Seed germination and seedling survival of *Drosera rotundifolia* (L.) cultivated on *Sphagnum*: Influence of cultivation methods and conditions, seed density, *Sphagnum* species and vascular plant cover

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SUMMARY

Round-leaved sundew (*Drosera rotundifolia* L.) is a rare bog species that is commonly collected for the European herbal market in the wild, leading to the destruction of its natural populations. The aim of this study is to compare sundew cultivation methods on *Sphagnum* lawn that meet the requirements of the pharmaceutical industry and could promote a sustainable commercial cultivation. Seed germination and seedling survival of *D. rotundifolia* were studied in biodegradable cellulose pots, paper mesh bags or directly sown (cultivation methods) in a natural, a semi-natural Sphagnum farming and a greenhouse environment (cultivation conditions); using different seed densities. *Drosera* was cultivated on *Sphagnum palustre* or *S. papillosum* lawn and with or without co-occurring vascular plants. Best seed germination for all cultivation methods was recorded in the greenhouse, most successful were cellulose pots (≤ 26 %). Cellulose pots were also most successful under semi-natural (≤ 15 %) and natural (≤ 7 %) conditions. Lowest seed germination rates (< 1 %) were found for direct sowing under semi-natural and natural conditions, indicating that large-scale cultivation by direct sowing requires large quantities of seeds. High survival rates were observed for all cultivation methods in the second year of growth (mean 70 %). The removal of co-occurring vascular plants showed a positive correlation with the number of *Drosera* seedlings in the first year and led to a higher number of surviving *Drosera* plants in the second year. Cultivation of *D. rotundifolia* in biodegradable cellulose pots and direct seed sowing on *Sphagnum* lawns meet the cultivation requirements of the pharmaceutical industry and have many ecological benefits compared to collection in the wild.

KEY WORDS: Droserae herba, paludiculture, pharmaceutical requirements, Sphagnum farming, sundew

INTRODUCTION

The European *Drosera* species have a long history in their use for treating respiratory diseases (Šamaj *et al*. 1999, Babula *et al*. 2009), with the drug 'Droserae herba' being traditionally prepared from the dried above-ground parts of *D. rotundifolia* (Egan & van der Kooy 2013, Baranyai & Joosten 2016). Severe decline has resulted in all native European *Drosera* species currently being endangered. Consequently, mainly non-European *Drosera* species are now being used for pharmaceutical purposes, despite their substantially lower content of pharmacologically active compounds (Krenn *et al*. 1995, Baranyai & Joosten 2016).

The increasing difficulties in procuring high-quality *Drosera* drugs has in recent years led to various indoor and outdoor experiments to cultivate *Drosera* (Wawrosch *et al*. 1996, Galambosi *et al*. 1998, 2000; Šamaj *et al*. 1999, Galambosi 2002, Krenn *et al*. 2005, Bruzzese *et al*. 2010, Banasiuk *et al*. 2012, Galambosi & Galambosi 2013, Baranyai & Joosten 2016), but these trials have not yet resulted in widespread sundew cultivation. An important reason for this is the requirements set by the pharmaceutical industry (Table A1 in the Appendix). Other reasons include the high costs and time expenditure of sundew propagation and cultivation (Baranyai *et al*. 2016).

Over the last decade, the cultivation of *Sphagnum* has emerged as a climate- and environment-friendly land use alternative on rewetted, formerly drained and degraded bog land (Gaudig *et al*. 2014, 2018; Beyer & Höper 2015, Muster *et. al*. 2015, 2020; Gaudig & Krebs 2016, Günther *et al*. 2017, Vroom *et al*. 2020). *Sphagnum* cultivation ('Sphagnum farming', Gaudig *et al*. 2014, Krebs *et al*. 2014) has created large artificial habitats where *D. rotundifolia*

occurs spontaneously. Furthermore, pilot studies have shown that *D. rotundifolia* grown on living *Sphagnum* develops better than grown on sand (B. Baranyai, unpublished data) and contains concentrations of bioactive compounds that exceed the required pharmacological minimum of 0.14 % dry weight (Baranyai *et al*. 2016).

This article reports on seed germination and seedling survival rates of *D. rotundifolia* cultivated on living *Sphagnum* using different cultivation variables. We hypothesise that Sphagnum farming areas constitute a suitable habitat for *Drosera* cultivation.

