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Investigations on plant functional traits, epidermal structures and the ecophysiology of the novel bioenergy species *Sida hermaphrodita* Rusby and *Silphium perfoliatum* L.

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Summary

A growth experiment was performed with different accessions of the novel bioenergy species *Sida hermaphrodita* Rusby (Fanpetals, Malvaceae) and *Silphium perfoliatum* L. (Cup Plant, Asteraceae) to study differences between the perennial shrubs and the variation among biotypes. Non-destructive assessments (phenology, leaf numbers, height, SPAD and gas exchange) and several harvests were conducted to examine growth, allometry and the quality of shoot material (C:N, raw ash, protein, fibre, fat, calorific value and leaf $\delta^{13}\text{C}$). In addition to the functional analyses, epidermal structures of two widely used accessions were addressed to give insight into anatomical properties of the species.

In the establishment phase, productivity was higher on average in *S. hermaphrodita* than in *S. perfoliatum* since latter remains in the rosette stage in the first year. While the accessions of *S. hermaphrodita* did not significantly differ in growth, functional traits and forage quality, *S. perfoliatum* showed large biotypic variation. Leaf water and ash contents, raw protein and fat levels were higher in latter species, while raw fibre contents were twice as high in *S. hermaphrodita*. At the end of the season, the calorific value of senesced stems of *S. hermaphrodita* proved to be higher than that of senesced leaves of *S. perfoliatum* pointing to its suitability of as a solid fuel.

Despite the shorter longevity, lower SPAD and smaller size, the delicate leaves of *S. hermaphrodita* had more stomata, higher photosynthetic rates and higher stomatal conductances than the robust leaves of *S. perfoliatum*. Less negative $\delta^{13}\text{C}$ signatures in *S. perfoliatum* (-27.3 ‰) compared to *S. hermaphrodita* (-30.24 ‰), point to the potentially higher water use efficiency of *S. perfoliatum*. Further investigations on the relationships between leaf properties, carbon acquisition and stomatal conductance under dry conditions may serve to select productive lines of the bioenergy species on marginal land thus avoiding conflicts with the farming of food crops.

Introduction

Among the many alternative bioenergy crops under discussion (see compilation by EL BASSAM, 2010), two species originally native to the cool temperate North-Eastern US, *Silphium perfoliatum* L. (Cup Plant or Rosinweed) and *Sida hermaphrodita* Rusby (Virginia Mallow or Fanpetals) are currently being introduced into temperate and sub-boreal Europe. Both species are perennial and remain highly productive over many years in managed permacultures. Under optimum agricultural practice yields of both species have been reported to reach a shoot dry mass of 9 to 20 t ha⁻¹ per annum (AURBACHER et al., 2012; BORKOWSKA and MOLAS, 2012).

S. perfoliatum is realized as an ideal feedstock for biogas systems in many of the so called bioenergy regions with currently over

250 hectares in Germany, according to BIERTÜMPFEL et al. (2012). The species is seen as a true alternative to maize monocultures which have created much concern due to high environmental burdens and reduced landscape esthetics. Green shoot mass (stems plus leaves) is harvested in autumn and the material is then used as a co-substrate in biogas plants. Methane production potentials have been shown to reach 320 NI per kg organic dry matter, which compares well to the results obtained with maize (BIERTÜMPFEL et al., 2012). In contrast, the lignocellulosic biomass of *S. hermaphrodita* is primarily used as a solid fuel. In Poland the species is cultivated on 750 ha momentarily (IGLINSKI et al., 2011), while in Germany it is grown in demonstration trials solely. Shoot mass of cultivated *S. hermaphrodita* is harvested in the winter and consists of dry leafless stems which are used in small combustion units. Energy contents of pellets made from its stems are comparable to those of pine wood chips with calorific values ranging from 16 to 18 MJ per kg dry mass (BORKOWSKA and MOLAS, 2012).

Although native to the Eastern US and Southeastern Canada, both plant species have been investigated in countries from the former Eastern Block first (SOKOLOV and GRITSAK, 1972; NEUMERKEL, 1980; BORKOWSKA, 2003, 2006). Only recently, the bioenergy potentials of the tall native grassland shrubs including the two species have been realized in the US (BOE et al., 2012; VOIGT et al., 2012), while the fodder value of *S. perfoliatum* had been highlighted before (STANFORD, 1990; ALBRECHT and GOLDSTEIN, 1997; LEHMKUHLER et al., 2007). In contrast to this species, the agronomic value of *S. hermaphrodita* has not yet been realized in Northern America, probably because it is nowadays a rare species (THOMAS, 1979; COSEWIC, 2010; BICKERTON, 2011). Both species prefer habitats in moist prairies and floodplains (for the ranges of the species see USDA Plants Database) but they can grow outside riverine ecosystems as well. While *S. perfoliatum* expands into the dry prairies west of the Mississippi, *S. hermaphrodita* is restricted to the Eastern US. However, it is unclear whether the more continental distribution of *S. perfoliatum* implies that it has a higher drought and frost resistance than *S. hermaphrodita*. For a detailed description of the anatomy and ecology of the two C3-species refer to USDA (2002), SPOONER et al. (1985) and BICKERTON (2011). *S. perfoliatum* belongs to the Composite (Asteraceae) Family and has large, dark-green fleshy ovate leaves (10 to 30 cm) that fuse oppositely around the four angled stem. *S. hermaphrodita* belongs to the Mallow (Malvaceae) Family and has smaller and more delicate leaves (5 to 20 cm). The light-green maple-shaped leaves are arranged alternately along the round stem.

Since no information on the growth and functional ecology of the assimilative organs of the two species and different biotypes (origins) was available from the literature, present study was set up to get new insights into leaf traits, chemical quality of the biomass and epidermal structures of *S. perfoliatum* and *S. hermaphrodita*. In order to compare the ecophysiological behavior of the species during the establishment phase, photosynthesis, stomatal conductance and ^{13}C discrimination were measured and related to various leaf traits.

