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A Note on the Flowering of *Ajuga orientalis* L. in Relation to Air Temperature in Mount Aenos (Cephalonia, Greece)

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Abstract

Ajuga orientalis L. is a widespread plant species in many countries, such as Greece, Italy and Turkey, with promising aesthetic value in the field and in landscape design, but nothing is known about its phenology, from a detailed, quantitatively, point of view, in relation to meteorological variables. Thus, under the aforementioned context, the purpose of our work is the elucidation of part of the phenology of this plant, especially concerning its flowering. To achieve this, the phenological stage 'Beginning of flowering', in terms of its start dates (julian days), was investigated in relation to average air temperature (T) of March in two areas, Roudi and Kaboulieri at north-northwest and south-southeast slopes, respectively, of Mount Aenos, Cephalonia, Greece, for three successive years (2014-2016). From the analysis of the T of March, it was confirmed that Kaboulieri area was significantly warmer ($P<0.05$) than Roudi area by 0.8 ^{0}C both in 2014 and 2015, with a significantly earlier appearance ($P<0.05$) of 'Beginning of flowering' of *A. orientalis* in Kaboulieri, ranging from 9.1 (2015) to 10.9 (2014) julian days. The findings of our study could be used for the planning of an efficient preservation program process of the aforementioned plant species in a vulnerable mountainous environment, such as the Mount Aenos environment, as well as for its further exploitation as a decorative plant.

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Introduction

Plant life cycle is related in a high degree to changes of meteorological variables[1], which may alter the structure and composition of the vegetation in natural ecosystems. Among various meteorological variables, air temperature (T) is considered to be the most critical driver[2-4] that affects the appearance of life cycle events, in other words, phenological stages, such as leaf unfolding, flowering and fruit ripening in plants.

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Flowering is a fundamental phenological stage of plants from the view of its confluence to their reproductive cycle. It has been reported that in mountainous environments and, especially at their higher altitudes (alts), flowering appears later, in comparison with their lower alts.[5] The aforementioned delay of flowering may be considered in a high degree as a consequence of a noticeable T decrease at higher elevations.[6, 7]

Apart from the effect of alt on flowering in mountainous environments, slope orientation has been shown to have an impact on this phenological stage, which has been reported to appear earlier on south-facing slopes compared to north-facing slopes.[8] It is well-known that south-facing slopes present greater T values[9], taking into consideration the greater amount of incoming solar radiation there[10], than north-facing slopes.

Ajuga orientalis L. is an interesting medicinal plant species[11] with noticeable biological activities and bioactive phytochemicals.[12] The global range of this species is large.[13] For example, it thrives in several countries of the mediterranean basin, such as Greece, Italy and Turkey.[14] It is worth mentioning that this species shows potential for landscape design because of its aesthetic appearance in the field[15], partially, due to its flowering.

The flowering period of *A. orientalis* depends on its habitat. For instance, it has been reported that the plant blooms in April in Greece[16] and Israel[17], as well as, in summer in Turkey[12]; however, to our knowledge, literature presents a lack of information about the flowering of this plant in relation to meteorological variables. Such information would be considered valuable concerning the *in situ* preservation of *A. orientalis* in vulnerable environments and its further exploitation as an ornamental plant in landscape design.

Therefore, the purpose of the present work is the elucidation of part of the flowering of *A. orientalis*, in terms of the start dates of its phenological stage 'Beginning of flowering', in a vulnerable environment, the national park of Mount (Mt) Aenos and its greater area, testing the hypothesis of the different response of the aforementioned stage to different T, derived from different surroundings.

Materials And Methods

Region of Investigation and Sites of Measurement

Research was carried out in the wider area of the national park of Mt Aenos in the island of Cephalonia which belongs to the Municipality of Cephalonia, Periphery of Ionian Islands, western Greece (Fig.1 a, b), during March of three consecutive years (2014-2016). The aforementioned mountain, the highest one in the Ionian Islands group, with a northwestern-southeastern orientation, is situated in the southern part of the Cephalonia island.[18] The attractive national park of Mt Aenos occupies an area of 28,620 Km^2 with rich flora and interesting fauna and has been characterized as a European Biogenetic Reserve.[19]

In our study, two areas (Fig.1 c, d) were selected, considering primarily two main criteria, the different orientation of each area from the other and the sufficient presence of the examined plant species, *A. orientalis*, in both areas. Therefore, there was one measurement site, NNW1 ($38^0$11´38.9˝N, $020^0$36´55.0˝E, alt of 816 m) on the north-northwest (NNW) facing slope of Mt Aenos range (Aenos National Park) at the first study area (Roudi location) and another measurement site, SSE2 ($38^0$06´44.4˝N, $020^0$42´34.6˝E, alt of 877 m), about 13 Km away from NNW1, on the south-southeast (SSE) slope of Mt Aenos (wider area of Aenos National Park) at the second study area (Kaboulieri location).

