

Article

Evaluation of the Intra- and Interspecific Development of Different Accessions of *Silphium perfoliatum* L. and *Silphium integrifolium* Michx.

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Abstract: For higher sustainability in biomass production the use of new perennial species can lead to sustainable progress in the energy production and manufacturing industry. During the last decades, two different species of *Silphium* were discussed for biomass production. However, some questions regarding their cultivation and different uses are still to be answered. In this study, two accessions of *Silphium perfoliatum* L. and *Silphium integrifolium* Michx. were investigated during the year of establishment, and the first generative year for the phenotypic characteristics and suitability for cultivation, under Central European field conditions. Intra- and interspecific comparisons were made with special attention to their growth kinetics. While cup plant (*S. perfoliatum*) is well known as a potential biomass crop in Europe, silflower (*S. integrifolium*) is still unknown. In intraspecific comparison, *S. integrifolium* shows a more uniform development than *S. perfoliatum*. In parallel, the development of *S. perfoliatum* accessions is temporally shifted, so that the *S. perfoliatum* accessions differ in the length of their phases of generative growth and onset of senescence in comparison to *S. integrifolium*. To make these results applicable, an improvement proposal was made to the existing BBCH scale for *S. perfoliatum*. In addition, an adaptation was conducted on *S. integrifolium*.

Keywords: *Silphium perfoliatum* L.; cup plant; *Silphium integrifolium* Michx.; silflower; plant development; BBCH scale; perennial biomass crop; perennial oilseed crop; sustainable crop production; de novo domestication



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1. Introduction

The cultivation of plants as renewable resources for replacing fossil carbon, aiming for a bio-based economy, is growing. After the era of the usage of annual crops for biomass production, a new generation of crops was discussed. These crops are perennial [1] and may be even better as flexible multipurpose third-generation biomass crops [2].

At the moment, within the genus *Silphium* are two sister species [3] under evaluation for the development of such a new crop type: cup plant (*Silphium perfoliatum* L.) and silflower (*Silphium integrifolium* Michx.). The genus *Silphium* is widely spread across the eastern part of North America [4–6]. As both *Silphium* species are distributed over different geographical zones, there is a genetic variance [7,8]. It can be assumed that different *Silphium* accessions also vary phenotypically from each other [9]. According to Wever et al. (2019), an accession is defined as a population from one geographically characterized location [10]. The primary use of *S. perfoliatum* is for energy production,

while *S. integrifolium* is under domestication to becoming an oil seed crop [4,11]. Both belong to the Asteraceae tribe of Heliantheae, and originate from the eastern part of North America [12–15].

In *S. perfoliatum*, a yield of 15 to 25 Mg of dry matter per hectare is possible depending on soil and water availability [16,17]. In addition to its many agro-ecological benefits, e.g., in supporting biodiversity and as a food source for a variety of insects [10,13,14,18,19], *S. perfoliatum* has many interesting characteristics for a potential future non-food-crop with regard to its usability in agriculture, as a fiber plant, as well as in the use of ingredients and metabolites in pharmacy [13,20]. Furthermore, *S. perfoliatum* biomass can be utilized for multiple purposes [2]. At the moment, cup plant is mainly used as a biogas substrate, but could also be suitable for the production of fibers, chemical and pharmaceutical substances and insulating materials. Due to this, the agricultural value of *S. perfoliatum* can be versatile, therefore its growing area is increasing [13,19–23]. Depending on the type of use, the optimal harvesting time and the expected harvesting quantity will change. An early harvest (22 August) [24] of *S. perfoliatum* shows a relatively high proportion of quickly convertible carbohydrates, fats and proteins. At later harvest dates (towards the end of the growing season), the fiber, as well as the ash contents, increase [13,19–22].

A plant with a high proportion of quickly convertible carbohydrates is advantageous for the use of energy generation by fermentation in biogas plants [19]. To an extent, the same applies for the use as forage. *S. perfoliatum* shows comparable dry matter yield to alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.), with lower nutritional value. This can be optimized by dual harvest management. Thus, the contents of nutritive ingredients change depending on the stage of development or the harvest date [25,26].

In the case of silflower, two natural botanic variants are known and described: *S. integrifolium* var. *integrifolium* and *S. integrifolium* var. *laeve* [5]. The variant *S. integrifolium* var. *integrifolium* is known to be from the north-eastern part, while *S. integrifolium* var. *laeve* is known to be from the south-western part, of the natural distribution. Both variants of *S. integrifolium*, similar to *S. perfoliatum* as described above, show great environmental benefits in soil, water and biodiversity [27–29]. Interest in the plant, which is largely unknown in Europe, is growing steadily [15].

S. integrifolium var. *laeve*, in its pre-domesticated form, can be used as a perennial oilseed crop [15,30]. In this process, two breeding objectives were pursued: the pistillate ray florets per head were increased (feminization) and the lateral branching of stems was reduced [29]. The oil composition of the seeds of both investigated *Silphium* species is similar to that of sunflower [4,15,31]. *S. integrifolium* shows a maximum seed yield of 279.36 g per plant and year, whereby 118 to 253 g of oil can be obtained from 1 kg of seed. In another large-scale field experiment, Schiffner et al. (2020) found that different planting densities, as well as different nitrogen levels in the soil, affect biomass yield. The maximum biomass yields ranged from 8.2 to 15.9 Mg per hectare [32].

Due to its natural distribution in the prairie habitat of North America, and the complex root system of this perennial crop, the plants are suitable as an oilseed in arid and semi-arid regions [33,34]. In addition to their cultivation in Kansas, USA, there are field trials in the north of Patagonia, Argentina. Due to the natural adaptation of *S. integrifolium*, to arid conditions, the amount of water needed can be reduced compared to other oilseeds [34].

Given its high potential as an oilseed crop, *S. integrifolium* seems to be a promising wild plant for domestication [15]. A breeding approach was carried out in Kansas, USA [4,29].

Due to its missing or low domestication level, *Silphium* accessions show a high phenotypic plasticity. Wever et al. (2019) cultivated five different European *S. perfoliatum* accessions under field conditions at the same location to show differences in phenotypic appearance regarding plant height, stem diameter, number of shoots per plant, internodes per shoot and inflorescence [10]. However, based on these data, the development kinetics of the plants can only be represented indirectly, so quantitative data collection is necessary [35].

A BBCH scale is a good tool to translate developmental data into a useful scale and it standardizes the development kinetics of crops and weeds. In this way, it will be

possible to exploit the interdependencies that exist today in research, trade, production and services [36]. The characteristics of plant development, from germination to senescence, are recorded and divided into ten macro stages. In addition, a smaller-step classification based on ten micro stages is possible to precisely define developmental steps within each macro stage. The structure of the BBCH scale was deliberately based on Zadoks et al. (1974) [36,37]. The BBCH scale always refers to a single plant. A certain stage is only considered to be given when more than 50% of the examined plants show this stage [36].

For *S. perfoliatum*, a BBCH scale describing the general development of the plant is already available. This BBCH scale divides the development of *S. perfoliatum* into two parts. Thus, it differentiates between the first year (juvenile) and the second year (adult). In the first year, germination, leaf formation and row closure are evaluated. Beginning with the second year, vegetative development phases are assessed in terms of leaf formation and shoot elongation. Generative growth is assessed by bud formation, flowering, seed development, as well as the seed maturity and senescence process as described in Cumplido-Marin et al. (2020) [13].

The aims of this study are to identify the phenotypic plasticity of different *Silphium* species and accessions, the further development of the BBCH scale for *S. perfoliatum* and an adaptation of *S. integrifolium*. In order to describe the genetic variation between accessions in a BBCH scale, it is necessary to investigate different accessions of *S. perfoliatum* and *S. integrifolium*. For the crop management and breeding of both species, developmental stages have to be clearly defined and characterized [36].

2. Materials and Methods

2.1. Growing and Climate Conditions

The field trial presented in this study was conducted from April 2020 to November 2021 at the Field Lab Campus Klein-Altendorf near Rheinbach (N 50.617, E 6.983), University of Bonn, Germany. The predominant soil type on the main Rhine terrace is Luvisol, weakly eroded from loess [38].

The general average climatic conditions are characterized by an average temperature of 9.6 °C and an annual precipitation of 603 mm. Figure 1 shows the monthly average temperatures and the corresponding total precipitation. The average temperature in the year of trial establishment was 11.5 °C. The amount of precipitation was 492 mm. In the second year of the trial, the average temperature was 9.8 °C and there was an above-average amount of precipitation of 788 mm [39].

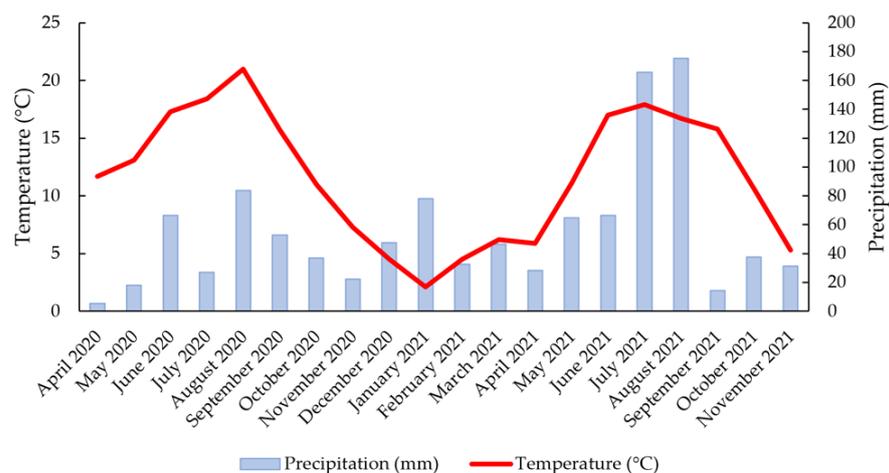


Figure 1. Monthly average temperature and precipitation over the experimental period [39].

2.2. Trial Setup

Two different accessions of each of *Silphium perfoliatum* and *Silphium integrifolium* were used. For this, it was ensured that the accessions were as geographically distinct as possible

and had the greatest genetic variance possible. A total of 36 plants per accession were placed in a 6 × 6 plot. Plants were sown at a planting density of four plants per square meter. For this, the seeds were treated in advance with a 0.05% gibberellic acid (GA₃) solution for 12 h at 4 °C to promote even germination [19]. In order to avoid missing plants due to lack of germination, five seeds of the corresponding accession were sown at each planting plot. Once germination was complete and cotyledons were fully formed, excess plants were mechanically removed.

For the study of *S. perfoliatum*, one accession from the southern (SPS) and one from the northern part (SPN) of the natural range were considered, both undomesticated. To evaluate the development of *S. integrifolium*, an accession from the western part of the native distribution area (Sii), and a pre-domesticated accession originating from the southern part of the native distribution area (Sil), were used (Table 1). Seeds for this trial were produced by a population grown at Campus Klein-Altendorf, (SPN and SPS), the botanical garden of the University of Düsseldorf, Germany (Sii) and The Land Institute in Salina, KS, USA (Sil).

Table 1. Locations of the natural habitat of *Silphium perfoliatum* and *Silphium integrifolium* accessions for this experiment. (* Origin of the accession. A pre-domestication was carried out at the Land Institute in Salina, KS, USA).

Accession Name	Accession Description	Latitude (N)	Longitude (E)
SPS	<i>Silphium perfoliatum</i> south	32.203	−89.254
SPN	<i>Silphium perfoliatum</i> north	41.857	−86.590
Sii	<i>Silphium integrifolium</i> var. <i>integrifolium</i>	41.776	−86.404
Sil	<i>Silphium integrifolium</i> var. <i>laeve</i>	39.106 *	−96.576 *

To avoid border effects and inter-plot competition, an additional two-row border of each accession was added around the whole experimental plot, as well as between the accessions, to reduce possible crowding out effects due to different competitive strengths [40].

During the phase of establishment, irrigation was necessary. Manual weed control procedures were carried out in the first year as well as at the beginning of the second year. Amounts of 50 kg N/ha were given at the beginning of each vegetation phase as maintenance fertilization.

2.3. Assistance for the Chronology of Incidents

In the evaluation of this experiment, all temporal data were given relative to the sowing date. In order to be able to put this information into a temporal context, Table 2 shows temporal translations to the day of the year, the Gregorian calendar and to the occurrences in the field trial.

Table 2. Temporal overview of the experimental period with translation into other forms of counting.

