei Silphie und Mais. In *Mitt. Ges. Pflanzenbauwiss.* (25), pp. 241–242.

Schoo, B.; Wittich, K.P.; Böttcher, U.; Kage, H.; Schittenhelm, S. (2017): Drought Tolerance and Water-Use Efficiency of Biogas Crops: A Comparison of Cup Plant, Maize and Lucerne-Grass. In *Journal of Agronomy and Crop Science* 203 (2), pp. 117–130. DOI: 10.1111/jac.12173.

Schorpp, Q.; Müller, A.L.; Schrader, S.; Dauber, J. (2016): Agrarökologisches Potential der Durchwachsenen Silphie (Silphium perfoliatum L.) aus Sicht biologischer Vielfalt. Themenheft Durchwachsene Silphie. Journal für Kulturpflanzen. In *Journal für Kulturpflanzen*. DOI: 10.5073/JfK.2016.12.12.

Schorpp, Q.; Schrader, S. (2016): Earthworm functional groups respond to the perennial energy cropping system of the cup plant (Silphium perfoliatum L.). In *Biomass and Bioenergy* 87, pp. 61–68. DOI: 10.1016/j.biombioe.2016.02.009.

Schrader, S.; Seibel, C. (2001): Impact of cultivation management in an agroecosystem on hot spot effects of earthworm middens. In *European Journal of Soil Biology* 37 (4), pp. 309–313. DOI: 10.1016/S1164-5563(01)01102-5.

Schulte, M.; Lewandowski, I.; Pude, R.; Wagner, M. (2021): Comparative life cycle assessment of bio-based insulation materials: Environmental and economic performances. In *GCB Bioenergy* 13 (6), pp. 979–998. DOI: 10.1111/gcbb.12825.

Schurr, U. (2017): Bioökonomie für Einsteiger. 1st ed. Edited by Joachim Pietzsch. Berlin, Heidelberg: Springer Spektrum.

Schwabe (2010): The Western Corn Rootworm (Diabrotica virgifera virgifera LeConte) – a danger to cultivation of corn in Europe. In *J. Cultiv. Plants* 62, p. 277.

Seth, R.S. (1990): Fibre Quality Factors in Papermaking — I The Importance of Fibre Length and Strength. In *MRS Proceedings* 197. DOI: 10.1557/PROC-197-125.

Settle, W.J. (1967): The chromosome morphology in the genus Silphium (compositae). In *The Ohio Journal of Science* 67 (1), p. 10. Available online at http://hdl.handle.net/1811/5260, checked on 8/13/2022.

Šiaudinis, G.; Jasinskas, A.; Šarauskis, E.; Steponavičius, D.; Karčauskienė, D.; Liaudanskienė, I. (2015): The assessment of Virginia mallow (Sida hermaphrodita Rusby) and cup plant (Silphium perfoliatum L.) productivity, physico–mechanical properties and energy expenses. In *Energy* 93, pp. 606–612. DOI: 10.1016/j.energy.2015.09.065.

SidaTim. Anbau und Kultiviering (2022). Available online at https://www.sidatim.eu/de/ueber-sida/anbau-und-kultiviering.html, updated on 2/3/2022, checked on 8/13/2022.

Signorell, A. (2022): Tools for Descriptive Statistics [R package DescTools version 0.99.45]: Comprehensive R Archive Network (CRAN). Available online at https://cran.r-project.org/web/packages/DescTools/index.html, checked on 8/13/2022.

Siwek, H.; Włodarczyk, M.; Możdżer, E.; Bury, M.; Kitczak, T. (2019): Chemical Composition and Biogas Formation potential of Sida hermaphrodita and Silphium perfoliatum. In *Applied Sciences* 9 (19), p. 4016. DOI: 10.3390/app9194016.

Slepetys, J.; Kadziuliene, Z.; Sarunaite, L.; Tilvikiene, V.; Kryzeviciene, A. (2012): Biomass potential of plants grown for bioenergy production. In *Renewable Energy and Energy Efficiency*.

Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. (2011): Determination of Structural Carbohydrates and Lignin in Biomass. In *National Renewable Energy Laboratory*.

Sokolov, V.S.; Gritsak, Z.I. (1972): Silphium – a valuable fodder and nectariferous crop. In *World Crops* 24, pp. 299–301. Available online at https://agris.fao.org/agris-search/search.do?recordID=US201303217287, checked on 8/13/2022.

Spooner, D.M.; Cusick, A.W.; Hall, G.F.; Baskin Jerry M. (1985): Observation on the Distribution and Ecology of Sida Hermaphrodita (L.) Rusby (Malvaceae). In *Sida, Contributions to Botany* 11 (2), pp. 215–225. Available online at www.jstor.org/stable/23909315.

Stanford, G. (1990): Silphium perfoliatum (cup-plant) as a new forage. In *Proceedings of the Twelfth North American Prairie Conference*, pp. 33–38.

StMELF (2022): Sida (*Sida hermaphrodita*). Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten. Available online at https://www.tfz.bayern.de/rohstoffpflanzen/mehrjaehrigekulturen/085998/index.php, updated on 2/10/2022, checked on 8/13/2022.

Stolarski, M.J.; Krzyżaniak, M.; Śnieg, M.; Słomińska, E.; Piórkowski, M.; Filipkowski, R. (2014): Thermophysical and Chemical Properties of Perennial Energy Crops Depending on Harvest Period. In *International Agrophysics* 28 (2), pp. 201–211. DOI: 10.2478/intag-2014-0009.

Stolzenburg, K.; Monkos, A.: Erste Versuchsergebnisse mit der Durchwachsenen Silphie (Silphium perfoliatum L.) in Baden-Württemberg. In *Landwirtschaftliches Technologiezentrum Augustenberg*. Available online at https://ltz.landwirtschaft-bw.de/pb/,Lfr,W-2/Startseite/Kulturpflanzen/Durchwachsene+Silphie?SORT=3&REVERSE=false, checked on 8/13/2022.

Subramanian, S. (2016): The effects of sample size on population genomic analyses--implications for the tests of neutrality. In *BMC Genomics* 17 (1), p. 123. DOI: 10.1186/s12864-016-2441-8.

Suttie, J.M.; Reynolds, S.G.; Batello, C. (2005): Grasslands of the world. Rome: Food and Agriculture Organization of the United Nations (FAO) (Plant production and protection series, no. 34).

Takkellapati, S.; Li, T.; Gonzalez, M.A. (2018): An Overview of Biorefinery Derived Platform Chemicals from a Cellulose and Hemicellulose Biorefinery. In *CLEAN TECHNOLOGIES AND ENVIRONMENTAL POLICY* 20 (7), pp. 1615–1630. DOI: 10.1007/s10098-018-1568-5.

Tavossi, H.M.; Tittmann, B.R.; Cohen-Tenoudji, F. (1999): Ultrasonice Characterization of Cement and Concrete. In *Review of Progress in Quantitative Nondestructive Evaluation* 18.

Terrer, C.; Phillips, R.P.; Hungate, B.A.; Rosende, J.; Pett-Ridge, J.; Craig, M.E. et al. (2021): A trade-off between plant and soil carbon storage under elevated CO2. In *Nature* 591 (7851), pp. 599–603. DOI: 10.1038/s41586-021-03306-8.

TFZ Bayern (2022): Sida (*Sida hermaphrodita*). Technologie- und Förderzentrum Bayern. Available online at https://www.tfz.bayern.de/rohstoffpflanzen/mehrjaehrigekulturen/085998/index.php, updated on 6/9/2022, checked on 8/13/2022.

Thykesson, M.; La Sjoberg; Ahlgren, P. (1998): Paper properties of grass and straw pulps Industrial Crops and Products. 7 (2-3), 351–362. DOI: https://doi.org/10.1016/S0926-6690(97)10001-2.

