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Antifungal potential of *Sphagnum* moss in growing media

Sphagnum-sammaleen sienilääkepotentiaali kasvualustoissa

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The cinnamon mould (*Peziza ostracoderma* Korf) and *Penicillium* spp. never grow on the surface of a living *Sphagnum* moss media. Antifungal effect of *Sphagnum* moss medium also works against the major soil- and seed-borne plant fungal diseases. Good examples of the suppressive effect are seedborne *Alternaria* damping-off on cabbage seedlings and soil-borne *Pythium* root diseases on the cucumber. The disease control effect varies considerably between mires. The most effective moss batch caused almost complete protection against *Pythium* damping-off. The best antifungal properties were found in almost pure *Sphagnum* moss mixtures that had formed in the last 11, 40 and 64 years after the previous moss harvest, and the dominant species were other than the most common specie of the raised bog, *Sphagnum fuscum* (Schimp) H. Klinggr. When the moss was dried at the temperature of 60 or 70 °C, the antifungal effect was significantly enhanced. The seedlings remained healthy for at least two weeks, while a large proportion of the seedlings became ill in untreated moss medium. When the *Sphagnum* moss was kept dry, the antifungal property remained unchanged for several years. The longest measured shelf life was six years. Microbial concentrations of the mosses showed that the moss, harvested at the depth of 10–30 cm or kept dry for 4 years, was practically free of living fungi and bacteria. The moss harvested from the upper 0–10 cm layer of the mires contained measurable levels of *Penicillium* spp. and bacteria, but concentrations were only a fraction of those found in Sphagnum peat. When oatmeal was added to the strongly suppressive moss, the disease suppression effect of the moss was completely lost. In the moss samples, which naturally had completely inhibited the activity of *Pythium* disease for two weeks, the *P. ultimum,* Trow fungus, was fully alive, when the moss sample was transferred to rich nutrient potato dextrose agar (PDA). Based on microbial assays and heat treatment tests, the antimicrobial property of the *Sphagnum* moss is based almost exclusively on chemicals produced by living moss itself and the mode of action on fungal diseases is antifungal.

Keywords: *Alternaria brassicicola, Penicillium* spp., *Peziza ostracoderma*, *Pythium ultimum, Sphagnum* moss harvesting

*A*vainsanat: *Alternaria brassicicola, Penicillium* spp.*, Peziza ostracoderma, Pythium ultimum,* Sfagnum-sammaleen korjuu

Introduction

Sphagnum moss growing medium, produced from 0–30 cm layer of the surface of mires, is an excellent growing medium because of its biological and physical properties ideal for plant growth. It can be used to produce all the growing media needed for cultivation. The water retention properties of the *Sphagnum* moss growing medium are ideal due to the pore space of the moss stem and crushed moss. Even with overirrigation, there is always enough air space and easily usable water for the plants holds about 10 percent of the volume of the growing medium. The decomposition of the moss as a growing medium is slow, so after another two years of cultivation, the physical structure is almost unchanged. Unsuitable anymore for professional use moss growing medium can be used for composting or adding humus to fields (Näkkilä et al. 2015; Silvan et al. 2019). There are about nine million hectares of peatland in Finland, of which about 280 000 hectares are suitable for moss production (Silvan et al. 2019). Calculatively, this area would be sufficient to cover increasing the growing medium needs of horticultural crops throughout Europe. The moss layer renews itself in 25–30 years to its original thickness, in which case the moss growing medium is a truly renewable, viable growing medium. Greenhouse growing media are most commonly peat and rockwool (Silvan et al. 2012; Silvan et al. 2019). There are no in-depth scientific publications on the antidisease properties of living *Sphagnum* moss. The most important similar studies have been carried out on Sphagnum peat (Tahvonen 1982).

Studies on the use of moss as a growing medium for horticultural plants have been launched in Finland since 2010 (Silvan et al. 2012). The very first study found that this medium has a strong inhibitory effect against saprophytic and plant disease fungi (Näkkilä et al. 2015). This phenomenon of suppression is very similar in its effect to the suppression of Sphagnum peat (Tahvonen 1982). These studies found that white peat has a high microbial activity, but the number of species was small. The most prominent antagonistic microbial genera were *Trichoderma* spp., *Penicillium* spp., and *Streptomyces griseoviridis* Anderson et al. From the last one, a biological preparation was made to control plant diseases (Tahvonen 1987). If the peat storage heats up and finally cools down, the bacterial and *Penicillium* levels rise sharply and rapidly decrease from peak levels. At the end of the fall, toxins are released into the substrate, which damage the plants and even prevent germination of seeds (Tahvonen and Kemppainen 2008).

In 2013 and 2014, growing boards and seedling pots for greenhouse cultivation were developed from living *Sphagnum* moss (Erkkilä et al. 2016). In previous studies it has been found a strong inhibitory effect of living moss on the growth of saprophytic molds (Näkkilä et al. 2015). For this reason, it was required that the antifungal phenomenon stays in the growing medium after industrial production, even though the moss must be dried in the warmest possible air for production technical reasons. For decades, growing boards from *Sphagnum* peat had been made for cucumber and tomato cultivation in Finland. These growing boards are dried in industrial production at about 80 °C. The cinnamon mould (*Osrtragoderma peziza* Korf) always grew very strongly in white peat growing medium already two weeks after the start of cultivation (Tahvonen 2014). The fungus mentioned above is never found on living *Sphagnum* moss in any of the culture experiments and only in very small amounts on unheated growing peat. For this reason, the first, quite simple test was conducted, where wet moss was kept in an open and closed container for two days at 70 °C. *Penicillium* fungus did not grow in the dried moss as in the wet treated moss (Figure 1). It immediately became clear in this experiment, that the moss can be dried up to a temperature of 70 °C without problems, but during drying, the moisture must evaporate unhindered from the moss. (Tahvonen 2013, unpublished).

Based on these observations, extended studies on *Sphagnum* moss antifungal effects were started: the effect of drying technology on the phenomenon, the importance of the origin of *Sphagnum* mosses and the variation of antifungal effect, the mechanism of the antifungal effect, especially on *Pythium* disease and the diversity of microbial populations of mosses. This extensive series of studies, covering several separate projects from 2013 to 2019, has been compiled

in this publication in the most important parts. This study comprises only *Sphagnum* moss, so for simplicity it is called "moss" throughout the text.

