RESEARCH ARTICLE

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Canopy effects on the distribution of *Ophrys insectifera* L. (Orchidaceae) – preliminary results from hemispherical imaging analysis

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ABSTRACT
Ophrys insectifera L. is the northernmost representative of the remarkable *Ophrys* genus with the predominantly Mediterranean distribution. Due to its specific biology and ecological requirements, it is among the most threatened vascular plants in Bulgaria. The study aimed to explore and evaluate canopy effects on the species distribution in some of the largest locations in Bulgaria. Exploration and investigation of few populations of the orchid *Ophrys insectifera* in few mountainous locations in South Bulgaria were conducted from 2013 to 2018. For the canopy effects evaluation on the plants' distribution, the geospatial data were collected and hemispherical photographs were made and analyzed. The results show that less open areas with the index of canopy openness less than 70% are more favorable for the distribution of the species. However, unlike the results and suggestions of the previous studies on the topic from north Europe in Bulgaria it was observed the opposite trend – the species dwells better in mid-shade to partly shady conditions. Howbeit further research is necessary for the determination of the relation to the other ecological factors. The study suggests that shading has some role as a limiter for the distribution of *Ophrys insectifera* L.

Key words: *Ophrys insectifera* L.; canopy effects; hemispherical imaging analysis

Introduction

Ophrys insectifera L. (fly orchid) is among the most threatened and rarest plants in Bulgaria. It is a tuberous perennial orchid that has two tubers of egg-like shape. Its stems are 15-40 (60) cm tall, having 2-5 basal leaves and 1-3 sheathing-leaves. In general, the flower resembles the body of a wasp, which are the main pollinators (Figure 1B). The flowering time is in May-June and the fruiting time is in June-July. It propagates by seeds (Delforge, 2006; Fay et al., 2015; Petrova, 2015; Popatanasov, 2018).

The necessary mycosymbionts for its successful development and growth most likely are from the polyphyletic Rhizoctonia group, precisely from the Tulasnellaceae family (Hofsten, 1973; Schweiger, et al., 2018), which may impose significant limitations upon its distribution and the possibilities for its expansion. However other factors may also be suspected to have a role in its distribution as promoters or limiters. During the field monitoring of some of the largest locations of *O. insectifera* near Yagodina village and Radomir town, A. Popatanasov noticed that this plant never grows in areas of the forest communities with well-developed understory layers, which does not seem to be a problem for other coexisting orchid species as *Goodyera repens* (L.) R. Br. (Popatanasov & Asenov, 2019), additionally in areas under predominately direct sunlight exposure some of the leaves are very pale even with "burnt" edges (Figure 1A). This led to the hypothesis that sunlight exposure can play a role of a limiter/promoter for the wellbeing and growth of this well adapted to boreal conditions species. Therefore, the investigation of the light shading as a factor for the distribution became the chief aim of the present study.

O. insectifera seems to be capable to inhabit rather diverse types of habitats such as grassland, wet meadows, fens, shrubs, wooded meadows, and open pinewoods, as well moss and sedge swamps and rarely on peat bogs, etc. (Popatanasov, 2018).

On a global scale, the species is endemic for Europe where it inhabits rather large but very disjunctive areal spreading in the east-west direction from Mt. Ural to Ireland and in the south-north direction from Bulgaria and Greece to North Russia (Fay et al., 2015; Popatanasov, 2018). In Bulgaria, the species has few very fragmented low numbered mountainous locations with an average altitude between 900-1200 m a.s.l. in the South (Znepole and Rhodope Mountain region) and a single location in the North at 300 m a.s.l. (Dragoevska Mountain) (Popatanasov, 2014; Petrova, 2015; Zahariev & Taneva, 2017; Popatanasov, 2018).

A B

Figure 1. *Ophrys insectifera L.at Radomir location (June 2017): A. Whole plant; B. Flowers; (photo A.Popatanasov).*

Globally regardless of the overall decline of the populations and its peculiar biology, this species is with the given status Least Concern (LC) in EU IUCN Red List, probably due to its yet vast area of distribution, which however at a closer look is rather disjunctive nowadays; additionally, it is included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Appendix II (Rankou, 2011; Popatanasov, 2018). Locally in Bulgaria, the plant is included in the Red Data Book and Red List of the Bulgarian vascular plants and also is protected by the Biodiversity Act since it fulfills the IUCN criteria for Critically Endangered species [CR C2a(i); D] (Petrova, 2009; Petrova, 2015). Such very threatened status demands from the biologists and ecologists to uncover with higher priority more of the biology and ecology of this critically endangered species to determine and develop better actions and plans for its bioconservation.

