

# Germination characteristic of *Silphium perfoliatum* L. seeds

## Keimfähigkeitscharakteristik von *Silphium perfoliatum* L.-Samen

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### Summary

*Silphium perfoliatum* L. is a perennial and flowering crop that has been investigated in recent years for its potential as an energy plant, particularly for biogas production. A stand establishment by sowing of seeds is complicated, owing to the low germination capacity of untreated *S. perfoliatum* L. seeds. Consequently, germination experiments were carried out with two- to four-factor levels to determine the effect of selected factors (medium, pretreatment, light, temperature, prechilling) on dormancy and germination of *S. perfoliatum* L. seeds and to achieve maximum germination rate. All factors had a highly significant effect on germination. Germination experiments displayed a primary and physiological dormancy. Germination could be significantly increased by using a 0.05% GA<sub>3</sub> solution during the imbibition phase, a light–dark cycle, alternating temperatures between 20°C and 30°C, and a wet stratification for seven days at 0°C. The experiments helped to fully exploit the germination potential and to develop a germination test method for *S. perfoliatum* L.

**Keywords:** bioenergy, cup plant, dormancy, seed production, seed treatment

### Zusammenfassung

*Silphium perfoliatum* L. ist eine ausdauernde und blühende Pflanze, die in den letzten Jahren hinsichtlich ihres Potentials als Energiepflanze für die Biogasproduktion untersucht wurde. Eine gesicherte Bestandesbegründung mittels Saat ist aufgrund der geringen Keimfähigkeit von unbehandelten *S. perfoliatum* L.-Samen schwierig. Folglich wurden Keimversuche mit zwei bis vier Faktorstufen durchgeführt, um die Wirkung ausgewählter Faktoren (Medium, Vorbehandlung, Licht, Temperatur, Vorkühlung) auf die Dormanz und die Keimung von *S. perfoliatum* L.-Samen zu bestimmen und die maximale Keimfähigkeit zu erreichen. Alle untersuchten Faktoren hatten einen signifikanten Effekt auf die Keimung. Die Keimfähigkeitsversuche ergaben eine primäre und physiologische Dormanz. Die Keimfähigkeit wurde durch den Einsatz einer 0,05% GA<sub>3</sub>-Lösung während der Quellungsphase, eines Licht- und Dunkelwechsels, einer alternierenden Temperatur zwischen 20 und 30°C und einer nassen Stratifikation von sieben Tagen bei 0°C signifikant erhöht. Damit wurde das Keimfähigkeitspotential ausgeschöpft und eine Keimfähigkeitsprüfmethode für *S. perfoliatum* L. entwickelt.

**Schlagworte:** Bioenergie, Becherpflanze, Dormanz, Saatgutproduktion, Saatgutbehandlung

## 1. Introduction

*Silphium perfoliatum* L. is a perennial, tall, yellow flowering  $C_3$  plant, characterized by a wide range of valuable practical traits and has been cultivated as a medical, melliferous, fodder, ornamental, and reclamation plant (Niqueux, 1981; Neumerkel and Märtin, 1982; Troxler and Daccord, 1982; Daniel and Rompf, 1994; Kowalski and Wolski, 2005; Kowalski and Kędzia, 2007; Zhang et al., 2010). In recent years, *S. perfoliatum* L. has been of interest as an energy crop, especially for biogas production (Vetter et al., 2010; Bauböck et al., 2014; Mast et al., 2014; Gansberger et al., 2015). The low care requirements (after the first year) compared to annual plants, the high yield potential of the biomass, and the ecological benefits make *S. perfoliatum* L. a valuable renewable raw material (Gansberger et al., 2015). The labor-intensive and expensive planting of pregrown seedlings is currently the common method of stand establishment. Sowing is not feasible owing to a lack of high-quality seeds (Bauböck et al., 2014; Franzaring et al., 2015; Gansberger et al., 2015) and inappropriate seed technology. Furthermore, little is known about the germination requirements of *S. perfoliatum* L. (Gansberger et al., 2015). To tap the full potential of the germination capacity, the necessary environmental conditions must be identified and the dormancy, if present, must be broken. According to Sokolov and Gritsak (1972), Troxler and Daccord (1982), and Vetter et al. (2010), untreated seeds of *S. perfoliatum* L. show a strong dormancy, leading to an uneven and sometimes highly delayed germination. Trölenberg et al. (2012) assumed that there was no dormancy in *S. perfoliatum* L., but instead, quiescence. Quiescent seeds are not dormant, but are metabolically inactive because of the absence of one or more environmental factors (Bewley, 1997; Baskin and Baskin, 2004). The aims of this work were to investigate the effect of individual factors and their levels on germination and dormancy and to determine the advisable conditions for germination, as well as to recommend a germination test method to evaluate the full germination capacity of *S. perfoliatum* L. seeds.