Studied Plant Species and Collection of Phenological Data

The examined plant species was considered, until recently, to be the cephalonian endemic subspecies *A. orientalis* L. subsp. *aenesia* (Heldr.) Phitos & Damboldt.[14, 20] However, according to other more recent reference sources[21-23], the accepted name of the examined taxon, currently, is *A. orientalis*. The studied plant is a herbaceous one with a height ranging between 10 and 40 cm. Its leaves are covered by soft, woody, greyish pubescence and the plant appears usually violet-purple bracts with sparse shallow teeth. Corolla presents a deep purple, occasionally bluish-violet colour.[14]

The flowers appear during March and April (*in situ* observations) and the development of capsule occurs from the end of April until May, according to Karagianni.[16] In recent years, a reduction

of the population of *A. orientalis* is noticed in the national park of Mt Aenos, mainly, due to human interference[14], a clear sign of vulnerability. In order to study a part of the phenology of *A. orientalis*, the phenological stage 'Beginning of flowering' was investigated.[1, 24, 25]

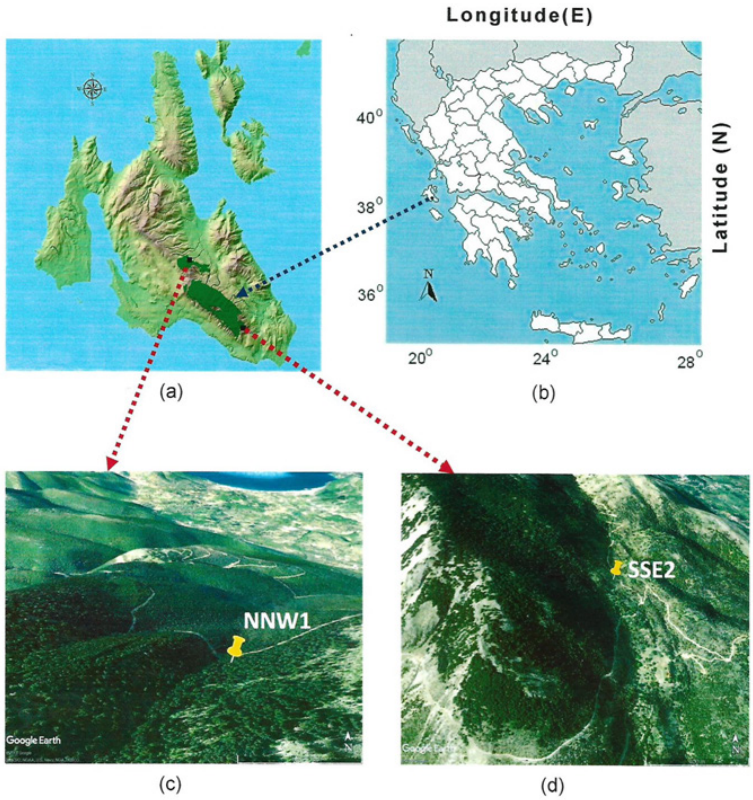

Fig. 1: Study locations (squares with black color) of the Aenos National Park (regions with dark green color) and its wider area of Cephalonia island (a), Greece (b) and measurement sites in (c) Roudi (NNW1) and (d) Kaboulieri (SSE2) areas, modified by Anonymous a[26] and b[27], respectively.

Therefore, in each study area sixty individual plants were selected and grouped randomly in six subareas (ten plants per subarea) with a maximum distance of 100 m around each measurement site. To evaluate the start date of 'Beginning of flowering' in each plant and subarea, the relevant phenological data were collected every five days[1, 5] during March of 2014-2016, where the examined phenological stage took place in both research areas. The average start date of 'Beginning of flowering' was estimated as the mean value of the sum of the relevant start dates of the plant for all subareas in each area and year.

Air Temperature Data Collection

Air temperature (T) data were recorded simultaneously every 10 minutes by sensors with dataloggers (Hobo Pro v2 U23-001, Onset Computer Corporation, USA, accuracy ± 0.2 ^{0}C over 0 ^{0}C to 50 ^{0}C), each one in each measurement site, for March of 2014-2016, that is, the month in which the examined phenological stage took place, as already mentioned, in order to form a more complete view of the thermal environment of the studied areas. The proper check and operation of the instruments were assured in agreement with other study.[5] From the recorded T data, the average T was estimated for March of each examined year for each area.

Statistical Analysis

The experiment was conducted according to the two-factor completely randomized design.[28] The first factor included two levels, each corresponding

to each studied area (Roudi and Kaboulieri) and the second factor included three levels, each corresponding to each studied year (2014-2016). The calculated means (julian days) of the start dates of 'Beginning of flowering' were used for analysis of variance (ANOVA). There were six replicates, each corresponding to each subarea.

Analysis revealed non significant interaction (P>0.05) of the two factors and thus, one-way ANOVA[29] was performed to all start dates of the examined phenological stage, including both studied areas and years, with means comparison by Tukey's HSD test[30] after the fulfillment of the necessary assumptions.[31]

In addition, to detect possible differences of T of March in each year and area, with regard to the examined areas and years, respectively, we analysed the relevant T data, deriving from mean daily T values, according to the two-sample t test for correlated data, after the appropriate adjustments.[32] The statistics was performed using MS Excel 2007[33] and IBM SPSS Statistics 21 with results to be considered significant at P≤0.05.

