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# Air humidity thresholds trigger active moss spore release to extend dispersal in space and time

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## Summary

**1.** Understanding the complete dispersal process is important for making realistic predictions of species distributions, but mechanisms for diaspore release in wind-dispersed species are often unknown. However, diaspore release under conditions that increase the probability of longer dispersal distances and mechanisms that extend dispersal events in time may have evolutionary advantages.

**2.** We quantified air humidity thresholds regulating spore release in the moss *Brachythecium rutabulum*. We also investigated the prevailing micrometeorological conditions when these thresholds occur in nature and how they affect dispersal distances up to 100 m, using a mechanistic dispersal model.

**3.** We show that moss spores were mainly released when the peristome teeth were opening, as relative air humidity (RH) decreased from high values to relatively low (mainly between 90% and 75% RH). This most often occurred in the morning, when wind speeds were relatively low. Surprisingly, the model predicted that an equally high proportion of the spores would travel distances beyond 100 m (horizontally) when released in the wind conditions prevailing during events of RH decrease in the morning, that lead to peristome opening, as in the highest wind speeds. Moreover, a higher proportion of the spores reached high altitudes when released at the lower wind speeds during the morning compared to the higher speeds later in the day, indicating a possibility for extended dispersal distances when released in the morning. Dispersal in the morning is enhanced by a combination of a more unstable atmospheric surface layer that promotes vertical dispersal, and a lower wind speed that decreases the spore deposition probability onto the ground, compared to later in the day.

**4.** Our study demonstrates an active spore release mechanism in response to diurnally changing air humidity. The mechanism may promote longer dispersal distances, because of enhanced vertical dispersal and because spores being released in the morning have more time to travel before the wind calms down at night. The mechanism also leads to a prolonged dispersal period over the season, which may be viewed as a risk spreading in time that ultimately also leads to a higher diversity of establishment conditions, dispersal distances and directions.

**Key-words:** abscission, atmospheric stability, bet-hedging, bryophyte, diaspore, Lagrangian stochastic model, wind dispersal

## Introduction

For wind-dispersed cryptogams and vascular plants, the dispersal process can be divided into release, transportation, and deposition of diaspores. Much of the literature focuses on the transportation phase, while the mechanisms triggering diaspore release have been less studied (Greene 2005; Kuparinen 2006). Dispersal models therefore often assume random diaspore release in relation to environmental conditions, such as wind speed and humidity (e.g. Tackenberg 2003; Kuparinen *et al.* 2007). For many

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species, this assumption is not true (e.g. Skarpaas, Auhl & Shea 2006; Borger *et al.* 2012; Pazos *et al.* 2013; Johansson *et al.* 2014; Savage, Borger & Renton 2014), and the conditions under which diaspores are released may strongly affect, for example their dispersal distances (Schippers & Jongejans 2005; Soons & Bullock 2008; Savage *et al.* 2012), which has implications for our understanding of, and the ability to predict, species distributions.

Plants that disperse diaspores over long distances may decrease competition with conspecifics and can better sample their surrounding environment to ensure colonization of new suitable habitats, which has evolutionary advantages (e.g. Howe & Smallwood 1982; Levin *et al.* 2003). Diaspore release mechanisms that have evolved under a selection pressure for long dispersal distances may work such that release thresholds in wind speed or turbulence filter dispersal events towards periods with high wind speed, which usually increase the probability of extended dispersal distances (Schippers & Jongejans 2005; Pazos *et al.* 2013; Johansson *et al.* 2014). Other examples are seed or pollen release in response to decreasing air humidity or plant wetness (Greene & Johnson 1992; Jackson & Lyford 1999; Jongejans *et al.* 2007), which also may extend dispersal distances as decreasing humidity often correlates with increasing wind speeds over 24 h (Greene, Quesada & Calogeropoulos 2008). For species living in variable environments, another strategy is to spread the risks in time, 'bet-hedging' (Seger & Brockmann 1987), where the classical example is delayed germination of seeds (e.g. Venable 2007), but it could also include extended diaspore release events in time (Bastida & Talavera 2002). However, for many species groups little is known about diaspore release mechanisms in response to environmental conditions and how they may affect dispersal in space and time, for example in mosses.

