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Research and Full Length Article:

Required Growing Degree-Days (GDDs) for each Phenological Stage of *Fritillaria imperialis*

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Abstract. Studying plant phenology is very important to regulate the rangeland utilization. Plant phenology can be determined by Growing Degree Days (GDDs). Since temperature varies yearly, the phenological stages may consequently differ yearly. Determining the base temperature is one of the key steps in the calculation of GDDs. The aim of this study was to calculate the required GDDs for each phenological stage to predict phenological stage of *Fritillaria imperialis* based on GDDs in the consequent years. To do so, *Fritillaria* bulbs were placed at constant temperatures of 0, 1, 2, 3 and 4°C. The length of sprouts as a growth index was measured as a function of temperature. Plant chronology was recorded in three day intervals from March 1st, 2016 in the field and the date of each phenological stage was recorded in the form of an index developed by combining Fick and Haun indices. Then, 20 plant individuals were selected in Dasht-e-Laleh of Kouhrang County, Iran and their growth stages were recorded. Meanwhile, the required amount of accumulated GDDs (AGDDs) to reach each phenological stage was also calculated. Results showed that *Fritillaria imperialis* began the sprouting (phase E) after receiving 130.75 GDDs on March 23rd, 2016. The emergence of leaves began on 27th March with 166.30 GDDs and finished on 9th April. Finally, this plant on 9th May received 560 AGDDs and went to senescence. Based on the phenological data, when the plant received 240 AGDDs, flowering stage started. The most appropriate time for tourist entrance is in the R₂ stage (flower opening) when the plant receives 298.8 AGDDs and the best exit time is when the plant receives 359.2 AGDDs. So, the relationship between phenological stages of the plant and GDDs will be useful in visiting the site in terms of ecotourism and determining proper visit times.

Keywords: Phenological Stages, GDD, Crown imperial (*Fritillaria imperialis*), Ecotourism

Introduction

Phenology is defined as the study of periodic plant and animal life cycle events and how these are influenced by seasonal and inter-annual variations in climate as well as such habitat factors as elevation (Morren, 1849). In other words, phenology is the scientific study of periodic biological phenomena such as germination, vegetative growth, flowering, breeding and migration in relation to climatic and habitat conditions. During the plant life, there are major events such as plant budding, growth, flower formation, fruit and seed propagation; the studying date and time of these events are called phenology. Simply, phenology is the calendar of events on the life of plants (Saeedfar and Rasti, 2000). Knowledge on phenology of rangeland plants as a complex ecological system affected by the changes in climatic factors, especially precipitation and temperature is so important for proper management of these valuable ecosystems. These factors are very important and influential in the phenology of plant species (Ehsani and Zand, 2015).

The presence or absence of vegetation in rangeland ecosystems are derived by environmental factors such as climate, soil and topography (Kolahi and Atri, 2014). Several researches indicated that temperature has the greatest impact on the occurrence time of plant phenological stages (e.g., Lambert *et al.*, 2010; Qelichnia, 2001; Weber, 2001; Shim *et al.*, 2017; Akbarzade and Mirhaj, 2002). Since temperature is an important factor on plant life cycle, changing weather conditions yearly (temperature) will consequently change the time occurrence of each plant phenological stage. Accordingly, the degree of day-to-day growth rate and subsequently phenological stages are predicted by temperature (Baghdadi *et al.*, 2013; Zarekia *et al.*, 2011). Determining and using the relationship between

phenological stages and Growing-Degree-Day (GDD) which is a criterion for the prediction of phenological stage are crucial especially in determining the grazing readiness of plants, modeling the desired rangeland and its management (Tarkesh, 2002). Moreover, this relation can be used to predict appropriate time of ecotourism site visit. Many researchers emphasized that the occurrence of each phenological stage is variable in different years; however, the amount of needed GDDs of each phenological stage is constant for every special species (Keneshloo and Ameri, 2012). As a result, a certain amount of heat is needed for plants to grow from one stage to another (e.g., from seedlings to 4-leaf stage) (Miller *et al.*, 2001).

