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Field Course in Behaviour and Ecology Project Report 2022

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Cirsium erisithales: Influence of Light Intensity on Degree of Droop of Flower Away from Horizon

Abstract

The species *Cirsium erisithales* is an under-studied angiosperm with a unique drooping morphology. There have been studies on other Angiosperms with similar 'nodding' or drooping morphologies, which have shown to protect pollen and other sexual organs from harmful UV-B, water, and predation. However, there is yet to be evidence for any of these factors influencing the amount of droop within *Cirsium erisithales.* Here we show there is no significant correlation, with a p-value of 0.777, between light intensity or area with the droop angle of *Cirsium erisithales.* There is therefor more scope for study and the reason for the drooping morphology of this species remains unclear. Further study which could be conducted is looking at other abiotic factors such as rain and wind or biotic factors such as predator avoidance or genetic variation.

Introduction

Some species of Angiosperms have been known to droop their flowers, shielding their pollen from the sky in harsh climates. This morphology has been shown to protect pollen from harmful factors, such as high temperatures and UV wavelengths (Chen *et al.*, 2013). These harmful effects can cause pollen to degrade which reduces success of fertilisation, thus reducing fitness. Other harm which pollen is shielded from is water and predation, including oviposition (Wise *et al.*, 2010). Chen *et al* (2013) looked at effects of the 'nodding capitulum' in *Cremanthodium campanulatum* and concluded that the drooping nature protected pollen and other sexual biology from UV-B radiation. They also discovered that erect, non-nodding individuals released fewer achnes and were thus less fit.

However, drooping is not completely advantageous. In some symbiotic pollinator-plant relationships it can prove to be detrimental. This is true for the species *Nicotiana attenuate*, which is pollinated by Sphingidae (hawkmoth) species. Haverkamp *et al* (2019) discovered that, even though attractive volatile compounds were distributed to the same extent (attracting hawkmoths), once the flowers were at a >90° angle, hawkmoths were no longer able to land and successfully pollinate the flowers.

The species *Cirsium erisithales* are known as 'Yellow Melancholy Thistles' and usually have drooping morphology, with their heads pointed in a downwards position. There is very limited research on this species, however some sources indicate that they mostly inhabit Eastern Europe and the Alps, and are found frequently in the Carnic Alps (botanica, n/a). Due to the lack of knowledge and research, the reason behind the drooping head morphology in the *Cirsium erisithales* is unknown. *Cirsium erisithales* are common within the Carnic Alps, being found in open meadows and in forested areas with varying levels of light intensity. This allows for the study of the influence of light intensity on the degree of droop of thistle flowers. Plants in the Carnic Alps are often at high altitudes and thus have higher exposure to harmful UV-B light, compared to plants at lower altitudes (Blumthaler, Ambach and Ellinger, 1997). This creates unfavourable conditions, which can lead to specialised adaptations in line with natural selection to increase fitness. In this example, it would be beneficial to shield sexual organs in a drooping morphology, in response to higher levels of light intensity, providing that plant-pollinator relationships could still exist. This study explored the relationship between light intensity and degree of droop, whilst accounting for thistle flower (head and bract) size. It was hypothesised that individuals of *Cirsium erisithales* being exposed to higher levels of light intensity would droop to a higher degree away from horizon, to shield sexual organs, compared to individuals in areas of lower levels of light intensity.

Materials and Methods

Methods

Study System

Cirsium erisithales is a poorly studied perennial herbaceous species belonging to the Asteraceae (thistle) family. They have long green stems which usually terminate after an arch very close to the tip, where there is a green bract and yellow thistle head. Upon observations, the height of plant seems to range from short (0.5<1) to tall (1<1.75m), which are inline with other species within the *Cirsium* genus (NPS, 2018). They appear to be able to withstand high altitudes, with them being found in study sites with altitudes over 1400m.

Site and Specimen Selection

In total, 5 study sites were visited within an 850m radius of Baita Torino Field Station (46.425995, 12.740744), as seen in Figure 1. They were studied around noon (12:00 – 14:00) to control for differential light intensities throughout the day. The wind forecasts were also checked to ensure the wind speeds were the same for each day. These sites were chosen in line with their accessibility by foot, along with their mixed open-meadow and dense-forest locations, which would offer varying levels of light intensity based on their being a canopy or not. A tool from GeoMidPoint.com allowed for two random points to be generated within a 50m radius of each site location. These location of sites and random points can be found in Table 1.

