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Interacciones positivas como determinantes del establecimiento y
desempeño de orquídeas en la zona Mediterránea
de Chile central

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a Ariel

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Capítulo 1: Introducción

1.1. Interacciones positivas entre plantas

Las interacciones entre plantas son muy importantes en los procesos de estructuración y organización de las comunidades vegetales ya que incrementan la probabilidad de coexistencia de las especies. Según su efecto en las comunidades, estas interacciones se dividen en: negativas o de competencia y positivas o de facilitación (Brooker 2006; Lambers *et al.* 2008) (Fig. 1.1). Por mucho tiempo, la única interacción considerada primordial en la supervivencia, desarrollo y reproducción vegetal fue la competencia (Callaway and Walker, 1997). Sin embargo, diversos estudios evidenciaron el rol que tiene la facilitación en la estructura y dinámica de muchos ecosistemas, y actualmente se la incluye en varios modelos ecológicos (Bruno *et al.* 2003; Michalet *et al.* 2006; Michalet and Pugnaire 2016; Bulleri *et al.* 2016). Entre las interacciones de facilitación, las más estudiadas son las que involucran especies llamadas “nodrizas” que generan condiciones microclimáticas menos estresantes para las plántulas que crecen bajo su dosel incrementando su supervivencia y desarrollo (Padilla and Pugnaire 2006).

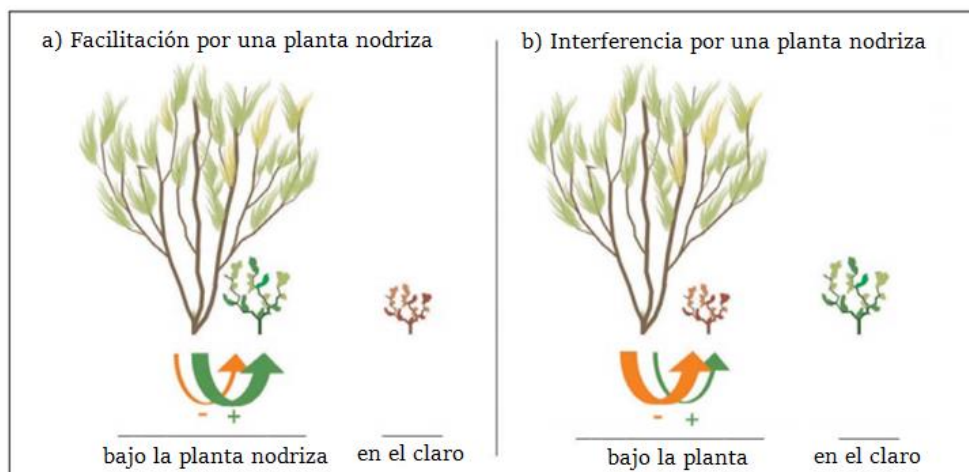


Fig. 1.1. Facilitación e interferencia bajo plantas nodriza; a) Los efectos positivos sobrepasan a los negativos ocasionando un incremento en el establecimiento y crecimiento de plántulas bajo la nodriza. b) Los efectos negativos sobrepasan a los positivos causando un menor desempeño en las plántulas bajo la nodriza (Padilla & Pugnaire, 2006).

El resultado entre las interacciones de competencia y facilitación está muy influenciado por las condiciones bióticas y abióticas, como lo señala la hipótesis del gradiente de estrés (Bertness and Callaway 1994). Esta hipótesis sostiene que las interacciones de facilitación aumentan a lo largo de un gradiente creciente de estrés, mientras que la importancia de la competencia disminuye. Esto fue observado por diferentes estudios realizados en zonas con elevado estrés como zonas de alta montaña (Cavieres *et al.* 2007), semiáridas (Maestre *et al.* 2001; Pugnaire and Luque 2001), desérticas (Franco and Nobel 1989; Drezner 2006), marismas (Bertness and Hacker 1994), árticas (Dormann and Brooker 2002) y antárticas (Molina-Montenegro *et al.* 2013). Sin

embargo, algunos estudios indican que el rol de la facilitación puede colapsar en ambientes excepcionalmente extremos y/o con condiciones muy severas (ver Michalet *et al.* 2006). Esto debido a que otros factores, además de las condiciones ambientales, pueden influenciar el resultado neto entre ambas interacciones, como ser rasgos de vida de las especies y tipo de estrés (Maestre *et al.* 2009).

1.2. Ecosistema mediterráneo chileno con estrés biótico y abiótico

El clima de tipo mediterráneo pertenece a la zona extra tropical y se encuentra representado sólo en un 2% del área terrestre. Estas regiones se hallan localizadas en los bordes occidentales de los continentes entre los 30 y 45° de latitud norte y sur, como consecuencia de patrones de circulación atmosférica y corrientes oceánicas frías (Jørgensen 2009). Cinco regiones del mundo presentan clima mediterráneo: centro sur de California, provincia del Cabo en Sudáfrica, sudoeste de Australia, cuenca mediterránea y centro de Chile (Blondel and Aronson 1999).

En Chile, el tipo bioclimático mediterráneo se halla extendido a lo largo de 5 regiones (**Tabla 1.1**), y se caracteriza por precipitaciones concentradas en invierno y un periodo de sequía durante 7 a 8 meses.

Tabla 1.1. Características del clima mediterráneo de Chile

| Clima mediterráneo en Chile | |
|------------------------------------|--|
| Localización | 32° a 38° latitud S |
| Regiones incluidas | Valparaíso, Metropolitana, O'Higgins, Maule y Bio-Bio |
| Temperatura | 16°C promedio anual |
| Precipitación | 560mm promedio anual |
| Vegetación | Norte: matorrales desérticos Centro: matorrales y bosques espinosos, esclerófilos y caducifolios Alta cordillera: matorrales y herbazales Sur: estepas y pastizales |

(Dallman 1998; Luebert and Pliscoff 2006)

El clima mediterráneo induce estrés en las plantas debido a cambios bruscos en los patrones climáticos que ocasionan una variación temporal en las condiciones abióticas y en la disponibilidad de recursos. El estrés abiótico más severo para las plantas que habitan estas zonas es la sequía, la cual limita el establecimiento y desempeño fisiológico, además de restringir la productividad, el crecimiento y la supervivencia (Jørgensen 2009). Por otro lado, un tipo de estrés biótico presente en este ecosistema es la herbivoría o remoción de partes de plantas por consumo animal (Gómez-Aparicio *et al.* 2008), con efectos en los procesos de dispersión, crecimiento, reproducción y morfología de las plantas (Blondel and Aronson 1999). Además, los efectos de la herbivoría son mayores cuando la planta está sometida a estrés abiótico. La tolerancia a la herbivoría es un mecanismo de las plantas para minimizar los potenciales efectos nocivos de los herbívoros, y puede estar compensada cuando las plantas se encuentran libres de interacciones de competencia y presentan buena disponibilidad de nutrientes y agua (Strauss and Agrawal 1999).

Una asociación espacial positiva en estas regiones estresantes sugeriría que las plantas presentan una interacción de facilitación. En la región mediterránea chilena se ha observado la presencia de orquídeas asociadas con arbustos del género *Baccharis*. Por ejemplo, la orquídea *Bipinnula fimbriata* (Fig. 1.2) creciendo bajo el dosel de arbustos de la especie *Baccharis macraei*.



Fig. 1.2. Especie de estudio y distribución geográfica. a) Inflorescencia de la orquídea endémica *Bipinnula fimbriata*, b) distribución (en rojo) de la orquídea a lo largo de la costa mediterránea chilena (Novoa *et al.* 2015).

