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Speed and force of spore ejection in *Selaginella martensii*

Jakob Schneller[1], Hans Gerber[2] and Alex Zuppiger[1]

[1] Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich; e-mail: schnell@systbot.uzh.ch
[2] Institute of Biomechanics, ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich; e-mail: hsgerber@ethz.ch

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Abstract

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Spores of the genus *Selaginella* are discharged through an ejection mechanism caused by the anatomical differentiation of the sporangium. To understand the evolution of these specialised dispersal mechanisms, it is important to know how effective they are, i.e. what distance is reached by the spores, how rapidly they are ejected, and what forces must be developed by the plants to achieve this speed. Here we present a method to determine these important variables. We observed the spore discharge process for *Selaginella martensii* using a high-speed camera, which allowed us to resolve the movement to 1/1000 s. The mico- and megasporangia opened slowly, separating into two ovoid valves, which subsequently dried and closed in a sudden, quick movement, ejecting the spores. The distances of spore dispersal were determined by placing single shoots of *Selaginella* on paper in the laboratory. Microspores reached up to 5 – 6 cm from the spore source, while megaspores reached up to 65 cm from the source, with a mean flight distance of 21.3 cm. Based on the flight trajectories observed on the photographs and the mean weight of a megaspore (1.4 μg), we calculated the speed and forces responsible for the ejection mechanism. The speed of the spores at ejection time was 0.6 m/s for microspores, and 4.5 m/s for megaspores. The initial impulse of one megaspore was estimated as 6.3 pNs. A force greater than 7 μN is necessary to accelerate the megaspore in less than 1 ms to this speed. Our new method to determine the impulse and initial speed of *Selaginella* spores makes possible more detailed studies about the role and function of biological structures responsible for spore ejection.

Key words: Biological movements, dispersal distance, dispersal mechanism, heterospory, Selaginellaceae, spore discharge.

Introduction

Heterospory denotes the separation of spore dispersal into two components, megaspore and microspore dispersal, which often occur through different mechanisms and over different distances. During the evolution of land plants, heterospory has evolved in eleven different clades, the most successful of which has led to the Gymnosperms and Angiosperms (Junker 1996). The Pteridophytes include both homosporous and heterosporous families. Separating the female (megaspore) and male (microspore) function has brought many advantages in plant evolution, e.g. greater genetic variability and more rapid adaptation, but also some constraints, such as the need to optimize two dispersal systems.

In the heterosporous Selaginellaceae (Lycopsida), wind is the dominant dispersal factor for both micro- and megaspores, however, the larger megaspores are transported less effectively by wind than the microspores. This disadvantage is partly corrected by an active ejection mechanism related to the anatomical differentiation of the sporangia (Mitchell 1910; Ingold 1939; Straka 1962; Webster 1995). The mechanism of spore discharge in *Selaginella* was first described at the beginning of the 20th century (Lyon 1901; Goebel 1901; Steinbrinck 1902). In particular, Goebel (1901) already gave a detailed description of the different phases of megasporangia opening and megaspore discharge. Knowledge about mechanisms of microspore discharge is more recent. Koller and Scheckler (1986) distinguished three types of microspore dispersal. The first type consists in a passive dispersal of microspores. The two valves of the sporangia separate along a dehiscence line and remain open. The spores passively fall out of the sporangium or are blown out by wind. This mechanism is found in *S. lepidophylla* Spring and many other species. The second type involves an active ejection of the microspores from the microsporangium during dehiscence. The two valves separate along the dehiscence line and remain attached at the bases. They bend away from each other up to an angle of ca. 150°, then the valves immediately snap back. Some spores may fall out before the sudden closing. The others are actively dispersed. Many *Selaginella* species belong to this type, including *S. martensii* Spring. With the third type of microspore dispersal, the open sporangia are ejected up to 20 cm from the sporophyll. Spores partly fall out before the sporangium has landed, but some remain in the sporangia. This mechanisms is found in *S. diffusa* Spring and a few other species.

