



## Seasonality effects on plant phenology and seed ecology in *Oritrophium peruvianum* (Asteraceae), a threatened tropical alpine species



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### ABSTRACT

*Oritrophium peruvianum* (Lam.) Cuatr. (Asteraceae) is a threatened perennial herb, endemic to the tropical alpine zone of the Andean páramos and used for medicinal purposes. In this study, its population structure, phenological patterns and seed viability and germination were analysed. In all of the three investigated populations, the proportion of flowering plants was smaller than that of non-flowering. Size structure differed among populations, with two following a normal and one a left-skewed distribution, with a higher percentage of young plants. Flowering was spread throughout the rainy season, with fruiting intensity highest at the end of the rainy season and at the beginning of the dry season. Seedling emergence was limited and appeared to be independent of the season, indicating opportunities for continuous seedling emergence and mortality throughout the year. Seed viability was higher in seeds collected at the end of the dry season compared with those harvested at the end of the rainy season. Laboratory studies suggest that under natural conditions, germination timing is likely to be independent from the season.

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### 1. Introduction

*Oritrophium peruvianum* (Lam.) Cuatr. (Asteraceae) is a perennial herb endemic to the tropical alpine zone of the northern Andes, commonly known as páramos (ca. 3000–4800 m a.s.l.). In Venezuela, where *O. peruvianum* is known as *Frailejón morado*, whole plants are pulled out from the wild, dried and used to prepare infusions, alone or in combination with other plants native of the páramos, for their widely acclaimed medicinal properties in treating breathing affections such as asthma and bronchitis (Aranguren et al., 1996; Gil-Otaiza, 1997). The biggest demand is a local one, coming from the rural and urban communities of the Venezuelan Andes, where it is primarily sold dry in bunches of a few plants; sometimes it is available as syrup, especially in tourist areas (Aranguren et al., 1996).

The species has a narrow distribution within the páramos, where it is restricted to azonal habitats such as riparian vegetation along streams, around lakes and on humid slopes. Wild populations of *O. peruvianum* are scarce and difficult to locate in the Venezuelan Andes and local people at the time of this study reported that these have been increasingly harvested in some areas, resulting in a decimation of the plant populations. Based on its actual and potential commercial demand this species is categorized as vulnerable in the Red Book of the Venezuelan flora

(Llamosas et al., 2003). However, the conservation of this species is also threatened by habitat loss and destruction due to cattle, fire and the advance of the agriculture frontier, as for the majority of the páramo vegetation (Aranguren et al., 1996). Furthermore, tropical alpine environments are identified as one of the most vulnerable to global environmental change, but remain among the least studied and described ecosystems in the world (Buytaert et al., 2011).

The provision of information on ecology and biology of species is considered crucial for the conservation of threatened species (Menges, 2000). In particular, knowledge of their life-cycle and reproductive traits is essential in identifying limits to regeneration, population growth and persistence (Bevill and Louda, 1999) and, among them, germination behaviour, is vital in understanding in situ conservation measures and promoting ex situ conservation options (Flores et al., 2008). Despite the low availability of wild populations of *O. peruvianum* and the increasing threats to its conservation in the Venezuelan Andes, there is a paucity of scientific information on the species. The only available information relates to taxonomy, morphology, ecological physiology and distribution (Aranguren et al., 1996; Torres et al., 1996; Cuatrecasas, 1997), but no data are available on population structure, phenology and seed ecology of this threatened species. Likewise, little is known on the ecology and biology of tropical alpine plants and studies refer mainly to giant rosette species (Silva et al., 2000). Some aspects of the population biology have been studied in the afroalpine *Senecio keniodendron* (Smith and Young, 1982, 1994) and in *Lobelia* species

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(Young, 1994), as well as in the Hawaiian *Argyroxiphium sandwicense* (Rundel and Witter, 1994). Several studies have also been made on populations of *Espeletia* in the high tropical Andes (Monasterio, 1986; Guariguata and Azócar, 1988; Estrada and Monasterio, 1991; Berry and Calvo, 1994), studying different aspects of its biology, whilst an integrated picture of giant rosette demography was made by Silva et al. (2000).

Little is known on the germination requirements of tropical alpine species (Baskin and Baskin, 1998). Seed germination of temperate alpine species is reported to be promoted by high (Mooney and Billings, 1961; Billings and Mooney, 1968) and alternating temperatures (Amen, 1966; Bliss, 1971). Winter dormancy released by wet-cold stratification seems to be a common requirement for these species (Baskin and Baskin, 1998). However, in the páramos, seasonal variability in temperature tends to be low because of the constant level of solar radiance throughout the year, whereas intraday variations dominate (Buytaert et al., 2011), leading to the “summer every day and winter every night” phenomenon (Hedberg, 1964). Under temperature-regulated, seasonal climates, seeds of many Asteraceae require cold stratification (over-wintering treatment) to break their physiological dormancy and increase germination (e.g. Baskin and Baskin, 2002; Brändel, 2004) and light to germinate (e.g. Merritt et al., 2006; Tobe et al., 2006; Galindez et al., 2009).

This study aims to investigate the biology and ecology of *O. peruvianum* with the intention of helping to inform the conservation of this species. The specific objectives were to: (1) analyse the population structure, its changes over time and among populations by sampling undisturbed populations and (2) conduct a first analysis of the reproductive characteristics of the species by analysing its phenology and seed viability and germination.

## 2. Materials and methods

### 2.1. Study area

The Páramo of Gavidia is located between 8°35' and 8°45' N and 70°52' and 70°58' W, in the Venezuelan Andes, within the Sierra National Park, in the state of Mérida. The area consists of several narrow glacial valleys, with altitudes between 3200 and ca. 4000 m. Soils are well-drained inceptisols of sandy-loam texture, very stony and acidic

(pH 4.3 to 5.5), and very rich in organic matter (up to 20%), but low in mineral nutrient content (Llambí and Sarmiento, 1998). The dominant vegetation consists of giant rosettes of the genus *Espeletia* and of shrubs of the genus *Hypericum*, *Baccharis*, *Hesperomeles*, *Stevia*, and *Acaena* (Sarmiento, 2000). The precipitation regime is unimodal, with a dry season between December and March and a peak of rainfall between June and July (Sarmiento, 2000). The mean air temperature ranges between 10 °C at 3200 to 6 °C at 3800 (Llambí et al., 2004), with soil surface temperatures ranging daily from ca. –5 °C to ca. 45 °C. The monthly solar radiation is around 500–700 MJ/m<sup>2</sup>. Dry and rainy seasons for the study period (2001–2002) were identified on the basis of the climatic data obtained from the weather station located in Gavidia, at 3400 m (Fig. 1). *O. peruvianum* was found above the human settlement of the Páramo of Gavidia (500 inhabitants), whose economy is based mainly on a long fallow agriculture and extensive cattle raising (Sarmiento, 2000). This páramo was chosen as the study area because, in accordance with information gathered from the local people, *O. peruvianum* was more abundant than in the other páramos and its harvesting was rare, so the populations could be considered as being un-harvested (Ulian, 2004).

