

# Growth Rates, Reproductive Phenology, and Pollination Ecology of *Espeletia grandiflora* (Asteraceae), a Giant Andean Caulescent Rosette

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**Abstract:** From March 2001 to December 2002, we studied the reproductive phenology, pollination ecology, and growth rates of *Espeletia grandiflora* Humb. and Bonpl. (Asteraceae), a giant caulescent rosette from the Páramos of the Eastern Andes of Colombia. *Espeletia grandiflora* was found to be predominantly allogamous and strongly self-incompatible. Bumblebees (*Bombus rubicundus* and *B. funebris*) were the major pollinators of *E. grandiflora*, although moths, hummingbirds, flies, and beetles also visited flowers. Inflorescence development began in March and continued through August to September. Plants flowered for 30–96 days with a peak from the beginning of October through November. The percentage of flowering plants strongly differed among size classes and between both years. Seed dispersal occurred as early as September through May of the following year. The average absolute growth rate for juveniles and adults rate was 7.6 cm/year. Given the scarcity of floral visitors at high altitudes due to climatic conditions, we suggest that even small contributions from a wide range of pollinators might be advantageous for pollination of *E. grandiflora*. Long-term studies on different populations of *E. grandiflora* are required to determine if the high growth rates are representative, to quantify the variation in the flowering behavior within and among populations, and to establish if nocturnal pollination is a trait that is exclusive to our population of *E. grandiflora*.

**Key words:** Asteraceae, *Espeletia grandiflora*, Colombia, reproductive phenology, pollination, growth rates, Páramo, bumblebees.

## Introduction

The genus *Espeletia* (Asteraceae) belongs to a complex of seven genera whose phylogenetic relationships remain unclear; together they contain about 100 species restricted to the north-western Andes, occurring from cloud forests, dry and wet Páramos to xeric periglacial habitats at approximately 2000–4600 m elevations (Rauscher, 2002). Many physiological, phenological, and morphological adaptations to high altitudes

have been documented for *Espeletia* s. str., including the leaf, flower, and fruit production during the wet season, low leaf osmotic potential, supercooling, and high photosynthetic rates. Among morphological adaptations are the densely pubescent, spirally arranged leaves, and a cloak of dead leaves covering the stem (e.g., Goldstein and Meinzer, 1983; Meinzer and Goldstein, 1985; Cabrera, 1996; Lüttge, 1997; Velez et al., 1998).

Most *Espeletia* biology is known from observations made in Venezuelan dry Páramos (i.e., < 1000 mm of mean annual rainfall), a region holding only 20% of the total known area of the Páramos. Almost all *Espeletia* species studied thus far are slow-growing plants (e.g., 1–2 cm/year and maturation at 20 years for *Espeletia spicata*), strongly self-incompatible and reproduce primarily through seed production (Smith, 1981; Berry and Calvo, 1989; Estrada and Monasterio, 1991; Cavalier et al., 1992). Bumblebees and hummingbirds are major pollinators of *Espeletia*, although some species inhabiting Páramos above 4000 m have adapted to wind pollination (Berry and Calvo, 1989, 1994). Nocturnal pollination has not been documented in *Espeletia* and it appears that this aspect of pollination has been generally overlooked in previous studies.

Herein we report data on the growth and general reproductive biology of *Espeletia grandiflora* Humb. and Bonpl. We chose to study *E. grandiflora* because it is an abundant, endemic species of the Eastern Andes of Colombia, occurring between 2900 to 4100 m altitude. We asked the following questions: What are the mortality and growth rates for *E. grandiflora*? What are the flowering and seed dispersal phenologies? What are the breeding and pollination systems? What are the major pollinators? Does nocturnal pollination occur?

## Materials and Methods

### Study site and species

This study was done from March 2001 to December 2002 on a single population of *Espeletia grandiflora* in Chingaza National Park (Páramo de Piedras Gordas) at 3200 m elevation in the Eastern Andes of Colombia (4°30'N, 73°40'W). Patches of *E. grandiflora* in our study population ranged from 0.45–1.23 plants per square meter ( $\bar{x}$  = 0.76 ± 0.4, n = 4 patches, 1359 plants). There are no climatic records for the study site, thus we used the rainfall and temperature data taken at the closest meteorological station (Palacio Station, Guasca, Cundi-

namarca; 4°44'N, 73°34'W, 3760 m). The rainy season is unimodal with maximum rainfall in June–July; December and January are the drier months of the year (Fig. 4). Frequent light rain, fog, and low clouds cover the sky during most of the day and sometimes at night in the study area, especially from the beginning of May until September. The mean annual rainfall is 1889 mm and the median annual temperature is 7°C (IGAC, 1996). The annual rainfall for 2001 and 2002 was 1946.8 mm and 1561.6 mm, respectively.