	Days after Sowing (DAS)	Gregorian Date	Developmental Occurrences
First year	0	22 April 2020	Sowing
	34	26 May 2020	Rosette development
	111	11 August 2020	Shoot development
	146	15 September 2020	Generative stage
	195	3 November 2020	Senescence
Second year	265	3 January 2021	Rosette development
	376	3 May 2021	Shoot development
	426	22 June 2021	Generative stage
	502	6 September 2021	Senescence
	580	23 November 2021	End of experiment

2.4. Description of the Germination

To describe the germination of plants, seeds of the accessions from the field trial were observed in rhizotrons. This allowed a precise description of the seed germination and root development of *S. perfoliatum* and *S. integrifolium*.

For this purpose, climatic conditions (day length and temperature profile) of the field trial during germination were replicated in a plant cabinet. Moisture was added as required. Fifty seeds from each accession were considered, which were treated in advance in a 0.05% GA₃ solution for 12 h at 4 °C according to Gansberger et al. (2017) as for the field trial [19].

2.5. Data Collection

Data from the germination experiment in the rhizotrons were collected over a period of 30 days. Within the stage of germination, different morphological sub-stages were recorded.

The data collection period of the field trial ranged from 26 May 2020 (34 days after sowing (DAS)) to 23 November 2021 (580 DAS), during which the development of the plant was documented weekly. Vegetative growth referred to the counting of nodes on the rosette as well as on the shoot. Here, the node was considered to be fully developed as soon as the corresponding leaves were fully formed. The corresponding data were collected on a pre-selected shoot of each plant. This selection was made at the beginning of the respective growing season and was not changed during the experimental period. In the following, this shoot is referred to as the main shoot. The latest fully developed node was always recorded. Node counts were performed continuously, so a cumulative nodule count of rosette nodes and shoot nodes was used for the evaluation to avoid neglecting node displacement effects, as described by Kadereit et al. (2014) [41]. The generative growth was recorded in the form of a rank-by-rank scoring of fully developed buds (fully elongated bud stalk of all buds belonging to the corresponding rank) and composite flowers (complete fertility of all flower heads of the corresponding rank), as well as seed development (seed filling completed in all heads of the corresponding rank, seed coat still green) and their maturity (brown and dry seedpods concerning the flower rank). The respective stage was considered to have been reached as soon as the respective rank had fully completed it. At the end of both growing seasons, the advancing senescence of the plants was estimated as chlorophyll breakdown in the whole plant in percentages. The experiment was terminated after completion of the second vegetation phase (580 DAS).

2.6. Data Processing

Data for each development stage were cumulated over the respective accession and presented in the form of heat maps. An intraspecies comparison was made between the two *S. perfoliatum* and *S. integrifolium* separately. Furthermore, interspecific development kinetics were evaluated. As soon as a relative frequency within a development characteristic had reached a maximum, a continuous presentation was disregarded. Statistical calculations are based on a 95% confidence level.

3. Results

The results of the experiment describe the development of the studied accessions of *Silphium perfoliatum* and *Silphium integrifolium*. The presentation of the results was made chronologically and can be divided into the year of establishment and the second year of development. The development steps occurring in each case, with the associated relative frequencies, are presented in heat maps. Furthermore, the statistically significant developmental differences in intraspecific comparisons were elaborated.

3.1. Development Description of the Establishment Year

3.1.1. Germination

The occurrence of the different development steps for the first 30 days of germination is shown in Figure 2. In order to be able to follow the underground development steps, germination was carried out separately in an additional experiment under simulated field

conditions in a plant cabinet. Initially, both accessions of both species demonstrated seed swelling. Seven days after sowing (DAS), the first seeds of Sil showed radicle emergence, while the other accessions lagged two to three days behind. The other developmental characteristics displayed the same pattern. The elongation of the radicle, the breaking through of the seed coat, the breaking through of the soil surface, as well as the development of cotyledons occurred, on average, three days earlier in Sil. Furthermore, it is noticeable that the germination of *S. perfoliatum*, in the late phase of the experimental period, had higher variance compared with *S. integrifolium*. Thus, it can be seen that, in a certain proportion of young seedlings, development stagnated during seed coat breakthrough or soil surface breakthrough. Furthermore, it showed a certain percentage of seeds in all accessions that did not progress beyond seed swelling (SPS: 16%; SPN: 36%; Sii: 22%; Sil: 10%).

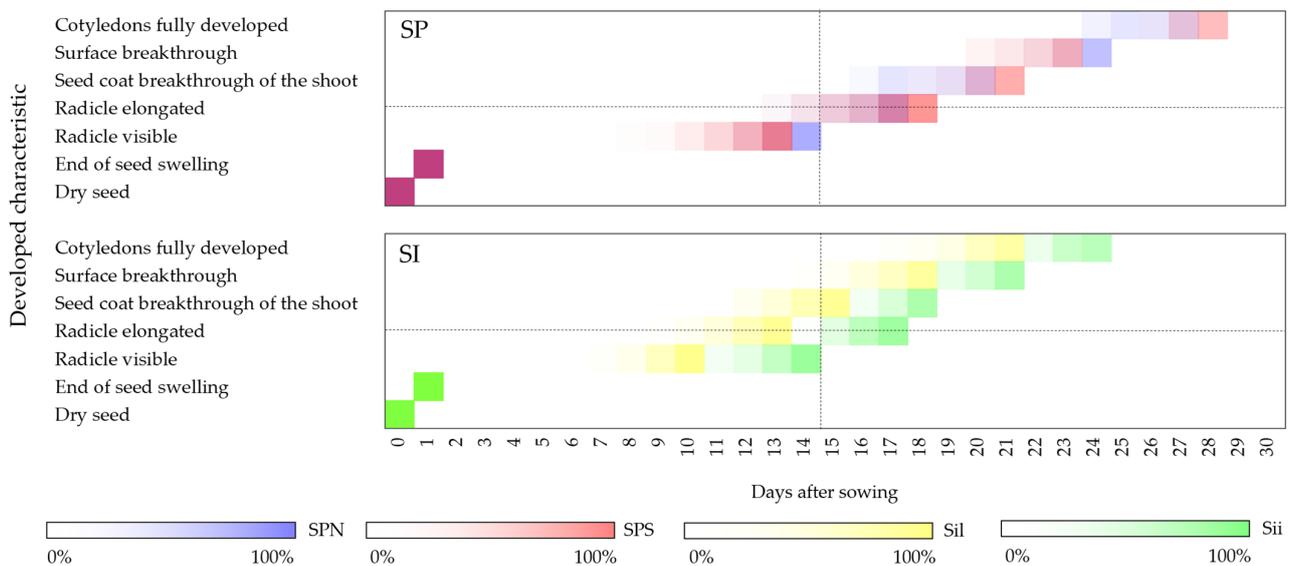


Figure 2. Heat map of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) germination in a rhizotron system over a period of 30 days. Heat map SP shows the germination behavior of both accessions of *Silphium perfoliatum* (SPN and SPS); heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) ($n = 50$). The intensity of the coloration shows the respective relative frequency (0–100%) of the complete expression of certain characteristics. After the achievement of the maximum level of development, the trait value will no longer be displayed. The climatic conditions were adapted from the corresponding climate during the field trial. The dashed lines serve as an orientation aid.

After 28 days of the experiment, the studied accessions reached the maximum germination rate (SPS: 52%; SPN: 42%; Sii: 54%; Sil: 70%).

3.1.2. Rosette Development

The development of the rosette of *S. perfoliatum* (SP) and *S. integrifolium* (SI), in the first year after sowing, is shown in Figure 3. In general, *S. integrifolium* showed a slightly faster development than *S. perfoliatum*. Sil formed a maximum of 20, Sii, SPS and SPN 14 nodes. The development of the individual plants of each accession becomes more heterogeneous over time.

Within *S. perfoliatum*, both accessions underwent a similar development. SPN was more inhomogeneous over time than SPS, so that the maximum frequencies of each fully developed rosette node were reached later. On two dates (139 and 160 DAS), a significant difference could be observed regarding the average number of nodes between the SPN and SPS accessions. Due to the relatively high p -values ($p = 0.041$) at these time points, and the

fact that there were no other differences between the studied accessions, no pattern can be identified, thus a random difference can be assumed.

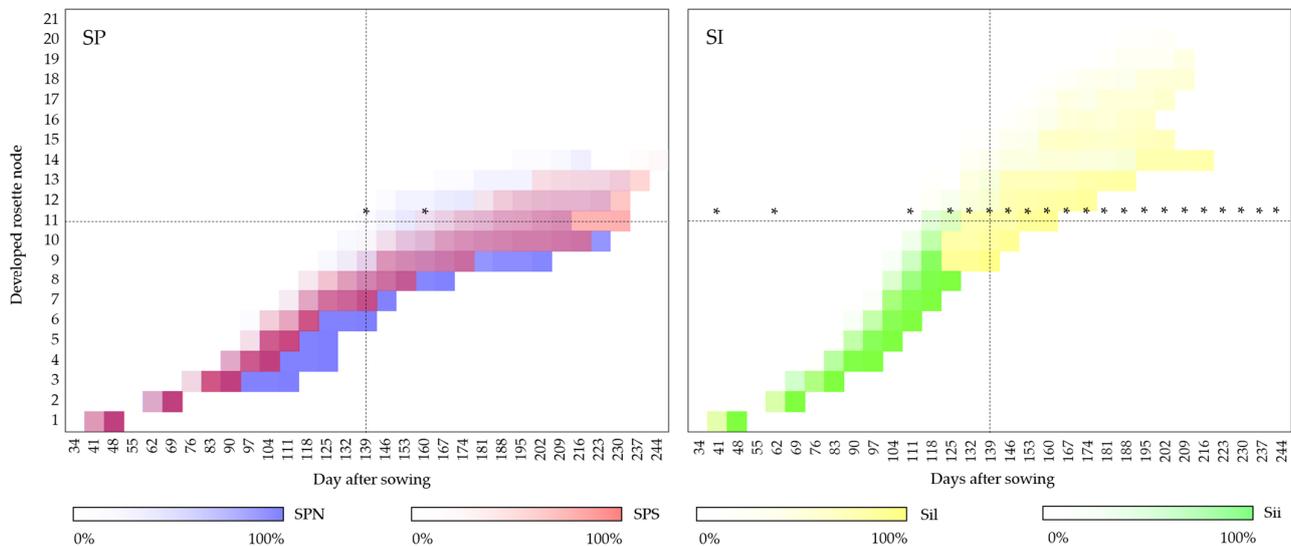


Figure 3. Heat map of rosette node development in the year of establishment of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 34 to 244 days after sowing. Heat map SP shows the rosette development of the first year of both accessions of *Silphium perfoliatum* (SPN and SPS); heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) ($n = 36$). The intensity of the coloration shows the respective relative frequency (0–100%) of the completely developed rosette node. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%. The dashed lines serve as an orientation aid.

The *S. integrifolium* accessions showed, equivalent to *S. perfoliatum*, a similar course in the first half of the growing period, with some significant differences at the beginning of plant growth. The development of Sii stagnated almost completely after 125 DAS, with a maximum of 14 nodes. In the second half of the developmental course, Sil showed progressive node formation, moving significantly steadily farther away from Sii. After 125 DAS, Sii showed an average of 10.26 ± 1.42 and Sil 10.91 ± 1.81 formed nodes ($p = 0.016$). After 216 DAS, there was no change in Sii, whereas Sil developed further and produced an average of 15.06 ± 4.53 nodes, making the difference between the studied accessions more significant ($p = 2 \times 10^{-8}$). Compared with this, *S. perfoliatum* showed an average of 7.52 ± 0.93 nodes after 126 DAS and 11.31 ± 2.66 nodes after 216 DAS.

3.1.3. Shoot Development

The shoot growth of the establishment year is shown in Figure 4, which is plotted with sequential numbering from Figure 3. In general, *S. integrifolium* shows a distinctly earlier shoot growth than *S. perfoliatum*. In *S. integrifolium*, the first shoot node could be detected after 118 DAS and in *S. perfoliatum* after 139 DAS. *S. perfoliatum* had a low tendency to form shoots in the accession SPS, as 22% of the plants formed rudimentary shoot growth in the first year. In SPN, there appeared no shoot formation. *S. integrifolium* showed shoot formation in both accessions. In Sii, 25% of the plants developed 25 cumulative shoot nodes; Sil achieved 27 cumulative shoot nodes on about 3% of the plants, after an experimental period of 265 DAS. The studied accessions of *S. perfoliatum* showed a significant difference for the first time after 160 DAS. SPS had an average of 1.06 shoot nodes ($p = 0.038$) at this time, while SPN did not form any shoot nodes. The studied accessions of *S. perfoliatum* showed a significant difference for the first time after 160 DAS. SPS had an average of 1.06 ± 2.14 nodes ($p = 0.038$) at this time, while SPN did not form any shoot nodes. Over

time, shoot node development continued in SPS so that, after 265 DAS, an average of 1.82 ± 3.43 shoot nodes had been formed and a significance increase ($p = 0.015$) was seen. The studied accessions of *S. integrifolium* showed a significant difference in the average number of shoot nodes from the beginning of shoot development. After 160 DAS, Sii had developed an average of 10.66 ± 4.57 shoot nodes and Sil 3.31 ± 5.8 shoot nodes ($p = 4.1 \times 10^{-7}$). At 265 DAS, Sil had formed an average of 12.81 ± 5.33 and Sii had formed 4.63 ± 6.99 shoot nodes. The p -value increased marginally to 1.7×10^{-6} at this time point.

