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*drology of Bog Ecosystems – Guidelines*

# 28 *Sphagnum* Regeneration – Toward an Optimisation of Bog Restoration

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## SUMMARY

**1.** Four common *Sphagnum* species (*S. magellanicum, S. nemoreum, S. angustifolium* and *S. papillosum*) found in Québec (eastern Canada) showed a very good ability to regenerate vegetatively from fragments. In extreme cases, even a stem section of only 1–2 mm long can regenerate.
**2.** A high water level close to the peat surface and mineral amendments (slow-release 12–12–12 NPK fertiliser and bone meal) enhanced *Sphagnum* establishment.
**3.** The addition of a shade cover did not prove to be an important factor in the spreading of the *Sphagnum* mosses.

## INTRODUCTION

With 170 million ha, Canada has more peatlands than any other country (Gorham, 1991). However, most of Canada's peatlands are fens or northern bogs affected by permafrost and are not usable for horticultural peat harvest. Hence, the southern ombrotrophic peatlands are under greater pressure of exploitation as only these bogs contain peat suitable for horticultural peat harvesting. At present the peat harvesting industry in Canada has reached a point in its development where certain bogs that have been harvested for the past decades are now largely depleted of their horticultural grade peat. When the harvesting operation ceases, the bog is abandoned, and, unfortunately, does not usually return to a natural cycle of bog formation. This situation is evident in New Brunswick, Québec, Manitoba, and Alberta, where such sites need to be restored to functional peatlands. However, there is little information available on North American peatland restoration. A long term goal is to implement a policy of no net loss of functions and values of wetlands in Canada (Lynch-Stewart, 1992).

Since the late 1960s, peat has been harvested by vacuum in Canada (Keys,

*Restoration of Temperate Wetlands*. Edited by B.D. Wheeler, S.C. Shaw, W.J. Fojt and R.A. Robertson

1992). Compared with the previously-used block-cutting method, the vacuum method leaves a flat, bare peat surface devoid of any *Sphagnum* diaspores. The recolonisation process of such peatlands is very slow compared with recolonisation of sites harvested with block-cutting methods (personal observations of 30 abandoned peatlands in eastern Canada). To facilitate the restoration of these bare, uniform substrata, different experiments were undertaken to investigate the regenerating ability of some common *Sphagnum* species found in North America. Our goal was to determine: (i) which part of *Sphagnum* can regenerate and how small a *Sphagnum* diaspore can be; (ii) the effect of the substratum humidity on the regeneration of *Sphagnum*; (iii) if the addition of minerals enhances the regeneration of *Sphagnum* mosses; and (iv) if the sole introduction of sparse *Sphagnum* diaspores in the field would initiate the recolonisation process. All these experiments were aimed at one goal: to minimise the amount of natural vegetation needed for peatland restoration. It would not be wise to destroy an equivalent surface of a natural peatland in order to restore an abandoned peat field. Thus, if we can reduce the size and density of diaspores needed to re-establish vegetation on a bare peat surface, the better it will be for the environment.

## MATERIAL AND METHODS

### 1. *Sphagnum* vegetative diaspore experiment

Two sets of experiments were performed. One set dealt with three *Sphagnum* species, namely *S. magellanicum* Brid., *S. rubellum* Wils. and *S. angustifolium* (C. Jens. ex Russ.) C. Jens. The living plants were collected in September 1992 at the Ste-Marguerite peatland (Mistassini, Qc.). Sectioning of living parts only was performed in sterile conditions using a laminar air-flow cabinet. The living plant organs were sterilised for 30 seconds with sodium hypochlorite 1.5% solution. Cultivation was done at 22 °C in closed cabinet, on sterile 7% agar in Petri dishes with (Rudolph & Voigt, 1986) medium added with 0.1% sucrose solution. Acidity was adjusted to pH 5.8. Light intensity was similar to daylight but continuous.

