

**LIFE HISTORY AND DEMOGRAPHIC
FEATURES OF *ASTRAGALUS NITIDIFLORUS*,
A CRITICALLY ENDANGERED SPECIES.**

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Abstract

Astragalus nitidiflorus is an endangered short-lived legume of SE Spain, which have been re-found after 100 years. To identify possible weak points that might contribute to its rarity and hamper its conservation, this paper presents data concerning location, habitat, demographic features and reproductive biology of the species. Censuses of three cohorts of seedlings show that the seedling stage is the most critical in the life cycle. The adult plants show a long flowering season with a high degree of synchrony. Despite the low reproductive success of the species, the annual seed production is very high due to the high floral production. The data show that *A. nitidiflorus* is a facultative xenogam species, but the presence of pollinators can enhance fruit set. The viability of the species is not limited by the flowering and fruiting process, however the maintenance of the habitat in the early successional stages seems to be the critical point for long-term survival of the species.

Keywords: conservation biology; endemic species; Fabaceae; population dynamics; reproductive success; Spain.

Introduction

The genus *Astragalus* is represented by ca. 3000 taxa in the world and is distributed in semi-arid steppe regions. In the Iberian Peninsula there are 41 described species of *Astragalus*, nine of which are endemics (Podlech, 1999). Among these endemic species, *Astragalus nitidiflorus* Jiménez Mun. et Pau (Fabaceae) is an endemism of the province of Murcia (SE Spain). It is a perennial herbaceous species that was collected in 1909 and described by Pau (1910). Since then it has not been recorded until a few individual plants were observed in 2003 near Cartagena (Murcia province). This species was first classified as extinct (Galicia and Sánchez, 2003) and is now listed as Critically Endangered (Sánchez et al., 2004) in accordance with IUCN criteria (2001). It is currently protected by a national law (BOE, 2005).

The reappearance of this species has awakened the interest of environmental managers because the reasons for its situation are unknown. Following Schemske et al. (1994), narrow endemics are susceptible to extinction for a variety of reasons, such as habitat destruction, biotic interactions and genetic collapse. Hence, those reasons need to be considered when designing conservation strategies because they may affect reproductive success (Godt and Hamrick, 1995). Several authors have described how many factors may affect reproductive success by influencing the processes of flowering, fruit and seed production: plant size (Bishop and Schemske, 1998; Torres et al., 2002; Albert et al., 2008), phenological traits (Sobrevilla, 1988; English-Loeb and Karban 1992; Gómez, 1993; Giménez-Benavides et al., 2007), the breeding system of species (Debandi et al., 2002; Torres et al., 2002; Albert et al., 2008; Hill et al., 2008) and the genetic structure (Hamrick and Godt, 1990).

For most plant populations with overlapping generations, mortality, survival and reproduction tend to vary with age or the size of individual plants (Harper, 1980; Hutchings, 1987; Hegazy, 1990). Quantitative data on population survival, mortality and reproduction throughout life may provide essential information on which conservation management can be based (Hegazy, 1992). However, no information exists about the effect of these factors on this species because it has been overlooked by investigators for almost a hundred years. In order to design and implement an effective conservation programme for this species, understanding demographics features and

insight into the life history of *A. nitidiflorus* is needed. In the present paper, the location, habitat, demography and reproductive biology of *A. nitidiflorus* is described and preliminary data are presented about biotic interactions with other species. More specifically, this study will aim to (1) describe the life history of the species, (2) describe the flowering and fruiting phenology, determining the reproductive success and the factors that influence it, (3) describe the patterns of seedling recruitment, and (4) discuss the implications for the management and conservation of the species.

Materials and methods

✓ Species and study site

Astragalus nitidiflorus is a short-lived herbaceous legume. Mature plants are generally < 30 cm high, with stems spreading from a caudex in a circular pattern up to 150 cm diameter. The leaves are imparipinnate and measure up to 14 cm and leaflets are villous. The flowers appear in racemes (until 300 racemes in the biggest plants) of up to 30 yellow flowers, with a corolla of up to 2.1 cm. Fruits are curved and acuminate legume of 18 x 7 mm, approximately.



Image 1. General appearance of *Astragalus nitidiflorus* (left) and detail of the inflorescence (right).

Currently there is only one population of *A. nitidiflorus*, located near Cartagena, in the Cabezos del Pericón Mountain Range (Murcia province) (Fig. 1). The site contains four subpopulations. The first subpopulation, found in 2003, is annotated in Fig. 1 as P1, and was chosen for the present study, which began in 2005. Subpopulation P2 was found in 2007 and its individuals have been monitored for demographic studies. Subpopulations P3 and P4 were found in June 2008 in previously unexplored habitats and they were not monitored for the study.

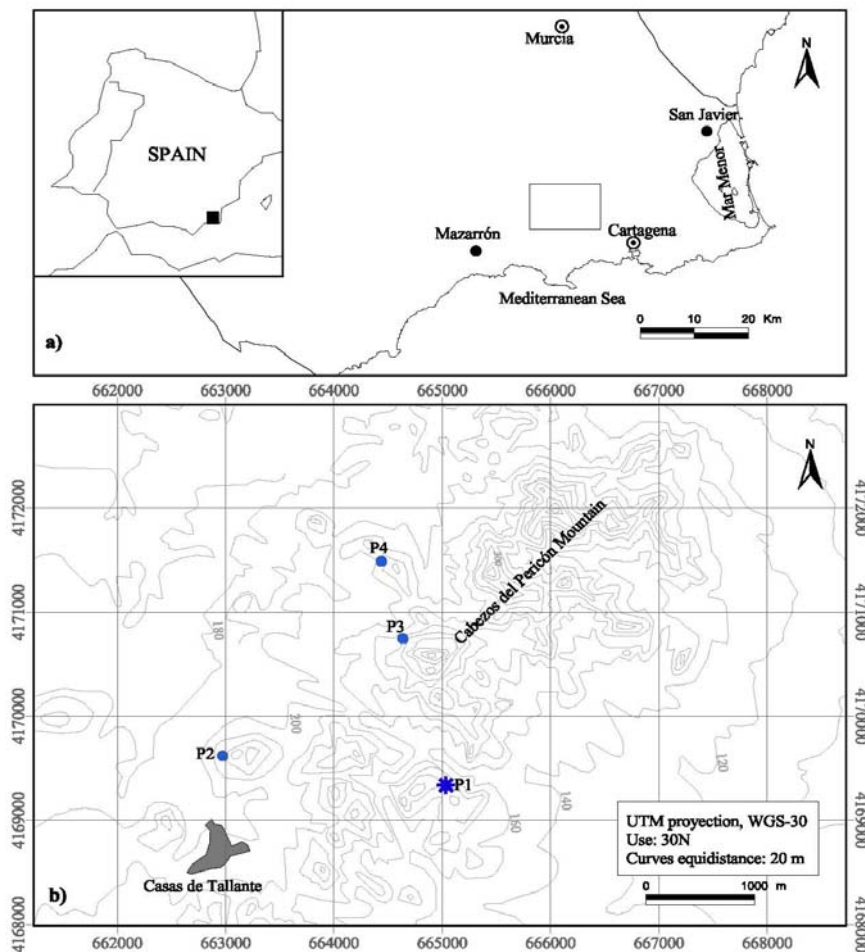


Figure 1. A) Geographical distribution range of *Astragalus nitidiflorus* in south-eastern Spain; B) Location of the population of *Astragalus nitidiflorus* subdivided into four subpopulations (P1, P2, P3, and P4).