Materials and Methods

Two of the locations of this critically endangered species having among the most numerous populations in Bulgaria were monitored. The first location is near Yagodina village at the slopes of Buynovsko gorge, Rhodope Mountains Central floristic region, and the second at the slopes of Mt Golo Bardo, near Radomir town (Znepole floristic region) (Popatanasov, 2018). The monitoring was performed during the flowering-fruiting season of *O. insectifera* (which for these locations was from May to July (August) for the years from 2013 to 2018 (Figure 2).

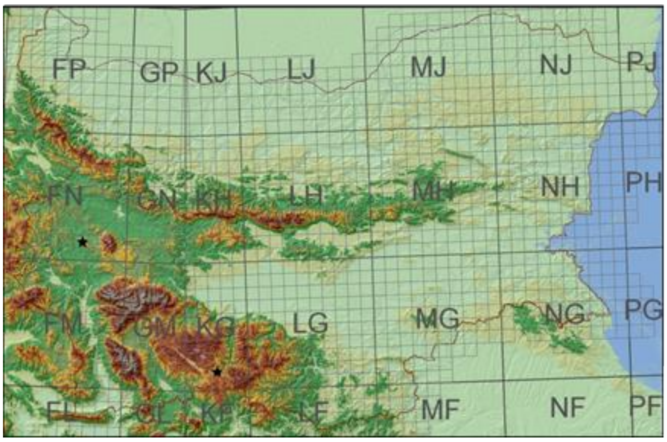

Figure 2. *The locations of the monitored populations of Ophrys insectifera at Yagodina village and Radomir town (marked with black stars). Grid is 10 km.*

The GPS coordinates were recorded with Garmin Colorado 400 GPS receiver and/or Solmeta 2 Pro Geotagger and GPS receiver were utilized for mapping the species distribution. To measure the distances and to map topographically the distribution of the software packages as Garmin BaseCamp ver. 4.6 and SASPlanet ver. 15 were utilized.

Microsoft Office ver. 2007 (Microsoft Corp., USA) was utilized for processing and presenting the acquired data and results.

The first step for the hemispherical image analysis was image acquisition. The hemispherical images were taken with a capacity to produce 24 MP pictures cameras. Furthermore, in dependence on the sensor size, the utilized lens was: 1/. Sigma 4.5mm f/2.8 EX DC Circular Fisheye HSM lens (Sigma Corp., Japan) (for 16 x 24mm size sensors); 2/. Belomo EWP 8mm f/3.5 Circular Fisheye MS lens (BelOMO-MMP, Belarus) or Nikon FC-E9 (Nikon Corp., Japan) (for 24 x 36mm size sensors). Since some of the moss ground cover layers where this orchid dwells are rather delicate and vulnerable, the specialized tripod as Benbo Trekker (Paterson Photographic Inc., UK) with a special attachment for low-level photography and Manfrotto 405 Pro Gear Head (Manfrotto, Italy) for precise positioning need to be utilized.

Initially, the hemispherical photographs were preprocessed with photo-editing software for general use as GIMP, and afterward, they were analyzed with specialized software like CanopOn2 (Takenaka, 2009a, Japan) according to the instructions in the manual (Takenaka, 2009b). In short, the converted greyscale images (Figure 3) were input in CanopOn2 software, where the canopy area was marked, and the type of projection of the used lens along with its field of view degrees was selected. Furthermore, according to the data from geotagger Solmeta Pro, the direction was adjusted and the canopy openness expressed as a percentage was measured.

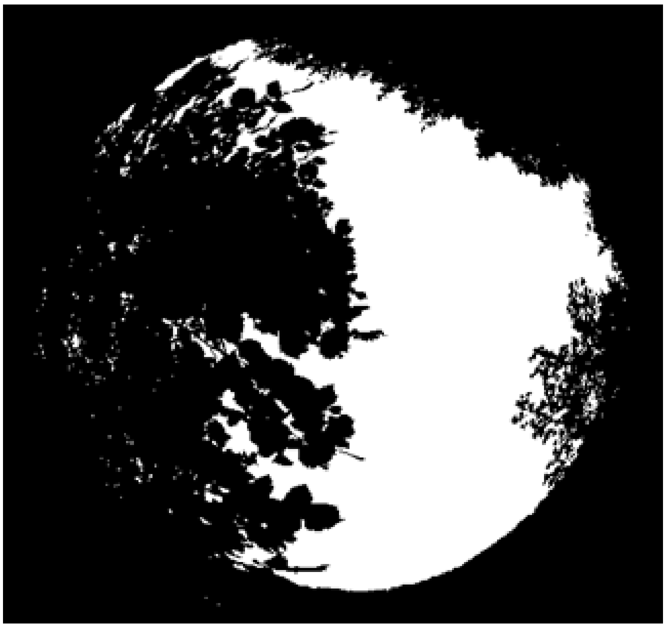

Figure 3. *Hemispherical photograph for canopy analysis (June 2017) (photo A. Popatanasov).*

The GPS coordinates of the central points of the locations as latitude, longitude, and altitude are presented in Table 1.