Studies of the grazing effects on the appearance of plant phenological stages showed that there is often a linear relationship between phenology and cumulative temperature, but grazing treatments have little effect on this relationship (Frank and Hofmann, 1989; and Sanadgol, 2003).

The attempts to introduce a comprehensive system of description and quantification of morphological forage development were not successful (Sanderson and Moore, 1999) but Haun (1973) and Kalu and Fick (1981) have suggested two criteria, the so called Haun index and Fick index for determining the phenological stages of perennial grasses and forbs, respectively. Frank *et al.*, (1993) conducted a study using Haun index and suggested that the GDD can be used to determine the appropriate time for grazing initiation. The functions and services of rangelands include but not limited to livestock production, industrial and pharmaceutical production plants, water conservation and aquifer recharge, wildlife and recreational uses (Moghadam, 2005).

Fritillaria genus from Liliaceae family of monocotyledons contains about 100 species and is distributed in mostly

temperate regions of the world. A few species were native to Cyprus, south of Turkey and Iran (Akhtar *et al.*, 2003). Fifteen perennial and bulbous herbaceous plants exist in Iran (Mozaffarian, 1996). Amongst them, Crown imperial (*Fritillaria imperialis*) grows in Chaharmahal-Va-Bakhtiari province, Iran and sometimes, it is dominant species among vegetation types, which is very beautiful for its downward looking crown flowers. One of its famous habitats is Dasht-e Lale in Kouhrang that attracts hundred-thousands of eco-tourists each year. Nonetheless, due to its short period of flowering period which is greatly affected by yearly weather conditions, finding appropriate time of tourism site visit is so difficult. By the way, like other plant species, the phenology of *Fritillaria* is influenced by temperature regime.

The knowledge of timing of phenological events and their variability influenced by yearly weather conditions can provide valuable data for planning, organizing and timely execution of managerial activities to preserve a plant species. Our understanding from the phenological stages of *Fritillaria imperialis* species is limited. To our knowledge, this is the first report describing the phenological growth stages of the crown imperial using GDDs indicator and perhaps, also the first report about the *Fritillaria* genus. Studying

phenological stages enables us to have a better understating of this plant life cycle that plays a major role in the management of natural areas (Keneshloo and Amer, 2012) and can be used in many rangeland management aspects including grazing readiness, ecotourism management and so on. Therefore, this study aims to investigate thermal needs of different growth stages of *Fritillaria imperialis* species and required GDD as an explanatory variable of phenological stages at Dasht-e Laleh of Kouhrang, Iran, which can be used for other habitats of this species.

Material and Methods

This study has been conducted in Dasht-eLaleh of Kouhrang County, Chaharmahal-va-Bakhtiary province, Iran. Based on Koppans' methodology of climatic classification, Kouhrang station is counted as the moderate-cold climate with arid and semi-arid summers. The station is located at 32.51445–32.66410 latitude and 50.11170–50.28516 longitude. The Kouhrang station is 2285 m above sea level. The absolute minimum and maximum temperatures observed in this station are -30.6 and 35.8 in the 15-year period of data collection (1987-2012), respectively. This station has also an average of 125 frosting days in a year.

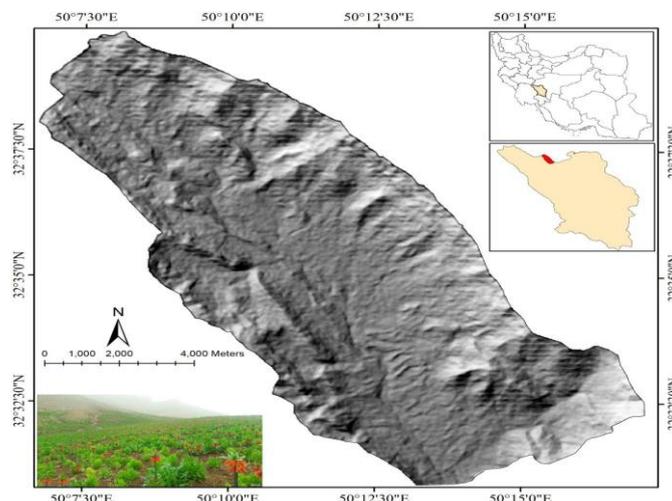


Fig. 1: Geographical location of Dasht-e-laleh in Chaharmahal-va-Bakhtiari province, Iran