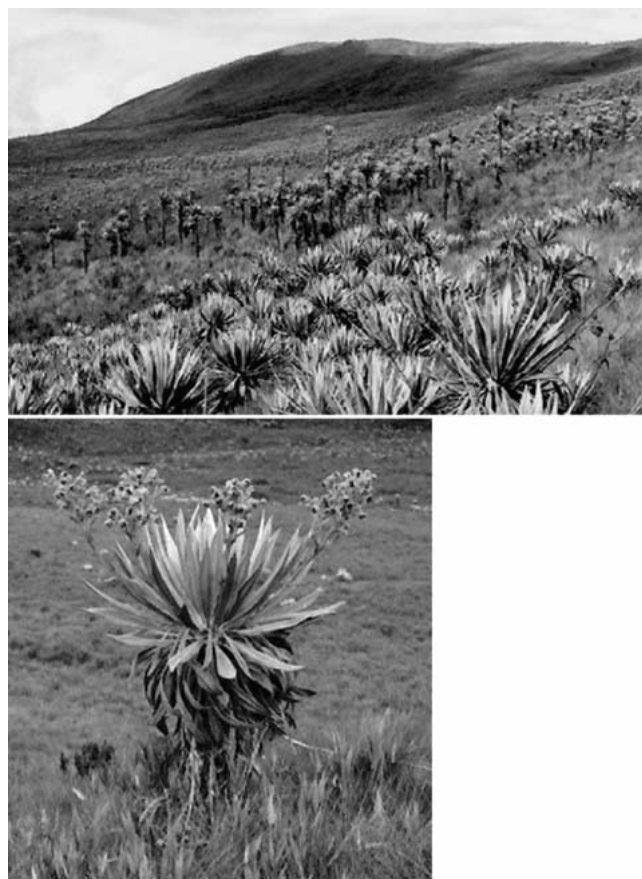
*Espeletia grandiflora* produces a woody, giant caulescent rosette that reaches over 2 m in height. It has highly pubescent leaves, and a stem densely covered with a thick layer of old leaves (necromass). Flowers are reduced and arranged into a disciform capitulum or head, which hangs from a long stalk (axillary inflorescences) at anthesis (Fig. 1). Each capitulum has two types of flowers. The majority of them are disk flowers, which are tubular, actinomorphic, have a sterile gynoeium, form the male part of the capitulum, and are located towards the centre of the capitulum. They are surrounded by the ligulate flowers, which are zygomorphic, with a long yellow ray, female, and are responsible for the showiness of the capitulum. Only male flowers have nectaries, which are located at the base of the corollas, surrounding the style; the five stamens have free filaments, and the anthers are fused, forming a tube around the style. The fruit is an achene about 2.5 mm long and lacks a pappus.

#### Mortality and growth rates

To estimate mortality rate of juveniles and adults, we marked with flagging tape a total of 1359 plants from four plots (each 30 × 15 m), which were randomly selected within the study area. Maximum height of each plant (measured from the ground to the uppermost tip of the plant), height of its stem (measured from the ground to base of the crown of leaves), length of its axillary inflorescence (from the stem to the outermost capitulum), reproductive stage, and overall condition (e.g., herbivory incidence) were recorded and followed every month from July 2001 to December 2002. Mortality rate was estimated as the percentage of marked individuals that died in the subsequent year. For seedling mortality, we followed the total number of seedlings (n = 96) found in a small area (3 × 15 m) within one of the four plots.

We used some of the marked individuals above to estimate growth rates. The annual absolute growth rate for juveniles (n = 12) and adults (n = 153) was calculated as the difference in total stem length between May 2001 and May 2002. The annual relative growth rate was calculated as the difference between the common logarithms of total stem length in May 2002 and 2001. We could not record the absolute growth rate for seedlings because none of the marked individuals survived to the next year.

To facilitate data analysis, we grouped individual plants primarily on the basis of stem length. Preliminary observations at the study site (C. Fagua, pers. obs.) indicated that the smallest plants with more than five leaves had an average stem length of 5 cm (± 0.52; n = 25), and that the smallest plants with a flowering stalk (i.e., mature plant) had an average stem length of 21 cm (± 4.60; n = 56). Therefore, we grouped individuals as seedlings (stem length ≤ 5 cm), juveniles (stem length



**Fig. 1** Páramo of Chingaza in the Eastern Andes of Colombia (3200 m) dominated by patches of *Espeletia grandiflora* (top) and a close up to an adult plant in bloom (bottom).

between 5 and 21 cm), and adults (stem length greater than 21 cm). There were eight classes of adults: adults I (22–38 cm), adults II (39–55 cm), adults III (56–72 cm), adults IV (73–89 cm), adults V (90–106 cm), adults VI (107–123 cm), adults VII (124–140 cm), and adults VIII (> 141 cm). To determine the adult size classes we used the following formula (Sturges, 1926):

$$C = (X_{\max} - X_{\min})/M,$$

where  $M = 1 + (3.3 \times \log N)$ ,  $N$  = total number of individuals,  $M$  = number of size classes,  $C$  = range of each size class,  $X$  = maximum and minimum stem length.

#### Reproductive phenology

To monitor the annual reproductive phenology of *E. grandiflora*, we randomly selected 200 adult plants along a transect. For each plant, twice a month, we recorded the developmental stage of the capitula and the blooming period through to seed dispersal. We recorded in detail the numbers of all capitula produced and the number of capitula in anthesis on 35 out of the 200 selected plants, from March 2001 through December 2002. The percentage of individuals that reproduced each year was estimated from the marked individuals used to estimate mortality.

### Floral biology

We observed three aspects of floral biology and development: 1) the pollen/ovule ratio per capitulum (average number of pollen grains per number of ovules per flower), 2) time of day when pollen and nectar were released in all male flowers in the capitulum, and 3) time of day of stigmatic receptivity of female flowers over the bloom time.

The average number of pollen grains per capitulum was estimated for five flowers from nine capitula ( $n = 45$  flowers) as follows: we collected flowers before anthesis to avoid pollen losses, and anthers were treated and preserved after acetolysis (Dafni, 1992). We then counted the number of pollen grains found in a suspension of known volume to estimate the total number of pollen grains per flower. Finally, we multiplied the average number of pollen grains by the average number of male flowers counted in the nine capitula examined.

