Monitoring plant species response to climate change

L. Gratani, L. Varone, M. F. Crescente, A. Bonito & G. Fabrini

Department of Environmental Biology, Sapienza University of Rome P. le A. Moro, 5 00185 Rome, Italy

Abstract

Plant traits and reproductive capability of the natural *Bidens cernua* population at Posta Fibreno Lake Reserve and a newly introduced population were analysed. *Bidens cernua* was included in the Red List of Italian Flora as a critically endangered species (CR) for *Latium* (Italy), because of its extremely local distribution and lack of representation in other protected areas in Italy. At phenological level, the life cycle phases of the new population (P), originating from the seed of the reintroduced plants, were not significantly different in length from those of the natural one (P_{nat}). However, P relative growth rate was 4 per cent higher than of the natural population and P plant dry mass was significantly higher than that of P_{nat}. Moreover, P produced 17 per cent more flower heads per plant than those of P_{nat}. The relatively high SLA (228 ± 27 cm^2 g^{-1}, mean value of P_{nat} and P) underlined *B. Cernua*'s strategy to invest mostly in leaf area over mass, thus increasing the potential capability of light interception. P had the potential to function in a manner that was demographically similar to P_{nat}. The newly created population of *B. cernua* will be monitored over subsequent years to evaluate plant traits and reproductive capability variations in the long term.

Keywords: conservation, growth analysis, reintroduction, threatened species

Introduction

Monitoring plant species' response to climate change has been identified as a crucial component of global change research programmes. Botanic gardens can play a major role in monitoring the conservation status of threatened species, outlining practices for reintroduction. The need for an integrated approach, utilizing both *in situ* and *ex situ* techniques to support wild populations has been promoted by botanic gardens and the European Botanic Garden Strategy underlines the need for specific regional conservation collections linked to protected area activities (Maunder *et al*., 2001). The reintroduction of species into natural or semi-natural habitats is becoming an increasingly popular strategy for conservation, especially when wild population number and size are small and habitat is fragmented (Münzbergová *et al*., 2005). Nevertheless, the success of plant species reintroduction remains a question of population viability, involving demographic, genetic, behavioural and ecological processes (Jusaitis *et al*., 2004; Münzbergová *et al*., 2005). Life history traits of wild species are rarely known, so reintroduction can be risky, leading to high mortality (Drayton & Primack, 2000). Reintroduction of threatened species can be used to either secure or improve the status of protected areas.

Numerous attempts have been made to create new populations of threatened species by translocation (Jusaitis, 1997; Mueck, 2000; Husband & Campbell, 2004). Nevertheless, only a few studies demonstrate their self-sustainability through growth capability, reproduction, and long-term persistence (Jusaitis *et al*., 2004). Knowledge of population trends is an important element in the evaluation of the real extinction risk (Kozlowski, 2008). Thus, the newly created populations should be monitored in subsequent years.

Bidens cernua L. is a nitrophilous species, growing on river banks, lakes and in wet ditches, preferring rich muddy soils, usually with its roots submerged: it is distributed in Europe, Asia and North America (Brändel, 2004). *B. cernua* is included in the Red List of Italian Flora as a critically endangered (CR) species for *Latium* (Italy) (Conti *et al*., 1997), because of its extremely local distribution and the lack of representation in other protected areas in Italy.

The main objective of this research was to compare plant traits and reproductive capability in the *Bidens cernua* natural population growing at Posta Fibreno Lake Reserve, and the reintroduced population in the same area. To this aim, we have also used data collected by Gratani *et al*. (2008) and Gratani *et al*. (2009). A significant threat to small, isolated populations such as this one, is their sensibility to environmental, genetic and demographic stochasticity, according to Morgan (2000). The establishment of plants in secure and managed conservation reserves is crucial both to the survival of many species as their habitats continue to be destroyed, and to increase the number of individuals in a population (Jusaitis *et al*., 2004).

Materials and Methods

Study site

The study was carried out at Posta Fibreno Lake Reserve (*Latium*, 41°42' N, 13°41' E; 290 m a.s.l.), and at the Botanic garden of Rome (41°53′53″ N, 12°28′46″ E; 53 m a.s.l.). *Bidens cernua* wild population grew in a limited area (46.7 m^2), localized along the eastern lake coast (‘Pantano Papiro’ area).

Climate

The climate of Posta Fibreno Lake Reserve is of Mediterranean type, and most of the total annual rainfall (1150 mm) occurs in autumn and winter (Meteorological Station of Arpino and Frosinone, Regional Agency for Development and Agricultural Innovation, data for the period 2004–2007). The mean annual air temperature was 14.6°C.

