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Reinforcement of the *Rhynchospora fusca* population in the Galbaniturri mire (Izki Natural Park, Álava, Spain): first results

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Abstract. *Rhynchospora fusca* (L.) W.T. Aiton is a Cyperaceae that lives in constantly wet zones in acid mires, whose European distribution reaches its southernmost limit in the Spanish Cantabrian corniche. It is a characteristic plant of Habitat 7150 “Depressions on peat substrates of the *Rhynchosporion*” in Annex I of the EU Habitats Directive of the European Union, and also listed in the Catalogue of Threatened Species of the Basque Autonomous Community under the “Endangered” category since an only locality within the Izki Natural Park is known, in the Galbaniturri-1 mire. Given the vulnerability of this only Basque population, and aiming to improve its conservation status, a reinforcement experience was set in 2011 within LIFE+ PRO-IZKI project. We describe the main results, including the plantation at five new points in the same mire (2013 and 2015), and the monitoring for survival, flowering and fruiting for 3 consecutive reproductive periods from 2014 to 2016. The plants used for the reinforcement were vegetatively generated from plants collected in the wild. The average survival rate for the first plants planted in 2013 was very low (28%), due to the choice of planting before the winter; a second plantation in spring 2015 obtained a much higher survival rate (79%). Plant growth by rhizome renewals has been increasing year after year in almost all plots. Flowering occurred for the first time in 2016 in 6 of the 9 plots, showing heterogeneous numbers among them. Seed production has been estimated for 4 of the 6 flowering plots, also showing considerable differences. As a whole, the initial phase of reinforcement for this species is considered successful, but a final evaluation can only be made on a long-term basis.
Keywords: plant conservation; threatened species; survival; growth; flowering; fruiting; seed production; Basque Country Autonomous Community; Spain.

Reforzamiento de la población de *Rhynchospora fusca* en el trampal de Galbaniturri (Parque Natural de Izki, Álava, España): primeros resultados

Resumen. *Rhynchospora fusca* (L.) W.T. Aiton es una ciperácea que vive en áreas constantemente húmedas en ciertos humedales higroturbosos, cuyo límite sur de distribución europea es la Cornisa Cantábrica española. Es una planta característica del Hábitat 7150 “Depresiones sobre sustratos turbosos del *Rhynchosporion*” incluida en el Anexo I de la UE Directiva Hábitats, y también incluida en el Catálogo de Especies Amenazadas de la Comunidad Autónoma Vasca, dentro de la categoría “En Peligro de Extinción” ya que sólo se conoce una única localidad dentro del Parque Natural de Izki, en el trampal de Galbaniturri-1. Dada la vulnerabilidad de esta única población vasca y al objeto de mejorar su estado de conservación, se inició una experiencia de reforzamiento en 2011 dentro del proyecto europeo LIFE+PROIZKI. Se describen sus principales resultados, incluyendo la plantación en cinco nuevos puntos (2013 y 2015), y el seguimiento establecido para determinar la supervivencia, floración y fructificación durante 3 periodos de reproducción consecutivos, desde 2014 a 2016. La planta para este reforzamiento fue producida a partir de material vegetativo recolectado en la población original. La tasa de supervivencia media en el primer ensayo en 2013 fue muy baja (28%), debido a haber hecho la plantación antes del invierno; la segunda plantación en la primavera de 2015 obtuvo una tasa mucho mayor (79%). El crecimiento a través de renuevos de rizoma ha ido incrementándose año tras año en casi todos los plots. La primera floración se dio en 2016, en 6 de los 9 plots, mostrando cifras heterogéneas entre ellos. La producción de semillas se ha estimado para 4 de los 6 plots que habían florecido, mostrando nuevamente diferencias considerables. En general, la fase inicial del reforzamiento de esta especie se considera exitosa, pero una evaluación final sólo podrá hacerse a largo plazo.
Palabras clave: conservación vegetal; especies amenazadas; supervivencia; crecimiento; floración; fructificación; producción de semillas; Comunidad Autónoma del País Vasco; España.

Introduction

Rhynchospora fusca (L.) W.T. Aiton is a Cyperaceae that lives in constantly wet areas in mires. It is a species with a narrow ecological plasticity which requires very specific environmental conditions and whose southernmost European populations are found in the Spanish Cantabrian corniche.

It is a characteristic plant of Habitat 7150 «Depressions on peat substrates of the *Rhynchosporion*» in Annex I of Habitats Directive of the European Union; it is included in the Red List of the Spanish Vascular Flora (Moreno *et al.*,

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2008) in the IUCN "Endangered" category, and it is present in several autonomous communities with populations in Asturias (Alejandre Sáez & *al*., 2017), Navarra (Balda, 2002, 2007-08), Galice (Rodríguez-Oubiña & Ortiz, 1986) and the Basque Autonomous Community (Aizpuru & *al*., 1997) where there is a population in Álava (Izki Natural Park) and references in the early XXth century from Gipuzkoa (Allorge & Allorge, 1941) but this latter population has not been observed in recent times despite prospection made.

