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The Possible Effect of Cypsela Morphology on Endemism in *Solidago shortii*

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Abstract

This project focuses on the differences between *Solidago altissima* and *S. shortii*, or the common and Short's goldenrods, respectively, and endeavors to provide an explanation for the narrow endemism of Short's goldenrod, relative to that of the common goldenrod. The common goldenrod has a range spanning from Southern Canada to Northern Mexico, while the Short's is found only in two locations in Kentucky and Indiana. They are rather similar to the untrained eye, but further observation reveals drastic morphological differences between the seeds of the two species, with those of the Short's being far larger than those of the common goldenrod, despite the two species having pappi, or parachute-like tufts of hair, of virtually identical size. This project examines data collected in the laboratory relating both to the morphology of the cypselae of the two species and behavior of the cypselae of the two species under conditions analogous to those seen in nature. The achenes of the Short's goldenrod were observed to be statistically significantly longer and wider than those of the common goldenrod, but no significant differences observed in the length of the pappi of the two species. The cypselae of the Short's goldenrod were observed to fall statistically significantly faster and be blown for a statistically significantly shorter distance than those of the common goldenrod. This difference in the ability of the cypselae to be disseminated via wind may contribute to the rarity and endemism of the Short's goldenrod.

Keywords: *Solidago shortii*, endemism, anemochory, cypsela

1. Background

First discovered at the Falls of the Ohio in the year 1840 by Dr. Charles Wilkins Short, a physician and botanist from Louisville, Kentucky and the man for whom it is named[1], Short's Goldenrod or *Solidago shortii* is - in the words of the Nature Conservancy - "one of the rarest plants in the world[2]." Though Dr. Short was a physician and professor of medicine by trade he was well trained in botany, and consulted some of his friends and colleagues in the discipline, including John Torrey and Asa Gray, in order to properly classify his new discovery as a distinct species[3].

Despite being closely related to the common goldenrod, or *Solidago altissima* (also known as *Solidago canadensis*), *S. shortii* is known to be endemic to only two known locations. The initial population discovered by Dr. Short on Rock Island near the Falls of the Ohio was extirpated sometime in the due to flooding of the site by impoundment. In 1939, another colony of the species was discovered in Northeastern Kentucky, in and around Blue Licks Battlefield State Park where there still exists a State Nature Preserve to which public access is very limited in order to protect and "enhance habitat for the goldenrod[4]."

Decades later another colony of *S. shortii* was found in Southern Indiana, in a state forest approximately 50 miles outside of Louisville[5]. It is possible that *S. shortii* could be found in other parts of the state. Some regions, in particular, "the South Central counties" because they "have been largely neglected by collectors and offer a high potential for new discoveries[6]." This is unlikely as it has been ~~strongly~~ suggested that *S. shortii* relied on the Eastern Woodland Bison to reduce competition as well as to spread its seeds. According to a report from the United States National Fish and Wildlife Service "it is possible that [populations of] *S. shortii* may occur along" the migration paths of the bison, which happen to connect Blue Licks, the Falls of the Ohio, and the population in Southern Indiana where *S. shortii* has been observed[7]. In contrast to its critically endangered cousin the common goldenrod is found throughout the continent, from Southern Canada to Northern Mexico. It is considered to be a weed by many, and can grow in diverse environments, including but not limited to "roadsides, railways, and city suburbs" in China where it has become a nuisance as an invasive species[8]It has also begun to become a problem in certain areas in Eastern Europe, concerning their "exceptionally successful[9]" invasion, and their "threat to biodiversity[10]."

The cypselae of both *S. shortii* and *S. altissima* are dispersed via an anemochorous mechanism, that is, after seeds are produced and matured they rely on the wind to blow them off of the parent plant and spread them to new territory. This is accomplished via pappi, or small tufts of hair-like material found on the tips of the achenes, or seeds, of the plants. An achene together with its associated pappus form the cypsela (plural: cypselae) upon germination will become a new plant. *S. altissima* is an organism with a wide range and large population. Research has found that "the chromosomal base number of *Solidago* is x=9" and *S. shortii* is known at ploidy levels of 2x and 4x, while *S. altissima* is known at the 4x and 6x levels[11]. A study on morphological variation of *S. altissima* concluded that while there is significant variation between some communities in the Western US and the rest of the population, the communities in the Southeastern US are consistent with the rest of the population, though this study did not examine seed morphology[12]. Research on native and invasive populations of *S. altissima* in Japan showed large genetic variability among *S. altissima* populations, but suggested that populations in Kentucky were rather similar to others in the Southeastern United States[13]. No such studies on genetic or morphological variation have been done on *S. shortii*.