The species grows in shallow soil from metamorphic and volcanic rocks in the interface between mountain and cultivated areas, from which it colonises old fields. Soils are Leptic Regosols (Eutric) (WRB, 2006) with pH values around 8 and a sandy loam texture. Altitude in the specific distribution area is from 200 to 260 meters above sea level. *Astragalus nitidiflorus* occurs in pastures with *Lotus edulis* L., *Astragalus*

sesameus L., *Scorpiurus sulcatus* L., *Brachypodium dystachion* (L.) Beauv., *Bellardia trixago* (L.) All., *Hyparrhenia synaica* (Delile) Llaurodó ex G. López, *Ononis natrix* L. among which are some chamaephytes such as *Lavandula multifida* L. and *Teucrium capitatum* L. and nanophanerophytes such as *Thymelaea hirsuta* (L.) Endl. The study area has a Mediterranean type climate with semiarid conditions. The mean annual rainfall is 246 mm and annual ETP of 1319 mm. The annual drought period lasts normally 5 months. The mean annual air temperature is 17.6° C, the warmest month being August (monthly mean temperature 26.1°C, mean maximal temperature 28.9°C, and mean minimal temperature 23.4°C), and the coldest month is January (mean monthly temperature 10.4°C, mean maximal temperature 15.26°C, and mean minimal temperature 6°C). Data of precipitation and temperature during the study period are shown in Fig. 2.

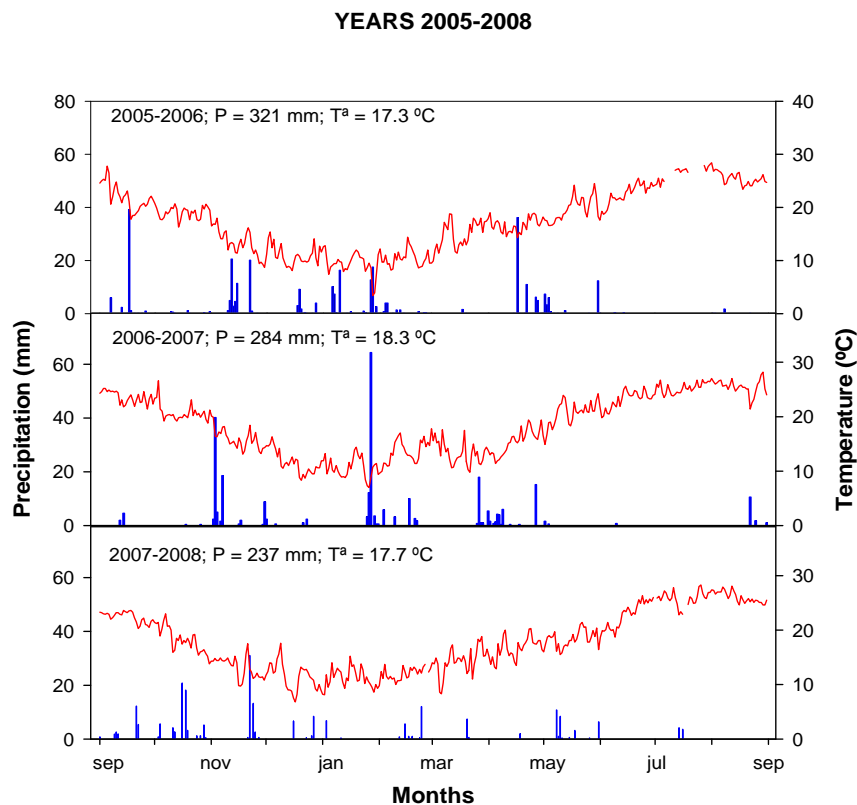


Figure 2. Daily precipitation amount (bars) and mean daily temperatures (lines) for the periods from September 2005 to August 2006; September 2006 to August 2007 and September 2007 to August 2008 in the studied area. P = total precipitation in each period; T = mean temperature for each period.

✓ **Demographic studies**

In order to know the life history and population dynamics of *A. nitidiflorus*, all individuals growing in subpopulation P1 were monitored (censused and labeled) from October 2005 to September 2009. In 2007 all plants of P2 were also censused. In order to register all individuals, several visits to the study area in the different seasons of the year were made. Seedlings and the autumn sprout of adult plants that appeared in autumn-winter were recorded and monitored until the end of the study. For the demographic study, plants monitored throughout the study were grouped into cohorts so that all the individuals that germinated in the same autumn-winter period belonged to the same cohort (e.g. all the individuals germinated in autumn 2006-winter 2007 belong to 2006 cohort). From the beginning of the study (September 2005) a total of 390 individuals were labeled and monitored, of which 20 were already adults when the study began. These 20 adult individuals were assigned to a specific cohort based on their phenological stage, so that one was assigned to 2003 cohort and the other nineteen to 2004 cohort (Table 1).

In order to analyse the population demographic data, and following Pielou (1977) and Hegazy (1992), a life table with the following parameters is shown:

Percentage of the original cohort surviving to the start of each stage (l_x), calculated as follows:

$$l_x = (N_x / N_1)100$$

where x is the age in years, N_1 is the original number of seedlings in each cohort and N_x the surviving individuals in each age.

