International Journal of Biodiversity and Conservation Vol. 4(8), pp. 316-325, 1 June, 2012
Available online http://www.academicjournals.org/IJBC
DOI: 10.5897/IJBC11.225
ISSN 2141-243X ©2012 Academic Journals

Full Length Research Paper

Distribution and abundance of terrestrial orchids of the genus *Bletia* in sites with different degrees of disturbance, in the Cupatitzio Natural Reserve, México

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Accepted 21 May, 2012

In this work, we determined the distribution and relative abundance of terrestrial orchids of the genus *Bletia* in the Cupatitzio Natural Reserve, Michoacán, México, during two flowering seasons. Four different sampling sites were identified according to degree of disturbance and site quality. Relative abundance was established with an arbitrary scale of four values: abundant (>40 individuals), medium (20 to 40 individuals), low (<20 individuals) and null. Three species of orchids were found: *Bletia roezlii* was the most abundant, with populations of over 40 specimens per 1000 m^2 in some sites (zones 1 and 3). It was present in all the reserve's sites, although it showed better development in areas near roads and/or in open sites. *Bletia purpurata* had medium distribution and abundance, with more of 30 plants per site, having more presence in conserves sites (zone 3). *Bletia punctata* had the lowest abundance and distribution, with populations of 10 or less individuals, presenting a mean abundance of low disturbance sites. The distribution of the specimens of *Bletia* spp. is determined by factors such as the degree of disturbance of the sites where they develop, the adaptability of the species, the amount and morphotype of the mycorrhizal fungus, which is associated with their dispersibility of them, among other factors.

Key words: Orchid distribution, relative abundance, disturbance.

INTRODUCTION

Orchidaceae is the most diverse of all angiosperm families, with estimates of <25000 species (Dressler, 1993; Mabberley, 1997; Cribb et al., 2003); and more than any other plant family they have a high proportion of threatened genera, with most containing threatened species. Two-thirds of orchid species are epiphytes and lithophytes, with terrestrial species comprising the remaining third, yet almost half of the extinct species according to The World Conservation Union (IUCN, 1999) are terrestrial herbaceous perennials. Terrestrial orchids thus represent a life form class likely to experience a greater extinction risk as a result of the multiplicity of threatening processes, particularly under current climatic change scenario (Swartz and Dixon, 2009).

An observation of ecological significance is that organisms differ greatly in distribution and abundance; consequently, rare species may be recognized as those

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of low numerical abundance compared with others (Pate and Hopper, 1993; Harper, 1981) classified rare species according to space, time or group relatedness. A space-dependent species may be locally abundant, but only occur in a limited number of sites, restricted due to high niche specificity or barriers reducing dispersal potential. These species are often local endemics, particularly vulnerable to threatening processes. A time-dependent rare species results from fluctuations in population numbers following adverse sporadic or cyclical events, such as drought or fire (Koopowitz et al., 2003). Populations of a rare species, occupying a specialized niche with a limited distribution, represent group-dependent rarity associated with certain ecotypes often at ecological frontiers for species. Orchids are found in all these classes. Although a significant literature exists on the many causes of rarity in plants, drivers of rarity in orchids are more often than not linked to their unique habitats and pollinator requirements. Ecological specialization has not only contributed to the great species diversity in Orchidaceae, but has also resulted in the high level of threat in this family (Cribb et al., 2003). However, it is the complexity of ecological specialization that makes orchids ideal model species for developing and testing conservation strategies.

In Mexico, terrestrial orchids are represented by genera such as *Spiranthes*, *Bletia*, *Govenia*, *Habenaria* and *Malaxis* (Soto, 1988). Among these, one of the most representative neotropical genera is *Bletia*, since its centre of diversification is in Mexico, with approximately 50 known species, out of which 13 have been described in the State of Michoacan, distributed throughout 19 municipalities (Hágsater et al., 2005; Sosa, 2007; data from Herbarium of Ecological Institute, Pátzcuaro, Michoacán, 2008).