Results and Discussion

The shoot number ranged from 20 to 40 shoots at each location as presented in Table 2. During the various years, it was observed variability of the annual number of shoots which in comparison to the best years could reach up to 25% reduction for the Yagodina village location and even more in Radomir town location - 56% (Table 2).

The results from the performed hemispherical image analysis showed slightly differing distributions at both locations concerning the lightening conditions as the canopy openness.

At the Yagodina village location, almost half of the shoots (over 48%) grow under lightening conditions with canopy openness between 40% and 60% (Figure 4). The range of the distribution was 47.3%, so all of the plants were under canopy cover whose openness is between 20% and 80%.

Table 2. *Total number of the monitored plants of Ophrys insectifera near Yagodina village and Radomir town for the period 2013 till 2018.*

Location	Total number shoots	Difference between the maximal and minimal annual shoot numbers over the monitored period
Yagodina location	29	7
Radomir location	39	22

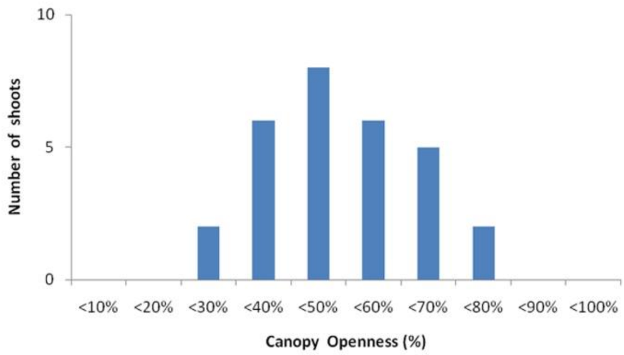

Figure 4. *Distribution of the shoots in regard to the percentage of canopy openness at Yagodina site.*

The largest Radomir town location has a different pattern of distribution according to the lighting conditions compared to the Yagodina village location. In this location, the greatest number of shoots (over 53%) grow under lightening conditions with canopy openness between 70% and 90% (Figure 5). The range of the distribution was 68,9%, so all of the plants dwell under canopy cover with canopy openness between 10% and 90%.

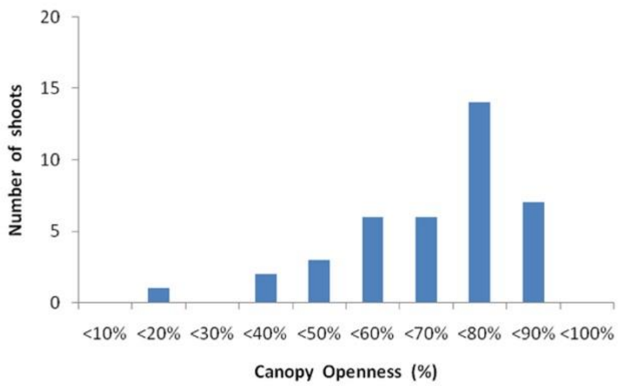

Figure 5. *Distribution of the shoots in regard to the percentage of canopy openness at Radomir site.*

Table 1. *GPS coordinates of the monitored locations of Ophrys insectifera near Yagodina village and Radomir town.*

Location	Latitude	Longitude	Altitude	Slope
Yagodina location	N41°37'58,86"	E24°20'29,77"	1100 m a.s.l.	North
Radomir location	N42°32'42,72"	E23°02'30,33"	990 m a.s.l.	North-North East

The distributions at the monitored locations were also shaped differently. The distribution at Yagodina village was slightly skewed to the right with skewness 0.34, while the one near Radomir town was highly skewed to the left with skewness -1,17. Additionally, the distribution at the former site is slightly platykurtic as showed by its kurtosis (-0.55), while the one at the later site is significantly leptokurtic (kurtosis is 1.35).

However, the combined results from both locations showed a more uniform distribution regarding the canopy openness (Figure 6). Half of the plants grew under canopy openness between 40% and 70%. The range of the distribution was 68.9%. The distribution is slightly skewed from the normal one with skewness -0.32 and slightly platykurtic with kurtosis (-0.68).