In the Threatened Species Catalogue of the Basque Autonomous Community, it is considered as 'Endangered', since an only locality is known inside Izki Natural Park, in the Galbaniturri-1 mire (BOPV/EHAA, 2011). This mire is formed of two branches (Figure 1) occupying two small valleys which converge in a larger one. Its centroid coordinates are UTM 30T0539124, 4727695, and it descends from 734 to 717 m asl. In all, this mire covers 28153 m^2. It is a very diverse site, lying on sandstones from the Late Cretaceous, where a mosaic of vegetation communities has been described, including: *Sparganium erectum* L. community on soaked soils with flowing waters by the stream; an herbaceous community dominated by *Molinia caerulea* (L.) Moench, placed over a moss lawn dominated by *Campylium stellatum* (Hedw.) Lange & C.E.O. Jensen; the *Rhynchosporion* community over wet soils with some free water, composed of *Rhynchospora alba* (L.) Vahl, *R. fusca* and *Drosera intermedia* Hayne in Dreves (this is the smallest community in terms of occupied area, around 4%, but it gathers the most interesting species); a community slightly over the waterlogged table, with *Sphagnum* spp., *Genista anglica* L. and *Calluna vulgaris* (L.) Hull; and finally a hydrophilous *Alnus glutinosa* (L.) Gaertn. forest with *Betula alba* L. and *Frangula alnus* Mill., over an underwood of *Carex paniculata* L. and sometimes *Sphagnum* spp. (Heras Pérez & Infante Sánchez, 2007).

The water feeding the mire comes on the one hand from the stream in the valley, and on the other hand from a series of lateral sources that contribute to the stream flow. The *R. fusca* population is found on one of these lateral sources, and this is of the utmost importance, since the source and its surroundings behave as an individual hydrological subunit, showing the lowest conductivity (42.7 – 103.2 µS/cm^2) and pH (4.8 – 5.1) values inside this mire.

The population of *R. fusca* occupies only 20 m^2, in a more or less continuous patch on this only lateral source, which makes it very vulnerable to external changes, even merely accidental. This is a most worrying situation since it is surrounded by a cattle breeding area, and so directly subject to grazing, trampling and eutrophication, and indirectly to impacts from pasture improving and access for farmers. For this reason, in 2011, under the European Union's LIFE+ PRO-IZKI project, and aiming at improving this population's conservation status in Izki Natural Park, a reinforcement experience was set up. Given its narrow ecological conditions, it was obvious that only a few sources could support *R. fusca*, so it was decided to create several new subpopulations inside the same mire, which could guarantee the persistence of *R. fusca* in case that the original population was damaged, and that these subpopulations could become viable and self-sustaining under minimal long-term management.

Material and Methods

Selection of sites for the reinforcement

On the basis of the studies conducted on Galbaniturri-1 mire in the late years (Heras Pérez & Infante Sánchez, 2007), every source point was identified in the mire. Among them, five sites were selected to install the plots (Figure 1) since they showed the maximum similarity to the characteristics of the original population site: source points with constant water flow, muddy substrate, permanent water with a pH ranging from 5 to 5.5, and a low plant cover, usually with the insectivore *Drosera intermedia* and *Rhynchospora alba*.

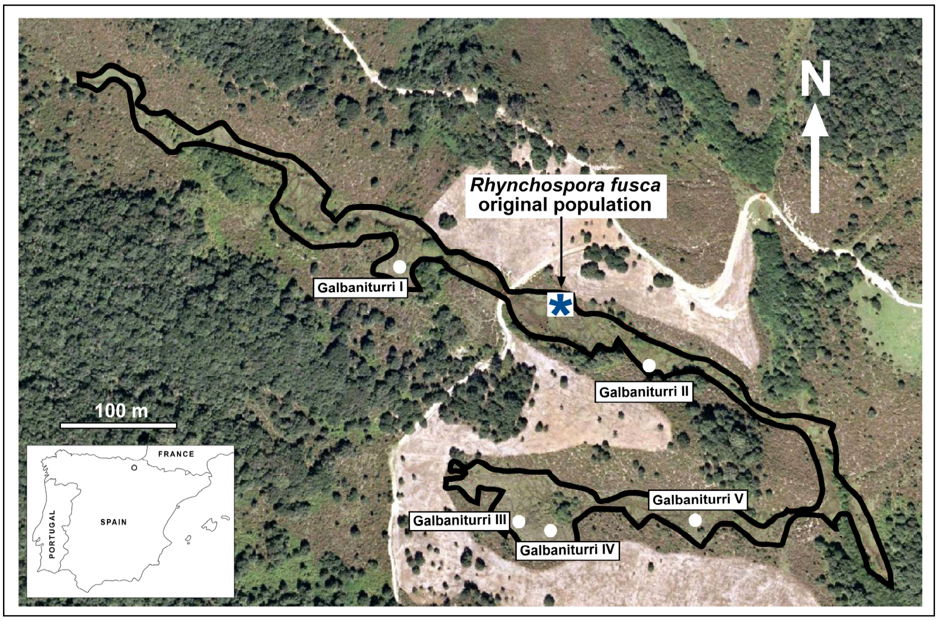

Figure 1. Location of Galbaniturri-1 mire, showing the original *Rhynchospora fusca* population and the sites selected for reinforcement.