While it may seem frivolous to spend time, energy, and other resources studying an obscure species only naturally found in two places in the world, "[t]he study of plant endemism is important because it could improve our knowledge of the flora of a region in at least two different respects … biogeography and evolution[14]." Beyond even this, researchers have determined that *S. shortii* produces "two new diterpenes" not previously observed in nature[15]. Though these particular compounds may not be of significant utility to humanity at the present moment, these and other compounds and enzymes produced by *S. shortii* and other endangered flora that are on the brink of extinction could quite possibly be of great use to humanity in the future, and thus must be protected for future use and research.

2. Materials and Methods

The cypselae of the two species were first measured to ascertain an estimate of their typical size. A dissecting microscope with an ocular micrometer in the eyepiece was used to conduct the measurements. The ocular micrometer was calibrated with a ruler after any adjustment.

50 cypselae of both *S. altissima* and *S. shortii* were randomly selected from a large sample of the cymes, or flowering branch tips, of both species collected in Berea, Kentucky. Each cypsela was measured using forceps, a pin to help hold the cypsela in place, and the ocular micrometer to actually make the measurement. Three measurements were made of each cypsela: the length of the achene portion of the cypsela, the width of the achene portion of the cypsela, and the length of the pappus portion of the cypsela. These metrics were recorded in "units" displayed on the ocular micrometer along with the value of mm per unit that had been calculated at the beginning of the session. The values were then converted into mm before statistical analyses were conducted.

As the masses of the cypselae are a very important metric when attempting to discern differences in the cypsela morphology between the two species, 5 samples of 100 cypselae of both *S. shortii* and *S. altissma* were randomly selected from samples collected in the field. They were counted using forceps and a tally counter. Each sample of 100 cypselae was placed into a small envelope that was then labelled. There was likely researcher error involved in calibrating the balance and recording the masses of the samples, as the results were highly variable and inconsistent with previous research done on the topic.

Given that "the number of diaspores [cypselae] is important for explaining the reproductive traits" of a species, the number of cypselae produced by each species was determined, in an attempt to ascertain whether or not either species produces more cypselae than the other[16]. The mean number of cypselae per involucre and the number of involucre per cyme were calculated, and then used to estimate the number of cypselae per cyme. Ten cymes from both *S. altissima* and *S. shortii* were randomly selected from samples that were collected in the field. Ten involucres were systematically randomly selected in order to ensure that samples were taken from all points along the cyme. The selected involucres were then removed and the number of cypselae on each was counted and recorded. Then, the number of involucres in 2 cm segment from the middle of the cyme was counted and recorded. Finally, the length of the cyme was measured, with the number and length of any branches that the cyme may have had. This data was used to estimate the mean number of cypselae produced per cyme of the plants of each of the two species being investigated.

As both *S. altissima* and *S. shortii* are anemochorous, or wind-dispersed, plants, an experiment was conducted in an attempt to determine whether or not the cypselae of the two plants behave differently under conditions similar to their natural wind dispersal. A small wind tunnel was constructed using three meter sticks - one acting as the floor of the channel and two making up the sides - and a 12 inch ruler was placed on top of the channel at the "starting end" to help direct the air current and minimize the number of cypselae that escaped the channel and thus making measurement more difficult. A pipette with a volume of 10.9 cm^3 was used to blow the cypselae. A group of five randomly selected cypsela of either *S. shortii* or *S. solidago* from a large sample of the cymes of both species collected in the field was placed in the channel exactly 2 cm away from the pipette, and after all of the cypselae had stopped moving, those that stayed in the channel were noted and their distance from the starting point was recorded. Cypselae that landed outside of the channel were noted but not included in the sample. This process was repeated until 50 cypselae of both *S. altissima* and *S. shortii* had been measured and recorded.