The second set of experiments dealt only with *Sphagnum papillosum* Lindb. The plants were collected in May 1993 at St-Etienne-de-Lauzon peatland (Lévis County, Qc.). Living plant parts were isolated under non-sterile conditions. Cultivation occurred at room temperature in non-sterile Petri dishes on Whatman No. 1 filter paper wetted with 3 ml of (Rudolph & Voigt, 1986) solution. Acidity was adjusted to pH 4.5 using HCl 1N. Enough bi-distillated water was added when the plants began to dry, to keep the filter paper wet. Cultures were kept at room temperature and were exposed to daylight, which averaged 2000 to 3000 lux.

### 2. Water levels experiment

This experiment was carried out in a glasshouse on vacuum-harvested peat in small containers (25 x 30 x 10 cm, split into two compartments). The living *Sphagnum* plant material was collected at the Ste-Marguerite peatland (Mistassini,

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Qc) in autumn 1992. Four species were investigated: *Sphagnum magellanicum* (section *Palustria*), *S. angustifolium* (section *Cuspidata*), and *S. fuscum* (Schimp.) Klinggr. and *S. nemoreum* Scop. (both of section *Acutifolia*). The Sphagna were dissected into four parts to test their powers of regeneration: capitulum only; 1 cm of stem below the capitulum; capitulum + stem (= 2.5 cm fragment), and branches only. A fixed number of pieces was evenly distributed on the peat surface: approximately 150 small spreading branches and 25 pieces for all the other fragments. The temperature was maintained around 20 °C with a 16 h photoperiod. The plants were left to grow for 3 months (December to March). Two different water levels, 8 cm and 1–2 cm below the peat surface, were maintained by differentially spraying with distilled water every two days. Water level was controlled with the aid of a small perforated PVC pipe inserted in every container. After 3 months of growth, the Sphagna cover of the bare surface was estimated and the number of capitula were counted. To be counted, a new capitulum needed to have at least 4–5 branches arranged in a rosette-like fashion. Two-way ANOVAs were computed for each species studied after verifying assumptions for normality and homogeneity of variances.

### 3. Mineral additions experiment

This experiment was designed to test the possibility of recolonisation by *Sphagnum nemoreum* on a peat substratum from a peatland abandoned in 1974 and which had not yet revegetated naturally. Nutrients were added to see if this would initiate colonisation. Six 60 litre containers (36.5 x 50 x 31 cm) were filled with a block of peat cut from the abandoned peatland. Three fertiliser treatments were applied: (i) control; (ii) addition of a slow release fertiliser (12–12–12 NPK – Grace Sierra), 10 g per container, limed with calcium carbonate and magnesium carbonate (9.25 g of each per container) in order to get a pH of 4.5; (iii) addition of bone meal (2–11–0 NPK; total $P_2O_5$: 15%, organic matter: 15%; So-Green Corp., Ont.), 8.55 g per container, and limed as above. The fertilisers and lime were incorporated only into the first 3 cm of the peat. Two methods of introducing the *Sphagnum* moss were tested. On one half of the surface, two cavities 3 cm deep were dug and a lump of *Sphagnum* at natural density was inserted into each one. This method was designed to simulate how *Sphagnum* would colonise a bare peat surface if bands of vegetation were left as source of diaspores during exploitation or if, for example, introducing 1 $m^3$ peatland vegetation plugs were introduced in the field for restoration. On the second half, pieces of mosses (0.5 to 1 cm long) were sprinkled on the surface. This method was designed to simulate the spreading of surface shredded peatland vegetation for large-scale bog restoration. The blocks were watered from the top with demineralised water and the water level was maintained at 10 cm below the surface. The containers were kept in a greenhouse, under natural light, at a temperature of 18–20 °C. The latter experiment lasted from November 1992 to May 1993. Observations of the development of the mosses were noted every two weeks, or each month towards

the end of the experiment. Diameter increment of the lumps of *Sphagnum* were measured once a month.