### 2.2. Study species

*Oritrophium* (H.B.K.) Cuatrec. (Asteraceae) is largely an Andean genus, with 19 species and 6 subspecies confirmed by Cuatrecasas (1997), ranging from the north of the Andean Cordillera in Colombia and Venezuela, southwards across Ecuador and Peru to southern Bolivia (Cuatrecasas, 1997).

*Oritrophium peruvianum* (Lam.) Cuatrec. subsp. *peruvianum* (hereafter *O. peruvianum*) is a small herbaceous perennial plant, growing in the life form of an acaulescent rosette, constituted by xeromorphic leaves, which protects plants from extreme low temperature, intense radiation, physiological water stress and strong wind (Hedberg and Hedberg, 1979; Torres et al., 1996). The below ground structure of the plants consists of a scarcely branched, short rhizome (Cuatrecasas, 1997), with attached fibrous roots. Capitula are solitary on the axillary scape and the inflorescence consists of fertile ray florets and sterile disc florets (Cuatrecasas, 1997). Cypselas are very small and light, ovate-oblong, 5-nervate and slightly hairy, measuring 3–4 mm in length and 0.5–1 mm in width (Aristeguieta, 1964; Cuatrecasas, 1997). These possess a pappus,

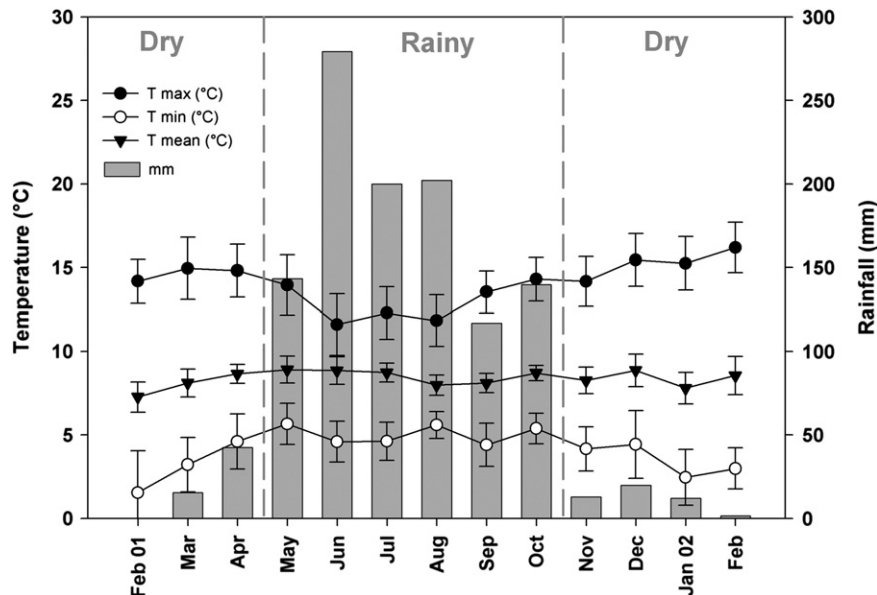


Fig. 1. Annual trend of temperatures (°C) and rainfall (mm), recorded for the study period in 2001 and 2002 at Gavidia (3400 m a.s.l.).

which promotes dispersal by wind. Reproduction occurs mainly by seeds, although vegetative propagation may rarely occur, as observed during the fieldwork for this study. *O. peruvianum* grows along the Cordillera in Colombia, Venezuela, Ecuador and Peru, between 3300 and 4600 m (Luteyn, 1999) and tends to occur in the wet parts of the páramos as do most *Oritrophium* species (Cuatrecasas, 1997).

### 2.3. Phenological observations

Seasonal phenology of *O. peruvianum* was followed in populations F1, F2, and F3 (Table 1). These populations were representative of the species distribution in the Páramo of Gavidia, and were chosen based on their relative accessibility, as they could be reached after about 2–3 h of trekking from the village. Data collection began on 11 and 15 February 2001 for populations F2 and F1, respectively and on 17 March for population F3. Data were collected each month (except in June 2001) over a one year period. At each site, eight, two and four plots, respectively of 1 × 2 m were randomly placed along a transect covering the population area (Table 1). The plots were marked and the data were collected in subplots by using a 50 × 50 cm metal frame. Between 100 and 200 plants were assessed each month per site. The number of plots required per site depended on the density of the plants in the plots (Table 1). Plant size was calculated according to the rosette diameter and 13 size classes were identified (from 2 to 26 cm of diameter). Plants with a rosette <2 cm were considered as seedlings. Plants with a rosette ≥2 cm and bearing flower buds, open flowers, fruiting stalks and dry flower stalks on remains of flower heads were considered as “reproductive plants”.

### 2.4. Germplasm collection

Fully ripened cypselas were collected directly from the plants during the dry (11 February 2002) and the rainy (25 October 2002) seasons. The wind and the rain quickly dispersed ripened cypselas, making harvesting difficult. Therefore cypselas collected in each season from the three investigated populations (F1, F2 and F3, see Table 1), but outside the sampled plots, were mixed in a unique seed lot, to allow a sufficient number of replicates for the experiments. Immature cypselas were not collected as they differed in colour from those containing mature seeds, appearing more green-brown instead of dark brown. Collected cypselas (hereafter, seeds) were then stored in paper bags at room temperature for less than one month, until the start of the experiments.

### 2.5. Seed viability

Seed viability of both seedlots was tested by soaking five replicates of 30 seeds each in a solution of 2,3,5-triphenyl-tetrazolium chloride (TTC), in darkness at room temperature for 72 h. At the conclusion of the viability tests, embryos were checked for staining, with a red colour indicating viable embryos.