Entonces, dado que la zona mediterránea de Chile central presenta un alto estrés abiótico y biótico, se espera que las orquídeas que crecen bajo el dosel de los arbustos tengan mayor supervivencia, crecimiento y desempeño fisiológico en comparación con aquellas que crecen en los espacios abiertos. Además, dado que las plantas que se encuentran en mejores condiciones abióticas son capaces de tolerar de mejor manera la herbivoría, se espera que las orquídeas facilitadas presenten mayor tolerancia a la herbivoría.

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Capítulo 2 : Top-down and bottom-up effects deployed by a nurse shrub facilitating an endemic Mediterranean Chilean orchid

El contenido de este capítulo está en revisión en la Revista "Perspectives in plant ecology, evolution and systematics" como: Baldelomar, M & Molina-Montenegro, M.A. (2018) Top-down and bottom-up effects deployed by a nurse shrub facilitating an endemic Mediterranean Chilean orchid

2.1. Abstract

Patterns of positive associations between seedlings of one species and sheltering adults of another species are known as the "nurse-plant syndrome". This facilitative association is specie-specific and can have direct top-down (canopy) or bottom-up (soil) effects. Besides these direct effects, the nurse plant can give indirect effects like protection against herbivory. The main objective of our study was to determine the positive interaction between the facilitated endemic Chilean orchid *Bipinnula fimbriata* with the nurse shrub *Baccharis macraei* and their direct and indirect facilitative effects in the Mediterranean Coastal ecosystem of Central Chile. To assess whether *B. macraei* acts as a nurse species, and the putative direct/indirect effects, we conducted field and greenhouse experiments. In the field, we realized manipulative experiments assessing the canopy and soil effects. We measured microclimatic variables and edaphic traits within and outside nurses, and assessed survival, growth and photochemical efficiency of PSII (Fv/Fm) in the facilitated species. In the greenhouse, we performed experiments with simulated herbivory in orchids individuals arranged mimicking the nurse and open spaces conditions. We measured survival, flowering percentage, Fv/Fm, growth rate and jasmonic acid in the facilitated orchid species. We found more soil moisture and nutrients under the nurses, and higher photosynthetically active radiation (PAR), soil and air temperature in open spaces. Survival, growth and Fv/Fm were significantly higher in plants under the nurses than in open spaces, with the top-down (canopy) effect induced by nurses was more important than the bottom-up (soil) effect. In the greenhouse experiment, we found higher survival, Fv/Fm, flowering and growth in orchids individuals in the nurse-like condition, independently of the imposed herbivory. Jasmonic acid was significantly higher in those individuals with herbivory and mainly in the open spaces condition. We conclude that *B. macraei* acts as a nurse for *B. fimbriata* as assessed by an improvement in microclimatic and edaphic conditions. In addition, nurses improved the fitness and physiological status in this native orchid as well as enhancing the tolerance against herbivory, by means of their top-down and bottom-up effects, resulting in an enhanced probability of establishment and spread of this native Mediterranean orchid.

Keywords: Facilitation, positive interactions, top-down, bottom-up, herbivory tolerance, Mediterranean ecosystem.

2.2. Introduction

Plant interactions have been pointed as a crucial driver for plant community composition and diversity. The role of positive interactions in population and community dynamics has received significant attention (Hacker and Gaines 1997; Stachowicz 2001; Brooker *et al.* 2008; McIntire and Fajardo 2014; Cavieres *et al.* 2015; Michalet and Pugnaire 2016) and is now widely recognized and included in many ecological models (Bruno *et al.* 2003; Michalet *et al.* 2006; Bulleri *et al.* 2016; Wright *et al.* 2017). Positive interactions between plants are usually stated as facilitation, which co-occur with competition within the same community and their interplay can drive population dynamics, community structure and ecosystem functions (Brooker *et al.* 2008, Pugnaire 2010). The balance among facilitation and competition within a community varies with the life stages (Amghar *et al.* 2016), physiology and life-history of the interacting species (Maestre *et al.* 2009), indirect interactions with other plants (Choler *et al.* 2001), and the intensity of abiotic and/or biotic stress (Callaway and Walker 1997; Michalet *et al.* 2006; Wright *et al.* 2015).

Facilitation has been defined as an interaction that benefits one species, and can have a beneficial, neutral or even harmful outcome to the facilitator specie of the same trophic level (Callaway 2007; Molina-Montenegro *et al.* 2016a), but as long as the effect on the other species is positive (*sensu* Bronstein 2009) it is called facilitation. Therefore, the modes of facilitation can be mutualistic (+/+) in a symmetric relationship or commensalistic (+/0) in an asymmetric relationship (Brooker *et al.* 2008; Lin *et al.* 2012). The best-known examples of plant-plant facilitation involve a plant that ameliorate the harsh environmental conditions compared with the surrounding environment and/or gives protection from biotic or abiotic stress, enhancing the germination, growth and/or survival of seedlings in their immediate proximity (Callaway 1995). Patterns of positive associations between seedlings of one species and sheltering adults of another species became commonly referred as the "nurse-plant syndrome" (see Callaway 1995). In these interactions, the seedlings benefit from the microenvironment created by the adult plant (Flores and Jurado 2003), which can alter its environment both above ('canopy or top-down effect') and below-ground ('soil or bottom-up effect') that can be interrelated (Gómez-Aparicio *et al.* 2005). The 'top-down effect' includes mainly microclimatic amelioration and protection from herbivores (Denyer *et al.* 2010), whereas the 'soil effect' involves the modifications that vegetation produces on chemical, physical and biological soil properties (Mihoč *et al.* 2016). The importance of canopy and soil effects can vary depending on the considered species, since the nurse-protégé interaction can have top-down or bottom-up effects that can differentially affect the physiological aspects of the beneficiary plant (Gómez-Aparicio *et al.* 2005). Besides the direct effect of the nurse over the amelioration of the abiotic stress, it is also important to consider the indirect effects that may arise because of enhanced tolerance against herbivory, due, for example, to increased nutrients when associated with the nurse. Therefore, to understand the real scope of the facilitation interaction, it is important to differentially study both direct and indirect effects. Surprisingly, the majority of studies consider only direct effects (but see Acuña-Rodríguez *et al.* 2006), but we understand the necessity of the evaluation of indirect effects, particularly in the Mediterranean zone, where biotic stress as those induced by herbivory is present.

Among the facilitation mechanisms, we can mention temperature buffer, enhanced soil moisture and nutrients, and physical protection from herbivory, which are included in both top-down and bottom-up effects (Callaway 1995; Flores and Jurado 2003). Microclimate under the

