

# The use of fungicide Nova to mitigate infection of *Sphagnum* by parasitic fungi in the greenhouse

J. Landry, C. Martinez, and L. Rochefort

**Abstract:** A common problem when growing *Sphagnum* mosses in the greenhouse is the propagation of parasitic fungi. Since no clear procedure is available to correct the situation, the aim of this experiment is to give scientists and growers a tool to control fungi invasions in the greenhouse. First, eight fungicides and the effect of temperature were tested on Petri dishes inoculated with two fungi commonly found in *Sphagnum*: *Lyophyllum palustre* (Peck) Singer and *Chaetomium* sp. To assess *Sphagnum* tolerance to fungicides, the four most efficient treatments were tested on healthy *Sphagnum* carpet, at maximum and minimum concentrations. Finally, the most promising fungicide, Nova (myclobutanil), was tested on *Sphagnum* carpets infected by *L. palustre* and *Chaetomium* sp. Since the concentration of this fungicide had no effect on biomass accumulation, the maximum concentration (0.54 g/L) was tested. Because of the high absorbency of *Sphagnum*, Nova was applied at the recommended dose (1 L/10 m<sup>2</sup>) and at three times the recommended dose (3 L/10 m<sup>2</sup>). An evaluation of infected *Sphagnum* individuals was carried out after a frequency of two and three applications. The recommendation for controlling the invasion of *Sphagnum* by *L. palustre* and *Chaetomium* sp. in the greenhouse is the application of Nova fungicide at three times the recommended dose. The frequency of applications had no significant effect.

**Key words:** *Sphagnum*, fungus, invasion, *Chaetomium*, *Lyophyllum palustre*.

**Résumé :** Lors de la culture de sphaignes en serres, un des problèmes fréquemment rencontrés est le développement de champignons parasites. Aucune procédure n'est disponible pour corriger cette situation. Le but de cette expérience est de donner aux scientifiques et aux producteurs un outil pour empêcher la croissance des champignons en serre. Premièrement, l'effet de huit fongicides et de la température a été testé en boîte de Pétri contre deux champignons, soient : *Lyophyllum palustre* (Peck) Singer et *Chaetomium* sp. Par la suite, afin de vérifier la tolérance des sphaignes, les quatre fongicides les plus efficaces ont été testés sur des sphaignes saines aux concentrations maximales et minimales recommandées par les manufacturiers. Enfin, le fongicide le plus prometteur, le Nova, a été expérimenté sur des tapis de sphaignes inoculés avec *L. palustre* et *Chaetomium* sp. Comme la concentration de ce fongicide n'a pas d'effet sur la biomasse des sphaignes, la concentration maximale (0,54 g/L) a été appliquée. De plus, en raison du fort potentiel d'absorption des sphaignes, le Nova a été administré à la dose recommandée (1 L/10 m<sup>2</sup>) et à trois fois cette dose (3 L/10 m<sup>2</sup>). Le niveau d'infection des sphaignes a été évalué après deux et trois applications de ces doses de fongicides. Ainsi la recommandation pour contrôler le développement de *L. palustre* et de *Chaetomium* sp. dans les sphaignes en serre, est l'utilisation de Nova à une dose de 3 L/10 m<sup>2</sup>. La fréquence des applications n'a pas d'effet.

**Mots-clés :** *Sphagnum*, champignons, invasion, *Chaetomium*, *Lyophyllum palustre*.

## Introduction

Interest in peatland ecosystems has grown considerably during the last decade. Numerous research projects have been conducted to better understand their development processes, their role in climate change, and their potential as a source of raw material. Many scientific experiments are better conducted in controlled environments such as greenhouses. In these cases, the quality of the results is strongly related to the ability to efficiently grow *Sphagnum*, the dominant species in many peatlands. Unfortunately, growing and maintaining healthy *Sphagnum* can be difficult in the greenhouse owing to infection by parasitic fungi that can lead to loss of material and ultimately limited opportunity for data publication.

Fungi are abundant in peatlands. Indeed, over 601 species have been identified in this habitat worldwide (Thormann and Rice 2007). They play a prevailing role in nutrient cycling and have been reported to contribute even more to decomposition of organic matter than bacteria (Andersen et al. 2006; Thormann 2006a). Even if fungi are commonly found in peatlands, they can become unwanted guests in greenhouse experiments or propagation involving living *Sphagnum* material collected in peatlands.

