Chemical control of the invasive non-native shrub murtilla *Ugni molinae* in mountain scrub on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile

Héctor Gutiérrez-Guzmán[1]*, Silvia R. Ziller[2] & Michele de S. Dechoum[2]

[1] *Oikonos Ecosystem Knowledge, Yerbas Buenas 498 – Valparaíso, Chile*
[2] *The Horus Institute for Environmental Conservation and Development, AC Baía Sul, Caixa Postal 4543 Florianopolis – SC, Brazil 88.047-971, Brazil*

SUMMARY

The Juan Fernandez Archipelago is a global biodiversity hotspot, where 67% of plant species are endemic, but competition with invasive plants threatens many native plant species with extinction. Murtilla *Ugni molinae* is a prevalent invasive plant in the archipelago which displaces native vegetation. This study aimed to determine an efficient one-time control method for murtilla that required little or no follow-up. We used an adaptive management framework to conduct chemical control trials of murtilla in order to identify an effective treatment. Eight different combinations of chemical treatments and manual cutting were tested in four trials between 2015 and 2017. The herbicides Rango (glyphosate) and Garlon 4 (triclopyr) were tested along with a surfactant, an emulsifier, ammonium sulphate and urea. Cutting stems at the base followed by stump application of triplocyr proved ineffective. All other treatments used foliar spraying. The most effective treatment was a foliar application of 3% triclopyr, 2% glyphosate and 15 g/l of urea diluted in water, which completely eliminated murtilla in 12 months. This treatment can be used for the control of murtilla over large areas and may also be useful to control other invasive shrubs that have leaves with thick cuticles resistant to herbicide absorption.

BACKGROUND

Oceanic islands are very important for the conservation of biodiversity because they often contain high percentages of endemic species resulting from thousands of years of isolated evolutionary processes. However, these ecosystems are vulnerable because their biological communities evolved in isolation and have not developed adaptations or the ability to compete with species introduced by humans (Estades 1998). Biological invasions have caused much damage on island ecosystems worldwide (Bellard *et al.* 2016, Spatz *et al.* 2017).

The Juan Fernandez Archipelago in Chile was made a National Park in 1935 and a Biosphere Reserve in 1977, and 67% of plant species on the islands are endemic (Penneckam-Furniel 2018). The archipelago has been identified as a global priority for conservation on several occasions and is considered a biodiversity hotspot (Mittemeier *et al.* 1999, Myers *et al.* 2000). Despite legal protection, however, the introduction of non-native animals and plants has been linked to the extinction and near-extinction of many species that are endemic to the archipelago (Matthei 1995, Cuevas & Van Leersum 2001, Greimler *et al.* 2002, Dirnböck *et al.* 2003, Cuevas *et al.* 2004, Danton 2004). Among the endemic plants, *Santalum fernandezianum* and *Eryngium sarcophyllum* are extinct, while *Dendroseris gigantea* is extinct in the wild, but a few live specimens remain in local nurseries. Only two specimens of *Dendroseris neriifolia* and *Greigia berteroi* are known to occur in the wild. Danton *et al.* (2006) noted that, of the 213 endemic plant species found on the archipelago, "8 disappeared (3.8% of the plant species), 173 are threatened at various levels (81.2%) and 32 are classified as least concern or data deficient (15.0%)".

Murtilla *Ugni molinae*, elmleaf blackberry *Rubus ulmifolius* and maqui *Aristotelia chilensis* are the most aggressive introduced plants in the archipelago and have severely affected the sustainability of native forests by preventing natural regeneration and monopolizing clearings formed when trees fall due to storms or old age (Dirnböck *et al.* 2003). Murtilla was probably introduced to Robinson Crusoe Island in the early 1800s for cultivation by colonizers (Johow 1896). The time of arrival of this species on Alejandro Selkirk Island, the westernmost island of the archipelago, is not known. As it is still restricted to few sites on this island, eradication might be achievable with an efficient control method.

Murtilla is an edible, fruit-bearing, evergreen shrub in the Myrtaceae family that grows on open terrain where shrubs and herbs are dominant, in forest undergrowth and along forest borders, and has become highly invasive in the Juan Fernandez Archipelago. Murtilla is native to central and southern continental Chile (Hoffmann 1997, Pastenes *et al.* 2003, Retamales *et al.* 2014). It grows on soils of low fertility and is known for outcompeting other species (Seguel *et al.* 2000).