Plants used for reinforcement

They have been obtained from vegetative plant material. In the spring of 2013, 6 10x10 cm tussocks, taken at six ends of the original population were collected. The samples were transferred to the Gipuzkoa Plant Germplasm Bank, where they were kept for two months in trays with black peat and saturated with water in order to acclimatize them. After flowering, one of these tussocks proved to be in fact *Rhynchospora alba*, and so it was discarded. The other 5 were used to separate vegetative material and produce plants in the nurseries in Arizmendi (Urnieta, Gipuzkoa). The survival rate of plants produced from the vegetative mats division was 100% (Garmendia & *al*., 2013).

Seeds from the original site were also collected from 2012 to 2016, which have been subject to specifically designed germination protocols, but plants obtained from seeds have not yet been used for introduction. However, the seeds are kept in the Active Bank (ultradried and at 5°C) (Arrieta & *al*., 2015); and a number of plants produced from vegetative material are stored in the nursery.

Plantation

The initial site where *R. fusca* was living before the reinforcement ('the original population') did not received new plants. The new plants were planted in 5 sites, herein called 'introduction sites' and named from Galbaniturri I to Galbaniturri V (see Figure 1). In total, 240 plants or vegetative units have been planted, in two different batches: the first in November 2013 with 120 units; as this first plantation was badly affected by water outbursts during the winter, a second plantation was estimated to be necessary and so, a second batch of 120 plants was introduced in the same 5 sites in April 2015, but this time in nine different plots instead of five (Table 1 and Figure 1). Before the plantation, no removing of the surrounding plant species was made. In the 2015 plantation, some of the plots were protected with a jute textile cover (Figure 2) to favour the rooting of the plants by smoothing the upwelling of the waters after heavy rains. No fencing has been used to protect the plants from cattle or wild animals.

Table 1. Number of plants introduced in 2013 and 2015. (* = protected by jute textile cover).

Site	08.11.2013	27.04.2015
Galbaniturri I	20	24*
Galbaniturri II	20	
Galbaniturri IIa		24*
Galbaniturri III	40	
Galbaniturri IIIa		12*
Galbaniturri IIIb		12*
Galbaniturri IV	20	24*
Galbaniturri V	20	
Galbaniturri Va		24
TOTAL	120	120

Figure 2. Plot Galbaniturri IIa protected by jute textile cover at the plantation in April 2015.

In situ monitoring and evaluation of reinforcement progress. The reinforcement evolution has been monitored *in situ* since 2014. A minimum of two visits, one at the beginning of the reproductive period and another at the end, have been made. At each visit and in each reinforcement plot, the surviving plants, the number of individuals, plant height and vigour are noted, also if flowering or fruiting has occurred, in addition to recording the water depth and taking a photo to document each plot.

The reinforcement progress has been assessed by survival, plant growth, flowering and fruiting rates of individuals for the first three reproductive periods (2014-16) for Galbaniturri I, II, III, IV and V; and for the first two reproductive periods (2015-2016) for Galbaniturri IIa, IIIa, IIIb and Va.

The survival rate has been calculated as the percentage of the number of introduced plants at the plantation date and the number at the end of the same reproductive period.

For plant growth, number of surviving plants has only been assessed in the first reproductive period after each plantation. The introduced plants of *R. fusca* quickly and easily produced rhizome renewals (Figure 5) and the caespitose growth form of these plants did not allow for individual monitoring beyond the first reproductive period. Therefore, the data for the second and third reproductive periods are counted in "individuals" (an emerging shoot with several leaves). This growth is represented as the number of "individuals" present at the end of one reproductive period.

For plant height, only the vegetative part height was measured, excluding inflorescence stems.

Regarding plant flowering, the individuals in flower, the number of inflorescences and finally the number of clusters per plot were counted. An inflorescence is considered as a flowering stem, usually 1-(2) are present in each individual; in each inflorescence there are typically two clusters of spikes, that have been named upper and lower clusters, sometimes there is just one cluster, or, even more rarely, three clusters. The clusters are formed of spikes; each spike contains typically 3 spikelets composed by a flower and its bract (Figure 3). The percentage of flowering individuals in each plot has been calculated, and as a vigour index for each reinforcement plot, the average number of flowering clusters per individual has been used.