Greenhouses are favourable environments for the rapid propagation of fungi, since they are closed environments with constant humidity and relatively warm temperatures. They can cause serious chlorosis, leading to the loss of capit-

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ula and, eventually, the death of the mosses. To our knowledge, no controlling tool has been clearly described to help growers and scientists deal with fungi invasion in greenhouse. This study focussed on two problematic fungi previously identified in greenhouse experiments. In 2008, *Chaetomium* sp. was found and identified in experiments by R. Pouliot (unpublished data). *Lyophyllum palustre* was also identified in greenhouse experiments (Rocheffort and Price 1998) and is known by other authors for infecting *Sphagnum* (Redhead 1981; Limpens et al. 2003).

The ascomycete *Chaetomium* sp. has been identified in minerotrophic and ombrotrophic peatlands (Thormann and Rice 2007) and is also commonly found in a broad variety of substrates such as soil, seeds, straw, manure, and fabric (Stamets and Chilton 1983; Webster and Weber 2007). It typically forms whitish colonies that may become yellow to yellow-green or copper in colour when mature and from which hair-like hyphae will erupt. The genus *Chaetomium* is a saprobe known in boreal peatlands as a simple polymer-degrading fungus (Thormann 2006b).

*Sphagnum* species are not the exclusive host for the majority of fungi found in peatlands. However, there are a few exceptions like the basidiomycete *L. palustre*, which is rather frequent in bogs of the northern hemisphere and has only been identified colonizing *Sphagnum* species (Redhead 1981; Untiedt and Müller 1985). *Lyophyllum palustre* is described as a parasitic necrotrophe, living on their host's dead tissue, leaving typical necrotic patches on *Sphagnum* carpet (Redhead 1981). Results show that *L. palustre* succeeds in infecting by altering the host cell protoplasm, allowing the fungus to penetrate *Sphagnum* cell walls (Untiedt and Müller 1985). The hyphae seem to infect preferentially meristematic regions such as the axil of leaf and branches as well as the capitulum apex causing the collapse of young cells (Redhead 1981).

*Sphagnum* is known to have a limited tolerance to desiccation (Murray et al. 1989; Sagot and Rocheffort 1996), to NaCl addition (Wilcox 1984), and to sulfur pollutants such as sulfate, sulfite, and sulfur dioxide (Ferguson et al. 1978), as they have no stomata and the exchanges with the environment are directly through the cells. Leaves of *Sphagnum* mosses are unistratose in structure, and they exchange all water and nutrients directly with the surrounding environment. Since *Sphagnum* is intolerant to a number of environmental stresses, determining the concentrations and dosages (quantity) of fungicides is challenging. Furthermore, all recommendations for the control of fungi in cultures are based on vascular plants and no indications exist for nonvascular plants like *Sphagnum*. The goal of this study was to find an appropriate fungicide concentration, dosage, and application frequency that prevents fungus development without affecting *Sphagnum* growth. In addition, the control of temperature to slow the rate of fungus development an alternative to the use of fungicide was also tested.

## Materials and methods

For the purpose of the experiment, *Sphagnum rubellum* Wilson moss material was collected near St-Charles de Bellechasse (Quebec) in a natural peatland (46°47'N 70°58'W) by removing the top 10 cm of a *Sphagnum* carpet. *Sphagnum ru-*

*bellum* fragments were spread in experimental plots in the greenhouse to cover the totality of the peat substrate, and the plots were watered two to three times a week with rain water to keep the water table as near as possible to the surface. After about 4 months in the greenhouse with a mean temperature of 20 °C during the day and 15 °C during the night, a photoperiod of 14 h of light, and relative humidity maintained around 70% using osmosis water, complete carpets of approximately 8–10 cm thick were obtained, and *Sphagnum* was ready to use for the experiments described below.