On Robinson Crusoe Island, murtilla occurs mostly between altitudes of 170 and 350 m, forming dense thickets that develop into impenetrable barriers (Cavieres *et al.* 2011). On these fragile, steep slopes it displaces native species such as *Blechnum cycadifolium* and *Gunnera bracteata* (Greimler *et al.* 2002). Approximately 116 ha on Robinson Crusoe are covered by murtilla and it has a very rapid rate of annual spread. In the seven years between 2003 and 2010, murtilla increased 3.43 ha in surface area, corresponding to an annual rate of spread of 0.49 ha/yr (Díaz-Vega 2013).

This study aimed to identify an efficient control method for murtilla in order to restore the vegetation on steep slopes invaded by this species in the Juan Fernandez Archipelago. No evidence was found in the Conservation Evidence database describing the effectiveness of chemical control of invasive shrubs, nor were there any studies of murtilla.

*To whom correspondence should be addressed: hgutierrez@oikonos.org

ISSN 1758-2067

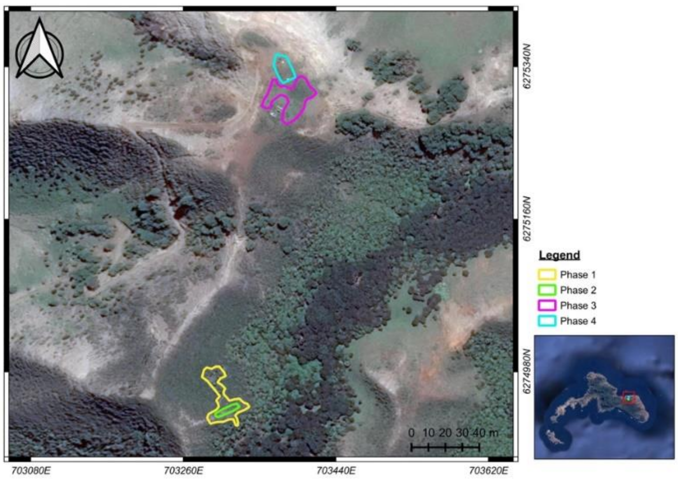

Figure 1. Location of murtilla control trials in Phases 1, 2, 3 and 4 on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile.

Figure 2. Slope invaded by murtilla, where trials were conducted in Phases 1 and 2 on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile.

ACTION

Control trials for murtilla were conducted on Cerro Centinela on Robinson Crusoe Island, in the Juan Fernandez Archipelago, about 667 km west of the San Antonio harbor in Central Chile, in the Pacific Ocean (33°38' S, 78°48' W; Figure 1). Four trial phases were conducted in mountain scrub on steep slopes, the first two in an area at 336 m above sea level., between December 2015 and May 2016, and the last two in another area at 328 m above sea level, between October 2016 and March 2017. These plots were monitored until February 2018. The trials aimed to identify an effective control method through an adaptive management approach. The information from each set of trials was used to improve the following trial until a satisfactory result was achieved.

The herbicides Garlon 4 (triclopyr) and Rango (glyphosate) were chosen for the trials because they degrade in a short time (half-life between 20 and 45 days) and are not exuded through the roots of plants. These characteristics are relevant for work in natural areas and fragile ecosystems because they help prevent soil and water contamination.

All plants were individually numbered with the number of the plot and the plant were using a permanent marker pen and plastic tape. The results of each treatment were monitored through direct observation of the plants and photographs of each individual, comparing the percentage of dead leaves. The treatments of the first two phases were monitored at month 3, 6 and 12. The treatments of Phase 3 were monitored in months 1,

Table 1. Different combinations of chemical treatments applied in Phases 1, 2, 3 and 4 of trials to control murtilla on Robinson Crusoe Island. All treatments were applied as a foliar spray diluted in water unless otherwise stated. AS = Ammonium sulphate.