Human activities such as logging and agriculture can severely damage forest ecosystems by changing forest structure, ecosystem function, and biodiversity. These changes may have long-lasting consequences, which influence forest recovery. The fact that loss of natural habitats due to changes in land use and other factors such as tree felling, contamination, introduction of exotic species, and collection of wild specimens, the populations of species of this genus, as of the rest of the orchids, have been reduced through time, and so their study is important for conservation purposes. Conservation through reserves alone is now considered unlikely to achieve protection of plant species necessary to mitigate direct losses of habitat and the pervasive impact of global climate change. Some of these species, like *Bletia campanulata* (La Llave are Lex), are able to grow in areas with scarce, disturbed soils, in reforested areas, or in areas with eroded soils. Others, like *Bletia reflexa* Lind., show very limited distribution within the Michoacán State, México (data from Herbarium of Ecological Institute, Pátzcuaro, Michoacán, 2008). Records of species found in different ecosystems are limited to taxonomic descriptions, and only a few studies conducted in Mexico offer information on abundance and distribution. This makes it hard to verify whether there has been an alarming reduction in their population through time, so as to be able carry out acts of conservation or legislation. In addition, it is important to determine the proficiency of different species to adapt the habitat transformation, in order to conserve those inhabited by orchids with the least ability to live in perturbed niches and which are therefore in greatest risk of being lost.

In spite of being a protected area, the Cupatitzio Natural Reserve, located in the municipality of Uruapan, Michoacán state, México (Figure 1), is affected by its proximity of the urban zone of Uruapan. Its neighbouring areas have undergone changes in vegetation cover due to being constantly stepped on by humans, to introduction of exotic species, to changes in land use from forests to horticultural orchards, mainly avocado and peach, and to the presence of plagues that force cutting down diseased trees, giving rise to areas that are more open and allow more light penetration. Whereas areas farthest from the locality and which have more restricted or difficult access, show vegetation that is better conserved and less disturbed.

Identification of sites with different characteristics within the reserve allowed studying the distribution and relative abundance of some species of the genus *Bletia*, with the objective of determining whether they showed changes in these parameters, related to different site quality and disturbance conditions. This will enable us to generate a first diagnosis, which will serve as a basis for conducting subsequent demographic studies that will help conserve the most threatened species and habitats in this reserve.

The study was based on the hypothesis that the species that were found would show broader distribution and relative abundance in less-disturbed sites, but they were also represented in disturbed sites within the same habitat, which reflects their capacity to adapt. Currently, only a few species of this genus are considered threatened or endangered according to Official Mexican Standard (NOM-ECOL-059-2001). Nevertheless, even if they are not officially considered to be threatened, some species show small populations and are scarce within Michoacán State (data from Herbarium of Ecological Institute, Pátzcuaro, Michoacán, 2008).

MATERIALS AND METHODS

Four zones with different degrees of disturbance within the reserve were established (zones 1, 2, 3 and 4) based on 6 recognition trips, marking the boundaries with 50 cm wooden stakes and by taking photographs (Sony DSC-W270 digital camera, 7.2 megapixels). The trips were made on July 29, August 29, September 6, October 10 and 24, and November 18, in 2008 and June16, September 9 and November 28, in 2009. The sampling sites were delimited considering previously described characteristics such as primary and secondary vegetation type (Bello and Madrigal, 1996; Gómez, 2005; Zavala, 2006), soil (Gómez, 1985), depth of humus, light penetration, altitude (GARMIN GPS unit), presence of exotic species, road routes and other infrastructure building sites.