Tilman, D.; Hill, J.; Lehman, C. (2006): Carbon-negative biofuels from low-input high-diversity grassland biomass. In *Science (New York, N.Y.)* 314 (5805), pp. 1598–1600. DOI: 10.1126/science.1133306.

Troxler J.; Daccord R. (1982): Silphium perfoliatum L.: an interesting fodder? In *Revue Suisse Agric* 14, p. 279. Available online at https://agris.fao.org/agris-search/search.do?recordID=CH8220253, checked on 8/13/2022.

Tsapekos, P.; Khoshnevisan, B.; Alvarado-Morales, M.; Symeonidis, A.; Kougias, P.G.; Angelidaki, Irini (2019): Environmental impacts of biogas production from grass: Role of co-digestion and pretreatment at harvesting time. In *Applied Energy* 252, p. 113467. DOI: 10.1016/j.apenergy.2019.113467.

Türk, O. (2014): Stoffliche Nutzung nachwachsender Rohstoffe. Grundlagen - Werkstoffe - Anwendungen. Wiesbaden: Springer Fachmedien Wiesbaden; Imprint; Springer Vieweg.

UBA (2013): Globale Landflächen und Biomasse - nachhaltig und ressourcenschonend nutzen. Umwelt Bundesamt. Available online at https://www.umweltbundesamt.de/publikationen/globale-landflaechen-biomasse, checked on 8/13/2022.

UBA (2018): Emissionsbilanz erneuerbarer Energieträger. With assistance of Lauf, T. Memmler, M. Schneider, S. Umwelt Bundesamt. Available online at https://www.umweltbundesamt.de/en/publikationen/emissionsbilanz-erneuerbarer-energietraeger-2018, checked on 8/13/2022.

United Nations (2009): Buildings and Climate Change. Summary for Decision-Makers. Paris. Available online at https://wedocs.unep.org/handle/20.500.11822/32152, checked on 8/13/2022.

USDA (2021): Plants Database. United States Department of Agriculture. Available online at https://plants.usda.gov/home/plantProfile?symbol=SIHE3, updated on 12/6/2021, checked on 8/13/2022.

Vacek, V.; Repka, R. (1992): Concise results of the experiment with Silphium perfoliatum L. In *Plant Genet. Resour. Charact. Util.*, p. 5. Available online at https://agris.fao.org/agris-search/search.do?recordID=SK1997000825, checked on 8/13/2022.

Van der Putten J.; Lesage K.; De Schutter G. (2016): Influcence of the particle shape on the packing density and pumpability of UHPC. Available online at https://www.researchgate.net/publication/312053582_Influence_of_the_particle_shape_on_the_packing_density_and_pumpability_of_UHPC.

van der Weijde, T.; Kiesel, A.; Iqbal, Y.; Muylle, H.; Dolstra, O.; Visser, R.G.F. et al. (2017): Evaluation of Miscanthus sinensis biomass quality as feedstock for conversion into different bioenergy products. In *GCB Bioenergy* 9 (1), pp. 176–190. DOI: 10.1111/gcbb.12355.

van Tassel, D.L.; Albrecht, K.A.; Bever, J.D.; Boe, A.A.; Brandvain, Y.; Crews, T.E. et al. (2017): Accelerating Domestication: An Opportunity to Develop New Crop Ideotypes and Breeding Strategies Informed by Multiple Disciplines. In *Crop Science* 57 (3), p. 1274. DOI: 10.2135/cropsci2016.10.0834.

van Tassel, D.L.; DeHaan, L.R.; Cox, T.S. (2010): Missing domesticated plant forms: can artificial selection fill the gap? In *Evolutionary Applications* 3 (5-6), pp. 434–452. DOI: 10.1111/j.1752-4571.2010.00132.x.

VDLUFA (Ed.) (2007): Die chemische Untersuchung von Futtermitteln. Band III. Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten e.V. 3rd ed.

VDLUFA (Ed.) (2011): Umweltanalytik. Band VII. Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten e.V. 4th ed.

Velthof, G.L.; Lesschen J.P.; Schils R.L.m., S.A. (2014): Grassland_2014_Final report.