Because female flowers have a single ovule, the number of ovules per capitulum corresponds to the number of female flowers. Pollen grains greatly outnumber the ovules within a capitulum, and the number of male flowers is not correlated with the number of female flowers (Pearson correlation coefficient,  $r = 0.06$ ;  $p > 0.05$ ); thus, to obtain a representative sample, we averaged the number of female flowers found in 103 capitula.

Pollen and nectar phenology and stigmatic receptiveness were studied on nine capitula randomly selected prior to blooming. Stigmatic receptivity was tested with hydrogen peroxide (Dafni, 1992).

### Breeding system

To determine the breeding system, we subjected capitula of *E. grandiflora* to five different treatments (Table 1). 1) To determine if plants were self-compatible, we hand self-pollinated flowers, using pollen from the same capitula ( $n = 28$  plants), and pollen from flowers from different capitula on the same plant ( $n = 25$ ). 2) To test for cross-pollination, we used pollen from capitula of plants separated by a distance of at least 10 m ( $n = 26$ ) to hand cross-pollinated flowers. This constitutes the minimum distance from *Espeletia schultzii* (Wedd), a morphologically similar species that does not share self-compatible alleles (Sobrevila, 1986). 3) To test for agamospermy, emasculations were done on capitula from 20 plants by covering the central disc of male flowers with silicone liquid to prevent them from blooming. In all three treatments above, we avoided contamination and predation by isolating capitula within a white, synthetic cloth bag about 3 months before anthesis and once the treatments were completed. 4) To test for the effect of bagging on the hand cross-pollination experiment, we left 19 capitula exposed after hand cross-pollination. Finally, 5) to serve as control with open pollination, we used 30 intact capitula. For all treatments, all plants and capitula were randomly selected from the population.

The effect of each pollination treatment was determined by measuring the percentage of viable seeds produced as follows: 2 months after the treatments, the number of flowers per capitulum were counted, fruits were removed from the capitulum and placed on water in a Petri dish until they germinated.

**Table 1** Mean ( $\pm$  S.E.) percentage of viable seeds produced in each pollination treatment in *Espeletia grandiflora*. Treatments not connected by the same symbol are significantly different (One-way ANOVA,  $p < 0.05$ ). N = sample size. Unless otherwise indicated, capitula were bagged once the treatments were completed

Treatments	Viable seeds	N plants	N seeds
Agamospermy	1.27 $\pm$ 0.71 $\Psi$	20	1448
Hand self-pollination within capitula	1.52 $\pm$ 0.24 $\Psi$	28	2080
Hand self-pollination among capitula	4.30 $\pm$ 0.35 $\Psi$	25	1964
Hand-cross pollination	79.3 $\pm$ 1.67 $\alpha$	26	2249
Hand-cross pollination (unbagged)	73.7 $\pm$ 1.15 $\beta$	19	1491
Control (open pollination)	60.2 $\pm$ 2.08 $\epsilon$	30	2452
Wind pollination	0.0	36	0.0
Small arthropods (< 1.3 mm)	1.71 $\pm$ 0.42 $\Psi$	36	2850
Diurnal open pollination	73.5 $\pm$ 3.85 *	17	1211
Nocturnal open pollination	24.5 $\pm$ 6.03 ‡	17	1120

Seeds that did not germinate were dissected under the stereomicroscope and the presence or absence of an embryo was recorded; when present, viability tests were performed on the embryo with triphenyl tetrazolium solution (1%) in darkness. Self-compatibility was estimated using the index of self-incompatibility (ISI), which is the number of viable seed produced by hand self-pollinations over the number of viable seed produced by hand cross-pollination (Cruden, 1977; Dafni, 1992).

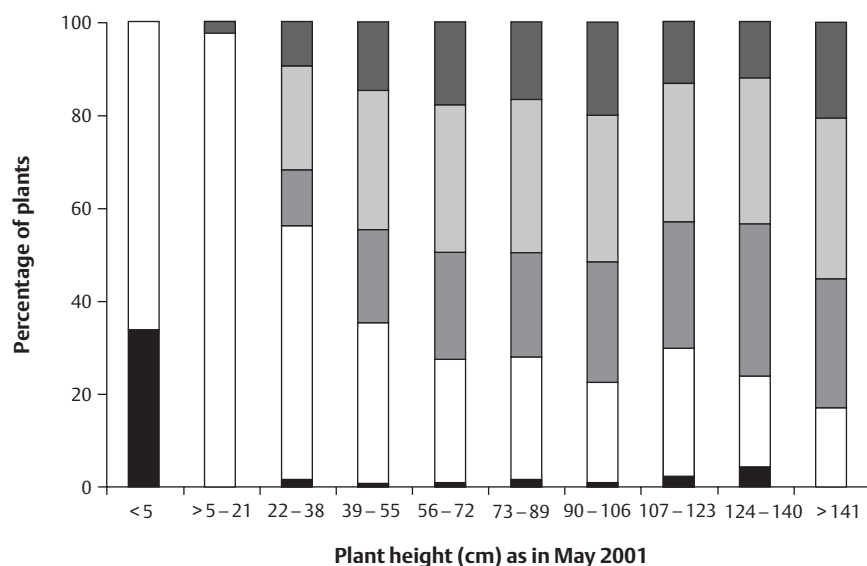
### Pollination mode

We tested for wind, nocturnal and diurnal pollination, and the effect of small arthropods on reproduction of *E. grandiflora*. Wind pollination was tested on 36 capitula from different plants, which were chosen from the population and isolated in the same fashion as for the breeding system treatments but using nylon mesh bags (openings of  $0.8 \times 0.8$  mm), thus excluding all possible pollinators but allowing the passage of air-borne pollen.