Treatment	Garlon 4 (ml/l) [triclopyr (%)]	Rango (ml/l) [glyphosate (%)]	Adjuvants
PHASE 1			
1	10 [0.67]	--	5 ml surfactant + 10 ml dye
2	20 [1.34]	--	5 ml surfactant + 10 ml dye
3	--	40 [2]	5 ml surfactant + 10 ml dye
4	30 [2.00]	--	5 ml surfactant + 10 ml dye
5	--	60 [3]	5 ml surfactant + 10 ml dye
Application on cut stump (cut with chainsaw) – dilution in vegetable oil			
6	10 [0.67]	--	10 ml emulsifier + 10 ml dye
7	30 [2.00]	--	10 ml emulsifier + 10 ml dye
Application on cut stump (cut manually) – dilution in vegetable oil			
8	10 [0.67]		10 ml emulsifier + 10 ml dye
PHASE 2			
9	60 [4.00]	0 [0.00]	10 g/l urea + 10 ml dye
10	60 [4.00]	60 [3.00]	10 g/l urea + 10 ml dye
PHASE 3			
11	60 [4.00]	60 [3.00]	10 g/l urea + 7.5 g/l AS + 10 ml dye
12	60 [4.00]	0 [0.00]	10 g/l urea + 7.5 g/l AS + 10 ml dye
13	60 [4.00]	60 [3.00]	10 g/l urea + 10 ml dye
14	60 [4.00]	0 [0.00]	10 g/l urea + 10 g/l AS + 10 ml dye
15	45 [3.00]	40 [2.00]	10 g/l urea + 10 ml dye
PHASE 4			
16	45 [3.00]	40 [2.00]	15 g/l urea + 10ml dye
17	45 [3.00]	40 [2.00]	20 g/l urea + 10ml dye

ISSN 1758-2067

Figure 3. Murtilla shrub with characteristic dry branches and some reddish leaves, before trials carried out on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile.

3, 5, 8, 15 and 17 after herbicide application. The treatments of Phase 4 were monitored at month 3, 10 and 12 after herbicide application.

All herbicide applications were conducted with personal protective equipment that included nitrile gloves, impermeable hooded aprons, eyewear, respirators and rubber boots Spraying was carried out with hand-held 1.5 L sprayers, as only small volumes of herbicide solutions were required for each treatment. The application of each treatment took between 6 and 8 h, which included setting up the trial plots and marking the plants. The total cost of trials and monitoring were estimated at US $185.00, including herbicides, personal protection equipment, transportation, sprayers and working hours for applications and monitoring.

Phase 1: Eight treatments using different combinations of herbicide solutions were conducted (Figure 2) on 4 and 7 December 2015 (Table 1).

Four plots with five plants each were established for each of the five foliar spray treatments, totaling 20 plants per treatment and 100 plants treated (Table 1). The height of all plants was recorded in three classes (0-20 cm, 20-40 cm and >40 cm) to ensure that efficiency in relation to plant size was considered in the evaluation of results. A minimum distance of 1.0-1.5 m was maintained between plots. The larger shrubs characteristically had some dry branches with a few reddish leaves before treatment (Figure 3).

Because the leaves of murtilla are covered by a thick, prominent cuticle on the upper surface (Retamales *et al.* 2014),

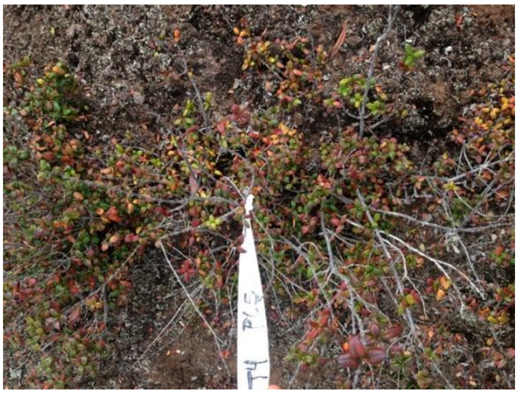

Figure 4. Murtilla on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile after Phase 1 treatment with triclopyr and glyphosate, with many reddish leaves.

Figure 5. Murtilla on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile after Phase 2 treatments including urea as well as triclopyr and glyphosate.

a surfactant (Dash© HC by BASF) was added to all herbicide solutions in Phase 1 in order to improve absorption. A blue dye (Hi Light by Rigrantec) was also added to mark the solution and increase application safety and efficiency. The solutions prepared for foliar spray were mixed in clean water.

Three additional treatments were tested on cut stumps of murtilla in Phase 1. In treatments 6 and 7, triclopyr was applied on stumps cut with a chainsaw, while in treatment 8 it was applied as each stem was cut manually with pruning shears (Table 1). The manual procedure took between one and two hours per shrub. These solutions were mixed in soybean oil with an emulsifier required for dilution of the dye.

Phase 2: A second trial was conducted on 19 April 2016. A new set of treatments was designed including urea, a low-cost product, to break the cuticle of leaves and allow herbicide absorption. The use of a surfactant and the cut stump method were both discontinued. The plants in two of the plots treated in Phase 1 were sprayed with the revised solutions diluted in water (Table 1).

As these treatments were applied before the fruiting period of murtilla, the local community was informed and asked not to collect fruit for consumption from the trial area.