Table 1. *The chosen site locations with the two randomly generated points.*

Site Number	Site location	Random locations
1	46.429000, 12.735861	46.428889, 12.737778 46.428889, 12.737222
2	46.428750, 12.743000	46.428889, 12.743333 46.428889, 12.743611
3	46.425505, 12.741767	46.425639, 12.742305 46.425358, 12.741355
4	46.422583, 12.744583	46.421008, 12.747430 46.421606, 12.746408
5	46.420298, 12.749701	46.419992, 12.749081 46.419444, 12.749444

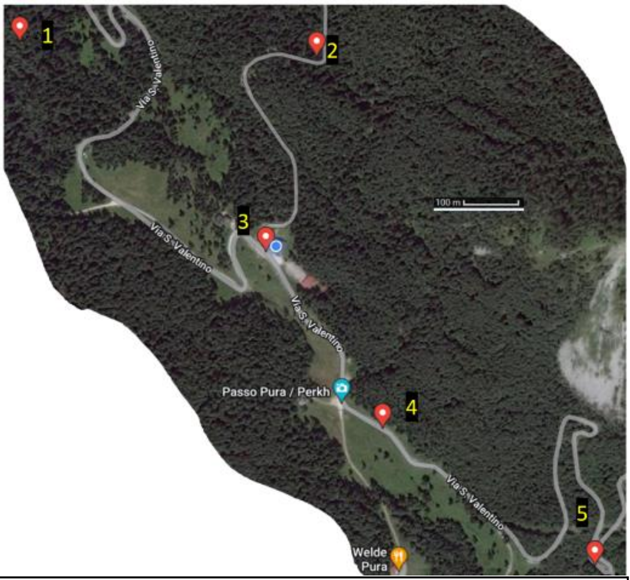

***Figure 1**. The chosen site locations on a satellite image map. (Google Maps, 2022)*

Up to four closest patches of *Cirsium erisithales,* from the random point, were identified using the tape measure. Patches were defined as groups of *Cirsium erisithales* individuals within an area with <1m between individuals. If there were over four thistles within a patch they were numbered in a clockwise circle and then a random number generator would be used to select four thistles to sample. To maintain a fair and consistent standard, the flower at the top of the dominant stem were selected. The dominant stem was defined as the thickest stem which continuously follows the main stem from the ground.

Data Collection

There are three measurements which need to be taken: flower area, flower droop angle and light intensity of each patch.

Light intensity of each patch was recorded using the Lux Light Pro V2.1.1 application on the iPhone X. The forward-facing sensor was selected, and the average was set to 0. Five measurements, in Lux, were taken within a 20s timeframe: 4 measurements at each corner of the patch and one in the middle – the average of these five measurements was recorded.

To record flower area and flower droop angle standardised photographs were taken. The Nikon D5200 had its settings set to F5.6 and ISO 500 with shutter speed being variable depending on lighting conditions. The Sigma 10-20mm lens was set to 20mm to reduce barrel distortion. The Velbon CX444 tripod was set up so that the lens was 50cm away from the specimen being recorded. The Camera was calibrated onto the tripod, so it was level with horizon, using Apple's 'Measure' application on the iPhone X. The Nikon D5200 4x4 grid was turned on and the specimen was framed with the flower in the middle 2x2 squares. The plane which was photographed was the one which intersected the middle of the flower and the stem, as shown in Figure 2. The photography plane was

***Figure 2.** The plane which was photographed.*

found using Apple's 'Compass' app, where the phone was placed above the flower with the speaker phone at the flower and the charging port at the stem. The bearing which the plane is on is then recorded and then the camera is calibrated to that bearing using the same app. The photography ruler was also be placed in the image, above the flower and inline with the same plane. The photo was then taken

ImageJ v1.53K, a software-based measuring tool was used to measure the droop angle and the flower area (Schneider, Rasband and Eliceiri, 2012). For droop angle, two lines were placed between the thickest point of the bract and across the stem just before the bract. The midpoint of these lines were connected to form the line to be measured. This line was measured against horizon to find the angle of droop, as seen in Figure 3. The polygon tool was used to measure the area of the flower. ImageJ was first set to scale using the photography ruler and then nine points joined up around the flower to measure the area. Four points were mirrored on each side of head and bract: either side of the stem before the bract; either side of the thickes part of the bract; either side of the bract before the head, either side of the thickest part of the bract and then one point at the tip of the flower. This totalled in nine anchor points, as shown by Figure 3.