### 4. Field reintroduction experiment

With this experiment we tested the ability of *S. magellanicum* and *Polytrichum strictum* Brid. to regenerate from fragments on a peatland abandoned for one year. The introduction plots were compared with similar plots where no treatment was applied to the bare peat surface (control) or where 250 ml of granular horticultural lime had been sprinkled on 1 $m^2$. The study was conducted at the Ste-Marguerite peatland (Mistassini, Qc) during the field seasons 1992 and 1993. Ste-Marguerite peatland is an ombrotrophic bog under the influence of a humid continental climate. Mean July and January temperature are 17.2 °C and –16.8 °C, respectively. Temperatures are below freezing point for at least five months of the year and the frost-free season is around 103 days (from 31 May to 12 September). Mean annual precipitation is 82 cm, of which 75% falls in the form of rain. Snow melts between March and April. During the growing season, the water level of the exploited and abandoned peatland was generally 5 cm lower than the level naturally occurring in the undisturbed part of the peatland (Figure 1).

Twenty-seven blocks of 4 x 1 $m^2$ quadrats were established on three abandoned harvesting bays on which the drainage ditches had been dammed in early spring 1992. *S. magellanicum* and *P. strictum* fragments (0.5–2 cm long) were shredded using a domestic blender. Approximately 750 ml of moss material was spread on each quadrat plot. The plant material was introduced during the beginning of July 1992. The number of individual capitula was recorded on 6 September 1992 and 8 October 1993.

Data were analysed by ANOVA in a randomised complete block design. The data for each year were analysed separately. In 1992, the numbers of capitula $m^{-2}$

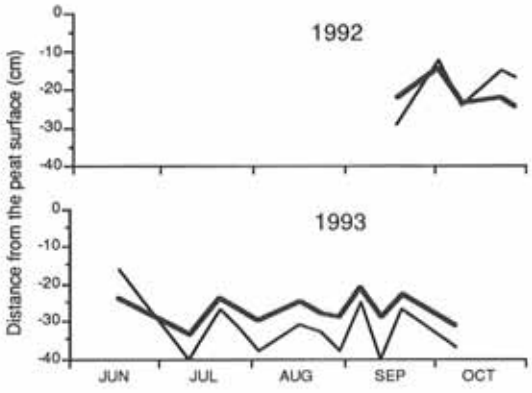


**Figure 1.** Fluctuation of the water table in the natural (thick line) and abandoned (thin line) part of the experimental peatland during 1992 and 1993.

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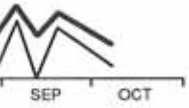


(thick line) and abandoned (thin line)
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for each of the four treatments were compared, based on data collected from the 27 blocks. In 1993, only 18 blocks were available to compare the effect of treatments in their second year; the other nine blocks were covered with a 40% shading cloth (and the lime and *Polytrichum* treatments received extra addition of *Sphagnum* fragments in spring 1993). Data were transformed ($\sqrt{(x+0.5)}$) prior to analysis, to reduce heterogeneity of variances. The analyses of variances were done using the GLM procedure of SAS (SAS, 1988). When the ANOVA showed a significant treatment effect at the 0.05 level, the means of treatments were compared using a Tukey test. For the *Sphagnum* treatments in 1993, we compared the number of capitula between the nine plots that were covered with a shading cloth with the 18 that were left uncovered using a T-test. Again the data were square-root transformed prior to analysis.

## RESULTS

### 1. *Sphagnum* vegetative diaspore experiment

Preliminary results (Table 1) show that despite a severe contamination of cultures, most *Sphagnum* organs, whether entire or in parts, possess the ability to regenerate when cultivated under sterile conditions with nutrients provided. Best results were obtained with *S. rubellum* apical buds and with *S. angustifolium* entire spreading branches. In general, both of these species show a better ability to regenerate than *S. magellanicum* despite a lower contamination rate with this species.