### 2.6. Seed germination experiments

Seed germination tests were carried out on both seedlots. Five replicates of 30 seeds each were randomly selected from each seedlot and sown in sterilized glass petri dishes, with filter paper moistened with 10 ml nitrogen fertilizer solution (1/4-strength Hoagland, pH = 6.5). Sterilized water was added, when necessary, during germination trials to maintain filter paper saturation. Seeds were tested for germination under three conditions: at room temperature (average low temperature, 16 °C; average high temperature, 24 °C), in a light room at the constant temperature of 26–27 °C (24 h of irradiance per day) and in a refrigerator at 6–7 °C, with clear glass door (i.e. daily natural irradiance). Nominal dark treatment, i.e. brief exposure to laboratory light levels for germination scoring only, was achieved by wrapping the germination dishes with aluminium foil. Dishes were checked at 3–4 day intervals and the number of new seedlings recorded. Each experiment was terminated within three weeks, by which germination was complete. At the end of the germination tests, a cut-test was carried out to determine the viability of any remaining seeds (ISTA, 2011) and the final germination percentage calculated as the mean of the replicates (±1 standard deviation) on the basis of the total number of filled seeds. The time to reach 50% of the maximum germination in one replicate (T50) was also calculated.

### 2.7. Statistical analysis

Annual trends of the number of flowering and fruiting stalks per reproductive plants and percentage of seedlings within the investigated period and in each investigated population were analysed for differences among populations by the nonparametric Kruskal–Wallis test and between seasons by the nonparametric Wilcoxon test by using R v. 2.12.1 (R Development Core Team, 2010). Seed viability between the dry and rainy seasons was compared by using PROC LOGISTIC and applying a one-way factorial model. Seed germination was tested in a three-way factorial model, using PROC LOGISTIC in SAS (SAS Institute Inc., 1985), with the main effects (treatments) being season (seeds collected during dry or rainy season), light (germination in light or nominal dark) and temperature. Final germination percentages were analysed using a maximum likelihood method with a logit model (Sokal and Rohlf, 1981). Differences in T50 values between seasons were checked by a one-way factorial model, by using R v. 2.12.1 (R Development Core Team, 2010).

## 3. Results

### 3.1. Population structure

In Fig. 2 the size structures of the three populations are reported for one month representative of the rainy season (July 2001) and one of the dry season (January 2002). The size structures of populations F1 and F2 were very similar, with a lower frequency of plants of smaller and larger sizes and a higher frequency of plants of middle sizes, whilst in population F3 the distribution was skewed to the left towards smaller sizes

**Table 1**  
General habitat characteristics of the sites sampled for phenological studies in the study area (Páramo de Gavidia). Number of plots and mean plant densities (plants/m<sup>2</sup>) in each plot are also reported.  $P < 0.01$  for mean plot density by One-way ANOVA; values with the same letters are not different at  $P > 0.05$ , by post hoc pairwise comparison Tukey's test.

Population	Site name	Altitude (m a.s.l.)	Coordinates	Aspect	Slope (°)	Number of plots	Mean plot density (±SD)
F1	Above Lake Brava	3912	N 08° 39' W 070° 53'	N	5	8	7.46 ± 3.57 <sup>a</sup>
F2	Llano del Parche	3840	N 08° 38' W 070° 55'	ENE	13	2	38.20 ± 17.00 <sup>b</sup>
F3	Below Alto S. Cristo	4021	N 08° 37' W 070° 55'	NE	0	4	31.42 ± 21.73 <sup>b</sup>

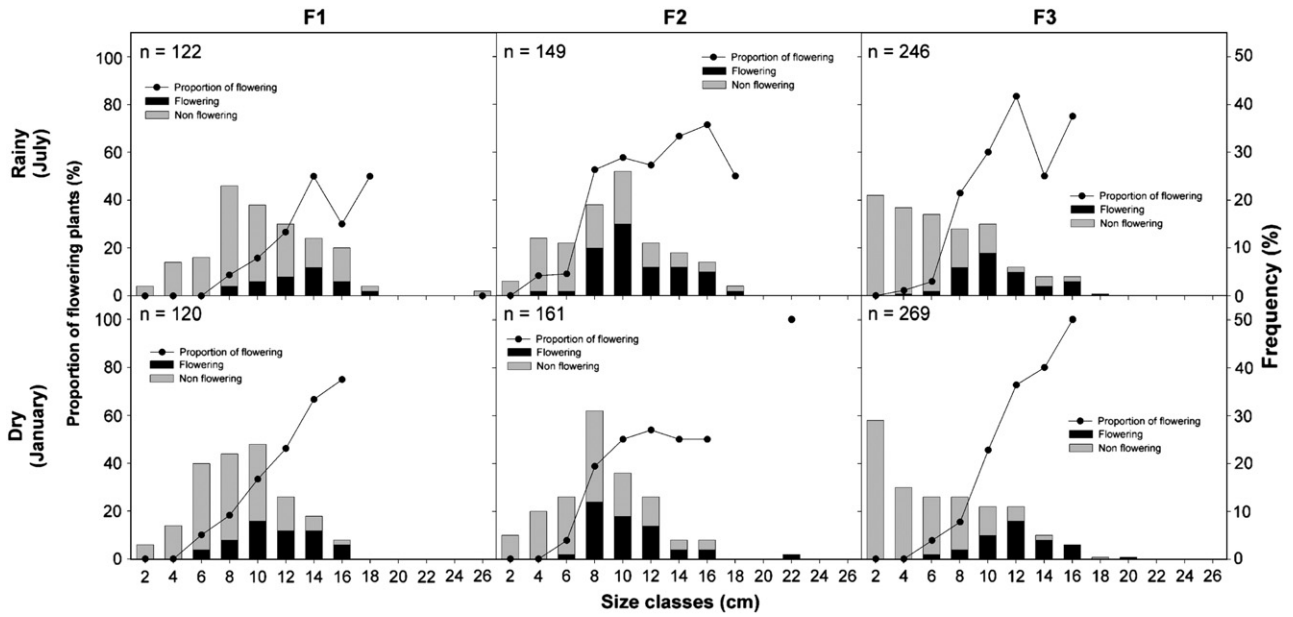


Fig. 2. Distributions of size classes of flowering and nonflowering plants for the three investigated populations, according to the rosette diameter (cm) and proportions of flowering plants in the rainy (July 2001) and dry (January 2002) seasons. See Table 1 for population code descriptions.

(Fig. 2). The proportion of flowering plants increased with increasing sizes for all the investigated populations (Fig. 2).