Percentage of the original cohort dying during each stage (d_x), calculated as:

$$d_x = (l_x - l_{x-1})100$$

Then, the stage-specific mortality percentage (q_x) was calculated as:

$$q_x = (d_x / l_x)100$$

The expectation of future life (e_x) in age units was estimated as:

$$e_x = \sum_{j=x}^{\infty} l_j / l_x$$

✓ **Growth and flowering attributes**

Plant diameters were measured at the end of the growing season for each tagged plant. This is considered an appropriate measure of size in plants that are prostrate and whose planar projection is roughly circular (Bishop and Schemske, 1998). To study the flowering phenology, in 2006, maximum flowering moment, flowering intensity, flowering duration and flowering synchrony were obtained in all nine reproductive adult plants in bloom in subpopulation P1. They were monitored every 3-4 days and all inflorescences were labeled when they appeared. Also, the number of flowers and fruits were monitored at each census, and, at the same time, the number of open flowers in each inflorescence was recorded to know the flowering intensity. In 2007, flowering duration and flowering synchrony were also studied in the eleven flowering plants.

For each plant, maximum flowering moment was calculated as the number of days from the first open flower in the population to the day of maximum flower count on each plant (Bishop and Schemske, 1998). Flowering duration was calculated as the number of days the plant remained in bloom. Intensity was estimated as the maximum number of simultaneously open flowers on one plant. Synchrony is described as the number of days when the flowering of one individual overlaps with the flowering of every other plant in the population (Gómez, 1993). This variable was calculated as follows:

$$S_i = \frac{1}{n-1} \left(\frac{1}{e_i} \right) \sum_{\substack{j=1 \\ j \neq i}}^n e_j$$

where: n is the number of plants in the population, e_j is the number of days in which i and j individuals flower simultaneously, and e_i is the number of days individual i is in flower. This index ranges from zero when there is no synchrony to one when flowering overlap is complete.

✓ **Reproductive success**

Following Charlesworth (1989), the reproductive success of a plant species is defined as the product of the number of fruits/number of flowers (fruit set) and number of seeds/number of ovules (seed set) ratios. These ratios range from 0 to 1. In 2006 and 2007 fruit set was calculated in the labeled inflorescences, in which the number of flowers and fruits were recorded. To know the seed set, fruits were opened to estimate the number of seeds, which were then related with the mean number of ovules per ovary previously obtained to calculate *P/O* ratio (number of pollen grains/number of ovules).

✓ **Breeding system**

To determine the breeding system of *A. nitidiflorus*, we calculated the Outcrossing Index (OCI) and the *P/O* ratio, following Cruden (1977). According to this author, the OCI is the sum of assigned values for three characteristics of the flower and floral behavior: 1) diameter of the flower (corolla up to one mm wide = 0; 1-2 mm wide = 1; 2-6 mm wide = 2; more than 6 mm wide = 3), 2) temporal separation of anther dehiscence and stigma receptivity (homogamy and protogyny = 0, protandry = 1) and 3) spatial relationship of stigma and anthers (if the stigmas and anthers were at the same level and contact between anther and stigma seemed possible = 0, if the stigmas and anthers were spatially separated and contact seemed unlikely = 1). Cruden took account also of the type of habitats: from highly disturbed (value 1) to late successional stages in which pollinator activity was unreliable (value 4).

To calculate pollen production and abundance, eight plants were randomly chosen, from which three inflorescences each with closed flowers were collected. Inflorescences were stored in FAA (formaldehyde acetic alcohol) and taken to the laboratory, where the three closed flowers were excised from each inflorescence for pollen measurements (Burne et al., 2003). The anthers were manually extracted and the pollen grains from each anther were washed into 2 ml soap solution (Tween 80) and then stirred to ensure adequate mixing of pollen in the solution. From this solution 0.01 mL was taken to calculate the amount of pollen per anther. Thereafter the *P/O* ratio was determined by dividing the average number of pollen grains by the average number of ovules per flower. The ovule number per ovary was counted under a stereo microscope in 30 randomly collected flowers.

Pollen viability was estimated using the method initially proposed by Heslop-Harrison and Heslop-Harrison (1970), based on the fluorochromatic reaction that viable pollen grains exhibit when they are incubated with fluorescein diacetate (FDA test). For this determination, 2 mg fluorescein diacetate and 1.71 g sucrose were dissolved in 10mL distilled water and the pollen was homogeneously immersed in this solution. All pollen grains, which fluoresced brightly in a fluorescence microscope were scored as viable.

Since some field observations indicated that the species might be self-compatible, three cultivated plants were individually bagged during their flowering period. At the end of this period, plants were uncovered and the average number of mature fruit in 126 randomly chosen inflorescences was calculated. Data were compared with those obtained from 119 inflorescences of uncovered plants. The average number of seeds per fruit was also recorded in these fruits.

✓ **Statistical analysis**

All statistical analyses were performed using the statistical package SPSS 15.0 for Windows (SPSS. Inc., 2006). ANOVA and GLM were used to test for differences in the growing, flowering and reproductive parameters. We used linear regression to test for an association of plant size with age, and plant size with flower production, fruit production, seed production and reproductive success. Before analysis, all the proportions were arcsine transformed to ensure homogeneity of variance. To describe the symmetry of the flowering curves the Weibull function was used, whereby the values lower and higher than 3.6 indicate asymmetry to the left or right, respectively.

Results

✓ **Life stage**

The plant occurs in the first stages of the plant succession in old fields and bordering intensively tilled almond crops. Tillage practice prevents the expansion of the species. When agricultural activity ceases, *A. nitidiflorus* forms part of the herbaceous

colonizer communities in the old fields. However, no *A. nitidiflorus* plants have been found when succession progresses to produce a dense matorral.

The life cycle of *A. nitidiflorus* begins with seeds, which germinate in autumn and winter. Seedlings (henceforth P1) develop until the summer. In summer, leaves and stems die and only a few buds remain at the base of the stem at ground level. After the autumn rains, the dormant buds of the P1 plants that have survived the summer sprout and begin a second stage of growth (P2). The life cycle continues in this way with successive periods of growth (P3, P4) until the plant dies. Some plants survive for four years (P4) (Fig. 3).

Figure 3. Life cycle of *Astragalus nitidiflorus*.

Until plants reach the stage P2, they are not able to reproduce. In the older plants flowering begins in March and continues until the end of May. The flowers are grouped into racemes that appear from the axillae of the apical leaves on the stem; new inflorescences appearing on the stem as it grows. During six to seven days (exceptionally up to 9 days), in each inflorescence two to four flowers appear per day.

Three days elapse between the time the flower corolla appears and the time it withers, so that the inflorescence has open flowers for a total of 9-10 days. The number of flowers per inflorescence is about 20, sometimes reaching 30. The flowers are visited by bees such as *Osmia tricornis* Latreille and *Apis mellifera* L. The fruits take about two months to ripen. Dispersion unit is the fruit, an indehiscent legume that contains up to 19 seeds. The prostrate plant growth habit and weight of the fruits on the infructescences bring these into contact with the soil and the mature fruits drop in the same place as the inflorescences develop (autochory). In this way, the fruit shadow occupies the whole area covered by the mother plant, under which fruits of different years can be found. Secondary agents (not studied here) must be responsible for fruits being transported far from the mother plant.