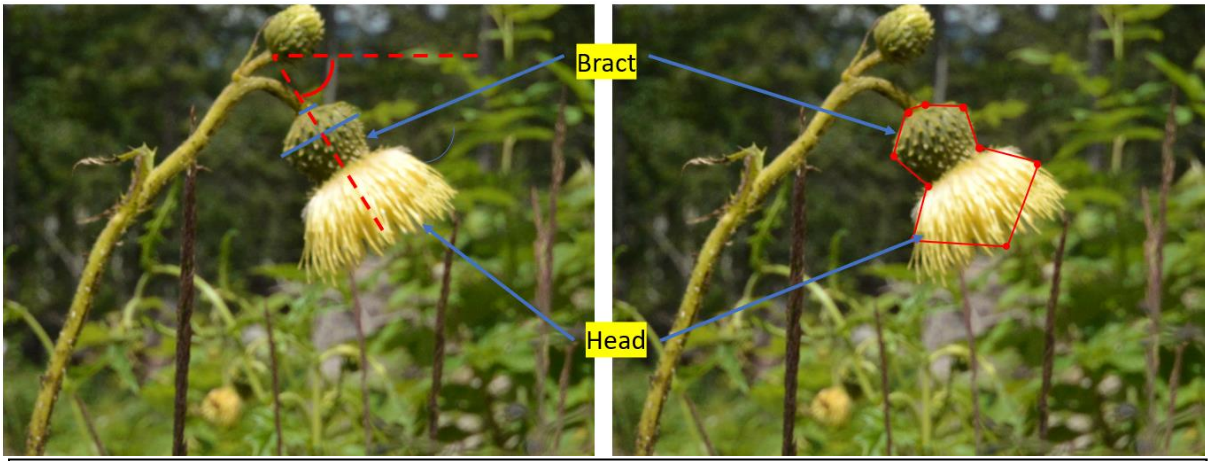

Figure 3. *ImageJ Measurement placements with head and bract labelled (L: angle R: area)*

Data analysis

The data was analysed using a linear mixed effects model which looked at the droop angle being dependent on the interaction of light intensity and flower area with the random effects of site, patch and position of patch in site. There was an issue with the 'Lux Light Pro' v1.2.2 iPhone application, where in some circumstances (3 occasions) the application recorded light intensity measurements of >120,000 Lux. This should not be possible as areas which have the highest outside light intensity rarely reach 120,000 Lux (Oregon, n/a). Therefore, these recordings were removed as they would not have been accurate and could have skewed data. The area of the flower was taken into consideration to account for different sizes of flower heads. Larger flower heads will have a higher mass and thus could influence droop angle so it was important to factor in.

Results

There was a variable degree of droop between different flowers, although most points were >40°. Running the linear mixed effects model resulted in a p-value of 0.777 for droop angle being dependent on the interaction of light intensity and flower area. The relationship between flower area and light intensity is shown in Figure 4. The data also showed no evidence that flower size, as measured by area, had no influence on flower droop angle, with a p-value of 0.705 with it being the only predictor.

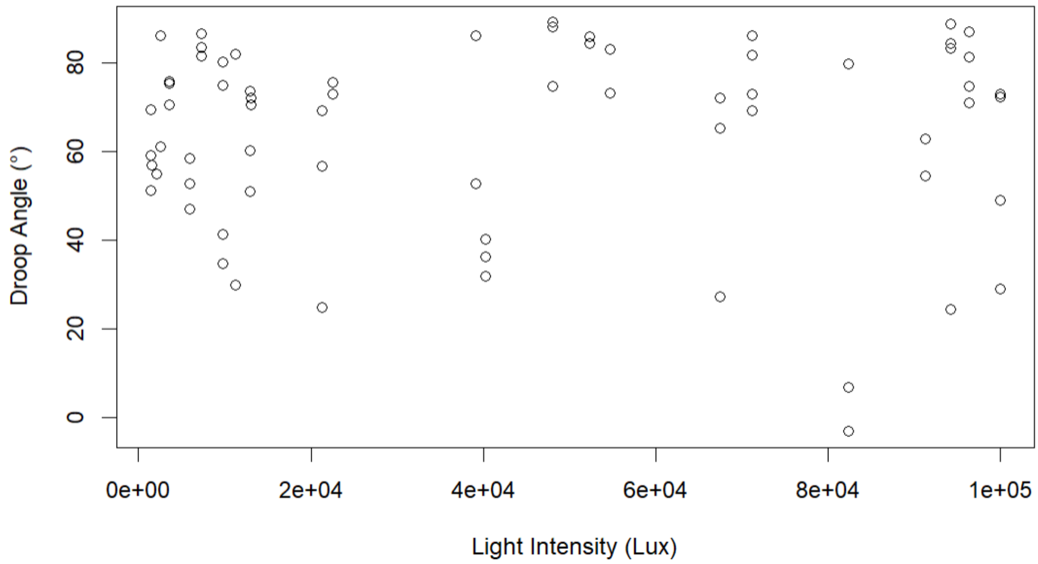

Figure 4. *The relationship between droop angle and light intensity, showing no significant correlation (p-value <0.05), with a p-value of 0.777*