METHODS

We cultivated *D. rotundifolia* under natural, semi-natural and greenhouse conditions from April 2014 to August 2015. Cultivation under greenhouse conditions was studied at the Botanical Garden of Greifswald University (NE Germany, 54º 09′ 38″ N, 13° 36′ 70″ E) using 35.5 × 22 × 6 cm containers. Each container was filled with a 1.5 cm thick bottom layer of medium-humified *Sphagnum* peat (von Post scale H5), followed by a 4.5 cm layer of *Sphagnum* biomass fragments from the Sphagnum farming site (Figure A1). Light levels were manually adjusted by lamps (Photosynthetic Photon Flux Density: in winter 79 ± 5 μmol m^{-2} s^{-1}, in summer 119 ± 8 μmol m^{-2} s^{-1}) to accommodate for the dormancy period of *D. rotundifolia*. Air temperatures were maintained constant at 24 ± 2.0 °C in summer and 15 ± 2.0 ºC in winter. Humidity was on average 87.2 ± 12.9 % and plants were well watered to simulate natural moist conditions.

Cultivation under semi-natural conditions was studied in a Sphagnum farming site on rewetted, former bog grassland near Rastede (NW Germany 53º 15′ 80″ N, 08º 16′ 05″ E, -0.5 m a.s.l, Gaudig *et al*. 2014, 2018, Temmink *et al*. 2017). Mean annual precipitation is 849 mm; mean annual temperature 9.8 ºC (Brust *et al.* 2018). The vegetation is dominated by *Sphagnum palustre* L., *S. papillosum* Lindb. and *S. fallax* H. Klinggr, with a total *Sphagnum* cover of 95 % (Wichmann *et al*. 2015). Co-occurring vascular plants include *Juncus effusus* L., *Drosera rotundifolia*, *D. intermedia*, *Juncus bulbosus* L., *Carex canescens* L., *Rhynchospora alba* (L.) Vahl, *Eriophorum angustifolium* Honck. and *Erica tetralix* L. (Gaudig & Krebs 2016). The Sphagnum farming site is equipped with an automatic irrigation system that keeps the water table constant around 5 cm below the *Sphagnum* surface during the whole year (Brust *et al.* 2018, Vroom *et al*. 2020). The vascular plants are regularly mown in the growing season (Wichmann *et al.* 2020).

Cultivation under natural conditions was studied in Fekete-tó (W Hungary, 46º 53′ 07″ N, 16º 53′ 07″ E, 275 m a.s.l.), a small transitional mire with a total size of 0.06 hectare (Kol 1967). Mean annual precipitation is 760–800 mm, mean annual temperature 9.1–9.8 ºC (Dövényi 2010). Two *Sphagnum* species dominate Fekete-tó: *Sphagnum palustre* growing mainly in higher (e.g. hummocks) and drier parts of the mire and *S. fallax* growing in lower and wetter parts. Accompanying plants are *Carex elata* All., *Eriophorum angustifolium* Honck, *Drosera rotundifolia*, *Calluna vulgaris* (L.) Hull and *Polytrichum formosum* (Hedw.) G.L.Sm. *Drosera rotundifolia* grows on *Sphagnum* hummocks with a low cover of vascular plants (10 %). The site is naturally subject to large water-level fluctuations during the year. In order to ensure comparable "*Sphagnum* lawn" conditions in the whole experiment the permanent quadrats in Fekete-tó were established only on *Sphagnum palustre*-dominated lawns (without hummocks) aside from the natural sundew populations (Figure A2).

Seed material of *D. rotundifolia* for the germination experiment was collected in September 2013 by hand from seedcases from 30 randomly selected mature plants *per* outdoor site (Table A2) and stored in a hermetically sealed flask at 4 ± 2 ºC. Seedcases were opened directly before the experiments began in April 2014. Seeds (mean length 1.78 ± 0.21 mm, mean width 0.20 ± 0.04 mm, n = 170) were cold stratified for 2 days at -18 ºC and counted at room temperature, mixed with dry ground *Sphagnum* biomass (1:10, Galambosi *et al*. 2000) and homogeneously sown on 10 × 10 cm plots (10, 50 or 100 seeds per plot) at all three sites.

Biodegradable cellulose Boller pots (Ø 4.5 cm, depth 5 cm; Romberg & Sohn GmbH, Germany) and biodegradable paper mesh bags (Ø 6 cm, depth 0.8 cm; with a 2 × 2 cm X-shaped incision in the centre of the adaxial surface; SENSEO® Pads, Douwe Egberts Retail Germany GmbH, Germany) were filled with 5–6 g of 100 % medium-humified *Sphagnum* peat. The seeds (3, 6 or 9 seeds per pot/bag) were placed homogeneously in the centre of the cellulose pots/paper mesh bags on the surface of the *Sphagnum* peat using tweezers.

In the outdoor experiments the pots and bags were carefully placed in the *Sphagnum* lawn to avoid disturbance of the lawn. In the greenhouse the pots and bags, and plots for direct seed sowing were placed in the containers: 6 pots or 6 bags per container with different numbers of seeds, and 3 plots per container with different numbers of seeds

(Figure A1). Pots, bags and plots were labelled with wooden sticks to allow easy retrieval.

