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Allelopathy in bryophytes – a review

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Allelopathy in bryophytes shapes ecosystems by influencing the species composition of both vascular plants and other bryophytes. Several allelopathically active chemicals in bryophytes have been discovered since the latter half of the 20th century and laboratory studies have showed their inhibiting impact on germination, growth and establishment of surrounding plants. However, other studies failed to demonstrate these effects. In the field, other properties of bryophytes might have stronger impacts, such as mechanical obstruction or alterations in temperature. In laboratory studies, water might not be an adequate extractant for active substances, since all of the chemicals claimed to be allelopathic are lipophilic with potentially longer retention times of the active substances in the soil when compared to water-soluble substances.

The plants collectively known as ‘bryophytes’, i.e. mosses (phylum Bryophyta), liverworts (phylum Marchantiophyta) and hornworts (phylum Anthocerotophyta) comprise the second largest group of plants after the angiosperms (Nozaki et al. 2007). Derived from algal ancestors, they represent the earliest divergent phylogenetic branches among terrestrial plants. As the first inhabitants of terrestrial habitats they were by default growing on bare ground or rock and many species are still primary or secondary colonizers. Their ancestors have been fighting for space for hundreds of millions of years, resulting in mechanisms which have evolved for both exploitive competition (monopolization of light and nutrients) and interference competition (by chemical warfare). In general, bryophytes display a low morphological complexity, but a high degree of chemical diversification (Huneck 1983, Asakawa et al. 2013), suggesting that secondary substances may play an important role in plant-to-plant interactions. Since bryophytes are abundant in many ecosystems, it is vital to understand how their production of allelopathic substances may influence the composition of communities.

The term ‘allelopathic chemical’ refers to certain secondary metabolites in plants, algae, bacteria, coral and fungi that are not required for basic metabolism. Such chemicals play important roles in defense against herbivory, plant–microbe relations and – as in the case of allelopathy – competition with other plants (Willis 2007). It should be noted that alle-

lopathic impacts upon lichens are beyond the remit of this paper. The competitive advantages of allelopathy affect species distributions, in extreme cases leading to dominance of a single species in a natural ecosystem or contributing to the invasive potential of introduced species (Koocheki et al. 2013). This might explain the dominance of bryophytes over vascular plants under certain conditions (Kato-Noguchi and Seki 2010).

Allelopathic interactions have been studied in vascular plants since the beginning of the 20th century, while the first studies of allelopathic chemicals in bryophytes followed several decades later (Watson 1981). Several substances isolated from liverworts were shown to be phytotoxic, inhibiting germination and growth of vascular plants in standard lab tests (Huneck 1983, Asakawa 1982, 1990, 1995, 2007). This toxicity inspired the search for economically valuable compounds, such as antitumor agents (Spjut et al. 1986) or natural sources of herbicides (Nozaki et al. 2007). In this review we focus on bryophyte–vascular plant and bryophyte–bryophyte allelopathic interactions and how such interactions may influence recruitment, competition and ultimately plant community assembly.

Methodology

The search for literature was non-systematic, but nonetheless comprehensive, using bibliographic data-bases including Google Scholar and Web of Science. In a second step, reference lists of retrieved articles were scanned, as well as manuals of bryophyte chemistry such as Huneck (1983) and Asakawa (1995, 2007) and Asakawa et al. (2013). The literature included in the review had to fulfill the conditions of treating the subject of both bryophytes and allelopathy in a wide sense (including phytotoxicity).

