· Article ·
Advances in Polar Science
doi: 10.13679/j.advps.2015.1.00024
March 2015 Vol. 26 No. 1: 24-29

Experimental culture of non-indigenous *Juncus bufonius* from King George Island, South Shetland Island, Antarctica

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Received 5 August 2014; accepted 11 March 2015

Abstract *Juncus bufonius* L. (Juncaceae) is recognized by the US Department of Agriculture as a weed or invasive plant. Recently, we reported on *J. bufonius* L. var. bufonius associated with the native vascular plants *Deschampsia antarctica* and *Colobanthus quitensis* in the environs of the Polish Arctowski Station, King George Island, in the Maritime Antarctica. In this study, we evaluated the developmental stages and morphological characteristics of *J. bufonius* plants cultivated in controlled conditions beginning with seeds obtained from plants of the Antarctic population. Germination occurred at 3 weeks and the germination percentage was low (22.5%). The average time between the anthesis and seed formation was 7 weeks, similar to that reported for other species in the Juncaceae. According to data reported in the literature, Antarctic individuals were significantly smaller than their relatives growing in other conditions, except for the number of inflorescences. The morphological characteristics of a species vary according to its distribution and the edaphoclimatic environment where it occur; cosmopolitan plants shuch as *J. bufonius* also have reduced stature in cold environments. The low percentage germination may have been due to water availability in the plant chamber in which the study was conducted. *J. bufonius* is intolerant of dry environments, and once it suffers hydric stress its recovery is very low; thus, a moister environment could be beneficial. *J. bufonius* has become established amongst native vegetation near Arctowski Station and without careful control or eradication; it may have the potential to spread far beyond the site, as has happened with the alien grass *Poa annua* as human disturbance and climate warming increase.

Keywords **Antarctica, invasive plants, *Juncus bufonius* (toad rush), non-native species, regional warming**

Citation: Cuba-Díaz M, Fuentes E, Rondanelli-Reyes M, et al. Experimental culture of non-indigenous *Juncus bufonius* from King George Island, South Shetland Island, Antarctica. Adv Polar Sci, 2015, 26: 24-29, doi: 10.13679/j.advps.2015.1.00024

1 Introduction

Antarctica is still a relatively pristine biome, but the increasing presence of Man combined with global climate change, which is accelerated in this region of the Earth, is causing substantial changes to the Antarctic ecosystem[1-5]. Regional warming is more pronounced in the adjacent Antarctic Peninsula and islands, where the temperature increase has been +0.56°C per decade from 1951 to 2000[6-7]. This has caused a major and accelerated retreat of the glaciers and thus a greater availability of ice-free areas, which could be colonized by local native plant species[8-10]. With the increase in human activity in many parts of Antarctica, the likelihood of other non-indigenous species arriving has also increased, and these may colonize and become invasive[11-15, 5].

There have been several reports of non-native plants in Antarctica, such as *Poa annua* L., which is recognized as

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the most widely distributed non-native species, and is able to colonize sites far distant from its introduction site. Recently was recorded in a new habitat away from of their original habitat, in the vicinity of Polish Antarctic Station Arctowski, on the deglaciated moraines of the Ecology Glacier, possibly having been dispersed by the wind[16]. *Poa pratensis* L. has been noted for its persistence, and is restricted only to its original site of introduction in Cierva Point (Punta Cierva), Danco Coast, northwest Antarctic Peninsula, because of the abiotic inhibition of its sexual reproduction and the low human activity where it is located[17].