Research Methodology

Phenological study was done and data were recorded during the growing season for the species of *Fritillaria imperialis* at Dasht-e Laleh Kouhrang.

We did not find any study on the quantification of this species phenology in the literature review. It is not possible to use the Han and Fick indices to record the phenological stages of this plant species because this plant is a grass-like species that do not follow phenological stages of neither grasses nor forbs completely. By examining the growth stages of the plant and following Miller *et al.* (2001) and Moore *et al.* (1991), we presented the growth index of this plant (Table 1). Each individual growth stage has both a mnemonic code and numerical index associated with it (Table 1). The codes were designed to be easily memorized and are useful for applying

the system in the field. Each code consists of two characters: a capital letter followed by a number. The letter denotes the primary growth stage and the number refers to the sub-stage within that primary stage. Numerical indices have been included so that the growth stages can be manipulated quantitatively. Numerical indices for the Germination, Emergence, Reproductive stage-Floral development, Seed development and Ripening Stage and Aging and Wilting Stage have been arbitrarily assigned. According to Miller *et al.* (2001), the leaf stage indices depend on the number of leaves pair (N) occurring in that stage for the species; according to the study done by Moore *et al.* (1991), they have been calculated as $2 + 0.9(n/N)$ where 2 is the primary stage number and n is the event number (Table 1).

Table 1: Designing phenological stages index for grass-like species (e.g., *Fritillaria imperialis*)

Stage	Numerical Index	Description
Germination		
G0	0	Appearing bulb bud
G1	0.5	Shoot elongation
G2	0.9	Appearing shoot from soil
Emergence		
E	1	Growing the plant
Leaf stage		
L0	2	Appearance the first pair leaf
L1	$2+0.9(2/N)$	Appearance the second pair leaf
L2	$2+0.9(3/N)$	Appearance the third pair leaf
L3	$2+0.9(4/N)$	Appearance the fourth pair leaf
L4	$2+0.9(5/N)$	Appearance the fifth pair leaf
Ln	$2+0.9(n/N)$	Appearance the nth pair leaf
Reproductive Stage-Floral Development		
R0	3.0	Inflorescence appearance
R1	3.3	Flower appearance
R2	3.6	Flower opening
R3	3.9	Capsule opening
Seed Development and Ripening Stage		
S0	4.0	Milking seeds
Aging and wilting Stage		
A0	5.0	Aging and wilting

Visiting the study area and phenological stage of the species was done every three days since March 1st to May 9th, 2016. For this end, about 20 base plants in each region were selected and numbered by wooden benchmark. Thus, at the end of the study period, the

history of each phenological stage was quantified.

In order to examine the stages of the plant phenological stages accurately, the Accumulated Growing Degree Days (AGDDs) index was used. AGDD in each sampling stage was calculated based on

the local meteorological data i.e., maximum and minimum temperatures as well as T base using Equation 1:

$$AGDD = \sum_{k=0}^n n \left[\frac{T_{\max} + T_{\min}}{2} \right] - T_b \quad (1)$$

Where:

AGDD is accumulated growing degree days,

T_{\max} is daily maximum temperature,

T_{\min} is daily minimum temperature,

T_b is base temperature and

n is the number of growing days.