## 2. Material and methods

### 2.1 Seeds

Seeds of two different origins were used for the germination experiments. Lot A was harvested in September 2012

in Vienna, Austria (48°15'23"N, 16°29'5"E). Lot B was obtained from a harvest in September 2011 in Rheinstetten-Forchheim, Germany (48°58'1"N, 8°20'3"E). The viability and the theoretical germination capacity of both seed lots (rep = 4 × 50) were determined by a tetrazolium test, which also counts seeds with a physiological dormancy as viable (Baskin and Baskin, 2014). The germination potential was  $97.5 \pm 2.2\%$  for lot A and  $98.5 \pm 0.9\%$  for lot B, providing an excellent basis for the subsequent tests.

### 2.2 Model of the germination experiment

The experimental design was based on several factors and factor levels. These were chosen based on literature values for germination requirements of achenes, other members of the Asteraceae, the results of preliminary experiments, and the climate conditions of *S. perfoliatum* L.'s natural habitat. A full-factorial randomized design (with a total of 144 combinations) was used with two- to four-factor levels of following factors:

- Medium: pleated paper (PP); top of paper (TP)
- Pretreatment: water;  $KNO_3$  – 200 mg/L;  $GA_3$  (Merck KGaA) – 500 mg/L
- Light (PAR between 400 and 700 nm): 24-h light; 12-h light
- Temperature: 20°C; 30°C; 20/30°C
- Prechilling: no prechilling; 7 days / 10°C; 7 days / 5°C; 7 days / 0°C

Each combination corresponded to a germination test method and was tested with seed lots A and B in a growth chamber (from the Kühlanlagenbau Fritz Lachmayr GesmbH, Kremsmünster, Austria). Based on sample size calculation (power 90%,  $\alpha = 5\%$ ,  $\delta = 2\%$ ), 144 analyses with eight replicates each were carried out for the two lots. Overall, 2304 observations ( $144 \times 2 \times 8$ ) were used and each replication consisted of 50 seeds.

The evaluation was carried out after 7, 14, and 21 days. The seedlings were classified as normal seedlings, abnormal seedlings, or un-germinated seeds according to the International Rules for Seed Testing (ISTA, 2015). However, for evaluating dormancy, normal and abnormal seeds were both counted as germinated seeds.

### 2.3 Statistical analyses

The data set was analyzed with the statistics software SPSS V22.0. The requirements of the statistical tests were

checked before and were fulfilled. Residuals were checked for normal distribution and homoscedasticity by using residual plots and statistical tests. An analysis of variance (ANOVA) was carried out with a mixed model approach to determine if the selected factors and their two-way interactions had a significant effect on germination capacity. The factors “medium, pretreatment, light, temperature and prechilling” were defined as fixed effects and the seed lot was defined as random effect. The significance level was chosen as 95%. Additionally, post-hoc analysis (Tukey-HSD) was applied for significant factors to separate the most effective factor levels.

Finally, the most promising factor levels were combined in a recommended germination test method and validated with the predicted expected values of the linear model with main effects and interactions.

### 3. Results and discussion

#### 3.1 Germination capacity

The results of the germination capacity experiments ranged between 0 and 96% germinated seeds. With an unfavorable combination of factors, no seeds germinated, and with favorable combinations, up to 96% germinated. This effectively corresponds to the full germination potential of around 98%, which was determined in advance by a tetrazolium test. In addition, these tests showed that the embryos were fully developed and that water could enter through the seed coat to the embryo. Thus, a morphological and physical dormancy could be excluded.

#### 3.2 Effect of selected factors and factor levels on germination

All main factors and also almost all two-way interactions had a highly significant effect on germination (Table 1). The interaction of the individual factors caused a large degree of fluctuation. The standard deviation was between 20% and 30% for all factors.

**Effect of various media on germination:** The mean value of germinated seeds with PP was about 1.7% higher than with TP (Table 2). Seeds in TP showed fungal infections. In contrast, the folds in the pleated paper (PP) served as a barrier, preventing fungi from growing from one seed to the next. PP seems better suited for the germination of *S. perfoliatum* L. seeds with their flat shape and large seed surface.