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Materials and methods

Growth experiments with different seed origins

In order to address differences in leaf traits, growth and shoot quality between the two species and between different accessions twelve seed lots in total (Tab. 1) were available in present study. The experiments followed the functional plant ecology protocol set up by CORNELISSEN et al. (2003) and were based on consecutive harvests of plants in which dry weights of different plant organs and leaf areas were determined. On 15 March 2012 seeds were sown in rows into trays filled with a standard earth (Fruhstorfer LD 80, Hawita GmbH Vechta). During the first four weeks trays were kept in climate chambers (Vötsch Bioline, Balingen) in which temperatures varied between 3 and 11 °C causing stratification and breaking of the seed dormancy.

After 50 days, ten seedlings of each of the ten origins that were able to germinate in high quantities were transferred into 1.7 L PE pots to follow growth for the following three months in a greenhouse trial. Soil substrate used in the pot experiment consisted of a mixture of LD 80 : river sand, 1:2 (v:v) resulting in an N-availability of 50 kg N ha⁻¹ which should suffice growth in the establishment phase. No extra light was supplied during the experiment and plants were regularly randomized to avoid shading and positioning effects. Temperatures in the greenhouse varied between 15 and 30 °C and plants were watered upon demand. In order to describe the variation in productivity and bioenergetic value within a population after the establishment phase, 65 plants of the accessions 3 and 8 were planted into 7 L plant containers using the same soil mixture like in the greenhouse trial. Plant containers had an N-availability of 100 kg N ha⁻¹ and were left outdoors. The two accessions were chosen, because their germplasm is widely used in bioenergy plantations in Germany and Poland. Aiming at the long term study of different origins, four seedlings per accession and square meter were also planted into an experimental garden to follow growth of the different origins outdoors in natural soil in the year after plant establishment.

During the greenhouse experiment, nondestructive methods included the weekly counting of leaf numbers, determining plant length and leaf greenness and the development of senescence (SPAD). Furthermore, photosynthetic rates (A) and stomatal conductances (gs) of the pot-grown plants were determined three times in June 2012 (92, 99 and 106 DAS) using a LI-6400 system (Li-Cor, Nebraska). Measurements were made under defined conditions (PAR 800 μmol m⁻²

s⁻¹, 24 °C, VPD 1.5 kPa). Only the plants to be harvested at the final harvest were used for these comparisons resulting in 16 plants of *S. hermaphrodita* and 24 plants of *S. perfoliatum*. Three harvests were performed fortnightly after 78, 92 and 106 days after sowing (DAS) in which leaf area and dry weights of the fractions leaves, stems and roots were determined. Samples were dried to constant weight at 80 °C and weighed thereafter. Shoot mass at the third harvest was used for the determination of C:N ratios, raw ash, raw protein, raw fat and raw fibre contents after the method specified in EC (2009). However, dry mass of the accessions 9 and 10 was not sufficient for these analyses.

Plants that were grown in larger plant containers were harvested when plants had completely senesced, i.e. 263 DAS for *S. hermaphrodita* and 279 DAS for *S. perfoliatum*. At that time the previous species had shed leaves completely and only the stem fraction was present, while in latter species the senesced rosette was harvested. Material was subjected to quality analyses (dry mass, ash contents and calorific value).

Stem mass of the four accessions of *S. hermaphrodita* that were planted into the experimental garden in 2012 was harvested in April 2013. Dry weights, ash contents and calorific values were determined in 16 individual plants to compare the bioenergy potentials of the different accessions and the variation thereof. An IKA[®] oxygen bomb calorimeter was used for the energy analyses. In contrast to the previous set of plants, these analyses were not performed in the six *S. perfoliatum* accessions since the species does not produce stems in the first year and its use is not intended as a fuel plant. Re-growth and length increments of the ten accessions were followed in the season of 2013 to investigate whether differences persisted in the second year. In July, green and senescing leaves were harvested to determine the relationships between SPAD, nitrogen and chlorophyll concentrations, δ¹³C as well as specific leaf area (SLA). Chlorophyll analyses followed INSKEEP and BLOOM (1985) while nitrogen, carbon and carbon isotope discrimination (δ¹³C) analyses were performed using isotope-ratio mass spectrometry (IRMS). Latter analyses followed FRANZARING et al. (2012).

Scanning electron microscopy of epidermal structures

For scanning electron microscopy (SEM), 1.0 cm x 0.5 cm pieces of medium aged leaves of *S. perfoliatum* and *S. hermaphrodita* (accessions no. 3 and 8) were frozen in liquid nitrogen, freeze dried

Tab. 1: Source of seed materials, harvest years (if known), origins (if known) of *S. hermaphrodita* and *S. perfoliatum* accessions used in the experiments. (○) marks the ten seed origins which germinated readily and were used in the greenhouse study on growth differences of different seed origins, (●) marks the two accessions that are primarily used in bioenergy plantations in Germany and Poland.

Nr.		Seed suppliers, country and harvest year (if known)	Population origin
<i>Sida hermaphrodita</i> L. Rusby			
1	○	B&T Seeds, Aigues-Vives (F)	unknown
2	○	Biomatwa, Dr. Bury, Mr. Kozakowski (PL), harvest 2003	unknown
3	○●	Biomatwa, Dr. Bury, Mr. Kozakowski (PL), harvest 2009	unknown
4	○	Botanical Garden Hohenheim (D)	unknown
5	○	Jelitto Schwarmstedt (D)	unknown
<i>Silphium perfoliatum</i> L.			
6	○	B&T Seeds, Aigues-Vives (F)	unknown
7	○	Bioenergieregion Hohenlohe (D)	unknown
8	○●	Chrestensen Erfurt (D)	unknown
9	○	Dr. K. Albrecht, University of Wisconsin (US)	Wisconsin
10	○	Dr. K. Albrecht, University of Wisconsin (US)	Wisconsin x Arkansas
11	○	Botanical Garden Hohenheim (D)	unknown
12	○	Dr. J. West, USDA National Plant Materials Center (US), 2008	Pearsons, Kansas

for 25 h (K750X, Quorum), mounted on Al-stubs and sputtered with gold-palladium (SCD, Blazers Union). Both sides of the leaves were investigated in a scanning electron microscope DSM 940 (Zeiss) at 5 kV.

Statistical analyses

Data from the growth experiments and the quality analyses were subjected to descriptive statistical analyses and graphically processed using various libraries of the free software environment for statistical computing and graphics (R Development Core Team, 2008). In order to identify and visualize significant differences between the different origins pair-wise multiple comparisons were performed using the library 'multcomp'. Distributions of growth and quality parameters determined in samples of the two selected populations were addressed using histograms and different measures of variance.