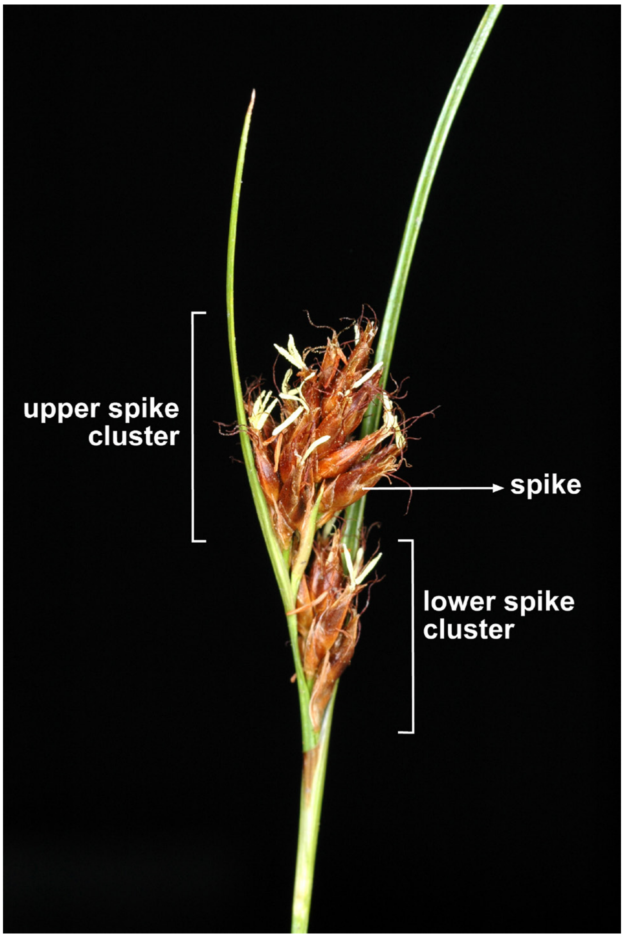

Figure 3. *Rhynchospora fusca* inflorescence, showing upper and lower clusters and spikes. (Photo: P. Heras).

To verify that the flowering in 2016 ended up producing seed and also to have an estimate of the seed production per plot, in August 31st, two inflorescences were collected in each four of the six plots of reinforcement that had fructified (Galbaniturri IIa, III, IIIa and IIIb); the other showed too few inflorescences so they were not sampled. A sample of two inflorescences was also taken from the original population. The contents of each spike were examined in the laboratory, to note the number of mature seeds and the number of aborted flowers or seeds. The average content of mature seeds per cluster was obtained for each of the sampled plots and as well for the original population.

Results

Plant survival. After the plantation in November 2013, in the first year 2014, survival rate in the five first plots (Galbaniturri I, II, III, IV and V) was at most 50% (Galbaniturri II), and it must be remarked how Galbaniturri I lost all its planted plants; in average for all plots, only 28% of all plants were alive at the end of the reproductive period 2014 (Figure 4).

In April 2015, a second set of plants was introduced, replacing Galbaniturri I and IV, and adding four new plots (IIa, IIIa, IIIb and Va; see Table 1); thus all nowadays plots have been produced by an only set of plants. Survival rates in the reproductive period in 2015 were much better, no plot was entirely lost, and plots Galbaniturri IIa and IIIb even reached a survival percentage over 90%. Average survival rate for all plots was set to 79% in 2015, in contrast to only 28% in 2014.

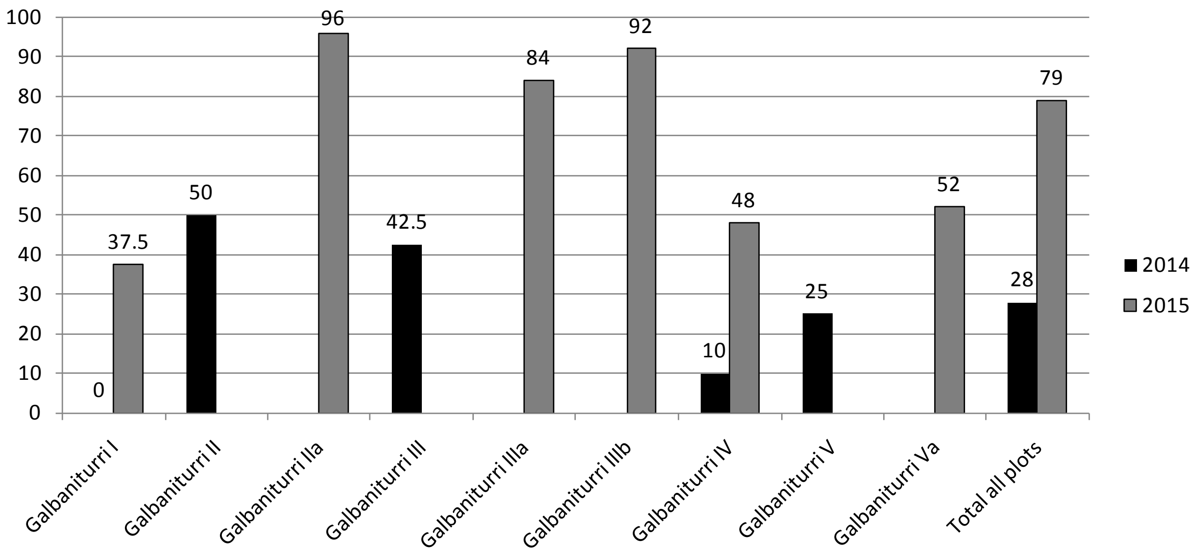

Figure 4. Plant survival rates.

Plant growth. Except for Galbaniturri V, all plots have increased their number of individuals in each of the reproductive periods. Plots Galbaniturri IIa and III have behaved particularly well. It is also worth noting that Galbaniturri II and III are the result of the first plantation in 2013, and thus, the only that count three reproductive periods in steady growth (Figure 6).

Growth in 2015 was limited in relation to that observed in 2016: Galbaniturri II only increased in 3 new individuals in 2015 while it more than doubled its effectives in 2016; Galbaniturri III increased in 10 individuals in 2015, while it increased in 35 individuals in 2016, again, more than doubled its effectives (Figure 6). The plot with the weakest performance is Galbaniturri V, which actually lost individuals in 2015, and regained in 2016 only to its departing level in 2014 (five individuals).