*Lyophyllum palustre* (DAOM 176547) was provided by the Canadian Collection of Fungal Cultures. *Chaetomium* sp. was initially found in a *Sphagnum* carpet from another experiment (R. Pouliot, unpublished data), identified, and purified by the Laboratoire de diagnostic en phytoprotection of MA-PAQ (Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec) and sent back to us in a Petri dish. *Lyophyllum palustre* and *Chaetomium* sp. were then propagated by introducing active mycelia on PDA medium (potato dextrose agar) at 27 °C for 2 weeks before using the actively growing fungi.

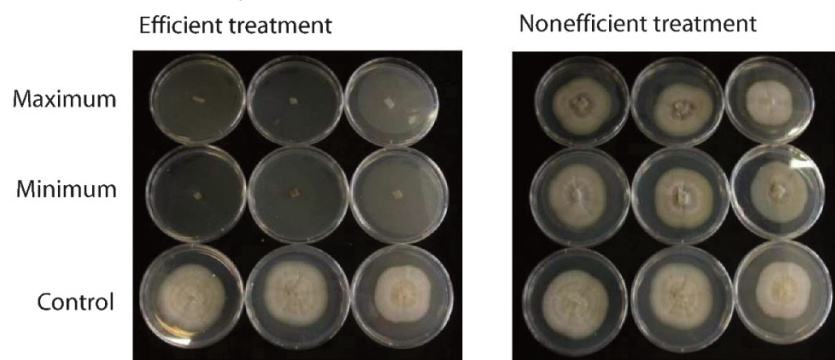
The study was divided into three experiments. The first experiment tested a variety of treatments (eight fungicides and one cold temperature treatment) believed to limit the growth of *Chaetomium* sp. and *L. palustre*. Fungicides are listed in Table 1. The goal of the second experiment was to identify which of these treatments do not affect *Sphagnum* growth. Finally, the goal of the third experiment was to find a fungicide that would limit fungal growth without affecting *Sphagnum* development, in a *Sphagnum* carpet infected by targeted fungi. Since no information exists on the use of fungicides for nonvascular plants, we used the highest (maximum) and the lowest (minimum) concentrations recommended for sensitive cultures such as flowers, fruits, and vegetables in all three experiments (Table 1). The dose of fungicides applied to *Sphagnum* carpets (experiments 2 and 3) corresponded to the dose of fungicides recommended by manufacturers for foliar application (1 L/10 m<sup>2</sup>).

### Experiment 1: growth of *Lyophyllum palustre* and *Chaetomium* sp. on Petri dishes (PDA medium) with the addition of eight fungicides and a cold treatment

The two fungi were evaluated for their sensitivity to the fungicides by in vitro radial growth assays. Mycelial growth of *Chaetomium* sp. and *L. palustre* was evaluated on PDA medium containing minimum and maximum concentrations of each fungicide, repeated three times, for a total of 48 Petri dishes. Commercial fungicides (Table 1) were incorporated into the medium after autoclaving, when the medium temperature was about 55 °C, just before the preparation of the dishes. Twelve Petri dishes containing PDA medium were kept without fungicide addition for the cold treatment and the control (repeated three times for both fungi). Petri dishes were inoculated by placing an agar disk covered with actively growing mycelium of both fungi in the middle of the dishes. Then, incubation of the fungicide treatments was conducted in a randomized design at room temperature (21 °C) and at 4 °C for the cold treatment. Radial growth was measured after 2 weeks; results are the means of two diameters measured in a cross in the middle of the dish (Fig. 1).

**Table 1.** Description of fungicides.

Fungicide trade name	Manufacturer	Systemic	Active ingredient	Concentration (g/L)	
				Minimum	Maximum
NOVA 40W	Dow AgroSciences	Yes	Myclobutanil	0.25	0.54
Previcur	Bayer Cropscience	Yes	Propamocarb	1.0	2.0
Senator 70W	Nippon Soda Co.	Yes	Thiophanate-methyl	0.42	2.25
Rovral WP	Bayer Cropscience	Yes	Iprodione	0.5	5
Maestro	Aresta corporation	No	Captan	1.25	4.5
Sulfur	Gowan Company	No	Sulfur	0.54	0.76
Truban 30W	Scotts-Sierra Crop Protection Company	No	Etridiazol	0.23	0.63
Botran	Gowan Company	No	Dichloran	1.0	1.5

**Fig. 1.** Example of efficient (Nova) and nonefficient (Previcur) treatments on the radial growth of *Chaetomium* sp. after 2 weeks of incubation. Maximum, minimum, and control refer to fungicide concentrations.