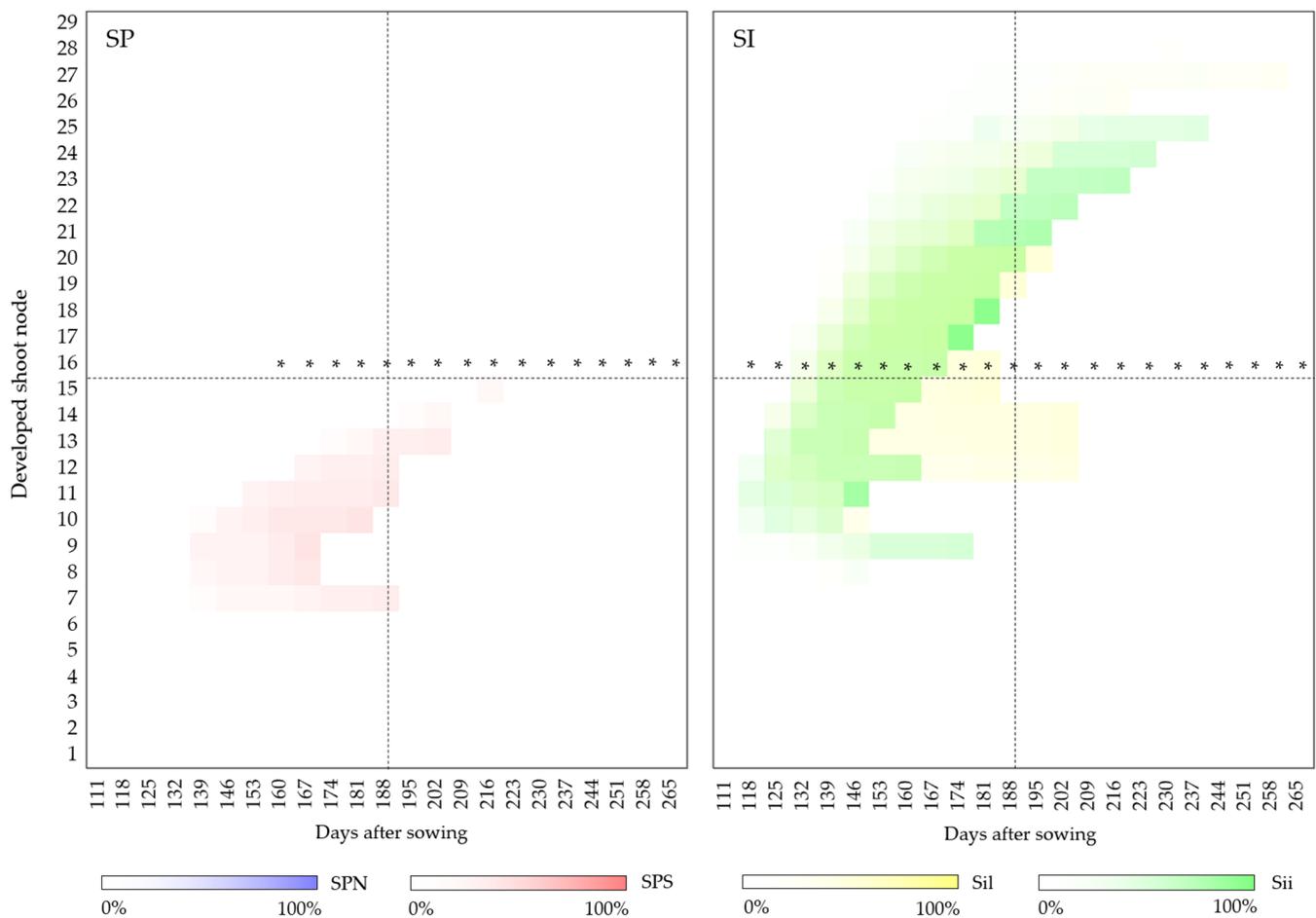


Figure 4. Heat map of shoot node development of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 111 to 265 days after sowing. Heat map SP shows the shoot development of the first year of both accessions of *Silphium perfoliatum* (SPN and SPS); heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) ($n = 36$). The intensity of the coloration shows the respective relative frequency (0–100%) of the completely developed shoot node. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%. The dashed lines serve as an orientation aid.

3.1.4. Generative Stage

The generative development of the year of establishment is shown in Figure 5. *S. perfoliatum* showed highly reduced generative growth in the first year of development. SPS showed first rudimentary bud development after 174 DAS. After 209 DAS, first rank buds were formed in 11% of the plants. From this point on, a significant difference was present between SPS and SPN ($p = 0.04$). Further generative growth was not detected in *S. perfoliatum*.

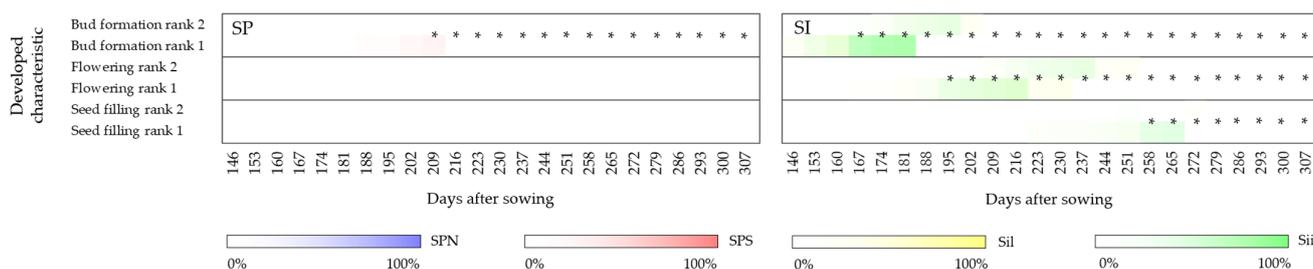


Figure 5. Heat map of the period of generative development of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 146 to 307 days after sowing. Heat map SP shows the generative development of the first year of both accessions of *Silphium perfoliatum* (SPN and SPS); heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) divided into bud formation, flowering and seed filling ($n = 36$). The intensity of the coloration shows the respective relative frequency (0–100%) of the completed development stage. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%.

S. integrifolium reached generative growth in both accessions studied. Both variants showed 146 DAS initial buds of the first rank. In Sil, 14% of the plants showed bud formation in the first and 9% in the second rank. An equivalent pattern arose during flower formation. Seed filling, in the first and second flowering rank, was completed in 6% and 3% of the plants, respectively. Bud development was reached in Sii of the first rank in 62% of the plants and in 34% of the second rank. Flowering was achieved in the first rank in 50% of the plants. In the second rank, 34% of plants attained this stage. Complete seed filling occurred in 21% of the first rank, and in 3% of the second rank, in Sii. The first significant differences in bud formation were seen after 167 DAS. On average, Sii had formed 0.47 ± 0.55 and Sil 0.14 ± 0.35 bud ranks at this time ($p = 0.003$). After completion of bud development after 202 DAS, an average of 0.86 ± 0.85 and 0.22 ± 0.58 bud ranks were formed by Sii and Sil, respectively. The difference at this time point also had a highly significant p -value of 5×10^{-4} . The first significant differences with respect to flower formation were evident after 195 DAS. At this time, an average of 0.28 ± 0.45 in Sii and 0.06 ± 0.23 in Sil flowering ranks were formed ($p = 0.011$). After the completion of flower formation after 251 DAS, significant differences between the studied accessions were still present with a p -value of 0.0016. At this time point, an average of 0.75 ± 0.89 and 0.17 ± 0.55 flowering ranks were formed in Sii and Sil, respectively. The first significant differences between the studied accessions were present after 258 DAS. At this time, Sii showed an average of 0.19 ± 0.46 filled seed ranks per plant in Sii and 0.06 ± 0.23 in Sil. At this point, a p -value of 0.0016 was evident, which did not change later in the time course.

Seed maturity did not occur in any accession.

3.1.5. Senescence

The course of senescence at the end of the vegetation phase of the first year is described in Figure 6. Senescence of the investigated plants was first detected after 195 DAS. The investigated accessions of *S. perfoliatum* showed a temporal offset, so that the senescence process in SPN ended after 300 DAS. SPS reached complete senescence in 81% of the examined plants after 307 DAS. Over the entire observation period, *S. perfoliatum* accessions showed a significant difference with an average p -value of 0.003.

The course of senescence of *S. integrifolium* was comparable referring to the two investigated accessions. The complete development of senescence occurred in Sii after 307 DAS and after 300 DAS for Sil. In the first half of the observation period, significant differences were present between the two studied accessions. Thus, Sii, after 195 DAS, showed an average senescence of $11.94\% \pm 3.95\%$ and Sil $16.7\% \pm 0.7\%$ with a p -value of 9.6×10^{-4} . As the course of senescence proceeded, the differences between the accessions became

smaller. Thus, after 244 DAS, the average senescence for Sii was $65.83\% \pm 15.7\%$ and for Sil $75.28\% \pm 17.56\%$, respectively, with a resulting p -value of 0.0204.

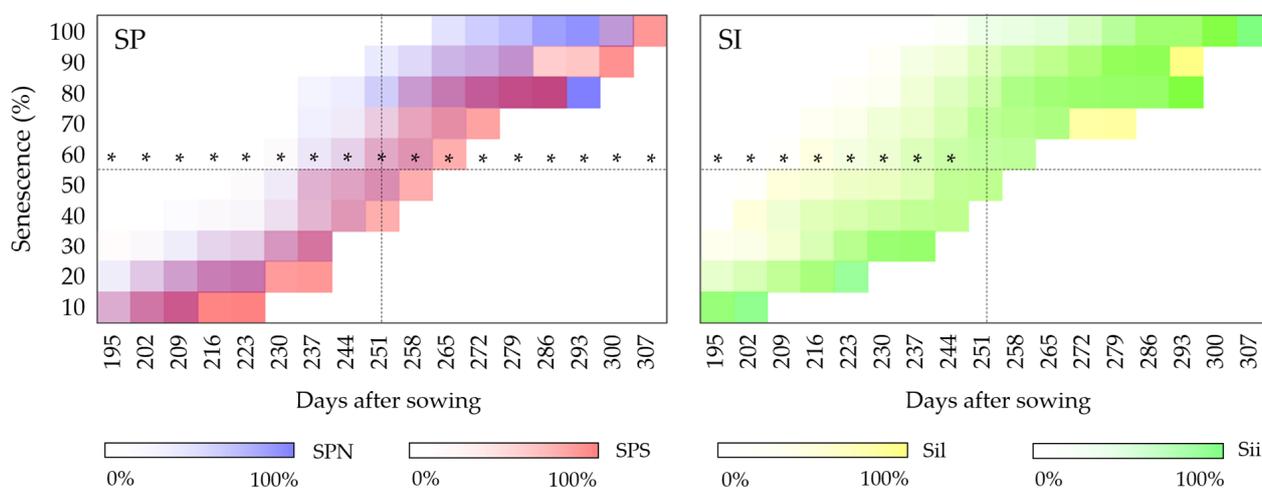


Figure 6. Heat map of the course of senescence progress of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 195 to 307 days after sowing. Heat map SP shows the senescence course of both accessions of *Silphium perfoliatum* (SPN and SPS) of the first year; heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) ($n = 30$). The intensity of the coloration shows the respective relative frequency (0–100%) of different senescence levels. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%. The dashed lines serve as an orientation aid.

3.2. Development Description of the Second Year of Development

3.2.1. Rosette Development

The development of rosette nodes in the second year is shown in Figure 7. The regrowth of the investigated plants started after 265 DAS. In general, the rosette node development in the second year of *S. perfoliatum* and *S. integrifolium* were comparable, because a similar progression of rosette formation occurred and it was terminated simultaneously in all accessions after 376 DAS. At the end of the observation period (384 DAS), SPS and SPN had formed a maximum of 9 and 11 rosette nodes, respectively. At the same time point, Sii and Sil formed a maximum of 10 and 13 rosette nodes, respectively.