Considering all individuals in all plots, the total number of individuals has risen from 32 at the end of the reproductive period 2014, to 120 in 2015 and finally 352 in 2016.

Figure 5. *Rhynchospora fusca* plant showing new shoots arising from rhizome. (Photo: P. Heras).

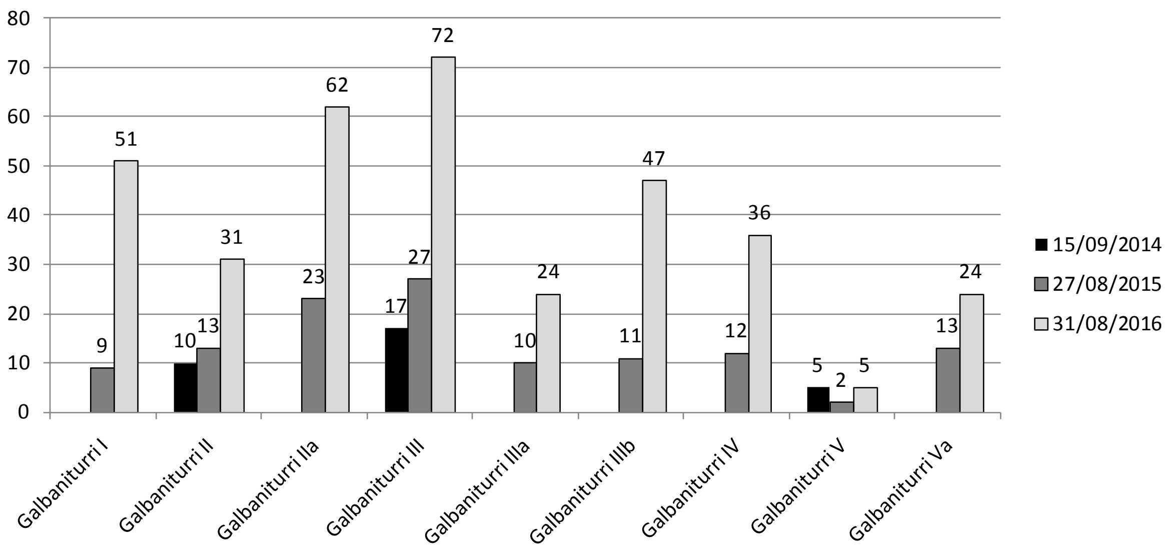

Figure 6. Growth as number of individuals present at the end of a reproductive period.

Plant height. Plant height varies in the different reinforcement plots, but in average individuals increase in 2.6 to 3 cm from June to September, at the end of the reproducing period. In 2014, plants had only developed to an average for all plots of 7.8 (SE±1.3) cm high, but in the second season 2015, they increased to 11.8 (SE±1.8) cm, similar to the height in the original population (Fig. 7). The highest plants have been registered in plots Galbaniturri II, IIa and III. Individuals in Galbaniturri IIIb and IV have decreased in height, while those in Galbaniturri V increased in 2015 but decreased in 2016.

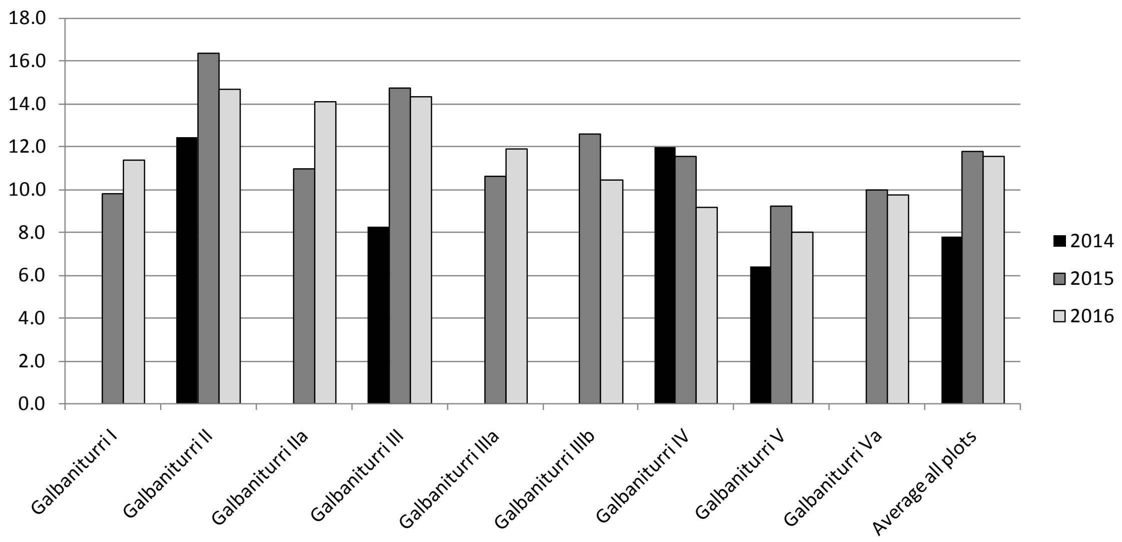

Figure 7. Average plant vegetative height in cm for the three reproductive periods.