Materials and methods

The natural antifungal effect of *Sphagnum* moss and the effect of drying

In all *Sphagnum* moss studies, the Neva-Lyly bog in Parkano, Western Finland, was used as a standard material of growing media by names Luke 1 from 2010 and Luke 2 from 2016. In this mire, 50% of the moss layer was *Sphagnum fuscum* (Schimp.) H. Klinggr. dominated and the rest covered by three other *Sphagnum* moss species (*S. medium* Limpr. 20%, *S. balticum* (Russow) C.E.O. Jensen 20% and *S. rubellum* Wilson 10%). Moss was collected from a 30 cm layer on the surface of the bog. After harvesting, all moss pieces were air-dried on tables in the greenhouse at a temperature of 15–20 °C, after which the moss was crushed with a tractor-powered device intended for crushing wood. Finally, the crushed moss was screened into three size classes according to the purpose of use, the maximum particle sizes of which were 10, 20 or 40 mm. The growing medium prepared in this way were limed and fertilized before the start of the plant tests with dolomite lime 4 g/50 g and peat complete nutrient fertilizer 1–1.5 g/50 g of crushed moss growing medium. The research conducted in years 2010–2014 and was focused on the basic properties of moss in cultivation technology, but observations and small-scale tests on microbiology and antifungal effect were also carried out. These preliminary findings are presented in this publication for the first time.

Substrates made from Luke 1 mosses were tested in years 2013–2014 in six different tests to prevent plant fungal diseases (*Pythium ultimum* Trow, *Alternaria brassicicola* (Schwein.) Wiltshire). The original purpose of these studies was to find an economical method for drying moss that still maintains the natural inhibition of saprophytic moulds. The determination of molds growing on mosses and other plant waste among the growing medium was done with a stereomicroscope.

Before starting the drying process, crushed moss was moistened with tap water, 400 ml/50 g. With this arrangement, we wanted to simulate industrial drying of moist moss after harvesting. In the first *Pythium* test, the moss was dried for two days at 70 °C, which was compared to untreated and wet disinfected moss at 80 °C in a closed container (Table 1, Figure 1). In the second, extensive drying test, temperatures were 50–80 °C in 10 degrees increments. The water concentrations at the beginning of moss drying were 300, 400 and 500 ml/l (1 liter = 50 g dry moss). Since these first moistures were not important for disease prevention, the disease results are presented as an average value of the moisture content (Table 2). Drying was done in circulating air in net-based boxes, where the thickness of the moss layer was 4–5 cm. Finally, the moss was limed, fertilized, moistened to the cultivation level (40%) and inoculated with *Pythium* mycelium. The fungus was grown on potato dextrose agar medium (PDA) for two weeks. The fungal culture was separated from the agar and homogenized into water. The *Pythium* suspension was inoculated to growing medium. The fungal suspension per 50 g of moss contained fungal mycelium from one of nine cm petri dish without agar medium. The inoculated moss was stored for one week at 16 °C before the start of the experiments. In the growing experiments of cucumber, there were four or six repetitions.

Microbes of living moss and disease suppression effect of moss harvested from different growth layers

The main goal of the 2016 research was to find out the disease suppression mechanisms of moss and the extent of the phenomenon. A detailed description of the research is in the master's thesis of Tomi Pousi (Pousi 2017). For this, moss samples were collected from six different bogs (Table 3). As much variation as possible was sought for the botanical composition of the bogs, including typical shrub plants. In addition, three moss samples were collected from the depths of 0–10; 10–20 and 20–30 cm. Suppressive effects of the samples were determined by the intensity of disease in cucumber and cauliflower. The micro-

bial population important in the control of fungal diseases was analysed like unheated Sphagnum peat (Tahvonen and Kemppainen 1998).

A. brassicicola and *P. ultimum* fungi were used as test diseases of suppressive features. *A. brassicicola* is the most common seed-borne damping-off of cabbage plants. *P. ultimum* causes aggressive damping-off, root and stem diseases in numerous horticulture plants. *Alternaria* was grown on PDA for two weeks, whereby an aqueous suspension was made from the mycelium and spores to inoculate onto cauliflower seeds. The dried seeds were sowed either in 12 cm pots (16 seeds/pot) or in cell dishes with one cell volume of one dl and one seed per cell. *Pythium* tests were performed as in previous experiments.

Selective medium was used for the detection of key microbes, *Trichoderma* spp. and *Penicillium* spp. (PDA + streptomycin sulfate), *Streptomyces* spp. (water agar) and bacteria (PDA + thiophanate-methyl and propamocarb hydrochloride). The microbial assays focused only on those microbes that had been assessed as the most important species in disease suppression in *Sphagnum* peat (Tahvonen 1982). In the laboratory tests, there were always three or four test dishes per one test member.

In Finland, the bait plant method has been used for *Pythium* studies for decades. The method (https://www.luke.fi/en/services/pythium-test) uses a mixture of oat flour and growing medium to detect and isolate *Pythium* disease from growing medium or water. This method was also used to find out the disease suppression effect of moss (Table 7).

The regeneration of the moss harvesting area and disease suppression

In 2019, there were 13 moss and peat samples (Figure 3), of which three batches were from areas where topmoss had been harvested 11, 40 and 65 years earlier. The composition of *Sphagnum* species of these bogs was (%): Tunkiosaloneva: *S. balticum* (Russow) C.E.O. Jensen, *S. fallax* (H. Klingger.) H. Köinggr. and *S. papillosum* Lindb. (60, 30, 10), Lylyneva: *S. fallax, S. papillosum, S. magellanicum* Brid. and *S. fuscum* (50, 20, 20, 10) and Aitoneva: *S. papillosum, S. magellanicum, S. pulchrum* (Lindb. ex Braithw.) Warnst., *S. fallax* and *S. fuscum* (30, 20, 20, 15, 15). These three bogs represented growing medium where exploitable moss had grown since the previous harvest. The original purpose of this moss research was to find out the quality of the moss as growing medium to produce plants. As an additional study, the variations in disease prevention of growing medium with the *Pythium* fungus were investigated. Of these mosses, three air-dried sub-samples were heated for 10 hours at 60 °C with the aim of increasing the variability of disease prevention between moss samples.

These mosses were also tested in the laboratory without *Pythium* disease in an attempt to develop a laboratory method to determine the strength of disease inhibition. Moist, healthy moss was shaped into balls and placed on PDA dishes (Figure 3). After three days, the *Pythium* fungus was inoculated in the center of the dish. It was hoped that the extent of the inhibition zone between the fungus and the moss would correlate with the strength of the disease inhibition, which was found in the cucumber seedling cultivation of the four-week plant experiment. At the end of the cucumber *Pythium* growth test, the mosses in the test pots (mosses 7, 11 and 12, Figure 3) were made into eight mm balls on PDA dishes, in which case the potato starch in the growing medium indicates the vitality of the *Pythium* fungus in the moss. The living fungus was examined two days after the start of the test, when the other microbes had not yet started to grow.