**Table 1.** Preliminary results of experiments with living *Sphagnum* vegetative parts in sterile culture on agar with Rudolph & Voight (1986) medium.

| Plant part | *S. magellanicum* | *S. rubellum* | *S. angustifolium* |
|---|---|---|---|
| Apical bud with leaf and branch primordia | 0/6* 0/6 0/6 1/1 | 4/5 4/4 4/5 4/4 | |
| Thin section of stem just below capitulum | 0/9 0/9 1/9 | | |
| Stem section between capitulum and first branch fascicle | 1/6 0/6 | | |
| Spreading branch with leaves | 0/3 0/3 0/4 | 0/6 1/5 1/5 | 3/6 4/6 1/6 |
| Spreading branch without leaves | 0/6 | 1/6 0/6 0/6 | 0/6 0/6 0/6 |
| Spreading branch leaf | 0/9 0/12 0/8 1/9 | 0/12 0/11 1/9 | 0/12 0/12 0/10 |
| Pendent branch with leaves | 0/6 0/6 0/6 | | |
| Pendent branch without leaves | 0/9 0/8 0/8 0/9 | | |
| Stem section between branch fascicles | 0/6 0/6 0/6 | | |

* Results are presented for each Petri dish as a replicate of each experiment within each species as $n_1/n_2$ = number of plant parts regenerating/total number of plant parts in the Petri dish. Variations with $n_2$ and number of replicates for each species are due to removal of plant parts contaminated with micro-organisms.

**Table 2.** Production of regeneration sites by some living *Sphagnum papillosum* detached organs as a function of time in non-sterile culture on filter papers wetted with Rudolph & Voight (1986) medium.

| Date of observation | | June | | | July | |
|---|---|---|---|---|---|---|
| | 6 | 16 | 22 | 28 | 7 | 26 |
| Plant parts | No. in culture | No. of regeneration sites | | | | |
| Capitulum branch | | | | | | |
| a) long | 43 | 53 | 77 | 77 | 77 | 77 |
| b) medium size | 31 | 13 | 18 | 26 | 27 | 32 |
| c) short | 43 | 0 | 1 | 3 | 5 | 7 |
| Apical bud | 3 | 0 | 0 | 0 | 0 | 0 |
| Stem section between capitulum and first branch fascicle | 3 | 0 | 0 | 0 | 0 | 0 |
| Stem section with a fascicle of branches | 20 | 21 | 23 | 34 | 38 | 40 |
| Stem section between branch fascicle | 28 | 0 | 0 | 3 | 8 | 16 |

The best results were obtained with sections of stem with attached fascicle of branches, as was expected from observations by Clymo & Duckett (1986). Here the number of regenerating sites doubled the number of stem sections put into culture. Good results were also obtained with fully elongated branches from the capitulum. The medium-sized and short branches from the capitulum continued their growth to full length before any regeneration site appeared. Regeneration on stem sections between branch fascicles was delayed, but these parts proved to possess good regeneration capacity. Total absence of regeneration from apical buds could be due to low nutrient availability compared with the results obtained with *S. rubellum* in sterile culture with high concentrations of nutrients.

The second experiment clearly shows that there is better regeneration (Table 2) in non-sterile culture than in sterile culture. Contamination by micro-organisms was also extremely small, probably due to the fact that the sterilisation process is too harsh for the moss tissues or that the non-sterile culture had no sugar. In addition, regeneration began within 10 or 20 days, a much shorter period than in the sterile culture of the first experiment. In the latter, one month and sometimes two months passed before the first sign of regeneration.

### 2. Water levels experiment

*Sphagnum fuscum*

*Sphagnum fuscum* regenerated best from fragments containing a capitulum still attached to the stem. After 3 months, the capitula almost tripled the initial number

ving *Sphagnum papillosum* detached
filter papers wetted with Rudolph &

| | | July | |
|---|---|---|---|
| 22 | 28 | 7 | 26 |
| No. of regeneration sites | | | |
| 77 | 77 | 77 | 77 |
| 18 | 26 | 27 | 32 |
| 1 | 3 | 5 | 7 |
| 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 |
| 23 | 34 | 38 | 40 |
| 0 | 3 | 8 | 16 |