To test for nocturnal pollination, just before anthesis, two capitula per plant were randomly selected in each of 17 individuals. One of the capitula was covered with a nylon mesh bag ( $< 0.8 \times 0.8$  mm) during the daytime (6:00–18:00 h) and exposed at night during the anthesis period. The other capitulum was tested for diurnal pollination by switching exposure periods.

To examine the effect of small arthropods (< 1.3 mm) on reproduction, 36 capitula from different plants were also isolated with nylon mesh bags that had larger openings ( $1.3 \times 1.3$  mm).

The percentage of viable seeds produced in each treatment was evaluated as explained for the breeding system treatments.



**Fig. 2** Demographic structure of *Espeletia grandiflora* in the Páramo of Chingaza showing the percentage of plants that reproduced and died between July 2001 and July 2002. The sample size for each size class was as followed (for explanation see "Materials and Methods"): seedlings (< 5) = 3840, juveniles (> 5–21) = 130, adults I (22–38) = 175, adults II (39–55) = 299, adults III (56–72) = 322, adults IV (73–89) = 254, adults V (90–106) = 118, adults VI (107–123) = 34, adults VII (124–140) = 14, adults VIII (> 141) = 13. (■) Flor-01 and 02; (◐) Flor-02; (◑) Flor-01; (□) vegetative; (■) mortality.

### Visitors and pollinators

To determine the flower visitors and pollinators of *E. grandiflora*, we made direct and detailed observations on blooming inflorescences of 11 plants on five, non-consecutive 24-h periods during anthesis at peak bloom. Behavioral observations of flower-visiting animals were made for 30 min every hour during the day (6:00–18:00 h) and every 2 h at night (45 h in total). Nocturnal observations were done using a red flashlight in order to avoid disturbing visitors. Foraging behavior of animals on the inflorescences of 11 plants was studied in terms of: 1) frequency and duration of visits to a capitulum, 2) numbers of plants and capitula visited, and 3) atmospheric conditions (temperature, relative humidity, and cloud cover).

We defined a visit as only when a visitor approached and landed on a capitulum or contacted a single flower (i.e., hummingbirds). Stigma contact was qualitatively considered high when a visitor's body touched more than 50% of the male part of the capitulum, and low when it touched less than 50%. Foraging range of the visitors was extracted from both the literature and direct observations.

### Data analysis

We used Pearson correlation analysis to test for association between relative growth rate and plant height. We used linear regression analysis to test the effect of plant height and relative growth rate on the following variables: 1) inflorescence length, 2) number of inflorescence, and 3) number of capitula per plant, as well as the effect of plant density on the percentage of flowering plants. Finally, we used a Chi-squared test to compare: 1) the percentage of plant mortality and percentage of flowering plants falling into the different plant size classes, 2) percentage of flowering plants between years 2001 and 2002, and between the number of plants that reproduced in both years and those that reproduced in either year. We used one-way ANOVA to detect differences among breeding system treatments, and between nocturnal and diurnal pollination experiments.

Sample sizes varied between experiments depending on the available material at the time (i.e., not all plants or all capitula flowered at the same time on a single plant at any time, even during the flowering peak; see results below). Unless otherwise indicated, we used a different set of plants and capitula for each experiment, and in all instances, plants, capitula, and flowers were randomly selected. When possible, mean values are given with standard errors.

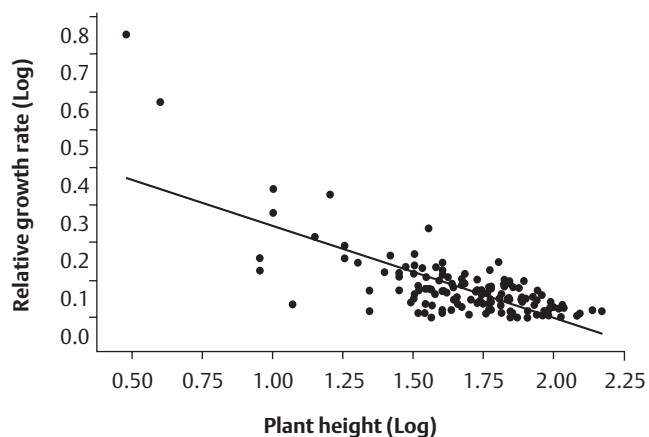
## Results

### Mortality and growth rates

Mortality was significantly higher in seedlings than in adults (Fig. 2;  $X^2_{.001[9]} = 312.6$ ,  $p < 0.001$ ,  $X^2$  test). About half (49 of 96) of the marked seedlings died during the study year, most of them (~85%) during the dry season (December–January). In contrast, overall adult mortality was quite low (~1.7%). Although the cause of death was unknown for both seedlings and adults, in at least one adult, the loss of structural stability caused one old, tall individual to fall to the ground. Adults of an unidentified burrowing beetle (Scolityidae, Coleoptera) and larvae of *Epistrophus* (Curculionidae, Coleoptera) were frequently found inside live stems and might have killed some plants, but we did not quantify the effect of these beetles on the plants. Finally, the average absolute growth rate for juveniles and adults was 7.6 cm/year ( $\pm 0.34$ ,  $n = 165$ ); relative growth rate and plant height were inversely correlated ( $r = -0.73$ ;  $p < 0.001$ ) as shown in Fig. 3.