In years 2010–2019, dozens of tests and studies were conducted, of which the most representative disease prevention studies are presented in this article. At the same time, extensive cultivation experiments were carried out with moss, in which simple unpublished observations on the microbiology of moss were also made (Näkkilä et al. 2015; Erkkilä et al. 2016). These findings were never in conflict with the results presented in this study. The most significant findings were naturally put to good use when studying the control of fungal diseases with *Sphagnum* moss.

Results

When examining the suitability of moss as a growing medium, the surface of the moss was never covered by saprophytic fungi, but the heat dried commercial peat growing medium used as the control medium always contained a large amount of cinnamon mould (*Ostragoderma peziza)*. When peat and moss were mixed, 25% moss reduced the cinnamon mould. Mixing 50% moss with peat prevented the growth of saprophytic like fungi on moss growing medium. Drying the moss at 40, 50, 60 and 70 °C did not increase the number of saprophytic moulds compared to the control moss, but keeping the moss wet at 70 °C caused several fungi growth (Figure 1). Saprophytic fungi, *Penicillium* spp. and *Ostragoderma* sp. grew on pieces of twigs and hay on the surface of moss, but they did not spread to the moss. The corresponding plant parts were fungi-free inside the *Sphagnum* moss.

In the first *Pythium* test (Table 1), the cucumber seeds germinated perfectly, and the seedlings only started to get the disease after the first week of growing, when the moss was dried at 70 °C. In the peat dried at the same temperature and in the moss dried in the greenhouse, germination was approx. 40% and the seedlings also died within two days. In another study (Table 2), mosses were dried at temperatures of 50–80 °C from three different humidity levels. Drying the moss at 60 or 70 °C before the *Pythium* inoculation of the growing medium protected almost completely the germination of seeds and young seedlings from damping-off (Figure 2). When the drying was done at 50 °C or 80 °C, germination was approx. 35% and the germinated plants died quickly after germination. Moisture level of moss medium was

Table 1. Germination of cucumber seeds and the number of healthy seedlings during cultivation. Drying moss and white peat at 70 °C for two days. *Pythium* inoculum was done directly in the growing medium one week before sowing (1.4.2014). Five seeds in a 0.6 liter pot/3 repetitions. The average number of healthy seedlings per test pot is shown.

Taulukko 1. Kurkun siementen itävyys ja terveiden taimien lukumäärä viljelyn aikana. Sammalen ja vaalean kasvuturpeen kuivaus 70 °C:ssa kaksi päivää. Pythium-lisäys on tehty suoraan kasvualustaan viikko ennen kylvöä (1.4.2014). Kylvetty viisi siementä 0,6 litran ruukuun, toistoja oli 3. Esitetty terveiden taimien keskimääräinen lukumäärä testiruukkua kohti.

Drying temperatures of growth media, °C ***Kasvualustojen kuivauslämpötilat, °C***	**6.4.2014 Emergence of seeds** ***Itäneitä siemeniä***	**8.4.2014 Healthy cucumber seedlings** ***Terveitä kurkun-taimia***	**16.4.2014 Healthy cucumber seedlings** ***Terveitä kurkun-taimia***
Moss - *Sammal* 20 °C	2.0	0.0	0.0
Moss - *Sammal* 70 °C	5.0	5.0	2.0
Sphagnum peat - *Vaalea kasvuturve* 70 °C	2.3	0.0	0.0

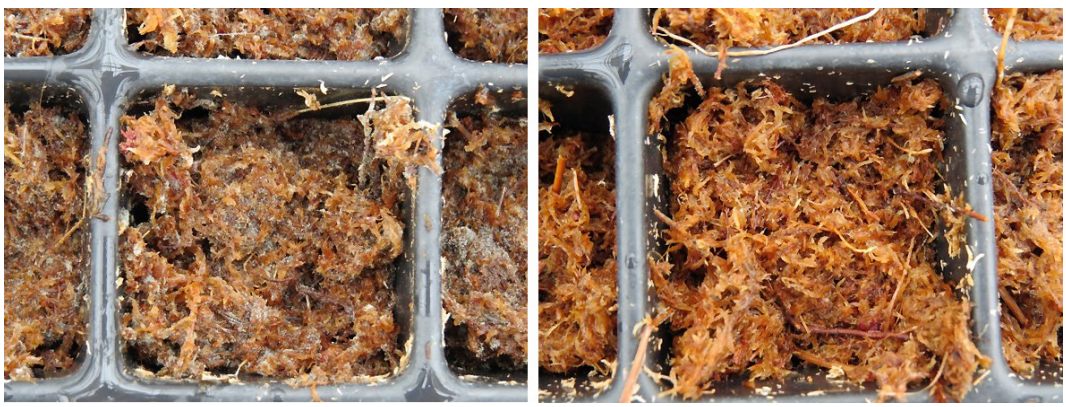

Figure 1. On the left moss heated for 2 days at 70 °C in a moist, closed container. On the right moss dried in an open container for 2 days at 70 °C. Photo taken after 3 days of incubation in the greenhouse.

Kuva 1. Vasemmalla rahkasammalta, jota on lämmitetty 2 päivää 70 °C:ssa kosteassa, suljetussa astiassa. Oikealla sammalta kuivattu avoimessa astiassa 2 päivää 70 °C. Kuva otettu 3 päivän inkuboinnin jälkeen kasvihuoneessa.

Table 2. Seed germination % of 45 cucumber seeds and the number of 2-week-old healthy seedlings/pot. Nine replications. Moss samples were dried at a temperature of 50–80 °C for 2 days. *Pythium* disease was inoculated into the growing media five days before sowing.

Taulukko 2. Siementen itävyys % 45 kurkun siemenestä ja 2 viikon ikäisten terveiden taimien määrä/ruukku. Yhdeksän toistoa. Sammalnäytteitä kuivattiin 50–80 °C:n lämpötilassa 2 päivää. Pythium-tauti siirrostettiin kasvualustaan viisi päivää ennen kylvöä.

Moss drying temperature Sammalen kuivauslämpötila	***Emergence, % Taimettumis-%***	**Healthy seedlings*) *Terveitä taimia**)**
50 °C	35.6	1.44a
60 °C	77.8	2.78b
70 °C	73.4	3.33b
80 °C	35.6	1.44a
20 °C, Peat, no *Pythium* sp. Turve, ei *Pythium*-sientä	100	5.00c

*) At a risk of P < 0.05, the difference is not significant when the same letter

**) Riskillä P < 0,05 ero ei ole merkittävä, kun sama kirjain*

not important from the point of view of intensity of disease. For this reason the results are presented as averages of three moisture levels. A 50 % share of moss in peat reduced the *Alternaria* disease amount of cauliflower to the moss level

Figure 2. *Pythium* sp. damping-off of cucumber seedlings sown in moss, 5 seeds in a pot. In the upper row moss dried at 70 °C and in the lower row moss dried on greenhouse tables at 20 °C. See Table 1.