Experiments were done on *Sphagnum* lawns dominated by *Sphagnum palustre* or *S. papillosum* (under greenhouse and semi-natural conditions) or *S. palustre* (under natural conditions).

Two permanent quadrats per *Sphagnum* species were established under natural and semi-natural conditions (one permanent quadrat: 1.7 × 1.7 m). In each quadrat pair one was left unmown while in the other the vascular plant material was cut monthly just above the *Sphagnum* lawn surface (Figure 1) using hedge shears and removed. In the greenhouse, vascular plants hardly occurred, but were anyhow removed.

As birds (e.g. carrion crow or Eurasian magpie) may destroy pots and paper mesh bags (B. Baranyai, unpublished data), the outdoor permanent quadrats were laid out with a wooden frame (1.7 × 1.5 × 1.7 m) and protected with a bird net (Figures A2 and A3).

Six replicates per treatment were installed randomly within the permanent quadrats at the natural and semi-natural sites and in the greenhouse (random distribution of all treatments within one container replicated for six containers) (Table 1).

Spontaneously established *D. rotundifolia* plants were removed in all permanent quadrats and containers. The replicates covered 7,200 cm^2 (direct seed sowing), 1,458 cm^2 (cellulose pots) and 2,035 cm^2 (paper mesh bags) in the semi-natural setting, and 3,600 cm^2, 729 cm^2 and 1,017 cm^2, respectively, in the greenhouse and natural settings. All results are expressed in m^2. Average plant density was calculated as the number of surviving plants (Table 2) per total area of replicates (pots, bags, and plots, see above) for all cultivation methods and conditions.

The experiment started in April 2014. The number of seedlings was monitored monthly from May to September 2014 and seedlings/plants survival from April to August 2015. The germination rate is the number of seedlings as a percentage of the number of deployed *D. rotundifolia* seeds. The survival rate is the percentage of seedlings as of September 2014 that were again recorded in August 2015.

Data exploration was carried out before the actual data analysis (after Zuur *et al*. 2009). Group differences for germination and survival were analysed with the non-parametric Kruskal Wallis test and a multiple comparison test after Siegel & Castellan (1988), and the R package pgirmess after Giraudoux (2010).

Considering the high number of seeds that did not germinate, the effects of treatments were analysed using a generalised linear model with a Poisson distribution. We analysed the response variable 'germination rate' (percentage of deployed seeds that germinated) with the following explanatory variables: cultivation conditions (greenhouse, natural or semi-natural), cultivation methods (cellulose pots, paper mesh bags or direct sowing), number of seeds (3, 6 or 9 seeds; 10, 50 or 100 seeds), *Sphagnum* species (*S. papillosum* or *S. palustre*), and co-occurring vascular plants (removed or not removed).

We applied the Spearman's rank correlation test to analyse the relation of survival rate and number of germinated plantlets for each of the three cultivation methods.

Statistical data exploration, computation and figure design were done with the software R (v3.1.3, R Development Core Team 2009) and the packages AED (Zuur *et al*. 2009), pgirmess (Giraudoux 2010) and stats (R Development Core Team 2009).

Figure 1. Permanent quadrats on the Sphagnum farming site in Rastede, autumn 2014: with vascular plants (left) and without vascular plants (right). Photos: Balázs Baranyai.

Table 1. Structure of the experiment. × performed treatment combinations, - not performed; vascular plants no = removed, vascular plants yes = not removed. In the greenhouse conditions containers were used (35.5 × 22 × 5 cm) and in semi-natural and natural conditions, permanent quadrats (1.7 × 1.7 m).

Cultivation condition				greenhouse				semi-natural				natural			
Sphagnum species				*S. palustre*		*S. papillosum*		*S. palustre*		*S. papillosum*		*S. palustre*		*S. papillosum*	
Vascular plants				no	yes	no	yes	no	yes	no	yes	no	yes	no	yes
Cultivation method	Description	Replicates	Seed number												
seed sowing	plots 10 × 10 cm	6	10	×	-	×	-	×	×	×	×	×	×	-	-
		6	50	×	-	×	-	×	×	×	×	×	×	-	-
		6	100	×	-	×	-	×	×	×	×	×	×	-	-
cellulose pots	Ø 4.5 cm, depth 5 cm	6	3	×	-	×	-	×	×	×	×	×	×	-	-
		6	6	×	-	×	-	×	×	×	×	×	×	-	-
		6	9	×	-	×	-	×	×	×	×	×	×	-	-
paper mesh bags	Ø 6 cm, depth 0.8 cm	6	3	×	-	×	-	×	×	×	×	×	×	-	-
		6	6	×	-	×	-	×	×	×	×	×	×	-	-
		6	9	×	-	×	-	×	×	×	×	×	×	-	-

Table 2. Total numbers of deployed and germinated *Drosera rotundifolia* seeds, germinated seeds and germination rate in the same year, and surviving plants and survival rates in the following year for the various cultivation conditions and methods.