### 3.2. Phenological patterns

In population F2 the proportion of flowering plants/non-flowering plants was higher than in populations F1 and F3, ranging from 32 to 50% of the plants in February and May 2001, respectively. In population F1, the percentage of flowering plants varied from 16% in March to 31% in November, and in population F3 from 17% in March to 35% in February of the following year (data not shown). In general, the changes in percentages of non-flowering and flowering plants throughout the year did not show a clear pattern. In population F1, the highest proportions of flowering plants were found between September and January 2002. In population F2 there was a slight decrease of flowering plants from February to May 2001 and then an increase again during July and August. In population F3, the proportion of flowering plants decreased from March to May (data not shown).

Although there were not substantial variations in the proportion of the flowering plants over the year, common phenological patterns of flowering and fruiting periods were detected for all three populations (Fig. 3). No differences in the mean number of flowering stalks per reproductive plant were detected among populations over the year ( $P > 0.05$ ), with mean values of  $0.4 \pm 0.2$ ,  $0.4 \pm 0.6$  and  $0.5 \pm 0.4$  for F1, F2 and F3, respectively. The annual trends showed a peak of flowering during the rainy season for all the three populations (Fig. 3A), as detected by significant differences on mean number of flowering stalks between dry and rainy seasons, for all the populations ( $P < 0.001$ ; Fig. 3B).

The numbers of fruiting stalks per reproductive plant were higher from October to November, i.e. throughout the end of the rainy season and the beginning of the dry season (Fig. 3A). However, the three populations showed inter-population differences in the mean number of fruiting stalks over the year, with mean values of  $1.4 \pm 0.4$ ,  $2.2 \pm 0.7$  and  $3.2 \pm 0.6$ , for F1, F2 and F3, respectively ( $P < 0.001$ ; Fig. 3A). In populations F2 and F3, there was a noticeable peak of fruiting plants in November, whilst in population F1 the fruiting season was late in comparison to the other two populations, since the highest number of fruiting plants was found between November and January 2002 (Fig. 3A). The fruiting peak in the dry season for all the populations

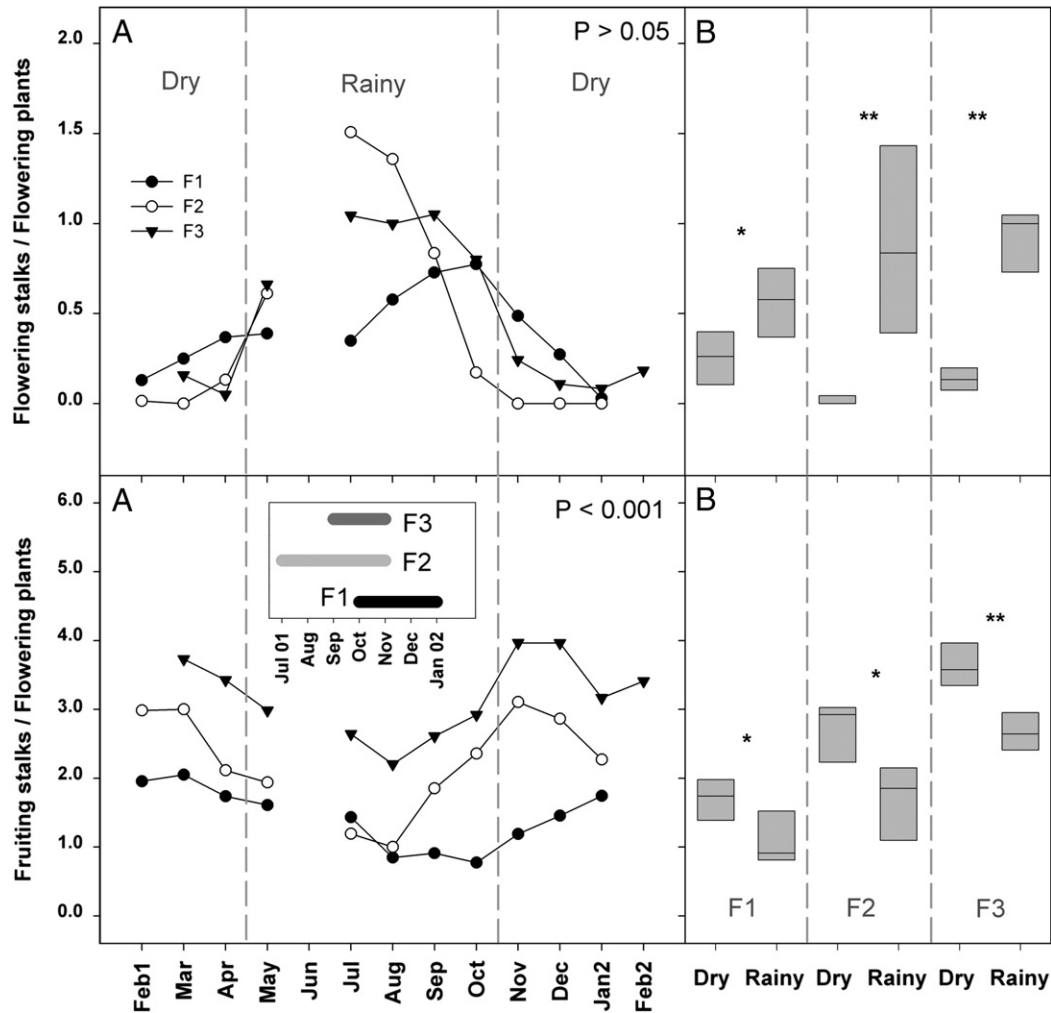
was confirmed by the comparative analysis between seasons on the mean number of fruits, with the latter being statistically higher ( $P < 0.001$ ) in the dry season (Fig. 3B).

Inter-population differences on the flowering and fruiting peaks determined a different time of seed development in the three populations, both in their duration (ranging from 2 months in F3 to 5 months in F2) and in the season, being mainly in the rainy season for F2 and F3 and mainly in the dry season for F1 (inset plot in Fig. 3). Field observations indicated that dispersal occurs quickly after fruit maturation and may be accelerated by wind or rain.

Higher percentages of seedlings in the population were recorded over the year in F3 ( $25.8 \pm 4.3\%$ ), than in F1 ( $2.6 \pm 1.7\%$ ) and F2 ( $4.5 \pm 2.8\%$ ) populations, with this difference being statistically significant ( $P < 0.001$ ; Fig. 4A). In F2 and F3 populations, the percentage of seedlings increased from the end of the dry season towards the end of the rainy season, ranging from 1% and 19%, in March 2001, to 10%, in September, and to 32%, in November, respectively, with peaks in September and November for population F2 and in April and November for F3 (Fig. 4A). In contrast, in population F1, the highest percentage of seedlings was found in February 2001 (7%) and the lowest percentages in November and December (1%; Fig. 4A). However, the comparative analysis on the percentages of seedlings between seasons showed no statistically differences for all the three populations ( $P > 0.05$ ; Fig. 4B).