### Experiment 2: effect of two concentrations of fungicides on *Sphagnum* health and biomass

This experiment was carried out between December 2009 and May 2010, for a total of 170 days, in a greenhouse complex at Laval University. Conditions in the greenhouse were the same as previously described. The treatments included a control and the four most effective fungicides obtained in experiment 1 (Nova, Botran, Maestro, Senator). Fungicides were sprayed on *Sphagnum* carpets at maximum and minimum concentrations (Table 1), and the same quantity of distilled water was used on the control. Each combination of fungicide and concentration was considered an independent treatment. Treatments and control were repeated four times and were distributed in pots (approximately 175 cm<sup>2</sup>) of pre-established *S. rubellum* carpet with a completely randomized design.

Application of fungicide was done twice once a complete carpet was formed (108 days after introduction of *Sphagnum* fragments) by spraying at 2-week intervals. The dose applied on each *Sphagnum* carpet was the dose recommended by manufacturers (1 L/10 m<sup>2</sup>), corresponding to 1.75 mL for the size of the pot. A visual examination of *Sphagnum* health was made 1 week after each application. Any change in the *Sphagnum* carpet color or shape was noted. At the end of the experiment (48 days after the last fungicide application), *Sphagnum* biomass for every pot was collected by taking the whole carpet down to peat substrate. Then, the biomass was oven-dried at 70 °C for 48 h and dry mass was measured.

### Experiment 3: effect of Nova fungicide on *Sphagnum* infected by *Lyophyllum palustre* and *Chaetomium* sp.

In February 2010, after obtaining the results of experi-

ments 1 and 2, the Nova fungicide was selected to test its effect on fungus-infected *Sphagnum* carpet. Since it was an exploratory study and since the quantity of infected *Sphagnum* carpet was limited, only the maximum concentration (0.54 g/L) was tested.

The first step was to induce infection in *Sphagnum* carpets. *Lyophyllum palustre* and *Chaetomium* sp. were duplicated on Petri dishes, and when a sufficient quantity of fungus was available, pieces of agar covered with mycelia were introduced directly into *Sphagnum* carpets. The *Sphagnum* carpets were inoculated three times in a time frame of approximately 9 months before infection really took effect. During this period, watering was more frequent, and temperature was set to 23 °C during the day and 18 °C at night to stimulate fungus propagation.

Long ago it was observed that *Sphagnum* species have a high capacity to retain water, almost like a sponge (Grout 1908). To compensate for the high absorbency of *Sphagnum* and to insure that the fungicide would penetrate the carpet through the infected zone (typically 1–3 cm below the capitula for *L. palustre*, as observed by Limpens et al. (2003)), three doses were tested: (i) zero (control), where distilled water was used in the same quantity as the recommended dose; (ii) the dose recommended by the manufacturer (1 L/10 m<sup>2</sup>); and (iii) three times the dose recommended by the manufacturer (3 L/10 m<sup>2</sup>). To complete this factorial design, frequency of application was also tested.

We based our schedule of application on a good practice of fungicide application in the greenhouse that suggests 10- to 14-day intervals between Nova applications. No more than six applications of Nova fungicides are recommended, and commonly one or two applications are used to control



**Fig. 2.** Signs of infection on a *Sphagnum rubellum* individual showing defoliated stem parts and presence of mycelium.



fungus in a greenhouse. We used a 10-day interval between applications and tested for a frequency of two and three applications. To evaluate the efficiency of applications, *Sphagnum* individuals were systematically selected in each plot and carefully pulled out of the *Sphagnum* carpet. Individuals were then observed under a 16× binocular for signs of infection. Individuals were classified as infected following the description by Limpens et al. (2003) when either mycelium was detected around the individual or when there were visible signs of defoliation (Fig. 2).