As regards biotic interactions in the species, apart from the presence of the above mentioned pollinators, pre-dispersive predation of the seeds by *Bruchophagus astragalii* Fedoseeva, a parasitic hymenoptera whose larvae develop inside the seeds, has been observed. At the beginning of vegetative growth (autumn), the leaves are consumed by larvae of the butterfly *Colias crocea* Furcroy, although little damage results. Generally, cattle avoid the plant, as do small wild rodents such as rabbit.

✓ **Demographic studies**

From October 2005 to the end of the study period the seedling emergence varied according to the year, 64 seedlings per population appearing in 2005 cohort, 292 in 2006 cohort, 14 in 2007 and 8 in 2008 cohort (N_1 in Table 1). At the end of the first growing period, only 20.9% of the 378 seedlings developed from October 2005 to September 2009 survived (see *Total cohorts 2005-2008* in Table 1). At the end of the second growing period, only 10.1 % of those seedlings had survived, and at the end of the third period 1.9 % of the original 378 seedlings were still alive. The highest mortality rate (q_x) was recorded during the seedling stage and at the end of the third growing stage (P3). In all cohorts (2005-2008), the P2 plants show lower mortality rates than any other stage and greater life expectancy than P1 and P3 (Table 1). In the analysis of the data for individual cohorts, the seedling stage was the most critical, with

mortality reaching 100% in some years (Table 1). Life expectancy also differs according to the cohort to which an individual belongs.

✓ **Growth and flowering attributes**

Plant size increased with plant age, and there was a positive relationship between both parameters ($r^2 = 0.536$, $P = 0.0001$). Seedlings (P1) showed a mean size of 9.1 ± 4.4 cm (range 3-17.5 cm, median = 9.5) and P2 plants showed a mean size of 51.9 ± 27.6 cm (range 14-106.5 cm, median = 49.5). P3 individuals were significantly larger than P2 ($F = 35.88$; $P = 0.000$) reaching a size of 80.4 ± 33.2 cm (range 20-153.5 cm, median = 83). P4 plants did not differ in size from P3 (98.14 ± 30.4 cm, with a range from 51 to 134 cm, median = 109.5). All the adult plants studied (from P2 stage onwards, $N=28$) flowered in 2006 and 2007.

The population showed marked interannual differences for the flowering period (Fig. 4). In 2006 the flowering season started on 21 March and lasted until 19 May (60 days). However, in 2007 the flowering period was much longer (84 days) because it started 13 days earlier (8 March) than in 2006 and finished 11 days later (30 May). In 2006, 100% of plants flowered simultaneously during 7 days (8 to 14 April), while in 2007 the simultaneous flowering was observed for a longer period (two weeks, approximately from 19 April to 3 May) (Fig. 4). The pattern of the percentage of flowering plants through time was symmetrical in 2006 ($c = 5.50$ in the Weibull distribution function), but asymmetrical to the left in 2007 ($c = 2.44$).

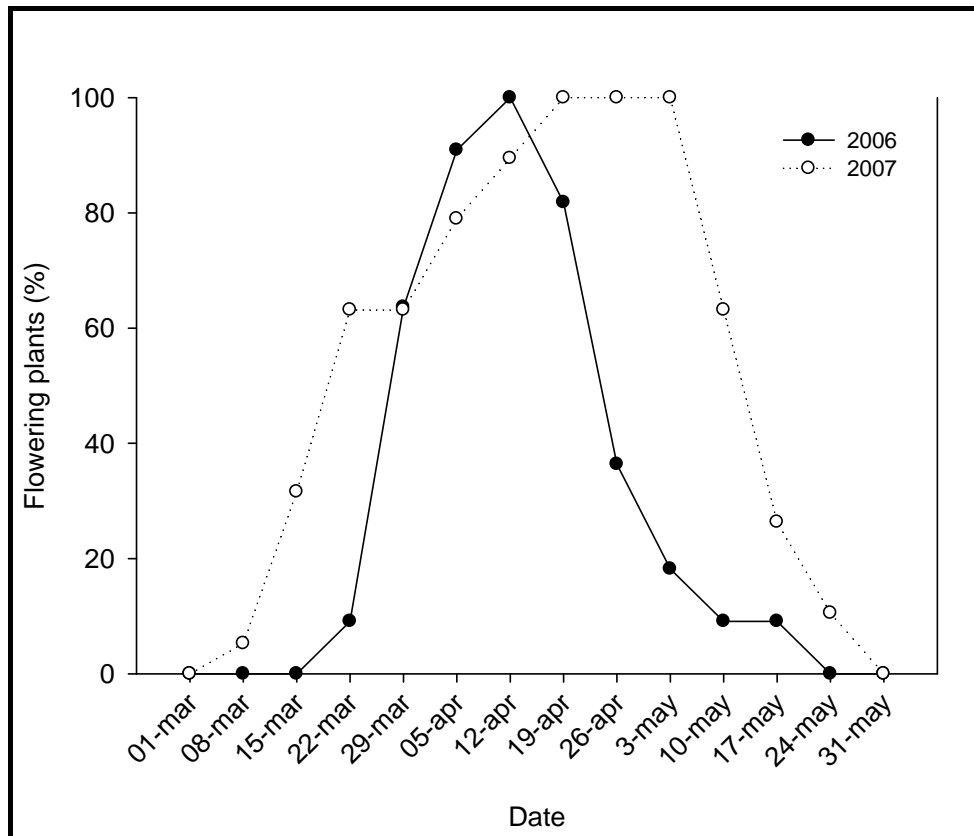


Figure 4. Flowering phenology in the studied population of *Astragalus nitidiflorus* in 2006 (closed circles) and 2007 (open circles).

Mean duration of the flowering period in P2 plants varied significantly ($F = 9.99$; $P = 0.006$, $N = 19$) from 27.9 ± 9.6 days in 2006 to 47.2 ± 15.1 days in 2007 (Table 2). The only P3 plant in the population in 2006 flowered for 36 days, and in 2007 the P3 plants flowered for a mean of 59.7 ± 5.9 days ($N=7$). There were no significant differences between P2 ($N=11$) and P3 ($N=7$) in 2007 ($F = 4.30$; $P = 0.054$). Flowering duration of the only plant P4 was 64 days (Table 2).

Maximum flowering moment occurred between 3 and 13 April (13 and 24 days after flowering started (Table 2), a few days after the middle of the mean flowering period.