Recently, another non-native vascular plant, *Juncus bufonius* L. was isolated in the laboratory from plant associations with *Colobanthus quitensis* and *Deschampsia antarctica*, the two native species in this biome[18]. Nevertheless, to date it has not been possible to specify either the size of the population or how long this species has been in Maritime Antarctica. In a recent visit has been identified probable site where *J. bufonius* were collected, in an area approximate of 250–270 m^2 between meteorological building and tourist shop. This area is full of human activity, high animal influence (mainly penguin), very close to the sea and gravel soil with abundant plants in the rockery that characterizes this area. *J bufonius* commonly known as toad rush, is a monocotyledon angiosperm belonging to the Juncaceae family, and is recognized by the US Department of Agriculture as a weed or invasive plant (plants.usda.gov). *Juncus* and *Luzula* are the only genera in this family that have species with an annual life cycle strategy, *J. bufonius* is possibly the only annual Juncus[19-20]. The Juncaceae have colonized all moist environments, particularly in temperate regions, and are wind-pollinated[21]. In Western Europe five species belonging to the same complex as *J. bufonius* have been recognized, with *J. bufonius* being the only polyploid[22-24]. Various studies have shown that this species is derived from the hybridization of diploid species belonging to the complex[24], which may have given it greater genetic and physiological variability, affording it greater adaptability to variable abiotic conditions. *J. bufonius* has a cosmopolitan distribution[25], including mainly sandy coastal habitats in cool and cold climates zones or mountainous regions with moderately high rainfall[20]; it can also tolerate saline conditions. Although usually growing in mixed-species communities, it may also develop pure stands[26]. It is expected that because of the morphological variability and adaptability of *J. bufonius* to different environmental conditions and the more favorable conditions in the Antarctic ecosystem caused by regional warming, this species could successfully established in Maritime Antarctica.

The aim of this work was to study the developmental stages and morphological characteristics of *Juncus bufonius* plants cultivated in controlled conditions beginning with seeds obtained from plants of the Antarctic population in order to shed light regarding its potential success in the current conditions in Maritime Antarctica due to regional warming.

2 Materials and methods

2.1 Plant material

In this study we used seeds of *Juncus bufonius* L. obtained in the Biotechnology and Environmental Studies Laboratory of the University of Concepción, Los Ángeles Campus, from material collected from the environs of the Polish Arctowski Station on the western coast of Almiralty Bay, King George Island, South Shetland Islands[18]. The laboratory plants were obtained from at least three plants from field and an indeterminate numbers of seed from field that germinated under controlled condition in the laboratory.

2.2 Developmental stages

In order to study the phenology of *J. bufonius* in controlled conditions, 200 seeds were placed on a substrate of earth:peat:perlite (3:2:1), in a plant growth chamber with a photoperiod of 16/8 h light/dark, a temperature of 15±2°C, a photosynthetic photon flux density (PPFD) of 75±20 μmol·m^{-2}·s^{-1}, and a relative humidity of 80%±5%, with manual irrigation with tap water, without nutrients addition. Parameters such as germination percentage, germination time, time between anthesis and seed formation, as well as the development time of each of the age stages described according the classical work of Gatzuk et al.[27]

2.3 Morphological measurements

Morphological parameters were measured using 10 plants at 20 weeks of age from 50 seeds germinated under the conditions described above. With a Vernier caliper (Mitutoyo, USA), total plant length, stem length, leaf length, number of leaves per plant, leaf width, root length, crown diameter and number of reproductive leaves per plant were measured.

2.4 Statistical analyses

The data from the morphological measurements are shown descriptively with the mean ± standard error (SE) according to a previously established formula[28].

3 Results

3.1 Developmental cycle of Antarctic *J. bufonius* growing in controlled conditions

Under the culture conditions used in this study, the germination percentage was low (22.5%). The time taken from sowing to the different developmental states is shown in Figure 1. In three weeks was observed that seedlings already had 4–5 foliar buds or leaves (seedling state, PL) and that the complete development cycle took 7 months (G3 state). The young generative state (G1) was observed at 4 months after sowing, and the time taken from anthesis to seed formation was approximately seven weeks, thus, individuals considered to be in the middle-aged generative state (G2) were observed 6 months after sowing.

3.2 Morphological characteristics of Antarctic *J. bufonius* growing in controlled conditions

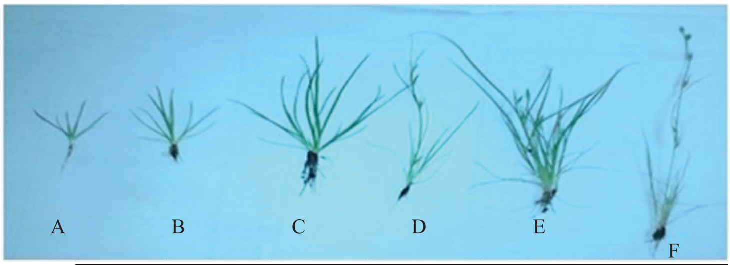
Table 1 shows that the *J. bufonius* plants had greater stems (6.76 cm) than roots development (3.30 cm) and that the leaves were long, spanning more than half of the aboveground part (3.70 cm). The plants had an equivalent number of vegetative tissues (3.50 leaves per plant) and reproductive tissues (3.07 leaves with inflorescences per plant).