First, a survey of infected *Sphagnum* individuals was done prior to fungicide application. Since infection was not homogeneous throughout the plots prior to application, the 27 plots (17 cm × 26 cm) were blocked in reference to their initial infection percentages. Nine blocks were created, inside of which the three doses were randomly assigned. To test the effect of frequency of application, a survey of infected *Sphagnum* individuals as described above was carried out after two applications (after 20 days) and after three applications of fungicide (after 30 days).

### Statistical analyses

Data analyses were carried out using the GLM procedure in SAS software (SAS Institute Inc. 2003). All data (experiments 1–3) were tested for homogeneity and normality. No transformations were required, and results were considered statistically significant at  $P < 0.05$ . In experiment 1, protected least significant difference tests were conducted to reveal the effect of fungicide on *L. palustre* and *Chaetomium* sp. Each combination of fungicide and concentration (e.g., Nova minimum concentration and Nova maximum concentration) was considered an independent treatment. To take into account the multiple comparisons between treatments, the Bonferroni correction was included in the analysis. To evaluate whether the application of fungicide had an effect on *Sphagnum* biomass (experiment 2), a series of predetermined contrasts were conducted. Finally, in experiment 3, a two-way ANOVA was performed to test the significance of doses

and frequency of Nova fungicide application on infected *Sphagnum* carpet.

## Results

### Experiment 1: growth of *Lyophyllum palustre* and *Chaetomium* sp. on Petri dishes (PDA medium) with the addition of eight fungicides and a cold treatment

For both fungi, the most efficient fungicide was Nova, which completely inhibited their growth on Petri dishes ( $P < 0.0001$ ). The cold treatment was not the best treatment, but did limit the growth of both fungi compared with the control (Fig. 3).

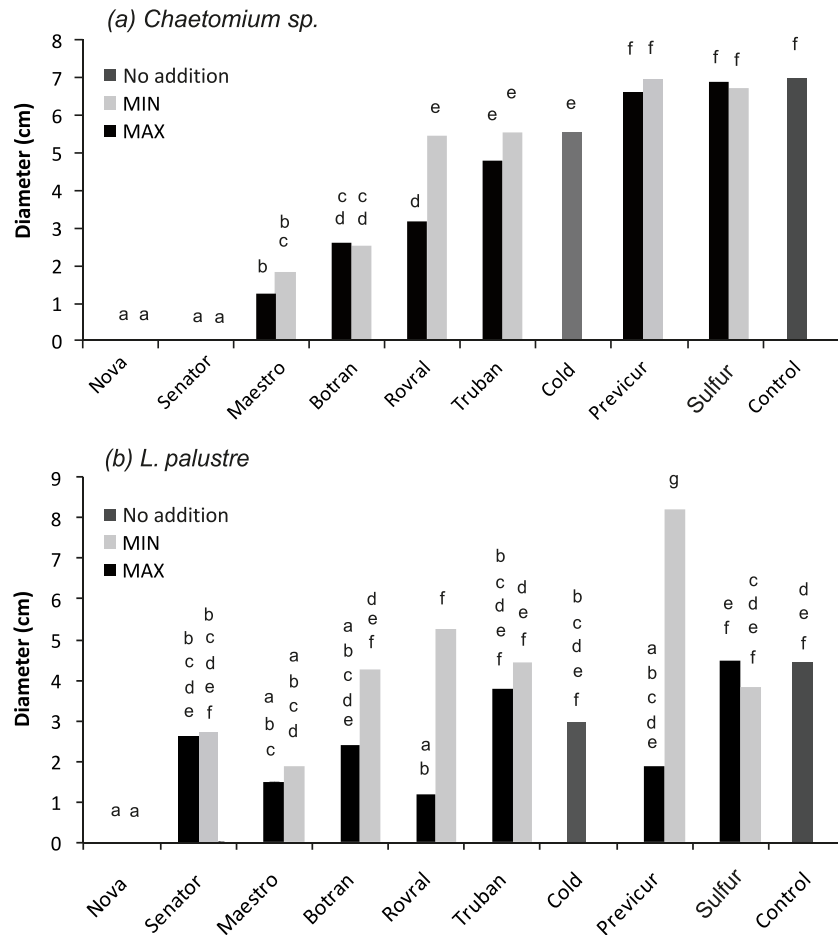
For *Chaetomium* (Fig. 3a), the four most efficient fungicides that had a considerable impact on fungal growth were Nova, Senator, Maestro, and Botran. The least effective treatments were Previcur and Sulfur, whose effects were similar to those of the control.