The climate of Rome is also of Mediterranean type, and most of the total annual rainfall (676 mm) occurs in autumn and winter (Meteorological Station of the Collegio Romano, data for the period 1995–2007). The mean annual air temperature was 16.8 °C.

Seed collection and seedling cultivation

Freshly-matured seeds of *Bidens cernua* L. were collected at the beginning of November 2005 from 100 representative plants growing naturally at Posta Fibreno Lake Reserve, in the ‘Pantano Papiro’ area, and were transported to the Botanic Garden of Rome.

The collected seeds were stratified in a cold chamber at 5 °C for 4 months. Then they were placed in 4 fine-mesh polyester cloth bags, 30 seeds in each, and the bags were buried in moist sand in 9 x 9 cm diameter plastic pots with drainage holes (Brändel, 2004). Seed germination was carried out in light- and temperature-controlled chambers (Type CC7, Amcota), at 15–30 °C and 12-hour day-length, equipped with cool-white fluorescent tubes providing a PFD of 80 µmol m^{-2} s^{-1}.

150 randomly selected seedlings were grown and cultivated in a greenhouse, in black polyethylene plastic pots filled with peat (pH 7). At the beginning of May 2006 they were placed in 5 pools (1 m in diameter and 0.30 m deep), filled with water and put outdoors (Baskin *et al*.,1999).

Reintroduction

At the beginning of June 2006, 150 cultivated seedlings were transferred back to the original area, near the provenance population at Posta Fibreno Lake Reserve (‘Pantano Papiro’ area). Three plots (1 x 1 m), subdivided into 100 grids (10 x 10 cm), were used as a planting template (Jusaitis *et al*., 2004).

New population monitoring

In 2007, the new population (P) originated from the seeds produced by the reintroduced plants was analysed. The phenological cycle, structural plant traits, morphological leaf traits, and reproductive capacity of P were analysed and compared with those of the natural population (P_{nat}) (Morgan, 2000).

Phenology

Phenological observations were carried out weekly, on 30 selected plants of P and P_{nat}, respectively. The times of seedling emergence, stem elongation, flowering, fruiting, and plant senescence were observed.

Flowering head production was analysed at the end of September by counting the number of heads on 100 plants each of P and P_{nat}, respectively. Seed production was determined in November by counting the number of seeds on 50 flowering heads each of P and P_{nat}, respectively.

Growth analysis

Observations were carried out weekly, on 30 selected plants, during the study period. Plant trait measurement included height (H, cm), total plant dry mass (PDM, g), and total leaf area per plant (TLA, cm^2) of P and P_{nat}, respectively. H was measured until its maximum was attained (end of August) in both P and P_{nat}.

At the beginning of September, 30 plants each of P and P_{nat} were harvested and separated into stems, leaves, and roots, and they were transported immediately to the Botanic Garden. Stems, leaves and roots dry mass was measured after oven drying at 80°C to constant mass, and PDM was determined. The above/below dry mass ratio was calculated.

TLA: calculated by multiplying total leaf number per plant by mean leaf area (LA, cm^2). LA was measured with the Image Analysis System (Delta-T Devices, UK).
Specific leaf area (SLA, $cm^2\ g^{-1}$): calculated as LA to leaf dry mass (LM, g).
The relative growth rate in plant height (RGR_H , $cm\ cm^{-1}\ d^{-1}$): calculated, as $RGR_H = \ln H_2 - \ln H_1/t_2 - t_1$, where H_1 and H_2 were plant height at time t_1 (seedling emergence) and t_2 (maximum plant height).
The relative growth rate in plant dry mass (RGR_m, $g\ g^{-1} d^{-1}$): calculated as: $RGR_m = \ln PDM_2 - \ln PDM_1 / t_2 - t_1$ where PDM_1 and PDM_2 were the total plant dry mass at time t_1 (emergences) and t_2 (maximum total plant dry mass).

Statistics

Differences in the considered variables were determined by the analysis of variance (ANOVA), and Tukey test for multiple comparisons. All statistical tests were performed using a statistical software package (Statistica, Statsoft, USA).

Results

Phenology

There were no significant differences in the length of phenological phases between P and P_{nat}: seedling emergence took place in early May, when mean minimum air temperature was ≥ 10.0 °C. Leaves were produced from mid May to end August, when mean maximum air temperature was 29.8 ± 2.6 °C. Flowering started at the end of August, and fruiting from end September to end November. Seed dispersion started at the end of October and the senescence phase was from end October to mid December. Flower head production was significantly ($p \leq 0.05$) higher in P than in P_{nat} (4.2 ± 0.3 and 3.6 ± 0.2 flower head per plant, respectively). Seed production per flower head was not significantly different in P and P_{nat} (116 ± 11 seeds per flower head, mean value) (Table 1).