4 Discussion

The inflorescences in this species can occupy 50% or more of the aboveground part of the plant[29], and have actinomorphic, bisexual flowers, and seeds with an ovoid to ellipsoidal shape, field germination rates are quite high, especially in moist, preferably waterlogged soils in open, unshaded situations without competition from other plants[22]. The low germination percentage we obtained (22.5%) may be related to water availability, since it has been suggested that *J. bufonius* is intolerant of dry atmosphere, and once drought-induced stress is initiated in these plants their recovery is very low[22], affecting mainly germination. Previous studies have reported that germination in *J. bufonius* can reach 78% for seeds that have been hermetically preserved for 12 months at 5°C in dry air conditions[30], occasionally with up to 85%−90% germination of seeds stored in various conditions and for various durations.

The cooling system in the plant chamber used in this study provides a dry cold air intake and manual irrigation and the programmed irrigation regime maintained the relative humidity inside the chamber never above 75%, it may have produced areas and short periods where the substrate was drier. Therefore, more studies that control the soil moisture more than the environmental relative humidity of the growth chamber are required.

According to reported data for the Juncaceae family, the time from anthesis to seed formation is approximately 5−6 weeks, when the first floral buds appear[31], which is consistent with what was observed in this study, where this cycle was completed in 7 weeks. The average duration of the life cycle stages of each plant species is genetically determined but can vary considerably because of environmental conditions, so that different individuals can reach certain stages at different times[27]. Gatsuk et al.[27] suggested that in different ecological conditions it is better to determine developmental stages rather than the calendar age. In this study, the different developmental stages for Antarctic *J. bufonius* were defined, and it was cultivated in controlled conditions as per the description established for the classical work of Gatzuk et al., where the authors defined the age's stage of plants of various growth forms[27]. Similar developmental stage characteristics have been observed in *D.*



	A	B	C	D	E	F
Age states	Seeding	Juvenile	Virginal	Young generative	Middle-aged generative	Old generative
Abbreviation	PL	I	V	G_1	G_2	G_3
Time after sowing	3 weeks	1 month	2 months	4 months	6 months	7 months

Figure 1 Developmental stages of *Juncus bufonius* grown in controlled conditions in the laboratory.

Table 1 Morphological characteristics of Antarctic *J. bufonius* L grown in controlled conditions in the laboratory (Mean of 10 plants)

Characteristic	Mean±SE
Total plant length/cm	10.08±7.20
Stem length/cm	6.76±5.70
Leaf length/cm	3.70±0.66
Number of vegetative leaves per plant	3.50±0.30
Leaf width/mm	1.35±2.02
Root length/cm	3.30±2.80
Number of reproductive leaves per plant	3.07±0.65

antarctica[10], plant still being another family (Poaceae) have similarity in morphological appearance with *J. bufonius*[18]. No reports have been in the literature on *Juncus* phenology. It has been reported that in natural conditions the flowering period occurs from March to September in the Northern Hemisphere[32], but there is no information regarding the duration of the different developmental stages of the species. Therefore, this approach may allow us to compare the developmental times of the different ontogenic stages of *J. bufonius* individuals of different origins.

According to the parameters measured in this study, the Antarctic *J. bufonius* cultivated in controlled growth conditions (growth chamber, 15±2°C, 16/8 h light/dark, 75%±5% RH) were smaller than individuals of the species described for other geographical regions growing in natural conditions[22,33]. Descriptive and quantitative data obtained from literature sources indicate that *J. bufonius* can reach a length between 5 and 20 cm[34], or between 2 and 50 cm[35]. Antarctic plants grown in controlled conditions reached 10 cm on average, which is an intermediate value compared with what has been reported in the literature. Likewise, other parameters such as leaf length and width were lower than reported in the literature, 3.7 cm and 1.35 mm versus 15 cm and between 0.5 and 5 mm, respectively[35]. It has been reported that the morphological characteristics of the species can vary according to its distribution and the edaphoclimatic characteristics[36]. Therefore, the smaller size of these plants could be related to the climatic conditions of Antarctica with its very low temperatures even during the species' reproductive season in the Antarctic spring-summer. This species has been described in temperate and warmer areas (varying between 1°C minimum to 20°C in Western Europe[22] and until 30ºC in Asia[33]), where it has been possible to determine that both low and high temperatures can inhibit its growth[22].