Results and discussion

Relationships between SPAD, chlorophyll and N, SLA and isotopic discrimination

Leaf greenness (SPAD units) was related to chlorophyll concentrations determined in the same leaf disks to derive calibration functions for the two species. In addition, SPAD measurements in green and senescing leaves were also related to specific leaf area (SLA), nitrogen concentrations and $\delta^{13}\text{C}$ signatures of the sampled leaves to identify species-specific calibration functions and differences in the isotope discrimination. Fig. 1a shows the leaf chlorophyll concentrations in relation to the SPAD values. Coefficients of determination were high for both species and chlorophyll concentrations increased linearly with increasing SPAD.

However, the slope of the relationship was much steeper in the dark green leafed *S. perfoliatum* as compared to the light green and thin leafed *S. hermaphrodita*. In contrast to the linear relationship between SPAD and leaf chlorophyll over a wide range found in the two shrubs, UDDLING et al. (2007) observed non-linear but exponential relationships between the parameters in birch, wheat and potato which could at least in part be due to the use of the less powerful solvent acetone in the chlorophyll extraction. As has been shown by INSKEEP and BLOOM (1985) pigment extraction with dimethylformamide (DMF) is superior to the use of acetone since extraction is more complete and pigments are more stable in DMF.

Chlorophyll degradation in senescing leaves of both species was paralleled by leaf thinning, which is indicated by the negative relationship between SLA and SPAD (data not shown). These findings are in contrast to the increasing leaf density (lowering of SLA) during the first weeks of plant growth (see Fig. 3) due to the consecutive accumulation of assimilates. At the same time, N and C concentrations were inversely related to SLA (data not shown) in older plants indicating an export of soluble carbohydrates and proteins from the senescing leaf. While C:N showed only a slight relationship to SLA, high coefficients of determination were found for the relationship between SPAD and C:N (Fig. 1c), confirming that leaf ageing leads to a remobilization of nitrogen and a relative accumulation of carbon. However, the feed value and probably the fermentation quality of old leaves will be reduced with increasing C:N but optimal harvest times are still under discussion.

Variation of leaf $\delta^{13}\text{C}$ showed relationships to several leaf traits (leaf N% and C% and SPAD values), but highest coefficients of determination were found between SLA and carbon isotope discrimination

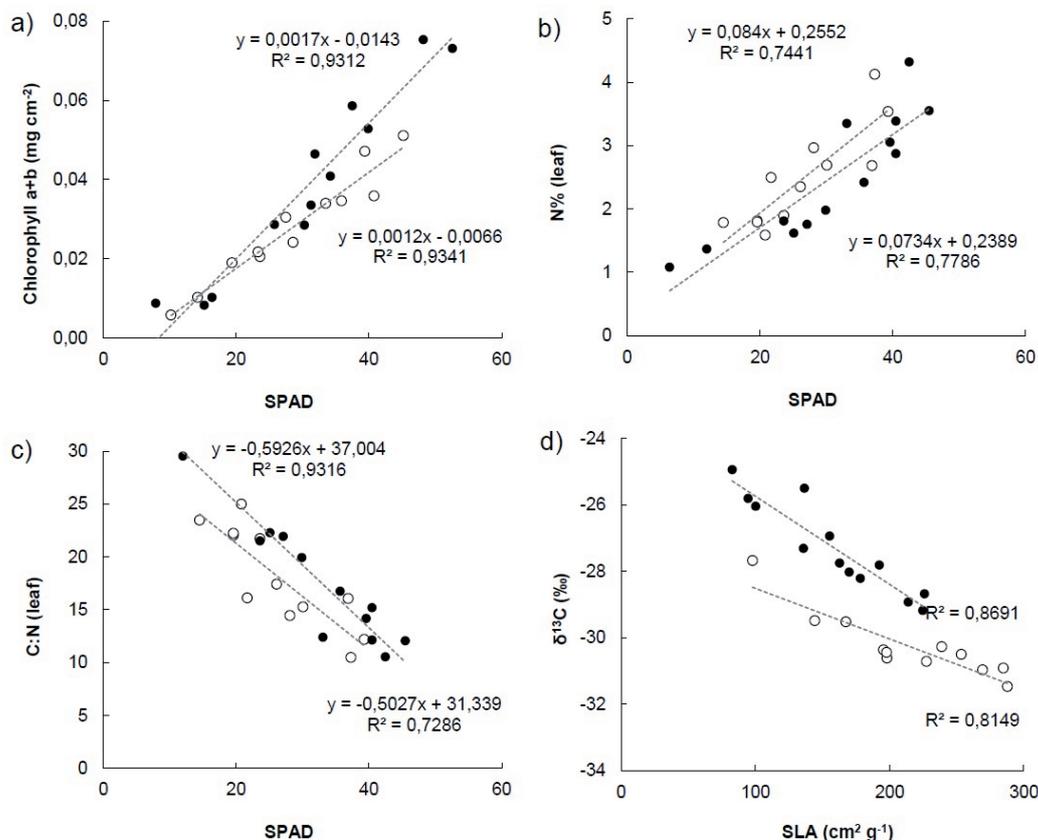


Fig. 1: Relationships between SPAD and leaf chlorophyll concentrations (a), leaf N concentration (b) and C:N (c) and relationships between specific leaf area and $\delta^{13}\text{C}$ signatures (d) in *S. hermaphrodita* (○) and *S. perfoliatum* (●).

(CID) in both species. With increasing SLA in the older leaves CID became stronger (i.e. $\delta^{13}\text{C}$ decreased, Fig. 1d), indicating the cumulative effect of stomatal discrimination with leaf age and probably, post-photosynthetic fractionation. However, carbon isotopic fractionation in non-photosynthetic tissues due to respiration and carbon remobilization to other plant parts are currently poorly understood (CERNUSAK et al., 2009). The same sort of relationship between SLA and CID was also found by SCHULZE et al. (2006) in *Eucalyptus* species along a rainfall gradient. Leaves in drier climates were thicker (lowered their SLA) and also reduced stomatal conductivity, which in turn resulted in a lower CID (less negative $\delta^{13}\text{C}$ -values).