Plant flowering. In 2016, six of the nine reinforcement plots have finally flowered (plots Galbaniturri II, IIa, III, IIIa, IIIb and Va). The plots with greater flowering have been Galbaniturri IIa and IIIb, showing 33 and 54 individuals in bloom (Figure 8). The percentage of flowering individuals in each plot has been calculated (Table 2); where again the best behaving plots are Galbaniturri IIa and IIIb, and in all an average of 41 (SE±13) has been attained.

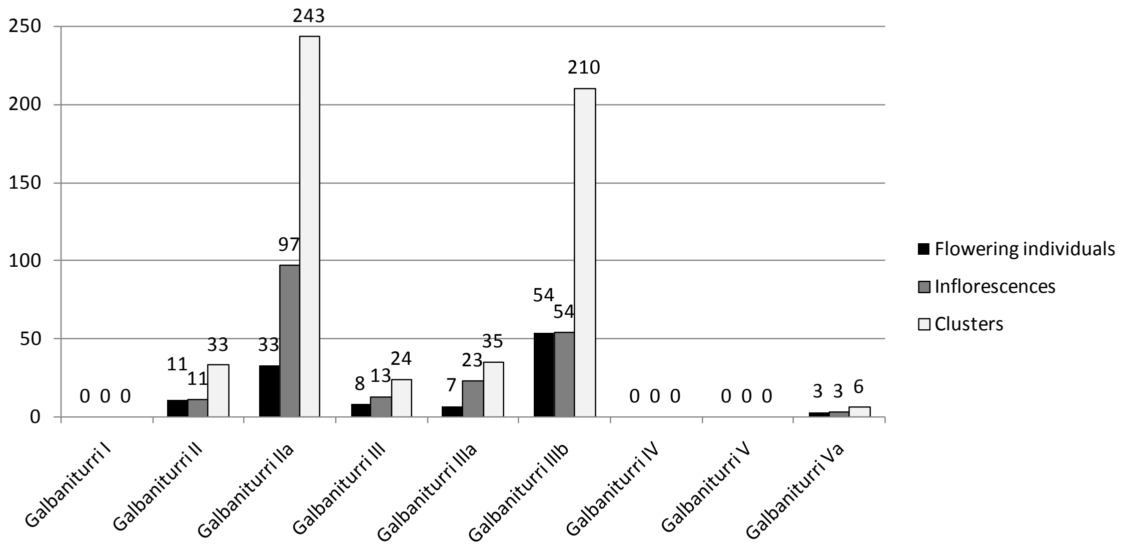

Figure 8. Total numbers of flowering individuals, number of inflorescences and number of spike clusters per reinforcement plot (11.06.2016).

Plant fruiting and seed production. It must be remarked that these results are merely estimates from a very short number of inflorescences, as a consequence of the small number of inflorescences produced, and should be taken with due care.

The average of seeds produced in each cluster varies among the different reinforcement plots, from 8.3 (SE±0.62) in plot Galbaniturri IIa to 14.0 (SE±0.7) in plot Galbaniturri IIIb; the latter being almost the same as the average production observed in the original population. It is also noteworthy that none of the clusters studied in plot Va contained any seeds and that their flowers had aborted (Figure 9).

Table 2. Percentage of flowering individuals per plot.

	11.06.2016
Galbaniturri I	0
Galbaniturri II	46
Galbaniturri IIa	67
Galbaniturri III	10
Galbaniturri IIIa	25
Galbaniturri IIIb	89
Galbaniturri IV	0
Galbaniturri V	0
Galbaniturri Va	11
Average for flowering plots	41 (SE±13)

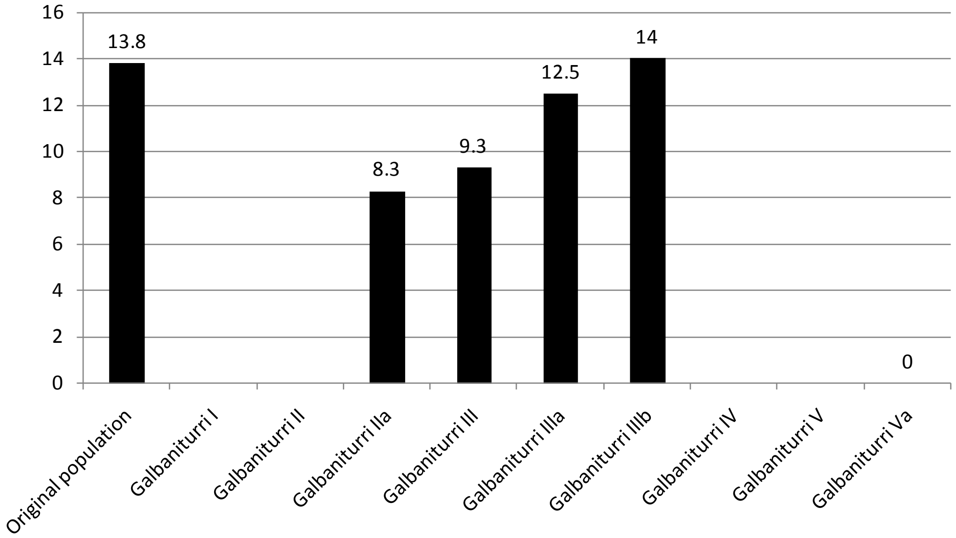

Figure 9. Average number of mature seeds per cluster in the different plots
Plot Galbaniturri II had also flowered but it was not sampled.