The maximum number of open flowers per plant (flowering intensity) showed a mean of 101 ± 70.4 flowers in P2, with a very variable range of 27-219 flowers (Table 2). The only P3 plant in 2006 showed a flowering intensity of 479 flowers. There was a strong positive relationship between flower production and flowering intensity ($r^2 =$

0.964, $P = 0.0001$, $N = 9$). In most of the plants the patterns of the flower production were significantly asymmetrical to the left ($c < 3.6$ in the Weibull distribution function), showing a sudden increase in flower production at the beginning of the flowering period, reaching a peak and then a gradual descent until the end of the flowering period (Fig. 5).

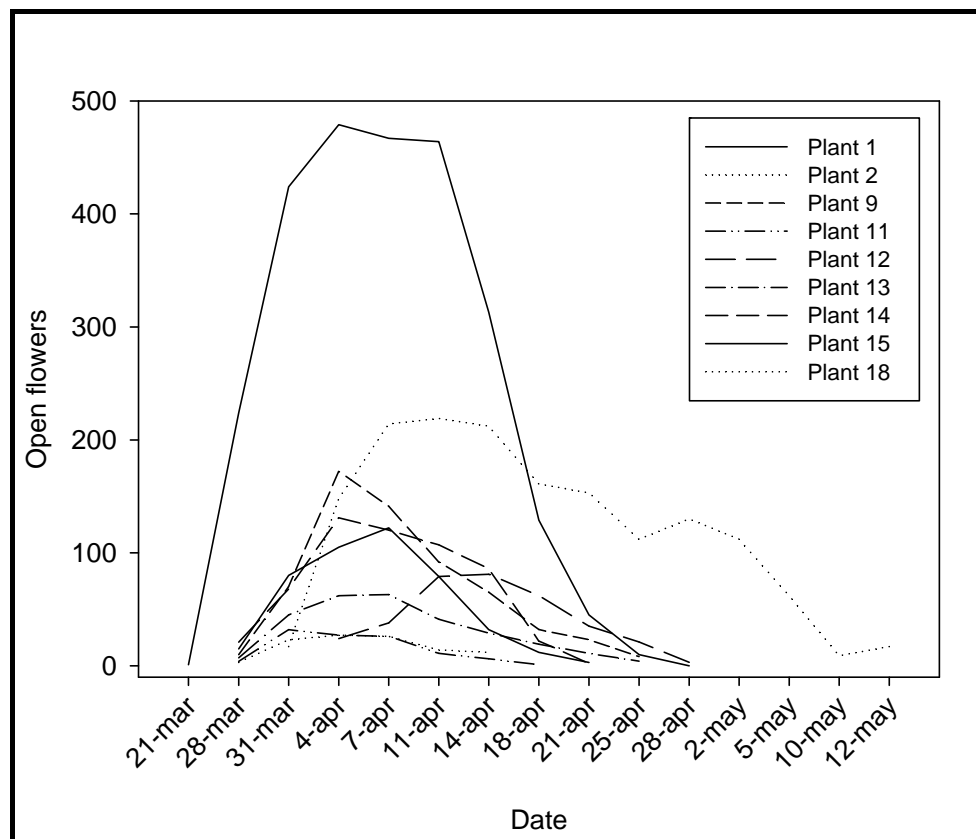


Figure 5. Flowering curves of *Astragalus nitidiflorus* individuals in studied population in 2006.

As regards the flowering synchrony values there were not significant differences between years ($F = 0.01$; $P = 0.913$) and adult types ($F = 1.41$; $P = 0.252$), with values of between 0.77 and 0.82 (Table 2).

✓ **Reproductive success**

Mean number of inflorescences in P2 plants varied from 33.9 to 32.5, with very high range of variation within the same year (from 2 to 118 per plant, median = 13), but

no differences between years (Table 3). In P3 plants from 2007, the mean value reached 179.5 ± 108 inflorescences (range 36-339, median = 163.5). The only P3 plant alive in 2006 produced 219 inflorescences, and 166 in the following year when the plant had become P4 (Table 3), both values within the range shown by P3 in 2007.

The number of flowers per plant varied greatly within each adult type studied. In P2 plants, the number of flowers per plant ranged between 690 ± 539.8 and 673.3 ± 843 (in 2006 and 2007 respectively, with no significant differences), while in P3 plants the mean value was significantly higher ($F = 24.97$; $P = 0.000$) reaching 3715.6 ± 2236.3 flowers per plant. The P4 plant produced 3436 flowers, which was within the range of variation shown by P3 plants (Table 3).

The mean number of ripe fruits per inflorescence (3.5-4.5) did not depend on the age of the plants (Table 3). The number of fruits produced by P2 plants hardly reached the mean value of 150 in either of the years studied (133.5 ± 114.3 and 148.5 ± 201.2 in 2006 and 2007 respectively). P3 plants produced a significantly higher number of fruit than P2 (800.2 ± 617) ($F = 16.06$; $P = 0.001$), with a very high range of variation (132 to 2017 fruits per plant) (Table 3).

The proportion of flowers setting fruits (fruit set) was always very low (mean values varying between 0.173 ± 0.06 and 0.214 ± 0.09), with no significant differences between years and type of individual. The number of seeds per fruit did not depend on the plant age, either, ranging from 10.1 ± 2.9 to 13.3 ± 2.8 seeds (Table 3). The number of ovules per ovary did not vary greatly (15-18 ovules; mean value 16.6 ± 0.93). The average proportion of ovules setting seed in each ripe fruit (seed set) was similar among adult plant types and years, varying between 0.788 and 0.610. Reproductive success was very low and was also very similar among adult plant types (mean values from 0.109 to 0.177) (Table 3). The seed production capacity of plants varied. In P2 plants, values ranged from 577 to 7741 seeds, while in P3 the range was 1386 to 27855. The estimated production for the only P4 plant studied in 2007 was 6238 seeds (Table 3).