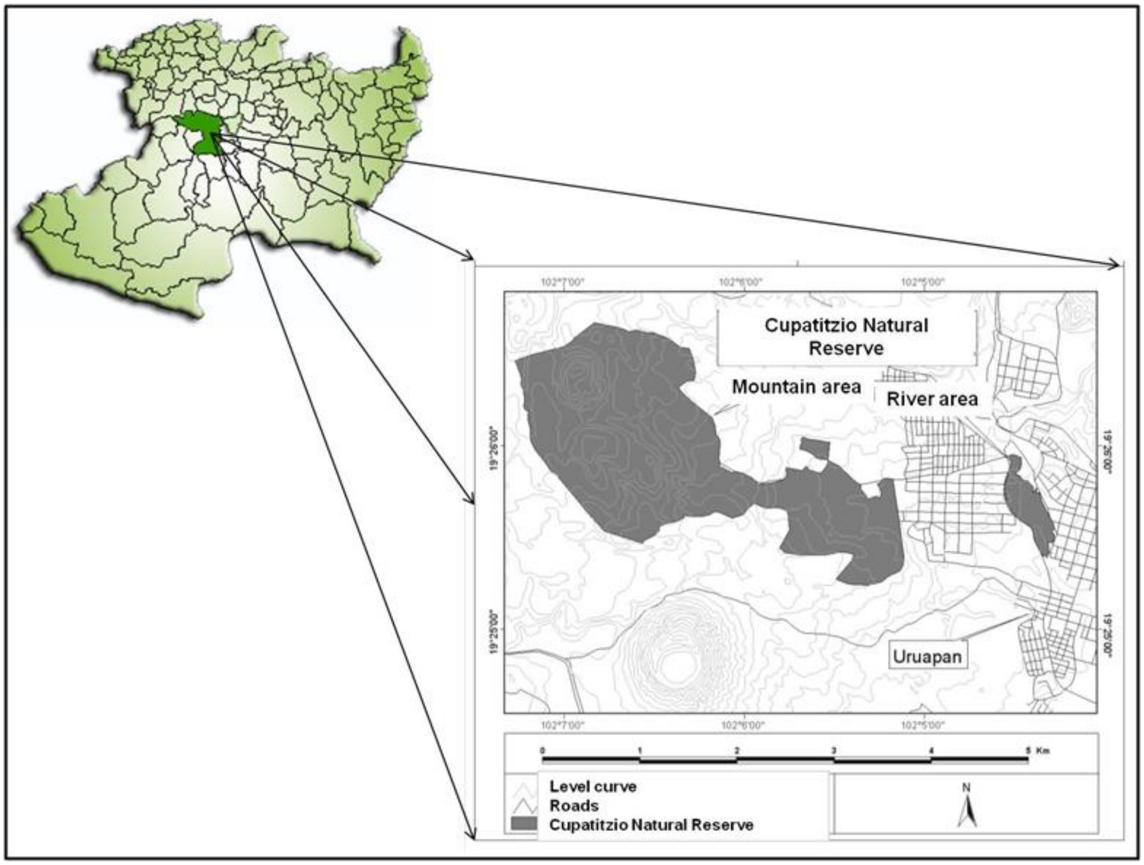

Figure 1. Location of the Cupatitzio Natural Reserve. Dark green represents the municipality of the Reserve. The gray areas represent all the reserve. (Source: Topographic map 1: 50 000 and map of the state of Michoacán 1: 75 000, INEGI).

Boundaries between sites were established considering the main and secondary roads that exist within the reserve (Figure 2).

Sites identified as 1 and 4 are adjacent to main roads that serve as transit route for people visiting the reserve and for the city's inhabitants, in addition to being close to the main access and the urban area. These sites include the orchards and the cabins, which accommodate tourists and researchers (Figures 2 and 3). This has resulted in changes in secondary vegetation, increased light penetration, soil erosion and therefore a greater degree of disturbance.

The sites identified as 2 and 3 are located in the portion of reserve area, near the main road, which is less travelled due to being far and hard to access, where vegetation is denser and less disturbed (Figures 2 to 4).

The four sites show the presence of land formed by volcanic rock types, however this is more abundant in site 4. These soils are defined as ground generated by the rapid cooling and solidification of volcanic lava and one for this characteristics is that they are considered as eroded soils with little water content, and their surface is very rough and it is difficult to navigate through them (Table 1).