Venable, D.L. (2007): Bet hedging in a guild of desert annuals. In *Ecology* 88 (5), pp. 1086–1090. DOI: 10.1890/06-1495.

Vetter, A.; Biertümpfel, A.; Conrad, M. (2010): Abschlussbericht: Optimierung des Anbauverfahrens für Durchwachsene Silphie (Silphium perfoliatum) als Kofermentpflanze in Biogasanlagen sowie Überführung in die landwirtschaftliche Praxis. Thüringer Landesanstalt für Landwirtschaft. Available online at http://www.fnr-server.de/ftp/pdf/berichte/22004307.pdf, checked on 8/13/2022.

Vico, G.; Brunsell, N.A. (2018): Tradeoffs between water requirements and yield stability in annual vs. perennial crops. In *Advances in Water Resources* 112, pp. 189–202. DOI: 10.1016/j.advwatres.2017.12.014.

Vo, L.T.; Navard, P. (2016): Treatments of plant biomass for cementitious building materials – A review. In *Construction and Building Materials* 121, pp. 161–176. DOI: 10.1016/j.conbuildmat.

Warnasooriya, S.N.; Brutnell, T.P. (2014): Enhancing the productivity of grasses under high-density planting by engineering light responses: from model systems to feedstocks. In *Journal of Experimental Botany* 65 (11), pp. 2825–2834. DOI: 10.1093/jxb/eru221.

Weaver, J.E.; Stoddart, L.A.; Noll, W. (1935): Response of the Prairie to the Great Drought of 1934. In *Ecology* 16 (4), p. 612. DOI: 10.2307/1932592.

Weir, B.S.; Cockerham, C.C. (1984): Estimating F-statistics for the analysis of population structure, evolution. In *Int. J. Organic Evol.* 38, p. 1358. DOI: 10.2307/2408641.

Westhoek, H.; Lesschen, J.P.; Rood, T.; Wagner, S.; Marco, A. de; Murphy-Bokern, D. et al. (2014): Food choices, health and environment: Effects of cutting Europe's meat and dairy intake. In *Global Environmental Change* 26, pp. 196–205. DOI: 10.1016/j.gloenvcha.2014.02.004.

Wever, C.; Becker, L. (2016): Erfolgreiche Forschungsreise hat viele Follower. Available online at https://news.cision.com/de/heinrich-heine-universitat-dusseldorf/r/erfolgreiche-forschungsreise-hat-viele-follower,c2090038, updated on 8/11/2022, checked on 8/13/2022.

Wever, C.; Höller, M.; Becker, L.; Biertümpfel, A.; Köhler, J.; van Inghelandt, D. et al. (2019): Towards high-biomass yielding bioenergy crop Silphium perfoliatum L.: phenotypic and genotypic evaluation of five cultivated populations. In *Biomass and Bioenergy* 124, pp. 102–113. DOI: 10.1016/j.biombioe.2019.03.016.

Wever, C.; van Tassel, D.L.; Pude, R. (2020): Third-Generation Biomass Crops in the New Era of De Novo Domestication. In *Agronomy* 10 (9), p. 1322. DOI: 10.3390/agronomy10091322.

Wickham, H.; Averick, M.; Bryan, J.; Chang, W.; McGowan, L.; François, R. et al. (2019): Welcome to the Tidyverse. In *Journal of Open Source Software* 4 (43), p. 1686. DOI: 10.21105/joss.01686.

Wickham, J.H.; Francois, R.; Bryan, J.; Bearrows, S. (2022): Read Rectangular Text Data [R package readr version 2.1.2]: Comprehensive R Archive Network (CRAN). Available online at https://cran.r-project.org/web/packages/readr/index.html, checked on 8/13/2022.

Willett, W.; Rockström, J.; Loken, B.; Springmann, M.; Lang, T.; Vermeulen, S. et al. (2019): Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. In *The Lancet* 393 (10170), pp. 447–492. DOI: 10.1016/S0140-6736(18)31788-4.