Considering the number of clusters counted at the end of August 2016 in each flowered plot and the average of seeds obtained per cluster, the total seed production in each plot has been estimated. The most productive are plot Galbaniturri IIa, with an estimated production of 1,791 seeds, and the plot Galbaniturri IIIb, with 1,960 seeds. (Figure 10).

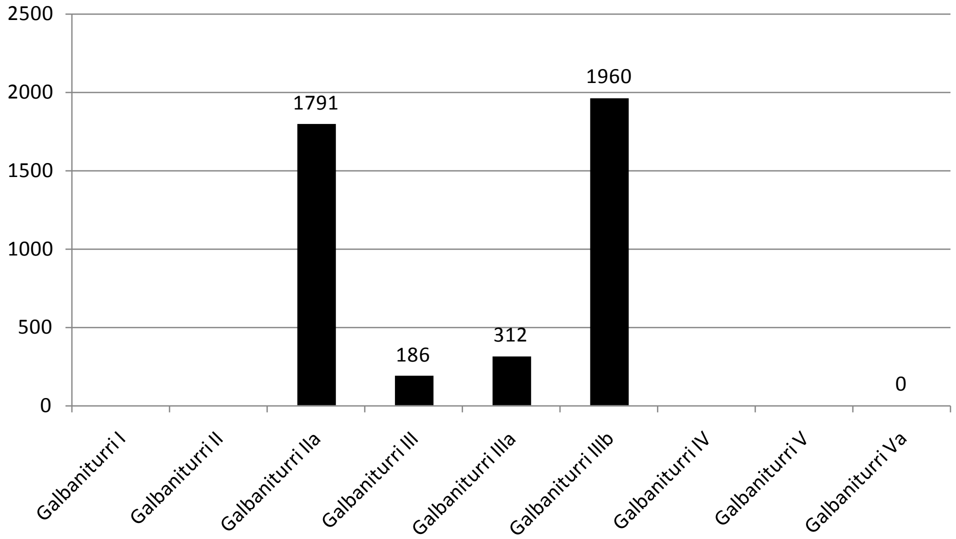

Figure 10. Total estimated number of mature seeds produced in the different plots.
Plot Galbaniturri II had also flowered but it was not sampled.

Discussion

The results show that there are obvious behavior differences among the nine plots. Regarding survival after plantation, results in the set of 2013 are considered a failure. In our opinion this was due to the decision of introducing the plants just before the winter. The newly planted plants were supposed to acclimatize during the non-growing season, but instead, they were not able to fix themselves enough to the substrate to tolerate the water outbursts in the source points after heavy winter rains. It must be stressed that the cause of these losses was strictly mechanical, since no weakened or dead plants were observed, something that could have pointed to a cause other than being dragged by water. It is also worth noting that this winter 2013-14 was not particularly harsh or rainy (Figure 11), so these water outbursts must be considered as usual, but weakly rooted plants are not able to overcome them. It must be noted how, apart winter water upwelling, no other serious problems have threatened the plots: just 3 dead plants were seen after the second plantation; as for the cattle, a slight consumption of the plants has been recorded, but it has not resulted in the loss of individuals so far.

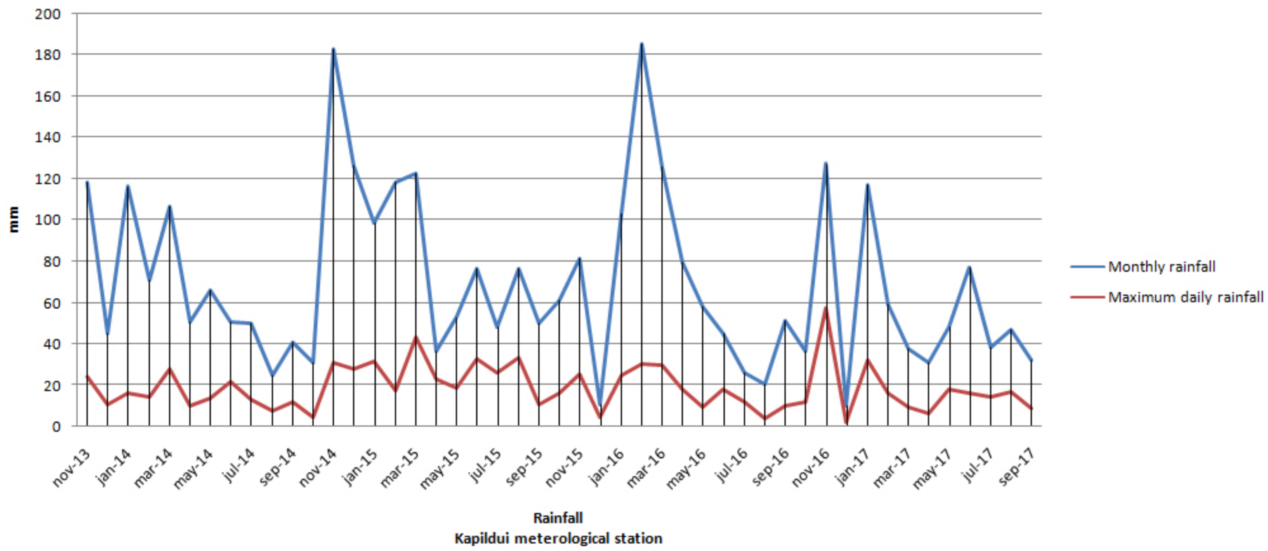

Figure 11. Monthly and maximum daily rainfall from November 2013 to September 2017.