Kuva 2. Kurkun taimien Pythium-taimipoltetta sammalalustassa, ruukussa 5 siementä. Ylärivissä sammal kuivattiin 70 °C:ssa ja alarivissä kuivaus kasvihuonepöydillä 20 °C. Katso taulukko 1.

Table 3. Intensity of *Alternaria*-disease on cauliflower seedlings, that grew in mixtures of peat and moss. Seeds were infected with *Alternaria* brassicicola fungus. Disease index, 0–5 (0 = healthy, 5 = dead).

Taulukko 3. Alternaria-taudin intensiteetti turpeen ja sammaleen seoksissa kasvaneilla kukkakaalitaimilla. Alternaria brassicicola -sienellä saastututetut siemenet. Sairausindeksi, 0–5 (0 = terve, 5 = kuollut).

Peat-% / Moss-%, *Turve-% / Sammal-%*	**100/0**	**75/25**	**50/50**	**0%/100%**	**Uninfected seeds *Saastutetamaton siemen***
Disease index, 0–5	2.4	2.1	1.4	1.3	0.2

(Table 3). Cauliflower seedlings in moss always had significantly less disease symptoms than in peat. None of the mosses was completely inactive towards cauliflower disease (Table 4).

There were great differences between the mosses in the control of *Pythium* disease (Table 4, Figure 3). If the moss contained large amounts of remains of dwarf shrubs and sedges (especially *Eriophorum vaginatum*), the disease control was lower than that of almost pure *Sphagnum* moss. These low-quality mosses also caused phytotoxic symptoms in cauliflower and cucumber. Symptoms included chlorotic spots and a sharp decline in growth. Moss samples in the 0–10 cm depth profile contained plenty of other mire plants, such as crowberry (*Empetrum nigrum* L.) and cranberry (*Vaccinium oxycoccos* L.). In the moss samples of the uppermost depth profiles of Isoneva and Konttisuo mires, cranberry and cloudberry (*Rubus chamaemorus* L.) and other than *Sphagnum* moss species, such as red bear moss (*Polytrichum commune* Hedw.) were present. At a depth of 10–30 centimetres, there was almost no other mire plants besides *Sphagnum* mosses, or their proportion remained very small. Especially for mosses that inhibit *Pythium* and *Alternaria* fungi well, it was typical that the stems of *Sphagnum* mosses were weakly grounded, pale and easily distinguishable up to the depth of 20–30 cm. In addition, in the two lowest depth profiles, 10–30 cm, hardly any other plant relicts could be observed. The weights of healthy-looking plants were significantly higher in deeper moss layers than in the topmost layer (Table 5). When the layers of three different moss

Table 4. Number of healthy cauliflower and cucumber seedlings on mosses of different origins. Cauliflower seeds inoculated with *Alternaria brassicicola* fungus and growth of cucumber seedlings on growing media inoculated with *Pythium ultimum* fungus. Origin of growth media: 1. Louhineva mire, 2. Isoneva mire, 3. Pahkaneva mire, 4. Saarineva mire, 5. Konttisuo mire, 6. Neva-Lyly mire, 7. disinfected peat, 8. peat, 9. Peat, no disease inoculation into the growth media or on seeds.

Taulukko 4. Terveiden kukkakaalin ja kurkun taimien lukumäärä eri alkuperää olevilla sammalilla. Alternaria brassicicola sienellä ympätyt kukkakaalin siemenet ja kurkkun taimien kasvatus Pythium ultimum -sienellä ympätyissä kasvualustoissa. Kasvualustojen alkuperä: 1. Louhineva, 2. Isoneva, 3. Pahkaneva, 4. Saarineva, 5. Konttisuo, 6. Neva-Lyly, 7. desinfioitu turve, 8. turve, 9. Turve, ei taudin siirrostusta kasvualustaan tai siemeniin.

Growth substrate no, ***Kasvualusta no***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Cauliflower, *Alternaria* sp., *Kukkakaali*									
Healthy seedlings %, *Terveitä taimia %*	56	78	83	44	81	69	25*	59	88*
Variation +/-%, LSD 5%, *Vaihtelu +/- 5 %:n riskillä*	8	5	0.7	9.3	6.8	9.9	5	6.8	3.1
Cucumber, *Pythium* sp., *Kurkku*									
Healthy seedlings/pot, *Terveitä taimia/ruukku*	4.3**	3.1*	3.8	4.4**	1.0***	4.5***	4.7***	3.5	5.0
Healthy seedlings % from seeds, *Terveitä taimia % siemenistä*	80	53	70	80	20	93	93a)	70	100
Range of healthy seedlings/pot, *Terveiden taimien vaihteluväli / ruukku*	1–5	1–4	2–5	3–5	0–2	4–5	3–5	2–5	5–5

*P<0.05, **P<0.01, ***P<0.0001, comparison with peat. *Vertailu turpeeseen*
a) *Streptomyces griseoviridis* biocontrol agent (Mycostop) contaminated the steamed peat with high content. *Streptomyces griseoviridis biotorjuntaeliö (Mycostop) levinnyt desinfioituun turpeeseen korkeana pitoisuutena*

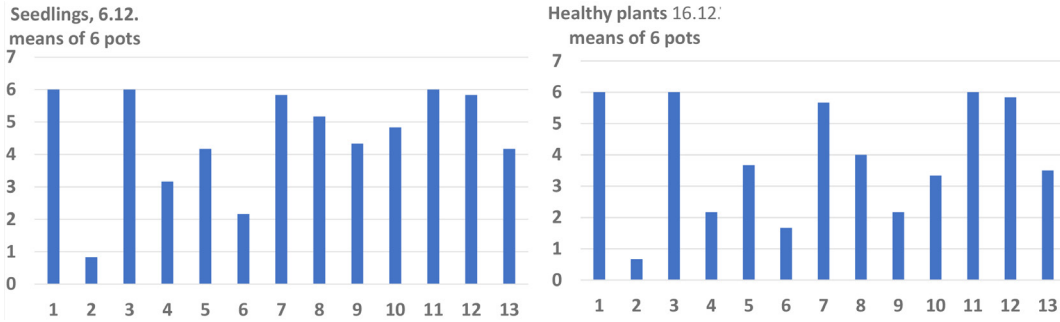

Figure 3. Germination of cucumber seeds and number of healthy seedlings 10 days after germination in 13 different growth media. *Pythium* fungus inoculated into growth media 2–13. Growth medium 2 was heated for 10 h in moist and growth media 5, 6 and 13 were heated air dry at 60 °C for 10 h. Moss batch 7 has grown years 11 years and moss batch 11 has grown 40 years, moss batches 12 and 13 have grown 65 years since the previous moss harvest. Six repetions of the experiment, 6 seeds in a test pot. Origins of growth media: 1. White peat, without *Pythium*-inoculation, 2. White peat (in moist 60 °C 10 h), 3. Luke 1, 4. Luke 2, 5. Luke 1 (in 60 °C 10 h), 6. Luke 2 (in 60 °C 10 h), 7. Tunkiosaloneva mire 11 years old, 8. Konttisuo mire, 9. Louhineva mire, 10. Isoneva mire, 11. Lylyneva mire 40 years old, 12. Aitoneva mire 65 years old, 13. Aitoneva mire 65 years old (in 60 °C 10 h).