Cultivation condition	Cultivation method	Deployed seeds	Germinated seeds (germination rate)	Surviving plants (survival rate)
greenhouse	cellulose pot	216	55 (26 %)	50 (91 %)
	paper mesh bag	216	43 (20 %)	30 (70 %)
	direct seed sowing	1920	188 (10 %)	135 (72 %)
semi-natural	cellulose pot	432	64 (15 %)	32 (50 %)
	paper mesh bag	432	37 (9 %)	18 (49 %)
	direct seed sowing	3840	29 (0.8 %)	27 (93 %)
natural	cellulose pot	216	15 (7 %)	11 (73 %)
	paper mesh bag	216	7 (3 %)	4 (57 %)
	direct seed sowing	1920	3 (0.2 %)	2 (67 %)
Total	All	9408	441 (5 %)	309 (70 %)

RESULTS

Germination

In total, only a small fraction of seeds (4.7 %) germinated, i.e. 12.2 % under greenhouse, 2.8 % under semi-natural and 1.1 % under natural conditions (Table 2). Mean germination rates with cellulose pots were higher than with paper mesh bags and direct seed sowing (Figure 2).

Direct seed sowing showed a substantially lower germination rate under semi-natural and natural conditions compared to greenhouse conditions (Figure 2). The differences between all cultivation conditions and methods are statistically significant (Table A3).

For cellulose pots and paper mesh bags, generally higher germination rates were observed with 3 and 6 seeds than with 9 seeds (Figure 3), but differences were not statistically significant (n = 287, Kruskal-Wallis, χ^2 = 2.93, d.f. = 2, P = 0.23). For direct seed sowing under natural conditions, no seeds were found to germinate in the 10 and 50 seeds variants, whereas for 100 seeds a very small germination rate of 0.3 ± 0.2 % was recorded (Figure 3).

Under greenhouse and semi-natural conditions, proportionally more seedlings were observed for direct seed sowing with 10 seeds, but these differences were not significant in comparison to 50 or 100 seed variants (Figure 3). The germination rate of *D. rotundifolia* did not significantly differ between *Sphagnum palustre* and *S. papillosum* lawn for the various cultivation conditions and methods (Table A3).

Under natural and semi-natural conditions, significantly larger germination rates were found when co-occurring vascular plants had been removed, with 2.5 times higher rates in cellulose pots (Tables A3 and A4). In contrast, three times more seeds germinated in paper mesh bags when vascular plants were present (except for one treatment under semi-natural condition on *S. papillosum*) (Table A4).

Survival

The survival rates for *D. rotundifolia* seedlings after one year were highest under semi-natural conditions with direct sowing (93 %), in the greenhouse with cellulose pots (91 %), and under natural conditions quite similar between direct sowing (67 %) and cellulose pots (73 %) (Table 2). No relation was found between *D. rotundifolia* survival rate and seed number (3, 6 and 9, Kruskal-Wallis test χ^2 = 0.58, d.f. = 2, P = 0.75 and 10, 50, 100 seeds, Kruskal-Wallis test χ^2 = 1.84, d.f. = 2, P = 0.39). Likewise, no relation was found between the survival rate and the number of germinated plantlets (Spearman rank correlation: cellulose pots P = 0.37, paper mesh bags P = 0.84 and direct seed sowing P = 0.39). Removal of vascular plants increased seedling survival significantly, e.g. in semi-natural conditions (Kruskal-Wallis test χ^2 = 7.33, d.f. = 1, P = 0.01).