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Pioneering observations during exploration of bryophyte secondary chemistry

Early studies focused on the detection of the chemical nature of allelochemicals. In a series of bioassays Huneck and Schreiber (1972) tested the effects of secondary substances derived from liverworts (gymnocolin, drimenol, longiborneol, longifolen, lunularic acid and scapanin; Table 1.) on the growth of *Lepidium sativum* L. (cress) roots, *Avena sativa* L. (oat) seedlings and oat coleoptiles. Growth was compared against controls with no substance added. They found that some substances consistently either decreased or increased the growth relative to controls. Other substances decreased growth in high concentration (10^{-3} M) but promoted growth in lower concentrations. The effects of the substances were not consistent across assays. For example, the inhibitory effects on growth were more pronounced for cress than for the other assays, and some substances had inhibitory effect on cress growth, but promoted growth of oats, relative to the control. Several other substances have been subject to standard assays in vitro, which suggest more or less strongly inhibitory effects. For example, several sesquiterpene lactones extracted from the thalloid liverwort *Conocephalum conicum* displayed complete inhibitory activity towards germination and growth of rice at concentrations down to 50–200 ppm (Asakawa and Takemoto 1979). The sesquiterpenoid (+)-vitrenal isolated from the liverwort *Lepidozia vitrea* inhibited growth of leaves and roots on *Oryza sativa* L. (rice) seedlings at a concentration of 25 ppm (Matsuo et al. 1980). Asakawa (1995) showed inhibitory effects of isobicyclogermaacrenal, lepidozenal and (+)-vitrenal on rice seedling growth. In a later manual (2007), he even went as far as to state that almost all crude extracts which contain “bitter or pungent” substances also have phytotoxic properties. In these early studies the replication is often poor and the data is tabulated without statistical tests. The results are accordingly difficult to evaluate, especially considering that the studies do not yet reveal to what extent the substances are actually released to the environment in biologically active concentrations.

Indications of bryophyte–bryophyte interactions

Bryophyte species have been shown to inhibit one another and also conspecifics. A ground-breaking experiment was undertaken by Watson (1981). Based on field observations, she proposed that the distribution of six *Polytrichum* species was due to “differential aggressiveness amongst juveniles” rather than competition between adults. This motivated her to sow spores of the moss *Funaria hygrometrica* at different distances on agar plates to observe protonemal development. She could see that the developing protonema released a “factor” that prevented different clones of conspecific protonema to grow into each other. This factor gradually accumulated with protonemal age and spores sown at the same time in the same spot were subject to minimal amounts of the substance and therefore grew normally to form a composite colony. This was the first concrete evidence of direct negative interactions between developing bryophytes, taken as proof of allelopathy, and the growth factor was identified as “cytokinin-like”, tentatively called factor H. Little is known about this factor H and one or more factors with similar effects may exist (Glime 2015) – few modern comparable studies have been conducted and the documentation is generally poor.

In the following years similar in-vitro experiments as well as field studies were carried out to explore how widespread such interactions could be. Sporadic observations summarized in Newton and Mishler (1994) suggested that spores are prevented from germination in mature moss colonies. Kimmerer (1991) observed that gemmae and spores of the fugitive species *Tetraphis pellucida* Hedw. germinated in patches of bare decaying wood but not in patches occupied by the competitors *Dicranum flagellare* Hedw. or *Hypnum imponens* Hedw. Longton and Miles (1982) noted that conspecific spores persisted un-germinated but viable for a year in cushions of the moss *Grimmia pulvinata* (Hedw.) Sm.

Cronberg et al. (2006) analyzed the clonal identity of all shoots in a large colony of *Hylocomium splendens* (Hedw.) Schimp. known to have produced spores annually for at least five years and found that the whole colony consisted of

Table 1. Examples of allelopathic chemicals in bryophytes. A somewhat larger number of substances have been claimed to have phytotoxic effects, but we have chosen to include those that have a comparatively solid documentation.

