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#### EASTERN KENTUCKY UNIVERSITY

The Possible Effect of Cypsela Morphology on Endemism in Solidago shortii

Honors Thesis

Spring 2017

By

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Faculty Mentor

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#### Abstract

This project centers around the differences between the *Solidago altissima* and the *S. shortii*, or Common and Short's Goldenrods, respectively, and endeavors to provide an explanation for the narrow endemism of *shortii*, relative to that of *altissima*. The Common Goldenrod has a range spanning from Southern Canada to Northern Mexico, while the Short's is found only in two isolated 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.

There is not a large body of academic work regarding the Short's goldenrod, and the majority of it focuses merely on describing the morphology and habitat of the species, rather than providing potential explanations as to why it is so rare. This project will primarily rely on data collected on the two species, in particular their cypselae, to attempt to provide a plausible explanation. Data collected shows that there is a very large difference in the size of the achenes of the two species but virtually none in the length of their pappi. Furthermore, the cypselae of *S. shortii* were demonstrated to have travelled approximately 12% farther and fall approximately 3 times as rapidly as those of the *S. altissima* under controlled laboratory conditions. This suggests that the morphology of the cypselae of the *S. shortii* are maladaptive to the anemochory upon which it must now rely to disperse its offspring.

## **Table of Contents**

ibstracti
ist of Figuresi
Acknowledgements
ackground2
Interials and Methods
lesults10
Discussion1
Conclusion19
Sibliography

## List of Figures

Figure 1. Photograph of the Short's Goldenrod2
Figure 2. Photograph of the Common Goldenrod
Figure 3. Ocular Micrometer
Figure 4. Photograph of <i>S. shortii</i> cypsela under ocular micrometer
Figure 5. Photograph of wind tunnel used to measure behavior of cypselae
Figure 6. Photograph of setup used to measure speed of cypselae falling9
Figure 7. Chart comparing achene length between <i>S. shortii &amp; S. altissima</i> 10
Figure 8. Chart comparing achene width between S. shortii & S. altissima11
Figure 9. Chart comparing pappus length between S. shortii & S. altissima11
Figure 10. Chart comparing cypselae per involucre in S. shortii & S. altissima13
Figure 11. Chart comparing involucres per cm of cyme in <i>S. shortii &amp; S. altissima</i> 13
Figure 12. Chart comparing mean cyme length in S. shortii & S. altissima14
Figure 13. Chart comparing distance travelled by S. shortii & S. altissima
Figure 14. Chart comparing drop time for cypselae of <i>S. shortii</i> & <i>S. altissima</i> 16

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#### 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 (Baskin, Walck, Baskin, and Buchlele, 2000), the Short's Goldenrod or *Solidago shortii* is - in the words of the Nature Conservancy - "one of the rarest plants in the world" (Nature Conservancy). 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 (Beck, Naczi, & Calie 2001).



Figure 1. Photograph of Short's Goldenrod. Taken by Dr. Patrick J. Calie

Despite being a close cousin of the Common Goldenrod, or *Solidago altissima* (also known as the *Solidago canadensis*), the *S. shortii* is known to be endemic to only two known locations, and is extinct at one locality. The initial population discovered by

Dr. Short on Rock Island near the Falls of the Ohio is believed to have gone extinct sometime in the 1920s, but the species was later reintroduced to a site nearby (Baskin, Walck, Baskin, and Buchlele, 2000). In the 1930s, another colony of the species was discovered in Northeastern Kentucky, in and around Blue Licks Battlefield State Park (Braun, 1941) 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" (Kentucky State Nature Preserves Commission).



Figure 2. Photograph of the Common Goldenrod: http://delawarewildflowers.org/images/solidago\_canadensis\_scabra.jpg

Decades later another colony of *S. shortii* was found in Southern Indiana, in and around a nature preserve not far from Louisville (Homoya & Abrell, 2005). It is possible that the *S. shortii* may be found in other parts of the state, as some regions, especially "the South Central counties" because they "have been largely neglected by collectors and offer a high potential for new discoveries" (Medley, Thieret, & Cranfill 1983). This is,

however, unlikely as it has been strongly suggested that the *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 nature preserve in Southern Indiana where *S. shortii* has been observed (USFWS 1988). In contrast to its critically endangered cousin the Common Goldenrod is found all over the continent, from Southern Canada to Northern Mexico. It is considered to be a weed by many, and can grow in all sorts of environments, including but certainly not limited to "roadsides, railways, and city suburbs" in China where it has become a nuisance as an invasive species (Zhao, Sun, Gituru, Chen, & Wang, 2014). It has also begun to become a problem in certain areas in Eastern Europe, where much research has been done on their "exceptionally successful" invasion, and their "threat to biodiversity" (Szymura & Szymura, & Śweirszcz 2016).