nurse canopy is characterized by less extreme irradiance (Semchenko *et al.* 2012), less evaporative water losses (Molina-Montenegro *et al.* 2005), and higher soil nutrient content (Cavieres *et al.* 2008). In addition, canopies of benefactor species can diminish damage to beneficiary species by repelling herbivores with specialized structures such as thorns, or by physically hiding them (Callaway 1995; García and Obeso 2003; Louthan *et al.* 2014). Besides physical protection against herbivores, the nurse plant can also enhance the plant tolerance. Plant tolerance to herbivory has been defined as the ability to reduce the impact of damage on plant fitness (*sensu* Strauss and Agrawal 1999), and it can be measured using either naturally occurring or manipulated herbivore damage (see Tiffin and Inouye 2000). In this way, nurse plants might affect the tolerance of the beneficiary species in a positive way given that the highest level of tolerance is achieved in environments released from competition and/or with low abiotic stress (Strauss and Agrawal 1999, but see Wise and Abrahamson 2005 for an alternative idea). The potential mechanisms of tolerance given by the nurses include increased leaf level photosynthetic activity, compensatory growth, activation of dormant meristems, utilization of stored reserves and phenological changes (Tiffin 2000; Milchunas and Noy-Meir 2002). In addition, jasmonic acid (JA) has been shown as a damage-inducible compound that elicits a diverse suite of defense response when a plant is exposed to herbivory or mechanical damage (McCloud and Baldwin 1997). Facilitation is expected to be more important in plant communities with high abiotic stress or consumer pressure (Bertness and Callaway 1994). Therefore, is thought to be more frequent in harsh than in mild environments (but see Holmgren and Scheffer 2010) and appears to occur frequently in deserts and arid and semi-arid biomes (Flores and Jurado 2003). The Chilean Mediterranean ecosystem is one of the 25 biodiversity hotspots (Myers *et al.* 2000), and because of its bioclimatic conditions, like summer drought (Cowling *et al.* 1996; Rundel *et al.* 2016) and high herbivory (Quinn 1986; Gutiérrez *et al.* 2010), is considered a zone with high abiotic and biotic stress. Additionally, this ecosystem has been extensively altered by human activities and is subjected to erosion, plant cover removal, and presence of introduced herbivores like rabbits and cattle (Mancilla-Leytón 2014). Because of the relevance of drought as a stress factor, the amelioration of extreme summer climatic conditions by shrub canopies has been proposed as a key facilitation mechanism in many Mediterranean ecosystems (Gómez-Aparicio *et al.* 2008; Molina-Montenegro *et al.* 2016b). The *Baccharis* genus has many species that act as nurses in different places, particularly, in Mediterranean Chile (Macek *et al.* 2017, Cuevas *et al.* 2013). It was previously observed (Atala *et al.*, unpublished data) a positive spatial association between the endemic orchid *Bipinnula fimbriata* and native shrubs of the *B. macraei* species in the Coastal areas of the Mediterranean zone of Chile, therefore, suggesting that these shrubs are acting as nurses for this endemic orchid.

The main goal of this study was to determine whether the nurse species *B. macraei* facilitate to the native orchids *B. fimbriata* in the Mediterranean region of Chile by direct (top-down and/or bottom-up) and indirect (increased tolerance to herbivory) effects. To accomplish this goal, we conducted manipulative experiments in field and greenhouse. Specifically, in the field, we performed a manipulative experiment with division between canopy and soil effects. We analyzed the amelioration of microclimatic conditions and the enhancement of fitness and physiological status in orchid individuals growing inside nurses or in open areas. In the greenhouse, we performed an experiment with simulated herbivory in orchids plants located in nurse-like and open spaces conditions to assess the indirect nurse effect by the enhanced tolerance against herbivory.

2.3. Materials and methods

2.3.1. Study site and target species

We conducted our study in the coastal cliffs zone of Laguna Verde, Central Chile (33°06'S, 71°40'W). This zone has a Mediterranean climate characterized by dry summers and rainfall concentrated during the mild winter, with mean annual temperatures ranging from 15°C to 20°C and annual precipitation ranging from 413.1 to 460mm with frequent water condensation in the mornings due to sea water evaporation and low clouds (Dallman 1998, Luebert and Pliscoff 2006, www.meteochile.cl). The dominant vegetation in the zone is represented by Coastal Mediterranean sclerophyllous forest dominated by *Lithrea caustica*, *Cryptocarya alba* and *Peumus boldus*. The shrub-like stratum is mainly composed of *Sophora* sp., *Lobelia* sp., *Puya* sp., *Crinodendron* sp., *Jubaea* sp., *Retanilla* sp. and *Baccharis* sp. (Pliscoff and Fuentes 2008). Other plants observed in the zone include terrestrial bromeliads like *Puya* spp., cacti and other annual species. However, the zone is found usually degraded with presence of *Pinus* sp. plantations and introduced *Acacia* and *Eucalyptus* species.

The facilitated specie is the orchid *Bipinnula fimbriata* which is endemic to Chile with a distribution of 800 km along the central coastline (between 30° and 36°S) (Novoa *et al.* 2015). This species is perennial herb with a basal rosette and a flower spike that can reach 70 cm height containing many flowers. Such flowers present fimbriae sepals (hence is Spanish common name “flor del bigote”, meaning “moustache flower”) and greenish spots-like coloration in the label (Novoa *et al.* 2015). The study was done during its growing season between April and November 2017, when the aerial development of the orchid was visible. The nurse species is the evergreen dioic shrub *Baccharis macraei* belonging to the Asteraceae family with simple and alternate leaves, capitulate white flowers and achene fruits (Riedemann *et al.* 2014).

2.3.2. Microclimatic measurements

To assess whether *B. macraei* modifies the microclimatic conditions under its canopy, we measured air and soil temperature, soil moisture, and photosynthetic active radiation (PAR) within and outside nurses. In addition, we measured the nutrient content, pH and organic matter in the soil within and outside nurses. The temperature measurements were realized 10 cm above ground and 10 cm below ground with a digital thermometer (Vichy, Model DM6804A+) every month for four months with 10 repetitions per microsite. Soil moisture was measured 15 cm below ground with a soil moisture sensor (Model ISR-300, Irrrometer Company, Riverside, California-USA) after a 2h period of stabilization once a month for four months. We measured PAR with a light meter (Li-cor model Li-250) once per month for four months, every measurement was done at 14:00 pm with 20 repetitions per microsite.

To analyze soil characteristics between microsities (under and outside nurses) we sampled 300 g of soil at 10 cm depth with 5 repetitions per microsite. Afterward, we analyzed: pH, organic matter, nitrates, ammonium, available nitrogen, available potassium, interchangeable potassium and phosphorus in all soil samples. All soil measurements were conducted in the Laboratory of Soils Analyses at the Universidad de Concepción, Chillán.

2.3.3. Manipulative field experiment

We conducted a transplant experiment to evaluate the nurse effect by *B. macraei* shrubs on ecophysiological performance and fitness-traits of the native orchid. All the 100 rosettes considered for the study were excavated and left without the soil around the roots. Although there was a difference between the initial sizes of the rosettes, we represented all sizes within each treatment and was not difference in the initial size among treatments ($F_{96,3} = 2.13$; $p = 0,62$). We performed 4 treatments: i) rosettes with nurses' soil put in open spaces (ON), ii) rosettes with nurses' soil put under the nurses (NN), iii) rosettes with open spaces soil put in open spaces (OO), and iv) rosettes with open spaces soil put under the nurses (NO), following the transplant methodology realized by Molina-Montenegro *et al.* (2016). We put each rosette inside plastic pots with 3 kg of the correspondent soil, according to the treatment, resulting in 25 plants per treatment. We selected 25 visually healthy nurses, similar in diameter (between 1,0 - 1,5 m of diameter), with enough space underneath their canopies to fit the pots. We located 2 pots at the middle portion between the trunk and the edge of canopy projection of each nurse and 2 pots in open spaces near the nurses (between 2 - 3 m from canopy edge of any nurse). The nurse shrubs were separated approximately 5 m apart, and each pot in both under canopy and in open spaces had 30 cm between them to avoid overlapping. The treatments located outside the shrubs were protected with a wire mesh cylinder (1 m diameter x 1 m tall, 25 mm mesh) supported by wood sticks buried in the ground. This was done to imitate the shrub canopy protection against natural herbivory in the zone and avoid damage to experimental plants. Final measurement showed not differences between percentages of aerial herbivory between treatments located inside and outside canopies ($F_{3,1} = 11,8$; $p = 0,29$). The four treatments were considered as a group with ten replicates per each group. The transplants were realized at the beginning of the growing season.

The experiment lasted for 7 months and survival and growth data were assessed as fitness parameters and photochemical efficiency of PSII (F_v/F_m) was considered as a proxy of physiological status. The survival was recorded monthly, considering that the plant was dead if it presented more than 90% of damaged tissue. Growth was calculated as the difference between the initial and final rosette leaves diameter, we took photographs of the rosettes and estimate the diameters with the program Image J. The F_v/F_m ; where $F_v = [F_m - F_0]$, F_m = maximum fluorescence yield, and F_0 = minimum fluorescence yield) (Molina-Montenegro *et al.* 2013) was estimated with a fluorometer (Handy and Pocket PEA Chlorophyll, Hansatech Instruments) in one visually healthy leaf per each individual at midday every month.