On average, $\delta^{13}\text{C}$ -values averaged to -27.3‰ in *S. perfoliatum* and to -30.24‰ in *S. hermaphrodita*. Both signatures lie within the typical range for C3-species, in which isotopic fractionation is dominated by carboxylation. Since *S. hermaphrodita* tends to discriminate ^{13}C more strongly than *S. perfoliatum* it may be expected that its leaves lead to a higher stomatal fractionation of the heavier isotope and probably to a lower water use efficiency than in the other species. Differences between leaf properties and gas exchange characteristics of the two species are addressed below and relationships between CID and leaf properties are discussed.

Growth and plant quality in the establishment phase

In the greenhouse experiment, which lasted until 106 DAS (i.e. the establishment phase), pot-grown plants of *S. hermaphrodita* produced 13.3 g dry mass per plant across different seed origins while *S. perfoliatum* produced only 6.8 g dry mass on average. However, the accessions primarily used in European bioenergy plantations did not differ in productivity. A reason for the higher productivity of *S. hermaphrodita* within the first three months may be that it soon forms a stem with new leaves while *S. perfoliatum* plants remain in the rosette stadium in the first season. At the final harvest of *S. hermaphrodita*, the leaf fraction accounted for 41% of the shoot dry mass. At that time, no leaf had turned senescent. For an overview of the results from the final harvest and general differences in leaf traits and leaf quality between the species refer to Tab. 2, while a graphical representation of total biomass increments from the first to the third harvest is given in Fig. 2.

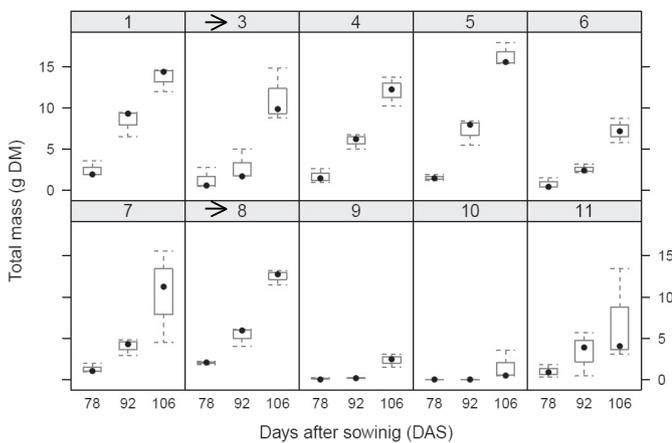


Fig. 2: Total biomass (shoot plus root) of four accessions of *S. hermaphrodita* (1, 3, 4 and 5) and six biotypes of *S. perfoliatum* (6 to 11) observed at three harvests performed at 78, 92 and 106 days after sowing. Numbers of origins (panel labels) refer to those given in Tab. 1. The presence of significant differences between different accessions at the final harvest is noted in Tab. 2. Arrows mark those accessions, which are the widely planted genetic material of the two species.

In contrast to the absence of significant differences in plant growth between the four origins of *S. hermaphrodita*, the six *S. perfoliatum* origins featured a highly variable growth (Fig. 2 and Tab. 2). As can be seen in Fig. 3b leaves of *S. perfoliatum* continuously increased their size especially in the productive biotypes. In contrast, leaves of *S. hermaphrodita* reached a maximum leaf size of about 100 cm^2 but over the first three months produced twice as many leaves as *S. perfoliatum*. Owing to the differences in specific leaf weight (inverse of SLA) and the formation of stems in *S. hermaphrodita*, the relationship between leaf area and shoot mass accumulation differed between the species (Fig. 3a), i.e. with an almost linear relationship between the parameters in *S. perfoliatum* and a curvi-linear relationship in *S. hermaphrodita*. As the plants got older, SLA and ash contents decreased due to the accumulation of photoassimilates (Fig. 3c, d). At the same time SLA and leaf water and SLA and shoot ash contents were positively associated (Fig. 3e, f) in both species.

Despite the thinner and more delicate assimilation organs leaves of *S. hermaphrodita* had lower leaf water contents on average than the fleshy leaves of *S. perfoliatum*. On the other hand raw ash contents of leaves of the latter species were higher than the shoot ash concentrations of *S. hermaphrodita*. Further differences in shoot quality between different accessions and between the two species at the end of the establishment phase (106 DAS) are presented in Tab. 2. As can be seen, leaves of *S. perfoliatum* had a higher concentration of raw protein and raw fat than *S. hermaphrodita*, while the shoot mass of latter had a higher raw fibre content than *S. perfoliatum* reaching 23% at the end of the establishment phase. At the same time the shoot material of *S. hermaphrodita* had a lower nitrogen concentration and a higher C:N than that of *S. perfoliatum*, while C contents did not differ between the two species.

Growth, ash contents and calorific values after the first season

In order to also study the productivity, ash contents and energetic values at the end of the first season shoot material of the two widely used accessions 3 and 8 was collected in the late autumn. Since leaves of *S. hermaphrodita* had already completely been shed, shoot mass of the available 61 pot-grown plants only consisted of stems. In contrast, shoot mass of the 64 individual pot-grown plants of *S. perfoliatum* consisted of leaves only. Histograms showing the variation between individual plants and descriptive statistics for the three variables are presented in Fig. 4. When comparing the differences in productivity between plants of the same accessions at the end of the establishment phase (106 DAS) and at the end of the first season, *S. perfoliatum* increased (tripled) its leaf mass from 8.1 g DM per plant to 26.6 g DM per plant. At the same time, stem mass in *S. hermaphrodita* only doubled from 3.9 at the end of the establishment phase to 8.4 g DM per plant at the end of the vegetation period. These findings indicate that growth of *S. hermaphrodita* slows down in the autumn, while *S. perfoliatum* is able to continue with the production of new rosette leaves in its first year. Although no information on the quantity of shed leaves was available in *S. hermaphrodita* (leaves and flowers will make up less than 50% of the shoot biomass) *S. perfoliatum* will have a higher biomass at the end of the first season compared to *S. hermaphrodita*. Nevertheless, leaves of *S. perfoliatum* will also be shed during the winter leading to a remobilization of nutrients.

In line with the findings during the establishment phase ash contents after the first season were higher in leaves of *S. perfoliatum* than in stems of *S. hermaphrodita* and the material of latter species also had higher energetic values than *S. perfoliatum*. Relationships between ash contents and calorific values are shown in Fig. 5 indicating that material with a high mineral density will have a lower energetic value.