Mosses (division Bryophyta *sensu stricto*) contain roughly 12 000 species. They are abundant on the ground or on stones, dead wood and living trees in most terrestrial ecosystems of the globe, and contribute to nutrient cycling, water retention and create habitat complexity (Vanderpoorten & Goffinet 2009). They disperse their spores from a capsule at the top of a seta that is often elevated above the moss shoot. Even if the density of deposited spores is highest within a few tens of metres from the source (Lönnell *et al.* 2015), several studies suggest that moss spores may be able to travel tens (Hutsemékers, Dopagne & Vanderpoorten 2008; Lönnell *et al.* 2012) or even hundreds of kilometres (Sundberg 2013). The potential for long dispersal distances may be affected by spore size (Wilkinson *et al.* 2012), but also by the spore release conditions. However, spore release mechanisms in mosses are still poorly understood, but we know that the release is regulated differently among species. In some species, spores may be passively released by the wind when the sporophyte starts to vibrate (Johansson *et al.* 2014) or just fall out when the capsule decomposes (Vanderpoorten & Goffinet 2009), while others have a more active release mechanism in which the spores are violently discharged (Sundberg 2010; Whitaker & Edwards 2010). Wind-induced vibrations and violent discharge, which lead to an instant release of spores, have been suggested to increase dispersal distances, but the first may only occur in species having capsules exposed to strong wind and the second is only found in one genus (*Sphagnum*). However, many species have a peristome in the capsule opening (Fig. 1) that reacts hygroscopically to changes in air humidity (cf. Mueller & Neumann 1988). In most species, the peristome teeth close in response to increasing humidity, and thus cover the capsule opening, but the reason for this is still unclear. It has been suggested that the main reason is to prevent spore release under wet conditions, and only allow dispersal during dry conditions when the peristome is open and spores can be shaken out by forces such as the wind (Ingold 1965), while others have suggested that the peristome movement in itself actively promotes spore release (Lazarenko 1957; Mueller 1973). The literature on the subject is old (cf. Mueller & Neumann 1988), and many of the observations are of anecdotal character. Quantifications of humidity thresholds for peristome movements, their relations to spore release, and how they affect dispersal distances are lacking. Such quantifications are important for understanding under what circumstances spores are released in nature. They increase the possibility of making accurate predictions of spore dispersal patterns, and to understand the evolution of dispersal traits.

The aim of this study was to (i) investigate the importance of peristome movements for spore release, (ii) quantify the air humidity thresholds for when the peristome opens and closes, (iii) investigate when these thresholds occur in nature and their relations to micrometeorological conditions, and (iv) examine how these conditions may affect spore dispersal distances. We do this based on spore release measurements in a humidity chamber (i, ii), micrometeorological field measurements (iii), and spore dispersal simulations using a mechanistic dispersal model (iv).

## Materials and methods

### STUDY SPECIES

All experiments were carried out using *Brachythecium rutabulum* (Hedw.) Schimp., a pleurocarpous moss in the family Brachytheciaceae. The species is common and has a wide habitat range from open to closed habitats, but is especially common on wood and rocks in deciduous forests. Fertilization occurs during the summer, the sporophyte matures during the autumn and winter and starts to release its spores, from the orthogonally oriented capsule (*sensu* Hedenäs 2007), after the lid dehisces during the spring (Arnell 1875). The capsule has two rings of peristome teeth in the opening (i.e. a double peristome; Fig. 1), which is a common type of peristome being found in roughly half (5000–6000) of the moss species globally (Frey, Stech & Fischer 2009). The capsule persists with an intact peristome over the summer and starts to decompose during the autumn. The spherical spores are around 18 μm in diameter and should therefore have a settling velocity of approximately 0·8 cm $s^{-1}$ (McCubbin 1944). The number of spores per capsule is

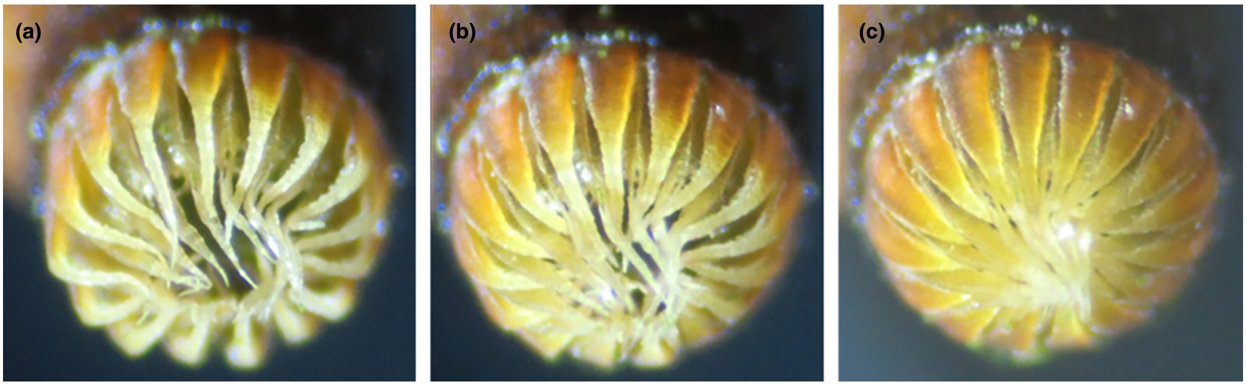

**Fig. 1.** The peristome movements of the sporophyte in a fresh *Brachythecium rutabulum* specimen from the greenhouse in response to relative air humidity (RH), when (a) open (RH; 40%), (b) closing (RH; 75%) and (c) closed (RH; 90%).

roughly 250 000, based on spore counts from five capsules using a Bürker counting chamber.

Capsule-bearing shoots of the study species were collected in November 2013 from a natural population in east-central Sweden (N59°, E18°), and were kept in a greenhouse where we followed the maturation process over the winter. We considered the capsules to have mature spores, ready to be released, when the capsule lost its lid. All experiments were carried out during March–May 2014.