In order to best study the behavior of the cypselae falling through the air 3-meter long plexiglass tubes were obtained in the hopes of eliminating any effect of air currents on the falling cypselae in an attempt to emulate earlier successful experiments on cypsela dispersal ability[17]. Unfortunately, electrostatic interactions between the pappi of the cypselae and the plexiglass tubing interfered too much. This is likely due to the small diameter of the plexiglass tubing available; the previous researchers used tubing with a 15cm diameter. The interior of the tubes was lubricated with Rain-X® (polysiloxane) in order to negate the effect of the static cling, but the attempt was unsuccessful. Instead, emulating other research done on anemochorous cypsela dispersal in slightly less controlled, more natural conditions, a corner of a laboratory between two walls and a shelf was selected in an attempt to minimize the effect of any air currents on the results[18]. A white sheet was placed on the ground in order to ensure that it would be apparent when the cypselae reached the ground. A group of 25 randomly selected cypselae of each species was collected. They were dropped from a height of 2 meters and the time required to fall to the ground was recorded. The results were analyzed using a confidence test with a Student's T distribution.

3. Results

The analysis of the data collected from cypsela measurements found that there was a statistically significant difference ($\alpha \leq 0.001$) between the lengths of the achenes of the two species, with the achenes of *S. altissima* being approximately only half as long as those of *S. shortii* (mean length for *S. altissima*:1.05 mm; *S. shortii*: 2.18 mm). Achene width was also found to be statistically significantly different ($\alpha \leq 0.001$), with the achenes of *S. altissima* averaging at 0.32 mm wide and those of *S. shortii* at 0.53 mm wide. There was no significant difference found in the average length of the pappi of the two species ($\alpha > 0.05$), with mean pappus length for *S. shortii* being 2.65 mm and mean pappus length for *S. altissima* being 2.76 mm.

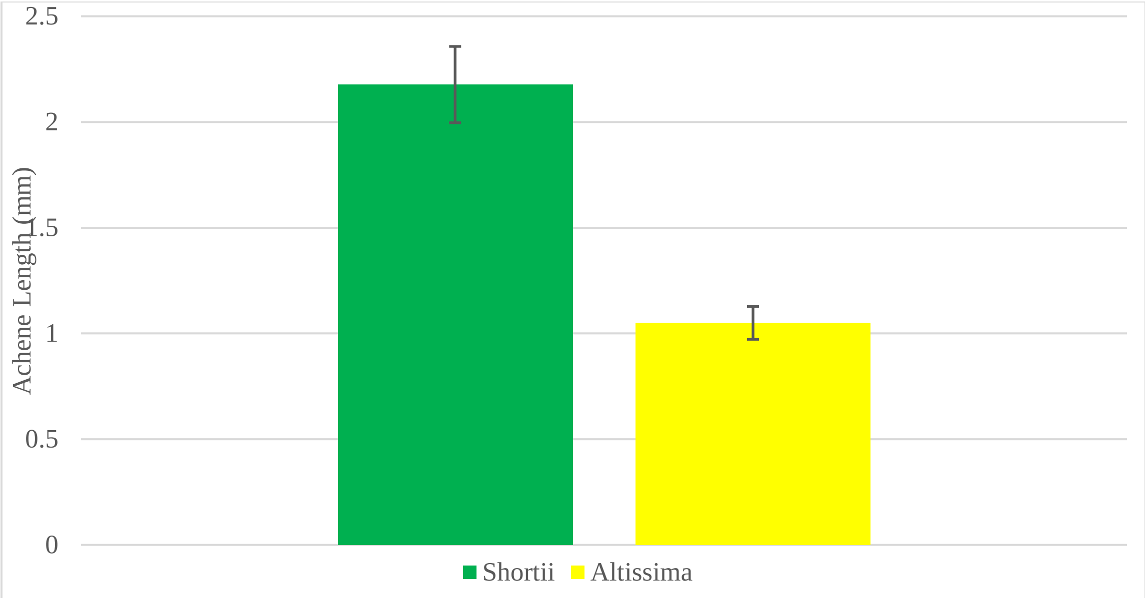

Figure 1. A chart comparing achene length between *Solidago shortii* & *S. altissima*.

