Humidity changes in dead fern fronds drives unique timing of spore dispersal in a widespread fern species

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Senesced fertile leaf from last year, alongside a new developing fertile leaf from the current year. The current year's developing fertile leaf will eventually senesce and look like the adjacent leaf from last year. Credit: Jacob Suissa

The sensitive fern—named due to its sensitivity to drought and frost—is a widespread species found throughout eastern North America and eastern Asia. It is a dimorphic plant because it has two distinct types of leaves—one for photosynthesis and one for reproduction. While most fern species in temperate regions produce and disperse their spores in the summer, the sensitive fern has an atypical timing of spore dispersal. In the early summer they produce heavily modified spore-bearing fronds (or leaves) with leaflets tightly enveloping their sporangia and spores. These fronds deteriorate with age, yet continue to persist above ground during the winter as dead mature structures. In the early spring their leaflets open to disperse spores. While the timing of spore dispersal has been observed for over 120 years, the structural mechanisms driving this phenology have remained elusive.

In a new study published in the *Annals of Botany* Jacob Suissa, Ph.D. candidate in The Department of Organismic and Evolutionary Biology and Fellow of the Arnold Arboretum at Harvard University, reveals that the unique timing of spore dispersal in the sensitive fern, known as Onoclea sensibilis, is determined by a structural mechanism of humidity-driven movement in spore bearing leaves.

When COVID-19 began Suissa could not access the labs to do his work so he took daily walks through Harvard's Arnold Arboretum. It was on one of his walks, in late winter with the ground frozen, that he noticed a green plant from the previous summer was now a dead leaf sticking up out of the snow. Wanting to learn more, Suissa grabbed a handful of fronds off of the plant and returned home.

"I started reading everything," Suissa said, "I read papers and books from 1960, then 1950, and all the way back to 1907 and 1890." He realized that scientists had long known about the timing of spore dispersal in the sensitive fern, but he could find nothing on what mechanism drove this phenology. With his doctoral work on hold, Suissa determined to find out how this odd fern achieves this unique phenology.

"I began conducting simple experiments in my house on the dead leaves I'd collected," Suissa said. He put some of the leaves in the oven at the lowest temperatures. Some he put in his freezer and some he put in plastic bags with a moist paper towel.

Suissa discovered that these dead mature leaflets seemed to open when they were dried out and close again when introduced to water. He learned that essentially the dead leaves move in direct response to changes in humidity and that it is a reversible process that can be repeated hundreds of time. "These are dead structures," Suissa said, "it's not like a Venus Flytrap or a Sensitive Plant where movement occurs in living plant tissues. I knew there had to be something structural that allowed this non-living material to move in response to changes in water status."

But that was as far as the study could go without access to lab equipment. So Suissa ran more experiments in his home until he could access high-tech equipment later in the summer. Once the labs reopened, he put the spore-bearing leaves in growth chambers adjusting the humidity by ten percent increments starting with zero percent. Just as he had observed in his home experiments, the leaflets opened when dry and closed when wet. Suissa repeated this experiment many times with the same results. This produced a fine scale resolution that demonstrated how small incremental changes in humidity led to small incremental changes in leaf opening. With this confirmation he then turned his attention to the underlying structural mechanism driving the movement.

Suissa sliced the leaf in half and examined it under the high-powered electron microscope. He saw that cells were constructed differently on the upper and lower side of the leaflet. Plants use cellulose to build their cells and the cellulose is usually arranged in bundles or fibers. Suissa found that in the cells of the upper side of the leaflet these fibers were oriented perpendicular to the length of the cell, while the fibers in the cells of the lower side of the leaf were oriented parallel to the length of the cell. The orientation of these fibers is very important in dictating how, or if, a structure will move because these fibers can expand when they absorb water.

Dead fern leaves sticking up out of the snow. Credit: Jacob Suissa

"Basically, because the bundles are oriented perpendicular to the length of the cell in the upper portion of the leaflet, when it is hydrated those cells can expand like an accordion," Suissa said, "the top layer of cells expands, but the bottom layer doesn't and this differential expansion leads to the leaflet closing when it's wet and opening when it's dry. Interestingly, this is the first time this mechanism has been documented in a whole fern leaf."

The finding also shows convergent evolution as it is the exact mechanism found in pine cones, which are also dead structures when they are fully mature. If you put a fully mature pine cone in the oven it will open up. Pop it into a plastic bag with a moist paper towel and it will close. "What's really interesting is that you have two identical structures—a pine cone and a fern leaf, but remarkably the evolutionary time that separates these two lineages is vast. They've been evolving independently for more than 300 million years," Suissa said.

These humidity-responsive structures are not unique to only ferns and pine cones. It occurs in a variety of flowering plants such as geranium fruit and wheat awns. While the process is not unique it likely has evolved to regulate the timing of the dispersal of seed, spore, or fruits.

Humidity-responsive biological structures have also inspired engineers to design materials that mimic these marvels of green engineering. The key aspect of movement in these structures is that they don't require significant energy inputs to move. This has led to environmentally friendly designs of patio roofs and art installations that open and close depending on the weather—open when dry and closed when raining. They move in response to the change in humidity requiring no energy input. Hopefully, work like Suissa's that reveals the biology of these structures can help inspire future architects and engineers to build climate-conscious structures that require little energy input.

Green vegetative or photosynthetic leaf from the current growing season, with senesced (dead) fertile leaf persisting from previous year. Credit: Jacob Suissa

Suissa is a botanist that specializes in understanding how plants are constructed, how they work, and how they have evolved across deep time. In particular, he focusses on ferns, a diverse group of land plants with over 11,000 species and a long evolutionary history. During his doctoral work, he has focused on how ferns move water through their vascular system. With his advisor Professor Ned Friedman in the Department of Organismic and Evolutionary Biology and director of the Arnold Arboretum, he is working to uncover how the vascular system has evolved over the 400 million years of fern evolutionary history.

"At the end of the day I threw these plants I found while walking into different humidities and looked at them under a microscope and I discovered something new about the natural world," Suissa said.

More information: Fern fronds that move like pine cones: humidity-driven motion of fertile leaflets governs the timing of spore dispersal in a widespread fern species, *Annals Of Botany* (2021). DOI: 10.1093/aob/mcab137

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