### SPORE RELEASE

To study spore release in relation to humidity, we used a dissecting microscope having its lens immersed into a plastic humidity chamber (Appendix S1, Supporting information). Humidity was regulated with a Beurer LB12 ultrasonic humidifier (Beurer, Ulm, Germany). The wind speed created by the humidified air entering the chamber was $<0{\cdot}1$ m s$^{-1}$. We quantified spore release for 15 fresh capsules from the greenhouse. For each sporophyte, the seta was attached vertically in the chamber, so that the capsule was horizontally oriented with the opening facing sideways, as in nature (Appendix S1). We filmed spore release (Appendix S2) during one opening of the peristome (that took approximately 1 min) per capsule through the dissecting microscope using a Canon IXUS 500 HS full HD digital camera (24 frames per second). From these films, we then roughly estimated the number of spores released. As it was difficult to assess the exact number of spores, especially when many were released, we rounded the numbers as follows: 1–10 (every spore), 10–100 (in whole tens), 100–1000 (in whole hundreds) and >1000.

We also tested the number of spores being released from the capsule in response to induced vibrations, when the peristome was closed (wet conditions) and after it had been open for at least 1 h (dry conditions). This was done by clicking on the adjustment sticks (Appendix S1) three times which has been shown to trigger substantial spore release in other species that do not have a peristome that closes in response to humidity (Johansson *et al*. 2014).

### PERISTOME MOVEMENTS

To quantify humidity thresholds for the peristome to close and open, we used the same humidity chamber as described above. The relative air humidity (RH; %) was recorded using the hygrometer Swema 3000 with the HygroClip2 HC2-S probe (Swema, Farsta, Sweden) located 3-cm sideways of the sporophyte. First, we gradually increased the RH and noted the value when the outer peristome teeth started to bend inwards (i.e. started to close). Secondly, we increased the RH until the peristome became completely closed, and then decreased it again and noted at which value it started to open. In total, we tested 45 sporophytes (one by one), 15 fresh from the greenhouse, 15 fresh from the field and 15 specimens from a herbarium (the Swedish Museum of Natural History, Stockholm). The fresh sporophytes from the field were collected in May 2014 and were included to investigate whether sporophytes grown in the greenhouse responded differently to humidity from field-collected sporophytes. The herbarium material was included to investigate whether the peristome movements are persistent, and respond to humidity in the same way as fresh material. This could simplify future studies of peristome movements by allowing the use of herbarium specimens, which may be more accessible than field material. We used herbarium material collected during the season of spore release and included three capsules from each of five different specimens (in total 15) that were collected between 1967 and 2011 (from the end of February to May). For the herbarium material, we increased the humidity (to ~95%) a couple of times before starting the measurements.

### HUMIDITY AND WIND CONDITIONS IN NATURE AND THEIR EFFECTS ON SPORE DISPERSAL DISTANCES

To illustrate when spores are most likely to be released in nature, we use data from another study on moss dispersal (Lönnell *et al*. 2015). In that study, relative air humidity and wind speed were recorded at 20 cm above the ground during 15 days in the beginning of May 2011, in a relatively open habitat with scattered trees. We also calculated the atmospheric stability parameter at one metre above the ground based on the Obukhov length (L) as 1/L. The Obukhov length is a widely used measure to characterize the stability of the atmospheric surface layer (ASL), the lower part of the atmospheric boundary layer (ABL), as it can be assumed to be constant within the ASL. The Obukhov length is defined via the vertical fluxes of temperature and momentum at the ground surface (for more details see e.g. Kaimal & Finnigan 1994). For simplicity, we only divided the stability into two categories: stable ($1/L > 0$) and unstable ($1/L < 0$). An unstable ASL occurs during daytime, when surface heating generates upward movements of heated air (convection), which may uplift spores and greatly facilitate their dispersal in the air compared to in the stable ASL that occurs at night (Stull 1988). Here, we present mean values from 10-min periods during these 15 days and relate them to humidity thresholds for the opening and closing of the peristome of our study species.

To illustrate how dispersal distance may differ when spores are released by the peristome opening as compared to a release mechanism that is triggered by high wind speed (e.g. Johansson *et al.* 2014), we modelled spore dispersal using a mechanistic dispersal model (Norros *et al.* 2014; Lönnell *et al.* 2015). We randomly chose one 10-min period from each of the 15 days when the RH was in the same range as when the peristome started to open (RH; 75–89%) and the period having the highest wind speed for each day (i.e. 15 other periods than for suitable RH). We then simulated spore dispersal given that spores were released under the conditions prevailing in each of these 30 periods. For each period, we performed Lagrangian stochastic trajectory modelling of air parcels carrying the 18-μm-diameter spores, released 5 cm above the ground. Such air parcels are assumed to be marked fluid elements containing a number of spores that is proportional to the spore release strength. However, in this study, we present the fractional dispersion statistics, and therefore, the spore release was assumed to have unit strength in all periods. Turbulence in the simulation domain was assumed to follow the ASL similarity scaling, and spore collection by ground vegetation and deposition to the surface was modelled following Norros *et al.* (2014) by using the dry deposition model by Petroff *et al.* (2008). Ensemble average dispersal and deposition statistics were inferred from 100 000 trajectories simulated for each 10-min period. Turbulent dispersal within each such period was driven by turbulence conditions determined by the measured heat and momentum fluxes and the variances of the three wind speed components; for each trajectory, the coordinates and the travel time counted from the release was calculated. During trajectory simulations, we also collected statistics for the vertical distribution of spores along the horizontal distance. Lagrangian trajectories were followed up to 100 m horizontally in all directions from the source. This maximum distance was chosen because (i) our model was parameterized based on the turbulence statistics in the ASL and therefore was not capable to predict the long-distance dispersal depending on the development of the ABL state and larger scale meteorology; and (ii) we know that the model performs well up to 100 m, since model predictions have been validated by empirically observed colonization patterns of another ground living moss to that distance (Lönnell *et al.* 2015). The model is more thoroughly described in Appendix S3, and all the details on the modelling approach can be found in Lönnell *et al.* (2015).