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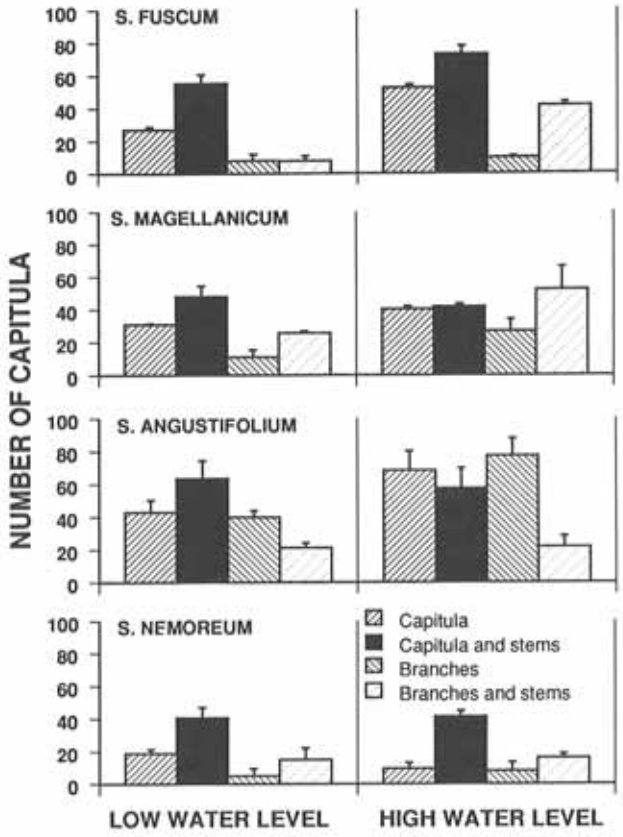



**Figure 2**. Regeneration of vegetative parts of four *Sphagnum* species: the number of capitula present after a 3-month incubation time in relation to two water levels (1–2 cm and 8 cm below the peat surface). The initial number of fragments was 150 for the small branches and 25 for the three other types.

(from 25 to 75; $F_{3,16} = 67.8$; $P < 0.0001$; Figure 2). Overall, the higher water level slightly increased the success of *S. fuscum* regeneration (minimum $F_{1,16} = 13.8$; $P = 0.0019$) in terms of innovations produced and coverage. Even though several new capitula were produced within the 3 months of growth, this species spread very little (barely one-fifth of the peat surface; Figure 3).

*Sphagnum magellanicum*

For this species, most parts regenerated well with the exception of branches alone (minimum $F_{3,16} = 6.3$; $P = 0.005$; Figures 2 & 3). After 3 months, *S. magellanicum* was beginning to form little cushions. A good coverage of the bare peat surface (Figure 3) was achieved more by the capitula which tended to increase in size rather than the number of newly formed regenerants (compare with graphs for

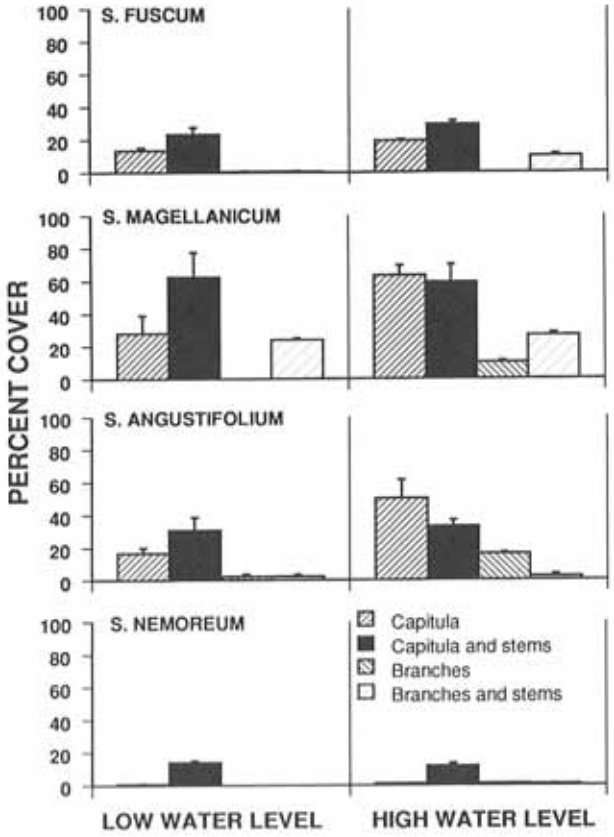
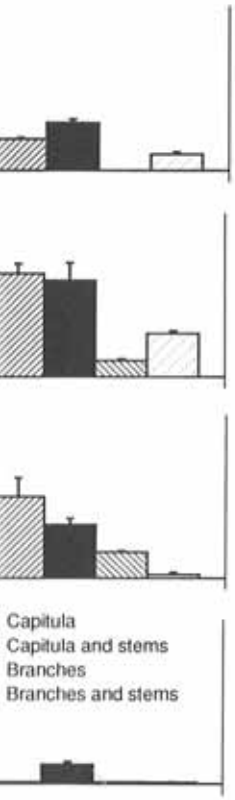