Aromatics	Flavonoids	vitexin, apigenin, apigenin-7-O-triglycoside, luteolin-7-O-neohesperidoside, saponarine, lucenin-2, bartramiaflavone	Basile et al. 2003
	Bibenzyl derivative	lunularic acid	Huneck and Schreiber 1972
	Phenolics	not specified	Soudzilovskaia et al. 2010
Terpenoids	Diterpenoids	gymnocolin, scapanin, 16 α -kaurenol perottetianal momilactone A and B	Huneck and Schreiber 1972 Asakawa 1990 Nozaki et al. 2007
	Sesquiterpenoids	drimenol, longiborneol, longifolene	Huneck and Schreiber 1972
	Sesquiterpenoids, drimanes	polygodial	Asakawa 1982, 1990
	Sesquiterpenoids	germacrane, isobicycligermaacrenal, lepidozane, lepidozenal, (+)-vitrenal	Ando and Matsuo 1984
	Sesquiterpene lactones, eudesmanolides	guaianolides, deoxyzaluzanin, diphyllolide, 7 α -hydroxydiphyllolide, 3-oxodiphyllin, frullanolide	Asakawa and Takemoto 1979 Asakawa 1982, 1990
	Sesquiterpene lactones, germacranolides	zaluzanin C, zaluzanin D, 8 α -acetoxyzaluzanin C, 8 α -acetoxyzaluzanin D,	Asakawa 1982, 1990
	Sesquiterpene secoaromadendrane-type	plagiochiline A, plagiochiline C, ovalifolienal, ovalifolienalone, 9 α -acetoxyovalifolienal	Ando and Matsuo 1984
	Trinorsesquiterpenoid	3-hydroxy- β -ionone	Kato-Noguchi and Seki 2010

no more than three clones that were only distantly related. Spore germination may be prevented because the mature shoot is monopolizing available nutrients, but several observations suggest that some form of more advanced chemical interaction is involved.

In an important study, Mishler and Newton (1988) documented strong inhibition of both spore germination and fragment growth in a material consisting of several species of the genus *Tortula* (since then transferred to the genus *Syntrichia* as *S. ruralis* (Hedw.) F. Weber & D. Mohr, *S. princeps* Mitten, *S. norwegica* Weber and *S. laevipila* Bridel) when applied onto living clumps of either *Tortula* itself or onto the unrelated species *Dicranum scoparium* Hedw. They were able to repeat the results when filter-sterilized water extracts of each species were applied onto spores on agar. Patterns of inhibition were similar to the preceding experiment, suggesting that the effects were mediated by chemical interaction rather than physical properties of the moss clumps. Attempts to document the presence of bioactive chemicals by application of substances dissolved from mature moss shoots on germinating spores rendered unclear results. This could have several different explanations, amongst them that the chemical leachate may be labile and have to be produced continuously to exert an effect (Newton and Mishler 1994).

Recent research suggests that bryophytes discriminate between species in their allelopathic reaction. Many mosses belonging to several unrelated lineages have a breeding system involving tiny dwarf males, which cling onto the shoots of normal-sized females where they assist in fertilization of the females. Rosengren and Cronberg (2015) showed by experimental application of spores that female shoots of the pleurocarpous moss *Homalothecium lutescens* allowed germination and development of dwarf males from conspecific spores as well as spores of the closely related species *H. sericeum* (Hedw.) Schimp., whereas spores of the unrelated species *Isoetecium alopecuroides* (Dubois) Isov. failed to develop dwarf males. In theory, this selective development of dwarf males could be related to e.g. differences in pH, but more likely some more complex allelopathic recognition system is involved. With this breeding system the female is actually promoting germination of conspecific male spores, in contradiction to the cases cited above which suggest that gametophytes in general suppress spore germination whether conspecific or not.

Experimental studies on bryophyte–vascular plant interactions

Further investigations of bryophytes showed that allelopathy targets not only other bryophytes, but also the germination of seeds of vascular plants (Hein 1966, van Tooren 1990, Basile et al. 2003, Löbel et al. 2006, Soudzilovskaia et al. 2010). Many of these studies appear to be triggered by the search for plants that produce substances that could potentially be used as natural herbicides, e.g. a number of Chinese articles (reviewed by Liu 2014). The studies are typically undertaken by soaking bryophytes in water and subjecting the seeds to water extracts. In many cases such water extracts produce results that are difficult to interpret or counterintuitive.