The cypselae of both the *S. shortii* and *S. altissima* are dispersed via an anemochorous mechanism, which is to say that after seeds are produced and matured they rely on the wind to blow the off of the parent plant and spread them to new territory. This is accomplished via pappi, or small tufts of hair found on the tips of the achenes, or seeds, of the plants. An achene together with its associated pappus together form the cypsela (plural: cypselae) that will eventually become a new plant. The Short's Goldenrod is thus said to be very narrowly endemic, due to the extreme isolation of its natural habitat, as compared to the widespread Common Goldenrod.

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" (Chiapella & Demaio, 2015). Beyond even this, researchers have determined that the *S. shortii* produces "two new diterpenes" not previously observed in nature (Williams, Du, Norman, Goering, O'Neil-Johnson, Woodbury, & Starks 2014). Though these particular compounds may not be of significant utility to humanity at the present moment, these and other compounds and enzymes produced by the *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.

#### **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 their natural habitats. 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

(minimization) and a spectra in a factor of a standard and and a standard and a standard a standard a standard

Figure 3. Ocular Micrometer https://www.aliexpress.com/price/ocularmicrometer\_price.html 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 isolated 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 some sort of researcher error involved in calibrating the balance and



Figure 4. Photo of *Solidago shortii* cypsela under ocular micrometer. Taken by Evan Singleton

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 (Šerá, 2008). The mean number of cypselae per involucre, 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, as was 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<sup>3</sup> 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.



Figure 5. Photograph of wind tunnel used to measure behavior of cypselae

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 (Meyer & Carlson 2001). 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 (Jongejans & Telenius, 2001). 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 were collected. They were dropped from a height of 2 meters and the time taken to fall to the ground was recorded.

#### Results

The analysis of the data collected from cypsela measurements found that there was a statistically significant difference ( $\alpha \le 0.001$ ) 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 ( $\alpha \le 0.001$ ), with the achenes of *S. altissima* averaging at 0.32 mm wide and those of *S. shortii* at 0.53



Figure 6. Photograph of setup used to measure speed of cypselae falling

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 7. Chart comparing achene length between S. shortii and S. altissima









Analysis of cypsela mass showed a mean mass for the cypsela of the *S. shortii* was approximately 277  $\mu$ g, and approximately 217  $\mu$ g 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* (Walck, Baskin, & Baskin, 2001). This data strongly suggests researcher error and will not be used in any further analysis.

Analysis of the quantity of the cypselae produced by each plant determined that there was 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. Similarly, 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. Finally, 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.











#### Figure 12. Chart comparing mean cyme length in S. shortii and S. altissima

Analysis of the distance travelled by the cypselae of each plant when dispersed by wind determined that there was a statistically significant difference between the distances that the cypselae of the two species travel when dispersed by wind ( $\alpha \le 0.001$ ). 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  $10.9 \text{ cm}^3$  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 13. Chart comparing distance travelled by cypselae of S. shortii and 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 ( $\alpha \le 0.001$ ). The cypselae of the *S. altissima* took an average of 14.02 seconds to fall to the ground from a height of 2 meters, while the cypselae of the *S. shortii* took only 5.22 seconds to fall to the ground from the same height.



#### Discussion

In all likelihood there is almost certainly a panoply of reasons for the extremely narrow endemism of *S. shortii* relative to *S. altissima*, and any attempt to exalt one factor over all others will ultimately leave out a big part of the story. While there has not been an enormous amount of research done on the issue the existing body of research has both ruled out possible explanations, such as the possibility of an abnormal germination pattern (Buchele, Baskin, & Baskin 1991) and put forth potential contributors, such as the idea that *S. shortii* "is a relatively young species" when compared with *S. altissima* 

(Walck, Baskin, & Baskin 2001). 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 shows that 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. This means that 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*.

First, the achenes of *S. shortii* were significantly larger than those of the *S. altissima*, despite the fact that the size of the pappi of the cypselae were virtually the same in the two species. This strongly suggests that the cypselae of the *S. shortii* would have a harder time travelling in the wind than those of the *S. altissima*, because their pappi, which are the same size as those of the *S. altissima*, have to carry 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 the *S. shortii*, at least when compared with the *S. altissima*.

This is even more strongly supported by the data from the experiments on the behavior of the cypselae falling through the air and being blown by the wind. The cypselae of the *S. altissima* travelled approximately 6 cm farther than those of the *S. shortii* on average, and took more than twice as long 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*.

In nature, it is likely that the differing behavior of the cypselae of the two species in lab translates to different behavior when released by the parent plant and when dispersed by the wind. The longer drop time of the *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 the Autumn, when the cypselae are mature and ready to be 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" (Knapp, Stadler, Harpke, & Klotz 2016).