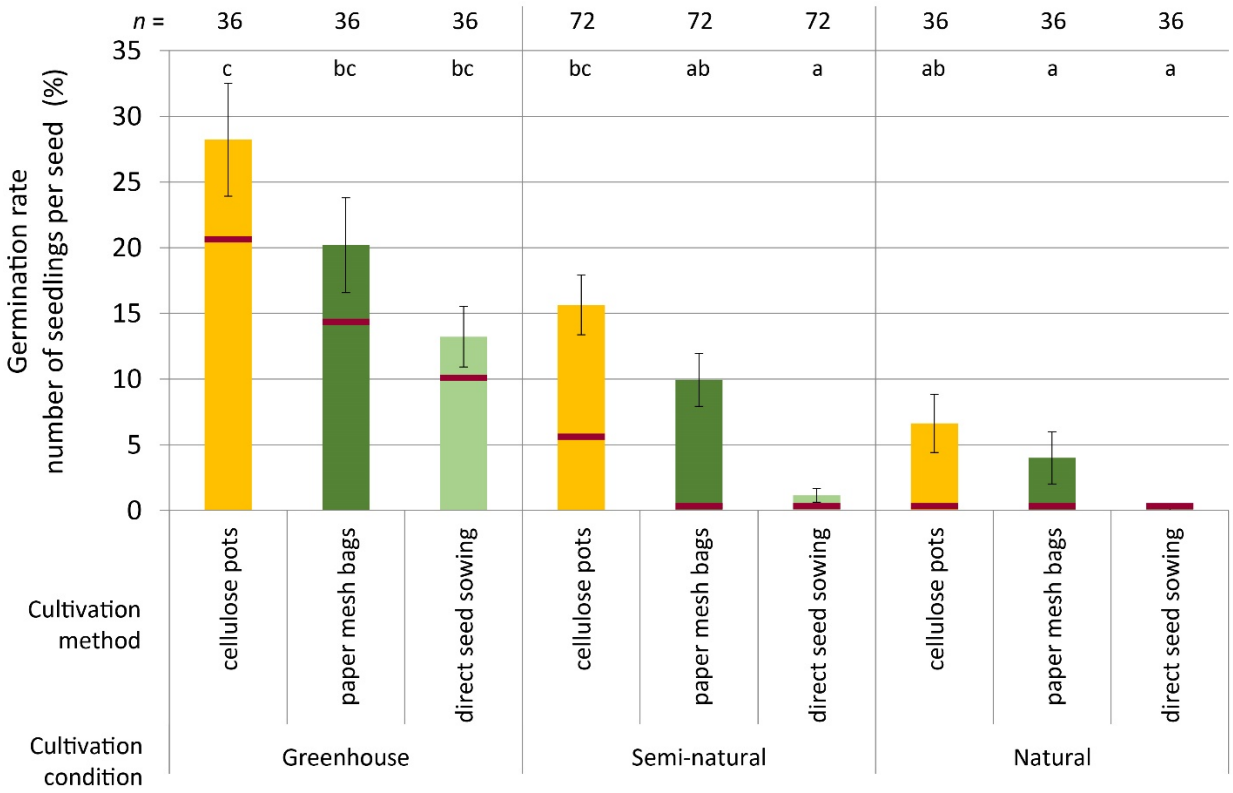

Figure 2. Germination rate of *Drosera rotundifolia* for the different cultivation conditions and methods. Vertical bars show the means, the whiskers the SE, the red lines the medians. Number of replicates is written above each bar. Values with different letters differ significantly ($P \leq 0.05$).

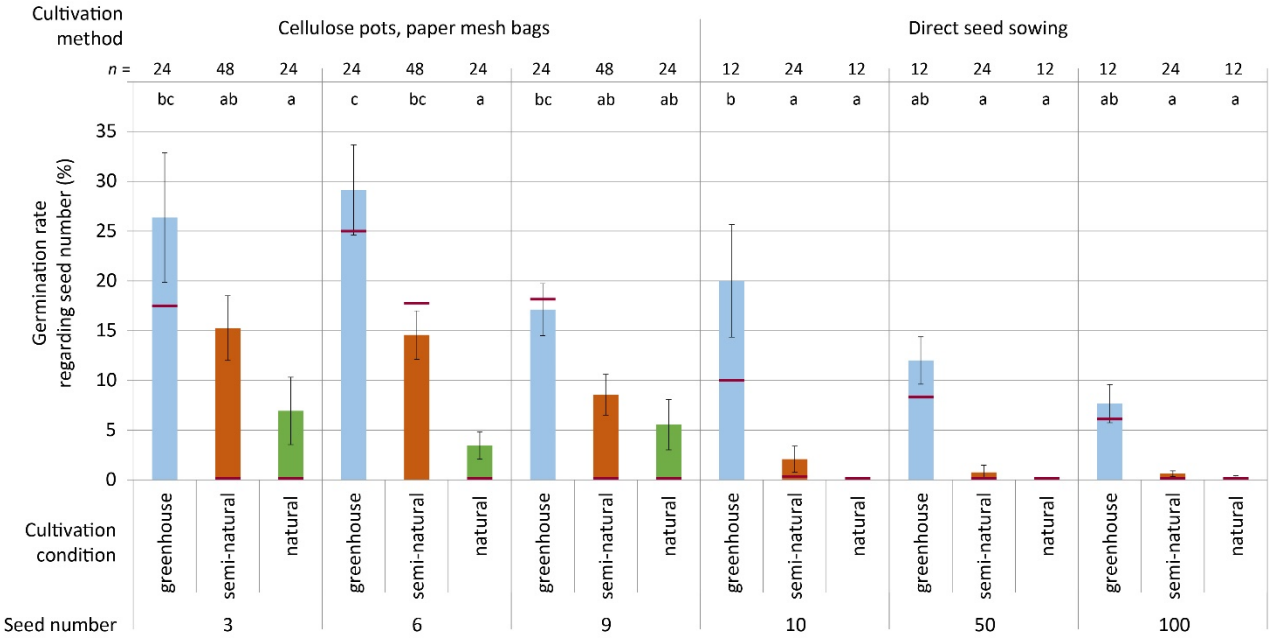

Figure 3. Mean germination rate of *Drosera rotundifolia* for the different treatments, i.e. cultivation conditions and methods, and seed number. Vertical bars show the means, the whiskers the SE, the red lines the medians. Number of replicates is written above each bar. Values with different letters differ significantly ($P \leq 0.05$).