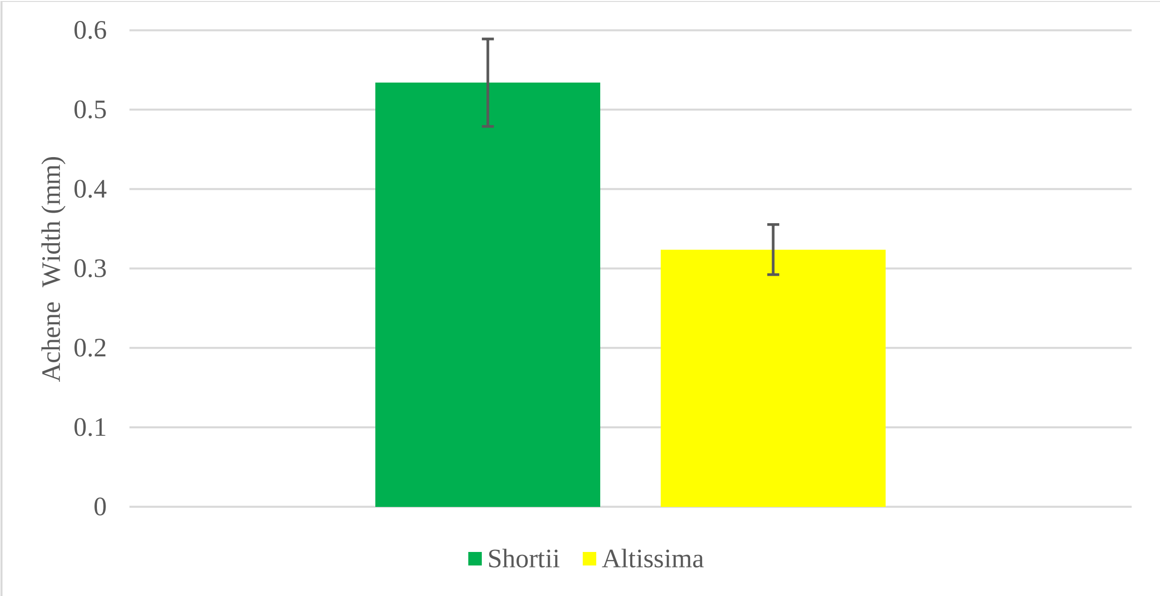

Figure 2. A chart comparing achene width between *Solidago shortii* & *S. altissima*.

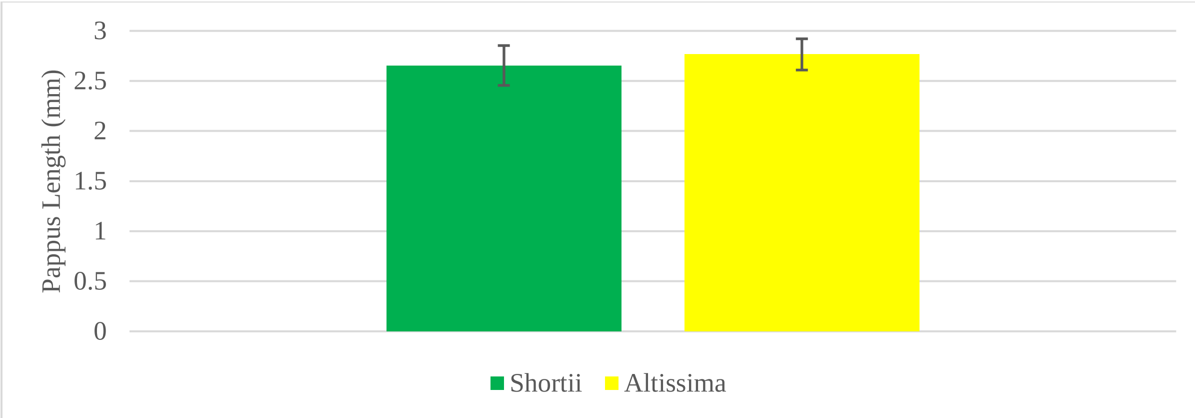

Figure 3. A chart comparing pappus length between *Solidago shortii* & *S. altissima*.

Analysis of cypsela mass showed a mean mass for the cypsela of *S. shortii* was approximately 277 µg, and approximately 217 µg for the cypselae of the *S. altissima*. These results were not statistically significant and had enormous variability. In addition, these results differ wildly from the data collected by earlier researchers who determined the mean cypsela mass for *S. shortii* to be approximately 370 µg and 60-70 µg for *S. altissima*[19]. These data strongly suggest researcher error and will not be used in any further analysis.