### Reproductive phenology

Individuals of *E. grandiflora* had 1–19 axillary inflorescences ( $\bar{x} = 6.3 \pm 3.8$ ,  $n = 116$ ) with an average length of 85 cm ( $\pm 12$ , 61.3–111.6,  $n = 174$ ). Inflorescence development began in March and continued through August–September. Plants flowered for 30–96 days ( $\bar{x} = 65 \pm 15.9$ ,  $n = 35$  plants), with a peak (> 25% of blooming capitula) from the beginning of October through November. Seed dispersal occurred as early as September through May (Fig. 4). Not all capitula flowered at



**Fig. 3** Relationship between plant height and relative growth rate of *Espeletia grandiflora* in the Páramo of Chingaza (Pearson Correlation coefficient,  $r = -0.73$ ;  $p < 0.001$ ,  $n = 165$ ). Data were collected between May 2001 and May 2002.

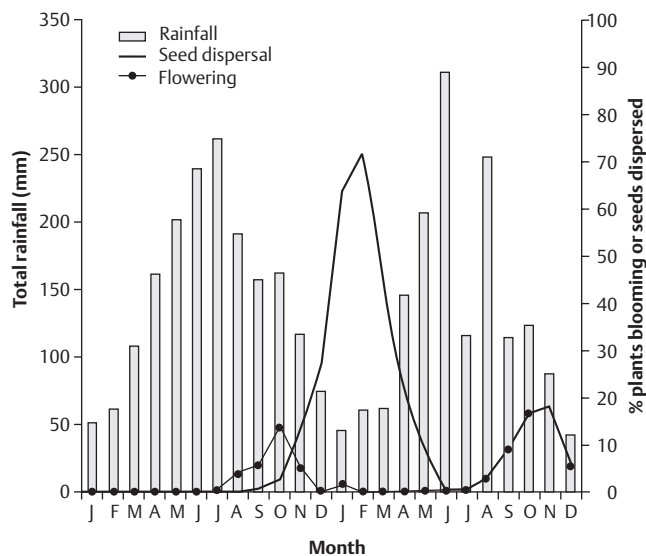
the same time on a single plant. An average of 24.9% ( $\pm 1.25$ ,  $n = 35$  plants) of the total number of capitula per plant flowered at the same time, but with slightly higher figures during peak blooming ( $\bar{x} = 41.4\% \pm 2.2$ ,  $n = 35$  plants). The percentage of flowering plants differed strongly among size classes (Fig. 2;  $X^2_{.001[24]} = 320.6$ ,  $p < 0.001$ ) and between the two years (Fig. 4;  $\bar{x}_{2001} = 41.7\% \pm 7.53$ ;  $\bar{x}_{2002} = 64.5\% \pm 4.72$ ,  $n = 1229$  plants). About 27.2% of plants reproduced in both years, whereas 9.3% and 25.8% flowered either in the first or second year, respectively, and 37.6% remained vegetative during the study; however, there were no significant differences between the number of plants that reproduced in both years and those that reproduced in either year ( $X^2_{.001[16]} = 25.4$ ,  $p = 0.063$ ). Thus, flowering in one year did not decrease the probability of a plant flowering in the following year. Finally, there was no significant effect ( $p > 0.05$ ) of plant density on the percentage of flowering plants.

#### Floral biology

Plants averaged 86 capitula ( $\pm 62$ ,  $n = 36$  plants), which ranged in diameter (including the ray length) from 5.0–6.2 cm ( $\bar{x} = 5.7 \pm 0.4$ ,  $n = 12$ ). The diameter of the male central disc ranged from 2.0–2.8 cm ( $\bar{x} = 2.5 \pm 0.3$ ,  $n = 12$ ), that is, 45% of the total area of the capitulum. The number of male flowers per capitulum was on average 2.5-times greater than the number of female flowers ( $\bar{x} = 199.4 \pm 6.2$ ,  $n = 9$ ;  $\bar{x} = 77.3 \pm 18.7$ ,  $n = 103$ ).

The average number of pollen grains produced by a male flower was 3422 ( $\pm 81.9$ ,  $n = 45$ ) (about 682170 pollen grains per capitulum [ $n = 9$ ]). Therefore, the average pollen/ovule ratio per capitulum was 8825. There was no significant effect ( $p > 0.05$ ) of plant height or relative growth rate on the number of inflorescences and capitula and inflorescence length.

The bracts that protect the capitulum remained attached to it even after seed dispersal. These bracts kept the capitulum closed for 5 to 7 days; about 4 days later, the ligules of the female flowers completed development. Subsequently, individual male flowers entered anthesis for about 24 h each, and in a centripetal fashion within the capitula for 12–15 days ( $\bar{x} = 9 \pm 0.9$ ). Stigmatic receptivity coincided with the peak of



**Fig. 4** Reproductive phenology of *Espeletia grandiflora* in the Páramo of Chingaza from March 2001 through December 2002 ( $n = 35$  plants). Rainfall data were taken from the weather station of "Palacio" (Guasca, Cundinamarca) from January 2001 to December 2002. Annual rainfall for 2001 and 2002 was 1946.8 mm and 1561.6 mm, respectively.

pollen presentation by male flowers, usually 4–12 days after anthesis. Unlike male flowers, female flowers developed synchronously and remained receptive for several days. Finally, the capitulum bent downwards and seed dispersal began about 8 weeks later.