As for plant growth, we note the ease with which *R. fusca* propagates vegetatively by shoot formation from the rhizome. This ability has multiplied the number of individuals in almost all plots (except for Galbaniturri V), and this indicates how plants in most plots are getting stabilized and spread, although still only vegetatively.

Plant height has an obvious relative increase from the beginning to the end of the reproducing period. From year to year, average for all plots in 2014 was rather low, but in the second year 2015, both the plots introduced in 2013 and those introduced in 2015, reached much higher, over 12 cm, and comparable to the height observed in the original population. This fact was interpreted as the announcing of a possible bloom in 2016, as it occurred.

Behaviour among plots regarding plant height is rather heterogeneous, but there are three which have registered the highest plants, Galbaniturri II, IIa and III, that must be noted. Nonetheless, it is evident that climate parametres in each year may affect the height of individuals, which has not been studied.

For conservation translocations, success can be defined as the ability of the population to persist and reproduce. In 2016, six of the nine reinforcement plots finally flowered (plots Galbaniturri II, IIa, III, IIIa, IIIb and Va). It is noteworthy that the plants from the first plantation (November 2013) and those of the second (April 2015) have flowered at the same time, proving how decisive it is to introduce a plant in spring with a certain amount of substrate, and so better protected from the violence of the upwelling of water and the harsh winter weather.

The percentage rate flowering/non-flowering individuals per plot is considered rather low when regarding average of the flowering plots, it does not even reach half of the individuals (41%). Galbaniturri IIa and IIIb are the plots with a higher percentage of flowering individuals, and at the same time, those with a higher absolute number of flowering individuals.

Flowering resulted in effective fruiting and seed production in all flowering plots except for Galbaniturri Va. Considerable differences have been observed in the number of mature seeds per cluster among the plots, ranging from 8,3 to 14,0; and only in Galbaniturri IIIb was comparable to that of the original population. All the rest is lower, what could be pointing to a still certain weakness of the plant vigour in most of the flowering plots. Finally, seed production has only been significant in Galbaniturri IIa and IIIb.

The non-fruiting plots (Galbaniturri I, IV, V and Va) cannot still be considered successful, even if most of them have registered positive growth. The plots that have flowered and fruited and therefore considered successful so far, are those at only two of the five sites chosen for reinforcement: site II (plots Galbaniturri II and IIa) and site III (plots III, IIIa and IIIb). Among these last plots, Galbaniturri IIa and IIIb show the highest scores for flowering and fruiting, and may be thus considered the most successful.

The reasons for these differences have not been studied so far, although water flow dynamics and depth would be *a priori* the most probable explanations.

Conclusions

Each reintroduction project is unique with regard to the species involved, questions asked, intended purposes and external circumstances in which the work is conducted (Guerrant & Kaye, 2007). Although mires are generally very vulnerable to both natural and human-induced environmental changes, on this occasion, after years of study and monitoring of the Galbaniturri 1 mire, it was decided to take action on only one species, *R. fusca*, because of its rarity at the Iberian peninsula level, and because it is characteristic of a very specific habitat (7150 *Rhynchosporion*), indeed the only example in the Basque Country Autonomous Community. Since the original population is stable but fragile given its small size and location in a mire surrounded by a cattle breeding area, subject to eventual trampling and grazing, the decision was made to create new sites within the same mire, which would multiply its survival chances in the event of destruction of the original population.

As a helophyte, *Rhynchospora fusca* presented specific additional difficulties in this population reinforcement. Water is always a difficult element to foresee and manage, and especially when it is in constant movement.

One of the most important conclusions of this experience relates to the planting season: planting in spring has proved being much more effective than planting in autumn, so that plants have a chance to root and grow before winter harsh weather. Planting in spring has allowed plants to flower at their second reproductive period, while those planted in autumn had to wait to the third reproductive period.

It seems obvious that site selection was quite good, but it is also obvious that there are better sites (II and III) than the rest (I, IV and V). This stresses how important, but yet difficult, it is to acquire enough knowledge of the conditions that affect plant life.

Nowadays, all discussed aspects lead us to believe that at least five of the nine plots (II, IIa, IIb, III, IIIa), situated in two of the five selected sites, exhibit a real potential to persist over time, due to their results in terms of plant growth, height and most importantly, flowering and fruiting. Nevertheless, long-term monitoring is necessary, since good initial results are often followed by reversals over time (Godefroid & *al*., 2011).

For the future, since used plants have been obtained from vegetative material, this could pose the problem of subtraction of material from the original population, and the production of genetically identical clones. During the current year 2017, the convenience of proceeding with a third plantation is being assessed according to the evolution of the plots, and the feasibility of introducing the individuals that have already be obtained from seed at the Gipuzkoa Plant Germplasm Bank.

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