Huneck and Meinunger (1990) used a very basic approach. They added seeds of kress to fresh, moistened bryophytes (52 mosses and 29 liverworts) and measured the lengths of the kress roots and shoots after five days at room temperature. They found three different kinds of reactions: 1) bryophytes that promote growth of the shoot, 2) bryophytes that promote growth of the root and 3) bryophytes that retard growth of both roots and shoots. Frahm et al. (2012) noted that there was a risk that the reactions were caused by microorganism associated with the bryophytes rather than the bryophytes themselves. To avoid this problem, they prepared liquid extracts by soaking some bryophytes (*Porcella platyphylla*, *Eurhynchium striatum*, *Dicranodontium denudatum* and *Brachythecium rutabulum* in tap water and distilled water for 12 h prior to germination tests involving kress and *Lactuca sativa* (lettuce). The bryophytes had been stored in a dry condition before the experiment, which may have influenced the leaking of substances. Like Huneck and Meinunger they found indications of both negative and positive influence on the germinating seeds, but the effects were sometimes indifferent to variations in concentration of the extracts. Extracts in distilled water gave the strongest effect, which was explained by higher dissolving capacity. They made a second experiment where they compared extracts from the liverwort *Bazzania trilobata* when soaked in water and in ethanol. They found somewhat stronger reactions with ethanol than with water when the same concentrations were compared.

Yet more sophisticated, Tsubota et al. (2006) used an assay called the sandwich technique to test *Dicranum japonicum*, *Hypnum plumaeforme*, *Racomitrium japonicum* and *Sphagnum palustre* for allelopathic activity at germination of lettuce seeds. They dried the moss material either at room temperature (with silica gel) or at 80°C, ground the dry mosses to a rough powder and embedded powders of individuals species within agar in micro-well plates, with a second agar layer atop to avoid direct contact between the moss tissue and the seed. They found significantly reduced *Lactuca* radicle elongation compared to the control for all species with exception for *R. japonicum*, with *D. japonicum* showing the strongest effect. Hypocotyl elongation was unaffected or promoted for all species with exception for *D. japonicum*, which again showed a strong inhibitory effect. The effects (positive or negative) were in general somewhat stronger for replicates dried by silica gel as compared to replicates dried in 80°C, suggesting that potential allelopathic substances were mildly sensitive to heat.

Basile et al. (2003) studied the impacts of gametophyte extracts of the moss *Tortula muralis* upon conspecific spore germination and protonemal development as well as on seed germination and root development of the angiosperm *Raphanus sativus* L. Seven flavonoids were extracted (Table 1) and it was found that each one of these induced a slow-down of the growth of *Tortula* and a significant decrease in germination percentage of its spores. Interestingly, both of these influences were found to be dosage dependent. Other signs of retarded protonemal growth occurred in relation to flavonoid presence; swollen tips as well as swollen and shortened intercalary cells were seen, particularly during the first days of culture. Early occurrence of brood cells on protonemal filaments, a common response to environmental stress, was

also seen in *Tortula*. Similar to *Tortula*, the percentage of *R. sativus* seeds germinating was reduced by flavonoid presence. Root elongation and root hair growth were also inhibited, with the flavonoid saponarin having the most dramatic effect.

Highlighting specific bioactive substances

Over time research progressed from mostly effect-oriented studies to characterizing the chemicals. The first allelochemical compounds to be described in mosses were claimed to be the terpenes momilactone A and B in a study by Nozaki et al. (2007; Table 1). Momilactone A and B were previously known as allelopathic chemicals from *Oryza sativa* (rice). Nozaki et al. realized that *H. plumaeforme*, like liverworts, contains oil bodies in the leaf cells, while mosses at this point were thought to be devoid of distinct oil bodies. This led them to examine inhibitory effects of ethyl acetate extracts of *Hypnum plumaeforme* on different plant species (*Arabidopsis thaliana* (L.) Heynh., *Nicotiana tabacum* L., *Jungermannia subulata* A. Evans, *Physcomitrella patens* (Hedw.) Bruch & Schimp. and *H. plumaeforme* itself) and comparing them with the effects of momilactone B. Both extracts and momilactone B showed inhibition of other species, but not of *H. plumaeforme* itself, pointing explicitly to an allelochemical effect of momilactone B.