To understand the evolution of these specialised dispersal mechanisms, we need to know how effective they are. For megaspores, the maximum horizontal distance reached by ejected megaspores under laboratory conditions was found to be 59 and 65 cm by Page (1989) and Filippini-DeGeorgi et al. (1997), respectively. Under field conditions, the dispersal distance can be much higher. For example, Burrows (1988) simulated the ballistic trajectories of small spherical particles representing spores and showed that projection to the largest possible vertical downstream altitude as well as wind motion are important factors increasing the horizontal distance reached by the spores. In any case, spores must be accelerated to a considerable speed to cover such distances, which means that a substantial force must be developed by the plant to achieve this acceleration. The ejection speed of spores and the force acting upon them would therefore be essential variables to determine in studies of dispersal mechanisms.

However, this is technically challenging, and to our knowledge, such measurements have not yet been done.

In this study we documented the first steps of spore ejection from micro- and megasporangia of *Selaginella martensii* using a high speed camera. The direct measurement of spore trajectories allowed us for the first time to calculate the speed and the forces necessary for the observed spore dispersal.

Materials and Methods

Selaginella martensii is a tropical species, which under natural conditions grows on the floor of cloud forests and mountainous regions. It has ascending stems, with strobili between a few cm and about 30 cm above the soil surface. Both microspores and megaspores are dispersed actively. In the case of microspores, tetrads are the dispersal units (Koller and Scheckler 1986).

We collected fresh fertile parts of *S. martensii* from the greenhouse in the Botanical Garden of the University of Zürich. Plants were kept moist in plastic bags until they were used in the experiments. To measure the distance reached by active spore dispersal, a fertile shoot with mega- and microsporangia (ca. 1 cm) was mounted horizontally on a glass slide and placed at the edge of a horizontally spread paper sheet of 70×110 cm^2, with the tip of the fertile strobilus directed towards the centre. Separate experiments were run to determine megaspore and microspore distribution, using black paper for megaspores and white paper for microspores. All experiments were carried out under quiet air conditions in the laboratory.

The distribution of microspores was recorded by drawing the outer border of the "cloud" of spread spores. The experiment was run ten times, and an average dispersal area was determined qualitatively by overlaying the ten individual clouds. To determine the distribution of dispersal distances, the number of microspores in an area of 1 cm^2 was counted at different distances from the tip of the strobilus in the direction of the shoot.

The distribution of megaspores was recorded more precisely by measuring the position of each single megaspore in each of 40 runs. The source point (tip of the strobilus) was taken as 0 in a rectangular coordinate system. The position of each spore was then given by two values (x and y). With these values, the dispersal distances and their frequency distribution were also calculated.

To directly observe the ejection process, strobili were placed on a Zeiss dissecting microscope (Type Semi SV 11), and the opening of sporangia and spore release were filmed with a high speed camera. We used a SpeedCAMpro camera with a macro lens and took up to 1000 pictures per second (512×512 pixels, black and white, electronic shutter). The camera allows to film continuously with 1000 Hz but only the last 4 s are stored in the memory. The shutter time used of less then 1 ms required a strong light source. The heat of the light was a good trigger for the dispersal events because plants dried relatively fast, inducing spore dispersal. The sporangia were continuously filmed; shortly after the spore ejection, filming was stopped, and the film sequence was stored. The initial speed was measured using the pictures taken by the SpeedCAMpro camera.

To verify whether these measurements yielded realistic values for the initial speed of the spores, the flight path of megaspores was simulated using the two following equations. The first equation describes the drag (resistance to air flow) of a spore moving in still air. The effect of the drag is to slow down the movement of the spore, and

its value depends on the current speed. The second equation describes the reduction in flight speed and the change in flight direction (downward bending) which result from the combined effects of gravity and drag.

$$\vec{D}(t) = -\frac{c_w \cdot A \cdot \rho_l}{2} \cdot v(t)^2 \cdot \vec{e}_v(t) \quad (1)$$

$$\dot{\vec{v}}(t) = -\vec{g} + \frac{\vec{D}(t)}{m}; \quad (2)$$

Equation 1 is the Navier-Stokes equation modified for particles with Reynold's number >> 1 (Oseen 1927); equation 2 is the Newtonian differential equation of translational motion of a particle; $\vec{D}(t)$ = Drag of the spore at time t after ejection, $\vec{v}(t)$ = speed of the spore at time t, A = cross section of the spore, m = mass of the spore, c_w = drag coefficient (approximated by a constant value of 1 based on Wieselsberger 1921), ρ_l = density of air (estimated to 1.2 kg/m^3), $\vec{g}$ = acceleration of gravity (9.81 m/s^2), $\vec{e}(t)$ = unit vector in direction of speed at time t.