**Figure 3.** Regeneration of vegetative parts of four *Sphagnum* species: the *Sphagnum* cover of the bare peat surface after a 3-month incubation time in relation to two water levels (1–2 cm and 8 cm below the peat surface). The initial number of fragments was 150 for the small branches and 25 for the three other types. .

*S. fuscum* and *S. angustifolium*). *S. magellanicum* produced more innovations under a higher water level regime ($F_{1,16} = 6.7$; $P = 0.02$).

*Sphagnum angustifolium*

The types of fragment differed significantly in terms of success of regeneration (minimum $F_{3,16} = 5.2$; $P = 0.03$). In terms of production of innovations, only *S. angustifolium* showed a good ability to regenerate from fragments composed of branches alone (Figure 2). Regeneration from branches did not spread as much on the peat surface (Figure 3) as when a capitulum was introduced but it was more successful than when the branches were still attached to a stem (Figures 2 & 3; for comparison 1 cm of stem of *S. angustifolium* had *c.* 8 branches x 25 = 200 branches). In general, the maintenance of a water level closer to the peat surface

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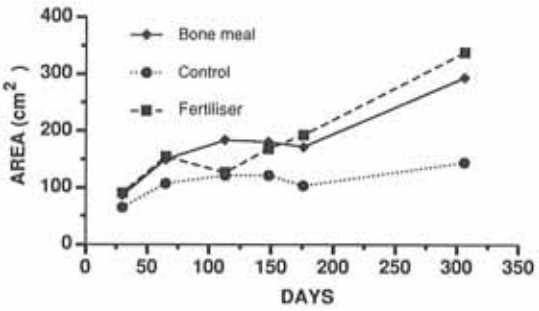



**Figure 4.** Growth of *Sphagnum nemoreum* on peat treated with slow-release fertiliser (12–12–12), bone meal (2–11–0) or no fertiliser.

favoured a better growth for all parts (minimum $F_{1,16} = 5.2$; $P = 0.037$) for both plant parameters measured.

*Sphagnum nemoreum*

With this species, only the diaspores composed of a capitulum attached to a stem succeeded in increasing its number of regenerants (from 25 to 40; $F_{3,16} = 20.3$; $P = 0.0001$; Figure 2). This species was neither vigorous nor influenced by the water level. Overall, it regenerated very poorly on the bare peat substratum (Figure 3).

### 3. Mineral additions experiment

Both fertiliser treatments enhanced the growth and the expansion of *S. nemoreum* compared with the control, where the peat substratum was as it occurs naturally in the bog (Figure 4). Nevertheless, differences between these two treatments were noted. Within a month, algae had developed on all the peat surfaces which had been treated with the slow release 12–12–12 fertiliser whereas, on peat where bone meal was applied, very few algae were observed and only on small areas. The algae, in some cases, covered the *Sphagnum* mosses and smothered them, reducing their total growth. However, given time, the development of the *Sphagnum* did not seem to be influenced by the algal growth, since its development in the fertilised treatment caught up with the bone meal treatment.