Kuva 3. Kurkun siementen itävyys ja terveiden taimien lukumäärä 10 päivää itämisen jälkeen. Pythium-sieni sekoitettu kasvualustoihin 2–13. Kasvualustaa 2 kuumennettiin 10 tuntia kosteana ja kasvualustoja 5, 6 ja 13 kuumennettiin ilmakuivaksi 60 °C:ssa 10 tuntia. Sammalerää 7 on kasvatettu 11 vuotta, sammalerää 11 kasvanut 40 vuotta, sammalerää 12 ja 13 kasvatettu 65 vuotta edellisestä sammalen korjuusta. 6 toistoa kokeessa. 6 siementä testiruukussa. Kasvualustojen alkuperät: 1. Vaalea kasvuturve, ilman Pythium-sekoitusta, 2. Vaalea kasvuturve (kostea 60 °C 10 h), 3. Luke 1, 4. Luke 2, 5. Luke 1 (60 °C 10 h), 6. Luke 2 (60 °C 10 h), 7. Tunkiosaloneva 11 v, 8. Konttisuo, 9. Louhineva, 10. Isoneva, 11. Lylyneva 40 vuotta, 12. Aitoneva 65 vuotta, 13. Aitoneva 65 vuotta (60 °C 10 h).

Table 5. The variation of disease prevention in mosses of four different bogs, when samples were taken from three different depth profiles, 0–10, 10–20, 20–30 cm. The test diseases are *Pythium ultimum* on cucumber and *Alternaria brassicicola* on cauliflower.

Taulukko 5. Taudinehkäisyn vaihtelu neljän eri suon sammalilla, kun näytteitä otettiin kolmesta eri syvyysprofiilista, 0–10, 10–20, 20–30 cm. Testitaudit ovat kurkulla Pythium ultimum ja kukkakaalilla Alternaria brassicicola.

***P. ultimum*, cucumber** [1)] fresh weight of plants, g/treatment *P. ultimum, kurkku* [1)] *kasvien tuorepaino, g/käsittely*	**0–10 cm**	**10–20 cm**	**20–30 cm**	**Peat, disinfected** ***Turve desinfioitu***	**Peat** ***Turve***	**Peat, no disease** ***Turve ei tautia***
Neva-Lyly	3.7	14.3*	8.3	7.7	7.7	23.3*
Alternaria brassicicola, cauliflower, fresh weight of plants, g/treatment *Alternaria brassicicola, kukkakaali, kasvien tuorepaino, g/käsittely*						
Neva-Lyly	38.7***	51.2	49.7			
Isoneva	35.6***	48.4	40.7***	36.5***	53.8	61.7
Pahkaneva	24.7***	14.8***	53.6			
Konttisuo	20.0***	51.6	55.8			

1) only Neva-Lyly moss was effective against *Pythium* sp. *P<0.05, **P<0.01, ***P<0.0001, comparison with peat
*1) vain Neva-Lyly sammal oli tehokas Pythium-sieneen. *P<0,05, **P<0,01, ***P<0,0001, vertailu turpeeseen*

profiles were mixed, similarly significant differences in disease reduction and plant weights could not be observed (Pousi 2017). In the experiment shown in Table 4, an interesting *S. griseoviridis* contamination occurred on the disinfected peat during the preparation of the substrates, in which case the disease prevention was as strong as the best mosses. *S. griseoviridis* is the active bacteria in the biological control product "Mycostop" (Tahvonen 1987).

Fungal and bacterial concentrations of living moss were very low compared with the new, non-cultivated *Sphagnum* peat sample. When the dry moss was stored for four years, the moss was practically free of living bacteria and fungi (Figure 4), but the antifungal property remained (Table 4 and Figure 3). Statistically significantly more *Penicillium* species were found in the Neva-Lyly moss compared with other moss samples (P<0.0001). The content of *Penicillium* fungi in

Table 6. Colony forming unit counts (CFU) per milligram of growth media obtained from *Penicillium*, *Streptomyces* and bacterial density assays. CFU counts are averages of different dilutions and replicates. P-values indicated by the GENMOD statistical analysis model have been calculated using all dilutions and replicates, compared to white peat (*Penicillium* test n=101, *Streptomyces* test n=46, total bacterial concentration test: n=93).

Taulukko 6. Pesäkkeitä muodostavien yksiköiden määrät (cfu) laskettuna milligrammassa kasvualustaa, mikä on saatu Penicillium-, Streptomyces- ja bakteeritiheysmäärityksistä. Pesäkkeitä muodostavien yksiköiden lukumäärät ovat eri laimennosten ja toistojen keskiarvoja. Tilastollisen analyysin GENMOD-mallin osoittamat P-arvot on laskettu käyttämällä kaikkia laimennoksia ja toistoja, verrattuna kasvuturpeeseen (Penicillium-testi n = 101, Streptomyces-testi n = 46, kokonaisbakteeripitoisuustesti: n = 93).