Recording method of the GDD for determining the developmental stage was conducted as follows (Frank *et al.*, 1993):

- 1- Daily record of minimum and maximum temperatures and calculating GDD using temperature data of daily weather stations (Temperature data were obtained from the Kouhrang weather station located approximately at 9 km far from the study site).
- 2- The starting date to calculate GDDs was considered to be the first day after the average air temperature was more than the base temperature for 5 consecutive days. If the daily average temperature is below the base temperature, no GDD values will be recorded for that day.
- 3- Summing up GDDs for each day from growing starting date and calculating AGDDs.

The first parameter for calculating GDDs is T base or the base temperature that is measured using regression model (see Eq. 1).

Base temperature also called minimum

temperature or biological zero is the temperature; germination rate is zero and above that plants start germinating, which is accounted as the required temperature for starting plant growth.

To determine the base temperature for *Fritillaria imperialis* species, the non-germinated bulbs were first prepared, and the bulbs were gathered from the original site of study. The bulbs were planted in containers with a ratio of 1: 1 from soil and wind sand. Then, they were transferred to incubator with temperature of 0 to 4 (for each temperature, 5 replicates were used). For 20 days, the daily amount of growth was measured for every bulb. Then, the required cumulative GDDs for growing stages were measured while counting the number of leaves and determining the phenological stage of 20 plants by comparing the cumulative daily GDDs. Finally, the regression equation between growth length of germinated plants and temperature of treatments was made and used to estimate base temperature. Afterwards, a linear trend line was applied between the observed values of growth length and temperature and the position that this trend line crossed the X axis (i.e., temperature) was regarded as the T base (see Prentice *et al.*, 1992).

Results

a) Calculating base temperature

Results of planted *Fritillaria imperialis*' bulbs under temperature treatments (0-1-2-3-4°C) showed that the plant at temperature of 0°C does not have any growth. The results of determining the base temperature are shown in Fig. 2:

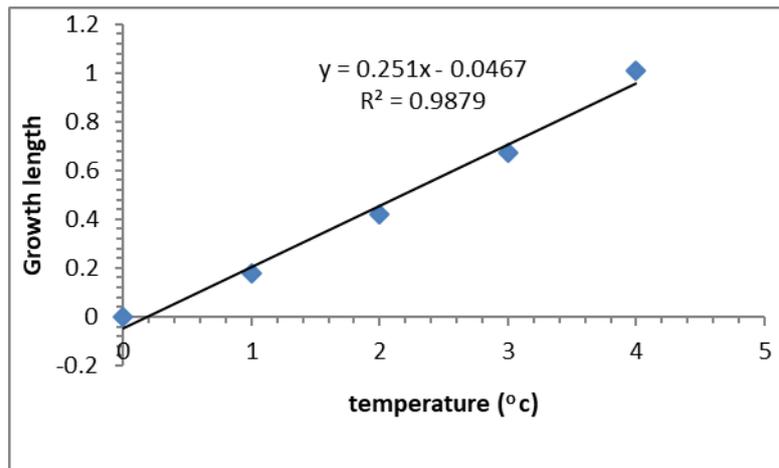


Fig. 2: Determining of basic temperature for *Fritillaria imperialis* using regression curve

There is a significant relationship between the temperature values and the plant growth length. Values of $R^2=0.98$ and $Sig = 0.001$ have been estimated (Fig. 2). Results of Table 2 indicate that increasing temperature significantly increases growth length of germinated plants. As indicated in Fig.2, the resulting regression equation of predicting growth length based on temperature is as follows:

$$Y = 0.251x - 0.0467 \quad (2)$$

Where:

X=temperature and Y= plant growth rate. By taking into account 0 for Y, the X would equal to 0.34 that is consistent with the results observed in the laboratory. This amount is considered as the base temperature for starting plant growth

Table 2: Surveying the coefficient of determination between growth length of germinated bulbs variation of *Fritillaria imperialis* and temperature

Equation	Model summarize				Estimated parameters	
	R ²	F	Df	Sig.	slope	constant
linear regression	0.983	244.99	3	0.001	0.251	-.047

b) Results of surveying plant phenology

During the three consecutive months, the phenological stages of 20 plants at Dasht-e-Laleh were recorded with an

interval of three days. The result of calculating AGDDs was recorded and phenological stages of *Fritillaria imperialis* with temperature in Dasht-e-Laleh for 2016 were shown in Fig. 3.