### 4. Field reintroduction experiment

In 1992, differences in the number of capitula $m^{-2}$ were significant between treatments (($F_{3,78} = 59.1$; $P = 0.0001$. The *Sphagnum* treatment differed from all other treatments, while the three other treatments were not significantly different from each other at the 0.05 level. In 1993, the results were similar ($F_{3,51} = 9.8$; $P =$

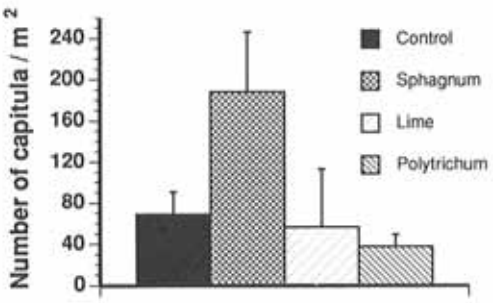



**Figure 5.** The effect of the addition of external *Sphagnum magellanicum* diaspores, lime and companion species (*Polytricum strictum)* on *Sphagnum* regeneration on an abandoned harvested peatland.

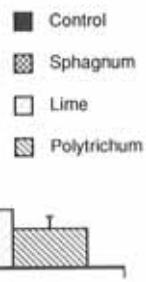
0.0001 for differences between treatments; Figure 5). In other terms, these results indicate that the addition of *Sphagnum* diaspores to a bare peat surface enhance the chance of *Sphagnum* recolonisation, as, even after two summers and one winter in the field, a number of the introduced diaspores were still alive. Covering the plots in the second summer did not result in a higher density of *Sphagnum* capitula in the quadrats (P = 0.216).

## DISCUSSION

To restore the vegetation of boreal bogs after peat extraction, we need to know how to reintroduce *Sphagnum* species into an abandoned peaty field and to minimise the disturbance of natural bog vegetation from where diaspores will be taken. As a partial answer, we have investigated which parts of different *Sphagnum* species can regenerate and how small a fragment can regenerate.

The growth of *Sphagnum* parts on agar plates (exp. 1) indicates that all four of the North American *Sphagnum* species tested had a high power of vegetative regeneration, as was also found by Poschlod & Pfadenhauer (1989). These authors did not succeed in getting new innovations from *Sphagnum* leaves. However, as previously found by Oehlmann (1898), we observed that very small parts of *Sphagnum* such as a single branch leaf or a cross-section of a stem were able to produce a new shoot. Now that we know that almost any vegetative parts of *Sphagnum* can regenerate into new individuals, we need to obtain more information on the growing conditions which can promote this regeneration on a larger scale.

The growth of *Sphagnum* parts on bare peat substratum (exp. 2) confirms that at least three species of *Sphagnum* can readily reproduce vegetatively and established themselves on peat. The level of regeneration achieved by the *Sphagnum* fragments tested during a 3-month period was very similar to the maximum

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n achieved by the *Sphagnum*
very similar to the maximum



numbers of new innovations observed by Wilcox & Andrus (1987) during a 112-day incubation (from 8 to 70 for similar species). A water level close to the surface of the peat substratum favoured the regeneration of all the species and types of fragment (except for *S. nemoreum*, which did very poorly). For *S. magellanicum*, our results are not directly consistent with the findings of Wallén *et al.* (1988), where a better rate of carbon fixation was obtained when the water level was maintained 10 cm below the surface compared with 2 cm. Based on observation of cores at natural density, we should have expected more biomass production of *S. magellanicum* at our low water level.

The growing conditions of the last two experiments were closer to the conditions in which natural *Sphagnum* regeneration will have to take place. With the results obtained from these experiments, we can conclude that an external source of diaspores will significantly enhance *Sphagnum* establishment and mineral additions will help it to spread more rapidly. The effect of a cover did not influence *Sphagnum* colonisation of already established capitula. However, more work is needed to assess if it could improve the conditions at the onset of fragment establishment.

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