### 3.3. Seed viability and germination ecology

The results of the TTC test (Fig. 5) showed a significantly higher viability ( $P < 0.001$ ) for seeds collected in the dry season ( $89.0 \pm 4.0\%$ ) than for those collected in the rainy season ( $76.0 \pm 2.0\%$ ). Maximum germination was achieved, for both seedlots, after sowing at room temperature in the light (RT-L; Fig. 5). Seeds collected in the dry season achieved  $88.0 \pm 6.0\%$  total germination, whilst seeds collected during the rainy season  $71.0 \pm 10.0\%$  ( $P < 0.001$ ; Fig. 5). These percentages were very similar to the related estimates of seed viability assessed by TTC, therefore only a small number of viable seeds (1–5%) did not germinate in each seedlot (Fig. 5). Lowest germination was recorded at HT-D treatment (Fig. 5). Seeds of both seedlots did not germinate at 6–7 °C (LT treatment; Fig. 5). Significant differences ( $P < 0.001$ ) in final germination percentages were also detected within each seedlot among germination conditions. The logistic analysis demonstrated



**Fig. 3.** Annual trend (A) of number of flowering (flower buds and open flowers) and fruiting (fruits and dispersed) stalks per reproductive plants in each investigated population (F1, F2, F3) and per season (B). Nonparametric Kruskal–Wallis test was used for detecting differences among populations (A) and nonparametric Wilcoxon test for differences between seasons (B); \* =  $P < 0.05$ , \*\* =  $P < 0.01$ . In the inset plot, time between the flowering and fruiting peaks for each population is reported. See Table 1 for population code descriptions.

highly significant differences for each of the main effects (season, light, temperature;  $P < 0.001$ ), whilst none of the interaction terms were found to be significant ( $P > 0.05$ ).

Seeds collected in the dry season germinated significantly faster ( $P < 0.001$ ) than seeds from the rainy season at all the tested conditions. T50 was reached from  $2.80 \pm 0.61$  days (RT-L treatment) to  $7.39 \pm 2.1$  days (HT-D treatment) in seeds collected in the dry season, whilst seeds collected in the rainy season needed from  $8.70 \pm 0.76$  days (RT-L treatment) to  $17.37 \pm 7.47$  days (HT-D treatment) to reach these values (Fig. 5).

## 4. Discussion

### 4.1. Population structure

In all the three populations, the proportion of flowering plants was smaller than the proportion of non-flowering plants. In general, this characteristic was maintained throughout the year, with F2 having the highest proportion of flowering plants (maximum of 50% in February 2001). Similarly, size structure of each population did not show big variations indicating limited seasonal demographic fluctuations throughout the year, as a result of the effects of the slow leaf growth combined with plant size loss caused by the leaves wilting. Smith (1981), in his study describing the seasonal variations in growth of *Espeletia* spp. in the Venezuelan Andes, found negative mean annual

growth rates, based on changes in plant height. The negative values were attributed to the slow leaf re-growth during the next wet season, too slow to permit full recovery from the wilting of the older leaves. However, he found that plant growth occurred primarily in the wet season and that the wilting of older leaves in the dry season caused a reduction in size even for plants that showed a net increase in size over the entire year.

Size structure differed among the populations and revealed differences in 'shape' giving some insights into their demographic status. The frequency distribution of plants, based on the rosette diameter and their reproductive status, followed a normal distribution in populations F1 and F2, whilst it had a left-skewed distribution in population F3, with a higher percentage of plants with smaller rosette diameters. In demographic terms, F3 is a more 'dynamic' population, compared to the other populations on the basis that the higher number of small plants reflects new recruits into the population (Oostermeijer et al., 1994).

### 4.2. Phenological patterns

Common phenological patterns of flowering and fruiting periods were detected for all the three populations, with some differences among populations. Flowering was spread throughout the rainy season (May to October) with different peaks of flowering during the rainy season for the three populations and it extended into the dry season

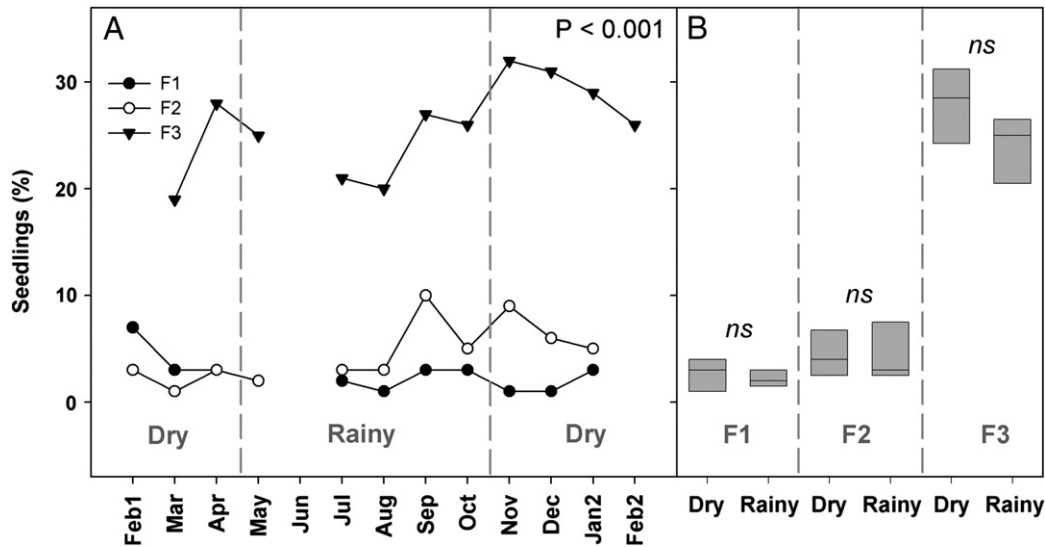


Fig. 4. Annual trend (A) of seedlings (%) in each investigated population (F1, F2, F3) and per season (B). Nonparametric Kruskal–Wallis test was used for detecting differences among populations (A) and nonparametric Wilcoxon test for differences between seasons (B); ns =  $P > 0.05$ . See Table 1 for population code descriptions.

with a few scattered individuals. Similar flowering patterns were observed for *Oritrophium limnophilum* by Smith (1975) in the Andean Páramo and all four *Espeletia* species studied in the Venezuelan Andes

flowered during the wet season and in the wet–dry season transition period (Smith, 1981). Synchronous, superannual flowering cycles were documented in *Espeletia timotensis* and in the monocarpic *E. floccosa*. In the high páramos, Monasterio (1986) demonstrated the existence of irregular, superannual flowering episodes in *E. spicata* and *E. timotensis* and suggested that four co-occurring species maintained separate flowering periods probably to avoid pollinator competition. Berry and Calvo (1994) suggested that the extended flowering periods recorded for *Espeletia* spp. (2–6 months) in the Venezuelan Andes were in part due to variability between individuals in the populations. However, *O. peruvianum* was found to have an extended flowering period into the dry season and this is likely due to the humid habitat where it grows, which might enable a few individuals to flower also when water is scarce elsewhere.