Over the time course, *S. perfoliatum* showed significant differences in the average rosette node number from 307 DAS to the end of the observation period (384 DAS). Thus, an average of 1.64 ± 0.67 rosette nodes was formed in SPS after 307 DAS, and an average of 2.27 ± 0.61 rosette nodes were formed in SPN. After 384 DAS, SPS had formed an average of 6.25 ± 1.23 and SPN an average of 7.97 ± 1.07 rosette nodes. As a result, the p -value increased from an initial 8.5×10^{-5} to 0.0037 over time. The significant differences between 279 and 386 DAS showed no pattern and are therefore negligible.

S. integrifolium fulfilled a rather comparable development between the studied accessions. Significant differences between Sii and Sil were present between 286 DAS and 351 DAS. On average, Sii showed 0.22 ± 0.48 and Sil 0.61 ± 0.68 nodes after 286 DAS ($p = 0.0071$). After 351 DAS, Sii had formed an average of 4.84 ± 0.7 and Sil 5.42 ± 1.16 nodes. At this point, there was a p -value of 0.0096. At the end of the observation period, Sii showed an average of 7.06 ± 1.13 and Sil 8.03 ± 1.64 rosette nodes, respectively. There is no significant difference at this point.

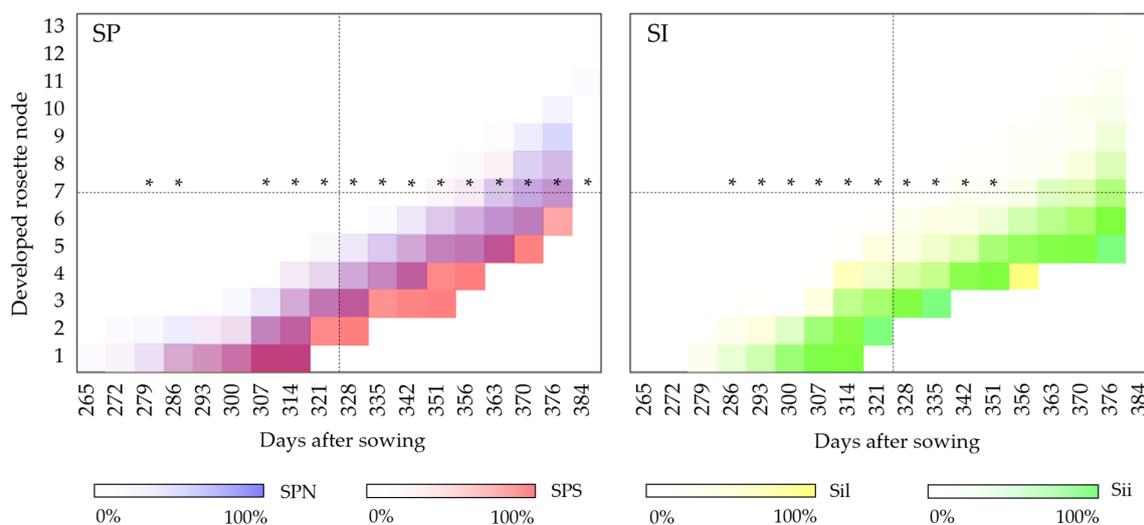


Figure 7. Heat map of the course of rosette node development of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 265 to 384 days after sowing. Heat map SP shows rosette development of both accessions of *Silphium perfoliatum* (SPN and SPS) of the second year; heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) ($n = 36$). The intensity of the coloration shows the respective relative frequency (0–100%) of the completely developed rosette node. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%. The dashed lines serve as an orientation aid.

3.2.2. Shoot Development

The shoot growth of the second year of development is shown in Figure 8, which is plotted with sequential numbering from Figure 7. The considered accessions of *S. perfoliatum* demonstrated a comparable developmental course. The first shoot nodes became visible after 376 DAS. After 446 DAS, no further shoot nodes were formed. At this point, SPN reached a maximum of 17 shoot nodes, while SPS formed 16 shoot nodes at the peak. *S. integrifolium* showed a comparable initial phase of shoot development between the two accessions which was slightly earlier than the development of *S. perfoliatum*. Both accessions developed first shoot growth after 376 DAS. A maximum of 24 nodes was counted in Sii after 446 DAS. At the same time point, Sil reached up to 33 shoot nodes.

Shoot development in *S. perfoliatum* is subject to widely significant differences among the studied accessions. These can be detected for the first time after 391 DAS. At this time, SPS showed an average of 2.94 ± 0.62 and SPN an average of 3.72 ± 1.1 shoot nodes. This resulted in a p -value of 5×10^{-4} . At the end of the observation period, SPS produced an average of 9.0 ± 1.13 and SPN 9.94 ± 1.1 shoot nodes, resulting in a p -value of 7.3×10^{-4} . *S. integrifolium* showed sporadic significant differences between the studied accessions in the first half of the observation period, which do not seem to be subject to any pattern and are negligible. In the second half of the shoot development period, coherent significant differences were present among the studied accessions. Thus, after 412 DAS, Sii showed an average of 10.64 ± 1.75 and Sil 12.2 ± 2.71 shoot nodes, resulting in a p -value of 0.0057. After 446 DAS, Sii formed 16.55 ± 2.33 and Sil 19.64 ± 4.39 shoot nodes. This resulted in a p -value of 4.6×10^{-4} , which corresponded to a significant increase.

3.2.3. Generative Stage

The course of the generative development phase of *S. perfoliatum* and *S. integrifolium* divided into bud formation, flower formation, seed filling and seed ripening, in rank-wise presentation, is shown in Figure 9. In essence, *S. perfoliatum* and *S. integrifolium* show a generative phase in which the individual developmental steps build on each other in

a mutually dependent manner. In general, the time offset between bud development, flowering and the seed filling phase is less than that for seed ripening.

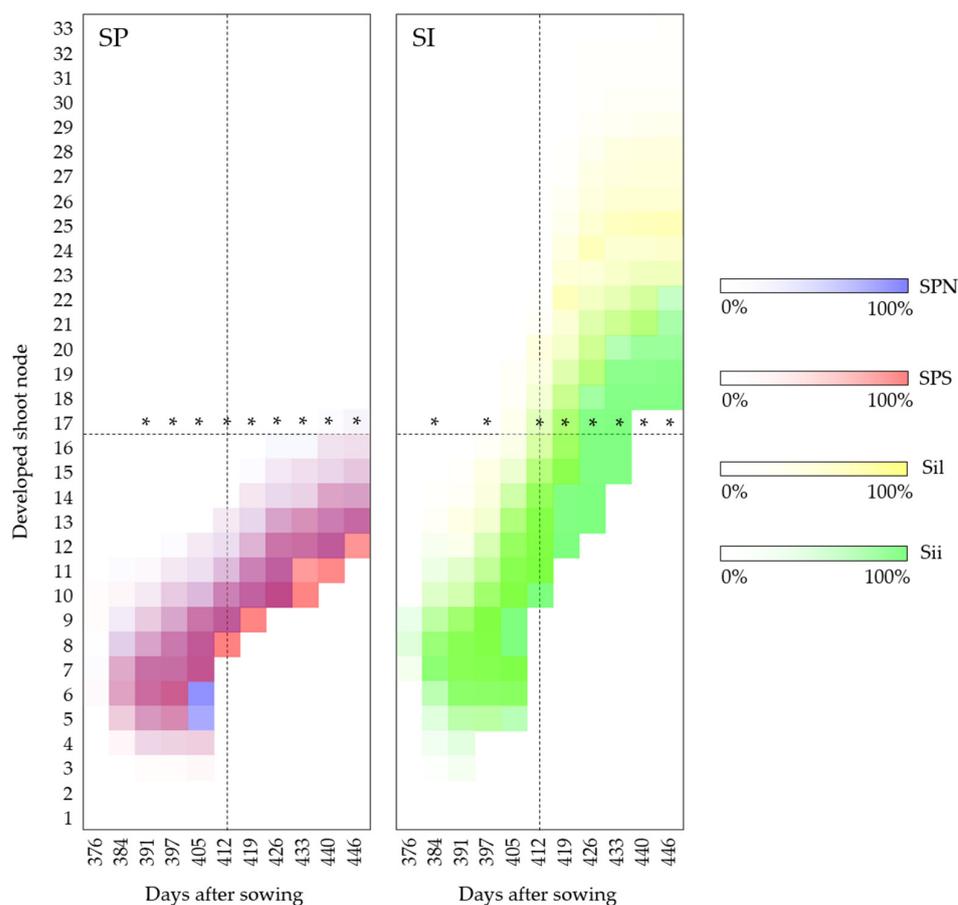


Figure 8. Heat map of the course of shoot node development (main shoot) of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 376 to 446 days after sowing. Heat map SP shows the shoot development of both accessions of *Silphium perfoliatum* (SPN and SPS) of the second year; heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) ($n = 36$). The intensity of the coloration shows the respective relative frequency (0–100%) of the completely developed shoot node. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%. The dashed lines serve as an orientation aid.

The bud formation in *S. perfoliatum* ranged up to 8 bud ranks in SPS and up to 6 bud ranks in SPN, respectively. On average, at the end of the observation period, SPS showed 6.5 ± 0.73 and SPN 5.75 ± 0.76 bud ranks. Between 440 and 482 DAS, there was a significant difference between the two accessions, with an average p -value of 9×10^{-4} . In *S. integrifolium*, Sii showed a maximum of 8 and Sil showed a maximum of 6 bud ranks at the end of the evaluation period. On average, 6.72 ± 0.61 bud ranks were formed in Sii and 5.82 ± 1.19 in Sil at this time. Except for 461, 469 and 482 DAS, there was a significant difference between the sampled accessions over the entire observation period. Since the non-significant time points did not reveal a pattern, these could be neglected. There was a resulting average p -value of 0.0032.

S. perfoliatum showed up to 7 flowering ranks during flowering in SPS. SPN flowered in a maximum of 6 ranks. At the end of the observation period, SPS had an average of 5.43 ± 0.72 and SPN 5.22 ± 0.77 flowering ranks. Over the flowering period, there was mostly a significant difference between the studied accessions. No significant difference was

present at 497 and 502 DAS, which is negligible due to non-detectable patterns. An average p -value of 0.0035 was present over the period. The flowering of *S. integrifolium* is shown in Sii up to 7 formed flowering ranks. Sil flowered in up to 6 ranks. On average, Sii flowered in 5.65 ± 0.64 flowering ranks and Sil in 4.8 ± 0.62 flowering ranks. Significant differences between the two accessions were at the end of flowering, due to flower development being more tapered in Sil than in Sii. No other significant differences were evident.