Data cards and herbarium specimens of the genus *Bletia* were consulted in the Institute of Ecology of the city of Patzcuaro, Michoacan and the Faculty of Biology of the Michoacana University, with the objective of determining the species that have been recorded near the reserve. Later, during the flowering season (June to November, 2008 and 2009), specimen marking was performed to identify the species and thus be able to determine which locations have populations of these orchids. Marking was conducted using aluminum plates containing number of specimen and species, tied to the plant's pseudobulb with a nylon string.

Species identification was conducted in the Orchid Garden of the of Morelia city (Michoacán, México). Plant geo-referencing was performed with GPS (Garmin® 3 m) during the trips for later positioning in digital images provided by Mexico's National Institute of Statistics and Geography (INEGI) using Autocad 2007 software, where the reserve borders and main topographic features were specified.

Relative abundance was determined quantitatively based on an arbitrary scale of four values conducted in four quadrat 20 × 50 m (1000 m^2) per site, selected on topographic maps based on physical features, where populations of over 40 specimens were considered abundant; 20 to 40 specimens, medium; less than 20 specimens, low; and null when no specimen. The results are reported in average values for each species per site and we performed statistical analyses of variance and Tukey test when the values showed significant differences, using the program JMP V.8.0s could be identified.

RESULTS

According to Gomez-Reyes (2005), four vegetation units were observed: a) arboretum, corresponding to a plantation zone with species of the genera *Eucalyptus, Cupressus* and *Pinus,* located at 2000 m altitude; b) avocado (*Persea americana* L.) and peach (*Prunus*