Plant densities

Average plant density calculated from the surviving plants (see Methods) was 295–686 plants *per* m^2 in the greenhouse, 38–219 plants *per* m^2 under semi-natural conditions, and 6–151 plants *per* m^2 under natural conditions. Under all cultivation conditions, the highest values were achieved with cellulose pots. In the greenhouse, average plant density on paper mesh bags (295 plants *per* m^2) was even lower than with direct seed sowing (375 plants *per* m^2). The lowest values with direct seed sowing were reported for semi-natural (38 plants *per* m^2) and natural conditions (6 plants *per* m^2).

DISCUSSION

Cultivation methods

The germination rates of *D. rotundifolia* in this study were much lower (< 26 %) than observed rates of cultivation on peat (30–95 %, Crowder *et al*. 1990). Reference data for germination rates on *Sphagnum* lawn are not available. The highest germination rates were obtained with cellulose pots, which were completely overgrown by *Sphagnum* within two months, whereas with bags this took at least one month longer. The fast overgrowth resulted in a micro-hollow around the small and deep cellulose pots (Ø 4.5 cm, depth 5 cm), which provided the seeds with better germination conditions, in the form of indirect sunlight and a warm-humid microclimate (Crowder *et al*. 1990, Baranyai & Joosten 2016).

In contrast, the paper mesh bags (Ø 6 cm, depth 0.8 cm) were not suitable. Their texture was too thin, so that a strong dehydration of the peat filling was observed in the greenhouse and outdoor. Outdoor most of the bags were easily lifted out of the *Sphagnum* lawn by upward-growing vascular plants (such as *Juncus* sp. and *Carex* sp.). In the greenhouse we even observed that the bags were lifted by upward-growing *Sphagnum*. This altogether hampered *Drosera* seed germination and lead to lower survival rates of seedlings/plants in paper mesh bags due to drought by intense sunlight exposure. Redbo-Torstensson (1994) explains the high mortality rates of *D. rotundifolia* seedlings and plants by their shallow root system, which is why drought events lead to immediate plant death.

Generally, germination rates with direct sowing were very low in contrast to cellulose pots and paper mesh bags. The reason for this is that *D. rotundifolia* seeds are very light, almost dust-like, so that they were probably washed by rain (outdoor) or artificial irrigation (greenhouse) into deeper layers of the *Sphagnum* lawn, where light intensity is insufficient for germination. This translocation of *Drosera* seeds into deeper layers of *Sphagnum* is also described by Redbo-Torstensson (1994) and Baranyai & Joosten (2016).

In general, survival rates of seedlings after one year were high (50–93 %) for all cultivation methods and conditions. The *Sphagnum* mosses ensured an optimal physical support structure for the seedlings, also in cellulose pots/paper mesh bags, which were overgrown by *Sphagnum* moss.

Cultivation conditions

The highest germination rates were recorded in the greenhouse, probably because of optimal temperature, irrigation, humidity and aeration and the absence of vascular plants. This may also explain why germination for direct seed sowing was much better in the greenhouse (10 %) compared to natural and semi-natural conditions (< 1 %). However, cultivation in a greenhouse does not comply with the pharmaceutical industry's cultivation regulations, which prohibit the artificial feeding necessary because of limited supply of prey in the greenhouse. The highest plant density under semi-natural conditions was achieved with cellulose pots (219 plants *per* m^2). Producing 1 kg fresh *D. rotundifolia* biomass (2,000–5,000 flowering individuals, Galambosi 2018) with cellulose pots in Sphagnum farming fields would require 5,000–12,500 cellulose pots in an area of 83-208 m^2, if all cups were placed 9 cm apart.

Low germination rates (< 1 %) were found under semi-natural and natural conditions with direct sowing, which could give the impression that *D. rotundifolia* hardly produces offspring on *Sphagnum* lawn. However, taking into account that one plant produces on average 716 seeds, the ~7 plants, which had delivered the 5333 seeds sowed *per* m^2, produced 8–40 seedlings in the first year, of which 6–38 plants survived in the second year (see Table 2). Apparently *D. rotundifolia* has adapted to low germination rates by producing sufficient seeds to secure survival on *Sphagnum*-dominated sites.