These results show the behavior of *J. bufonius* growing in controlled conditions for the first time, as well as in a more compact substrate than the sandy sites previously described[25]. More studies are needed to ascertain the behavior of this species in both controlled and natural conditions, where the abundance and behavior of the species is as yet unknown because of the accidental way in which it was discovered in maritime Antarctic[18]. It has been established that the success of non-native and invasive species depends on the number of propagules of the species introduced into the region, their capacity for colonization, extent of their settlement, and, once established, their ability to expand and alter the ecosystem[5].

The Antarctic terrestrial ecosystem is vulnerable to the introduction of non-native species because of its low diversity and the simple structure of its community[11,14,37]. It has been reported that the two vascular plants native to Antarctica (*C. quitensis* and *D. antarctica*) can reproduce in vegetative form, although rare in the case of *C. quitensis*. Notwithstanding an ample seed bank for both species has been reported[38-39], the seeds are not always viable. This is due mainly to the short summers and sub-optimal temperatures for the development of mature seeds, germination, and the lack of moisture and the shortage of nutrients favoring vegetative propagation over seed germination. Other authors have suggested that the regional increase in summer temperature may benefit plant growth, and the possibility of establishing seedlings and the expansion of established plants[40-41], and also increase sexual reproduction and germination from soil seed banks[37-39,42-43].

Recent climate change in the Antarctic Peninsula has created conditions for increased growth and biomass production of native and non-native species[7,40,43-46], gradually weakening the environmental barriers that limit the arrival and colonization of new species in this region[5,13]. This is supported by recent reports of non-indigenous species becoming established in the Antarctic terrestrial ecosystem[18,48-50] as well as the detection of seeds, diasporas and other propagules of vascular plants, bryophytes and lichens transported on clothing and footwear by visitors to the Antarctic[5,14-15]. These studies have also been able to ascertain the main plant families arriving to the Antarctic[14], the different visitor categories (tourists, scientists, and associated support personnel) transporting greater amounts of propagules[5,15], and the areas within the Antarctic that present the greatest risk of receiving propagules of potentially invasive species. In this respect, the most vulnerable sites are those associated with human occupation and casual visitation in the northern Antarctic Peninsula, Victoria Land and some extensive ice-free areas in Enderby Land.

Additionally, regional warming favors conditions for the establishment and spread of non-native species. Higher summer temperature and more frequent rain fall results in increasing ice-free habitat available for colonization, and increased soil moisture caused by longer periods of thawing of the permafrost and melting of snow patches and glaciers[43,51]. Such conditions also increase soil microbial activity, resulting in increased nutrient availability[52-53]. In an Antarctic context, species such as *J. bufonius* that exist in a wide global distribution and occupy similar climate environment and habitats, are likely to be well adapted to the more extreme climate of the maritime Antarctic. To date, the species occurs on some sub-Antarctic island, such as Navarino Island[54] and the Falkland Islands[55]. It could potentially reach the Antarctic Peninsula with relative via the various previously mentioned vectors and become established there under the current climate and soil regimes. According to paleoecological records, this would be quite homologous with the ecosystem described for the early Holocene period in Sub-Antarctic South Georgia, where a close relative, *J. scheuchzerioides* Gaudich., is widespread in bog communities overlying thick peat[56]. Further investigation is required to elucidate the status of *Juncus bufonius* at Arctowski Station on King George Island. Under Antarctic Treaty regulations all non-indigenous plant species are required to be removed from the Treaty Area. It is important that the presence and range of *J. bufonius* is carefully monitored by the Polish authorities, and appropriate measures are taken to eradicate the population as soon as possible.

Acknowledgments This work was financed by the INACH T_03-09 and INACH RG_02-13 projects of the Chilean Antarctic Institute. We thank INACH, the Chilean Navy and the Polish Antarctic Arctowski Station for their logistical support. We also thank H. Lowry for her assistance in revising the English and the two anonymous reviewers for their valuable comments.

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