Analysis of the quantity of the cypselae produced by each plant demonstrated no significant difference ($\alpha>0.05$) in the number of produced by the involucres of the two species, with the mean for *S. shortii* being 11.19 cypselae per involucre and the mean for *S. altissima* being 11.72 cypselae per involucre. There was no significant difference ($\alpha>0.05$) in the number of involucres per cm between the two species with the mean for *S. shortii* being 6.85 involucres per cm and the mean for *S. altissima* being 6.45 involucres per cm. There was no significant difference ($\alpha>0.05$) in the total cyme length (including branches) between the two species with the mean for *S. shortii* being 19.45 cm and the mean for *S. altissima* being 19.5 cm.

Analysis of the distance travelled by the cypselae of each plant when dispersed by wind demonstrated a statistically significant difference ($\alpha\leq0.001$). The cypselae of *S. altissima* travelled an average of 62.4 cm through a channel 2.5 cm wide and 1.75 cm deep when propelled by 10.9 cm^3 of air expelled from a pipette over a period of approximately 0.4 seconds, while the cypselae of *S. shortii* only travelled an average of 55.94 cm under identical conditions.

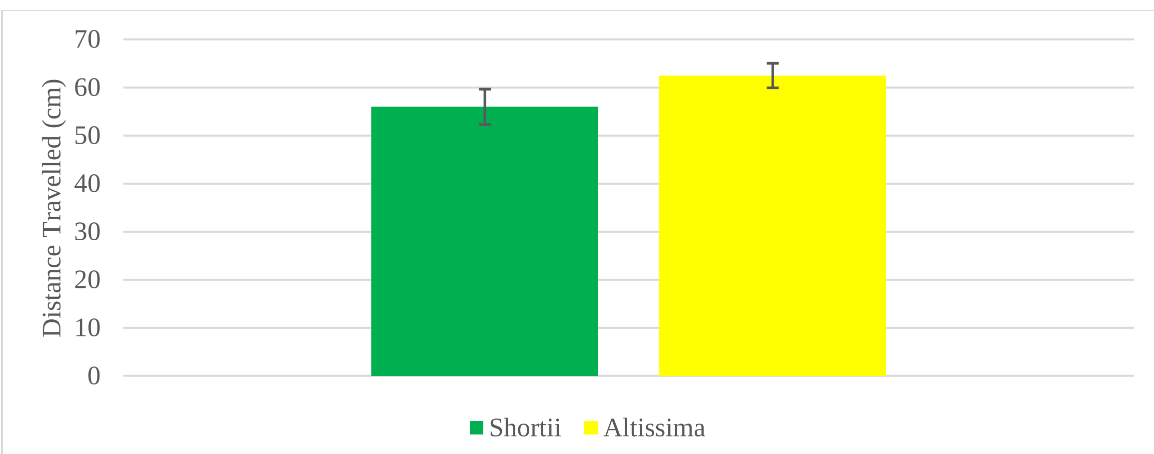

Figure 4. A chart comparing distance travelled by cypselae in a wind tunnel between *Solidago shortii* & *S. altissima*.

Analysis of the behavior of the cypselae falling freely through the air determined a statistically significant difference in the amount of time that it takes for the cypselae of the two species to fall with no outside forces acting upon them ($\alpha\leq0.001$). The cypselae of the *S. altissima* required an average of 14.02 seconds to fall to the ground from a height of 2 meters, while the cypselae of the *S. shortii* required 5.22 seconds.

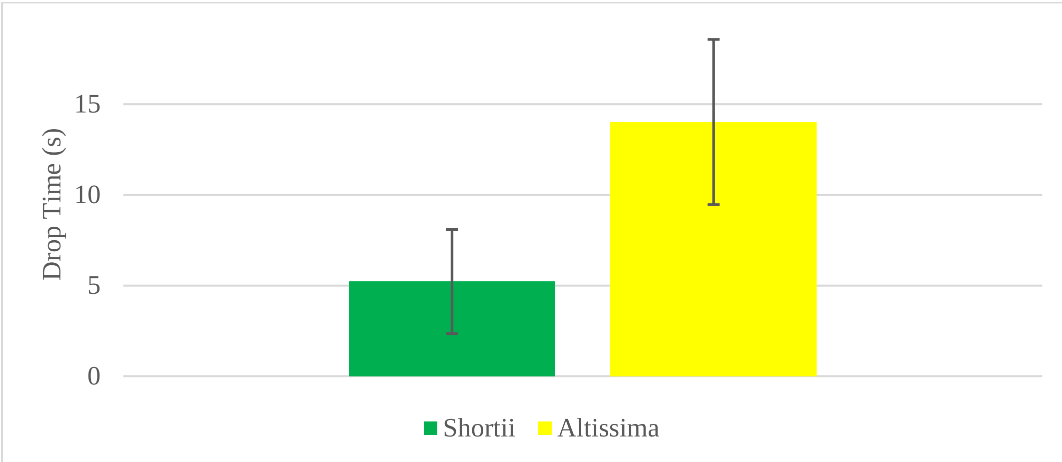

Figure 5. A chart comparing time required for the cypselae of *Solidago shortii* & *S. altissima* to fall 2 meters.