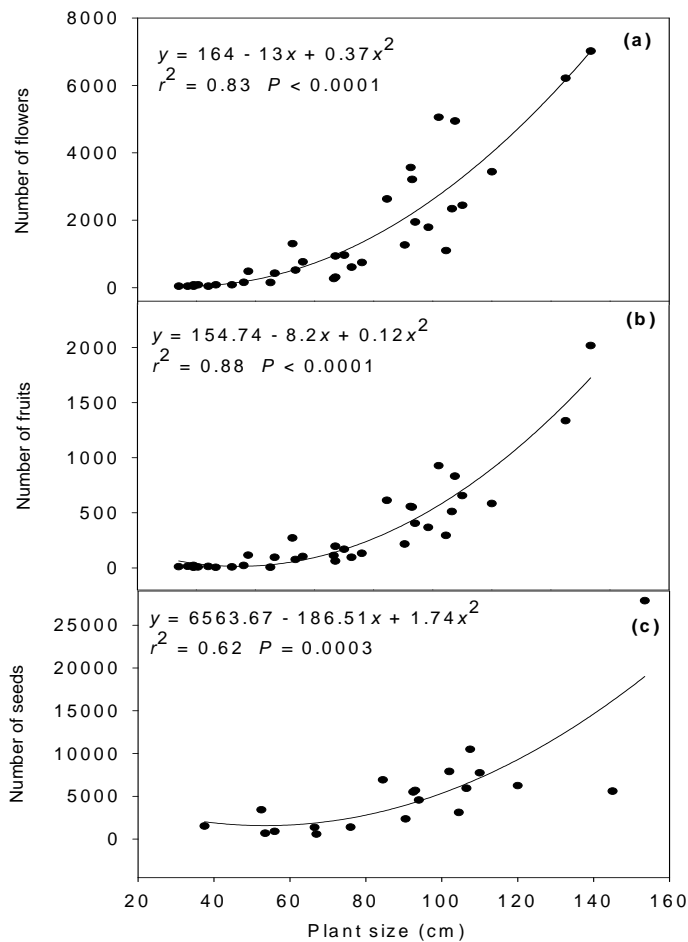


Figure 6. Flowers, fruits and seed production *Astragalus nitidiflorus* plants as a function of size of individuals.

✓ Breeding system

Taking into account that the corolla diameter of the species is slightly greater than 2 mm (value = 2 following Cruden (1977)), that there is no contact between anthers and stigma (value = 0) and that the species is typical of early plant successional stages (value = 2), the OCI value was equal 4.

The mean number of pollen grains per stamen was 1893 ± 174 . Taking into account that each flower has ten stamens, the number of pollen grains estimated per flower would be 18937. The mean number of ovules per ovary was 16.6 ± 0.93 , and the P/O ratio was 1,140.8. Pollen viability was 98.8 ± 1.33 %.

Uncovered plants produced a statistically higher number of fruits per inflorescence than covered plants (7.1 ± 4.34 compared with 4.6 ± 2.8 ; $F = 29.32$, $P =$

The absolute reproductive variables as number of flower per plant, number of fruits and number of seeds were related to the size of the individuals (Number of flowers = $164 - 13x + 0.37x^2$, $r^2 = 0.83$, $P < 0.0001$, $N = 34$; Number of fruits = $154.75 - 8.20x + 0.12x^2$, $r^2 = 0.88$, $P < 0.0001$, $N = 34$; Number of seeds = $6,563.67 - 186.51x + 1.74x^2$, $r^2 = 0.62$, $P = 0.0003$, $N = 20$) (Fig. 6). However, fruit set, seed set and reproductive success were not related to plant size ($r^2 = 0.09$, $P = 0.06$, $N = 35$; $r^2 = 0.02$, $P = 0.053$, $N = 20$ and $r^2 = 0.006$, $P = 0.73$, $N = 20$, respectively).

0.000). However, no significant differences were found in the seed set between flowers from covered plants (12 ± 3.1) or from uncovered plants (10.8 ± 3.7 ; $F = 3.53$, $P = 0.063$).

Discussion

✓ Life stage

Field observations indicate that *Astragalus nitidiflorus* must be considered a short-lived perennial herb (four years old and maximally three flowering periods) that lives in border strips of crops or in old-fields undisturbed by tilling for several years although often with sheep grazing. The species is unable to colonize arable lands that are frequently disturbed (at least twice every year), nor can it colonize areas that have developed late successional vegetation, with higher amounts of nanophanerophytes.

The species is adapted to severe summer drought conditions by forming vegetative buds at the base of the plant at ground level (hemicryptophyte). The vegetative period of the species begins after the first autumn rains (September-October) with the appearance of new seedlings from seeds germinating from the soil seed bank or the resprouting of individuals from previous years that have spent the summer with their dormant vegetative buds at ground level.

In all the cohorts, mortality at the seedling stage was very high (71-100% of seedlings died at the end of the first growth period with the arrival of the summer drought). Therefore this is the most vulnerable stage of the species' life cycle. Although a proper test was not carried out, field observations would suggest that only plants of the first year's cohort which have a sufficiently developed deep root system or which grow in the shade provided by bushes have any possibility of surviving beyond the beginning of summer, especially when spring rainfall has been scarce. Adult P2 plants show a much lower mortality rate, although this varies according to the year (11-64% according to the cohort). Another critical time is the end of the third growth stage (P3), when plants have exhausted their reserves following two intense flowering periods: approximately 82% of P3 plants did not survive the summer season. It is therefore very

difficult to find plants that have undergone four growth periods (P4) and which will flower for a third time (only 7 out of the 378 seedlings recorded since 2005 reached P4 stage). No P4 survives to begin another growth period.

Low plant recruitment due to high seedling mortality, probably as a result of the adverse arid conditions prevailing in the region, and the high mortality of adults leads to an age population structure in which adult plants are scarce. This low life expectancy of adult plants, together with the low or null recruitment of new individuals to the population in dry years, could lead to the extinction of individual patches or small subpopulations. Similar observations can be read in classic literature on rare *Astragalus* (Moseley and Popovich, 1995; Kaye, 1999). High germination rates are necessary to increase recruitment and to ensure the survival of a population. However, despite the production of a high number of viable seeds (ca. 100%), very few seedlings emerge, presumably due to the physical dormancy of the hard coated seeds, which, also, are protected inside an indehiscent fruit. The absence of seedlings recruitment can threaten the maintenance of the population, though the reproductive effort of the adults is high, as mentioned by Hegazy (1990, 1992) for other, similarly rare taxa.

The long time that *A. nitidiflorus* seedlings remains in a vulnerable juvenile stage, where mortality tends to be relatively high, is an adaptative disadvantage in face of therophytic species in Mediterranean areas with long dry summer (Hegazy, 1992). In the case of *A. nitidiflorus*, we have already mentioned the high number of plants that die in the first year of life before they can flower. This is a disadvantage when they are competing with autumn therophytic species that germinate at the same time as *A. nitidiflorus* and compete for the same scarce resource that water represents in spring. However, this could turn into an advantage for the few plants that survive the summer, since these plants sprout strongly after the first autumn rains and could compete advantageously against the newly generated therophytes, taking up sufficient reserves for a prolonged and intense flowering period. That is, to prepare for such a long flowering period, the plants sacrifice flowering the first year, since this would be of little benefit (indeed, poorly developed P2 plants produce hardly any seeds) and involve a high expenditure of their scant reserves, harming their capacity to resprout in the following growing season.