We found no significant differences in *D. rotundifolia* germination rates between *Sphagnum palustre* and *S. papillosum*. Cellulose pots and paper mesh bags work similarly well in lawns of both species. We observed that direct sowing under semi-natural conditions lead to higher germination rates in *S. palustre* compared to *S. papillosum*. This may relate to morphological differences between the species (Crum 1984) as the spreading branch leaves of *S. palustre* (Figure 4) provide a better footing for the small seeds of *D. rotundifolia* than the somewhat more imbricate leaves of *S. papillosum*.

Figure 4. *Drosera rotundifolia* seeds fixed between the leaves of *Sphagnum palustre* (left); germinating *D. rotundifolia* seed (right). Photos: Balázs Baranyai.

Whether *D. rotundifolia* seeds thus remain better in the upper parts of the *S. palustre* lawn, where better exposure to sunlight allows better germination, needs to be addressed in further research.

Regular removal of vascular plants in the first year generally led to higher germination rates and substantially increased survival rates in the second growing year. These findings are not surprising: *D. rotundifolia* is a small, shade-intolerant species (Stewart & Nilsen 1992), which needs direct light for seed germination (Schmid 1912), whereas faster growing species (e.g. graminoid species) restrict light availability (Stewart & Nilsen 1992). The constant removal of vascular plants in our study created optimal site conditions for *D. rotundifolia* growth – an open, bright and competition-free *Sphagnum* lawn. Such lawn also facilitates insect capture (nutrient uptake) and therewith plant growth (Schmid 1912, Crowder *et al*. 1990, Schulze & Schulze 1990) especially for older plants that reach the *Sphagnum* lawn surface in the second year.

CONCLUSION

This is the first study in which germination and survival rates of *Drosera rotundifolia* on *Sphagnum* moss as a growing substrate were studied from the perspective of producing sundew for industrial pharmacological applications.

Our cultivation experiment shows that *D. rotundifolia* can be successfully cultivated indoors and outdoors on living *Sphagnum.* The best method was using biodegradable cellulose pots with few seeds. However, direct seed sowing on *Sphagnum* farming sites may more practical, as it is easy to perform and less labour-intensive, although it requires a large amount of seeds. Whether the procurement of seed material will outweigh the effort of cultivating in cellulose pots can only be answered by further research.

Compared to greenhouse and natural conditions, the cultivation of *D. rotundifolia* on Sphagnum farming sites offers the best possibility for producing

sundew raw material. Greenhouse cultivation cannot meet the current requirements of the pharmaceutical industry (no artificial feeding), is labour-intensive, and lacks the ecosystem services that *D. rotundifolia* cultivation on Sphagnum farming sites may provide (e.g. peat preservation, water purification and retention, reduction of greenhouse gas emissions, conservation of bog species). Cultivation under natural conditions conflicts with the protection of natural mires. Furthermore, cultivation on Sphagnum farming sites is more advantageous than collection from the wild, because of higher plant density and the possibility to select cultivation areas without nature conservation restrictions, e.g. former peat extraction sites or drained peat meadows. Results of this study implicate that further research is necessary to increase germination and survival rates of *D. rotundifolia*, as well as optimal plant growth, on *Sphagnum* lawn as a cultivation substrate. Cultivation on Sphagnum farming sites provides new opportunities for the industrial production of sundew raw material and offers synergies with climate, peatland and biodiversity protection initiatives.

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AUTHOR CONTRIBUTIONS

BB performed the germination trials and the data recording. Data management and statistical analysis were carried out jointly by BB and MK. The manuscript was written by BB with considerable contributions from MK, CO and HJ.

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Appendix

Table A1. Requirements of the pharmaceutical industry for cultivated or wild (collected in nature) *Drosera* raw material in central Europe.

Requirements (cultivation)	Source
No genetically identical (cloned), unnatural and artificially fed *Drosera* plants	Baranyai & Joosten 2016
Drosera raw material cultivated on Sphagnum farming is accepted.	B. Baranyai, unpublished data
The plants should be bred from seeds (generative) on peat substrate	U. Westphal, personal communication 2013
No use of fertiliser nor any other chemical treatment	Länger & Schiller 2004
Full transparency and continuous documentation of the cultivation process	Franke 1999
Requirements (plant material)	
For fresh biomass: *Drosera* plants must be freshly collected and transported (≤ 24 h), whole (incl. roots) and very well cleaned; high preferred is the *Drosera* raw material from sustainable sources	B. Baranyai, unpublished data
For homeopathic remedy: *Drosera rotundifolia* L., *D. intermedia* Hayne and *D. anglica* Huds. plants should be collected in flower or at the beginning of flowering, alone or in a mixture	Blaschek 1998, HAB 2014
A minimum content of bioactive compounds (e.g. naphthoquinone: 0.14–0.22 %)	Wichtl 2009
High purity of the biomass (≤ 3 % plant material other than *Drosera*, and among *Drosera* ≤ 10 % plant material from *D. anglica* and/or *D. intermedia*)	ÖAB 9 1960, EB 6 1989
About 80–90 % of the picked *Drosera* plants should be in flower.	Schweitzer 1937

Figure A1. Installation of the greenhouse experiment in the Botanical Garden of Greifswald University in 2014. Photo: Balázs Baranyai.