## Results

Spores were almost only released during the opening of the peristome, and the release occurred instantaneously when the peristome teeth started to move. The estimated number of spores released during one opening of the peristome ranged from 20 to >1000 (median = 200) for the 15 tested capsules from the greenhouse. Most spores were released at the incipient opening, rather than towards the end. No spores were released in response to vibrations induced in sporophytes with closed peristome (high humidity), and only in two of 15 capsules spores (<10) were released from sporophytes with open peristome (low humidity) when being vibrated.

After being dried, the outer peristome started to close at a clear threshold of RH = 69·3% (SD = 2·53), and after being wet, it opened at a clear threshold of RH = 85·5% (SD = 2·39, Fig. 2) for the material from the greenhouse. The thresholds were very consistent between different types of fresh and herbarium material (Fig. 2). The corresponding thresholds in RH for fresh, field-collected material were 67·5% (SD = 1·72) and 86·4% (SD = 1·68), and for herbarium material 68·8% (SD = 2·93) and 87·4% (SD = 2·77). There was no difference between the groups for the process of closing ($F = 2{\cdot}32$, $P = 0{\cdot}11$) or opening ($F = 2{\cdot}42$, $P = 0{\cdot}10$).

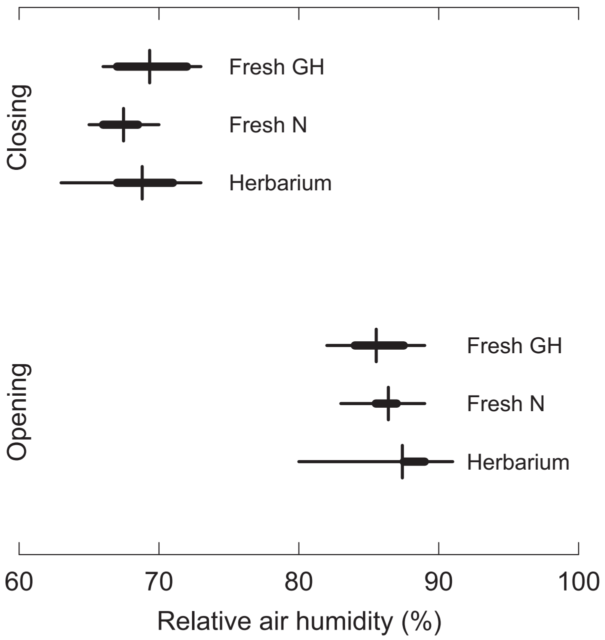

**Fig. 2.** The relative humidity (RH; %) when the peristome teeth of *Brachythecium rutabulum* start closing when humidity increases, and opening when humidity decreases, in fresh material, from a greenhouse (GH) and the field (N), and in herbarium material. Thin vertical lines show mean values, horizontal thin lines the full range of the data, and horizontal thick lines show the range between the 25th and 75th percentiles.

Based on micrometeorological field measurements at 20 cm above the ground, the RH-thresholds for peristome opening and closing were crossed mainly in the morning and in the evening, respectively (Fig. 3). When the RH-threshold for peristome opening was crossed in the morning, wind speeds were increasing but were generally lower than at noon and in the afternoon (there was a significant negative correlation between air humidity and wind speed; $r = -0{\cdot}55$, $t = -30{\cdot}7$, $P < 0{\cdot}001$, Fig. 4). The stability of the ASL switched from stable to unstable during this time, and these conditions then continued during the day (Fig. 3). The probability for an unstable ASL was 0·71 for periods with conditions suitable for peristome opening, while the probability was 0·64 for all other periods. For the highest wind speeds (the upper 95% quantile), the ASL was always unstable but on average less unstable ($1/L = -0{\cdot}013$) as compared to the unstable periods suitable for peristome opening ($1/L = -0{\cdot}18$).