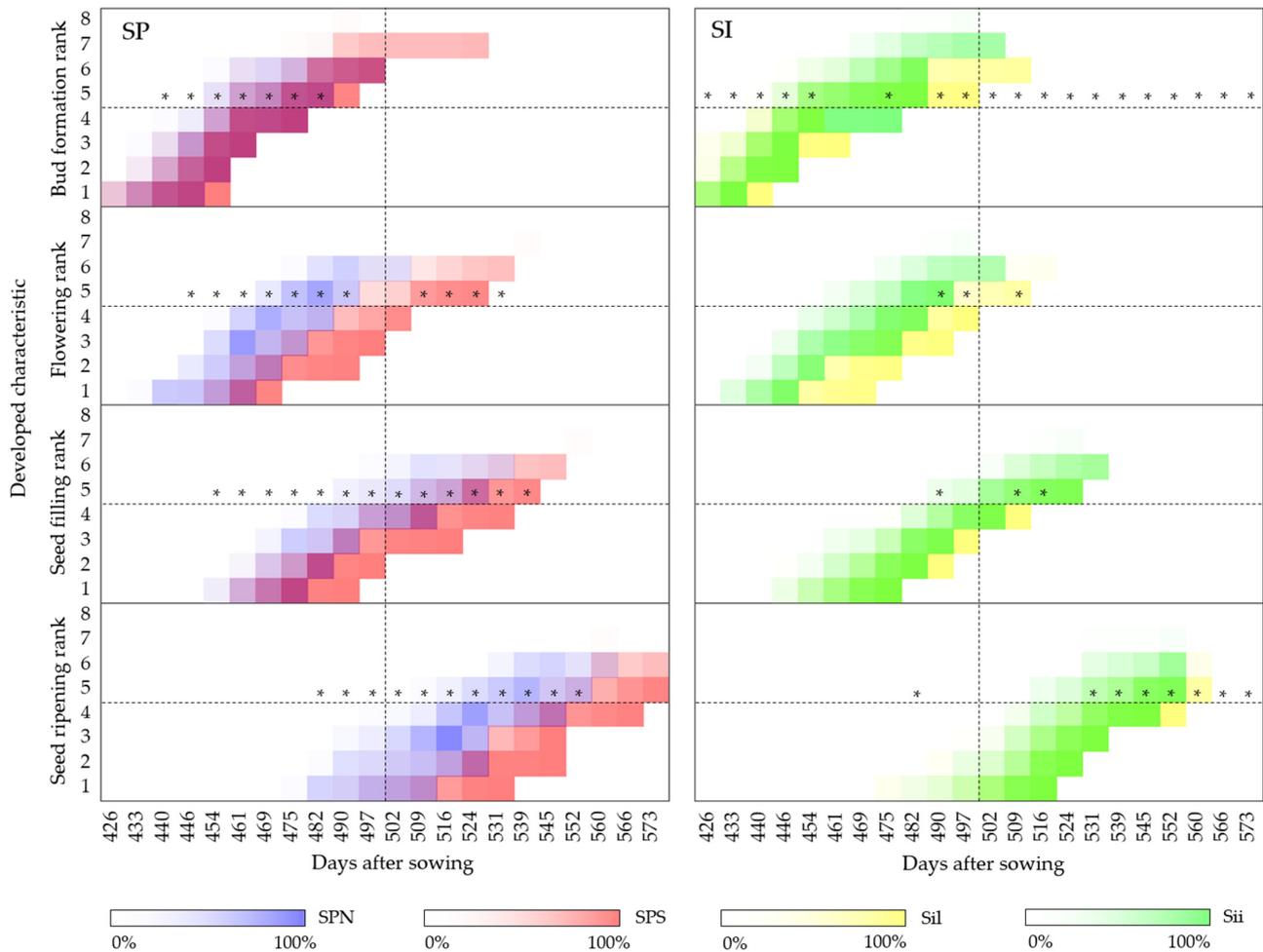


Figure 9. Heat map of the period of generative development of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 426 to 573 days after sowing. Heat map SP shows the generative development of the first year of both accessions of *Silphium perfoliatum* (SPN and SPS); heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) divided into bud formation, flowering and seed filling. ($n = 36$). The intensity of the coloration shows the respective relative frequency (0–100%) of the completed development stage. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%.

The seed filling phase in *S. perfoliatum* shows a maximum of 7 filled seed ranks in SPS. In SPN, up to 6 seed ranks were filled at the end of the observation period. On average, the number of filled seed strands in SPS was 5.19 ± 0.63 and in SPN was 5.22 ± 0.76 . Significant differences with an average p -value of 0.0028 were consistently present between the studied accessions over the observation period. In *S. integrifolium* up to 7 and, in Sil, up to 6 seed ranks were filled. On average, there were 5.5 ± 0.66 filled seed ranks in Sii and 4.72 ± 0.64 in Sil. Significant differences among the studied accessions were present at 490, 509 and

516 DAS. As no pattern was discernible, it could be assumed at this point that the alleged significant differences were negligible.

In the last part of the generative phase, *S. perfoliatum* in SPS showed up to 7 ripened seed ranks. In SPN, up to 6 seed strands had fully ripened. On average, in SPS 5.47 ± 0.73 and, in SPN, 5.28 ± 0.77 seed heads were fully matured. Between 482 and 552 DAS there were significant differences with an average p -value of 9×10^{-6} . The ripening characteristics of *S. integrifolium* showed a maximum of 7 fully ripened seed ranks in Sii and 6 fully ripened seed ranks in Sil. On average, 5.75 ± 0.6 and 4.63 ± 0.59 seed ranks were ripened in Sii and Sil, respectively. From 539 to 573 DAS, significant differences were observed between the studied accessions. An average p -value of 0.0025 was present. A single significant difference after 482 DAS could be neglected due to a missed pattern.

3.2.4. Senescence

The course of senescence after the end of the vegetation phase of the first year is described in Figure 10. The senescence evaluation began at 502 DAS. In general, the senescence progression of the two accessions of *S. integrifolium* (Sii and Sil) and SPN is comparable. The senescence process of SPS is successively delayed over the observation period after the same onset. Thus, complete senescence in SPN is reached after 566 DAS. Sii and Sil reach this state already after 560 DAS. SPS is completely senescent after 580 DAS.

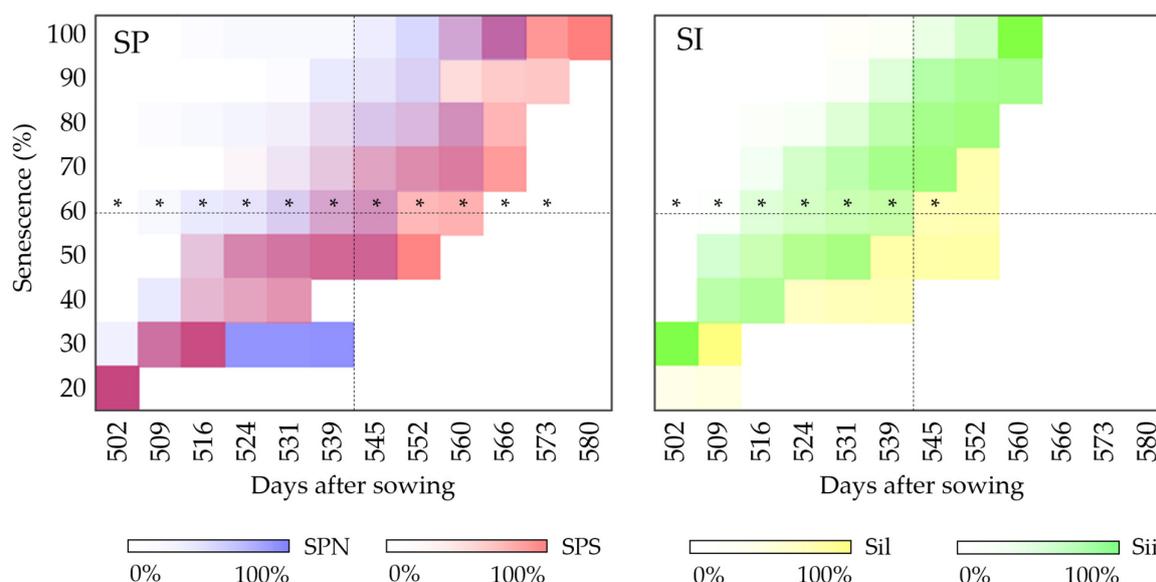


Figure 10. Heat map of the course of senescence progress of *Silphium perfoliatum* (SP) and *Silphium integrifolium* (SI) from 502 to 580 days after sowing. Heat map SP shows the senescence course of both accessions of *Silphium perfoliatum* (SPN and SPS) of the second year; heat map SI shows the equivalent of both *Silphium integrifolium* accessions (Sii and Sil) ($n = 36$). The intensity of the coloration shows the respective relative frequency (0–100%) of different senescence levels. After the achievement of the maximum level of development, the trait value will no longer be displayed. The star-shaped markings (*) indicate a significant difference in the average number of nodes between the accessions shown at the respective time point, with a confidence level of 95%. The dashed lines serve as an orientation aid.

In the intraspecific comparison, *S. perfoliatum* and *S. integrifolium* showed a significant difference between the studied accessions over the major part of the observation period. Thus, *S. perfoliatum* shows an average p -value of 0.0077 and *S. integrifolium* shows a p -value of 0.013 on average over this period. At the end of the experiment, the differences became non-significant due to the fact that complete senescence occurred in all accessions.

3.3. BBCH-Coding of the Phenological Development Stages of *Silphium perfoliatum* and *Silphium integrifolium*

In the following, an adaptation of the collected data to a BBCH scale, according to Hack (1992) and Meier (2018), was carried out for *Silphium perfoliatum* and *Silphium integrifolium* (Tables 3–7). For this purpose, only those stages that occurred on more than 50% of the examined plants were included [35,36]. Accordingly, the possible BBCH stages occurring for both species are shown below, with a brief description with the respective cumulative relative frequencies determined, in order to define the relevant stages for the establishment of a BBCH scale of both investigated accessions. Additionally, illustrations of *S. perfoliatum* and *S. integrifolium* of selected BBCH stages are presented (Figures 11–15).

- BBCH Macro Stage 0—Germination

Table 3. BBCH macro stage 0—Seed germination. Present stages of the different phases of seed germination, with the corresponding relative frequencies of each germination characteristic of *Silphium perfoliatum* and *Silphium integrifolium*, based on the collected data from the germination trial. ($n = 36$).

Code	Description	SP 1. Year	SI 1. Year	SP 2. Year	SI 2. Year
0 0	Dry seed	1.00	1.00	-	-
0 3	End of seed swelling	1.00	1.00	-	-
0 5	Radicle visible	0.84	0.90	-	-
0 6	Radicle elongated	0.82	0.82	-	-
0 7	Seed coat breakthrough of the cotyledons	0.64	0.82	-	-
0 8	Surface breakthrough	0.52	0.76	-	-
0 9	Cotyledons fully developed	0.52	0.70	-	-

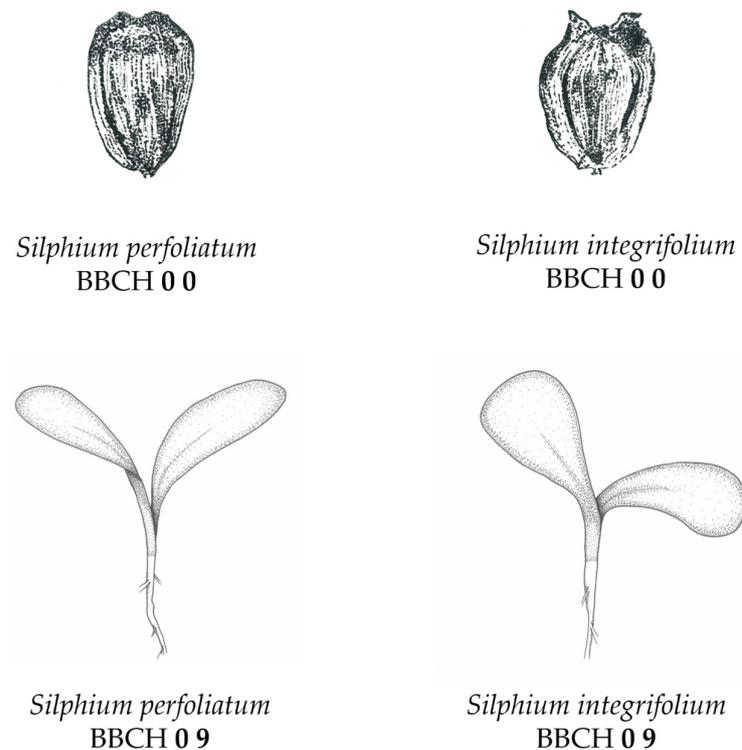


Figure 11. BBCH macro stage 0—Illustration of dry seeds and young seedlings of *Silphium* spp.

- BBCH Macro Stage 1—Rosette Development

Table 4. BBCH macro stage 1—Leave and rosette development. Present stages of rosette development based on rosette nodes or leaf pairs with the corresponding relative frequencies for the first, as well as the second, year of development of *Silphium perfoliatum* and *Silphium integrifolium* based on the collected data from the field trial. ($n = 36$).