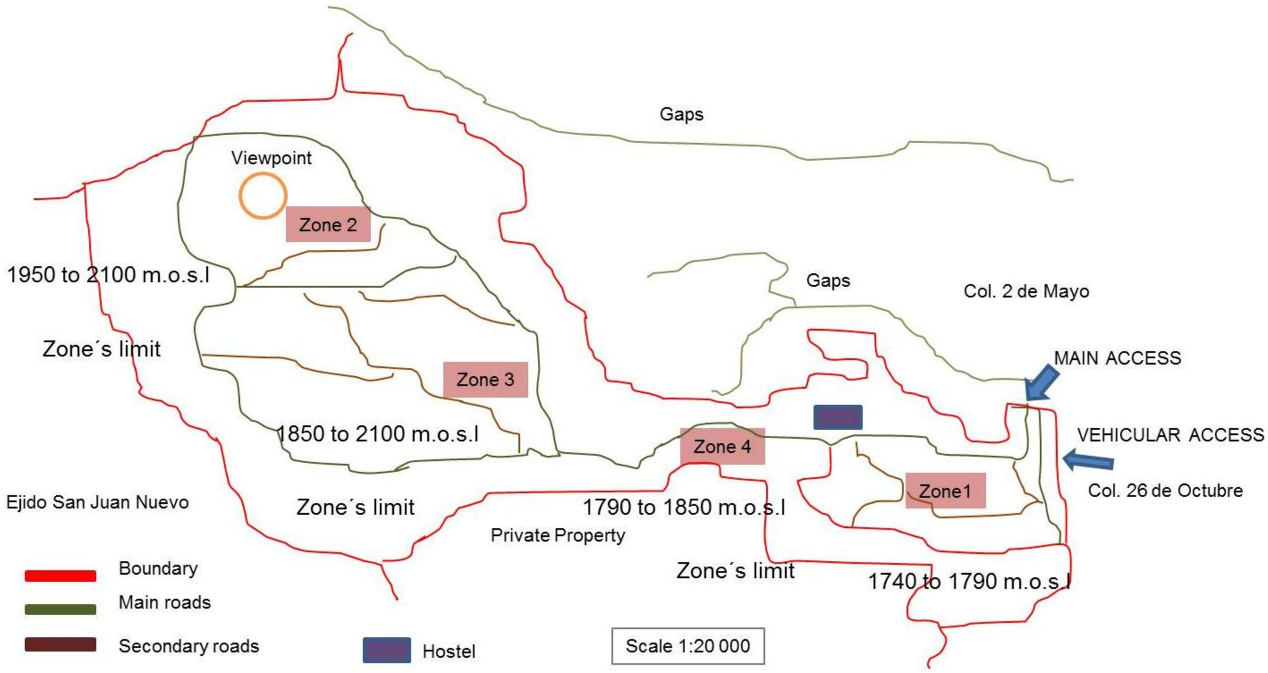

Figure 2. Delimitation of sampling areas in the Cupatitzio Natural Reserve (elaborated based on a Reserve Route Map).

Figure 3. Appearance of sites designated as disturbed (sites 1 and 4). Where roads are observed, the so-called secondary vegetation and badlands (bottom left).

Figure 4. Appearance of sites designated as preserved areas (sites 2 and 3). Where there is dense vegetation, less illuminated and secondary vegetation.

persica L. Stokes) orchards near the main access and the facilities; c) pine forests (*Pinus lawsonii, Pinus douglasiana, Pinus michoacana* and *Pinus leiophylla*), which are the dominant vegetation in the reserve; and d) pine-oak forests, considered to be the original vegetation throughout the reserve, dominated by the previously described pine species and by *P. michoacana* var. *cornuta* and *Quercus obtusata, Quercus castanea*, *Quercus candicans*, *Quercus magnoliifolia* and *Quercus resinosa*.

During the previously mentioned flowering season, only three species were found in the reserve areas: *Bletia purpurata* (A. Rich and Galleoti), *Bletia punctata* (Llave and Lex) and *Bletia roezlii* (Reichb. f. Linnaean) (Figure 5). 1360 specimens that had inflorescences, therefore allowing taxonomic identification, were marked. Plants that did not flower during the trips were not considered for abundance, and so the populations might be

Table 1. Characterization of sampling sites of the reserve area, according to site quality and degree of disturbance.

Zone	Vegetation	Soil (FAO)	Depth of humus	Altitude (m.a.s.l.)	Light penetration	Disturbance	Malpais presence
1	Pine, pine- oak, orchards, exotic vegetation	Lithosol and Andosol in equal proportions	From 1 to 15 cm in some areas outside the roads	1740 to 1770	High	Change of land use, nearby urbanization, introduction of exotic species, people passing through, and looting.	Yes
2	Pine, pine-oak and arboretum	Lithosol and Andosol	From 5 to 15 cm	1950 to 2050	Lower due to denser arboreal vegetation	Low disturbance due to its remoteness and hard access.	Yes
3	Pine-oak and pine	Lithosol	From 5 to 20 cm	1850 to 2100	Lower due to denser arboreal vegetation	Low disturbance, mostly preserves its original vegetation	Yes
4	Pine	Lithosol	From 1 to 10 cm	1790 to 1840	High	Moderate disturbance due to its proximity to the urban zone, little organic matter in soil, and abundant malpais.	Yes

Figure 5. Species found: a) *B. purpurata* (B. Rich and Galeotti), b) *B. roezlii* (B. Rchb. f. Linnaean) and c) *B. punctata* (La Llave and Lex).