Simulations of spore dispersal, based on the mechanistic dispersal model and the field-micrometeorological data, showed similar spore deposition patterns within 100 m horizontal distance, and that on average roughly the same proportion of spores (~27%) was transported beyond 100 m when being released in the highest wind speed

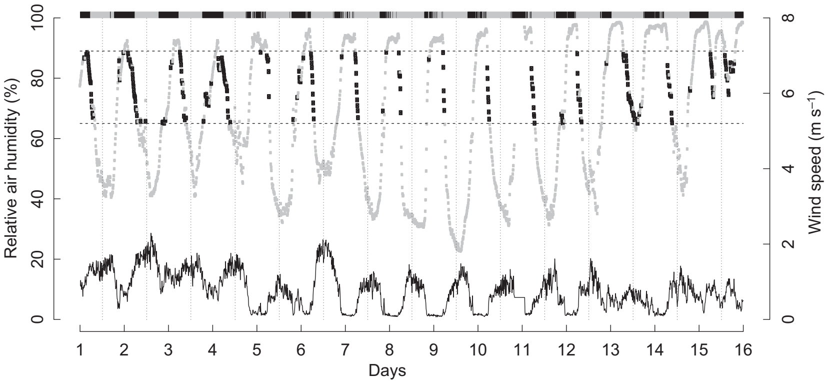

**Fig. 3.** Micrometeorological data from the field measured at 20 cm above the ground, showing relative air humidity (RH; dots), wind speed (lower continuous line) and ASL stability (black = stable, grey = unstable, at the top) in 10-min periods during 15 days in May, where vertical dotted lines indicate noon each day. Horizontal broken lines show the humidity threshold for the opening and the closing of the peristome, and big black dots indicate when spores are most likely to be released as the peristome opens (i.e. periods when the previous period had a higher RH that also was between the two thresholds).

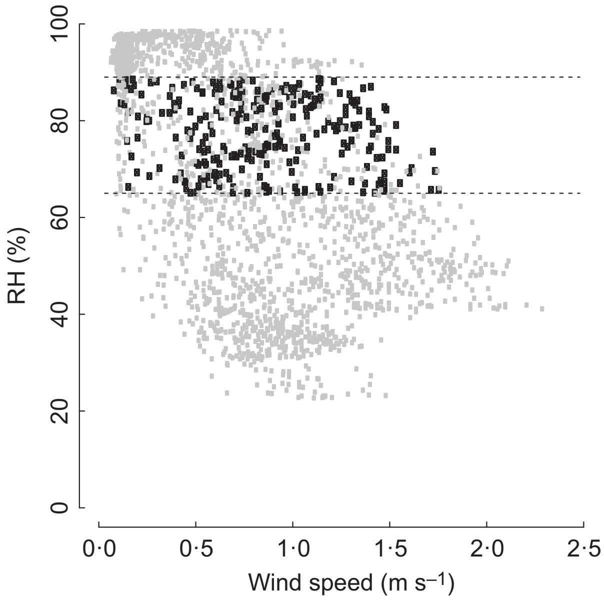

**Fig. 4.** Correlation scatter plot between horizontal wind speed and RH. Horizontal broken lines show the humidity threshold for the opening and the closing of the peristome, and black dots indicate when spores are most likely to be released as the peristome opens (i.e. 10-min periods when the previous period had a higher RH that also was between the two thresholds).

(mean of the highest wind speed class from the 15 days being 1·45 m s$^{-1}$) as in the wind conditions (mean 0·64 m s$^{-1}$) that prevailed during periods of peristome opening (Fig. 5a). However, the vertical distribution of spores at 100 m horizontal distance differed considerably (Fig. 5b); for spores being released during the RH-threshold of peristome opening, 62%, 34% and 16% exceeded 5 m, 10 m and 20 m above-ground, respectively, while the corresponding proportions for spores being released at the highest wind speed were 42%, 10% and 0·7%, respectively. The ASL was more unstable during the 15 periods that corresponded to the period of peristome opening (mean of 1/L = −0·29) as compared to the 15 periods with the highest wind speed each day (mean of 1/L = −0·026, Fig. 6).

## Discussion

We here describe and quantify the importance of an active release mechanism of moss spores that has been poorly understood, despite that it is probably very common among mosses, since roughly half of the 12 000 mosses possess a similar peristome. We show that spores are only released when the peristome teeth in the capsule mouth open in response to decreasing humidity, which usually occurs in the morning. During spore release, wind speeds are comparatively low, which contrasts mechanisms for seed release. Nevertheless, the release mechanism seems to enhance dispersal distances, compared to mechanisms that release spores at higher wind speeds, because release in the morning results in that a higher proportion of the light spores reach high altitudes. The mechanism may also extend the dispersal in time more than other known release mechanisms for moss spores.