4. Discussion

There is likely a panoply of reasons for the extremely narrow endemism of *S. shortii* relative to *S. altissima*, and any attempt to elevate one factor over all others will ultimately obscure a large part of the story. The existing body of research has both put forth potential contributors, such as the idea that *S. shortii* "is a relatively young species" when compared with *S. altissima*[19] and ruled out possible explanations, such as the possibility of an abnormal germination pattern[20]. Despite the roles that other factors play, the data collected and summarized here leaves little question as to the role that the morphology of the cypselae play in contributing to the endemism of *S. shortii* relative to *S. altissima*. The data demonstrate it is unlikely that one of the two species of goldenrod are producing more cypselae than the other, with there being no significant difference between the number of cypselae per involucre, the number of involucres per cm of cyme, or the length of the cyme between the two species. Neither species is likely to be producing significantly more offspring than the other, and suggests that differences in success of dispersion of cypselae might be playing a larger role in the relative endemism of *S. shortii* compared to *S. altissima*.

The achenes of *S. shortii* were significantly larger than those of *S. altissima*, despite the fact that the size of the pappi of the cypselae was identical in the two species. This suggests the cypselae of *S. shortii* would travel a shorter distance than those of *S. altissima*, because their pappi, which are the same size as those of *S. altissima*, possess an achene that is approximately twice as large, and according to the research done by earlier researchers, approximately six times as massive. This would almost certainly point to difficulty in dispersing seeds by wind on the part of *S. shortii*, at least when compared with *S. altissima*. However as stated previously, due to the morphological variability from region to region of the *S. altissima*, it is unclear if the samples collected can be considered representative of the entire species[12]. That said, as the research suggests that the Kentucky populations are similar to others in the Southeastern US, it can be assumed that the samples are representative of *S. altissima* individuals in this part of the nation[13].

This is supported by the data from the free fall and wind tunnel. The cypselae of *S. altissima* travelled approximately 6 cm farther than those of *S. shortii*, and required more than twice the time to fall from a height of 2 meters, presumably due to the advantage given to them by their pappus : achene ratio, which is much larger than that of *S. shortii*.

It is likely that the differing experimental behavior of the cypselae of the two species in lab translates to different behavior when released by the parent plant and dispersed by the wind. The longer drop time of *S. altissima* cypselae suggests that in nature, its cypselae will fall more slowly and have more opportunity to be blown about by wind. Similarly, the fact that the cypselae of *S. altissima* travel farther when blown by wind suggests that they may be able to travel much farther than those of *S. shortii* when exposed to the far stronger and more sustained force of wind in Autumn, when the cypselae are mature and released by their parent plants. This translates to lower levels of reproductive success, as "Dispersal potential and dispersal strategies, such as wind- or animal-dispersal, have been assumed to be highly relevant for the success of plant species[21]."

Beyond the disadvantage of not being able to spread their offspring over a larger area to propagate the species, another downfall of not widely dispersing cypselae is the risk of loss of seeds due to pre-dispersal predation, which researchers have determined can lead to the loss of approximately "97% of the seeds[22]" of the dandelion to certain invertebrates, as "consumers may cause seed mortality and thus influence population dynamics of the dandelion[23]" and are "crucial factors limiting dandelion populations[24]." As the dandelion is much more efficient and successful at dispersing cypselae via wind than *S. shortii*, such predation could prove devastating to the plant if some non-native predator were to invade one of its very few habitats.

All of this data suggests that the morphology of the cypselae of *S. shortii* contribute to its rarity as well as its narrow endemism when compared to its close relative, the extremely common and very widespread *S. altissima*.

Future research on this topic ought to analyze how strongly the cypselae of the two species are attached to their parent plants, and potentially following in the path of research on other anemochorous species by using all variables to create a mathematical model for the effect of cypsela morphology on anemochory[25].

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