Even 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" of the dandelion to certain invertebrates, as "consumers may cause seed mortality and thus influence population dynamics of the dandelion" and are "crucial factors limiting dandelion populations" (Honěk, Štys, & Martinková 2013, Honěk, Martinková, & Saska, 2005, Honěk, Martinková, Saska, & Koprdova 2009). As the dandelion is much more efficient and successful at dispersing cypselae via wind than the *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 cousin, 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 (Takana, Shibata, & Nakashizuka 2008).

#### Conclusion

Depending on one's viewpoint, it could seem puzzling that *S. shortii* has not either became extinct completely or adapted to its environment and begun to evolve a better mechanism with which to disperse its cypselae so that it stands a better chance of producing offspring that will spread and propagate the species. While research has shown that "rapid evolution is remarkably common," there is "no evidence for rapid evolution of seed dispersal," and there much being done to protect the species from further endangerment and extinction (Bartle, Moles, & Bonser 2013, Nature Conservancy).

As demonstrated conclusively by the data collected, the morphology of the cypselae of *S. shortii* is maladaptive to anemochory, which is the system of dispersal upon which it now relies in the absence of the Eastern Woodland Bison (Gelbart, 2015)

(US FW, 1988). This poses, however, an even larger problem for the Short's Goldenrod, as "long distance seed dispersal plays a *crucial* role in plant metapopulation persistence and *response to rapid climate change*" (Greene 2005) [empasis added]. Furthermore, as "[m]igration of plant populations is a potential survival response to climate change that depends critically on seed dispersal," a lack of successful seed dispersal could spell the end for a species as the climates in environments and fragile ecosystems across the globe continues to change rapidly (Thompson & Katul 2013).

The Intergovernmental Panel on Climate Change has concluded that "Human influence on the climate system is clear, and recent anthropogenic emissions of greenhouse gases are the highest in history. Recent climate changes have had widespread impacts on human and natural systems" (IPCC 2014). The drastic, continuing, and possibly irreversible effects of human behavior on the climate threaten to devastate, and potentially even wipe out populations of fragile species like *S. shortii*. Unfortunately, just as the human destruction of the Eastern Woodland Bison population proved devastating to species dependent on it, like the Short's Goldenrod and Buffalo Clover, so too could the loss of these more fragile species devastate other species within their ecosystems (Gelbart 2015).

Humanity relies on the proper order and balance of the natural world in order to subsist. Though mankind may find itself at the "top" of the food chain, reckless and unchecked human action has, and may unfortunately continue to wreak havoc upon the rest of the organisms and structures that make up the planetwide system of balance upon which it relies. Ultimately, it is not to preserve the Short's Goldenrod, the Eastern Woodland Bison, the Black Rhinoceros, the Polar Bear, or any other low or high profile endangered species that humanity must act to slow, stop, and attempt to reverse the devastating effects of climate change. As devastating as these losses are to the beauty and quality of life on Earth, ultimately it is to preserve human life that humanity must take action.

A 2012 report found "unequivocal evidence" for the assertion "that biodiversity loss" which is exemplified by the rapid loss of many endangered species, throwing off balance in ecosystems and leading to further species loss, "reduces the efficiency by which ecological communities capture biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients," all of which are crucial to the balance of life on the planet and to human life as it has been known since the advent of civilization (Cardinale, et. al. 2012).

Even major religious organizations have felt the need to speak out on the importance and immediacy of this issue, with Patriarch Bartholomew, leader of the Eastern Orthodox Churches stating that "biodiversity is the work of divine wisdom ... not granted to humanity for its unruly control," and that "to destroy the biological diversity of God's creation" is sinful (Bartholomew 2012). Similarly, Pope Francis, leader of the Catholic Church, in his encyclical warns that the effects of climate change will be particularly severe "for the poor," and even dedicated an entire chapter to our planet's "Loss of Biodiversity (Francis 2015). In this chapter, he decries not merely the "short sighted approaches" of humanity's abuse of the environment as the potential destruction of resources that could prove useful in the future, but also the mentality that portrays nature as full of "'resources' to be exploited," saying that due to human action, "thousands of species will no longer give glory to God by their very existence" (Francis 2015). Furthermore, he reminds the faithful that not merely "more visible" species like "mammals and birds" must be protected because many rare or obscure species may "play a critical role in maintaining the equilibrium" of ecosystems (Francis 2015). The Short's Goldenrod - one of the most of such species - has not escaped recognition for its roles in these such matters. In an article written for an online review of ecological theology, author Zeb Weese describes the *S. shortii* and defends its importance in the ecosystem and the importance of biodiversity overall, not for selfish reasons, but for its place of beauty in the natural order (Weese 2013).

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