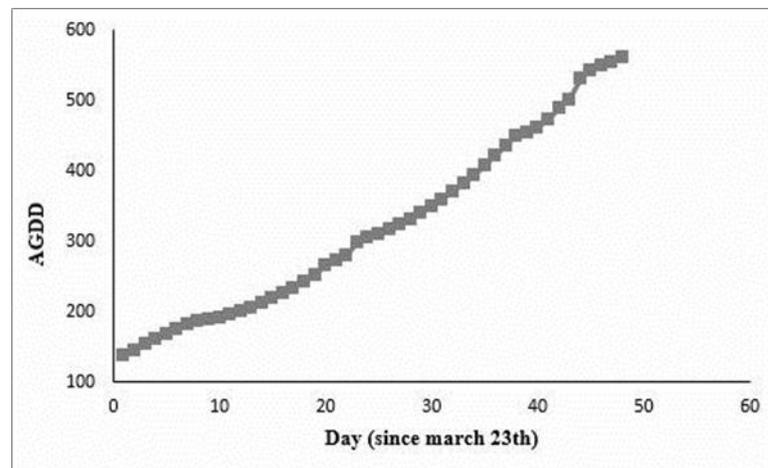


Fig. 3: Fitting phenological stages of *Fritillaria imperialis* with temperature variation in Dasht-e Laleh

Phenological calendar of species was shown in Table 3. This result indicates that in the study area, about 50% of *Fritillaria imperialis* plants began their sprouting (phase E) on March 23rd in 2016 when the minimum temperature for growth of the species is afforded. The amount of thermal units which plant has received in this date was about 130.75 degree-days. In other words, the phase E or sprouting phase needs 130.75 AGGDs.

As indicated in Table 3, the stage of L₇ reached on March 27th (with receiving a 166.30 AGGDs), stage of L₁₄ on March 31st (with receiving 204 AGGDs)

and stage of L₁₈ on April 6th (with receiving 216.60 AGGDs).

The emergence of inflorescence stems (R₀) coincides with the April 9th while receiving 240 AGGDs. The emergence of peduncle R₁ was on April 12th with receiving 270 AGGDs and it was altered to full flowering (R₂) on April 15th with receiving 298.8 AGGDs. Also, when the plant received 453.2 AGGDs on April 28th, the capsules (S₀) of flowers were observed. Finally, on May 9th, with receiving 560 AGGDs, the plants were seeded (A₀).

Table 3: The results of the study of phenology stages and description of occurrence of each stage of plant growth at Dashte-Lale

Stage	Description	Numerical index	Occurrence date	Accumulated GDD
E	Emergence stage	1.00	March 23 rd	130.75
L ₇	Leaf stage 7	2.31	March 27 th	166.30
L ₁₄	Leaf stage 14	2.63	March 31 st	204.00
L ₁₈	Leaf stage 18	2.80	April 6 th	216.60
R ₀	Reproductive Stage 0	3.00	April 9 th	240.00
R ₁	Reproductive Stage 1	3.30	April 12 th	270.00
R ₂	Reproductive Stage 2	3.60	April 15 th	298.80
R ₃	Reproductive Stage 3	3.90	April 23 th	359.20
S ₀	Seed Development 0	4.00	April 28 th	453.20
A ₀	Aging and wilting Stage 0	5.00	May 9 th	560.00