2.3.4. Greenhouse experiment

To assess if the nurse effect induced by the shrubs *B. macraei* enhance the tolerance to simulated herbivory in the native orchid *B. fimbriata*, we performed a greenhouse experiment with simulated herbivory damage to rosettes under nurse-like and open spaces conditions. The experiment consisted in 4 treatments (detailed below), differentiated by the moisture, edaphic factors and herbivory damage. We collected 48 rosettes with the soil around the roots from the study site and transported them to the greenhouse located at the Universidad de Talca, Chile.

Then, we put the rosettes in 0,5 L plastic pots filled with soil from each microsite and sand (2:1 v/v) and left them a week with daily irrigation for stabilization.

In order to generate both nurse-like and open spaces conditions, we tried to keep the conditions of each microsite and hence we brought the soil from beneath nurses and from open spaces. In addition, we determined that a watering with 40 ml every two days in 0,5 L pots would represent the soil moisture underneath the nurse, and 20 ml every two days in 0,5 L pots would represent the moisture from open spaces (see **Table 1**). To achieve these watering values, we conducted a manipulative experiment in the same 0,5 L pots filled with soil from different microsities and then watering with 5 different volumes (20, 40, 60, 80 and 100 ml) every two days for two months. Using a soils moisture sensor (Model ISR-300, Irrrometer Company, Riverside, California-USA) we determined soil moisture and thus selected the final watering amount for each treatment.

Half of the individuals ($n = 48$) were put in pots with soil from nurses and irrigated with 40 ml and the other half ($n = 48$) were put in pots with soil from open spaces and irrigated with 20 ml, thereby representing the nurse and open spaces conditions, respectively. Four weeks later, we imposed 25% of herbivory damage –mimicking the average herbivory percentage observed in the field (data not shown)- at half of the rosettes in both conditions (nurse-like and open spaces). To achieve this percentage of damage we cut 50% of the foliar tissue with scissors along the mid vein in the half of leaves of each rosette following the methodology used by Acuña-Rodríguez *et al.* (2006). Therefore obtaining 4 treatments: 12 orchids in nurse-like conditions without herbivory (N), 12 orchids in nurse-like conditions with herbivory (NH), 12 orchids in open spaces conditions without herbivory (O) and 12 orchids in open space conditions with herbivory (OH).

We measured every week the survival, flowering plants and the photochemical efficiency (Fv/Fm) as fitness parameters, as well as jasmonic acid concentration in the leaves and growth rate as a proxy of mechanisms of tolerance or compensation to herbivory damage. Jasmonic acid determination was done using approximately 50 mg of each lyophilized plant sample with addition of the 1 ml of pre-warmed (65°C) methanol and the extraction proceeded for 60 min at room temperature under gentle shaking (for more details, see Loba and Pollmann 2017). In brief, each sample was supplemented with 50 pmol [2H5]-JA (stable isotope-labelled internal standards). Cell-free supernatants were dried under vacuum and pre-purified for subsequent gas chromatography-mass spectrometry analysis (ISQ single 7K-VPI, Thermo Fisher Scientific Inc.). Finally, at the end of the experiment, we collect all tissue (root and shoot) of each orchid and over-dried at 60 °C for 96h to obtain the total dry biomass. All leaves fallen or dead (naturally dried) during the experiment were dried and added to the correspondent replicate. Inter-pot distance was 20 cm and all pots were randomly arranged each week inside the greenhouse to avoid overlapping in their canopies and any possible block effect, respectively.

2.3.5. Data analysis

Data from the microclimatic measurements (soil and air temperature, soil moisture and photosynthetic active radiation) were analyzed with a repeated measures ANOVA to compare the effect of the presence/ absence of the nurses along time. Soil nutrients were analyzed with a one-way ANOVA. Survival curves recorded in the field were analyzed with a Kaplan-Meier test and the statistical significance between them was estimated with a Cox-Mantel test (Fox 1993). Growth data in the field was compared with two-way ANOVAs. Fv/Fm data recorded in the field was analyzed with a two-way repeated measures ANOVA. On the other hand, survival percentages recorded in the greenhouse were analyzed with a Kaplan-Meier test and the statistical significance estimated with a Cox-Mantel test (Fox 1993). Jasmonic acid concentration and growth data in the greenhouse were analyzed and compared with two-way ANOVAs. Fv/Fm data recorded in the greenhouse was analyzed with a repeated measures ANOVA, and flowering data was analyzed with a Chi-square test. All response variables fulfilled ANOVA assumptions, normality and variances homogeneity which were analyzed with the Shapiro-Wilk and Bartlett tests, respectively (Sokal and Rohlf 1995). Comparisons between treatments were evaluated using a posteriori Tukey HSD tests. All ANOVAs were performed using the RStudio (v. 0.99.8967.0, Software) and the graphics were done using Statistica (v. 6.0; Statsoft, USA).

2.4. Results

2.4.1. Microclimatic measurements

From the four microclimatic variables registered in the field, three showed significant differences affected both by microsite and time (**Table 2.1 and 2.2**). Photosynthetically active radiation was the variable that differ the most between microsities, presenting values almost five-times higher in open spaces in comparison with the microclimate under the nurses (**Table 2.1 and 2.2**). Soil moisture also showed a significative difference, with 50% higher values under the nurses than in open spaces conditions (**Table 2.1 and 2.2**). Finally, soil and air temperature data presented significantly higher values in open spaces than beneath nurses (**Table 2.1 and 2.2**).

Table 2.1. One-way ANOVAs of the microclimatic variables measured under nurses and in open sites. Significant differences are highlighted in bold.

| Microclimatic variable | Under nurses | Open sites | F | p-value |
|--|--------------|------------|-------|------------------|
| Air temperature °C | 21±4.2 | 20.6±4.4 | 0.194 | 0.661 |
| Soil temperature °C | 13.4±2.2 | 21.4±11.3 | 9.559 | 0.004 |
| Soil moisture KPa | 12.8±7.3 | 25.4±6.9 | 7.859 | 0.023 |
| Radiation PAR $\mu\text{mol m}^{-2} \text{s}^{-1}$ | 227±353.5 | 1051.9±660 | 84.35 | <0.001 |

Table 2.2. Repeated measures ANOVAs of microclimatic variables (air temperature, soil temperature, moisture and radiation). The main effects were location (under nurses or open spaces) and time. Significant differences are highlighted in bold.

| Source of variation | df | MS | F | p value |
|---|-----|----------|---------|------------------|
| Air temperature °C | | | | |
| Location (L) | 1 | 10.65 | 5.35 | 0.023 |
| Time (T) | 3 | 569.57 | 285.91 | <0.001 |
| L X T | 3 | 6.05 | 3.03 | 0.032 |
| Residuals | 112 | 1.99 | | |
| Soil temperature °C | | | | |
| Location (L) | 1 | 786.43 | 1052.19 | <0.001 |
| Time (T) | 1 | 1044.17 | 1397.02 | <0.001 |
| L X T | 1 | 695.64 | 930.72 | <0.001 |
| Residuals | 26 | 0.75 | | |
| Soil moisture % | | | | |
| Location (L) | 1 | 396.90 | 17.13 | 0.014 |
| Time (T) | 2 | 41.03 | 1.77 | 0.281 |
| L X T | 2 | 114.63 | 4.95 | 0.083 |
| Residuals | 4 | 23.17 | | |
| Radiation ($\mu\text{mol m}^{-2}\text{s}^{-1}$) | | | | |
| Location (L) | 1 | 24811937 | 999.24 | <0.001 |
| Time (T) | 4 | 8362876 | 336.79 | <0.001 |
| L X T | 4 | 1449925 | 58.392 | <0.001 |
| Residuals | 137 | 24831 | | |

On the other hand, the soil traits that showed a significant difference between microsites were: pH, organic matter, nitrates, ammonium, available nitrogen, available potassium and interchangeable potassium; presenting higher values in the soil located under the nurses in comparison with open spaces (**Table 2.3**).