Tab. 2: Plant traits, growth and shoot quality of different accessions of *Sida hermaphrodita* and *Silphium perfoliatum* in the establishment phase (pot grown plants) and in plants that were grown for two seasons in a garden experiment. Pot grown plants were harvested 106 days after sowing (DAS) and weight and quality of stems of *S. hermaphrodita* was determined in field grown plants 395 DAS. Length increments and the onset of flowering of the ten accessions were followed in the second year in field grown plants. Different letters below the mean values indicate significant differences between the accessions (four replicates per origin) specified in Tab. 1. "DOY" indicates day of the year.

Accessions / Origins	<i>Sida hermaphrodita</i>				Mean	<i>Silphium perfoliatum</i>						Mean
	1	3	4	5		6	7	8	9	10	11	
Growth parameters of plants harvested 106 DAS												
Plant length (cm)	80.0	65.7	81.3	92.0	79.8	28.8	24.7	33.7	17.8	16.0	26.0	24.5
	a	a	a	a		a	a	a	a	a	a	
Number of leaves (n)	16.7	14.7	17.0	16.3	16.2	7.7	10.3	9.0	5.0	5.0	5.3	7.1
	ab	ab	b	a		a	a	a	a	a	a	
Leaf area (cm ² plant ⁻¹)	1378	1445	1404	1680	1477	849	1182	1265	354	257	790	783
	a	a	a	a		ab	ab	b	ab	a	ab	
Mean leaf area (cm ² leaf ⁻¹)	82.7	98.3	82.6	103.3	91.7	121.6	112.3	141.9	70.8	51.4	142.5	106.7
	a	a	a	a		a	a	a	a	a	a	
Leaf weight (g dm plant ⁻¹)	3.8	3.7	3.8	4.6	4.0	4.6	6.7	8.1	1.5	1.1	4.4	4.4
	a	a	a	a		ab	ab	a	ab	b	ab	
Specific leaf area (cm ² g ⁻¹)	365.1	394.2	366.5	367.4	373.3	187.4	177.2	155.6	238.8	275.7	189.7	204.1
	a	a	a	a		ab	ab	a	bc	c	ab	
Stem weight (g dm plant ⁻¹)	4.9	3.9	4.3	6.3	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	a	a	a	a								
Root weight (g dm plant ⁻¹)	5.0	3.6	3.9	5.5	4.5	2.7	3.7	4.4	0.8	0.4	2.5	2.4
	a	a	a	a		ab	ab	b	ab	a	ab	
Total mass (g dm plant ⁻¹)	13.6	11.1	12.1	16.3	13.3	7.2	10.5	12.5	2.4	1.5	6.9	6.8
	a	a	a	a		ab	ab	a	b	b	ab	
Shoot:root ratio	1.7	2.1	2.1	2.0	2.0	1.7	1.8	1.8	1.8	5.5	1.9	2.4
	a	a	a	a		b	b	ab	ab	ab	a	
Shoot quality of plants harvested 106 DAS												
Raw ash (% dm)	11.0	11.1	11.0	11.0	11.0	15.8	13.9	12.9	17.4	17.7	14.6	15.4
	a	a	a	a		ac	ab	b	c	c	ab	
Raw protein (% dm)	7.5	8.0	8.1	8.0	7.9	12.9	11.5	10.2	13.8	12.0	14.5	12.5
	a	a	a	a		a	a	a	a	a	a	
Raw fibre (% dm)	22.7	20.1	24.7	24.3	22.9	12.6	11.5	11.8			11.7	11.9
	a	a	a	a		a	a	a			a	
Raw fat (% dm)	2.5	2.4	2.4	2.1	2.3	3.5	7.4	4.5			3.6	4.8
	a	a	a	a		a	a	a			a	
C (% dm)	39.6	39.2	39.9	40.2	39.7	38.5	39.2	39.7			40.5	39.5
	a	a	a	a		a	ab	ab			b	
N (% dm)	1.2	1.3	1.4	1.2	1.3	2.2	2.1	1.6			2.8	2.2
	a	a	a	a		ab	ab	b			a	
CN	32.8	30.1	30.2	32.7	31.5	17.5	19.0	24.9			14.6	19.0
	a	a	a	a		a	ab	b			a	
Weight and quality of leafless stems after the winter (395 DAS, garden experiment)												
Stem weight (g dm plant ⁻¹)	22.7	31.4	29.9	30.1	28.6							
	a	a	a	a								
Raw ash (% dm)	4.09	4.49	5.37	4.48	4.6							
	a	a	a	a								
Calorific value (MJ kg ⁻¹)	16	15.78	15.84	16.17	16							
	a	a	a	a								
Plant performance in the second year (garden experiment)												
Final plant length (cm)	229	258	213	235	233	151	178	205	175	188	128	171
	a	a	a	a		ab	ab	b	ab	ab	a	
Onset of flowering (DOY)	199	198	203	201	200	203	198	193	204	202	207	201
	a	a	a	a		ab	ab	b	ab	ab	a	

Differences in stem mass, ash contents and calorific values of soil-grown plants were assessed in stems of the four accessions of *S. hermaphrodita* that were harvested in the end of the winter (395 DAS). In contrast, the leaf material from individual *S. perfoliatum* plants could not be harvested since it had almost been degraded at this time. Material of *S. hermaphrodita* only consisted of leafless stems which are used as a solid fuel in bioenergy production. No significant differences in productivity, raw ash contents and calorific values were seen between the accessions (for results see Tab. 2, last section), but mean raw ash concentrations were lower by 56% (4.6 against 11 %) than in the material which had been harvested at the end of the establishment phase. Still, raw ash contents in stems of *S. hermaphrodita* accession Nr. 3 were only slightly reduced between 263 and 395 DAS (5.2% vs. 4.9%), indicating that over the winter only few minerals were released from the stem. In contrast, the calorific

value was reduced from 17.4 to 15.8 MJ per kg DM from the early to the late winter, indicating that dead stems may lose energetic value during the winter.