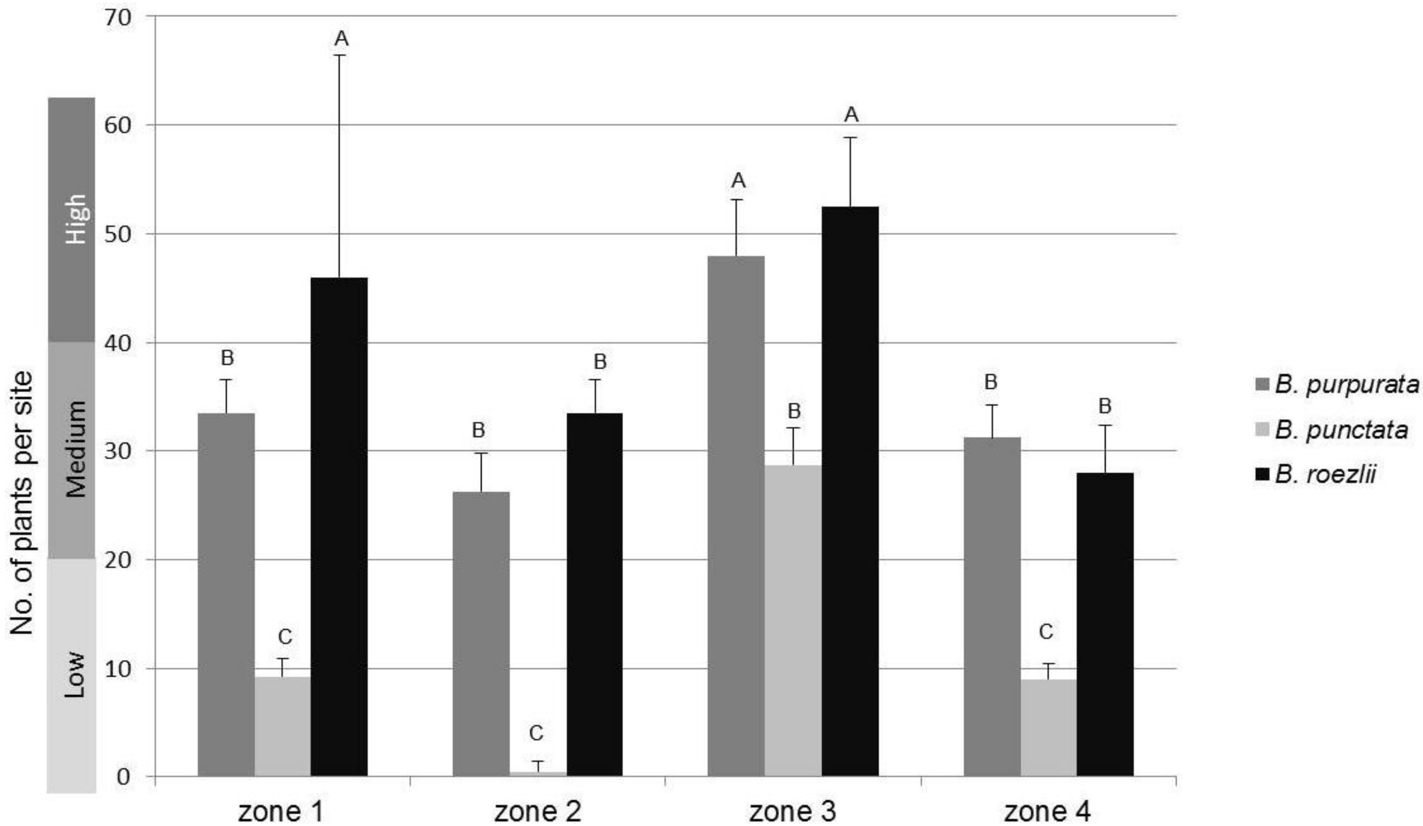

Figure 6. Relative abundance (abscissa) of the three species of the genus *Bletia* in the four study areas, in Cupatitzio Natural Reserve. We present mean values with standard deviation, different letters indicate significant differences (Tukey, P ≤ 0.05).

underestimated.

The most widely distributed and abundant species in the reserve area for the year of sampling was *B. roezlii* (B. Rchb. f. Linnaean), with populations of more than 35 plants per site in the four areas. This species developed well in both very disturbed areas, in sites near of roads or in open areas (zone 1) and of low disturbance areas (zone 3), with 46 plants and 53 plants, respectively (Figure 6). Espejo et al. (2002) mention it in pine-oak forests and in stony soils, as found by Hágsater et al. (2005) in temperate forests.