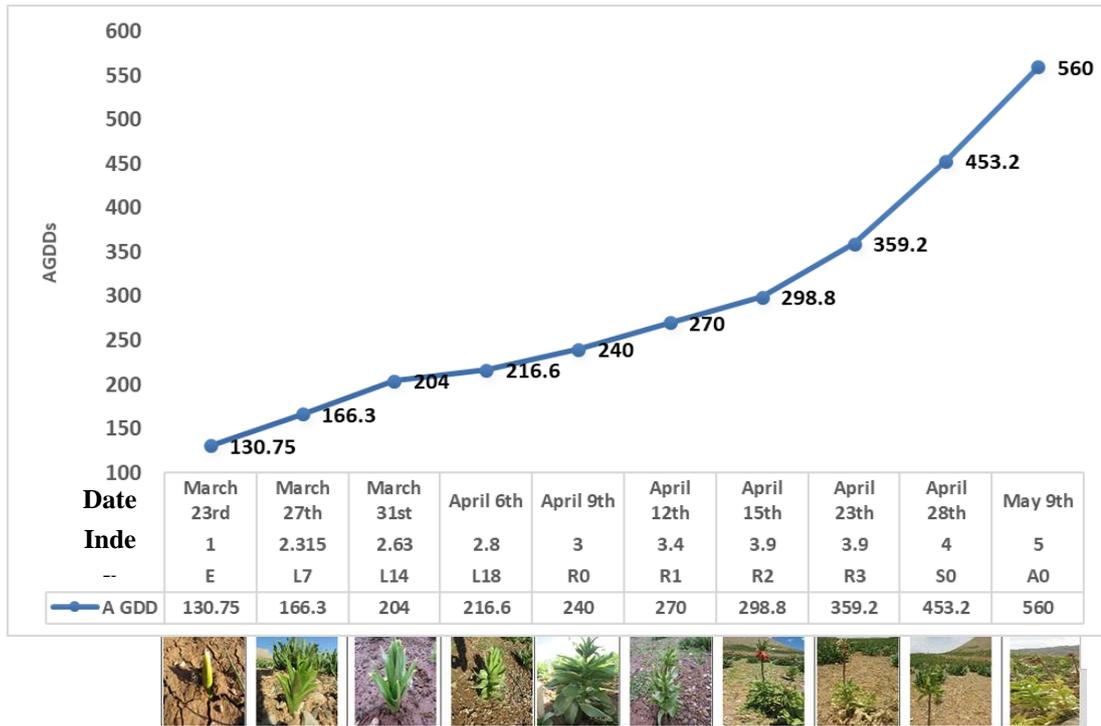


Fig. 4. Suitable phenological stages of *Fritillaria imperialis* for tourists visiting (full flowering) in Dasht-e Laleh

Phenological stages and the needed AGDDs for each phenological stage of *Fritillaria imperialis* in the Dasht-e Laleh are shown in Fig. 4.

In Fig. 4, the X-axis shows the phenological stages of *Fritillaria imperialis* including germination (E), stage of L7, stage of L14, stage of L18, the emergence of inflorescence stems (R0), the emergence of peduncle (R1) and the formation of flowers (R2). The Y axis shows the AGDDs for the occurrence of phenological stages in the Dasht-e Laleh. As it is shown in Fig. 4, from E to R2 phenological stages, a linear cumulative of AGDDs is observed whilst from R2 to A0, a rapid increase of AGDDs is perceived.

Discussion and Conclusion

According to the Society for Range Management (SRM), rangeland readiness refers to the appropriate time of rangeland exploitation (Perryman *et al.*, 2005) and it should be considered in management planning (Walker and Heitschmidt, 1986). Since the readiness of *Fritillaria imperialis* species in this

study area is important for tourists visit rather than grazing objectives, we should take into account the starting time of flowering as the appropriate time of tourist visit. Since a tourist should plan the site visit in advance and the starting time of flowering is different year by year, using AGDDs is an appropriate method to predict phenological stage of the plant. Since temperature that drives GDD influences the phenological development of plants, it can be used to forecast phenological stages of plants such as *Fritillaria imperialis* that is so important in tourist visit. In this case, AGDDs can be used to predict not only grazing readiness of rangelands (Frank and Hofmann, 1989) but also the developmental stages of an attractive species for ecotourism visitors. As indicated in Fig. 2, *Fritillaria imperialis* starts its emergence almost above 0.34°C, which is in accordance with the findings of Lambert *et al.* (2010) stating that the amount of base temperature for mountainous plants is relatively lower when snow start melting.