Table 2.3. Soil nutrients (pH, organic matter %, nitrates (N-NO₃) mg/Kg, ammonium (N-NH₄) mg/Kg, available nitrogen mg/Kg, Phosphorus Olsen mg/Kg, available potassium mg/Kg and interchangeable potassium cmol/Kg) under nurses and in open spaces. Significant differences are highlighted in bold.

| Soil variable | Under nurses | Open spaces | F | p-value |
|---------------------------|--------------|-------------|-------|---------------|
| pH | 7.07±0.2 | 6.63±0.16 | 15.34 | 0.0044 |
| Organic matter | 4.61±0.69 | 3.15±0.45 | 15.76 | 0.0041 |
| Nitrates | 4.32±1.28 | 2.46±0.27 | 10.06 | 0.0132 |
| Ammonium | 9.06±3.37 | 1.62±0.75 | 23.14 | 0.0013 |
| Available nitrogen | 13.38±4.30 | 4.06±0.98 | 22.38 | 0.0015 |
| Phosphorus | 6.34±2.64 | 4.28±0.83 | 2.77 | 0.1344 |
| Available potassium | 452.66±87.98 | 302.1±51.79 | 10.88 | 0.0109 |
| Interchangeable potassium | 1.16±0.22 | 0.77±0.13 | 11.02 | 0.0105 |

2.4.2. Manipulative field experiment

Survival of *B. fimbriata* individuals was significantly different in the four treatments (Cox-Mantel test = 72.3, $p < 0.001$). Orchids under nurse canopy and with soil from nurses presented the highest survival, followed by those individuals under nurse canopy with soil from open spaces. Finally, the rosettes transplanted with nurse soil and placed in the open spaces presented complete mortality at the end of the experiment as well as the orchids with soil from open spaces and placed in open spaces (Fig. 2.1).

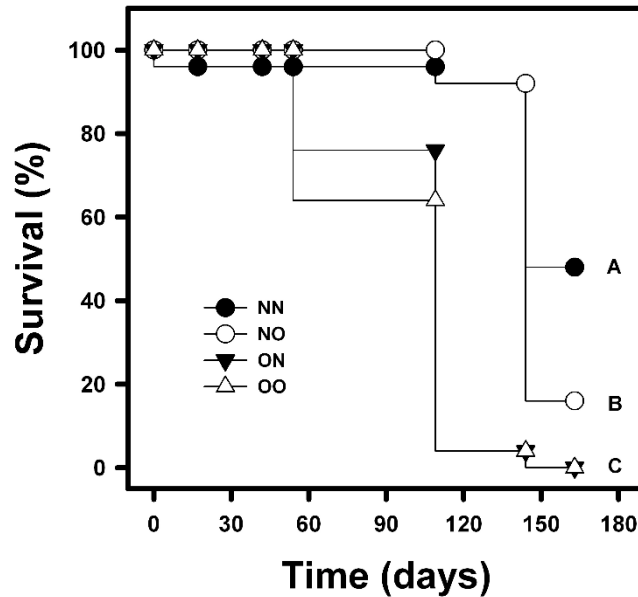


Figure 2.1. Mean survival percentage of *B. fimbriata* rosettes along time and under the four different treatments in the field: under nurse canopy + soil from nurses (NN), under nurse canopy + soil from open spaces (NO), in open spaces + soil from nurses (ON) and in open spaces + soil from open spaces (OO). Different letter indicates significant differences (Cox-Mantel test = 72.3, $p < 0.001$).

Orchids photochemical efficiency (Fv/Fm) was affected by nurse canopy, times and their interaction (ANOVA: $F_{1, 210} = 28.61$, $p < 0.001$; $F_{4, 210} = 9.95$, $p < 0.001$ and $F_{4, 210} = 3.61$, $p = 0.007$, respectively). Nonetheless, nurse soil did not affect orchids photochemical efficiency (ANOVA: $F_{1, 210} = 0.38$, $p = 0.54$). Fv/Fm was higher in the orchids underneath the nurse canopy, independently of the soil origin, in comparison with the orchids in open spaces that showed lower Fv/Fm on average and over time (Fig. 2.2).

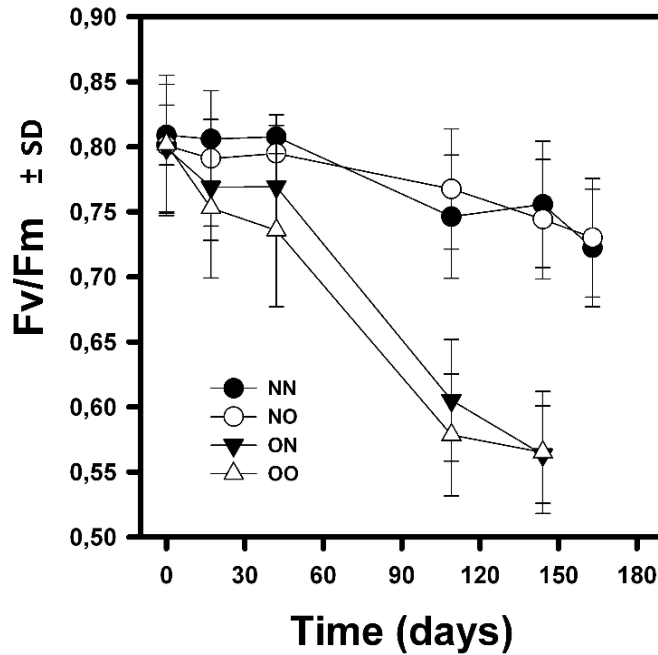


Figure 2.2. Mean (± 1 SD) of photochemical efficiency (Fv/Fm) during the recording time of *B. fimbriata* rosettes under the four treatments in the field: under nurse canopy + soil from nurses (NN), under nurse canopy + soil from open spaces (NO), in open spaces + soil from nurses (ON) and in open spaces + soil from open spaces (OO).

On the other hand, both nurse factors, top-down (i.e., canopy) and bottom-up (i.e., soil), had a significant effect on orchids growth (ANOVA: $F_{1, 94} = 38.28$, $p < 0.001$ and $F_{1, 94} = 9.11$, $p < 0.01$, respectively). Growth was significantly higher in orchids with nurse canopy and soil from nurses (**Fig. 2.3**). The orchids under nurse canopy with soil from open spaces and orchids in open spaces with soil from nurses showed similar growth. Finally, those individuals placed in open spaces with soil from open spaces had significantly the lower growth (**Fig. 2.3**).

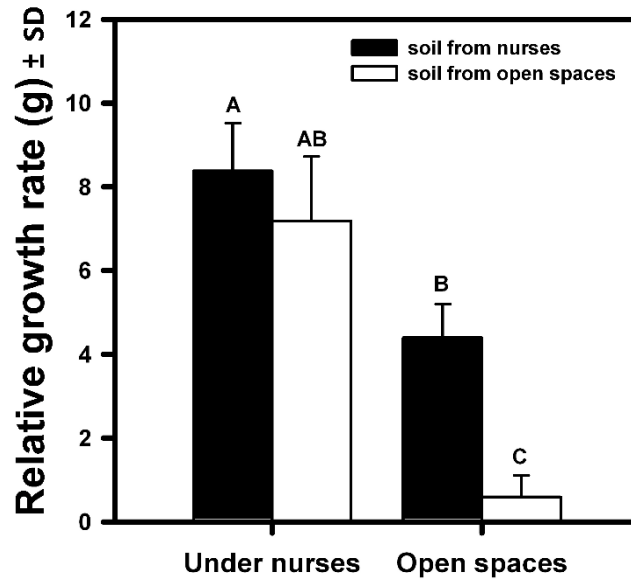


Figure 2.3. Relative growth rate (± 1 SD) of *B. fimbriata* rosettes under the four different treatments in the field: under nurse canopy + soil from nurses (NN), under nurse canopy + soil from open spaces (NO), in open spaces + soil from nurses (ON) and in open spaces + soil from open spaces (OO). Different letter indicates significant differences. Bars are means \pm SE. Different letters indicate significant differences ($P < 0.01$, Tukey test).