Figure A2. Permanent quadrats in "Fekete-tó" in 2014: installation of the experiment (left) and installed permanent quadrat before fixing the bird net (right). Photos: Balázs Baranyai.

Table A2. Collected seeds of *Drosera rotundifolia* for the experiment in semi-natural (Sphagnum farm) and natural (Fekete-tó) cultivation conditions in September 2013 – number of collected plants with its total number of seedcases, seeds and the average number of seedcase per plant and average number of seeds per seedcase.

Cultivation condition	Collected plants	Total number of seedcases	Number of seeds per plant (N ± SD)	Seedcases per plant (N ± SD)	Seeds per seedcase (N ± SD)
semi-natural	30	295	730 ± 313	11 ± 3	70 ± 31
natural	30	282	678 ± 260	9 ± 2	74 ± 27

Figure A3. Permanent quadrats on the Sphagnum farming site in Rastede 2014: at the back with vascular plants and in the front without (left) and a permanent quadrat without vascular plants (right). Photos: Balázs Baranyai.

Table A3. Results of the generalised linear modelling of the germination rate (percentage of deployed seeds germinated) for the treatments: cultivation conditions, cultivation method (direct seed sowing = DSS, cellulose pot = CP, paper mesh bag = PMB), number of seeds, *Sphagnum* species, vascular plants. The seed numbers differed between direct seed sowing (DSS, seed numbers: 10, 50 and 100) and the cellulose pot (CP) and paper mesh bag (PMB, with seed numbers: 3, 6 and 9). Seed numbers were classified as low (3 for CP and PMB and 10 for DSS); medium (6 for CP and PMB and 50 for DSS) and high (9 for CP and PMB and 100 for DSS). Presence/absence of vascular plants was only tested for natural and semi-natural conditions, as in the greenhouse all vascular plants had been removed. SE: standard error; *Z*: standard scores; P: level of significance. Null deviance: 10,783 on 431 degrees of freedom, Residual deviance: 7,730 on 423 degrees of freedom.

Treatment		Factor	Estimate of the slope	SE	Z	P
cultivation condition	greenhouse	natural	-1.57	0.055	-26.36	≤0.001
		semi-natural	-0.65	0.036	-18.41	≤0.001
cultivation method	cellulose pot	paper mesh bag	-0.40	0.032	-12.40	≤0.001
		direct seed sowing	-1.44	0.047	-30.73	≤0.001
seed number	low seed number	medium seed number	-0.09	0.034	-2.87	≤0.01
		high seed number	-0.54	0.039	-14.04	≤0.001
Sphagnum species	*S. palustre*	*S. papillosum*	-0.017	0.031	-0.56	0.58
vascular plants	present	removed	0.40	0.042	9.53	≤0.001

Table A4. Number of germinated seeds (GS) in September 2014 and number of surviving plants (SP) in August 2015 for the different cultivation conditions and methods. Total number of deployed *D. rotundifolia* seed per treatment = 108 (pots and bags) and 960 (seed sowing); vascular plants no = removed, vascular plants yes = not removed. In brackets: GR = germination rate; SR = survival rate.

	Cultivation method		cellulose pot		paper mesh bag		direct seed sowing	
cultivation condition	*Sphagnum* species	vascular plants	GS (GR)	SP (SR)	GS (GR)	SP (SR)	GS (GR)	SP (SR)
greenhouse	*S.palustre*	no	29 (27 %)	28 (96 %)	26 (24 %)	19 (73 %)	130 (14 %)	93 (72 %)
	S.papillosum	no	26 (24 %)	22 (85 %)	17 (16 %)	11 (64 %)	58 (6 %)	42 (72 %)
semi-natural	*S.palustre*	no	18 (17 %)	9 (50 %)	6 (6 %)	5 (83 %)	23 (2 %)	22 (96 %)
		yes	5 (5 %)	4 (80 %)	11 (10 %)	3 (27 %)	0 (0 %)	0 (0 %)
	S.papillosum	no	19 (18 %)	14 (74 %)	15 (14 %)	8 (53 %)	5 (0.5 %)	4 (80 %)
		yes	22 (20 %)	5 (23 %)	5 (5 %)	2 (40 %)	1 (0.1 %)	1 (100 %)
natural	*S.palustre*	no	14 (13 %)	10 (71 %)	3 (3 %)	2 (66 %)	2 (0.2 %)	2 (100 %)
		yes	1 (1 %)	1 (100 %)	4 (4 %)	2 (50 %)	1 (0.1 %)	0 (0 %)