Sharma et al. (2009) used aqueous and two different lipophilic extracts of eight bryophytes in order to find out more about the chemical nature of allelochemicals. The studied bryophytes were *Targionia hypophylla*, *Marchantia polymorpha*, *Plagiochasma appendiculatum*, *Brachythecium buchananii*, *Leucodon secundus*, *Timmia anomala*, *Rhodobryum roseum* and *Plagiomnium integrum*, the effected plant was *Bidens biternata* (Lour.) Merr. & Sherrif. They found that inhibition of germination of the vascular plant was strongest in methanol extract, followed by acetone extract, while effects of the aqueous extract were minor. The lack of hydrophilic allelochemicals is surprising at first as the chemicals could be released (via volatilization, leaching, decomposition of residues and root exudation) far more easily if they were hydrophilic (Sharma et al. 2009). However, they would also leach faster from the soil, which could be a possible disadvantage for the allelopathic activity and thus an explanation for their hydrophobic chemical nature.

3-hydroxy- β -ionone was stated to be another allelopathically active chemical. *Rhynchosyrium pallidifolium* inhibited cress grown on agar medium stronger at close proximity to the moss tissue than at a more remote position. This correlates with higher values of 3-hydroxy- β -ionone closer to the moss tissue (Kato-Noguchi and Seki 2010). Furthermore, 3-hydroxy- β -ionone applied exogenously showed inhibitory effects on cress. This suggests that the chemical is in fact allelopathically active.

Relating allelopathy to ecological aspects

Recent research brought progress in connecting effects of chemical compounds in bryophytes to ecological aspects.

Van Tooren (1990) found that the numbers of emerging seedlings of angiosperms were reduced up to 30% in the presence of a bryophyte layer, both in field conditions

and under controlled conditions in a greenhouse. This result could partially be explained by changed light environment in the moss carpet, increased risk for mortality of seed due to fungal infections or monopolization of nutrients by the high ion exchange capacity of bryophytes. However, allelopathic interaction was put forward as a supplementary explanation.

When studying the regeneration niche of *Pinus sylvestris* (Scots pine) in boreal forest, Steijlen et al. (1995) found reduced recruitment from seeds in sites dominated by *Pleurozium schreberi* as compared to sites dominated by *Cladonia* spp. In a follow-up lab experiment they found observed significant inhibition of *P. sylvestris* seeds when grown in petri dishes in contact with living shoots of *P. schreberi*. However, this effect was lost when pre-germinated seedlings (with 1-mm radicles) were tested and when they attempted to germinate seeds with water extracts from the moss. They interpreted that the negative influence of *P. schreberi* is a combined effect of moisture factors, chemical interaction and its ability to monopolize nutrients.

One important aspect of allelopathy is the way in which it is modified by environmental variation. Löbel et al. (2006) observed at periodically dry grasslands on Öland an increase in species richness of bryophytes at the expense of vascular plant cover. Of course, this may have been due simply to environmental conditions favouring bryophytes, but some further evidence pointed to stronger effects of allelopathy under dry conditions. Zamfir (2000) investigated the way in which environmental variation influenced the germination of seeds in 'bryophyte carpets' in a series of greenhouse experiments. Despite somewhat varying results, Zamfir concluded that there was a general trend towards bryophyte mats inhibiting seedling emergence of some grassland species (particularly *Veronica*), this tendency being exaggerated under dry conditions. Although these findings were placed in the context of allelopathy, Zamfir admits that the physical properties of the differing substrates may also have had a large influence on seed germination. In a contradiction of Zamfir's findings, Otsus and Zobel (2004) reported a positive effect of removal of bryophytes on vascular plant germination only in moister conditions.