In addition to the thermal units, the

base temperature plays an important role in germination of plants too (Arnold, 1959). Therefore, we can conclude that this species is quite coldness tolerant species. As a result, this species is appearing at the beginning of spring and passes its phenological stages so rapidly. There is a significant relationship between the temperature and the plant growth length of the species after emergence (Fig. 2 and Table 2). Therefore, the time span that this temperature is met is so important for starting the plant growth which varies yearly. Accordingly, we should focus on the time period that meets the minimum temperature when the bulbs of this species emerges and gets out of the soil needing almost 100 AGGDs. Results of the study showed that growth period of *Fritillaria imperialis* lasts about 45 days. Leaves were occurred in the third week of March and reached to its peak almost after 20 days of leaf appearance.

The flowering stage started in the first week of April and reached to its peaks almost in the second week of April in 2016 (Fig. 3). Flowering stage continued until the mid-April and then, in late-April, the capsules were formed. The seeding started from early May and extended to mid-May (Table 3).

Fig. 4 shows that there is a linear relationship between temperature and the growth process of *Fritillaria imperialis*. Effect of temperature on plant growth stages was revealed in other studies (e.g., Weber, 2001; Bertiller et al., 1991; Thompson, 1990 and Akbarzade and Mirhaj, 2002).

The results indicate that starting growth date of this plant at the Dasht-e Laleh is on 23th March in 2016 when the melting of snow is started and adequate temperature is afforded. Other researchers also endorsed that melting of snow is the determinant factor of primary flowering time of mountainous plants (Dunne, Harte and Taylor, 2003; Saavedra et al., 2003; Lambert et al.,

2010; Forrest et al., 2010).

However, from flower appearance to flower opening, capsule occurrence and capsule opening, the process of life cycle of *Fritillaria imperialis* passes so rapidly and causes a short period of flowering and consequently site visit. This is due to more GDDs afforded in these days (Fig. 3) and intrinsic characteristics of the species.

The results of this study can be used for tourism management. As indicated in Fig. 4, *Fritillaria imperialis* starts its flowering on 12th April (i.e., R1 stage with 3.3 index) which is the appropriate time for starting tourist visit with 270 AGDDs and lasts till the occurrence of capsule with 359.2 AGDDs on 23th April. The amount of AGDDs needed to complete the phenological stages of plant and reach to senescence is almost 560 GDDs. The AGDDs that each species needs to reach each phenological stage and complete its life cycle is fixed (Jordan and Haferkamp, 1989). Therefore, AGDDs can be used to model the phenological stages of *Fritillaria imperialis* and can be used for tourist management. In this regard, predicting flowering date of the species based on temperature (max, min) records of meteorological stations can guarantee the time of appropriate time of tourist entrance for site visit. Modelling the spatio-temporal of each phenological stage based on AGDDs can accurately predict flowering date of this species and the time span that flowering lasts, which helps decision makers to do ecotourism management. This method is promising due to its precision and simplicity that requires three parameters of maximum and minimum temperatures as well as base temperature and offers relatively acceptable outputs (Azarnivand et al., 2010).

In general, we can conclude that AGDDs can be used to predict occurrence of each phenological stage of *Fritillaria imperialis* as an attractive

ecotourism species, especially flowering stage that needs almost 270 AGDDs and the time span that flowering lasts until the occurrence of capsules with 359.2 AGDDs requirement which is the most appropriate time of visit. The results revealed that predicting and managing ecotourism site visit based on AGDDs can guarantee the satisfaction of visitors as an appropriate tool for forecasting proper time of plant flowering.