Wisur, H.; Sjöberg, L.-A.; Ahlgren, P. (1993): Selecting a potential Swedish fibre crop: fibres and fines in different crops as an indication of their usefulness in pulp and paper production. In *Industrial Crops and Products* 2 (1), pp. 39–45. DOI: 10.1016/0926-6690(93)90009-X.

Wrobel, M. (2013): Influence of degree of fragmentation on chosen quality parameters of briquette made from biomass of cup plant Silphium perfoliatum L. In *Engineering for Rural Development*, pp. 653–657. Available online at https://agris.fao.org/agris-search/search.do?recordID=LV2013000731, checked on 8/13/2022.

Wu, T.D.; Nacu, S. (2010): Fast and SNP-tolerant detection of complex variants and splicing in short reads. In *Bioinformatics* 26 (7), pp. 873–881. DOI: 10.1093/bioinformatics/btq057.

Wüstenberg, T. (2015): Cellulose and cellulose derivatives in the food industry. Weinheim: Wiley-VCH. Available online at https://books.google.de/books?id=ypI_BAAAQBAJ, checked on 8/13/2022.

Yancey, N.; Wright, C.T.; Westover, T.L. (2013): Optimizing hammer mill performance through screen selection and hammer design. In *Biofuels* 4 (1), pp. 85–94. DOI: 10.4155/bfs.12.77.

Ye, H.; Zhang, Y.; Yu, Z.; Mu, J. (2018): Effects of cellulose, hemicellulose, and lignin on the morphology and mechanical properties of metakaolin-based geopolymer. In *Construction and Building Materials* 173, pp. 10–16. DOI: 10.1016/j.conbuildmat.2018.04.028.

YIECP (2018): State Register of Plant Varieties Suitable for Dissemination in Ukraine. Kyiv, Ukraine. Available online at http://www.sops.gov.ua/uploads/page/5aa63108e441e.pdf, checked on 8/13/2022.

Yu, Q.L.; Spiesz, P.; Brouwers, H.J.H. (2013): Development of cement-based lightweight composites – Part 1: Mix design methodology and hardened properties. In *Cement & Concrete Composites* 44, pp. 17–29. DOI: 10.1016/j.cemconcomp.2013.03.030.

Yu, Y.; Koljonen, K.; Paulapuro, H. (2002): Surface chemical composition of some non-wood pulps. In *Industrial Crops and Products* 15 (2), pp. 123–130. DOI: 10.1016/S0926-6690(01)00102-9.

Zeldin, M.; Wynne, K.J.; Allcock, H.R. (1988): Inorganic and organometallic polymers. Macromolecules containing silicon, phosphorus, and other inorganic elements. Washington DC: American Chemical Society (ACS symposium series, 360).

Zhai, H., Lee, Z. (2006): Ultrastructure and topochemistry of delignification in alkaline pulping of wheat straw. In *Journal of Wood Chemistry and Technology* (9 (3)), 387–406. DOI: https://doi.org/10.1080/02773818908050306.

Zhang, H.; Shen, Z.; Yang, G.; An, T.; Sun, Q. (2011): Effect of chicken manure-amended copper mine tailings on growth of compositae Silphium perfoliatum and substrate properties. Available online at https://www.researchgate.net/publication/287469546_Effect_of_chicken_manure-amended_copper_mine_tailings_on_growth_of_three_leguminous_species_soil_microbial_biomass_and_enzyme_activities.

Zhang, Q.; Zhou, Q.; Ren, L.; Zhu, Y.; Sun, S. (2006): Ecological effects of crude oil residues on the functional diversity of soil microorganisms in three weed rhizospheres. In *Journal of Environmental Sciences* 18 (6), pp. 1101–1106. DOI: 10.1016/S1001-0742(06)60046-6.

Zhang, X.; Xia, H.; Li, Z.; Zhuang, P.; Gao, B. (2010a): Potential of four forage grasses in remediation of Cd and Zn contaminated soils. In *Bioresource Technology* 101 (6), pp. 2063–2066. DOI: 10.1016/j.biortech.2009.11.065.