Soudzilovskaia et al. (2010) studied effects of bryophyte traits, including release of phenolics (in a wide sense), on the germination of vascular plant seeds. They focused on differences between six bryophyte species (*Barbilophozia lycopodioides*, *Ptilidium ciliare*, *Dicranum scoparium*, *Hylocomium splendens*, *Pleurozium schreberi* and *Polytrichum strictum*) in their effect on 10 vascular plant species (*Dryas octopetala* L., *Empetrum nigrum* L., *Vaccinium myrtillus* L., *Betula pubescens* Erh., *Pinus sylvestris*, *Epilobium angustifolium* L., *Silene dioica* (L.) Clairv., *Solidago virgaurea* L., *Carex rostrata* Stokes and *Deschampsia flexuosa* (L.) Trin.) from a subarctic heath-woodland. In a field experiment, they collected cushions of the bryophyte species, replanted them and sowed seeds of the different species of vascular plants under the moss cushions. A laboratory experiment accompanied the field experiment, growing the bryophytes on glass fiber filters and analyzing the filters for total phenolic content using the Folin-Ciocalteu method. In the laboratory experiment they found that germination of vascular plants differed between bryophyte species and was reduced in relation to concentrations of phenolics. Bryophytes suppressed germination also in the field experiment, but not in relation to concentration of phenolics since

no difference was found between bryophytes species. The differences in seed germination were actually best explained by early spring soil temperature mediated by the insulating effect of bryophyte mats and their thicknesses. The discrepancy between the laboratory study and the field study regarding the phenolics may partially be explained by different approaches to collect the phenolic substances and possible degradation occurring in the field experiment. The authors highlight the difficulties of drawing conclusions about complex ecological processes based solely on laboratory experiments.

Lett et al. (2017) used the same Fiolin–Ciocalteu method to estimate total content of phenols in eight bryophytes in a study which compared performance of young seedlings of pine and birch under conditions simulating temperature conditions at the alpine tree line in current (7.0°C) and near future expected (9.2°C) temperature averages. They found that the amounts of phenols in water extracts from the bryophytes differed significantly between species with the highest amounts measured in *Pleurozium schreberi* and *Hylocomium splendens*. However, the variation was uncorrelated (birch) or positively correlated (pine) with the response variables (biomass, N-uptake), especially at the higher temperature. If anything a high content of phenols seemed to favour seedling growth, but other bryophyte traits appeared to be more important, similar to the conclusions in Soudzilovskaia et al. (2010). The authors noted that seedlings and seeds might respond differently to bryophyte traits.

Michel et al. (2011) could connect the effect of bryophyte chemicals on vascular plants shown in a laboratory experiment to field conditions. They tested the effects of 17 species of bryophytes (Supplementary material Appendix 1 Table A1) on three species of vascular plants (*Lactuca sativa*, *Melicytus ramiflorus* J. R. Forst & G. Forst. and *Fuchsia excorticata* (J. R. Forst & G. Forst) L.f.). Leachates soluble in water were obtained from dried bryophytes and their influences on seed germination and seedling growth were measured. The influence of the leachates was very variable between the three vascular plant species. The impact on seed germination ranges from minor (*L. sativa*) to both stimulatory and inhibitory depending on the bryophyte species (*M. ramiflorus*) and inhibitory effects (*F. excorticata*). Radicle growth was inhibited in all three species, but for *L. sativa* only after reaching a critical concentration of bryophyte leachates. In the field, patterns of seedling occurrence were investigated. They found that seedling density on bryophyte cushions varied between the bryophyte species being lowest in stands of *Dendrohypopterygium filiculiforme*. Michel et al. related their observations to allelopathy, however, they failed to take other properties of bryophytes into account as done by Soudzilovskaia et al. (2010).