Growing media, *Kasvualustat*	***Penicillium* spp.**		***Streptomyces* spp.**		**Bacteria**	
	cfu/mg	*P*-value	cfu/mg	*P*-value	cfu/mg	*P*-value
Louhinneva	3	<0.0001	0	1.0000	0	<0.0001
Isoneva	27	<0.0001	0	1.0000	0	<0.0001
Pahkaneva	10	<0.0001	1	0.2985	1	<0.0001
Saarineva	10	<0.0001	3	<0.0001	4	<0.0001
Konttisuo	33	<0.0001	0	1.0000	1	<0.0001
Luke 1	0	<0.0001	0	0.9283	0	<0.0001
Desinfcted peat, *Desinfioitu turve*	0	<0.0001	0	1.0000	0	<0.0001
White peat, *Kasvuturve*	668		0		865	

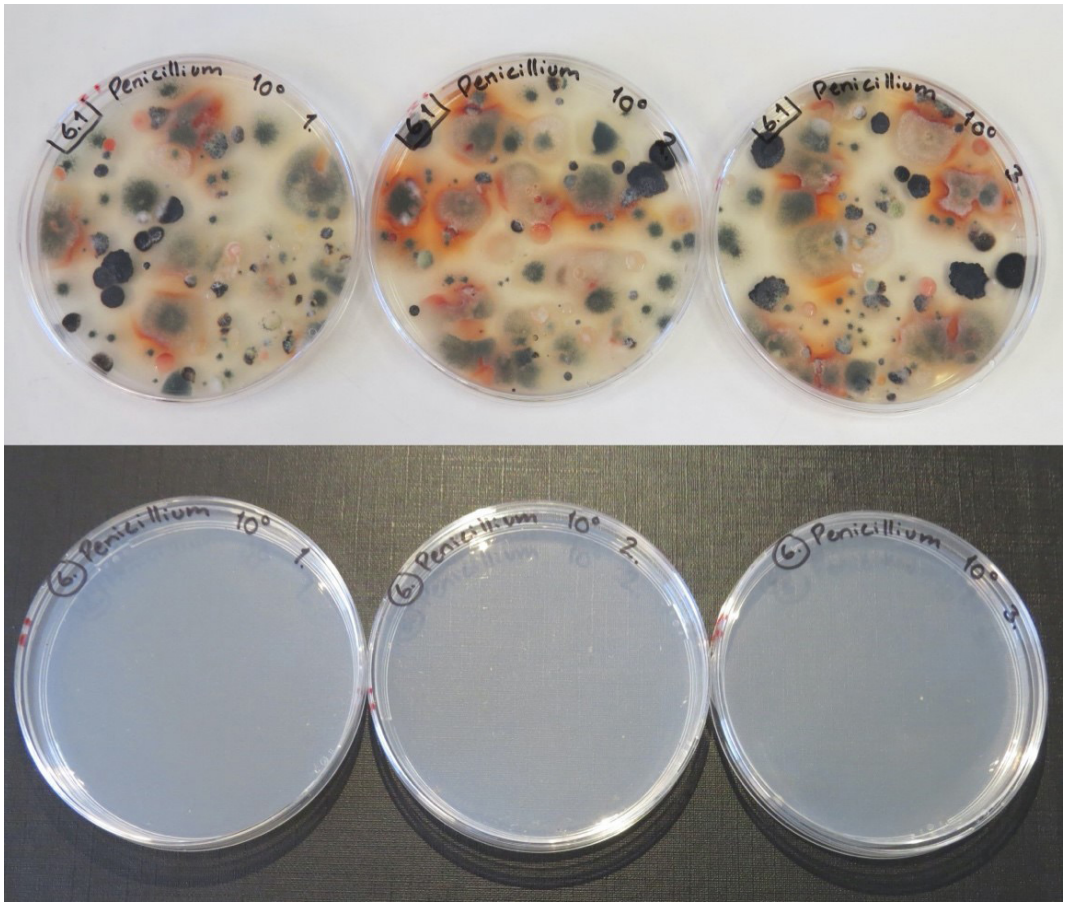

Figure 4. *Penicillium* test on the top 0–10 cm depth of profile in the 2016 Neva-Lyly moss (top) and the 2013 Neva-Lyly moss Penicillium test (below). The 2013 moss was stored dry for 4 years. Stretomycin sulfate in PDA medium 0.02%.

Kuva 4. Penicillium-testi suoprofiilin yläosan 0–10 cm:n syvyydeltä vuoden 2016 Neva-Lyly-sammalesta (ylhäällä) ja Penicillium-testi vuoden 2013 Neva-Lyly-sammalesta (alla). Vuoden 2013 sammalta oli säilytetty kuivana 4 vuotta. Stretomysiinisulfaatti PDA-alustassa 0,02 %.

the profile of 0 to 10 cm was 506 cfu/mg. In the 10–20 cm depth profile, the average *Penicillium* fungi were 228 cfu/mg and in the 20–30 cm depth profile 135 cfu/mg. These figures were still statistically lower than in *Sphagnum* peat, 668 cfu/mg ($P < 0.0001$). This moss had the best disease inhibitory effect in the middle profile (Table 5).

Already in the first studies (Table 1, 2 and 7) the working mechanisms of the disease prevention phenomenon were investigated. Drying temperatures of the moss were 60 and 70 °C for two days. The unequivocal result was that the phenomenon is not based on fungi and bacteria, but on the moss own activity. For this reason, oat flour was added as a stimulant to the growing medium as a detailed test in studies of the especially important *Pythium* disease. Five grams of oatmeal in 50 g of moss stimulate the disease very aggressively. The cucumber seeds did not germinate at all. In moss dried at 70 °C without oatmeal, all the seeds germinated, and the plants remained alive up to 10 days after sowing. In parallel samples of the earlier growing medium with oat flour, only two out of five seeds germinated, and they also died shortly after germination (Table 7). The moss quality study (Figure 3) included four exceptionally pure *Sphagnum* moss, numbers 3, 7, 11 and 12. Sample no. 3 had been stored for seven years in dry storage, when it did not hold living fungi and bacteria (Figure 4, Table 6). In

Table 7. The effect of oat flour on the aggressiveness of the *Pythium* disease of the cucumber in different growth media when the growth media had been dried at a temperature of 70 °C for 2 days. *Pythium* contamination was done directly in the growing medium one week before sowing. Five seeds in a 0.6 liter pot, with 3 repetitions. Average number of seedlings per test pot.

Taulukko 7. Kaurajauhon vaikutus kurkun Pythium-taudin aggressiivisuuteen eri kasvualustaissa, kun kasvualustaa oli kuivattu 70 °C:n lämpötilassa 2 vrk. Pythium-kontaminaatio suoraan kasvualustaan viikkoa ennen kylvöä. Viisi siementä 0,6 litran ruukussa, toistoja 3. Keskimääräinen taimien lukumäärä testiruukussa.