Flowering phenology and length increments of in total ten accessions were determined in the garden experiment to address differences between species and plant origins. All of the plants survived the rather cold winter and re-sprouted readily in spring 2013. In the end of the season, September 2013, *S. hermaphrodita* plants were taller on average than those of *S. perfoliatum* (233 compared to 171 cm), but onset of flowering did not differ between the species. Both species began to flower on day 200 (i.e. 18 July). Like with the parameters addressed before, shoot length and flowering phenology did not differ between the four tested accessions of *S. hermaphrodita*. It can thus be followed that the species shows rather uniform

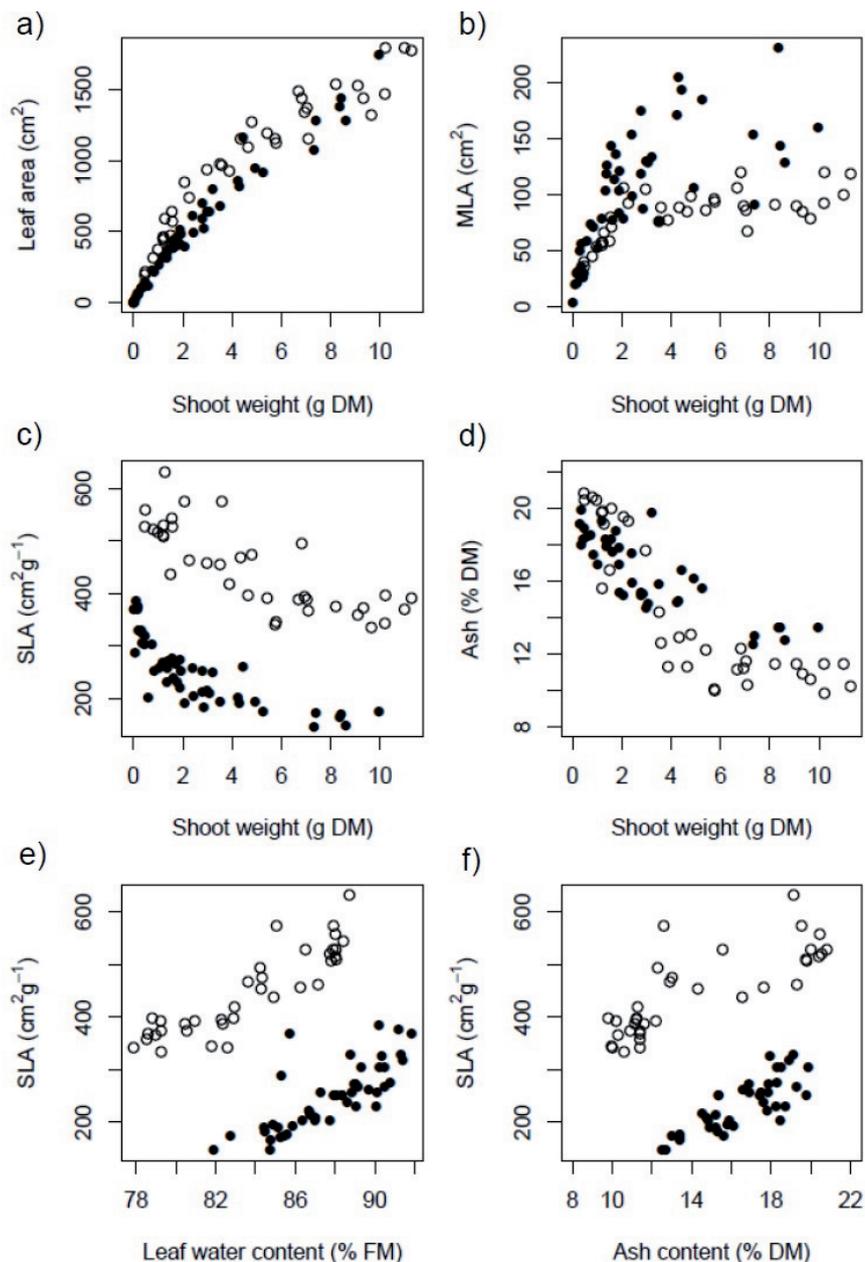


Fig. 3: Relationships between shoot mass and leaf area (a), mean leaf size (b), specific leaf area (c) and ash content (d) and relationships between specific leaf area and leaf water (e) and ash (f) contents in *S. hermaphrodita* (○) and *S. perfoliatum* (●) across different accessions determined at three consecutive harvests during the establishment phase (78, 92 and 106 DAS).