Plant traits

There were significant ($p \leq 0.05$) differences in H and TLA between P_{nat} and P, the latter having the higher values (47.1 ± .0 cm and 82.7 ± 6.0 cm^2, respectively) (Table 2). There were no significant differences of SLA in P and P_{nat} (228 ± 8 $cm^2\ g^{-1}$, mean value). PDM was 41 per cent higher in P than in P_{nat}, and the above/below dry mass ratio was 6.7 ± 0.4 and 4.7 ± 0.2 for P and P_{nat}, respectively (Table 2).

Growth analysis

RGR_H and RGR_m were significantly ($p \leq 0.05$) higher in P (0.047 ± 0.001 cm cm^{-1} d^{-1} and 0.057 ± 0.001 g g^{-1} d^{-1}, respectively) than in P_{nat} (0.045 ± 0.001 cm cm^{-1} d^{-1} and 0.055 ± 0.001 g g^{-1} d^{-1}, respectively) (Table 1).

Discussion

At phenological level, the life cycle phases of the newly created *B. cernua* population are not significantly different in length from those of the natural one. Seedling emergence occurs when mean minimum air temperature is ≥ 10.0 C°, and the maximum plant height is reached four months later. Vegetative activity finishes when mean maximum air temperature is 29.8 ± 2.6 °C. Plant senescence begins at the end of October and seedlings are completely dry by the middle of December. *B. cernua* flowering starts at the end of August and fruits from the end of September to the end of November. Nevertheless, the new population produces 17 per cent more flower heads per plant than those of the natural one, suggesting that it has some potential for longer-term persistence in the area, according to the results of Morgan (2000).

Plant growth is the result of the interaction between environmental factors and biomass allocation capability. RGR is a useful indicator of the extent to which a species is using its photosynthate for growth (Groeneveld, 1998). Plant species with a high intrinsic capacity to grow possess structures to efficiently capture and process resources from the environment (Cornelissen *et al*., 1998). The mean RGR, averaged over the assimilation period, can be used to characterize a species in terms of productivity (Larcher, 2003).

B. cernua RGR_m (0.055 ± 0.001 g g^{-1} d^{-1}, mean value over the study period of the natural population) is in accordance with the value calculated by Almeida-Cortez *et al*. (1999) for the same species. Nevertheless, RGR_H and RGR_m are 4 per cent higher in P than P_{nat}. Posta Fibreno Lake is a nutrient-rich environment (Montelucci, 1979) favouring a higher *B. cernua* dry mass allocation to leaves and stems (85 per cent of the total plant dry mass) than roots, according to the results of Müller *et al*. (2000). Moreover, the relatively high SLA (228 ± 27 cm^2 g^{-1}, mean value of P_{nat} and P) underlines *B. Cernua*'s strategy to invest more in leaf area than mass, increasing the potential capability of light interception, according to Gratani and Ghia (2002).

The results largely underline that the newly created population of *Bidens cernua*, characterized by a significantly higher plant dry mass than P_{nat}, has the potential to function in a manner demographically similar to the natural one at Posta Fibreno. The establishment of this new population is considered essential for long-term survival of *B. cernua* because its limited distribution in the *Latium* makes it vulnerable. Such a population would spread the risk of localized catastrophic failures, according to the results of Jusaitis *et al*. (2004) in other threatened plant species. The newly created *B. cernua* population will be monitored over subsequent years to evaluate plant traits and reproductive capability variations in the long term. Knowing the potential growth capability of the wild species is an asset in the conservation of native populations, monitoring the conservation status of threatened species and outlining practices for the reintroduction projects.

References

Almeida-Cortez, J.S., Shipley and B., Arnason, J.T. (1999). Do plant species with high relative growth rates have poorer chemical defences? *Functional Ecology* **13**: 819–27.

Baskin, C.C., Baskin, J.M. and Chester, E.W. (1999). Seed germination ecology of the annual grass *Leptochloa panicea* ssp. *mucronata* and comparison with *L. panicoides* and *L. fusca*. *Acta Oecologica* **20**: 571–7.

Brändel, M. (2004). The role of temperature in the regulation of dormancy and germination of two related summer-annual mudflat species. *Aquatic Botany* **79**: 15–32.

Conti, F., Manzi, A. and Pedrotti, F. (1997). *Liste rosse regionali delle piante d'Italia.* Roma: TIPAR.