Date, ***Päivämäärä***	**6.4.2014 Emergence** ***Taimettuminen***	**8.4.2014 Healthy plants** ***Terveitä taimia***	**16.4.2014 Healthy plants** ***Terveitä taimia***
Growing mediums without oat flour, *Kasvuaalusta ilman kaurajauhoa*			
Luke1 moss, *Luke 1 sammal*	2.0	0.0	0.0
Dried moss, *sammalen kuivatus,* 70 °C	5.0	5.0	2.0
Dried white peat, *kasvuturpeen kuivatus,* 70 °C	2.3	0.0	0.0
Oatmeal in the growing medium 5 g/l, *Kasvualustassa kaurajauhoa 5 g/l*			
Luke1 moss, *Luke 1 sammal*	0.0	0.0	0.0
Dried moss, *sammalen kuivatus*, 70 °C	0.0	0.0	0.0
Dried white peat, *kasvuturpeen kuivatus*, 70 °C	0.0	0.0	0.0

moss growing media 7, 11 and 12, all plants were completely healthy or only one plant showed symptoms of *Pythium* disease. In these moss samples, the dominant species were other *Sphagnum* than *S. fuscum*, which is the dominant species in the *Sphagnum* peat. On the growing media 3, 4 and 12, the plants remained healthy throughout seedling three weeks cultivation. Although the seedlings grew strongly in the *Pythium* infected growing media, no symptoms of the disease appeared in the cucumber roots and stems. *Pythium* fungus started to grow immediately when these mosses were inoculated onto PDA agars. This showed that the disease was fully alive among the growing media, but the mosses prevented the development of disease symptoms on the test plants.

The determination of the disease-inhibiting effect of moss on PDA medium did not give any useful result, as only one moss sample formed an inhibitory zone between the moss and the *Pythium* fungus (Figure 5). This moss had been stored in a dry place for 6 years, after which no viable microbes were detected in microbial analysis.

Discussion

In all greenhouse experiments, it was found that the amount of seedling diseases increased during cultivation. This result clearly showed that fungal diseases survive in moss, but that their ability to grow is severely retarded. Especially effective were the moss patches, which consisted of almost pure *Sphagnum* moss and where the moss stalks extended to a depth of almost 30 cm, controlled the disease as effectively as the fungicide. This result is completely different compared to similar studies with *Sphagnum* peat, where disease control is based on the peat's own antagonistic microbes (Tahvonen 1982). For this reason, fresh peat samples can hold both highly suppressive batches and susceptible to disease samples at the level of disinfected peat. Therefore, the use of peat in biological disease control has no commercial significance, but biocontrol has to be done with microbes that work in peat, for example *S. griseoviridis* (Tahvonen 1982). Moss harvested from a bog, where there are many shrubs among the moss, contain toxins that disturb the growth of plants. A similar phenomenon is also found in *Sphagnum* peat, but this characteristic develops from the toxins produced in connection with the self-heating of the peat storage and the subsequent cooling of the peat storage, when large amounts of *Penicillium* fungi and bacteria die (Tahvonen and Kemppainen 2008).

A small amount of oatmeal in moss eliminates completely the antifungal effect to *Pythium* sp. Based on this result, no dead, easily degradable organic matter should be added to the moss. Cauliflower experiments show that moss samples did a good or satisfactory job in reducing *Alternaria* disease. *A. brassicicola* silique infection is generally not very aggressive (Rotem 1998). This observation means that weak pathogens are very well controlled by suppressive moss. Soil-borne seedling pathogens are aggressive pathogens (Martin and Loper 1999; Agrios 2005). Now

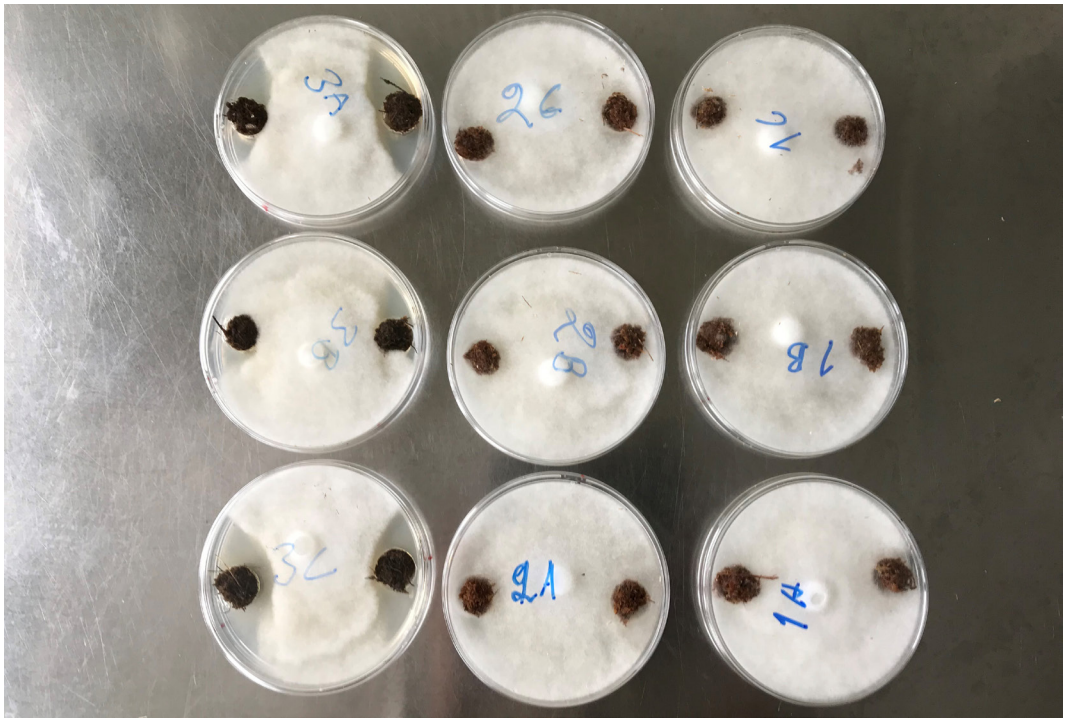

Figure 5. An attempt was made to develop a laboratory method to determine the antifungal effect of moss. *Pythium*-free samples were used in these experiments. The moss samples were shaped into balls with a diameter of approx. 6 mm. They were placed in holes made in agar. After 3 days, the *Pythium* fungus was inoculated in the center of the petri dish. Only one of the mosses tested showed an inhibition zone. This moss was Luke 1, which had been stored for 6 years. It was completely free of live microbes (see Figure 2). 1A–C = Moss peat, 2A–C = 80 °C, 10 h wet moss treatment, 3A–C = moss stored for six years Luke 1.

Kuva 5. Yritettiin kehittää laboratoriomenetelmä sammalen antifungaalisen vaikutuksen määrittämiseksi. Näissä kokeissa käytettiin Pythium-vapaita näytteitä. Sammalnäytteet muotoiltiin palloiksi, joiden halkaisija oli n. 8 mm. Ne laitettiin agariin tehtyihin reikiin. 3 päivän kuluttua Pythium-sieni ympättiin petrimaljan keskelle. Vain yksi testatuista sammalista osoitti estoalueen. Tämä sammal oli Luke 1, jota oli säilytetty 6 vuotta. Se oli täysin vapaa elävistä mikrobeista (katso kuva 2). 1A–C = vaalea kasvuturve, 2A–C = 80 °C, 10 h märkäkuumennettu vaalea kasvuturve, 3A–C = seitsemän vuotta varastoitu Sphagnum Moss Luke 1.

only a few samples of mss or moss dried at a special temperature had the ability to quickly and effectively suppress *Pythium* disease. The result of this implies that the moss batches intended for the control of severe diseases must be carefully selected for cultivation purposes.