B. purpurata (B. Rich and Galeotti) was also well represented in the four areas of the reserve, developing better in preserves areas (zone 3), with 48 plants. However, this species presented a medium abundance with 33 and 31 specimen in disturbed sites, zones 1 and 4, respectively (Figure 6). The species seems to adapt well to places with low soil development (Ortega-Larrocea and Rangel-Villafranco, 2007) and/or disturbance (data from the Herbarium of the Ecological Institute, Pátzcuaro).

B. punctata (La Llave and Lex) was hard to locate in most of the study sites and was not found in some of them. In zone 3, site of low disturbance, the abundance was medium with 29 plants. Nevertheless, in zone 2, as well considered a site of low disturbance, the abundance was low with only one plant. Less than 10 plants were found in sites with greater disturbance (zones 1 and 4) (Figure 6).

DISCUSSION

The reasons for the absence or reduced abundance of some species of *Bletia* in the reserve, like *B. punctata* in disturbed areas are not known.

Bletia establishment in these areas may be affected by a number of different factors. These factors can be intrinsic and extrinsic. The intrinsic factors related to limits on abundance and distribution as a result of natural factors. In the case of terrestrial orchids, for example, range and abundance may be driven by factors pertaining to the underground and above-ground life history phases of species (Woolcock and Woolcock, 1984; Clements, 1988; Dixon, 1989). The first need, represented in the underground phase, is a mycorrhizal association with a specific fungal endophyte (Warcup, 1971; Ramsay et al., 1986; Rasmussen, 2002).

A population decrease in *B. punctata* has been described in other habitats in the country where it was believed extinct (Hagsater et al., 2005), and so studies of its populations should be considered in samplings that are conducted over longer time periods. Other species such as *B. purpurata and B. roezlli* are more abundant, and it is possible that lower mycorrhizal specificity could favor their distribution over a wide range of habitats where different mycorrhizal partners are available.

Previous works report that the orchid *B. roezlii* was significantly more colonized than the other two species. There were no significant differences in any mycorrhizal colonization parameter among sites (Beltrán-Nambo et al., 2010). Additionally, this orchid was the most abundant at all sites. It seemed quite possible that the mycotrophic status of this species was not influenced by habitat transformation, resulting in a better adaptation of this species to a larger heterogeneity in soil, light and altitude, or possibly a lower specificity (McCormick et al., 2004).

Conversely, the orchid's *B. purpurata* and *B. punctata* were more affected in their mycotrophic as a consequence of habitat transformation, such as an increase in light resources when the forest was replaced by exotic species (Beltrán-Nambo et al., 2010). Habitat degradation is comprised not only of changes in plant coverage, but species composition and soil erosion processes. Indirect changes in light availability for orchids that grow in shade forest and their dependence on mycotrophic could be indirectly affected. The response of orchids to such transformations is not known or extensively documented.

The fact that some species were more abundant at all sites and that others seemed sensitive to habitat transformation could be due in part to changes in fungal partners or the status of mycorrhizal colonization. The abundance or distribution of orchids could be influenced by mycorrhizal dynamics and root colonization levels in response to several factors.

The second need is an effective pollination/fertilization in the above-ground phase (Stoutamire, 1983; Roberts, 2003). The great taxonomic diversity of Orchidaceae is often attributed to specialization of these two requirements, either independently or in combination, the effects of which place species on a theoretical risk continuum from low to high in the event of environmental and habitat change.

Orchids produce vast numbers of minute seeds lacking storage reserves, such as an endosperm, found in many other angiosperms (Arditti and Ghani, 2000; Batty et al., 2000). Seed recruitment success varies from species to species, with some temperate terrestrial species possessing a requirement for stratification or ageing in soil to release dormancy (Stoutamire, 1974).