**Effect of various pretreatments on germination:** GA<sub>3</sub> and KNO<sub>3</sub> solutions can affect the metabolic activity of seeds (Baskin and Baskin, 2004). Our experiments show that the treatment with GA<sub>3</sub> and KNO<sub>3</sub> solutions led to significantly higher germination than the untreated control with water (Table 2 and Figure 1). The greatest effect on germination capacity was achieved with GA<sub>3</sub>. GA<sub>3</sub> has the opposite effect to abscisic acid (ABA) and the exact ratio of GA<sub>3</sub> to ABA determines, on a plant-hormone level, whether a seed will start to germinate or remain dormant. ABA accumulates in the embryo during seed ripening and is responsible for primary dormancy (Kucera et al., 2005). In contrast, GA<sub>3</sub> induces growth of the embryo and increases the availability of nutrients (Koornneef et al., 2002; Hilhorst, 1995). The ratio of GA<sub>3</sub> and ABA at *S. perfoliatum* L. seeds has probably been shifted by adding GA<sub>3</sub> solution and consequently the germination capacity increased. This effect suggests the presence of a physiological dormancy. The experiments of Vetter et al. (2010) and Trölenberg et al. (2012) with *S. perfoliatum* L. seeds using

Table 1. Results of the mixed model analysis (ANOVA)  
Tabelle 1. Ergebnisse der gemischten Modellanalyse (ANOVA)

	<i>F</i>	<i>p</i> -value
Medium	14.6	<0.001
Light	221.3	<0.001
Temperature	6063.3	<0.001
Pretreatment	528.0	<0.001
Prechilling	540.7	<0.001
Lot	3213.7	<0.001
Medium × light	14.5	<0.001
Light × prechilling	18.0	<0.001
Light × pretreatment	2.7	0.065
Light × temperature	13.9	<0.001
Medium × prechilling	4.3	0.005
Medium × pretreatment	22.1	<0.001
Medium × temperature	75.3	<0.001
Pretreatment × prechilling	11.8	<0.001
Temperature × prechilling	39.8	<0.001
Temperature × pretreatment	11.5	<0.001

The seeds from lot A had a germination capacity (overall factors and factor levels) of 34.0<sup>a</sup> ± 26.1%. Lot B, with 56.2<sup>b</sup> ± 27.9%, had significantly higher germination rates than lot A.

Table 2. Mean value and standard deviation of germinated seeds for selected factor levels  
Tabelle 2. Mittelwert und Standardabweichung gekeimter Samen für ausgewählte Faktorstufen

Factors	Factor levels			
Medium	Pleated paper (PP)	Top of paper (TP)		
	48.3 <sup>b</sup> ± 30.7%	46.6 <sup>a</sup> ± 30.4%		
Pretreatment	Water	KNO <sub>3</sub> (200 mg/L)	GA <sub>3</sub> (500 mg/L)	
	39.3 <sup>a</sup> ± 29.8%	47.0 <sup>b</sup> ± 30.6%	55.9 <sup>c</sup> ± 28.9%	
Light	24-hours light	12-hours light		
	44.3 <sup>a</sup> ± 29.4%	50.6 <sup>b</sup> ± 31.4%		
Temperature	20°C	30°C	20/30°C	
	18.6 <sup>a</sup> ± 18.7%	48.5 <sup>b</sup> ± 21.0%	75.2 <sup>c</sup> ± 20.2%	
Prechilling	No prechilling	7 days / 10°C	7 days / 5°C	7 days / 0°C
	33.4 <sup>a</sup> ± 26.6%	48.8 <sup>b</sup> ± 31.1%	51.8 <sup>c</sup> ± 29.9%	55.7 <sup>d</sup> ± 29.6%

The factor levels marked with different letters (a–d) are significantly different ( $\alpha = 5\%$ ) by Tukey-HSD test. See additional data in Table 4.

GA<sub>3</sub> solution also showed a germination capacity close to the theoretically possible maximum.

#### Effect of 12 hours and 24 hours light on germination:

Light and its intensity can have a substantial effect on dormancy and germination (Oh et al., 2006). Light had a positive effect on the germination of *S. perfoliatum* L. during the preliminary experiments. To investigate this effect, 12- and 24-hour light variants were tested. The 12-hour light–dark cycle with a light intensity between 400 and 1200 lux resulted in significantly higher germination capacities than the 24-hour light variant (Table 2).

#### Effect of various temperatures on germination:

Favorable environmental conditions, like specific temperature ranges, are important for germination; otherwise the seeds cannot germinate (Baskin and Baskin, 2004) or fall into secondary dormancy (Hilhorst, 1998; Finch-Savage and Leubner-Metzger, 2006). If seeds have physiological dormancy, alternating temperatures can be used to break their dormancy (Long et al., 2014).