In the first studies, the moss samples came from only one bog, so the moss samples could contain moss species that do not belong to the most common bog types. In addition, the moss samples of the 2013 bog clearly differed by species from the moss samples of 2016 and 2019, so there is no reliable correlation between the antimicrobial effects of the mosses of that bog and the mosses of different depth profiles. However, the most important observation from the experiments was that the moss species only belonged to the *Sphagnum* genus. Although different moss samples had varying disease control properties, plants grown in moss were always significantly healthier than those grown in peat. This result is completely different from similar *Sphagnum* peat disease prevention studies (Tahvonen 1979, 1982). Variations in disease control between *Sphagnum* peat lots can be either fully susceptible or fully preventative. In *Sphagnum* peat, disease control is largely based on peat microbes. But some control can come from living moss that

has been mixed with peat during mechanical peat harvesting. This assumption is supported by tests of mixtures of moss and *Sphagnum* peat.

The dominant species in the surface layer of old *Sphagnum* bog and the underlying undecomposed material is *S. fuscum*. it (Laine et al. 2011). In this study, there were moss samples from raised bogs from which the surface layer had been harvested 11, 40 and 65 years earlier. The dominant species of mosses in these bogs were *S. balticum, S. fallax, S. magellanicum, S. papillosum* and *S. pulchrum*. The volumes of the shares varied between 60 and 20 percent. The growing medium made from the moss samples of these bogs completely controlled the *Pythium* disease of the cucumber. This research result is significant, as it shows that the regenerated moss is of the best possible quality of growing medium in prevention of plant diseases. The growing properties of the plants in regenerated moss are also good, even better than that of the *Sphagnum* peat (Silvan 2020). These results are of significant importance for the future in the production of good quality moss, because moss can be grown in a bog that has already been harvested once.

The effect of *Sphagnum* moss on plant diseases is based almost exclusively on the substances secreted by the living moss, which prevent or limit the activity of pathogens. This mechanism of action was highly relevant in the current studies. The findings included microbial assays, high temperature drying of mosses, plant disease tests, and laboratory and microscopic examinations of cauliflower and cucumber seedlings. One of the most significant research results was the phenomenon where drying *Sphagnum* moss at a temperature of around 60–70 °C multiplies the effectiveness of the disease control result. This technique has numerous cultivation applications to combat fungal diseases and prevent the growth of harmful fungi on the surface of the growing medium. For this reason, the drying method, which increases the prevention of diseases and the utilization of the phenomenon in various commercial applications, is protected by a patent (Tahvonen et al. 2020). The most special example is the control of liverwort (*Marchantia polymorpha* L.) in the cultivation of seedlings in nurseries, when a 1–2 cm layer of living moss is placed on the surface of the growing medium of seedling pots (Särkkä and Tahvonen 2020).

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Authors' contributions

Risto Tahvonen has written the manuscript, planned, and implemented studies in greenhouses and in the laboratory. Niko Silvan has mapped the mires suitable for research, collected moss samples and determined the key botanical compositions of the collected mosses.

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Sphagnum-sammaleen sienilääkepotentiaali kasvualustoissa

Kanelihome (*Peziza ostracoderma* Korf) ja *Penicillium* spp. ei koskaan kasva elävän *Sphagnum* sammalalustan pinnalla. *Sphagnum* sammalalustan sieniä estävä vaikutus toimii myös tärkeimpiä maaperän ja siemenistä leviäviä kasvien sienitauteja vastaan. Hyviä esimerkkejä estovaikutuksesta ovat siemenlevintäinen *Alternaria*-taimipoltteen torjunta kaalin taimista ja maaperässä leviävät *Pythium*-juuristotaudit kurkulla. Taudintorjuntavaikutus vaihtelee huomattavasti soiden välillä. Tehokkain sammalerä sai lähes täydellisen suojan *Pythium*-taimipoltetta vastaan. Parhaat antifungaaliset ominaisuudet löytyivät lähes puhtaista *Sphagnum* sammalseoksista, jotka olivat muodostuneet viimeisten 11, 40 ja 64 vuoden aikana edellisen sammalkorjuun jälkeen, ja hallitsevat lajit olivat muita kuin kohosuon yleisin laji *Sphagnum fuscum* (Schimp). H. Klinggr. Kun sammalta kuivattiin 60º tai 70 °C:n lämpötilassa, antifungaalinen vaikutus parani merkittävästi. Taimet pysyivät terveinä vähintään kaksi viikkoa, kun taas suuri osa taimista sairastui käsittelemättömissä sammaleissa. Kun *Sphagnum* sammalta pidettiin kuivana, antifungaalinen ominaisuus pysyi muuttumattomana useita vuosia. Pisin mitattu säilyvyysaika oli kuusi vuotta. Sammaleiden mikrobipitoisuudet osoittivat, että sammal, joka oli korjattu 10–30 cm:n syvyydestä tai pidetty kuivana 4 vuotta, oli käytännössä vapaa elävistä sienistä ja bakteereista. Soiden ylemmästä 0–10 cm:n kerroksesta korjattu sammal sisälsi mitattavissa pitoisuuksia *Penicillium* spp. sieniä ja bakteereita, mutta pitoisuudet olivat vain murto-osa vaalean *Sphagnum*-turpeen pitoisuuksista. Kun kaurajauhoa lisättiin voimakkaasti tauteja hillitsevään sammaleeseen, sammalen tauteja ehkäisevä vaikutus hävisi kokonaan. Sammalnäytteissä, jotka olivat luonnostaan täysin estäneet *Pythium*-taudin aktiivisuuden kahden viikon ajan, *P. ultimum* Trow -sieni oli täysin elossa, kun sammalnäyte siirrettiin runsaasti ravinteita sisältävään perunadekstroosiagariin (PDA). Mikrobimääritysten ja lämpökäsittelykokeiden perusteella *Sphagnum* sammalen antimikrobinen ominaisuus perustuu lähes yksinomaan elävän sammalen itsensä tuottamiin kemikaaleihin ja vaikutustapa sienitauteja vastaan on fungistaattinen (antifungaalien). Vain yksi sammalnäyte tuotti antibiootin kaltaisen estoalueen patogeenille PDA-maljalla. Tätä sammalta oli säilytetty kuivassa 6 vuotta, jolloin siinä ei ollut häiritseviä mikrobeja.