Code	Description	SP 1. Year	SI 1. Year	SP 2. Year	SI 2. Year
1 1	1st rosette node/1st pair of leaves fully developed	1.00	1.00	1.00	1.00
1 2	2nd rosette node/2nd pair of leaves fully developed	1.00	1.00	1.00	1.00
1 3	3rd rosette node/3rd pair of leaves fully developed	0.97	1.00	1.00	1.00
1 4	4th rosette node/4th pair of leaves fully developed	0.97	1.00	1.00	1.00
1 5	5th rosette node/5th pair of leaves fully developed	0.94	1.00	1.00	1.00
1 6	6th rosette node/6th pair of leaves fully developed	0.83	1.00	1.00	1.00
1 7	7th rosette node/7th pair of leaves fully developed	0.63	1.00	0.92	0.83
1 8	8th rosette node/8th pair of leaves fully developed	-	1.00	0.67	0.53
1 9	9th rosette node/9th pair of leaves fully developed	-	0.91	-	-
1 9	10th rosette node/10th pair of leaves fully developed	-	0.83	-	-
1 9	11th rosette node/11th pair of leaves fully developed	-	0.83	-	-
1 9	12th rosette node/12th pair of leaves fully developed	-	0.77	-	-
1 9	13th rosette node/13th pair of leaves fully developed	-	0.71	-	-
1 9	14th rosette node/14th pair of leaves fully developed	-	0.66	-	-
1 9	15th rosette node/15th pair of leaves fully developed	-	0.54	-	-

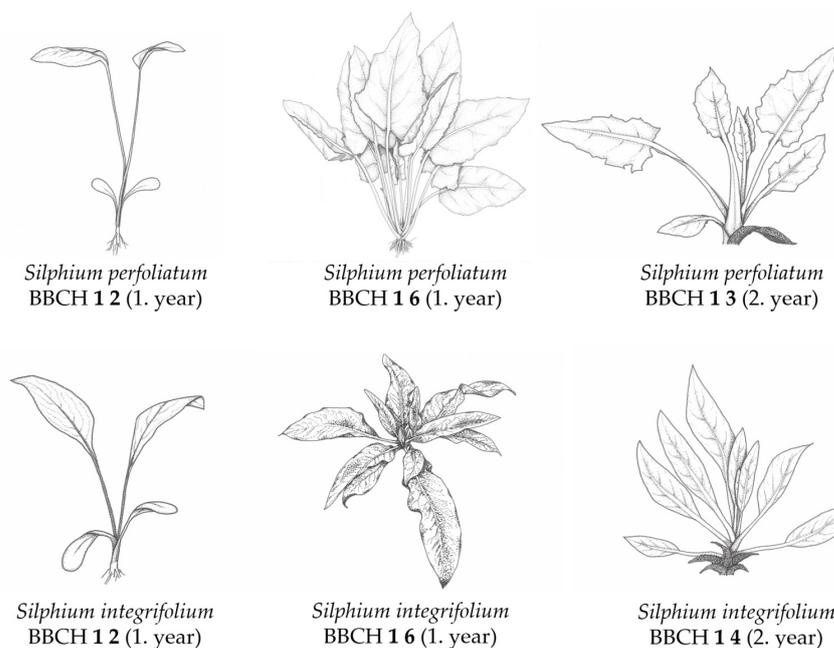


Figure 12. BBCH macro stage 1—Illustration of rosette development of *Silphium* spp.

- BBCH Macro Stage 3—Shoot Development

Table 5. BBCH macro stage 3—Shoot development (main shoot). Present stages of shoot development on the basis of shoot nodes or leaf pairs with the corresponding relative frequencies for the first, as well as the second, year of development of *Silphium perfoliatum* and *Silphium integrifolium* based on the collected data from the field trial. The consecutive numbering is to be understood as the subtracted respective rosette nodes. ($n = 36$).

Code	Description	SP 1. Year	SI 1. Year	SP 2. Year	SI 2. Year
3	1-x	1st shoot node/corresponding leaves fully developed	-	-	-
3	2-x	2nd shoot node/corresponding leaves fully developed	-	-	-
3	3-x	3rd shoot node/corresponding leaves fully developed	-	-	-
3	4-x	4th shoot node/corresponding leaves fully developed	-	-	-
3	5-x	5th shoot node/corresponding leaves fully developed	-	-	0.67
3	6-x	6th shoot node/corresponding leaves fully developed	-	-	0.86
3	7-x	7th shoot node/corresponding leaves fully developed	-	-	0.94
3	8-x	8th shoot node/corresponding leaves fully developed	-	-	0.97
3	9-x	9th shoot node/corresponding leaves fully developed	-	-	1.00
3	9-x	10th shoot node/corresponding leaves fully developed	-	0.57	1.00
3	9-x	11th shoot node/corresponding leaves fully developed	-	0.71	1.00
3	9-x	12th shoot node/corresponding leaves fully developed	-	0.89	1.00
3	9-x	13th shoot node/corresponding leaves fully developed	-	0.89	0.89
3	9-x	14th shoot node/corresponding leaves fully developed	-	0.89	0.61
3	9-x	15th shoot node/corresponding leaves fully developed	-	0.89	-
3	9-x	16th shoot node/corresponding leaves fully developed	-	0.89	-
3	9-x	17th shoot node/corresponding leaves fully developed	-	0.89	-
3	9-x	18th shoot node/corresponding leaves fully developed	-	0.89	-
3	9-x	19th shoot node/corresponding leaves fully developed	-	0.89	-
3	9-x	20th shoot node/corresponding leaves fully developed	-	0.89	-
3	9-x	21th shoot node/corresponding leaves fully developed	-	0.63	-
3	9-x	22th shoot node/corresponding leaves fully developed	-	0.54	-
3	9-x	23th shoot node/corresponding leaves fully developed	-	0.51	-
3	9-x	24th shoot node/corresponding leaves fully developed	-	-	-
3	9-x	25th shoot node/corresponding leaves fully developed	-	-	-

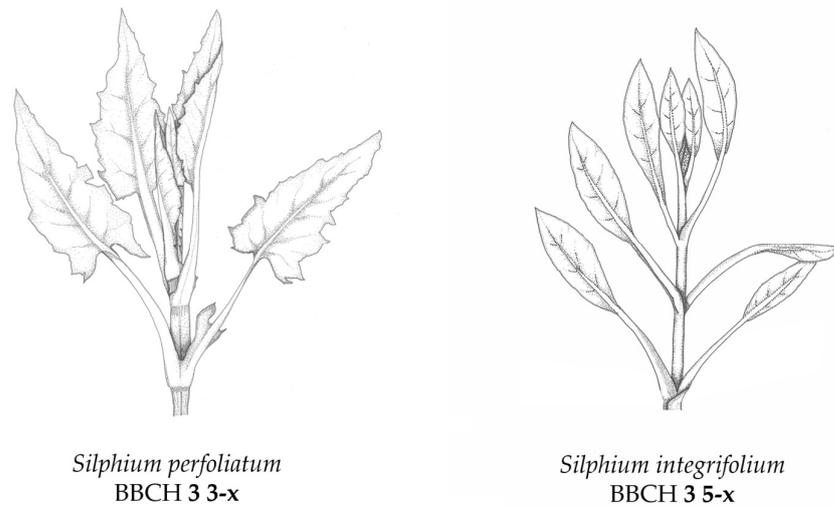


Figure 13. BBCH macro stage 3—Illustration of shoot and leaf development of *Silphium* spp.

- BBCH Macro Stage 5–8—Generative Stage

As the generative phase of *S. perfoliatum* or *S. integrifolium* occurs rank-wise and independently nested, several BBCH stages occur in parallel. It is therefore recommended to define all occurring BBCH stages.

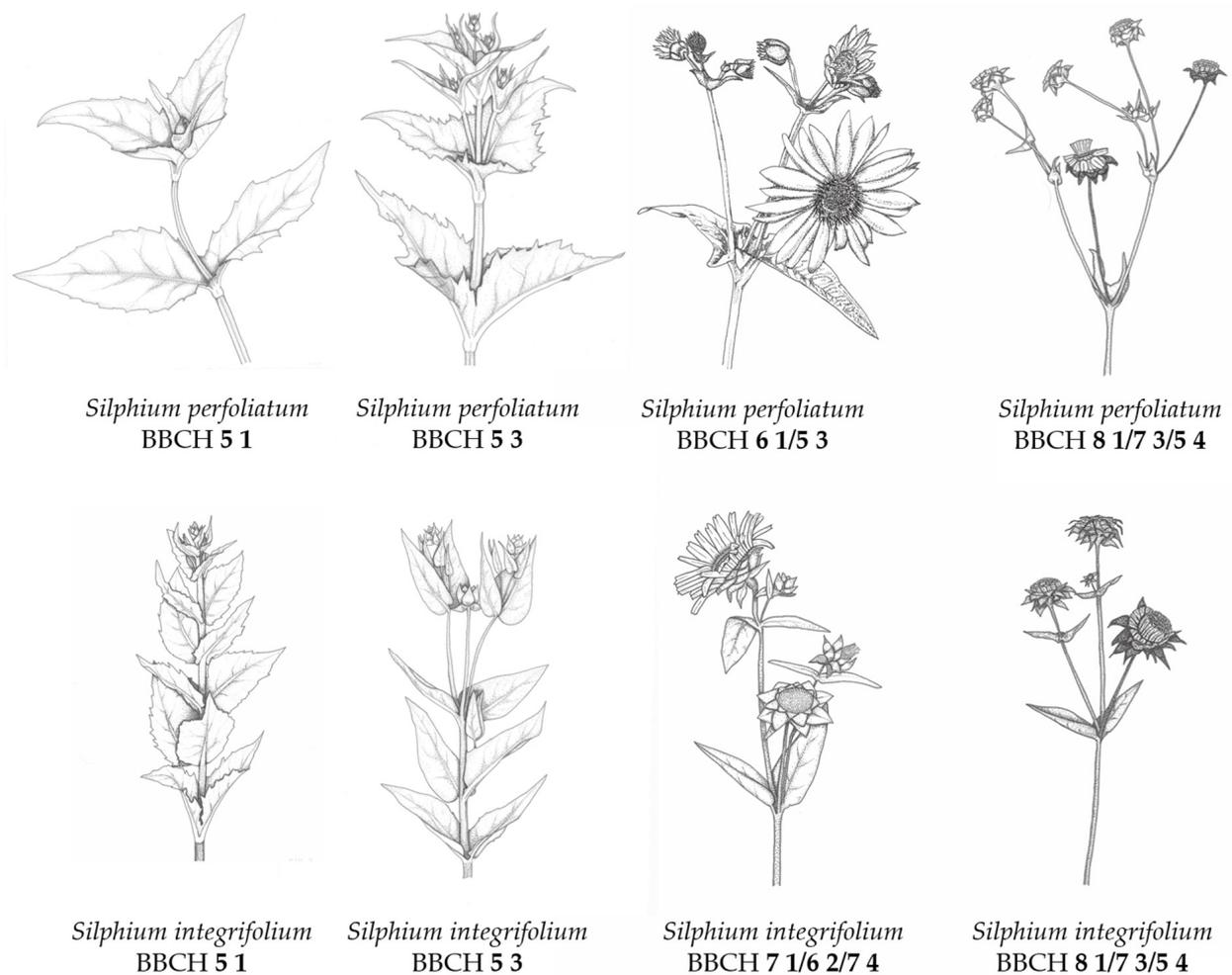


Figure 14. BBCH macro stage 5–8—Illustration of generative development of *Silphium* spp.

Table 6. BBCH macro stage 5–8—Generative development. Present stages of the generative phase based on bud and flower development as well as seed filling and ripening with the corresponding relative frequencies for the first as well as the second year of development of *Silphium perfoliatum* and *Silphium integrifolium*. ($n = 36$).

Macro Stage 5—Bud Formation						
Code		Description	SP 1. Year	SI 1. Year	SP 2. Year	SI 2. Year
5	1	1st rank: bud fully developed	-	0.63	1.00	1.00
5	2	2nd rank: buds fully developed	-	-	1.00	1.00
5	3	3rd rank: buds fully developed	-	-	1.00	1.00
5	4	4th rank: buds fully developed	-	-	1.00	1.00
5	5	5th rank: buds fully developed	-	-	1.00	1.00
5	6	6th rank: buds fully developed	-	-	0.94	0.97
5	7	7th rank: buds fully developed	-	-	0.56	0.69
Macro stage 6—Flowering						
6	1	1st rank: Flower fully developed	-	0.50	1.00	0.97
6	2	2nd rank: Flowers fully developed	-	-	0.97	0.94
6	3	3rd rank: Flowers fully developed	-	-	1.00	1.00
6	4	4th rank: Flowers fully developed	-	-	0.97	1.00
6	5	5th rank: Flowers fully developed	-	-	0.92	0.97
6	6	6th rank: Flowers fully developed	-	-	0.50	0.61
Macro stage 7—Seed filling						
7	1	1st rank: Seed filling fully completed	-	-	1.00	1.00
7	2	2nd rank: Seed filling fully completed	-	-	1.00	1.00
7	3	3rd rank: Seed filling fully completed	-	-	1.00	1.00
7	4	4th rank: Seed filling fully completed	-	-	1.00	1.00
7	5	5th rank: Seed filling fully completed	-	-	0.92	0.97
7	6	6th rank: Seed filling fully completed	-	-	0.53	0.69
Macro stage 8—Seed ripening						
8	1	1st rank: Seed ripening completed	-	-	1.00	1.00
8	2	2nd rank: Seed ripening completed	-	-	1.00	1.00
8	3	3rd rank: Seed ripening completed	-	-	1.00	1.00
8	4	4th rank: Seed ripening completed	-	-	1.00	1.00
8	5	5th rank: Seed ripening completed	-	-	0.94	0.97
8	6	6th rank: Seed ripening completed	-	-	0.53	0.72

- BBCH Macro Stage 9—Senescence

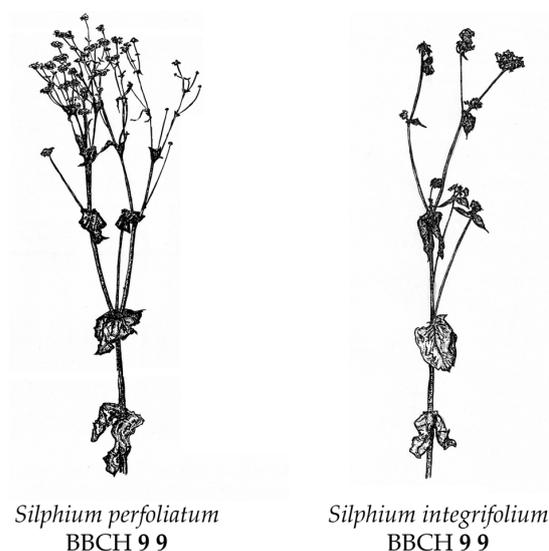


Figure 15. BBCH macro stage 9—Illustration of senescence of *Silphium* spp.