By integrating the equation system over time, we simulated the flight trajectory of a spore and calculated the flight distance at which the trajectory reaches the ground. The simulation was run with the initial speed of megaspores based on the film sequence as well as measured values for A and m. Good agreement between the simulated distance and the directly observed distance would indicate that the value for initial speed was correct. To determine A, the two-dimensional projection of 50 megaspores (which is approximately a circle) was determined from diameter measurements, and the mean was used for A. To determine m, 500 megaspores were counted and weighed, which resulted in an average mass of 1.4 μg per spore.

Results

Our direct observations of the ejection process showed that the microspores of a sporangium are often but not always shot simultaneously. The two valves sometimes showed a repeated opening and closing, and sometimes jerky opening movements, which induced some spores to leave the sporangium. However, when there was a second or third closing movement, this was not as effective as the first one. In most cases the sporangia were nearly or completely empty at the end of the ejection process.

Microspores were dispersed at most 5–6 cm in still air (Fig. 1a), and the large majority of the spores landed within 1 cm of the source (Fig. 1b). According to the spore trajectories recorded by the high-speed camera, the initial speed of the crowded microspores was about 0.6 m/s. A cloud of numerous spores were fired together. Under quiet air conditions, they were soon retarded by air drag and slowly hovered down to the ground.

Megaspores were ejected up to 65 cm (Fig. 2a,b). The average dispersal distance was 21.3 cm, and the distribution of dispersal distances was nearly uniform from 0 to 45 cm (Fig. 2b). Figure 3 shows the first steps of spore dispersal out of the megasporangium. At the resolution of 1000 pictures/s and a shutter time of 0.8 ms the spores are still seen as short lines. We estimated the initial speed of the megaspores at a maximum of about 4.5 m/s. Using this speed in the computer simulation of the flight path results in a calculated dispersal distance of about 60 cm, which corresponds well to the maximal flight distance observed in the experiment.

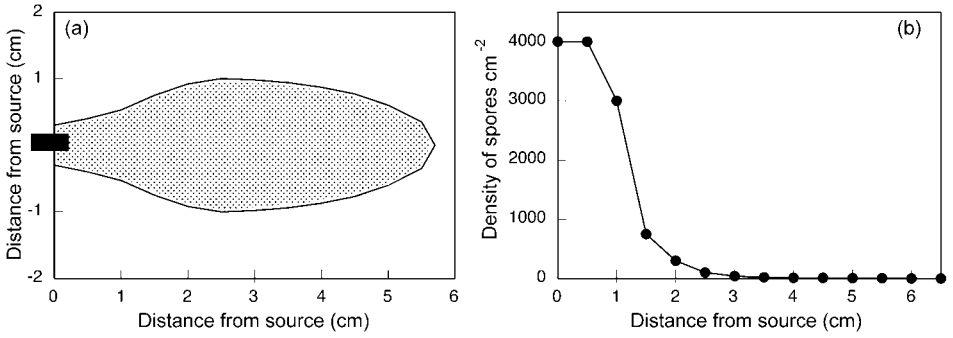

Fig. 1. Microspore dispersal of *Selaginella martensii* in quiet air. Spores were released by a single fertile shoot of c. 1 cm length and collected on a large sheet of paper. (a) Dispersal area of the microspores (approximate shape of the spore cloud). (b) Spore density (number of spores on 1 cm^2 paper surface) at various distances from the spore source.