Zhang, Z.; Ersoz, E.; Lai, C.-Q.; Todhunter, R.J.; Tiwari, H.K.; Gore, M.A. et al. (2010b): Mixed linear model approach adapted for genome-wide association studies. In *Nature Genetics* 42 (4), pp. 355–360. DOI: 10.1038/ng.546.

Zheng, X.; Levine, D.; Shen, J.; Gogarten, S.M.; Laurie, C.; Weir, B.S. (2012): A high-performance computing toolset for relatedness and principal component analysis of SNP data. In *Bioinformatics* 28 (24), pp. 3326–3328. DOI: 10.1093/bioinformatics/bts606.

DIN 6167, 1980-01: Description of yellowness of near-white or near-colourless materials. Available online at https://www.din.de/de/mitwirken/normenausschuesse/fnf/veroeffentlichungen/wdc-beuth:din21:544142.

NREL TP-510-42618, 2012: Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP) (Revised July 2011). Available online at https://www.nrel.gov/docs/gen/fy13/42618.pdf, checked on 8/13/2022.

DIN EN ISO 536, 1996-08: Paper and board - Determination of grammage. Available online at https://www.din.de/de/mitwirken/normenausschuesse/npa/veroeffentlichungen/wdc-beuth:din21:312886884.

DIN EN ISO 534, 2005-05: Paper and board – Determination of thickness, density and specific volume. Available online at https://www.din.de/de/mitwirken/normenausschuesse/navp/wdc-beuth:din21:145713171.

ISO 1762, 2001-12: Paper, board and pulps — Determination of residue (ash) on ignition at 525 °C. Available online at https://www.din.de/de/mitwirken/normenausschuesse/npa/veroeffentlichungen/wdc-beuth:din21:311580245.

ISO 187, 1990-12: Paper, board and pulps; standard atmosphere for conditioning and testing and procedure for monitoring the atmosphere and conditioning of samples. Available online at https://www.din.de/de/mitwirken/normenausschuesse/npa/veroeffentlichungen/wdc-beuth:din21:984678.

ISO 2144, 1997: Paper, board, pulps and cellulose nanomaterials - Determination of residue (ash content) on ignition at 900 °C. Available online at https://www.din.de/de/mitwirken/normenausschuesse/npa/veroeffentlichungen/wdc-beuth:din21:310726314.

ISO 16065-1, 2014-04: Pulps — Determination of fibre length by automated optical analysis — Part 1: Polarized light method. Available online at https://www.din.de/de/mitwirken/normenausschuesse/npa/veroeffentlichungen/wdc-beuth:din21:204672328.

ISO 5264-2, 2011-02: Pulps - Laboratory beating. Available online at https://www.din.de/en/getting-involved/standards-committees/npa/publications/wdc-beuth:din21:139233913?destinationLanguage=&sourceLanguage=.

ISO 5269-2, 2005-05: Pulps – Preparation of laboratory sheets for physical testing. Available online at https://www.din.de/en/getting-involved/standards-committees/npa/publications/wdc-beuth:din21:76864665?destinationLanguage=&sourceLanguage=.

ISO 18134-3, 2015: Solid biofuels — Determination of moisture content — Oven dry method — Part 3: Moisture in general analysis sample. Available online at https://www.din.de/en/getting-involved/standards-committees/nmp/publications/wdc-beuth:din21:242039782.

ISO 12625-3, 2005-09: Tissue paper and tissue products. Available online at https://www.din.de/en/getting-involved/standards-committees/npa/publications/wdc-beuth:din21:203861149.

ISO 12625-4, 2016: Tissue paper and tissue products. Available online at https://www.din.de/en/getting-involved/standards-committees/npa/publications/wdc-beuth:din21:266872530.

ISO 12625-7, 2014-08: Tissue paper and tissue products. Available online at https://www.din.de/en/getting-involved/standards-committees/npa/publications/wdc-beuth:din21:345140598.

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