Fruiting intensified towards the end of the rainy season and at the beginning of the dry season for all the populations, although with significant differences in fruiting peaks. Populations F2 and F3 had fruiting peaks in November, whilst population F1 peaked two months later (January 2002). Similarly, in the study by Smith (1981), fruit dispersal from *Espeletia* species began in the wet season and continued throughout the dry season. Berry and Calvo (1994) found that in *Espeletia* fruit dispersal was more extended than flowering, and peak dispersal periods occurred at the end of the wet season and during the dry season. In *O. peruvianum* populations, the fruiting interval was shorter than the flowering period. On reaching maturation, cypselas were easily and quickly air-borne, aided by the pappus. However, mortality of the flower heads might occur and affect the reproductive output.

Reproductive capacity varied among populations, with slight seasonal differences as explained above, whereas seedling emergence did not show a clear seasonal pattern, extending over much of the year. However, seedling percentages did fluctuate throughout the year, indicating continuous seedling emergence and mortality throughout the year. In the Venezuelan Andes, seedling recruitment of *Espeletia* spp. (Smith, 1981) occurred entirely in the wet season, beginning in the early wet season, but most seedlings were destroyed by frost as soon as they germinated. Frequent occurrence of night freezing temperatures throughout the year is a common feature in high tropical mountain environments (Hedberg, 1964) therefore, seedling mortality might occur continually. The alternate freezing and thawing of the soil (solifluction; Rundel, 1994) were found to be a major source of seedling mortality for plants at the highest elevations in the northern Andes and on Mount Kenya (Smith, 1984; Smith and Young, 1994). In the dry season, low water availability was considered to be the main factor limiting

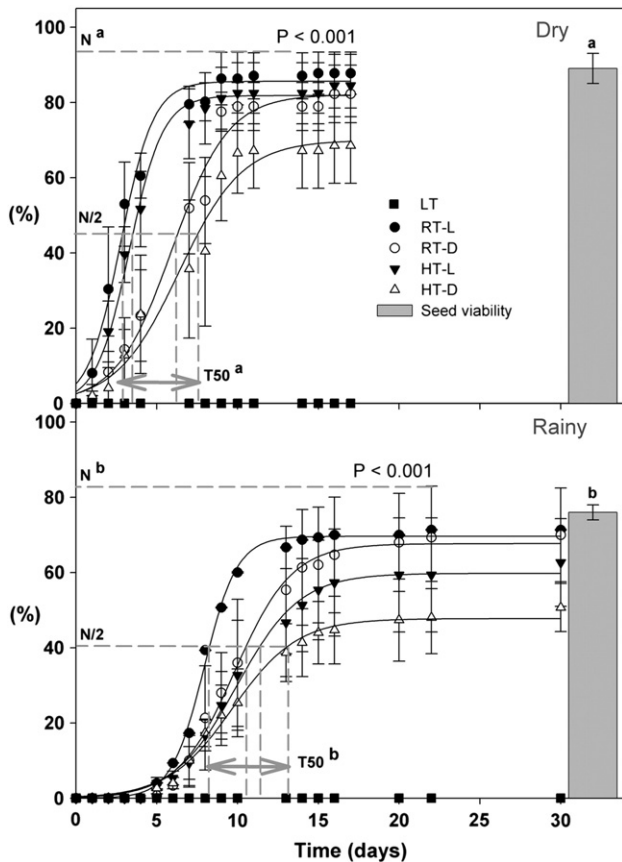


Fig. 5. Seed viability (grey bars) and germination curves for the two seedlots collected in the dry and rainy seasons. LT: 6–7 °C; RT-L: room temperature (ca. 24/16 °C) in the light; RT-D: room temperature (ca. 24/16 °C) in nominal dark; HT-L: constant 26–27 °C in continuous light; HT-D: constant 26–27 °C in nominal dark. Data are the mean of five replicates  $\pm$  standard deviation. Parameters with different letters were significantly different ( $P < 0.001$ ) between seasons by One-way ANOVA (seed viability and maximum germination) and nonparametric Wilcoxon test ( $T_{50}$  values).  $P < 0.001$  for final germination percentages among germination conditions by One-way ANOVA within each season.

germination and seedling establishment (Smith, 1981; Smith and Young, 1982). Repeated surveys of the Afroalpine giant rosette *Senecio keniodendron* (Smith and Young, 1994) showed that seedling recruitment was interrupted during the dry season, except in seepage slope and stream banks, and most seedlings died within a few months of germination, with most mortality concentrated in periods of low rainfall. In this sense, *O. peruvianum* distribution is restricted to azonal habitats such as riparian vegetation along streams, around lakes and on humid slopes and this might explain its unclear pattern of seedling emergence.

#### 4.3. Seasonality effect on seed viability and germination

*Oritrophium peruvianum* showed a high overall seed viability (>75%), with seeds collected at the end of the dry season being significantly more viable than those collected at the end of the rainy one. During the rainy season, seeds are subjected to high levels of humidity that together with the high day temperatures (up to 45 °C) might accelerate seed ageing.

Seeds of both seedlots reached high germination percentages (>60%) at all the tested conditions, suggesting that *O. peruvianum* seeds are nondormant, as also recently reported for seeds of another tropical alpine plant (*Polypsis besseri*) distributed at 3000–4000 m a.s.l. in the Andes (Gareca et al., 2012). However, dry after ripening studies should test the presence of dormancy in freshly harvested seeds. Seeds collected at the end of the rainy season showed significantly lower germination percentages and a slower germination rate at all the tested conditions, which may have resulted from in situ deterioration (Ellis and Roberts, 1981).