2.4.3. Greenhouse experiment

Survival of *B. fimbriata* rosettes was significantly different between the treatments (Cox-Mantel test = 12.9, $p = 0.047$; Fig. 2.4). Those individuals with nurse-like conditions showed significantly higher survival percentage compared with those placed in open spaces conditions independently of the herbivory damage (Fig. 2.4).

Rosettes photochemical efficiency (Fv/Fm) was affected by condition (nurse-like or open spaces), herbivory and time (ANOVA: $F_{1, 264} = 163.14$, $p < 0.0001$; $F_{1, 264} = 15.56$, $p < 0.0001$ and $F_{5, 264} = 168.37$, $p < 0.0001$, respectively). It was also affected by the interaction between condition (nurse-like or open spaces) and time (ANOVA: $F_{5, 264} = 29.12$, $p < 0.0001$). Fv/Fm was significantly higher in the rosettes under nurse-like conditions, presenting the highest Fv/Fm values in the orchids without imposed herbivory. Although orchids of the four treatments showed a decreased over time, orchids in open spaces conditions presented significantly lower values at the end of the experiment (Fig. 2.5).

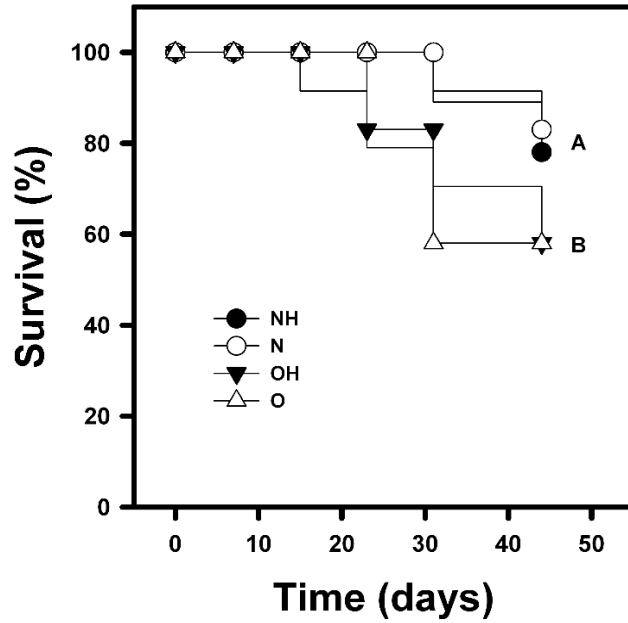


Figure 2.4. Mean survival percentage of *B. fimbriata* rosettes under the four treatments in the greenhouse: nurse-like conditions with (NH) and without (N) herbivory, and open spaces conditions with (OH) and without (O) herbivory. Different letters indicate significant differences (Cox-Mantel test = 12.9, $p = 0.047$).

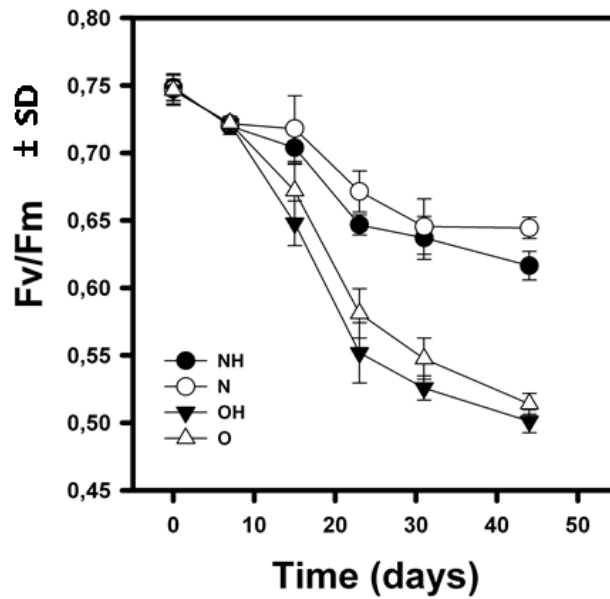


Figure 2.5. Mean (± 1 SD) photochemical efficiency (Fv/Fm) during the recording time of *B. fimbriata* rosettes under the four treatments in the greenhouse: nurse-like conditions with (NH) and without (N) herbivory, and open spaces conditions with (OH) and without (O) herbivory.

Similarly, jasmonic acid concentration in the leaves was affected by condition (nurse-like or open spaces), herbivory and time (ANOVA: $F_{1,95} = 24.36$, $p < 0.0001$; $F_{1,95} = 347.16$, $p < 0.0001$ and $F_{6,95} = 86.85$, $p < 0.0001$, respectively). It was also affected by the interactions of the three factors. Jasmonic acid concentration remained low in individuals without imposed herbivory. In opposite way, plants subjected to herbivory showed significant increase in jasmonic acid seven days after herbivory, with the rosettes in the open spaces conditions presenting the highest increase. Finally, after 44 days, the jasmonic acid concentration in these rosettes decreased, reaching the initial values (Fig. 2.6).

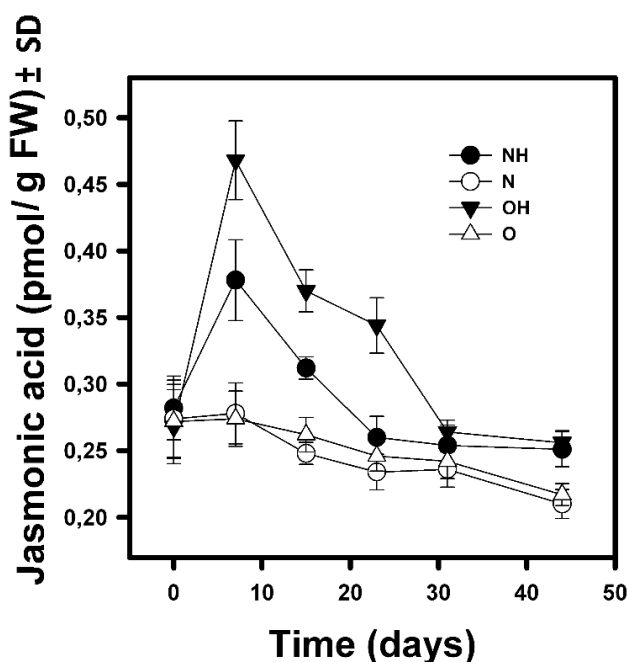


Figure 2.6. Mean (± 1 SD) of jasmonic acid concentration (pmol/g FW) in *B. fimbriata* rosettes under the four treatments in the greenhouse: nurse-like conditions with (NH) and without (N) herbivory, and open spaces conditions with (OH) and without (O) herbivory.

Growth rate of the orchids was affected both by the condition (nurse-like or open spaces) and the imposed herbivory (ANOVA: $F_{1,27} = 22.37$, $p < 0.0001$ and $F_{1,27} = 8.31$, $p = 0.008$, respectively). The orchids under nurse-like conditions presented higher growth in comparison with the orchids in open spaces conditions, with higher values in the orchids without imposed herbivory (Fig. 2.7). In addition, those plants in the open spaces condition and with imposed herbivory showed significantly lower growth (Fig. 2.7).

