Cirsium erisithales: Influence of Light on Drooping Angle Away from Horizon

Research Question

Does light intensity affect the angle at which individuals of *Cirsium erisithales* flower heads droops from the horizon?

Introduction and Background Information

Some Angiosperm species have been known to droop their pollen-holding flowers, hanging them away from the sky in harsh climates. This has been shown to protect the pollen from harmful factors such as UV-B wavelengths and high temperatures (Chen *et al.*, 2013). These factors can degrade a plant's pollen, reducing success and thus lowering fitness (Schmucki and Philipona, 2002). Drooping also protects from the harm caused by water, oviposition and other forms of predatory behaviour (Wise *et al.*, 2010).

However, drooping is not always advantageous, and in some symbiotic relationships can be disadvantageous. This is true for the species *Nicotiana attenuate,* which is pollinated by Sphingidae (hawkmoth) species. Haverkamp *et al* (2019) found that, even though the attractive volatile compounds were distributed to the same effect, once the flowers were positioned past a ~ 90°C angle, hawkmoths could no longer land and pollinate flowers.

Cirsium erisithales are known as 'yellow melancholy thistles' and often have their head pointed in a downwards position. These thistles seem to have limited research around them and the reason for their downwards-pointing flower does not seem to be known. However, the before mentioned study by Chen *et al* (2013) answered a similar question for *Cremanthodium campanulatum*. They concluded that the plant drooping was beneficial for that species as it decreased exposure to UV-B, which is dangerous and harmful to pollen. Thus, a hypothesis could be drawn from this for *Cirsium erisithales*.

Thus, light intensity is being considered the main factor for *Cirsium erisithales* as they are found in the carnic alps at high altitudes and thus are exposed to more UV-B light compared to plants at lower altitudes (Blumthaler *et al.*, 1997). UV-B has been shown to degrade pollen and cause a reduction in fitness (Zhang *et al.*, 2014). Thus, if drooping angle increases with light intensity, it could indicate that *Cirsium eristhales* are adapted to high UV as they would be shielding their pollen away from UV wavelengths.

Hypothesis

I hypothesise that *Cirsium eristhales* individuals will droop to a greater extent, at a more obtuse angle to horizon as light intensity increases.

Data Collection

I am collecting 2 variables – light intensity and angle of droop of the flower. I will collect data within the area surrounding the Baita Torino field station, which is positioned between Ampezzo and Sauris in the Italian Carnic Alps.

Materials and Methods

Materials

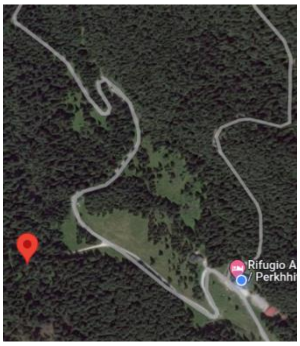
- 1x Apple iPhone X with 'Lux Light Pro' and apple's 'Measure' applications installed
- 1x 30m Measuring tape
- 1x Nikon D5200 with Sigma 10-20mm lens
- 1x Computer with 'ImageJ' software installed
- 1x Velbon CX 444 camera tripod
- 1x 30cm Ruler

Method

Cirsium erisithales grow in small patches, never usually alone or singular. Thus, I am going to visit 6 locations and sample up to 9 patches in the close area (50m radius). These locations are all similar altitudes, being within 60m (vertically) of each other. A-C are all under canopy, whereas D-F are all out in meadows or clearings where there is no cover from light. These locations can be seen in Figure 1 with coordinates labelled below:

A 46°25'43.5"N 12°44'34.8"E

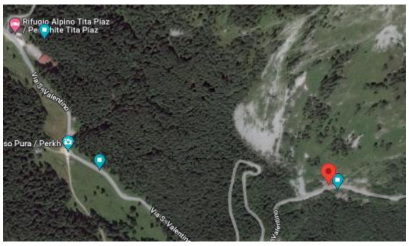
B 46°25'04.8"N 12°44'56.4"E



C 46°25'33.4"N 12°44'09.1"E

D 46°25'21.3"N 12°44'40.5"E

E 46°25'44.4"N 12°44'14.6"E



F 46°25'20.0"N 12°45'07.4"E

Figure 1 *– The locations where patches of Cirsium erisithales will be sampled. A-C are in areas of forest with limited light penetration through the canopy, whereas D-F are in open field.*

An online software called GeoMidPoint will be used to find 3 random coordinates within a 50m radius of these points – this software can be found here: https://www.geomidpoint.com/random/. From these random points, the closest 3 patches of thistles will be assessed using a tape measure. If there are more than 4 thistles in this patch, they will be numbered in a clockwise order, spiralling inwards if there are any in the middle of the patch. A random number generator will then randomly select the 4 thistles which will be sampled. *Cirsium erisithales* often has multiple flowers for each individual. To maintain a fair and consistent standard, the flower at the top of the dominant stem will be used. If there are less than 4 thistles, the only thistles present will be sampled. The maximum number of thistles measured will be 216 (6 points * 3 random locations * 3 patches * 4 thistles)

Once the flowers have been randomly selected, the light intensity above them will be measured. This is using the 'Lux Light Pro' iPhone application. The front-facing light sensor will be selected using the app. The iPhone X is then placed screen upwards towards the sky, 5cm above the flower being measured and the light intensity will be recorded in Lux.