### DIASPORE RELEASE IN RESPONSE TO CHANGING HUMIDITY AND ITS EFFECT ON DISPERSAL DISTANCE

We show that moss spores can be released in response to a changing humidity, which agrees with release studies of seeds (Greene & Johnson 1992; Jongejans *et al.* 2007; Greene, Quesada & Calogeropoulos 2008; Marchetto *et al.* 2012) and fungal spores (e.g. Rockett & Kramer 1974). However, the relationships to humidity and the release mechanisms or mechanisms regulating under which conditions release could take place differ among organism groups. We show an active mechanism triggering moss spore release within a rather tight span of air humidity. Spores are mainly released when the humidity is still rather high, which to some extent agrees with many fungi (e.g. Rockett & Kramer 1974), even if the regulating mechanism

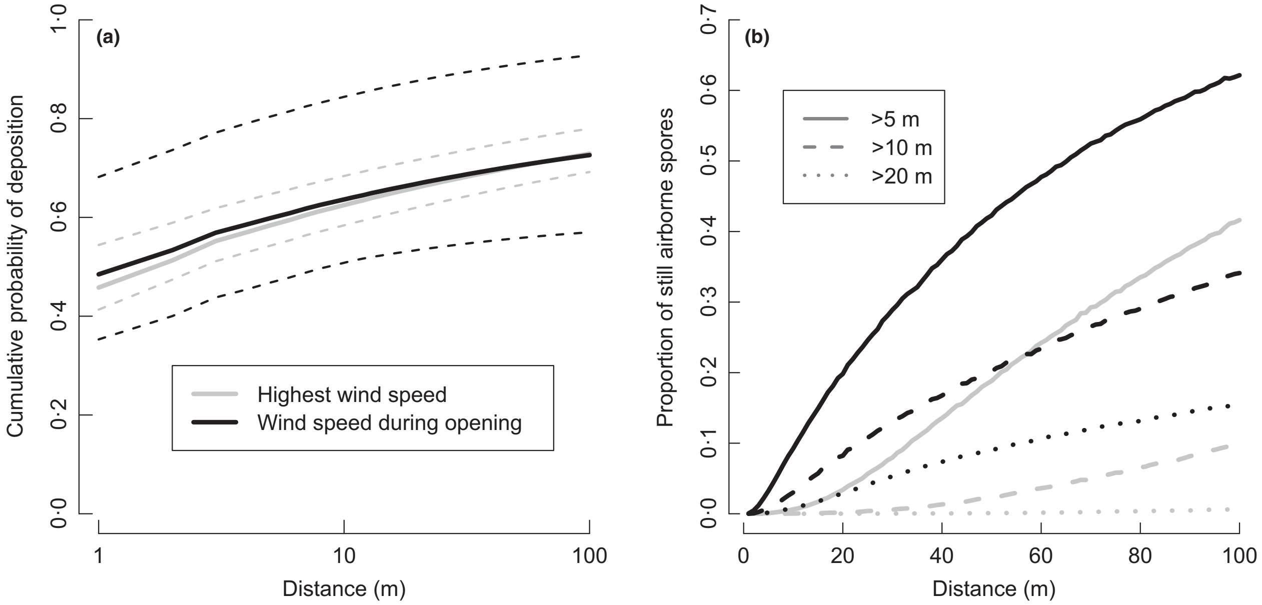

**Fig. 5.** (a) The cumulative spore deposition up to 100 m when spores (18 μm in diameter) are released in (i) the highest wind speed each day (mean from the 15 days = 1·45 m s$^{-1}$), and (ii) the wind conditions (mean = 0·64 m s$^{-1}$) prevailing when the decrease in RH leads to peristome opening and spore release (RH; 75-89%), based on a mechanistic dispersal model and micrometeorological data from a relatively open habitat with scattered trees during 15 days in May 2011 (Lönnell *et al.* 2015). Solid thick lines represent the mean, and broken thin lines the 95% confidence interval based on the means from the 15 periods. The ASL was on average unstable for both (i) and (ii), but it was more unstable for (ii); mean of 1/L was −0·026 and −0·29, respectively. Note the logarithmic ($\log_{10}$) *x*-axis. (b) The average proportion of spores, among the ones that are still airborne, exceeding three threshold heights above the ground, at increasing distance from the source, when released in (i) and (ii).

is completely different (Money 1998). In contrast, seed release seems to increase with decreasing humidity (Greene & Johnson 1992; Jongejans *et al.* 2007; Greene, Quesada & Calogeropoulos 2008; Marchetto *et al.* 2012). Compared to light spores, the heavier seeds may benefit more from being released during dry and windy conditions, which usually correlate over 24 h (Greene, Quesada & Calogeropoulos 2008; Fig. 3). Several studies suggest that seed release at high wind speed and turbulence promotes longer dispersal distances (e.g. Schippers & Jongejans 2005; Soons & Bullock 2008), which has also been suggested for spores (e.g. Savage *et al.* 2012; Johansson *et al.* 2014). However, according to our results, the wind conditions prevailing during the opening of the peristome in the morning lead to an equally large proportion of spores being dispersed beyond 100 m as for the highest wind speeds each day (Fig. 5a; during midday or afternoon), even though the mean horizontal wind speed is lower in the morning. The primary reason for this is the stability of the ASL, which switches from stable to unstable during the morning when the sun starts to heat the ground (Stull 1988). Unstable atmospheric conditions drive the vertical dispersal, which promotes longer dispersal distances by lifting the light spores higher up. The increasing wind speed later in the day generates more mechanical turbulence and the conditions become less unstable (Fig. 6). Thus, the increasing wind speed does not increase the proportion of spores dispersing beyond 100 m (Fig. 5a), because such conditions promote spore deposition also at shorter distances onto vegetation (Petroff *et al.* 2008) even though shorter residence times of the air parcels within ground vegetation layer is expected. High wind speed may thus not be the main driver of long dispersal of spores but rather the enhanced vertical mixing under unstable conditions which promotes transport higher up in the atmosphere and then consequently to larger horizontal distances (Fig. 6). The vertical distribution of the still airborne spores at 100 m suggests that spore release at the lower wind speeds during the morning may actually promote dispersal of spores beyond 100 m, as they have a higher vertical distribution compared to spores released later in the day (Fig. 5b). For example, given no vertical wind movement, a spore of *Brachythecium rutabulum* at 10 and at 20 m above-ground would settle in roughly 21 and 42 min, respectively, while at the higher altitude the spore would also experience higher horizontal wind speed and at the same time run a reduced risk that its trajectory would be stopped by obstacles. Moreover, spores entering the transportation phase early in the day have more time for dispersal over long distances, if reaching higher air masses, before the wind conditions calm down at night (Sundberg 2013).