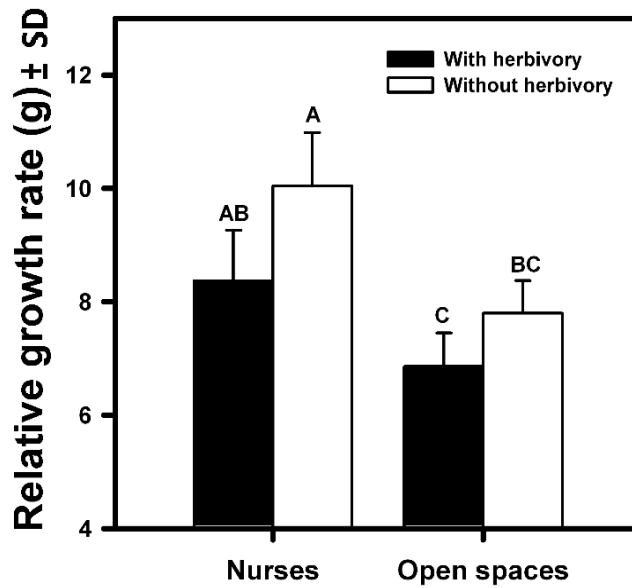


Figure 2.7. Relative growth rate (± 1 SD) of *B. fimbriata* rosettes in the four greenhouse treatments: nurse-like conditions with (black bar) and without (white bar) herbivory, and open spaces conditions with (black bar) and without (white bar) herbivory. Different letters indicate significant differences. Bars are means \pm SE. Different letters indicate significant differences ($P < 0.01$, Tukey test).

The flowering of the orchids was different between the four treatments (Chi-square: $\chi^2 = 20.8$, $p = 0.001$). It was higher in plants under nurse-like conditions, independently of the imposed herbivory damage, presenting 50% flowering the orchids without herbivory and 33,3% the orchids with herbivory. The orchids in the open space condition without herbivory presented only 16,7% flowering, and individuals in the open space conditions with herbivory did not flower at all.

2.5. Discussion

Facilitation promotes diversity at medium to high environmental stress levels, expanding the realized niche of species, however, when the stress level becomes extremely severe, the positive effects of the benefactors dwindle (Michalet *et al.* 2006). Moreover, the possible facilitative interaction could be due to top-down or/ and bottom-up effects that would differentially affect the development of the beneficiary species. It is important to clarify that the interaction considered in this study is between plants that belong to the same trophic level. In our study, we found a facilitative interaction in the stressful Mediterranean zone, and we found that the canopy of the nurse was the main responsible for the amelioration of the stress, suggesting a strong top-down effect. Mediterranean climates impose high temperatures with elevate irradiance, causing a summer drought stress in plants growing in this ecosystem (Dallman 1998). Therefore, it is not surprising that higher moisture and a lower PAR, as well as milder soil and air temperatures (top-down effect), are associated with a higher establishment of orchids, particularly *B. fimbriata*.

Although we found considerably less PAR under the nurses, it seems to bring enough light through its canopy to support the establishment and survival of the rosettes and allow them a higher growth and photochemical efficiency than in open spaces. Although light is inhibitory to germination in many terrestrial orchid species and even some epiphytic species, the correlation of canopy coverage with successful germination and seedling development could be attributed to light avoidance and preference of a mesic shaded microclimate (Kartzinel *et al.* 2013). Besides the microclimatic amelioration at canopy level of the shrubs, nutrients also have been indicated to be more abundant in the soil under the nurses (Mihoč *et al.* 2016). Similarly, in our study zone, we found more organic matter, nitrates, ammonium, available nitrogen and potassium underneath the nurses in comparison with soil from open spaces. Even though we didn't consider the difference in microorganisms between the soils, it is expected that a change in soil chemistry would entail a variation in soil microorganisms. The higher growth observed in the rosettes located under the nurse of our study may have been because the soil presented more moisture and nutrients or because the leaves increased the area to receive enough light for photosynthesis, or both (García-cervigón *et al.* 2015). Considering that the PAR under the nurses was lower and that Fv/Fm was higher in the rosettes underneath the nurse, we could assume that the growth in the rosettes leaves was not (only) a response to shading. Moreover, the Fv/Fm decreases in the presence of stress, like photoinhibition, and it can also affect the growth of the plants (Stancato *et al.* 2002), but that was not the case in our study because orchids that were growing underneath the canopy of the nurse presented values of Fv/Fm consistent with no- or low stress.

It is known that besides the direct effect that a nurse can have on the beneficiary plant, there could be indirect positive effects that could also improve the performance of the plants protected by the nurse. Orchids have symbiotic association with mycorrhizal fungi, usually orchidoid fungi, which allow their seeds to germinate (Brundrett 2002; Valadares *et al.* 2012). The association is crucial since they are mycoheterotrophic at early stages (Arditti and Ghani 2000; Rasmussen 2002; Smith and Read 2008), lacking reserves in their very small seeds (Paudel and Pant 2012), but there is also mycorrhizal association in adult individuals (Pereira *et al.* 2014; Atala *et al.* 2015). This association is considered the key event in the evolution of the Orchidaceae and was originated close to 100 Myr ago during the Cretaceous (Brundrett 2002). *B. fimbriata* was found to be associated with at least five different mycorrhizal fungi colonizing roots and to have seeds completely dependent on them (Steinfert *et al.* 2010; Herrera *et al.* 2017). Besides the biotic interaction with the mycorrhizas, orchid germination is affected also by abiotic factors such as substrate moisture and temperature. The effect of the abiotic factors could be direct on the orchid seeds/seedlings or indirect through the availability of fungi and carbon source (Rasmussen *et al.* 2015). Therefore, the better physiological status of the rosettes underneath the nurses in our study could be not only due to the direct effect of the nurse on the orchids but also to the indirect effect in mycorrhizal diversity and/or abundance, as observed in a semiarid Mediterranean system, also in Chile (Molina-Montenegro *et al.* 2016b).

Regarding the tolerance experiments in the greenhouse, we found that *B. fimbriata* plants under nurse-like conditions (more water availability) showed enhanced tolerance to simulated herbivory damage. The mechanisms of tolerance observed in our study were: increased survival, increased photochemical efficiency, and compensatory growth observed as greater flowering percentage and higher growth, results that fit with the tolerance mechanisms reported for plants

(Tiffin 2000; Milchunas and Noy-Meir 2002). Nevertheless, this tolerant response was observed for the average percentage of herbivory in natural conditions and may vary if the 25% herbivory level considered rises. Although our plants did not experience the timing, distribution, and pattern of damage that they would experience in a natural population, the manipulative herbivory imposed in our study allowed us to control the level of damage (25% as observed in natural conditions) and to estimate the tolerance more accurately (Tiffin and Inouye 2000). In addition, jasmonic acid production induced by the biotic stress was higher in the herbivory treatments, in agreement with the literature (Baldwin *et al.* 1997; Howe and Schaller 2008). Nevertheless, the triggered response was higher in orchids that were in the open spaces/stressful conditions. One possible explanation for this is that the expression of induced plant defense responses is mediated by responses to abiotic stresses and jasmonic acid, in particular, is a common key signaling molecule, involved both in biotic and abiotic stress (Ramegowda and Senthil-Kumar 2015). The exposure of plants to simultaneous abiotic (i.e. water limitation and soil properties) and biotic (i.e. herbivory) stress can result in tolerance to both stresses due to inherent ability of plants to induce unique tailored strategies, or contrarily, it can make plants more susceptible to both the stresses due to exacerbation of damage caused by one stress on other (Ramegowda and Senthil-Kumar 2015). Therefore, in our study, the previous stress exposure of the orchids (less moisture) could have had an additive effect on the jasmonic acid production so that the orchids could deal with both stresses simultaneously. But then, it is clear that experiments assessing the jasmonic acid quantity before and after the stresses are needed to confirm this supposition. Thus, the compensation for the removed tissue or herbivory in the orchids is seen in the similar fitness and tolerance between the orchids under nurse-like conditions with herbivory and the orchids under open spaces conditions without herbivory, meaning that the herbivory has no negative effect in the survival and development of the orchids that are benefit by the nurse (considering the 25% herbivory average level in natural conditions).