Results And Discussion

One-way ANOVA showed that there were significant differences concerning the start dates (julian days) of 'Beginning of flowering' of *A. orientalis* (P<0.05), which grew in the examined areas (Roudi and Kaboulieri) for the whole time period of the experimentation (March of 2014-2016). Specifically, the impact of area on the start dates of the examined phenological stage in each year was apparent in the majority of the cases (2014 and 2015), with significant differences between the relevant start dates (P<0.05) after their comparison against each other for the above mentioned years (Table 1). The earlier appearance of 'Beginning of flowering'

Table 1: Effect of area (Roudi and Kaboulieri) on average start dates of 'Beginning of flowering' of *Ajuga orientalis* L. at Cephalonia, Greece, for the whole examined period (2014-2016)

Year	2014		2015		2016	
	Area 1[a]	Area 2[b]	Area 1	Area 2	Area 1	Area 2
	Average start dates (Julian days)					
	79.2 a[c]	68.3 b	80.8 a[c]	71.7 b	76.8 a[c]	71.0 a

[a]: Roudi, [b]: Kaboulieri, [c]: For each year, entries with different letters indicate significant differences of the means concerning the different areas at P<0.05 by Tukey's HSD test.

of the examined plant species took place on the SSE slope of Mt Aenos (Kaboulieri) in comparison to its NNW slope (Roudi) with differences ranging from 5.8 (2016) to 10.9 (2014) julian days (Table 1). Regarding the impact of year on the start dates of the aforementioned phenological stage in each studied area, it was found that there were no significant differences (P>0.05) among the examined years for both SSE and NNW slopes of Mt Aenos.

As regards the average T of March, Kaboulieri area was warmer than Roudi area by 0.8 ^{0}C in both 2014 and 2015 with this difference being significant, contrary to 2016, where the two areas exhibited almost the same thermal behavior with T difference being not more than 0.2 ^{0}C (Table 2). Concerning the impact of year on the average T of March in each study area, no significant T differences (P>0.05) were confirmed for both Roudi and Kaboulieri locations among the three successive years.

The significant differences of the start dates of the studied phenological stage between the two areas, for 2014 and 2015, which are characterized by different orientation and alt, but not more than 61 m, could be attributed to the respective significant

Table 2 : Effect of area (Roudi and Kaboulieri) on average air temperature of March at Cephalonia, Greece, for the whole examined period (2014-2016)

Year	2014		2015		2016	
	Area 1[a]	Area 2[b]	Area 1	Area 2	Area 1	Area 2
	Average Air Temperature (^{0}C)					
	6.5 a[c]	7.3 b	5.8 a[c]	6.6 b	6.5 a[c]	6.7 a

[a]: Roudi, [b]: Kaboulieri, [c]: For each year, entries with different letters indicate significant differences of the means concerning the different areas at $P<0.05$ by two-sample t test.

differences of average T of March. That is to say, from a statistical point of view, the warmer area of Kaboulieri in the SSE slope of Mt Aenos, compared to Roudi (NNW slope of Aenos), in terms of average T of March in 2014 and 2015, may justify the earlier appearance of the examined phenological stage in the area with south-southeast orientation (Kaboulieri). As already mentioned above, a T decrease results in a late flowering of plants, e.g. Chronopoulou-Sereli and Flocas.[1] Also, it has been reported that spring flowering plant species, as our examined plant species *A. orientalis*, are correlated, in a high degree, with temperature.[34]

The increased average T values of March in Kaboulieri, that is, warmer conditions there, when compared to Roudi, may be attributed to the fact of its south-southeast orientation, in contrast to the north-northwest orientation of Roudi, taking seriously into account that the south-facing slopes, in general, receive noticeably increased amounts of solar radiation than the north-facing slopes[35], shaping in this way distinct microclimatic conditions.[10, 34]

The confirmed variability of the start dates of 'Beginning of flowering' of the studied plant, in relation with the orientation, combined with its aesthetic value, offer the perspective of its use on the configuration of the natural landscape. An additional advantage is the fact that the examined plant belongs to perennial plants[36], so its capsules, through which this plant propagates, may create around it a cluster of plants with special ornamental value. The aesthetic value of the plant may offer the growers a new perspective for its propagation and its further use in landscaping.

From our findings, it was possible to form a detailed view, for the first time, on the flowering (in terms of 'Beginning of flowering') of an interesting plant species, *Ajuga orientalis* L., in relation to air temperature. Undoubtedly, a more complete view on phenology of this species requires more years of research including also other phenological stages.

Conclusion

Kaboulieri area (south-southeast slope of Mount Aenos) was significantly warmer than Roudi area (north-northwest slope of Aenos) by 0.8 ^{0}C in both 2014 and 2015, in terms of average T of March, where a significantly earlier appearance of 'Beginning of flowering' of *Ajuga orientalis* L. took place (Kaboulieri), ranging from 9.1 (2015) to 10.9 (2014) julian days. The findings of our study could be a first step for the efficient preservation of the aforementioned plant species in a vulnerable natural environment and for its further exploitation as an ornamental plant.

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