Temperature had the greatest effect on germination capacity over all the five factors in our experiment. The differences between the temperature variants were highly significant. As Table 2 and Figure 1 show, the temperature cycles of 20/30°C every 12 hours showed distinctly more germination than constant temperatures at 20°C or 30°C. In combination with the GA<sub>3</sub> solution, an average germination capacity of over 85% was achieved.

Trölenberg et al. (2012) extensively studied the effect of various constant and changing temperatures on the germination of *S. perfoliatum* L. seeds at a temperature-gradient germination table. The results showed that germination was positively influenced by higher temperatures up to 30°C and by alternating temperatures with higher amplitude.

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#### Effect of various prechilling variants on germination:

Several decades ago, Sokolov and Gritsak (1972) and Troxler and Daccord (1982) recommended sowing *S. perfoliatum* L. seeds in late autumn, at the latest 15–20 days before the first night frost, using freshly gathered seeds or in spring with seeds that had been stratified for 2 months. Accordingly, the factor “prechilling” was included in the model, as prechilling is particularly suitable to break the physiological dormancy (Long et al., 2014).

The results confirmed that prechilling has a positive effect on the germination capacity. The germination capacity of the variants with prechilling (7 days / 10°C; 7 days / 5°C; 7 days / 0°C) was highly significantly higher than the untreated control (no prechilling). The differences between the individual prechilling temperatures were not pronounced, but still significant. Therefore, a prechilling phase at 0°C over 7 days is advisable to achieve a high germination capacity and to break the physiological dormancy.

The results are consistent with the conclusions of Vetter et al. (2010) and Trölenberg et al. (2012). They recommend a prechilling phase at 5°C over 5 and 7 days respectively. Franzaring et al. (2014) used a four-week prechilling period in climate chambers for their growth experiments with *S. perfoliatum* L. The temperatures varied between 3°C and 11°C, causing stratification and breaking of the seed dormancy.

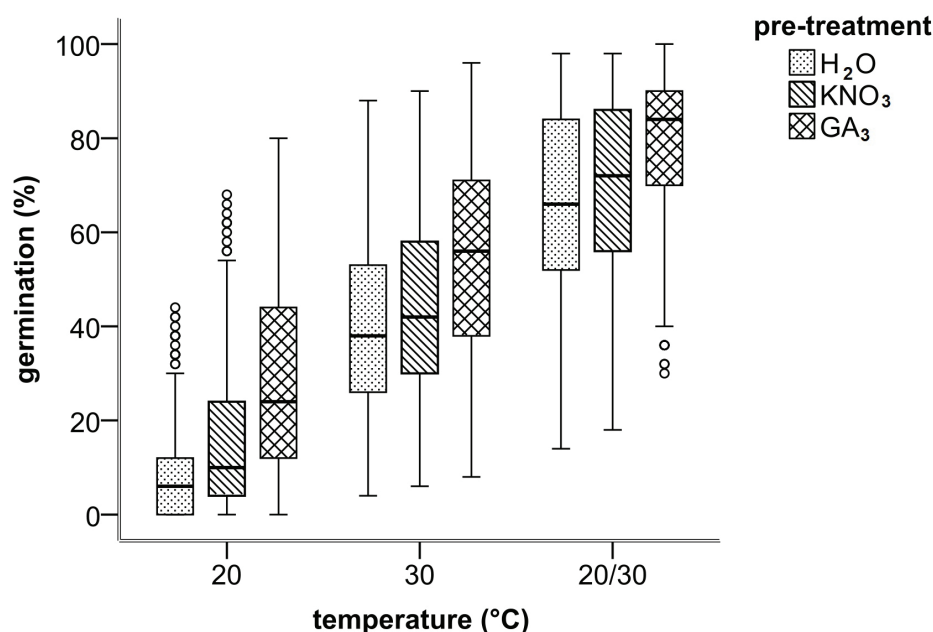


Figure 1. Effect of various temperatures (20°C; 30°C; 20/30°C) and pretreatments (H<sub>2</sub>O; KNO<sub>3</sub>; GA<sub>3</sub>) on the germination of *Silphium perfoliatum* L.

Abbildung 1. Effekt von unterschiedlichen Temperaturen (20°C; 30°C; 20/30°C) und Vorbehandlungen (H<sub>2</sub>O; KNO<sub>3</sub>; GA<sub>3</sub>) auf die Keimfähigkeit von *Silphium perfoliatum* L.