Table 7. BBCH macro stage 9—Senescence. Present stages of senescence with the corresponding relative frequencies for the first, as well as the second, year of development of *Silphium perfoliatum* and *Silphium integrifolium*. ($n = 36$).

Code	Description	SP 1. Year	SI 1. Year	SP 2. Year	SI 2. Year
9 1	10% of the aboveground plant parts are senescent	0.97	0.86	-	-
9 2	20% of the aboveground plant parts are senescent	0.89	0.78	1.00	-
9 3	30% of the aboveground plant parts are senescent	0.75	0.81	0.97	1.00
9 4	40% of the aboveground plant parts are senescent	0.64	0.72	0.61	0.61
9 5	50% of the aboveground plant parts are senescent	0.64	0.75	0.94	0.69
9 6	60% of the aboveground plant parts are senescent	0.64	0.58	0.61	0.60
9 7	Leaves completely senescent	0.72	0.72	0.78	0.75
9 9	Fully senescent above ground plant parts	1.00	1.00	1.00	1.00

4. Discussion

In the present experiment, *Silphium perfoliatum* and *Silphium integrifolium*, each species with two different geographic accessions, were investigated with regard to their development kinetics. The aim was to identify the phenotypic plasticity for both species regarding agricultural traits. It was noticed that there are differences between, as well as within, the investigated species and accessions. In a further step, possibilities for the further development of the BBCH scale for *S. perfoliatum* of Cumplido-Marin et al. (2020) will be discussed [13]. In addition, an adaptation for *S. integrifolium* will be carried out.

4.1. Germination Kinetics of Both *Silphium* Species

The emergence rate of Sil was distinctly higher than the emergence rates of the other *Silphium* accessions, which occurred due to unintended selection during the domestication process [42], as is well known from several crops of lower domestication level [43]. Furthermore, it is worth mentioning that the southern accessions of both species behaved comparably with respect to the higher emergence rate compared to the western/northern variants. This can be projected accordingly to the results from the overall germination kinetics in the interspecific comparison. The *S. integrifolium* accessions show a higher relative frequency of the respective trait in all described developmental stages. Especially in Sil, this domestication syndrome was detectable, which showed up in the form of a higher germination rate [44]. Intraspecific differences in germination and emergence characteristics are visible between the southern and northern variants of *S. perfoliatum* within all developmental stages. They become more pronounced with increasing growth stages. In contrast, a decrease in the differences of germination characteristics between the southern and the western accession can be observed in *S. integrifolium* over time.

Based on established methods, and in order to exclude possible influencing factors, the seeds were selected by hand and underwent a 0.05% gibberellic acid treatment. Compared to untreated or pelleted seed, this method achieved significantly higher and faster field emergence in *S. perfoliatum* than in previous studies [45]. The omission of a seed treatment by means of light and dark phases, as well as a temperature change during the swelling phase, in combination with a wet stratification of seven days at 0 °C can be seen as a weak point of this study; however, this does not limit the validity of the observations. However, Gansberger et al. (2017) showed that a significant increase in emergence rates can be achieved with this combination of methods used [19]. This being said, the same study shows relatively high standard deviations in the mentioned factors of 20% to 30%, which can support the relatively low germination rate of SPN. Transferred to the establishment of *S. perfoliatum* in field conditions, further research is needed with regard to emergence rates [19]. Regarding the germination behavior of *S. integrifolium*, a study was conducted by Reinert et al. (2018) whereby different pretreatments were carried out on a chemical and thermal basis. Among other results, a treatment with Ethephon and potassium nitrate in combination with a drying process showed significant increases in the germination rate of different *S. integrifolium* accessions [46]. Since these treatment options are also applied

to sunflowers [47], it can be assumed that the same treatments would also be a useful instrument for *Silphium*.

Adaption to BBCH Code—Macro Stage 0 [36]

In accordance to the phenotyping results, the interspecific comparison clearly shows that the *S. integrifolium* accessions tend to reach a higher degree of formation (%) at all micro stages (BBCH 0 0 to 0 9). Additionally, it can be stated that, on both studied species, the achievement of successive micro stages decreases with the increasing degree of formation. Intraspecific differences in the germination and emergence process are visible between the southern and western/northern accessions of *S. perfoliatum* and *S. integrifolium* within all micro stages, which increase with progressing growth stages. Regarding germination traits, both accessions of *S. perfoliatum* showed a high conformity with the BBCH scale established by Cumplido-Marin et al. (2020) [13]. Furthermore, an adaptation to the BBCH scale for *Miscanthus × giganteus*, according to Pude (1998), was omitted at this point, since resprouting in the second year of development could be clearly described on the basis of leaf formation (BBCH macro stage 1 (leaf formation)) [48]. A differentiation within this macro stage between actual seed germination and resprouting, as was carried out for the BBCH scale of *Sida hermaphrodita* L., was, accordingly, omitted [49].

4.2. Rosette Development

4.2.1. Year of Establishment

Rosette development was defined by the fully developed node, contrary to the method used in the literature of considering the single leaf, since each node usually expresses two opposite leaves [50,51]. The descriptions in the literature differ from the data collected from the phenotyping conducted in this experiment, but tend to accurately describe the difference between *S. perfoliatum* and *S. integrifolium*. Accordingly, a formation of an average of 26 leaves (13 rosette nodes), rather than 12–17 foliage leaves [52], was observed in *S. perfoliatum* during this field experiment. In *S. integrifolium*, an average of 34 leaves (17 rosette nodes) was counted instead of 32 deciduous leaves [50,52]. An adaptation to the environmental conditions regarding the leaf and root architecture, biochemical pathways and defense mechanisms of the respective origin seem clear here [4].

Franzaring et al. (2014) show that the results from a greenhouse experiment in which *S. perfoliatum* accessions from different geographic origins were evaluated for foliage number up to three months after sowing were also exceeded. After an experimental period of 106 days, an average of 7.1 leaves per plant were observed [53]. The data underlying this study show a mean rosette node number of 5.67 ± 0.67 at a comparable time point (104 DAS) for *S. perfoliatum*. Thus, a mean leaf number of 10 to 12 leaves per plant can be assumed. Projected on *S. integrifolium*, Sii and Sil showed an average of 8.1 ± 1.08 rosette nodes which is equal to 16–18 rosette leaves.

4.2.2. Second Year of Development

The phenotyping in the context of rosette node development shows that all accessions formed fewer rosette nodes, so that shoot formation occurred distinctly earlier than in the previous year. The fact that Sil developed the most rosette nodes compared to all other accessions could be related to a domestication syndrome that occurred incidentally due to breeding activity on this accession [44]. Whether this was a breeding goal, or arose incidentally, remains to be seen. The temporal divergence between Sil and Sii is less pronounced than in the *S. perfoliatum* accessions studied. After 376 DAS, all accessions showed an end of rosette formation. From this time on, shoot formation started.

4.2.3. Adaption to BBCH Code—Macro Stage 1 [36]

Using the BBCH code of *S. perfoliatum* according to Cumplido-Marin et al. (2020), the evaluation was also conducted up to the 9th nodes or 18th leaf [13]. Accompanying the higher number of fully developed nodes by the *S. integrifolium* accessions, a higher number

of micro stages, as well as higher proportions of fully developed micro stages, could be scored on them using the modified BBCH macro stage “leaf development”. Since the development of *Silphium* is continuous at this point, a progressive description of more than nine nodes (18 true leaves) is not absolutely necessary, provided that a node-dependent counting method is available. With respect to rosette development, a maximum BBCH-stage of 19, describing 9 or more rosette nodes, results for both species studied.

4.3. General Shoot Development

The shoot forms the largest part of the total yield in the biomass-oriented culture of *S. perfoliatum*. At the end of the growing period, the biomass in the leaves amounts to 30.94% of the total biomass. After the completion of senescence, this percentage is reduced to 8.14% [54]. The relative proportion of biomass formed in the shoot behaves accordingly. Depending on the intended use and the expected biomass yield, the ratio of shoot to leaf biomass can be varied considerably by adjusting the harvest date. The biomass uses of *S. integrifolium* are to be assessed as secondary, since the main focus here is on its use as a perennial oilseed [4,34]. Accordingly, less importance is attributed to shoot formation. However, the shoot development of both species will be discussed below, as it is important for the general plant development.

The shoot development of *S. perfoliatum* and *S. integrifolium* is generally characterized by the shoot tip forming a shoot through the formation of elongated internodes and internode elongation within the already-formed rosette. A simultaneous longitudinal growth allows the rapid shoot formation [41]. Thus, supposedly, rosette-bearing nodes become shoot-bearing nodes. Retrospectively, originally rosette-bearing nodes can be identified on the shoot by the lack of cupped leaf formation. Due to this fact, it is necessary to count continuously for nodes in the rosette and nodes on the shoot.

4.3.1. Year of Establishment

Contrary to the statement of Gansberger et al. (2015), *S. perfoliatum* showed a sporadically developed shoot formation in the field trial conducted in the first year of cultivation [55]. The tendency of *S. integrifolium* for shoot formation is in agreement with the statements of Kowalski (2004), who noted that *S. integrifolium* can reach a growth height of 20 to 69 cm in the first year [50]. In a previous field experiment conducted by Conrad et al. (2010), the influence of shoot formation on individual plants in the first growing season was observed with an unfavorable growth behavior of *S. perfoliatum* in the following year, so that only 0.853 Mg dry yield per hectare could be harvested [56]. Accordingly, it can be assumed that differences between the previous year's shoot-forming and non-shoot-forming plants could also occur in this field trial, in the second growing season, with regard to stand closure, shoot formation as well as stand stability. As shown in the previous chapter, “Rosette development—Year of establishment”, *S. perfoliatum* accessions tended to differ less than the *S. integrifolium* accessions studied with respect to rosette formation. However, SPN was the only accession characterized by a lower emergence rate and by slower emergence kinetics compared to the other *Silphium* accessions. Thus, SPN tended to show slower germination kinetics and delayed rosette development by about 21 days, which may result in failure to shoot. Other factors may also have an influence on this result [56]. Furthermore, *S. perfoliatum* and *S. integrifolium* showed large morphological differences [50]. For instance, the average fresh mass yield of *S. integrifolium* fluctuated at 345 g per plant in the first year [50]. *S. perfoliatum* formed about 50% more biomass in the first year with an average of 519 g per plant [50,57].

4.3.2. Second Year of Development

After the simultaneous start of shoot formation in all analyzed plants, phenotypic differences were observed between and within both species. Thus, the difference regarding shoot node development in the intraspecific comparison between the studied *S. perfoliatum* accessions was characterized by a slight temporal divergence. *S. integrifolium* showed,

in addition to a larger temporal discrepancy, a much larger number of formed nodes on the shoot. Sil formed significantly more nodes than Sii. Sii showed comparable results to other studies [50]. Due to breeding progress and divergent parent material, Sil showed a higher number of nodes and, at this point, was apparently subject to a domestication syndrome [44]. *S. perfoliatum* showed a comparable numbers of nodes within the shoot formation of European accessions in a previous study under the same field conditions [10]. In contrast to the first year of development, a similar picture tends to be seen in the *S. integrifolium* accessions studied, with all plants now showing shoot growth. Based on the fact that shoot growth is a necessary step for generative growth in *Silphium*, it can be shown that this has also been observed elsewhere [58]. In *S. perfoliatum*, this picture is clearer, since, in the first year of cultivation, the proportion of shooting plants was lower than or completely absent in the *S. integrifolium* accessions studied. This allows for the hypothesis that *S. perfoliatum* is more dependent on vernalization than *S. integrifolium* or that both species show different strategies for generative propagation [26,58].