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محاسبه درجه روز-رشد لازم برای مراحل رویشی گونه *Fritillaria imperialis*

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چکیده. بررسی فنولوژی گیاهان برای تنظیم برنامه‌های بهره‌برداری از مراتع بسیار مهم است. با توجه به اینکه فنولوژی گیاهان متأثر از درجه حرارت می‌باشد و درجه حرارت نیز از سالی به سال دیگر دستخوش تغییراتی می‌گردد، استفاده از معیار درجه روز رشد، برای تعیین تاریخ وقوع مراحل فنولوژی گیاهان و پیش‌بینی آن حائز اهمیت زیادی است. هدف از این مطالعه، محاسبه ارزش‌های GDD برای هر مرحله فنولوژی گونه لاله واژگون *Fritillaria imperialis* به منظور پیش‌بینی مراحل وقوع فنولوژی گیاه بر مبنای درجه روز رشد تجمعی (AGDDs) می‌باشد. تعیین دمای پایه، یکی از مراحل اصلی طراحی مدل-های GDD است. برای این منظور، ثبت کروئولوژی گیاه با فواصل سه روزه و تاریخ وقوع هر مرحله فنولوژی در قالب ترکیب شاخص اصلاح شده فیک و هان برای گیاهان شبه گندمی (اصلاح شده توسط نویسندگان) از تاریخ ۲۵ اسفند در سال ۱۳۹۴ در منطقه دشت لاله های واژگون کوهرنگ استان چهارمحال و بختیاری انجام شد. پیازهای گیاهان در محیطی کنترل شده در درجه حرارت‌های ثابت (۰-۱-۲-۳-۴ درجه سانتی‌گراد) کشت و تغییرات طول رشد جوانه های گیاهی بصورت تابعی از درجه حرارت تعیین گردید. در تحقیق حاضر به منظور بررسی ویژگی‌های فنولوژیکی *Fritillaria imperialis* مراحل رشد این گیاه در عرصه ثبت شد. به منظور مطالعه فنولوژی گونه گیاهی یاد شده، ۲۰ پایه از گیاه در مناطق مورد بررسی انتخاب و همزمان با ثبت مراحل رویش گیاه نسبت به تعیین مقدار AGDD مورد نیاز برای رسیدن به هر مرحله فنولوژیکی این گونه اقدام شد. نتایج نشان داد که *Fritillaria imperialis* پس از به دست آوردن ۱۳۰/۷۵ درجه-روز-رشد در تاریخ ۴ فروردین ۱۳۹۵، مرحله‌ی جوانه زنی (مرحله‌ی E) را آغاز کرد. مرحله برگدگی این گیاه در تاریخ ۸ فروردین (با دریافت ۱۶۶,۳۰ درجه-روز-رشد) آغاز شد و این مرحله پس از دریافت ۲۴۰ درجه-روز-رشد در ۲۱ فروردین ۱۳۹۵ به اتمام رسید. در این تاریخ، مرحله تولید مثل آغاز شد. در نهایت این گیاه در ۲۰ اردیبهشت ماه ۱۳۹۵ و با دریافت ۵۶۰ درجه-روز-رشد به پایان دوره رویشی خود رسید. در نتیجه، براساس اطلاعات فنولوژیکی *Fritillaria imperialis* و تاریخی که گیاه ۲۴۰ GDD (مرحله گلدهی) دریافت کرد، تعیین زمان مناسب برای ورود گردشگران و با دریافت ۳۵۹,۲ درجه روز رشد گلدهی آن پایان و زمانیکه ۴۵۳/۲۰ GDD دریافت نمود ظهور کپسول به وقوع پیوست که زمان خروج گردشگر از دشت لاله محسوب می‌شود. بنابراین تعیین رابطه بین مراحل فنولوژیکی گیاه و درجه روز رشد در بکارگیری اصول مدیریت گردشگری مناطق دارای این گونه گیاهی و همچنین تعیین آمادگی مرتع برای ورود گردشگر و مدل‌سازی مراحل رشد سودمند خواهد بود.

کلمات کلیدی: مراحل فنولوژی، درجه روز-رشد، *Fritillaria imperialis*، اکوتوریسم