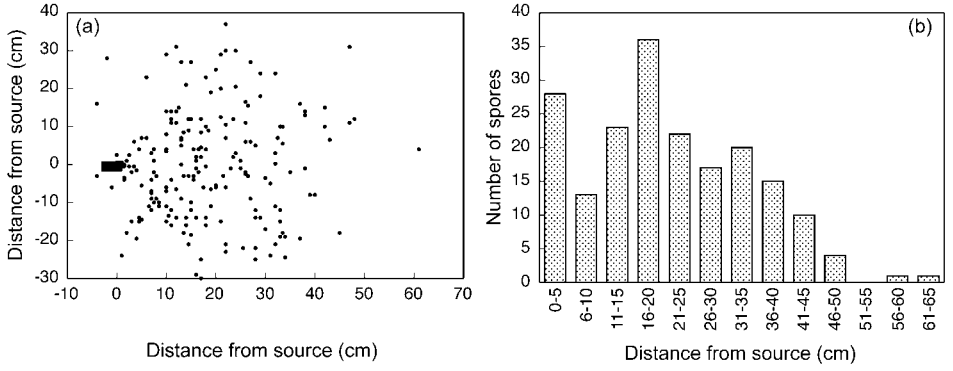

Fig. 2. Megaspore dispersal of *Selaginella martensii* in quiet air. In each of 40 runs, spores were released by a single fertile shoot of c. 1 cm length and collected on a large sheet of paper; pooled data from these 40 runs are shown. (a) Distribution of spores on the paper. (b) Frequency distribution of dispersal distances.

The four megaspores were released in pairs but sometimes all four spores together were released in less than 1 ms. There was always a release of two opposite megaspores, never of two spores lying side by side. Obviously this avoids loss of momentum of each of the megaspores due to recoil. The estimated impulse (= mass×speed) of one megaspore is 6.3 pNs (v = 4.5 m/s, m = 1.4 μg). This means that a force (= impulse/time) greater than 7 μN is necessary to accelerate the megaspore in less than 1 ms to its ejection speed.

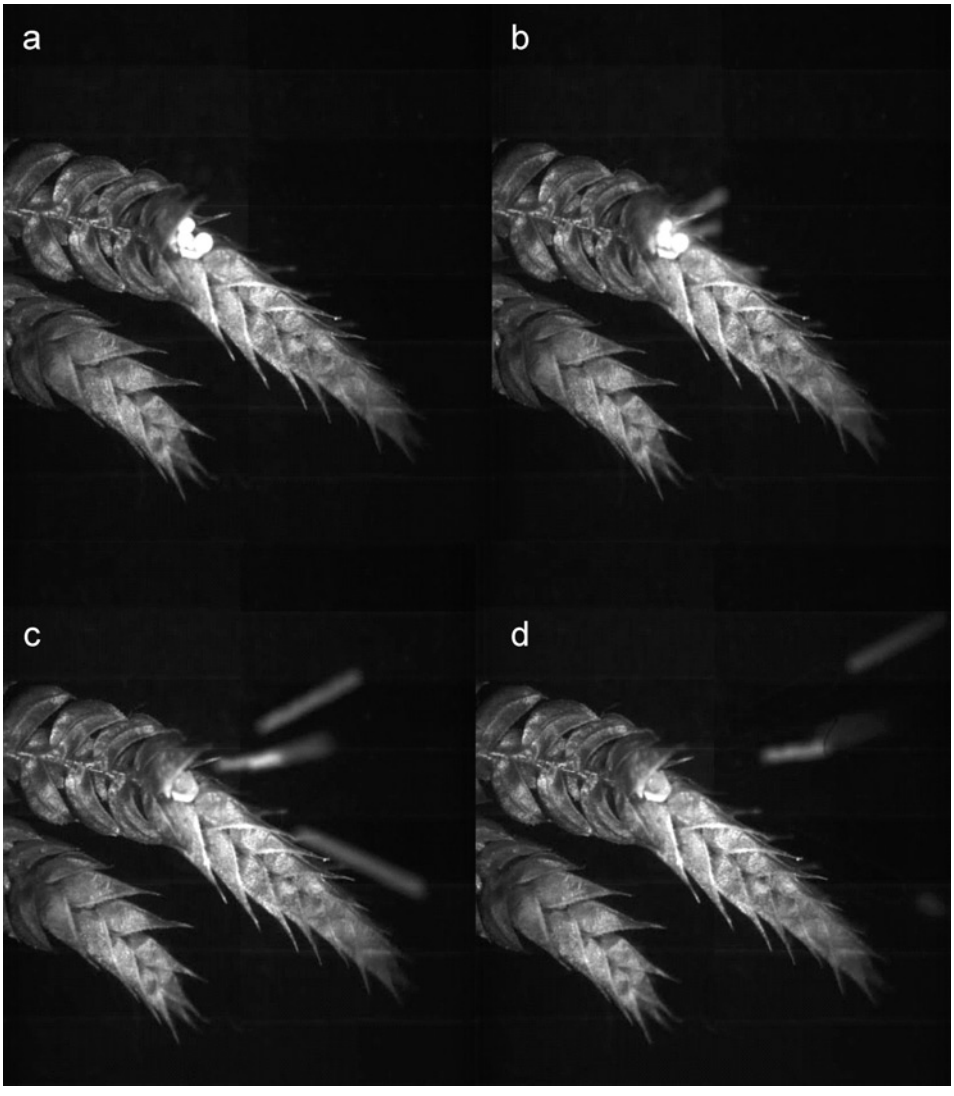

Fig. 3. Megaspore dispersal from a fertile shoot of *Selaginella martensii,* recorded with a high-speed camera. The time between sucessive photographs (a, b, c, d) was 1 ms, the shutter time 0.8 ms.