### 3.3 Germination characteristic and germination test method of *S. perfoliatum* L.

The most promising factor levels were combined in a germination test method, which allows the assessment of the germination capacity of *S. perfoliatum* L. seeds (Table 3). These results were confirmed by using the predicted outcome of the linear model with main effects and interactions. The only deviation between the results of the ANOVA and post-hoc tests and the linear model could be found for the factor “type of paper.” However, the difference between the mean values of the factor medium was minor (Table 2). Consequently, the choice between the paper substrates seems to be of less importance. The use of PP is recommended, due to the reduced fungal growth and the smaller number of abnormal seedlings in comparison to TP.

### 4. Conclusion

The results of the germination characteristic of *S. perfoliatum* L. indicate that a targeted and optimized pretreatment is required for a fast and complete germination. The germination test method (Table 3) includes various treatments for breaking the physiological dormancy. The germination capacity was increased considerably with the use of GA<sub>3</sub> solutions, light, alternating temperatures and prechilling, and their modes of action suggest a pronounced physiological dormancy in fresh *S. perfoliatum* L. seeds. In future experiments, for example, the influence of seed moisture content, harvest date and seed age on dormancy and different seed preparation should be tested and the laboratory-scale results need to be confirmed at field scale. Therefore

Table 3. Resulting germination test method for *Silphium perfoliatum* L. to exploit the germination potential

Tabelle 3. Resultierende Keimfähigkeitstestmethode für *Silphium perfoliatum* L. zur Ausschöpfung des Keimfähigkeitspotentials

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Recommendations for breaking dormancy	Additional directions	Additional advice
<i>S. perfoliatum</i> L.	PP	20/30*	7	21	GA <sub>3</sub> ; prechill	-	prechill 7 days / 0°C; L/D*

\* Alternating temperature and light regime: first temperature 12 hours with light, second temperature 12 hours without light.

applying additional pretreatments, like other biologically active compounds, priming and pelleting, are of great interest. For this, the results of this work can be incorporated.

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Table 4. Multiple Comparisons of the selected factors temperature, pretreatment and prechilling  
Tabelle 4. Mehrfachvergleiche der ausgewählten Faktoren Temperatur, Vorbehandlung und Vorkühlung

(I) temperature	(J) temperature	Mean Diff. (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
20°C	30°C	-29.87*	0.514	<0.001	-31.08	-28.67
	20<=>30°C	-56.56	0.514	<0.001	-57.77	-55.35
30°C	20°C	29.87	0.514	<0.001	28.67	31.08
	20<=>30°C	-26.69	0.514	<0.001	-27.89	-25.48
20<=>30°C	20°C	56.56	0.514	<0.001	55.35	57.77
	30°C	26.69	0.514	<0.001	25.48	27.89
(I) pretreatment	(J) pretreatment					
H <sub>2</sub> O	KNO <sub>3</sub>	-7.76	0.514	<0.001	-8.96	-6.55
	GA <sub>3</sub>	-16.68	0.514	<0.001	-17.89	-15.48
KNO <sub>3</sub>	H <sub>2</sub> O	7.76	0.514	<0.001	6.55	8.96
	GA <sub>3</sub>	-8.93	0.514	<0.001	-10.13	-7.72
GA <sub>3</sub>	H <sub>2</sub> O	16.68	0.514	<0.001	15.48	17.89
	KNO <sub>3</sub>	8.93	0.514	<0.001	7.72	10.13
(I) prechilling	(J) prechilling					
no prechilling	7d / 0°C	-22.28	0.593	<0.001	-23.81	-20.76
	7d / 5°C	-18.39	0.593	<0.001	-19.91	-16.86
	7d / 10°C	-15.31	0.593	<0.001	-16.84	-13.79
7d / 0°C	no prechilling	22.28	0.593	<0.001	20.76	23.81
	7d / 5°C	3.90	0.593	<0.001	2.37	5.42
	7d / 10°C	6.97	0.593	<0.001	5.45	8.50
7d / 5°C	no prechilling	18.39	0.593	<0.001	16.86	19.91
	7d / 0°C	-3.90	0.593	<0.001	-5.42	-2.37
	7d / 10°C	3.07	0.593	<0.001	1.55	4.60
7d / 10°C	no prechilling	15.31	0.593	<0.001	13.79	16.84
	7d / 0°C	-6.97	0.593	<0.001	-8.50	-5.45
	7d / 5°C	-3.07	0.593	<0.001	-4.60	-1.55

Based on observed means.

The error term is Mean Square(Error) = 101.407.

\*) The mean difference is significant at the 0.05 level.

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