### ACTIVE MOSS SPORE RELEASE BY PERISTOME MOVEMENTS THAT EXTENDS THE DISPERSAL PERIOD

We show that moss spores are actively released by the peristome movements, as the outer peristome teeth dig into the spore mass when closing and drag out spores when

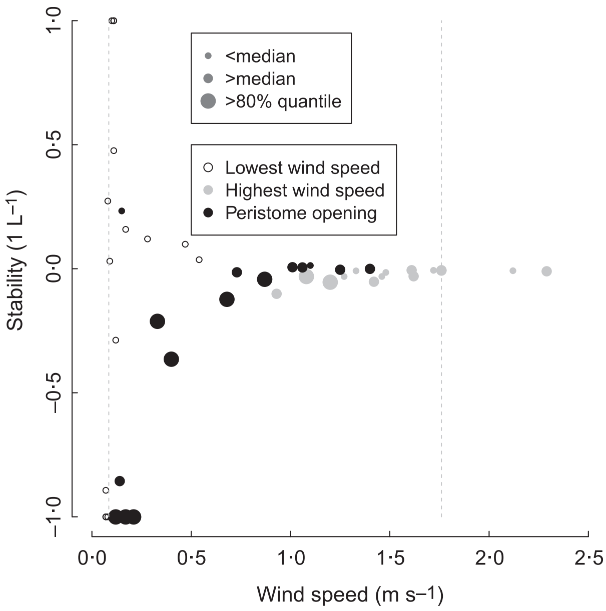

**Fig. 6.** The relationship between horizontal wind speed and the stability of the atmospheric surface layer, and how these variables relate to the probability of spores being dispersed long distance and to high altitude for spores released in (i) conditions prevailing during peristome opening ($N = 15$), (ii) the highest wind speed ($N = 15$), and, to also include periods with a more stable surface layer and low wind speeds (Appendix S3), (iii) the periods representing the lowest wind speed for each day ($N = 15$). The probability of spores being dispersed long and high is calculated as the product of the probability of dispersing beyond 100 m and the probability of being >10 m above-ground at that distance for each period, and the size of the dots show probabilities that are below the median, above the median, and above the 80% quantile among all 45 periods. Positive values of stability correspond to a stable atmospheric surface layer and negative values to an unstable layer. Vertical broken lines indicate the range of wind speeds within which spores may be released by peristome opening.

opening, which agrees with some earlier observations (Ingold 1959; Mueller 1973). The inner sides of the outer teeth have small ridges, and the tips of the teeth are hook-shaped, which are two morphological features that both may aid in the collection of spores from inside the capsule. Our results suggest that this may be the main mechanism for spore release in this species. We did not see any spore release when vibrating capsules at high humidity, which is rather obvious from Fig. 1c since the capsule opening is closed, but in contrast to our expectations, the same was also observed for most capsules when the peristome was open in dry conditions. The reason could be that the inner peristome keeps the opening rather narrow and cone-shaped when the outer peristome has opened (Fig. 1a). However, we believe that very strong vibrations of the capsule may still cause substantial spore release, as shown for species having a peristome that is always open (Johansson *et al.* 2014), and for the release of seeds (Kadereit & Leins 1988) and pollen (Urzay *et al.* 2009; Timerman *et al.* 2014). However, in our study species, such strong vibrations are unlikely to be triggered by wind, since the capsules are not much elevated above the ground, which decreases the probability of wind-induced vibrations (Johansson *et al.* 2014). Vibrations may instead occur, for example in contact with animals. Hence, based on our results it seems like the peristome movements are a prerequisite for spore release.