Depending on the weather conditions, pollen presentation and nectar secretion started early in the morning (5:00 h), and extended until 8:00–13:00 h. In rainy conditions, flowers released pollen once rain stopped or when temperatures increased to about  $9^\circ\text{C}$  ( $\pm 0.5$ ,  $8.5 - 10$ ,  $n = 5$  days). Nectar production was synchronized with pollen presentation and lasted for about 18 h. Flowers began to release pollen into the inner tube formed by the fused anthers, sometimes even before flowers opened. At this point, the style was relatively short. During style elongation, the collecting hairs, located at the style tip, pushed the pollen grains up through the tube, thus exposing them to pollinators. Pollen was gradually released throughout the day.

#### Breeding system

The pollination tests showed that *E. grandiflora* is predominantly allogamous and self-incompatible (ISI = 0.018 and 0.071 for self-compatibility tests within and among capitula of the same plant, respectively). The percentage of viable seeds formed by hand cross-pollination was significantly higher than those produced by the self-pollination treatments and controls (Table 1).

#### Pollination mode

No viable seeds were produced when blooming capitula were exposed exclusively to pollination by wind. In capitula exposed to animal pollinators, both nocturnal and diurnal pollination occurred; however, the latter produced a significantly

**Table 2** Major pollinators of *Espeletia grandiflora* in the Páramo of Chingaza, Colombia, based on 45 observation-hours of 11 plants during peak flowering. Pollinators: Col = Coleoptera [Chr = Chrysomelidae, Cle = Cleroidea], Dip = Diptera (Bibionidae), Hym = Hymenoptera (Apidae), Lep = Lepidoptera. F: frequencies of approaches to the observed patch (n = 117 observations). Mean visit duration: mean ( $\pm$  S.E.) duration of visits on a capitulum (min). SC: stigmatic contact. FA: foraging activity time. FR: foraging range (a foraging range was considered large when visitors move more than 5 km a day, small when their foraging range is less than 5 km, and very low when daily foraging spanned only single capitula or adjacent capitula. Foraging range for *Bombus* was taken from Goulson and Stout [2001] because there is no biological data on Andean bumblebee species [Gonzalez and Engel, 2004]). L = low, H = high, VL = very low, D = diurnal, N = nocturnal

Pollinators	F	Mean visit duration (min)	SC	FA	FR
<b>Insects</b>					
Syrphidae (Dip)	0.08	58 ( $\pm$ 28.7, n = 9)	L	D	VL
<i>Dhilophus espeleninae</i> (Dip, Bib)	0.04	72 ( $\pm$ 42, n = 5)	L	D	VL
<i>Phyllotrupes</i> sp. (Col, Chr)	0.01	6 (n = 1)	L	D	VL
<i>Haplamaurus kiesewetteri</i> (Col, Cle)	0.01	13 (n = 1)	L	D	VL
<i>Bombus funebris</i> Smith, 1854 (Hym, Ap)	0.28	1.52 ( $\pm$ 1.9, n = 33)	H	D	H
<i>Bombus rubicundus</i> Smith, 1854 (Hym, Ap)	0.32	0.88 ( $\pm$ 1.6, n = 38)	H	D	H
Noctuidae sp. 1 (Lep)	0.03	56 ( $\pm$ 55, n = 3)	H	N	H
Noctuidae sp. 2 (Lep)	0.06	30.9 ( $\pm$ 9.1, n = 7)	H	N	H
<b>Hummingbirds (Trochilidae)</b>					
<i>Chalcostigma heteropogon</i>	0.15	0.13 ( $\pm$ 0.2, n = 18)	L	D	L
<i>Rhamphomicron microrhincus</i>	0.01	0.12 (n = 1)	L	D	L
<i>Oxygogon guerinii</i>	0.01	0.09 (n = 1)	L	D	L

higher number of viable seeds. Viable seeds due to pollination by small arthropods (< 1.3 mm) such as *Phyllostox aristidis* (Coleoptera, Curculionidae), accounted only for 1.71% of the total number of produced seeds and was not significantly different from any of the self-compatibility treatments. Observations on the behavior of *P. aristidis* also revealed that adults rarely moved between capitula (Table 1); therefore, despite their abundance in the capitula (> 40 individuals per capitulum), adult *P. aristidis* are not significant pollinators of *E. grandiflora*.

#### Visitors and pollinators

Four hummingbird species, three arachnid species, and 29 insect species were associated with capitula of *E. grandiflora*. The bumblebees *Bombus rubicundus* and *B. funebris* were the most important pollinators, as indicated by their high visitation frequencies, longest daily movement among plants, and their frequent contact with several stigmata in a capitulum. Bees visited *E. grandiflora* from about 7:00–18:00 h, however, the visitation peak of *B. rubicundus* was 9:00–11:00 h and of *B. funebris* 12:00 and 13:00 h. Both peaks occurred during the warmest periods of the day. Other diurnal pollinators, including flies, beetles, and hummingbirds, were also observed, although their overall contributions to *E. grandiflora* pollination were relatively low (Table 2). Hummingbirds did not perch at the inflorescences to take nectar; instead they hovered in front of the capitula and rarely touched the stigma. Moths belonging to the families Pyralidae, Noctuidae, and Geometridae were the only nocturnal pollinators observed; two unidentified Noctuidae species exhibited a peak visitation period between 19:00–22:00 h. There were no visits between 1:00–5:00 h.