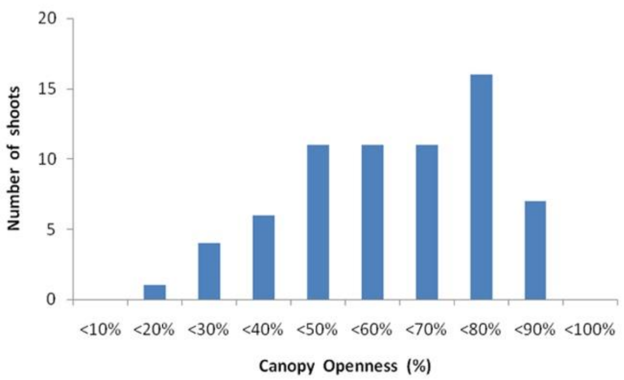

Figure 6. *Combined distribution of the shoots regarding the percentage of canopy openness.*

The presented results suggest that canopy coverage plays some role in the distribution and wellbeing of this species. Previous studies from the West Northernmost border of the global areal of the species from West North and middle North region of Europe as the Netherlands, UK, and Denmark showed that more light availability is beneficial for this species in this geographic region (Wolff, 1951; Dorland & Willems, 2002; Skipp, 2017). However, the presented results acquired under the specific climate and microclimate conditions in Bulgaria show an opposite trend than the one observed in North Europe. This species in the Bulgarian climate has a preference for the more moderately shaded environment as we can see from the combined results according to which 66% of the shoots dwell under canopy openness below 70%. Nevertheless, when we look closer at each location separately, then for the largest location in Bulgaria near Radomir town we may conclude that the majority of plants prefer more sunny areas. Howbeit if take into account the greater inter-annual variability of the shoot numbers and some of the populational parameters of wellbeing presented in the previous study on these populations (Popatanasov, 2018) then we can conclude that at the sunnier Radomir site, many of the plants fade away or reside in the dormant state indicating a more suppressed existence of the population. Moreover, we may speculate here that partly this suppressed state can be because almost half of the plants live near the limits of their solar exposure tolerance under the Bulgarian climate as we can deduce from the combined distribution results. However, let's not forget that Bulgaria is the southernmost border of the global areal of this species (Rankou, 2011; Popatanasov, 2018), which means that it more often can reach the limits of its environmental tolerance towards some of the ecological factors, that more easily can put it in physiologically suppressed and/or dormant state.

On the other side, at the Yagodina village location, the distribution of the shoots is more normal and less spread, positioned at the center of the combined one, which is with the concordance with the less pronounced interannual variability in shoot numbers and the better state of the local population as the previous study showed (Popatanasov, 2018).

Such a complex and even contradictory picture, compared to the previous studies on the ecology of this species concerning the light regime (Wolff, 1951; Dorland & Willems, 2002; Skipp, 2017), probably can be explained if we take into consideration some of the other abiotic or biotic factors, such as temperature, humidity and other plant species in the community that can also impact the distribution and wellbeing of this orchid. Northern Europe has a cooler and moister climate. Therefore, the more exposure to the sun sites often means less cool and moist microenvironmental conditions, while in the Mediterranean type habitats in Bulgaria as the one at Radomir location, the more exposure to the sun site would mean drier and hotter microscale local conditions (Schönwiese & Rapp, 1997). At both locations, A. Popatanasov (Popatanasov, 2018) recently described the presence of more xerothermic Mediterranean elements in the vegetation, while this species has more mesic environmental requirements. Thus, the observed preference for partial shading at the Bulgarian locations of this orchid probably counteracts to some extent the more xerothermic micro-environmental conditions caused to some extent by the direct and prolonged solar irradiation.

Furthermore, little is known about the environmental requirements and tolerance of the crucial mycosymbiont partner/s of this orchid. Currently, we know that they belong to the Tulasnellaceae family as mentioned earlier. A long-term study (Heinemeyer et al., 2004) showed that light regime and soil temperature can impact the spatial distribution and abundance of the mycorrhizal soil fungi, which also can jointly facilitate or limit the orchid distribution.

Further research is needed to determine better the combined interaction and impact of these factors upon the growth and distribution of this critically endangered orchid.

Conclusion

The presented data suggest that light exposure can impact the distribution and wellbeing of *O. insectifera* L.

The study suggests that special care should be taken at the locations inhabited by this species when it is necessary to be performed habitat maintenance activities as woodcutting that can affect the light regime of this critically endangered orchid. Further research to relate the role of the other abiotic and biotic factors like temperature, humidity and species composition at the sites would reveal new features in the preferences, adaptation, and distribution of this critically endangered species.

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