It may seem unrealistic that the peristome opening mechanism alone could empty the huge amount of spores available in a capsule, and further studies are needed to reveal the complete mechanism. However, we can speculate in how it might be possible based on our observations and knowledge of the species. First, our data on spore release during one single opening of the peristome may strongly underestimate the number of spores released during 1 day. The reason is that in our experiments the humidity was decreased rather steadily during approximately 1 min, while the same decrease may occur under sometimes several hours in nature (Fig. 3). Even if the humidity in nature seems to decrease steadily, when summarized over 10-min periods, there may be fluctuations in humidity during this time, which might lead to several openings each morning and, thus, more spore release. Secondly, the capsules remain in nature for months which together with the previous suggestion would result in numerous openings. The peristome release mechanism, hence, extends spore release events in time as compared to species with a more instant release (e.g. Whitaker & Edwards 2010; Johansson *et al.* 2014). However, one question that needs more investigation is how spores are transported towards the capsule opening. We believe that the spores may fall towards the opening when the outer spores are being removed by the peristome teeth as long as the horizontally arranged capsules contain decent amounts of spores. It has also been suggested that capsules shrink when drying, which forces spores closer to the opening where they can be caught by the peristome teeth (Mueller 1973), but if this can completely empty capsules is unclear. Another suggestion is that raindrops vibrate the capsules when they are closed so that spores are transported forward (the first raindrops could even shake out spores before the peristome closes in response to increased humidity). Perhaps capsules are rarely completely emptied, and the remaining spores are released on-site when the capsules decompose.

### EVOLUTIONARY ADVANTAGES OF LONG DISPERSAL DISTANCES AND DISPERSAL IN TIME

Plant species with specific habitat requirements, especially if the substrate is short-lived, may live under a selection pressure for traits that increase their possibility for extended dispersal distances, so they can better sample their environments and ensure that at least some diaspores reach suitable habitats (e.g. Howe & Smallwood 1982; Levin *et al.* 2003). We show a release mechanism for moss spores that may have evolved under this pressure; mosses are often restricted to delimited and dynamic patches of suitable habitat. Other examples are mechanisms that violently discharge spores up in the air where they can more easily be caught by the wind (in *Sphagnum*; Whitaker & Edwards 2010) and thresholds in seta vibration that only allow spore release in turbulent winds (in Polytrichaceae; Johansson *et al.* 2014). In contrast, epiphytic mosses in several clades have evolved a reduced peristome that opens instead of closes in response to increasing humidity (Mueller & Neumann 1988; Goffinet & Shaw 2008), which may not be ideal for long-distance dispersal. One reason may be that for these more wind-exposed species, establishment (which occurs at high humidity) is a stronger evolutionary bottleneck than dispersal distance. We thus suggest that dispersal kernels of mosses are the result of simultaneous evolution of several traits, such as peristome structure, seta length, capsule orientation, spore size and shape, as well as release mechanism.

The mechanism we study does not only lead to longer dispersal distances but also to a dispersal that is extended in time. This may be viewed as a 'bet-hedging strategy' for species living in variable environments (Seger & Brockmann 1987). The classical example of bet-hedging in plants is seed dormancy (e.g. Venable 2007), but it could also be extending seed release over the season (Bastida & Talavera 2002). By slowly portioning out spores with peristome movements, the surrounding environment and the conditions suitable for establishment are sampled over time, as compared to species with a more instant spore release (e.g. *Sphagnum*; Whitaker & Edwards 2010). Spreading the dispersal events in time should also ultimately result in a higher diversity of dispersal distances and directions.

### CONCLUSIONS AND FUTURE RESEARCH

Our study emphasizes the importance of studying diaspore release mechanisms in relation to environmental conditions, for an understanding of dispersal distances. Most mechanistic dispersal models do not take release conditions into account (Tackenberg 2003; Kuparinen *et al.* 2007), which may strongly affect the predicted dispersal distance (Schippers & Jongejans 2005; Soons & Bullock 2008; Savage *et al.* 2012), and the area over which diaspores are deposited (Savage *et al.* 2010). We show a non-random release mechanism of moss spores in response to changing humidity, suggesting that spores are mainly released when the humidity drops from high values, which usually occurs during the morning (Fig. 3). This release mechanism may lead to longer dispersal distances because more spores reach high altitudes which, since release happens in the morning, have the whole day to travel before the wind calms down at night. The mechanism also leads to a dispersal extension in time, which may be viewed as a risk spreading. Spore release by peristome movements should be common, because many mosses have a peristome that opens in decreasing humidity (Goffinet & Shaw 2008). However, the air humidity thresholds, and the significance of the peristome movements for spore release, may differ depending on, for example the peristome structure and capsule orientation. Moreover, the timing of spore release may differ among habitats; in forests, air humidity is usually higher than in open habitats, which may lead to spore release somewhat later in the day in the forest. Hence, there are several aspects to investigate before fully understanding the release of moss spores and the evolution of dispersal traits, and we therefore suggest focusing more research on the release process. This has also been pointed out for other wind-dispersed organisms (Kuparinen 2006; Nathan *et al.* 2011), to give a more complete understanding of when and where diaspores are dispersed, and hence, our ability to make realistic predictions of species distribution patterns and population dynamics.

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## Data accessibility

Peristome movement data, spore release data and micrometeorological data are deposited in Dryad Digital Repository http://dx.doi.org/10.5061/dryad.mv346 (Johansson *et al.* 2015).

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## Supporting Information

Additional Supporting information may be found in the online version of this article:

**Appendix S1.** Description of the humidity chamber.

**Appendix S2.** An example film from the spore release experiment.

**Appendix S3.** Description of the mechanistic dispersal model and the meteorological data.