✓ **Flowering period**

The population showed a long flowering period lasting two to three months. The flowering period began in late winter and finished in late spring, with an optimum in April. This may be considered as an adaptive advantage in Mediterranean ecosystems, where water stress would restrict the reproductive success of later-flowering plants (Johnson, 1992; Copete et al., 2008). In fact, the data we have concerning fructification in 2008 (not shown) indicate a reproductive failure due to an earlier drought period that occurred in 2008. The rainfall may be extremely scarce both during the flowering period, and in the period between the beginning of sprouting (September) and the time when flowering begins.

An extended blooming period can increase the individual's chance of having a large number of mates both as pollen donor and recipient (Torres et al., 2002) and it reduces the risk of reproductive failure (Bawa, 1983). Furthermore, the individual flowering pattern produced right-skewed flower production curves, which implies the rapid appearance of flowers at the beginning of the flowering season and a gradual, slowly falling away towards the end of the flowering period. This phenological pattern has also been documented in other species (Thomson, 1980; Torres et al., 2002) and it has been suggested that it may be an adaptive response to attract pollinators that usually visit other species (Thompson, 1980). A high flowering intensity (up to 479 flowers open at the same time) positively correlated with flower production is very attractive for a pollinator, and can be added to the strategy that the above mentioned flowering pattern represents (Iriando et al., 1998).

The high degree of flowering synchrony in the species also may be related with attracting pollinators or simply due to the fact that plants live in very homogeneous habitats as regards ecological conditions (Thompson, 1980). Strong flowering synchrony implies that each plant can exchange genes with most plants of the population, increasing the genetic diversity of the same.

✓ **Reproductive success**

The reproductive success of the species is very low and does not depend on plant size. Individual analysis of the components of reproductive success showed that the proportions of flowers setting fruits (fruit set) were always very low regardless of the year and age of the plants (no more than 17% of the flowers produced ripe fruit). However, the average proportion of ovules setting seeds (seed set) was relatively high and was also independent of the year studied and age of the plants (ca. 60 to 80%). Despite the low reproductive success of the species as a result of a low fruit set, seed production capacity may be very high.

This high level of seed production, despite the poor reproductive success of the plants, can be explained by the high number of flowers produced. Large floral displays represent larger energy investments in reproduction (Udovic, 1981), but they attract more pollinators, allow versatility of the plants in face of variable pollinator and improve male fitness of hermaphroditic flowers (Stephenson, 1981; Sutherland, 1986). The low dispersal capacity of the seeds, which are contained inside indehiscent fruits, implies a high concentration of seeds in the immediate surroundings of the plant. Taking into account the low emergence rate of seedlings near the mother plant (personal observation) and the fact that the seeds have a hard water-proof cover that hinders germination, the seeds must be concentrated in the soil seed bank, as preliminary data suggest (data not shown). The fact that not all the seeds dispersed in one year germinate the following year may prevent high seedling mortality in a year with a favourable autumn but unfavourable (dry) spring, which would lead to the extinction of the population, particularly if we remember that the plants do not produce seeds until the second year of life.

✓ **Breeding system**

According to Cruden (1977), a P/O ranging from 800 to 5800 would normally point to facultative xenogamy, but OCI values of 4 would indicate obligate xenogamy. The OCI calculated for *A. nitidiflorus* is 4 and the P/O ratio of 1140 suggests that the species is xenogamous. In obligate xenogamous plants, pollination occurs when the pollen from the stamens of a plant reach the stigma of another plant. However, facultative xenogamous plants are regularly self-compatible and although some species

require in any case a pollinator, most are able to self-pollination when the flowers close (Cruden, 1977). The mean value of flowering synchrony in *A. nitidiflorus* (ca. 80%) would also suggest xenogamy, although the high fruit set in inflorescences of plants that flower alone during some time (e.g. plant 18 in Fig. 5) would indicate the facultative character of this crossing system. Also, the exclusion of pollinators from *A. nitidiflorus* inflorescences reduced fruit set per inflorescence but not seed set per fruit, suggesting that plants are facultative xenogamous (typically outcrossed, but genetically self-compatible). The same suggestion has been made for other common and rare species of *Astragalus* (Green and Bohart, 1975; Karron, 1989; Kaye, 1999). This phenomenon is facilitated by the simultaneous ripening of anthers and stigmas in the genus *Astragalus* (Barneby, 1964). Among the evolutionary advantages of self-compatibility, Bawa (1973) mentions that it increases the probability of successful pollination and represents a lower cost of reproduction because the fruit set ratio is much higher in self-compatible than in self-incompatible species. Therefore, selective pressure for high levels of seed production may also be responsible for the evolution of self-compatibility. This self-compatibility may provoke an inbreeding depression in seedlings (although not observed in *A. nitidiflorus* seedlings growing in the greenhouse). However, when population sizes are small the genetic load may be largely eliminated (Lande and Schemske, 1985; Schemske and Lande, 1985) and self-compatibility may increase. Then, self-compatibility, which is a vital life-history strategy for many rare and locally endemic species that might otherwise be particularly vulnerable to inconsistent availability of pollinators (Karron, 1987; Stebbins, 1957), may be the result of natural selection during repeated population bottlenecks (Kaye, 1999), and the phenomenon of self-compatibility in *A. nitidiflorus* suggests that the taxon might be able to survive future bottlenecks.

✓ **Implications for conservation and management**

In this paper we provide evidence for a facultative xenogamous breeding system in *A. nitidiflorus*, which suggests that the plant does not exclusively depend on pollinators, although their activity would favour fruit production. The results also show that the investigated population of *A. nitidiflorus* is presently not limited by the flowering and

fruiting process. Plants are able to produce a sufficiently high number of viable seeds, which because of their low germination rate and hard coats, will form a seed bank which helps dampen the effect of missing recruitment in “bad” years. Detailed studies on the role of the soil seed bank are necessary to fully understand the life cycle of the species. Nevertheless, due to the small size of the subpopulations of the species, demographic stochasticity and extremes of natural climatic conditions (e.g. severe drought periods) could easily lead to a decrease in the number of individuals or even the disappearance of small population patches. In addition, the restricted range of the species makes it vulnerable to catastrophic events.