## Discussion

### Mortality and growth rates

Mortality was higher in seedlings than in adults because, unlike adults, they lack morphological adaptations to survive low temperatures. Thus, a high growth rate is very important for very small plants because individuals that grow quickly and/or retain old leaves on their stems (necromass) are more likely to survive cold and drought, as observed in other *Espeletia* (Smith, 1979, 1981; Goldstein et al., 1985; Monasterio, 1986; Mora-Osejo and Sturm, 1994). Consequently, growth rate decreased with increasing plant size because once nutrients are no longer intensively used in vegetative growth they could be relocated to the construction of reproductive structures (e.g., Cavalier et al., 1992), such as shown in Fig. 2.

The average absolute growth rate of juveniles and adults of *E. grandiflora* (7.6 cm/year) is the fastest growth rate thus far reported for any *Espeletia* species. Although we do not have data on the growth rate of seedlings, even if it takes several years (2–5) for a seedling to reach a stem length of 5 cm to be considered a juvenile, it would take at least another 3–4 years to first reproduction (~21 cm of stem length); this overall corresponds to about 10 years total to maturation. Based on our limited data, this is a conservative estimation that contrasts with the widely accepted generalization that all *Espeletia* are slow-growing plants, which reach maturation at 20–50 years (Estrada and Monasterio, 1991; Cavalier et al., 1992). Therefore, tall *E. grandiflora* individuals ( $\geq$  2 m) are perhaps only about 40–45 years old, not a century old or much older as previously believed. We do not know exactly what conditions favour such high growth rates in *E. grandiflora*. Also, we have data from only a single year and do not know if growth rates are comparable over time.

### Reproductive phenology

Plant phenologies are the result of interactions among biotic (e.g., plant morphological and physiological adaptations, and pollinators), climatic (e.g., photoperiod, temperature, rainfall), and phylogenetic factors that, through natural selection, determine the most efficient timing for growth and reproduction (Van Schaik et al., 1993; Wright and Calderon, 1995). The genus *Espeletia* shows considerable interannual variation in flowering behavior within and among species; some species are monocarpous, many species are mast-flowering, and some are even aperiodic, remaining entirely vegetative for several consecutive years (Monasterio, 1986). This variability has been hypothesized as an adaptation that leads to more varied mating patterns by increasing the spatial-temporal diversity in reproductive populations (e.g., Bawa, 1983; Berry and Calvo, 1989).

Our observations over two years indicate that *E. grandiflora* also exhibits variability in interannual flowering behavior, with individuals blooming only one year, both years, or remaining vegetative for at least two consecutive years. Although the underlying mechanisms that trigger a differential flowering response in some individuals in the population are not yet fully understood, several factors including age, growth rate, rainfall, genotype, resource availability, shading, soil moisture, and density-dependent interactions might influence flowering behavior in *Espeletia* (e.g., Rathcke and Lacey, 1985; Monasterio, 1986; Widén, 1991; Cavalier et al., 1992; Marques et al., 2004). Furthermore, even if most or all individuals were able to produce flowers, fecundity rates would be low because of the low pollinator availability in the Páramos.

We found evidence that the percentage of flowering plants in *E. grandiflora* differed significantly among size classes (i.e., tall individuals are more likely to reproduce than small ones; Fig. 2), that there was not a significant effect of plant density on the percentage of flowering plants, and that flowering in one year did not significantly decrease the probability of flowering the following year. In addition, more plants reproduced in 2002, when annual rainfall decreased to 19.8% of that in 2001; this observation contrasts with the expected direct relationship between annual rainfall and blooming found in other *Espeletia* (e.g., *E. barclayana*; Cavalier et al., 1992). Therefore, growth rate that is inversely correlated with plant size (Fig. 3), might be a good predictor of flowering behavior in *E. grandiflora*. Long-term studies on *E. grandiflora* are required to determine the plasticity of this behavior within and among populations, and to what extent it is influenced by rainfall.

Phenological adaptations that may have evolved in *E. grandiflora* to compensate for reduced pollinator availability and to increase cross-pollination success in Páramos include the synchronous flowering peak in the population, individual interannual variation in flowering behavior, long individual flowering period (65 days), low percentage of capitula in anthesis per plant, and flowers with long periods of stigmatic receptivity and pollen presentation (12–15 days). Such adaptations have been previously suggested for other *Espeletia* species and, in general, for plants growing at high altitudes (Primack, 1985; Monasterio, 1986; Berry and Calvo, 1989, 1994; Monasterio and Sarmiento, 1991; Seres and Ramirez, 1995; Medan, 2003; Somanathan et al., 2004).