Discussion

The hypothesis that light affects droop angle was unsupported by the data. By accounting for area of the flower, the data also finds no evidence that flower size influences flower droop angle. Thus, for *Cirsium erisithales*, there must be alternative factors which has influence on droop angle. There could be environmental and abiotic factors influencing droop, such as wind and water availability. Wind has been known to cause stress and influence growth in plants, causing them to grow in directions to support themselves (Gardiner *et al.*, 2016). It is also known that at high altitudes, such as the sites in this study, wind tends to be stronger (Arya, 1988). This could mean that perhaps it is wind which influences droop angle. Water availability could also be an influencing factor as limited water availability and over-availability of water have been known to affect plant growth (Ritchie, 1981). Perhaps the water availability causes the droopiness in the *Cirsium erisithales*, however there would need to be further study into this within the Carnic Alps for both these factors.

Limitations and Constraints

There were some limitations within the study which were unable to be accounted for or mitigated due to them being unforeseen. There were some abiotic factors which were unavoidable, such as the location of one site. Initially, the study was going to visit six sites, however one site was located on a cliff edge and was thus inaccessible. Furthermore, upon visiting this site, there were no individuals of *Cirsium erisithales* to sample, even if it was accessible. There was also one random location which had to be generated again due to there being no thistles in the immediate area (<120m). If this study was to be conducted again, the sites should be assessed for any nearby inaccessible areas which do not require specialist skill or equipment to access, unless it was available. Another limitation was logged areas, which could have influenced light intensity measurements, depending on how recent the logging was. For areas which were logged after the thistles had grown, the light intensity may have been lower for majority the thistles life. If the study was done again, more research should be done into what areas have been targeted for logging, in order to avoid them.

There is also the possibility that the areas which were visited had light intensities which did not vary enough. The light intensities did show normal distribution, however there were specimens which were under thick canopy and thus causing a very low light intensity. If conducted again, there would be study sites located deeper into forests with thicker canopies.

The main constraint on this study was time. With only four data collection days, there was limited time to find new sites, as set out by contingency. Furthermore, the tripod setup and calibration of camera required more time than was expected. This meant that less samples could be taken within the allocated time.

Further study areas

Due to the lack of significant results, the factor which influences droop angle is still unknown and unproven, and further study needs to be done to explore factors, other than light intensity and flower size.

Genetic variation of plant growth hormone regulation, for example auxin, could be investigated using molecular analysis to test for a correlation between certain genes and droop angle. Auxin, particularly indole-3-acetic acid, is very well understood and pathways and regulation is fairly well-studied, as set out by Woodward and Bartel, 2005. Upon observations of specimens, all dominant heads and bracts appear to droop past horizon, with the exception of one. This indicates that the drooping nature could be a typical morphology of *Cirsium erisithales* and could be influenced by genetic variation, perhaps alone.

Investigating the drooping morphology as an adaptation to predator avoidance could also give more insight into *Cirsium erisithales*. Other drooping Angiosperms have been studied and shown to have protection from predation. For example, Wise *et al* (2010) showed that, by drooping or ‘nodding’, the species, *Solidago altissima,* had protection from the oviposition of *Eurosta solidaginis*, thus improving fitness. Similar studies could be done into *Cirsium erisithales* to investigate whether the nodding morphology is an adaptation to protect from certain types of predation. *Cirsium arvense*, a species of the same genus, has been known to be vulnerable to predators such as the *Hadroplontus litura* Clark *et al* (2020), thus there may be similar predators for *Cirsium erisithales*, which the drooping protects against.

Summary

The knowledge and understanding of *Cirsium erisithales* is still limited and the reason behind the drooping morphology is yet to be known. However, light intensity and flower size can now be discounted as predictors for droop angle. Wind and water availability, along with more complex interactions, such as genetics and adaptation to predation, can now be explored.

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