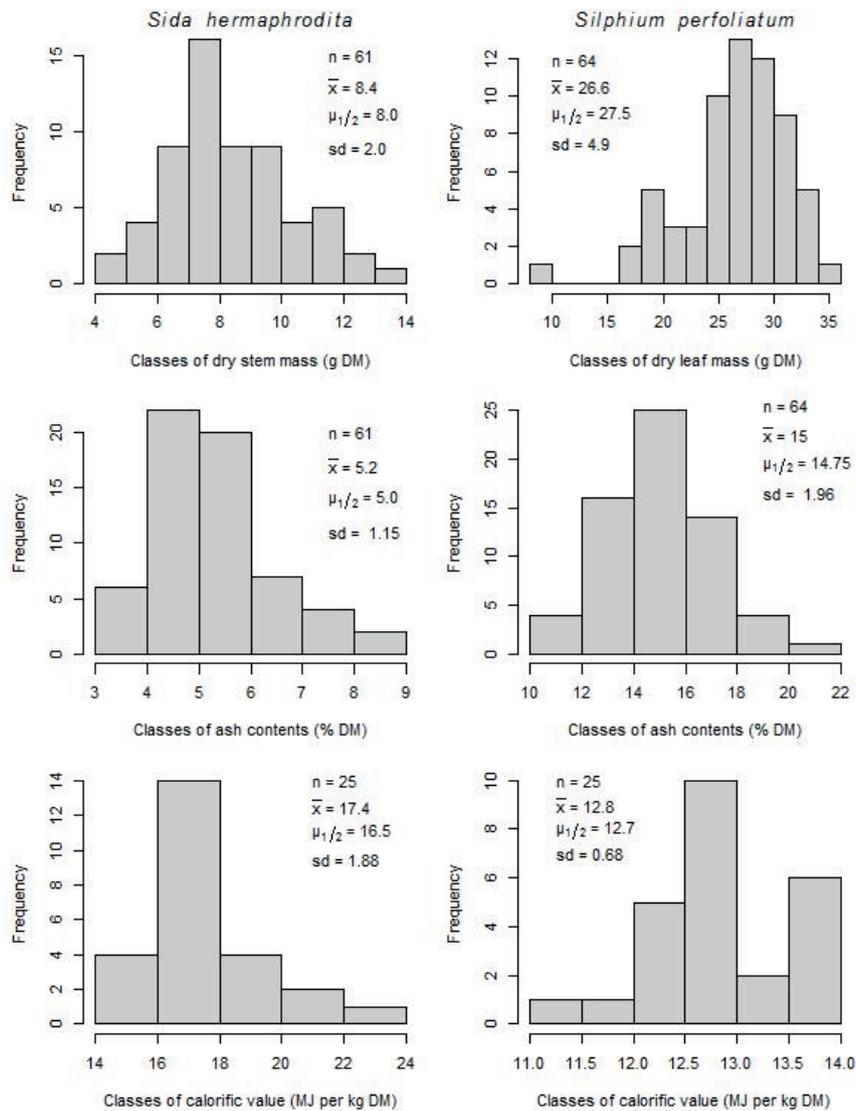


Fig. 4: Histograms of shoot mass, ash contents and calorific values of pot-grown plants of *Sida hermaphrodita* (left, accession Nr. 3) and *S. perfoliatum* (right, accession Nr. 8) harvested 263 and 279 DAS, respectively. Note: shoot mass consisted only of stems in *S. hermaphrodita* and only of leaves in *S. perfoliatum*.

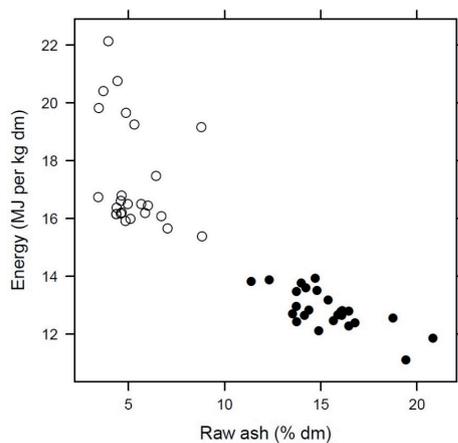


Fig. 5: Dependence of calorific values on raw ash contents of 25 randomly selected plants each of *S. hermaphrodita* accession number 3 (○) and *S. perfoliatum* accession number 8 (●) that were harvested when plants had completely senesced, i.e. 263 and 279 DAS, respectively.

behavior across different accessions. In contrast, the six accessions of *S. perfoliatum* varied to a certain extent with regard to the onset of flowering and length increment (Tab. 2) with strongest differences between accessions 8 (tallest) and 11. Latter accession, however, showed strong leaf wilting and necrotic leaf spots, which were probably due to a bacterial infection by *Pseudomonas syringae*. Similar pathogenic responses right before flowering and consecutive growth reductions have been shown by STOLZENBURG (2012).

Foliar epidermal anatomy, photosynthesis and stomatal conductance

In *S. hermaphrodita* the often silvery-shiny leaf surface and velvet like feel of the surface especially of the upper leaves point to the presence of hairy structures, while hairs on leaves of *S. perfoliatum* especially on midribs are visible to the eye. Since no information on the leaf structures of the two plant species was available, scanning electron microscopic (SEM) photos were taken from middle-aged leaves of *S. hermaphrodita* accession no. 3 and *S. perfoliatum* accession no. 8.

Both species have hairs on both leaf sides. In *S. hermaphrodita* trichomes are stellate with four to five hairs of an individual length of 200 μm (Fig. 6). In line with this observation, SHAHEEN et al. (2009) found star-shaped trichomes in seven other species of the genus *Sida*. In contrast, *S. perfoliatum* possesses simple, mostly non-glandular trichomes with a length of 200 μm . Similar trichomatic structures were observed in the related species *S. trifoliatum* and *S. integrifolium* by KOWALSKI (2008). Trichomes protect plants from insect attacks and reduce palatability, but also serve as an important feature to increase reflectivity and to reduce the air flow on the leaf surface. Latter may affect the gas exchange and the loss of water by transpiration.

Besides from being amphitrichomatic, both species have stomata on ad- and abaxial leaf sides. While the length of stomata is 20 μm in *S. hermaphrodita* and 30 μm in *S. perfoliatum*, stomatal density in *S. hermaphrodita* is somewhat higher than in *S. perfoliatum*. Exemplary counts of abaxial stomata yielded 530 per mm^2 for *S. hermaphrodita* and 220 per mm^2 for *S. perfoliatum*. Latter value compares to the density of 274 stomata per mm^2 given for the close relative *S. terebinthinaceum* by POULSON and DELUCIA (1993). However, detailed comparisons of stomatal densities and characteristics between

different species would have to be based on standardized protocols using fresh instead of lyophilized material.

Based on exemplary measurements under controlled conditions on leaves during the establishment phase a relationship between photosynthesis and stomatal conductance was observed in both species. Photosynthetic rate and stomatal conductance were higher on average in *S. hermaphrodita* than in *S. perfoliatum*. The steeper slope of this relationship in *S. hermaphrodita* than in *S. perfoliatum* (Fig. 7) indicates a lower intrinsic water use efficiency of *S. hermaphrodita*, which is paralleled by the higher stomatal number in the species as compared to *S. perfoliatum*. However, relationships between water use efficiencies, gas exchange and the number, shape and size of stomata in the two species would have to be tested under a different water supply to better understand plant water relations.