4.3.3. Adaption to BBCH Code—Macro Stage 3 [36]

In the context of this study, only the development of a predefined main shoot of the respective plant was described, since the development of the secondary shoots occurs in parallel and these were equal. Accordingly, the BBCH macro stage 2 (side shoot formation) postulated by Meier (2018) was omitted. A description of crop closure at BBCH macro stage 2, which was carried out here for *S. perfoliatum*, in the first year [13], and *Sida hermaphrodita* [49], is not provided in the extended BBCH scale according to Meier [36].

After the shoot development phase was completed, the generative phase of *S. perfoliatum* and *S. integrifolium* was initiated. An overlapping of shoot growth and generative growth did not occur on the individual plant.

Delayed internode elongation [41] in the rosette leads to a shift in shoot node numbering. As mentioned above, this leads to a subtraction of the remaining rosette nodes and results in an optional extension of the BBCH scale. Thus, for BBCH macro stage 3, the result is an indication from $3\ 1-x$ to $3\ 9-x$, where the sequential number describes the micro stages present and the variable x describes the nodes remaining in the rosette. Here, BBCH $3\ 9-x$ defines 9 or more fully formed shoot nodes, less the nodes remaining in the rosette. The classification of micro stages, as performed according to Cumplido-Marin et al. (2020), using the relative proportions of final shoot height as the determining measure, may present difficulties, as this can only be carried out retrospectively. Furthermore, macro stage 4 (development of vegetative plant parts), as postulated by Meier (2018), was also not included, as this stage also did not occur in the actual sense [36].

4.4. Generative Phase

4.4.1. Year of Establishment

Generative growth was not documented in the first year of cultivation of *S. perfoliatum* by another study. Equally, no generative development of *S. perfoliatum* is described in the summary of the literature [55,59]. Contrary to these statements, some of the *S. perfoliatum* cultivated during this field experiment showed a generative growth phase in the first year. In terms of further development, neither the development of a flower, nor the formation of seeds, occurred. Another possible reason could be that the plants received on-demand irrigation at the beginning of their development and, in combination with the excellent soils and climatic conditions, partial flower induction occurred in SPS. This hypothesis is clear, but is not described in the literature. SPN, corresponding to the absence of shoot formation in the first year, showed no tendency towards generative growth. *S. integrifolium* showed a tendency to enter a generative phase of development equivalent to shoot formation in both accessions studied. Kowalski et al. (2004) documented no generative development in the first year in their developmental description of *S. integrifolium* [50]. Contrary to this, Schiffner et al. (2021) describes that *S. integrifolium* also shows generative development depending on the sowing date, and the available development time, until the end of the

vegetative phase of the first year [58]. Sil shows a higher tendency towards generative growth than Sii whereas, in both accessions, the seed filling phase no longer occurred completely. Since these developmental steps occurred distinctly later than usual [55], it can be assumed that the performance of pollinating insects was diminished because of the flowering in winter.

4.4.2. Second Year of Development

In the second year of the field experiment, all plants showed full generative growth. The same was reported in other studies [50,60,61]. The first buds were visible in all accessions 426 DAS and, in the course of generative growth, an intraspecific variation of the respective accessions developed. This was more evident in *S. perfoliatum* and increased over the entire generative development, so that the seed ripening of SPN of each flowering rank occurred 21 days earlier than SPS. *S. integrifolium* did not show this behavior, so that a more comparable development of the generative phase in both accessions was present. Conspicuous were the far larger flowers appearing in Sil, due to the breeding activity and feminization. This increases the number of seeds formed in each flower [29].

Across both *Silphium* species, all investigated accessions were showed at the last flower rank just at the bud stage. A further development of the last rank in each case did not occur.

4.4.3. Adaption to BBCH Code—Macro Stage 5–8 [36]

A classification in the BBCH scale according to Meyer et al. (2018) is possible in the macro stage 5 to 8, which describes the generative development phase of the plant [36]. Within the existing BBCH scale, a methodology is used giving the most precise description possible [13]. A difficulty to be considered is that, especially in the higher ranks, there are certain shifts in the individual inflorescences in each stage, which makes it impossible to give an accurate description of the plant as a whole. One possibility to describe the inflorescence would be by using a percentage of the advanced inflorescence, as it was established within the BBCH scale of rape seed (*Brassica napus* L.) [36]. As the number of inflorescences multiplies with increasing rank, this method can be misleading, because of the problems in estimating the exact global developmental stage of the plant. A rank-by-rank assessment of progressive flowering seems to be the most appropriate method. The number of flowers per rank is fixed, and each rank has a distinct designation.

4.5. Senescence

4.5.1. Year of Establishment

Senescence occurred but was delayed in the first year of cultivation. Complete senescence in all accessions was seen after 307 DAS. This was probably due to the extremely mild climatic conditions until then. A cold period in the end of January provided the onset of complete senescence. *S. perfoliatum* showed a general offset of the senescence progress, so that SPN, in general, had a head start of one week, which was due to the climatic conditions of the place of origin in the northern part of the distribution area. This adaptation to climatic conditions was also found in European wild types of *Arabidopsis thaliana* [62]. Thus, it can be assumed that the southern accession (SPS) goes through a longer vegetative phase, due to its natural habitat, than a northern accession (SPN), and thus reaches full senescence later. Since the studied accessions of *S. integrifolium* originate from similar latitudes, divergence, with respect to the senescence progression between them, is not likely. Furthermore, the senescence of individual plants of *S. integrifolium* is more inhomogeneous than within the accessions of *S. perfoliatum*, as both accessions show a greater dispersion of the senescence degrees.

4.5.2. Second Year of Development

The development of senescence in the second year is more differentiated in *S. perfoliatum* than in the first year. Equivalent to the first year, this behavior can be explained by the different climatic conditions of the natural origin [62].

The *S. integrifolium* accessions studied showed a much similar senescence behavior. A tendency of earlier onset of senescence of Sii showed no influence on the achievement of complete senescence. Both accessions studied showed complete senescence at 560 DAS, which was due to the similar latitude of the origin.

4.5.3. Adaption to BBCH Code—Macro Stage 9 [36]

A classification in the BBCH scale according to Meyer et al. (2018) is possible in macro stage 9 [36]. An initial classification in percentage terms is given, up to a degree of senescence of 60%. The use of the BBCH scale according to Cumplido-Marin et al. (2020) is only of partial use at this point, since senescence may not proceed in the order indicated [13]. The foliage of the plants located at the lower part of the shoot had already become necrotic while the plant was still in the generative phase, because the light intensity there was no longer sufficient. This had already occurred after about 461 DAS (no data shown). However, actual senescence was not detected until 502 DAS. Since the course of senescence is simultaneous on almost all plant parts, a percentage representation of the expression of senescence up to and including BBCH 9 6 is reasonable. BBCH 9 7 describes the complete senescence of the foliage. BBCH 9 9 describes the complete senescence of the aboveground emergence of the plants [13].

4.6. Classification of Phenotypic Traits and Use

Harvesting, as well as utilization, of any kind, in the first year of cultivation, should generally be avoided due to low yields and possible damage to the plants. A versatile usage of the second year biomass is possible [10,23,55]. Often, the time of harvest and the associated developmental stage or maturity is crucial for the use [19,23].

Here, the agronomic usability of *Silphium* maturing at different rates is an important aspect. Depending on the site conditions and climate, it makes sense to select a suitable accession. The results show that SPN may be of interest for use in the case of large weed pressure, because it is characterized by a high growth rate in rosette development in the second year of emergence in the first stages. It develops many leaves quickly and thus can take light away from competing weeds more quickly than other *Silphium* accessions through early stand closure. Soil type can also be a criterion for selecting a certain *Silphium* accession. A high clay and fine silt content, along with high water content, provide difficult conditions for trafficability as well as soil compaction [63]. Thus, in terms of harvest timing, which accession is chosen and when it matures may be critical for a specific use. Since SPS finished maturing over two weeks later than SPN in the trial, this should be taken into consideration when choosing an accession.

With regard to economic use, the harvesting times should be clearly defined. Harvesting times should generally distinguish between green biomass and dry biomass. For practical use, it is useful to label the harvest time in BBCH scales and to map the associated dry matter content. A common classification of maturity dates for corn are the FAO numbers. These represent a use-specific maturity number that considers dry matter contents. The intended use defines the stage of maturity and, respectively, the harvest date [64].

Green *Silphium* biomass is mainly harvested for energy use in biogas plants [55,65]. Plants used for fiber production for the packaging industry also have the same maturity period as those for energy use, since a cascade use is possible here [66]. The plants should be cut for harvest when the stems are not fully lignified. An advanced or even shot down lignification of the fibers is also detrimental with respect to convertibility in biogas production [67]. To maximize methane yield, a harvest in summer at the end of flowering in BBCH 6 6 is envisaged. Dry matter should then range from 20% to 25% [55,65]. If *Silphium* is to be harvested together with maize (*Zea mays* L.), a late-maturing accession, such as SPS, would be suitable here. If necessary, an early-maturing maize should be used here to generate a further convergence of the harvest date [67].

If the plant is to be used as a building material, the aim is to achieve a dry matter content as high as possible, and lignification that is preferably high, so that the plant is

not harvested until BBCH 99. This development stage occurs at the end of the vegetation period. Harvesting in winter reduces the moisture content. After 596 DAS, a harvest of aboveground biomass was conducted. A mean dry matter content of $63.65\% \pm 12.73\%$ was determined. Longer maturity in the field would suggest higher dry matter contents. It may be necessary to consider the amounts of different structures or the contents of different carbohydrates in the respective harvested material. Moll et al. (2022) found that certain *Silphium* accessions had different ratios of cortex to parenchyma, which could imply different benefits of biomass [23].

S. integrifolium can also be harvested as a biomass crop from mid-June to mid-August. Schiffner et al. (2020) described a dual use of *S. integrifolium* [32]. Due to the different maturation dates of biomass and seeds, and the resulting discrepancy between the optimal harvest dates, an adaptation of the optimal harvest date should be carried out. Therefore, a differentiation should be made between its use for animal feed and use for oilseeds. Accessions with a safe maturity are critical for yield in oilseed use. Late *Silphium* accessions cannot realize their high yield potential in Central European climatic conditions. A higher yield can thus be generated at early harvesting times [68]. For oilseed use, the studied pre-domesticated accession Sil is the most promising. Thus, the average number of seeds per flower was distinctly increased [4]. Since *Silphium* still shows natural seed shattering, the seeds drop out as soon as a complete maturation of the seed pod occurs. As maturation progresses continuously across the different flower ranks, an optimal harvest date must be found to ensure the highest possible yield with the best quality possible. The utilization of biomass in late spring requires high forage quality. However, the seed yield from the regrowing biomass is reduced by up to 45%, which is equivalent to a utilization gap [68]. Furthermore, an adaptation of the ripening behavior to that of forage maize would be advisable, as a simultaneous harvest would offer great economic advantages. The harvesting technique and the silage process for both crops are almost identical [69].

5. Conclusions

Different genotypes of *Silphium integrifolium* and *Silphium perfoliatum* were investigated, with two accessions each, during their establishment phase and the second year of their cultivation. Both the interspecific and intraspecific variations in the quantitative phenotypic traits and growth kinetics were analyzed. *S. integrifolium* shows distinctly more developed nodes, and a stronger tendency towards flower formation, than *S. perfoliatum* in the first year. In the second year of cultivation, the geographical difference of the places of origin of the two *S. perfoliatum* accessions studied becomes apparent by a discrepancy that increases over the course of development. *S. integrifolium* shows this to a lesser extent.

Furthermore, a BBCH scale based on genotypes with high genetic distance was developed within this study and was adapted to *S. integrifolium*. In addition, botanical drawings of the growing parts of the plant were made to provide references for exemplary developmental stages.

The suitability of differently developing accessions of *S. perfoliatum* and *S. integrifolium* were discussed. Here, different forms of usage and corresponding harvesting times were addressed. The genus *Silphium* represents two species that are good alternatives to conventional annual crops for industries of many kinds. The usage of this plant family for oil and biomass production has been intensively researched in the recent years and, for *S. integrifolium*, domestication has already started. The phenotypic variation within the exploited accessions is still at a high level, which affects the uniformity of the crop in the field. Breeding for varieties with specific uses would be crucial for further crop development and would accelerate the domestication process in both species.

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