Cornelissen, J.H.C. Castro-Díez, P. and Carnelli, A.L. (1998). Variation in relative growth rate among woody species. In: Lambers, H., Poorter, H. and Van Vuuren, M.M.I. (Eds.): *Inherent Variation in Plant Growth. Physiological Mechanism and Ecological Consequences*. pp. 363–92. Backhuys Publishers – Leiden.

Drayton, B. and Primack, R. (2000). Rates of success in the reintroduction by four methods of several perennial plant species in Eastern Massachusetts. *Rhodora* **102**: 299–331.

Gratani, L. and Ghia, E. (2002). Changes in morphological and physiological traits during leaf expansion of *Arbutus unedo*. *Environmental Experimental Botany* **48**: 51–60.

Gratani, L., Crescente, M. F., Fabrini, G. and Varone, L. (2008). Growth pattern of *Bidens cernua* L.: relationships between relative growth rate and its physiological and morphological components. *Photosynthetica* **46**: 179–84.

Gratani, L., Crescente, M. F., Fabrini, G., Bonito A. and Varone, L. (2009). Growth analysis of a reestablished population versus a natural population of *Bidens cernua* L. *Plant Biosystems* **143**: S63–68.

Groeneveld, H.W. (1998). Measuring the RGR of individual grass plant. *Annals of Botany* **82**: 803–8.

Husband, B. and Campbell, L. (2004). Population responses to novel environments: implications for ex situ plant conservation. In: Guerrant, E., Havens, K. and Maunder, M. (Eds). *Ex situ plant conservation: supporting species survival in the wild*. Washington: Island Press. pp. 231–66.

Jusaitis, M. (1997). Experimental translocations: implications for the recovery of endangered plants. In: Touchell, D.H., Dixon, K.W., George, A.S. and Wills, A.T. (Eds). *Proceedings of the 4th International Botanic Gardens Conservation Congress, Perth, Western Australia*. pp. 181–196.

Jusaitis, M., Polomka, L. and Sorensen, B. (2004). Habitat specificity, seed germination and experimental translocation of the endangered herb *Brachycome muelleri* (Asteraceae). *Biological Conservation* **116**: 251–66.

Kozlowski, G. (2008). Is the global conservation status assessment of a threatened taxon a utopia? *Biodiversity Conservation* **17**: 445–8.

Larcher, W. (2003) *Physiological Plant Ecology.* Springer-Verlag, Berlin-Heidelberg.

Maunder, M., Lytea, B., Dransfield, J. and Baker, W. (2001) The conservation value of botanic garden palm collections. *Biological Conservation* **98**: 259–71.

Montelucci, G. (1979). Aspetti botanici del Lago della Posta-Fibreno (Lazio). *Lav. Soc. Ital. Biogeogr.* **6**: 1–16.

Morgan, J.W. (2000). Reproductive success in reestablished versus natural populations of a threatened grassland daisy (*Rutidosis leptorrhynchoides*). *Conservation Biology* **14**, 780–5.

Mueck, S.G. (2000). Translocation of the Plains Rice-flower (*Pimelea spinescens* ssp. *spinescens*), Laverton, Victoria. *Ecological Management & Restoration* **1**: 111–16.

Müller, I., Schmid, B. and Weiner, J. (2000). The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspectives in Plant Ecology Evolution and Systematic*: 3: 115–27.

Münzbergová, Z, Mildén, M, Ehrlén, J. and Herben, T. (2005). Population viability and reintroduction strategies: a spatially explicit landscape-level approach. *Ecological Applications* **15**: 1377–86.

Table 1 Mean values (± standard error) of plant traits in *Bidens cernua* newly created population (P) and the natural population (P_{nat})

	Population	
	P	P_{nat}
RGR_H (cm cm^{-1} d^{-1})	0.047±0.001	0.045±0.001
RGR_m (g g^{-1} d^{-1})	0.057±0.001	0.055±0.001
Number of flower head	4.2±0.3	3.6±0.2
Number of seeds per flower head	116±10	115±12*

RGR_H = relative growth rate in plant height, RGR_m = relative growth rate in plant dry mass. Mean values are significantly different ($p \leq 0.05$), except *

Table 2 Mean values (± standard error)of plant traits of *Bidens cernua* in new created population (P) and natural population (P_{nat})

	Population	
	P	P_{nat}
H (cm)	47.1±9.0	41.1±5.4
TLA (cm^2)	82.7±6.0	71.2±10.5
SLA (cm^2 g^{-1})	222±19	233±32*
PDM (g)	2.09±0.3	1.48±0.2
Above/below plant biomass ratio	6.7±0.4	4.7±0.2

H = plant height, TLA = total leaf area per plant, SLA = specific leaf area, PDM = total plant dry mass. Mean values are significantly different ($p \leq 0.05$), except *