Light was more favourable than darkness for germination of *O. peruvianum* seeds, as previously reported for many other Asteraceae (e.g. Merritt et al., 2006; Tobe et al., 2006), also from Venezuelan Andes species such as *Espeletia timotensis* and *E. schultzei* (Pannier, 1969; Guariguata and Azocar, 1988.). However, germination occurred also in the dark, suggesting that seeds of this species are able to emerge from greater soil depths than light can penetrate to and, therefore, that this species is unlikely to form a persistent soil seed bank. The higher germination percentages detected at room temperature (i.e. alternating temperature regime) than at the constant temperature of 25–26 °C, reflected the adaptation of seeds of this species to the extreme daily temperature fluctuation, typical of the páramo environment (Hedberg, 1964). However, the detected seed responses to temperature and light should be confirmed by further studies without using the nitrogen fertilizer solution, as nitrate may influence the sensitivity of phytochrome receptors (Hilhorst, 1990) and affect the seed germination rate (Daws et al., 2002) of some species.

The relatively high germination detected under laboratory conditions and the constant percentages of seedlings retrieved throughout the year suggest that under natural conditions the timing of germination is likely to be correlated to favourable soil moisture conditions, independently from the season. This phenological pattern limits species distribution to the riparian vegetation along streams, around lakes and on humid slopes where it grows.

The seed production of this species (ca. 40 well developed cypselas per capitulum; Ulian, 2004) and its high seed viability and germination percentages detected in this study allow initiating balanced seed collections (sensu Menges et al., 2004) and subsequent long term seed conservation and plant multiplication in nurseries, to support integrated conservation of this species.

## 5. Conclusions

This study provides new information on the biology and ecology of *O. peruvianum* and its populations in the Venezuelan Andes. These data contribute towards building valuable knowledge around the life history of *O. peruvianum* and allow initiating seed conservation measures of this threatened tropical alpine species growing in the world's

most diverse high-altitude ecosystem threatened by human impact and climate change (Luteyn, 1999; Buytaert et al., 2011).

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## References

- Amen, R.D., 1966. The extent and role of seed dormancy in alpine plants. *The Quarterly Review of Biology* 41, 271–281.
- Aranguren, A., Márquez, N.J., Prato, R., Lesenfans, Y., 1996. Use, collection, commercialization, and vulnerability of two species of the genus *Oritrophium* (*O. venezuelense* and *O. peruvianum*, Compositae) in the Venezuelan Andes. *Acta Botánica Venezuelica* 19 (1), 16–38.
- Aristeguieta, L., 1964. Compositae, Flora de Venezuela, X. Parte primera, Instituto Botánico, MAC, Caracas.
- Baskin, C.C., Baskin, J.M., 1998. *Seeds. Ecology, biogeography, and evolution of dormancy and germination*. Academic Press, San Diego.
- Baskin, C.C., Baskin, J.M., 2002. Achene germination ecology of the federally threatened floodplain endemic *Boltonia decurrens* (Asteraceae). *American Midland Naturalist* 147, 16–24.
- Berry, P.E., Calvo, R., 1994. An overview of the reproductive biology of the *Espeletia* (Asteraceae) in the Venezuelan Andes. In: Rundel, P.W., Smith, A.P., Meinzer, F.C. (Eds.), *Tropical Alpine Environments: Plant Form and Function*. Cambridge University Press, Cambridge, pp. 229–249.
- Bevill, R.L., Louda, S.M., 1999. Comparisons of related and common species in the study of plant rarity. *Conservation Biology* 13, 493–498.
- Billings, W.D., Mooney, H.A., 1968. The ecology of arctic and alpine plants. *Biological Review* 59, 24–73.
- Bliss, L.C., 1971. Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics* 2, 405–438.
- Brändel, M., 2004. Dormancy and germination of heteromorphic achenes of *Bidens frondosa*. *Flora* 199, 228–233.
- Buytaert, W., Cuesta-Camacho, F., Tobón, C., 2011. Potential impacts of climate change on the environmental services of humid tropical alpine regions. *Global Ecology and Biogeography* 20, 19–33.
- Cuatrecasas, J., 1997. Synopsis of the Neotropical Genus *Oritrophium* (Asteraceae: Astereae) *BioLlania*, 6 (Edición Especial), 287–303.
- Daws, M.I., Burslem, D.F.R.P., Crabtree, L.M., Kirkman, P., Mullins, C.E., Dalling, J.W., 2002. Differences in seed germination responses may promote coexistence of four sympatric Piper species. *Functional Ecology* 16, 258–267.
- Ellis, R.H., Roberts, E.H., 1981. The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* 9, 373–409.
- Estrada, C., Monasterio, M., 1991. Comportamiento reproductivo de una roseta gigante, *Espeletia spicata* Sch. Bip. (Compositae) del Páramo Desértico. *Ecotropicos* 4 (1), 1–17.
- Flores, J., Jurado, E., Himenez-Bremont, J.F., 2008. Breaking seed dormancy in specially protected *Turbincarpus lophophoroides* and *Turbincarpus pseudopectinatus* (Cactaceae). *Plant Species Biology* 23, 43–46.
- Galíndez, G., Ortega-Baés, P., Daws, M.I., Sühling, S., Scopel, A., Pritchard, H.W., 2009. Seed mass and germination in Asteraceae species of Argentina. *Seed Science and Technology* 37, 786–790.
- Gareca, E.E., Vandeloock, F., Fernández, M., Hermy, M., Honnay, O., 2012. Seed germination, hydrothermal time models and the effects of global warming on a threatened high Andean tree species. *Seed Science Research* 22 (4), 287–298.
- Gil-Otaiza, R., 1997. Plantas usuales en la medicina popular venezolana. Universidad de Los Andes, Mérida.
- Guariguata, M.R., Azocar, A., 1988. Seed bank dynamics and germination ecology in *Espeletia timotensis* (Compositae), an Andean giant rosette. *Biotropica* 20 (1), 54–59.
- Hedberg, O., 1964. Features of afroalpine plant ecology. *Acta Phytogeographica Suecica* 49, 1–144.
- Hedberg, I., Hedberg, O., 1979. Tropical-alpine life forms of vascular plants. *Oikos* 33, 297–307.
- Hilhorst, H.W.M., 1990. Dose response analysis of factors involved in germination and secondary dormancy of *Sisymbrium officinale*. I. Phytochrome. *Plant Physiology* 94, 1090–1095.
- ISTA, 2011. *International Rules for Seed Testing*, Edition 2011. The International Seed Testing Association, Bassersdorf.