Discussion

This study has confirmed the principal mechanisms of micro- and megaspore dispersal in *Selaginella*, as described previously (Goebel 1901; Steinbrinck 1902; Page 1989; Webster 1995). In microsporangia, the very quick closing of the two valves seems to be responsible for the short distance dispersal of the microspores. It is mostly

compressed air between the valves, rather than the direct contact of spores to the wall of the valves, that produces air turbulences which disperse the numerous microspores. However, it is possible that some electrostatic forces are also involved in the observed events (Filippini-DeGeorgi et al. 1997; Hemsley and Griffith 2000).

The dispersal mechanism of the megasporangium consists of two main steps. First, the basal boat-like part constricts upon drying and presses the two upper spores together, until they are suddenly discharged. Then the bases of the two valves clash together, and the second spore pair is ejected. Both the high speed of the first steps of dispersal (less than 1 ms) and the force needed for megaspore ejection (> 7 μN) are impressive, considering that a human muscle fiber can only produce a force of about 0.3 μN.

The observed maximum distance of megaspores dispersal was 65 cm, as shown earlier by Page (1989). The much larger dispersal distance of megaspores compared to microspores (5–6 cm) is related to the special dispersal mechanism of megaspores and can be regarded as an early adaptation for a heterosporous plant to allow better establishment of new sporophytes by increasing the opportunity for cross-fertilization. Indeed, the short dispersal distance of microspores observed here only holds for quiet air. When there is wind, microspores are spread over larger distances and well mixed with the megaspores (Filippini-DeGiorgi et al. 1997). It is then likely that electrostatic forces may lead to a close contact between microspores and megaspores.

The ejection speed of spores could in principle be calculated from observed flight distances through computer simulations of flight trajectories (equations 1 and 2 above). However, there is large variation in flight distances (Figs 1 and 2), which can be due to numerous by post-ejection influences (Burrows 1988; Filippini-DeGiorgi et al. 1997; Hemsley and Griffiths 2000). As a result, simple simulations based on flight distances can only yield rough approximations for the initial speed. The direct observation of spore trajectories using a high-speed camera is therefore more reliable.

Because of the very fast movements of the first steps in spore dispersal, it would be interesting to use photography of even higher speed and resolution. This would allow a more precise measurement of these starting events, and it would then be possible to more precisely calculate the forces necessary for the observed dispersal distances. The new possibility to precisely determine the impulse and initial speed of *Selaginella* spores will stimulate more detailed studies about the role and function of biological structures responsible for spore ejection.

Zusammenfassung

Wir untersuchten die ersten Phasen der Sporenausbreitung von *Selaginella martensii* Spring, einer tropischen Moosfarnart. Die aktive Sporenausbreitung basiert auf anatomischen Besonderheiten des Sporangiums, dessen zwei Klappen sich langsam öffnen und dann plötzlich schliessen. Wir legten fertile Sprossabschnitte von *Selaginella* auf Papier und massen die Distanzen, über die die Sporen ausgebreitet wurden. Die Microsporen wurden bis zu 5–6 cm von der Quelle aktiv ausgebreitet, die Megasporen hingegen erreichten eine Distanz von bis zu 65 cm, und im Durchschnitt 20–25 cm. Mit der SpeedCAMpro-Kamera erreichten wir eine Auflösung von 1/1000 s. Aufgrund der Kamerabilder und dem mittleren Gewicht einer Megaspore konnten wir die Anfangsgeschwindigkeit und den Impuls der Megasporen sowie die durch den Schleudermechanismus erzeugte Kraft berechnen. Die Anfangsgeschwindigkeit

betrug 4.5 m/s, der Impuls 6.3 pNs. Die Kraft, die eine Megaspore in weniger als 1 ms erfährt, ist 7 μN.

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