Because traits that enhance competitive ability or stress-tolerance of co-existing species, and the type and level of stress can influence their net interaction, we analyzed these factors in our study to see if they met the predicted relationship suggested by Maestre *et al.* (2009) between abiotic stress and the outcome of a given plant–plant interaction according to their refined stress gradient hypothesis (SGH). Our study species show a stress tolerant type, according to the characteristics and reports. Shrubs of *B. macraei* are considered drought-tolerant (Grime 1977; Badano *et al.* 2005) and orchids are considered stress tolerant ruderals (Hágsater and Dumont 1996). Therefore, considering that the stress factors in our study are resource-driven (i.e. water), we could expect that the interaction outcome between our study species would be negative when the stress is low or high and positive in the medium-high stress scenario (Maestre *et al.* 2009). It is considered that our study site has bioclimatic characteristics that impose a medium stress to its biota. The study site is considered a semiarid ecosystem (Noy-Meir 1973) with an average annual precipitation ranging from 413.1 to 460 mm (Dallman 1998, www.meteochile.cl). Hence, the predicted positive interaction between two stress-tolerant species under medium resource stress was observed as a nurse effect between the studied species. Nonetheless, it is important to take into account that the net interaction could change if the intensity of the stress changes in the zone. A decrease in rainfall is predicted in the study region for the next years (Quintana and Aceituno 2012), which may turn the water stress from medium to high, increasing the facilitative interaction due the higher ecological relevance of nurses or even changing the net

interaction from a positive to negative if the stress level overpass the shelter action of nurses (see Michalet *et al.* 2006). Thus, more studies should be conducted considering future climate change scenarios to unravel these hypotheses and its consequences for this native orchid in the Mediterranean ecosystem.

2.6. Acknowledgements

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Capítulo 3 : Conclusiones

Las interacciones de facilitación entre plantas han sido incluidas en la teoría ecológica, y en los últimos años se están usando de forma aplicada en la restauración de ambientes degradados y en la reintroducción de especies (Gómez-Aparicio *et al.* 2004; Padilla and Pugnaire 2006; Lortie 2017). Los primeros estudios involucrando el uso de plantas nodriza con propósitos de restauración ecológica fueron a finales de los noventa, publicados en los trabajos de Castro *et al.* (2002) y Gasque & García-Fayos (2004). Desde entonces, se han venido realizando experimentos de facilitación con distintas especies y en diferentes ecosistemas, particularmente en los que presentan elevados niveles de estrés y de degradación antrópica (Padilla and Pugnaire 2006). Entre estos estudios de restauración se encuentran los realizados en bosques riparios tropicales (Galindo *et al.* 2017), bosques de pastoreo mediterráneos (Costa *et al.* 2017) y estepas patagónicas (González and Pérez 2017), bosques montanos post-incendio (Siles *et al.* 2010), zonas de minería a cielo abierto (Torroba-Balmori *et al.* 2015), sitios agrícolas contaminados (Domínguez *et al.* 2015) y suelos estériles (Navarro-Cano *et al.* 2015). Sin embargo, en la región mediterránea de Chile, considerada un hotspot de biodiversidad, a la fecha existen pocos trabajos que plantean esta técnica de restauración ecológica (Fleury *et al.* 2015; Root-Bernstein *et al.* 2017) y ninguno considera orquídeas.

Muchas especies de orquídeas en Chile son endémicas y se hallan en un hábitat con mucha intervención antrópica que podría generar problemas de conservación en el futuro (Novoa *et al.* 2015). Actualmente existen métodos para la producción asimbiótica en laboratorio de la especie considerada en este estudio (Pereira *et al.* 2015) y otras especies de orquídeas de la zona (Pereira *et al.* 2017), incluso técnicas para micorrizarlas posteriormente (Pereira *et al.* datos no publicados). Sin embargo, se considera que el punto crítico para la reintroducción de estas especies de orquídeas sería la mortalidad al trasplantar los individuos a campo debido a las características bióticas y abióticas estresantes de la zona. Así, el uso de técnicas de restauración ecológica podría ayudar en el establecimiento y desarrollo exitoso en esta fase crítica.

Los resultados del experimento de trasplante realizados por este estudio demuestran que los arbustos de la especie *Baccharis macraei* otorgan una interacción neta positiva a las orquídeas de la especie *Bipinnula fimbriata*, y permiten una observación de los cambios durante el ciclo de vida de la orquídea, lo que permite determinar en qué estadios de desarrollo de las orquídeas la facilitación es más relevante. Este estudio además de observar el efecto global dado por la planta nodriza, determinó que el mayor impacto positivo es dado por el dosel del arbusto, efecto top-down. Esta información sumada al hecho de que el nivel de facilitación de las nodrizas varía con la edad (Amghar *et al.* 2016), nos da una idea de las características de desarrollo que deben presentar los arbustos para ser considerados nodrizas apropiadas.

El éxito diferencial de los programas de restauración con plantas nodriza está influenciado tanto por las características propias de las especies interactuantes como por los niveles del estrés biótico o abiótico (Siles *et al.* 2010; Svriz *et al.* 2013). Entre las características, la ontogenia, tolerancia al estrés (Torroba-Balmori *et al.* 2015), capacidad competitiva y distancia filogenética (Carrión *et al.* 2017) de las especies son importantes para poder predecir el resultado de su interacción. En general, gran parte de los experimentos de restauración toman en cuenta la mayoría de estos factores, pero no realizan un experimento previo de trasplante que permita

conocer con certeza el resultado de la interacción, resultando en algunos casos en una interacción no deseada. Trabajos como el realizado en este proyecto de tesis, permiten no sólo tomar en consideración los aspectos arriba mencionados, sino que dan resultados confiables acerca del beneficio que otorga una planta nodriza durante las distintas etapas del desarrollo de la planta beneficiada, permitiendo una mejor planificación en un posible proyecto de restauración futuro.

El cambio de uso de suelo sumado al cambio climático sugiere que esta orquídea podría disminuir su tamaño poblacional ingresando en una de las categorías de “Amenaza” de la IUCN (IUCN, 2001) como ha sucedido con otras especies de orquídeas endémicas de la zona (Atala *et al.*, 2016). Por lo cual, es importante considerar su conservación *ex situ*. Otra posible aplicación de los resultados de este estudio incluye el uso del arbusto nodriza *Baccharis macraei* para la conservación *ex situ* de esta especie de orquídea endémica (Hágsater and Dumont 1996). Esta conservación podría realizarse tanto en jardines botánicos, parques nacionales, reservas privadas u otros espacios, ubicando a esta orquídea en cercanía a este arbusto nodriza para garantizar su establecimiento y supervivencia. Además, considerando que el arbusto nodriza es nativo, su introducción en estos espacios no representaría un problema ni una amenaza para otras especies existentes.

Finalmente, los resultados acerca de las condiciones adecuadas para la supervivencia y desarrollo de la orquídea (condiciones bajo la nodriza) pueden servir también para la producción en masa de estas orquídeas en invernadero con fines comerciales. Si bien en invernadero podría no necesitarse el uso del arbusto nodriza como tal, las modificaciones que realiza este arbusto al microclima nos permiten conocer los rangos adecuados de temperatura, humedad, luz y nutrientes que permitirían una propagación artificial exitosa de esta orquídea.

3.1. Referencias bibliográficas

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