Ingerpuu and Vellak (2013) investigated interspecific impacts in growth and morphology among three *Sphagnum* species *S. magellanicum*, *S. teres* and *S. wulfianum*. Only *S. wulfianum* showed significant changes in its morphology when grown together with neighbours (reduced height, higher weight, smaller capitula, and a denser branch arrangement). The authors came to the conclusion that this response is non-adaptive as the altered morphology conveyed no competitive advantage. A low competitive strength might explain the rarity of this moss; even in suitable habitats it has a sparse distribution. They discuss that this observation may explain

why some bryophytes (such as *S. wulfianum*) with a wide distribution range are still restricted to a few places within their respective ranges.

Bu et al. (2017) examined three common peatland moss species from the northern hemisphere; *Polytrichum strictum*, *Sphagnum palustre* and *S. magellanicum*. *Polytrichum strictum* is showing an increased presence in peatlands in China. They aimed to discover whether this was due to higher initial germination rates, or to allelopathy. In a laboratory experiment spores were stored in hummocks of all other mosses, after which germination success was assessed. They conclude that it was indeed allelopathy that conveyed the competitive advantage for two reasons. Firstly, *P. strictum* was found to be highly allelopathic, to the extent that even its own spores grow better in *Sphagnum* hummocks than in *P. strictum* hummocks. Secondly, *P. strictum* showed lower germination rates than *S. palustre*. It should however be considered that *P. strictum* produces more spores than *S. palustre* and *S. magellanicum*. *Sphagnum magellanicum* was shown to have the weakest allelopathic effect of the three. Bu et al. (2017) also investigated the effect of the water table upon germination, higher germination was found in the treatment with lower water availability. They suggest one possible explanation for this could be that allelopathic impacts are reduced in dryer conditions. This has interesting implications for future peatland development as many peatlands face water loss due to climate change.

Discussion

The impact of allelopathy on ecosystems is important to understand when assessing species distribution and competitive interactions. Despite this, there is still much work to be done on understanding to what extent allelopathic chemicals in bryophytes exert a relevant impact in the field. Although there are many other factors that influence the competitive ability of a plant species, it does not take long to find studies where the impact of allelopathic chemicals is neglected. For example, Rydin (1997) used morphological traits such as shoot size, shoot density and branching type to explain how different *Sphagnum* species became dominant under various environmental conditions. In the light of findings such as those of Watson (1981) about allelopathic interactions upon moss recruitment or those of Ingerpuu and Vellak (2013) about interspecific impact on morphology in peat mosses, it seems possible that elements of interference competitions through allelopathy is equally important as the more apparent and experimentally tractable effects of exploitation competition. Vice versa, the ignoring of certain factors in laboratory experiments decreases the use of the findings in the field. For example Michel et al. (2011) claim to be able to relate their results from the laboratory to field conditions, however they did not take into account the mechanical and physical obstructions that bryophyte-cushions can represent for vascular plant seedlings, such as the effects of temperature demonstrated by Soudzilovskaia et al. (2010).

Apart from the study by Huneck and Meinunger (1990) – which sampled a broad number of species for a rather narrow experimental scope – a small and strongly taxonomically biased group of bryophytes have been tested for allelopathy so far (Supplementary material Appendix 1 Table A1). For example,

Hypnales, Polytrichales and Sphagnales are overrepresented in the articles reviewed here. This attention could probably be explained by the fact that these orders contain many species that are dominant in the bottom layer of various habitats. However, more knowledge about representatives from other bryophyte lineages could bring interesting new insights and reveal to what extent the allelopathic substances are unique or diverse relative to the phylogenetic diversity.