Production of large numbers of small seeds favours high dispersal rates, plant fecundity and expression of genetic variability across geographical and ecological boundaries while minimizing parental investment per seed (Batty et al., 2002; Zettler et al., 2003). Although abundant seed is released, few germinate, and even fewer develop into mature plants. Under natural conditions, seeds of most terrestrial orchid species will germinate only in association with a compatible mycorrhizal fungus (Warcup, 1981; Ramsay et al., 1986; Arditti et al., 1990). Due to limited food reserves, orchid seeds have a complete dependency on nutrients supplied by the mycorrhizal association during early germination and seedling establishment phases (Rasmussen, 1995), although some species may substitute or acquire new mycorrhizal associates depending upon plant maturity (Bidartondo and Read, 2008).

Rangel-Villafranco and Ortega-Larrocea (2007) in a preliminary study for the mycorrhizal fungi diversity associated to germination seed in terrestrial orchids in southern Mexico City, found that it looks like some genera are highly specific for their mycobiont as the couples *Bletia* - *Epulorhiza, Dichromanthus* – *Ceratorhiza, Habenaria* – *Epulorhiza,* and *Malaxis* – *Epulorhiza*. More evidence is required to confirm whether this specificity always occurs in nature, due to the fact that some symbiotic cultures can be developed *in vitro* with different isolates. Bioassays confirm specificity at some levels, they noticed that *Bletia* species are less specific for both genus isolates than *Dichromanthus.* However, results for the *in vitro* propagation must be interpreted carefully and do not reflect the specificity in nature. *In situ* bioassays demonstrate that specificity can be developed through the life history of the plant. The *Bletia* spp. can be probably less dependent on fungi for *in vitro* germination and less specific because they photosynthesize rapidly; but in nature, populations are more endangered than *Dichromantthus* and seedlings are very difficult to observe where asexual corm propagation is common (Ortega and Rangel, 2007).

This agrees with the fact that of the three species found in *Bletia* in the sampling years (2008, 2009), *B. punctata* (Llave and Lex) was the one with lower distribution and abundance. It is possible that this species has a lower capacity to adapt to the transformation of their habitat because in sites that were identified with some degree of disturbance, its presence was little or none.

This coincides with that is the specie of lower distribution in the state. It has been described in only two municipalities. *B. roezlii* has been recorded in four municipalities and *B. purpurata* in eight municipalities (data from the Herbarium of Ecology Institute, Pátzcuaro, 2008; Herbarium of Biology Faculty, Michoacana University, 2008). All these factors may influence the distribution of the three species of *Bletia* in the reserve, and these results can be compared with the data obtained from Bergman et al. (2006), who described the analysis of the *W. calcarata's* abundance in the different historic canopy cover class areas that showed that the orchid was most abundant in areas which had been minimally impacted by human activity like the result in the present study.

Conclusions

This study allows concluding that disturbance affects the distribution of three *Bletia* species (*B. punctata, B, purpurata* and *B. roezlli*). They showed the highest distribution and abundance in sites with a low disturbance and a low number of plants in sites with higher degree of disturbance*. B. punctata* presented the lower distribution and abundance. It is possible that this species is less able to adapt to habitat transformation, since its presence was very low or null in areas with some degree of disturbance. An understanding of ecosystem dynamics and the role of interactions between plants and their environment are essential to plant conservation (Schemske et al., 1994). In the case of *Bletia*, we have found that land-use history dramatically affects the suitability of sites for the occurrence of the orchid.

Anthropogenic processes often also accelerate environmental and habitat change (kick-on effects), adversely impacting environmental conditions necessary for sustaining orchid populations. These include such factors as spread of disease and pests, changed fire regimes, salinization and desertification (Sahagian, 2000). Extrinsic factors with knock-on effects pose some of the most significant and pervasive of all threats to orchid conservation, particularly in the face of climate change (Dixon et al., 2003).

Prospective studies such as this one are important because they provide basic information on the relative abundance and adaptation degree exhibited by some species of orchids. However, population studies and interactions with other organism must be designed through the time, so as to generate useful data for future conservation and/or restoration practices.

ACKNOWLEDGEMENTS

We dedicate our cordial thanks to Biol. Nuria Gómez Dorantes and Q.F.B Yolanda Mendez Sántiz for their support during field trips.

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