In the case of *L. palustre* (Fig. 3b), results were not as clear as for *Chaetomium*, but the patterns were similar. The most effective fungicides still include Nova and Maestro, but also the maximum concentration of Rovral. The least effective treatment was still Sulfur, but also the minimum concentration of Previcur, Rovral, and Botran.

### Experiment 2: effect of two concentrations of fungicides on *Sphagnum* health and biomass

Neither the minimum nor maximum concentration of Nova fungicide (the most effective treatment in experiment 1) had an effect on *Sphagnum* biomass (Table 2, contrasts C4 and C5). The only fungicide having a significant effect on *Sphagnum* biomass ( $P = 0.02$ ) was Senator applied at maximum concentration (Table 2, contrast C7). In general, the concentration at which the fungicides were applied on the *Sphagnum* carpets (Table 2, contrast C1) had an impact on *Sphagnum* biomass ( $P = 0.01$ ). When fungicides were applied at maximum concentration, final biomass was lower than that when fungicides were applied at minimum concentration.

**Fig. 3.** Effect of eight fungicides and a cold treatment on *Chaetomium* sp. (a) and *Lyophyllum palustre* (b) growth after 2 weeks on a Petri dish (potato dextrose agar medium). The data presented are means ( $n = 3$ ). Letters indicate significant differences between treatments ( $P < 0.05$ ).



**Table 2.** A priori contrasts for analysis of variance for effects of selected fungicides (Nova, Maestro, Senator, Botran) on *Sphagnum* final biomass.

	df	F	P
Treatment	8	2.21	0.05
Error	31		
Total	39		
Contrasts			
C1: Max. vs. min.	1	8.08	0.01
C2: Min. doses vs. control	1	2.00	0.17
C3: Max. doses vs. control	1	0.82	0.37
C4: Nova min. vs. control	1	1.84	0.18
C5: Nova max. vs. control	1	0.12	0.73
C6: Maestro max. vs. control	1	1.25	0.27
C7: Senator max. vs. control	1	5.86	0.02
C8: Botran max. vs. control	1	0.39	0.54

During visual examinations of *Sphagnum* health, no apparent signs of distress were noticed on *Sphagnum* carpet, except in the case of Maestro fungicide. All plots treated with Maestro fungicide showed a 10% and 80% color change. After Maestro treatment (minimum and maximum concentrations), *S. rubellum* carpets, usually dark pink, changed to almost a fluorescent pink.

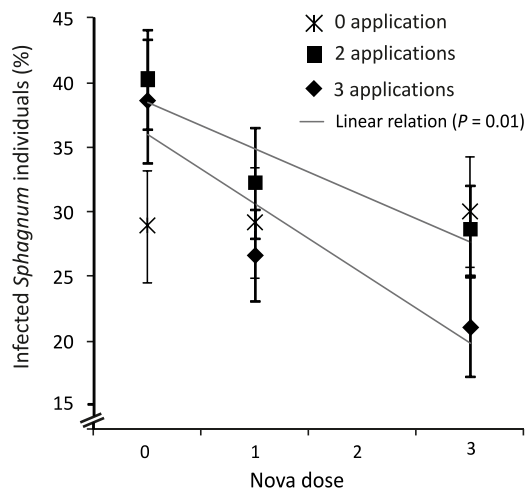
### Experiment 3: effect of Nova fungicide on *Sphagnum* infected by *Lyophyllum palustre* and *Chaetomium* sp.

The dose of Nova fungicide (zero corresponding to distilled water in the same quantity as dose 1, one and three times the recommended dose) applied on infected *Sphagnum* carpet had an effect ( $P = 0.02$ ) on the proliferation of infection on *Sphagnum* individuals. There was no interaction between dose and frequency. The increase of Nova dose resulted in a linear ( $P = 0.01$ ) decrease in the percentage of infected *Sphagnum* individuals (Fig. 4). When no fungicide (only distilled water) was applied on the infected *Sphagnum* carpets, fungus infection increased by 11% in 30 days (from  $29\% \pm 4\%$  (day 0) to  $39\% \pm 5\%$  (day 30)). After three applications (30 days), when three times the recommended dose was applied, infection decreased by almost half ( $21\% \pm 4\%$ ) in comparison with infection of the *Sphagnum* carpets receiving only distilled water (dose 0) ( $39\% \pm 5\%$ ) (Fig. 4). Frequency (number of application) did not have a significant impact on the reduction of the number of *Sphagnum* individuals infected ( $P = 0.28$ ).