Despite the pollinator constraints at high altitudes, the blooming peak of *E. grandiflora* occurred about 2 months before the dry season; this is during the wet season, when atmospheric conditions were not optimal for pollinators (Fig. 4). A similar blooming pattern has also been reported for other *Espeletia* species in Venezuela (Smith, 1981; Berry and Calvo, 1994), and was observed in three other species in Chingaza that are sympatric with *E. grandiflora* and bloom asynchronously with it, namely *E. killipii* and *E. argentea* (C. Fagua, pers. obs.). Thus, given that drastic daily changes in temperature that may damage flowers are more frequent during the dry than rainy season (Sarmiento, 1986), there is likely a trade-off between avoiding capitula damage and blooming in favourable conditions for pollinator flight activity. Unlike vegetative parts, capitula lack anatomical adaptations, except for the bracts of the involucre, to resist cold (Smith, 1979; Mora-Osejo and Sturm, 1994; Mora-Osejo, 2001). In fact, capitula damage during the dry season in *E. grandiflora* occurred in some capitula that lasted until December, and has also been reported for *E. schultzii* (Smith, 1979). If capitula showiness is an important trait to attract pollinators such as bees, changes in shape, size, or even colouration of the ray florets due to freezing temperatures could influence their attractiveness and reduce plant fitness. Therefore, blooming prior to the dry season could reduce capitula damage as well as permit increased flower showiness and attraction to pollinators (although these factors were not evaluated here). Another advantage in flowering prior to the dry season is to ensure that sufficient resources (e.g., water) are available for seed maturation.

Finally, the seed dispersal behavior of *E. grandiflora* is similar to that of other *Espeletia* species in that it occurs during the dry season, prior to the next rainy season (e.g., Monasterio, 1986; Guariguata and Azocar, 1988). The strong winds during the dry season might facilitate release and dispersal of seeds from capitula because, as in other species, *E. grandiflora* achenes lack a pappus.

### Breeding system and pollination mode

The breeding and pollination systems of *E. grandiflora* are similar to those described for most other *Espeletia* species (e.g., Berry and Calvo, 1989, 1994); that is, individuals in our population were predominantly allogamous, strongly self-incompatible, and pollinated mainly by bumblebees. The spatial distribution of male and female flowers within the capitulum and the high pollen/ovule ratio in the capitula of *E. grandiflora* are also characteristic of obligate outcrossing and incompatibility (Cruden, 1977; Richards, 1986; Dafni, 1992; Proctor et al., 1996).

Observations of nocturnal pollination of *E. grandiflora* by moths have not been previously documented in *Espeletia*. Although seed set under nocturnal pollination only was considerably lower than under diurnal pollination (24.5% vs. 73.5%), it is comparable to seed set under diurnal pollination in some Venezuelan *Espeletia* species (Berry and Calvo, 1989, 1994). Thus, the contribution of nocturnal pollination to seed production in *E. grandiflora*, albeit low, is not insignificant. Further studies are required to determine if nocturnal pollination is a trait that is exclusive to our population of *E. grandiflora* or if it is a widespread phenomenon within and among *Espeletia* spe-

cies, since it appears that this aspect of pollination has been generally overlooked in previous studies.

Sturm (1990) hypothesized that the beetle *Phyllotrox aristidis* was a major pollinator of *E. grandiflora* because of its abundance inside the capitula. This hypothesis, however, is not supported by our behavioral observations on *P. aristidis* adults nor by the low percentage of viable seeds produced in the treatment with nylon mesh bags, allowing access by small invertebrates (Table 1); nevertheless, it is possible that the presence of the mesh bag could have affected the beetles' behavior.

#### Visitors and pollinators

In addition to our observations on bumblebee foraging behavior, namely high visitation frequency, longest daily movement among plants, and frequent contact with several stigmata in a capitulum, the high nectar concentration (47.2%, G. Stiles 1992, pers. comm.), as well as the shape, size, and coloration of the capitula of *E. grandiflora* are features that reinforce bee pollination over other pollination syndromes (Kearns and Inouye, 1994).

Some hummingbird species are major pollinators of certain *Espeletia* in Venezuelan Páramos (e.g., *Oxyopogon guerinii* on *E. schultzei*, Berry and Calvo [1989, 1994]). However, our observations on the foraging behavior of the hummingbird species that visited *E. grandiflora* did not indicate such an important role. Birds hovered in front of the capitula to take nectar and rarely touched the stigma, but we do not know if such behavior could vary through the flowering season, among populations of *E. grandiflora*, or in areas with low bumblebee populations. Considering the low availability of pollinators at high elevations (e.g., Primack, 1985), specialization on a particular type of pollinator does not seem like the "best strategy". The higher percentage of seeds produced by hand cross-pollination when compared to control (open) pollination (Table 1) suggests that low pollinator numbers may limit seed production in *E. grandiflora*. In addition, the high pollen/ovule ratios as found here in *E. grandiflora* are usually associated with pollination by a wide range of pollinators and a high incidence of pollen-eating insects (Mani and Saravanan, 1999). Thus, even the small contributions we observed from a wide range of pollinators may be advantageous for plants such as *E. grandiflora* at high elevations.

#### Conclusions

The biology of *E. grandiflora* reported here is similar to that of other *Espeletia* species from the dry Páramos in Venezuela, except for the high growth rate found for juveniles and adults, and the occurrence of nocturnal pollination by moths. *Espeletia grandiflora* also exhibited similar phenological adaptations (i.e., synchronous flowering peak, plasticity in flowering behavior, and long flowering periods), which most likely function to compensate for reduced pollinator availability and to increase cross-pollination success in the Páramos. Although floral morphology reinforces bumblebee pollination over other pollination syndromes, given the climatic constraints for flight activity of pollinators in the Páramos, we suggest that even small contributions from a wide range of pollinators might be advantageous to *E. grandiflora*. Long-term studies on different populations of *E. grandiflora* are required to determine if the

high growth rates for the small plants are representative of the species, to quantify the plasticity of its flowering behavior within and among populations, and to establish if nocturnal pollination is a trait that is exclusive to our population of *E. grandiflora* or if it is a widespread phenomenon within and among *Espeletia* species.

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