Despite these limitations, a picture of the usage of allelochemicals by bryophytes is starting to emerge. Since the beginning of research into this topic several chemicals with effects under lab conditions on both other bryophytes and vascular plants have been identified. Allelopathic effects could be induced by hormones or hormone-like substances such as “factor H” (Watson 1981) that are actively or passively released to the surroundings. Such substances are likely to exert a similar influence on conspecific and alien shoots. More efficient would be production of substances that are specifically targeted against alien plants, such as in the case of momilactone B and *Hypnum plumaeforme* (Nozaki et al. 2007). At present, most of the substances claimed to have allelopathic effects are not known as plant hormones. The fact that some observations point to a promoting effect in low concentrations but a retarding or inhibitory effect in high concentrations may indicate that these substances behave like hormones.

All of the putatively allelochemical substances in the papers reviewed here are lipophilic and the amounts released from the plants to the soil can vary with environmental conditions. Otsus and Zobel (2004) suggest that inhibition of vascular plants by bryophytes is increased by moist conditions. This appears to be contradictory to the findings of Löbel et al. (2006) and Zamfir (2000) who propose allelopathy is accelerated by drought. However, the effects of the first study could be due to non-allelopathic obstructions (with bryophyte cushions growing bigger and tighter in moist conditions). Furthermore, the latter found allelopathy to be exacerbated when followed by heavy rain. Bryophytes are well known to be poikilohydric (unable to regulate cytosol water content under dry conditions) and a rain following a drought episode can wash an excess of secondary chemicals into the soil (Michel et al. 2011). In the soil the lipophilic nature of the substances may be advantageous as such substances are far less mobile than hydrophilic compounds. As a result they may reside longer in the soil and remain closer to the releasing plant. From this perspective, it is unfortunate that it has been common practice to use aqueous extracts in the assessment of allelopathic chemicals, especially since Sharma et al. (2009) showed that polarity of the extracting medium actually mattered. Soudzilovskaia et al. (2010) found a way to reproduce close-to-natural conditions by using living plants and collecting the exudates in filters that they used as substrates for growth. It might be interesting to try to bring experiment designs even closer to natural conditions by inclusion of variation in water accessibility. Such experiments might reveal the conditions that promote leakage of allelopathic substances.

The research covered in this review is of great potential practical interest, since observations of bryophyte allelopathy could lead to novel natural herbicides for agricultural use. To some extent, the optimism in this respect seems to have faded, herbicidal effects are only briefly mentioned in

recent compilations of bryophyte secondary chemistry, e.g. Asakawa et al. (2013), maybe because of somewhat conflicting results and the fact that high and low concentrations of many substances appear to have divergent effects on growth of vascular plants. On the other hand, Frahm et al. (2012) points to another potentially interesting use in the commercial seed industry, in which bryophyte allelochemicals may serve the double function of promoting seed germination and providing protecting against fungal attack.

An area not covered in this review is the growing evidence that bryophyte growth and development is influenced by symbiotic or otherwise associated fungi and other microorganisms. It should be noted that such hidden interactions could have profound effects on experimental studies. To control for this, it is necessary to perform experiments under axenic conditions, which is tractable for bryophytes since sterile cultures are possible to obtain from surface-sterilized spore capsules (Duckett et al. (2004).

Conclusions

Bryophytes produce a wide array of secondary substances, some of which have been shown to have phytotoxic activity even in low concentrations. Given this fact, allelopathy remains relatively understudied. There is documented knowledge about the impact of bryophyte allelopathic chemicals on some vascular plants under laboratory conditions, but less about impacts upon other bryophytes. Research in this area has just begun in the last years and focused on a few related groups. In particular, we know little about the mechanisms controlling selective spore germination of conspecific and alien spores in contact with mature gametophytes or developing protonema. It is necessary to undertake both in-vitro studies in controlled environments and well-designed field experiments in order to understand the importance of allelopathic substances in relation to other bryophyte traits. As demonstrated most research so far focused on a few related groups of bryophytes. Therefore another scope for future research would be to investigate the less well studied majority of bryophyte groups.

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Supplementary material (available online as Appendix L-1097 at <www.lindbergia.org/readers/appendix>). Appendix 1.