### Discussion

Our results point to Nova fungicide as the most efficient treatment, able to inhibit *L. palustre* and *Chaetomium* sp.

**Fig. 4.** Effect of the frequency of application at maximum concentration: 0 applications (mean infection per treatment before any application), 2 applications (10-day interval between applications, so 20 days after the beginning of experiment), and 3 applications (30 days after the beginning of the experiment) in relation to the dose of Nova fungicide. 0 = control (distilled water in the same quantity as the recommended dose); 1 = dose recommended by the manufacturer; 3 = 3 times the dose recommended by the manufacturer. The data presented are means and standard errors ( $n = 9$ ). Dose had a significant impact on infection of *Sphagnum* individuals ( $P = 0.02$ ).



growth, without compromising *Sphagnum* development. Nova fungicide applied at three times the dose recommended by the manufacturer (3 L/10 m<sup>2</sup>) appears to be a good tool to control fungus invasion of *Sphagnum* carpet in greenhouse experiments. This fungicide is a systemic fungicide with both eradicant and protectant properties. The contact and protectant (nonsystemic) fungicides did not efficiently prevent fungal growth. It is probable that the effect of a systemic fungicide was necessary to limit the propagation of both fungi into the *Sphagnum* cells. Systemic fungicides are taken up by the plant and distributed through the tissues for a certain period of time, preventing further infection in healthy plant shoots, and therefore plants have the potential to recover after proper treatment. Moreover, *Chaetomium* and *Lyophyllum* belong to the divisions of Ascomycota and Basidiomycota, respectively, and Nova has proven to be efficient against fungi from both those divisions, including *Spaerotheca*, *Erysiphe* and *Puccinia*.

Since *Sphagnum* is known to be intolerant to a number of stress factors (Ferguson et al. 1978; Wilcox 1984; Murray et al. 1989; Sagot and Rochefort 1996), we were sceptical about the ability of *Sphagnum* to support fungicide addition. Only Maestro fungicide caused noticeable harm to the *Sphagnum* carpet, and only Senator affected the final biomass. Therefore, these fungicides were automatically excluded as possible treatments.

The cold treatment slowed the growth of both fungi. Consequently, to conduct greenhouse experiments in the winter or when a fungus invasion occurs in a greenhouse, lowering the temperature could help contain the infection. During the trials to infect *Sphagnum* with *L. palustre* and *Chaetomium* sp., we noticed a peak in necrotic patches (fungus occurrence) when the temperature was higher than 20 °C.

This study was the first attempt to find a solution to a recurrent problem; more investigation is needed to refine the suggested guideline. For instance, the maximum concentration of Nova fungicide (0.54 g/L) had no effect on *Sphagnum* final biomass (experiment 2), and that is why it was chosen in experiment 3. It is to be noted that Nova fungicide is used against fungi on sensitive flowers like roses, poinsettias, and gerberas. It is possible that *Sphagnum* and those flowers have similar sensitivity to the chemical products. However, it would have been interesting to test the minimum concentration (0.25 g/L) of the Nova fungicide, since it was as efficient as the maximum concentration in inhibiting the growth of both fungi on Petri dishes. Furthermore, it would be interesting to further investigate the number of applications and the optimal doses.

In conclusion, the most efficient treatment against infection by *L. palustre* and *Chaetomium* sp. in *Sphagnum* cultures in a greenhouse is the Nova fungicide applied at a dose of 3 L/10 m<sup>2</sup> and a concentration of 0.54 g/L. Nova was the only treatment completely inhibiting the growth of both fungi on Petri dishes with no repercussions for *Sphagnum* biomass accumulation. Ultimately, after three applications it was proven to be efficient in reducing fungal infection by half in infected *Sphagnum* carpets.

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