The mean values for stomatal conductance and photosynthesis determined in *S. perfoliatum* are in the same range than those found in the related species *S. terebinthinaceum* by SMITH and ULLBERG (1989). While the authors addressed the potential effects of leaf orientation on gas exchange, leaf temperatures and water saving in the large leaved *Silphium* species, present study did not account for dif-

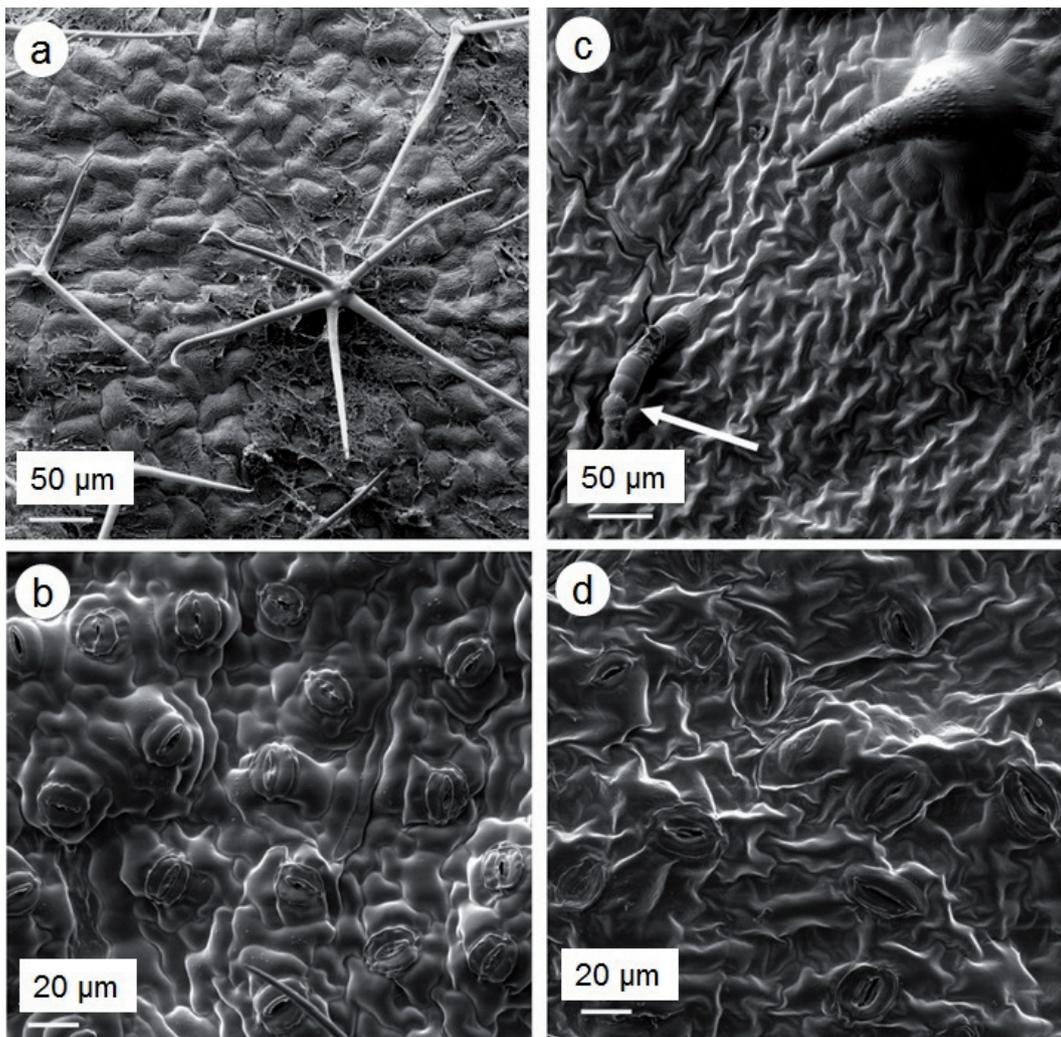


Fig. 6: Scanning electron micrographs of the epidermis of *S. hermaphrodita* (a, b) and *S. perfoliatum* (c, d). a: stellate trichomes on upper epidermis. b: lower epidermis with stomata. c: simple trichomes on upper epidermis (arrow indicates that trichomes of *S. perfoliatum* may have spiky as well as rounded tops). d: lower epidermis with stomata.

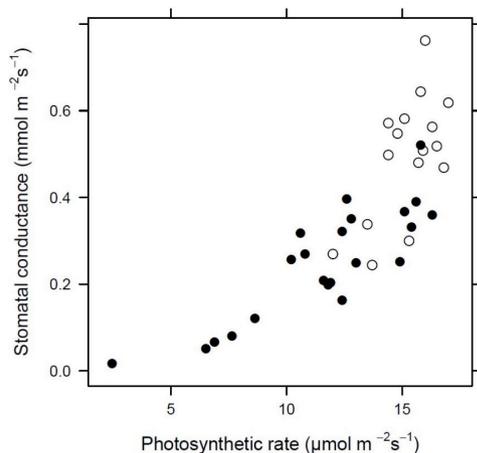


Fig. 7: Relationship between stomatal conductance and photosynthetic rate determined in leaves of *S. hermaphrodita* (○) and *S. perfoliatum* (●). Measurements were performed under controlled conditions on three days during the establishment phase (see Materials & Methods).

ferent leaf positions because *S. perfoliatum* does not belong to the group of prairie compass plants.

Since the gas exchange measurements were taken under optimum water supply at only three dates, it remains unclear whether the ecophysiological behavior of the two species will change under dry conditions and whether the differences in water use efficiency of leaves of the two species will be maintained throughout the season. Nevertheless, the above presented results on CID, i.e. the more negative $\delta^{13}\text{C}$ values, i.e. stronger discrimination of the heavier isotope in *S. hermaphrodita* confirms that it has innately lower water use efficiency than *S. perfoliatum*. These findings are also in line with the different ecology of the species with *S. perfoliatum* being able to thrive in drier prairies west of the Mississippi and *S. hermaphrodita* being restricted to the riverine habitats in the North-Eastern US.

Outlook

While different accessions of *S. hermaphrodita* did not differ much with regard to phenology, growth and chemical composition of the shoot material, seed origins of *S. perfoliatum* showed larger variation. Highest productivity was observed in accession 8, which is currently the most widely planted cultivar. In order to test plant performance under different climatic and edaphic conditions as well as under a different nutrient supply agronomic trials would have to be extended to different regions over multiple seasons. Besides agronomic traits and the occurrence of plant diseases research should focus on multiple harvests, optimum harvest dates (in *S. perfoliatum* late summer and in *S. hermaphrodita* late winter) and the energetic value (in *S. perfoliatum* methane production potentials of green material and in *S. hermaphrodita* calorific value of dry stems). Since more drought spells will occur in the future climate and the availability of productive land will be reduced permanent bioenergy cultures will have to be based on marginal agricultural production systems. The perennial species addressed here are potentially suited candidates because they have high water use efficiency and do not need to be grown under highly intensive conditions.

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