A tripod will be set up so that the camera lens is 1m away from the specimen and calibrated using the apple 'Measure' application to ensure it is level to horizon. This position should ensure that the photo is being taken perpendicular of the plane which intersects the stem and flower as it droops, as seen in Figure 2. A Nikon D5200 with a Sigma 10-20mm lens will then be set up on this tripod. The lens will be set to 20mm to reduce the barrel distortion caused by wide angle lenses. The image should be captured so that it droops to the left of the image, ensuring the right side of the plane is being photographed. This setup can be seen in Figure 3. The only setting which may be variable between different photos will be shutter speed. This is due to light intensity being variable for locations. However, shutter

Figure 2 *- the plane which needs to be captured. Correct framing is shown on the right-hand side.*

Figure 3 *– Nikon D5200 with Sigma 10-20mm lens set up 1m away from specimen.*

speed should not incur any distortion which would result in a non-standard image. ISO should be set to 500 and F-Stop set to F5.6 these settings will remain constant.

The Nikon D5200 should have a 4x4 grid turned on. The flower specimen should be positioned in the middle 3 squares of the grid, where any barrel distortion is constant between photos. This can be achieved using the height adjustment on the tripod. A photo scale can then be placed in the frame to allow for any future measurements. The photo is then captured. For each patch a new folder should be used to store the photos. Once a plant has been measured, it should be marked with small bit of sting on the stem as to not measure it again. This string should be removed after all points are measured at the 6 individual points.

ImageJ, a software-based measuring tool, will be used to measure the angle of droop (Schneider, Rasband and Eliceiri, 2012). The two intersecting lines will be a horizontal line, which is calibrated to the true horizon, and a line which intersects the middle of the flower head, as seen in Figure 4.

***Figure 4** – The droop angle which is being measured and horizon for context. Above horizon will be counted as negative, below horizon will be counted as positive.*

<u>Data Analysis</u>

The data collected will be analysed using linear regression. The data will be plotted on a graph with angle (-90-90° with 0 > -90° being above the horizon and 0 < 90°being below the horizon) being on the Y axis and light intensity (Lux) being on the X axis. If the hypothesis is correct there should be a positive correlation with droop angle increasing with light intensity.

Planned Timeline

Date	**Activity**	**Details**	**Wind forecast**
10.07.2022	Project proposal	N/A	N/A
11.07.2022	Project proposal	N/A	N/A
12.07.2022	Data collection 1	Collect data from location A + E	Wind – 5-8km/s (collect data at 12pm for 8km/s)
13.07.2022	Data collection 2	Collect data from locations F + B	Wind – 5-10km/s (Collect data at 12pm for 8km/s)
14.07.2022	Data collection 3	Collect data from locations C + D	Wind – 6-11km/s (collect data at 1pm for 8km/s)
15.07.2022	Data collection 4	Use Image J to calculate angles and collate data in Excel	N/A
16.07.2022	Stats	Stats	N/A
17.07.2022	Write up	Write up report	N/A
18.07.2022	Write up	Write up report and create oral presentation	N/A
19.07.2022	Oral presentation	Present orally	

Due to data needing to be collected at a similar time, the locations which are closest in proximity will be collected on the same days.

Limitations

This study involves a lot of data collection, by collecting a total of 216 photos and 216 measurements of light intensity. There is probability that, due to human error, the photos may not be taken perfectly. This would be hard to nullify as removing images because they don't appear correct could create bias.

There are also factors which may change the factors being measured.

- Some study sites are located near to roads or footpaths. This could mean that people or vehicles could have hit the potential specimens, altering the angle of droop.
- Wind could be an important factor which affects the angle of droop. A possible way to avoid this is using a wind barrier around the plant. However, it would be hard to know the exact natural state of the droop if this was done. The best way to avoid wind being a large issue is to ensure that data collection days are conducted on days with similar wind forecasts. However, due to time limitations this may not be possible.
- Some study locations may be hard to access and in some there may not be many of the thistles in the immediate area. This means it could take some time to find the closest patch, especially due to the tape measures capping at 30m. This increases the data collection time.
- Light intensity on different days may be different depending on the weather. Due to the time constraint, it is hard to ensure that everyday has the same weather forecast.

However, all data collection days will have data collected around the same time which should help minimise this limitation.

However, due to the large amount of data (216 of each light intensity measurements and angle measurements) any anomalies will have minimal impact and should not skew the final result.

Contingency

In the event that there is adverse weather or sites, or locations are unable to be accessed, new locations can be selected. This is possible as the immediate area has many areas which are under and not under canopy. If there is too much wind, a wind barrier may need to be created. However, the wind barrier should be used for every specimen which may require the re-capture of previous days data collection.

References

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