- Llambí, L.D., Sarmiento, L., 1998. Biomasa microbiana y otros parámetros edáficos en una sucesión secundaria de los páramos venezolanos. *Ecotropicos* 11, 1–14.
- Llambí, L.D., Law, R., Hodge, A., 2004. Temporal changes in local spatial structure of late-successional species: establishment of an Andean caulescent rosette plant. *Journal of Ecology* 92 (1), 122–131.
- Llamoza, S., Duno, R., Meier, W., Riina, R., Stauffer, F., Aymard, G., Huber, O., Ortíz, R. (Eds.), 2003. Libro Rojo de la Flora Venezolana. 1ª Edición. PROVITA, Fundación Empresas Polar, Fundación Instituto Botánico de Venezuela "Dr. Tobias Lasser", Conservación Internacional, Caracas.
- Luteyn, J.L., 1999. Páramos: A Checklist of Plant Diversity, Geographical Distribution, and Botanical Literature. *Memoirs of the New York Botanical Garden*. New York Botanical Garden, New York.
- Menges, E.S., 2000. Population viability analyses in plants: challenges and opportunities. *Trends in Ecology and Systematics* 15, 51–56.
- Menges, E.S., Guerrant, E.O., Hamze, S., 2004. Effects of seed collection on the extinction risk of perennial plants. In: Guerrant, E.O., Havens, K., Maunder, M. (Eds.), *Ex Situ Plant Conservation: Supporting Species Survival in the Wild*. Island Press, Covelo, pp. 305–324.
- Merritt, D.J., Kristiansen, M., Flematti, G.R., Turner, S.R., Ghisalberti, E.L., Trengrove, R.D., Dixon, K.W., 2006. Effects of a butenolide present in smoke on light-mediated germination of Australian Asteraceae. *Seed Science Research* 16, 29–35.
- Monasterio, M., 1986. Adaptive strategies of *Espeletia* in the Andean Desert Páramo. In: Vuilleumier, F., Monasterio, M. (Eds.), *High Altitude Tropical Biogeography*. Oxford University Press, Oxford, pp. 49–80.
- Mooney, H.A., Billings, W.D., 1961. Comparative physiological ecology of arctic and alpine populations of *Oxyria digyna*. *Ecological Monographs* 31, 1–29.
- Oostermeijer, J.G.B., Van't Veer, R., Den Nijs, J.C.M., 1994. Population structure of the rare, long-lived perennial *Gentiana pneumonanthe* in relation to vegetation and management in the Netherlands. *Journal of Applied Ecology* 31, 428–438.
- Pannier, F., 1969. Untersuchungen zur Keimung und Kultur von *Espeletia*, eines endemischen Megaphyten der alpinen zone (Paramos) der Venezolanischen Anden. *Berichte der Deutschen Botanischen Gesellschaft* 82, 559–571.
- R Development Core Team, 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [cited 2010 Aug 1]. Available from <http://www.R-project.org/>.
- Rundel, P.W., 1994. Tropical alpine climates. In: Rundel, P.W., Smith, A.P., Meinzer, F.C. (Eds.), *Tropical Alpine Environments: Plant Form and Function*. Cambridge University Press, Cambridge, pp. 21–44.
- Rundel, P.W., Witter, M.S., 1994. Population dynamics and flowering in a Hawaiian alpine rosette plant, *Argyroxiphium sandwicense*. In: Rundel, P.W., Smith, A.P., Meinzer, F.C. (Eds.), *Tropical Alpine Environments: Plant Form and Function*. Cambridge University Press, Cambridge, pp. 295–306.
- SAS Institute Inc., 1985. *SAS User's Guide Statistics Version 5*. SAS Institute Inc., Cary, North Carolina.
- Sarmiento, L., 2000. Water balance and soil loss under long fallow agriculture in the Venezuelan Andes. *Mountain Research and Development* 20 (3), 246–253.
- Silva, J.F., Trevisan, M.C., Estrada, C.A., Monasterio, M., 2000. Comparative demography of two giant caulescent rosettes (*Espeletia timotensis* and *E. spicata*) from the high tropical Andes. *Global Ecology and Biogeography* 9, 403–413.
- Smith, A.P., 1975. Insect pollinator and heliotropism in *Oritrophium limnophilium* (Compositae) of the Andean Páramo. *Biotropica* 7 (4), 284–286.
- Smith, A.P., 1981. Growth and population dynamics of *Espeletia* (Compositae) in the Venezuelan Andes. *Smithsonian Contributions to Botany* 48, 1–45.
- Smith, A.P., 1984. Postdispersal parent-offspring conflict in plants: antecedents and hypothesis from the Andes. *American Naturalist* 123, 354–370.
- Smith, A.P., Young, T.P., 1982. The cost of reproduction in *Senecio keniodendron*, a giant rosette species of Mt. Kenya. *Oecologia* 55, 243–247.
- Smith, A.P., Young, T.P., 1994. Population biology of *Senecio keniodendron* (Compositae), a giant African rosette species. In: Rundel, P.W., Smith, A.P., Meinzer, F.C. (Eds.), *Tropical Alpine Environments: Plant Form and Function*. Cambridge University Press, Cambridge, pp. 273–293.
- Sokal, R.R., Rohlf, F.J., 1981. *Biometry*. W.H. Freeman and Co., San Francisco.
- Tobe, K., Zhang, L., Omasa, K., 2006. Seed germination and seedling emergence of three *Artemisia* species (Asteraceae) inhabiting desert sand dunes in China. *Seed Science Research* 16, 61–69.
- Torres, F., Gavidia, J., Peraza, J., 1996. Anatomía foliar de *Oritrophium* (H.B.K.) Cuatrec. del Páramo de Sierra Nevada, Estación Loma Redonda. *Plántula* 1 (1), 65–73.
- Ulian, T., 2004. The demography of *Oritrophium peruvianum* (Lam.) Cuatr. and the impacts of harvesting on its populations in the Venezuelan Andes. (PhD Thesis) Natural Resources Institute, University of Greenwich, Chatham Maritime.
- Young, T.P., 1994. Population biology of Mount Kenya *Lobelias*. In: Rundel, P.W., Smith, A.P., Meinzer, F.C. (Eds.), *Tropical Alpine Environments: Plant Form and Function*. Cambridge University Press, Cambridge, pp. 251–272.