The intense tillage in the dispersal area of the species prevents the establishment of new patches that would ensure its continued presence. In addition, advanced stages of plant succession (dense formations of nanophanerophytic species) do not favour its presence. Given that *A. nitidiflorus* must be considered typical of early-successional stages, maintenance of this early successional habitat seems to be the critical point to preserving the species. So, suitable agricultural management practices would be needed to avoid both excessive disturbance of the soil and the prolonged advance of successional stages of the vegetation. Further studies are needed to look into other factors, both biotic (grazing, symbiosis, competition) and abiotic (land management), which may affect the conservation of the species.

Table1. Life table calculated for the studied population of *Astragalus nitidiflorus*: x , age in years; N_x , number of individual per stage; l_x , proportion of original cohort surviving to the start of each stage; d_x , proportion of the original cohort dying during each stage; q_x , stage-specific mortality rate; e_x , expectation of future life. The questions marks in Cohorts 2003 and 2004 indicate unknown number of seedlings (P1) in N_x column, and then incalculable values l_x and d_x columns.

Stage of cohort	Growth period	x (years)	N_x	l_x (%)	d_x (%)	q_x (%)	e_x (years)
<i>Cohort 2003</i>							
P1 (Seedlings)	oct-03/sep-04	1	?				
P2	oct-04/sep-05	2	?				
P3	oct-05/sep-06	3	1	?	?	0	
P4	oct-06/sep-07	4	1	?	?	100	
P5	oct-07/sep-08	5	0	?			
<i>Cohort 2004</i>							
P1 (Seedlings)	oct-04/sep-05	1	?				
P2	oct-05/sep-06	2	19	?	?	31.58	
P3	oct-06/sep-07	3	13	?	?	53.85	
P4	oct-07/sep-08	4	6	?	?	100	
P5	oct-08/sep-09	5	0	?			
<i>Cohort 2005</i>							
P1 (Seedlings)	oct-05/sep-06	1	64	100	71.87	71.87	1.58
P2	oct-06/sep-07	2	18	28.12	3.12	11.11	2.06
P3	oct-07/sep-08	3	16	25.00	20.31	81.25	1.19
P4	oct-08/sep-09	4	3	4.69	4.69	100	1
P5	oct-09/	5	0				
<i>Cohort 2006</i>							
P1 (Seedlings)	oct-06/sep-07	1	292	100	79.11	79.11	1.35
P2	oct-07/sep-08	2	61	20.89	13.36	63.93	1.67
P3	oct-08/sep-09	3	22	7.53	0.98	12.96	1.87
P4	oct-09	4	4	6.56			
<i>Cohort 2007</i>							
P1 (Seedlings)	oct-07/sep-08	1	14	100	100	100	1
P2	oct-08/sep-09	2	0	0			
P3	oct-09	3	0				
<i>Cohort 2008</i>							
P1 (Seedlings)	oct-08/sep-09	1	8	100	100	100	1
P2	oct-09/	2	0	0			
<i>Total cohorts (2005-08)</i>							
P1 (Seedlings)	1 th growth period	1	378	100	79.10	79.10	1.33
P2	2 th growth period	2	79	20.90	10.85	51.90	1.60
P3	3 th growth period	3	38	10.05	8.20	81.58	1.18
P4	4 th growth period	4	7	1.85	1.85	100	1
P5	5 th growth period	5	0	0			

Table 2. Values of phenological variables in different *Astragalus nitidiflorus* adult plants (P2, P3 and P4) in 2006 and 2007.

Flowering moment (days)		Flowering intensity (flowers)		Flowering duration (days)					Flowering synchrony				
2006		2006		2006		2007			2006		2007		
P2	P3	P2	P3	P2	P3	P2	P3	P4	P2	P3	P2	P3	P4
17	14	122	479	25	36	54	67	64	0.90	0.68	0.78	0.73	0.77
14	-	27	-	18	-	29	51	-	0.93	-	0.97	0.84	-
14	-	172	-	29	-	61	64	-	0.84	-	0.78	0.76	-
13	-	27	-	22	-	54	64	-	0.92	-	0.81	0.77	-
24	-	81	-	25	-	51	57	-	0.76	-	0.82	0.80	-
17	-	41	-	29	-	62	61	-	0.84	-	0.68	0.77	-
14	-	120	-	25	-	22	54	-	0.93	-	0.99	0.72	-
21	-	219	-	50	-	26	-	-	0.47	-	0.90	-	-
-	-	-	-	-	-	52	-	-	-	-	0.78	-	-
-	-	-	-	-	-	65	-	-	-	-	0.70	-	-
-	-	-	-	-	-	43	-	-	-	-	0.78	-	-
16.7±3.9	-	101±70.4	-	27.9±9.6	-	47.2±15.1	59.7±5.9	-	0.82±0.15	-	0.82±0.1	0.77±0.04	-

Table 3. Flower, fruit and seed production of different *Astragalus nitidiflorus* adult types (P2, P3 and P4) in 2006 and 2007 (mean \pm S.D and range of values between parentheses).

Plant Stage and year	Number of Inflorescences	Total flowers	Number fruits/ inflorescence	Total fruits	Fruit set	Number seeds/fruit	Seed set	Reproductive success	Total seeds per plant
P2 - 2006 (N=8)	33.9 \pm 27.0 (7-87)	690 \pm 539.8 (150-1790)	3.7 \pm 1.4 (0-14)	133.5 \pm 114.3 (6-367)	0.173 \pm 0.06 (0.04-0.239)	13.3 \pm 2.8 (6-18)	0.788*	0.177*	1519.6*
P2 - 2007 (N=17)	32.5 \pm 40.7 (2-118)	673.3 \pm 843 (41-2443)	4.5 \pm 2 (0-15)	148.5 \pm 201.2 (5-656)	0.214 \pm 0.09 (0.06-0.416)	10.6 \pm 1.3** (1-16)	0.636 \pm 0.08** (0.518-0.729)	0.174 \pm 0.08** (0.079-0.303)	3021 \pm 2558** (577-7741)
P3 - 2006 (N=1)	219	4939	4.0 \pm 4.1 (16-0)	832	0.168	12.6 \pm 1.7 (16-8)	0.757	0.109	10483.2
P3 - 2007 (N=8)	179.5 \pm 108 (36-339)	3715.6 \pm 2236.3 (745-7017)	4.2 \pm 0.9 (0-22)	800.5 \pm 617 (132-2017)	0.204 \pm 0.04 (0.156-0.287)	10.1 \pm 2.9 (1-19)	0.610 \pm 0.17 (0.252-0.832)	0.127 \pm 0.06 (0.054-0.239)	8031.2 \pm 8259.8 (1386-27855)
P4 - 2007 (N=1)	166	3436	3.5 \pm 3.2 (0-12)	583	0.170	10.7 \pm 2.9 (5-15)	0.645	0.110	6238

* N=1; ** N=7.

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