Phase 3: Further trials were conducted in a new area on Cerro Centinela on 21 October 2016. Five treatments were applied (Table 1) to four new plots, with 30 plants in each plot. This included five control plants per plot that were not sprayed. All treatments were applied by foliar spraying of herbicide solutions with different concentrations of active ingredients diluted in water and the addition of 10 g/l of urea.

Phase 4: Based on the results of the previous trials, two new treatments were applied on 31 March 2017 (Table 1). These solutions were diluted in clean water and applied on new plants that had not been previously treated in an area near the Phase 3 plots. The use of ammonium sulphate was discontinued and the concentration of urea was increased to 15 and 20 g/l.

CONSEQUENCES

The Phase 1 chemical control trial using triclopyr and glyphosate was carried out in December 2015. By March 2016, none of the treated plants had died, although part of the leaves of most plants had become reddish (Figure 4). The thick, waxy cuticle of murtilla apparently prevented the herbicide solutions from being absorbed. None of the plants dried up completely

ISSN 1758-2067

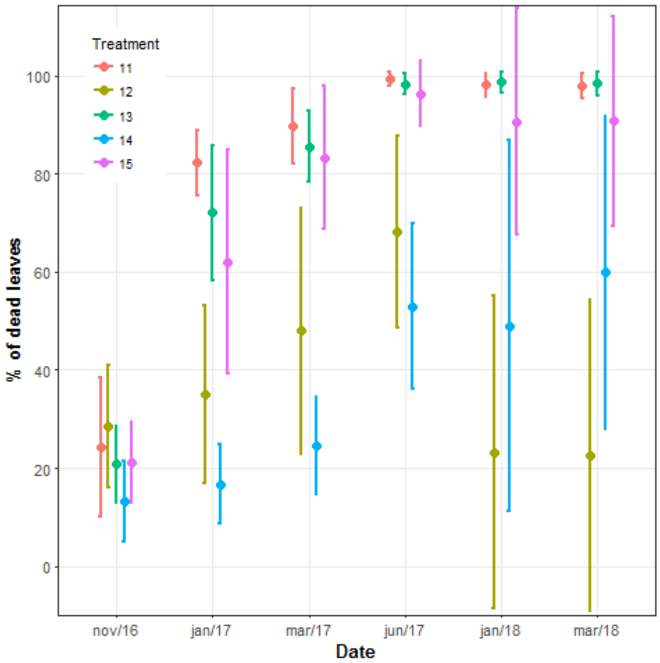

Figure 6. Percentage of dry murtilla leaves resulting from treatments 11 – 15 in Phase 3 of the trials to control murtilla with triclopyr, glyphosate and ammonium sulphate conducted on Cerro Centinela, Robinson Crusoe Island, Juan Fernandez Archipelago, Chile. Treatments were applied in October 2016.

and all were alive after 12 months. The shrubs cut manually or with a chainsaw re-sprouted from roots or the base of stumps, while the tiny stems that were not possible to treat by this method were not affected and continued to develop. Because the treatments were not effective, no detailed results were recorded. New trials were then planned based on these negative results.

In Phase 2, in which the same plants were treated with a herbicide mix containing urea as well as triclopyr and glyphosate (Figure 5), only five plants (25%) dried up completely (treatment 10). After six months, 45% plants had 90% dry leaves, 20% had 80% dry leaves and 10% had 70% dry leaves. Although this trial showed that the combination of herbicide plus urea was more effective than treatment with herbicide alone, it was not considered effective because the incomplete drying out meant that another follow-up treatment would be required. Based on these results, new trials were conducted in a different area, on plants that had not been treated before.

Ammonium sulphate was added to the treatment in Phase 3, to help break the thick cuticle of murtilla leaves. In this phase, none of the five treatments eliminated all the plants, regardless of size, and large variation was observed between treatments during the monitoring period (Figure 6). After 17 months the percentage of dried leaves on 100 plants treated varied between 0 and 100%. While 14 plants had 10% or less of the leaves affected, 22 plants dried up and the other 64 dried partially. Therefore, the use of ammonium sulphate did not increase the percentage of dead plants and was discontinued in the following trial phase. The plants in the untreated plot had no dry leaves, showing that external causes were not affecting the plants.

Finally, in Phase 4 two more treatments (16 and 17) were tested on a new set of plants in the same area on 31 March, 2017. The concentration of herbicide was reduced to 3% triclopyr and 2% glyphosate, while the amount of urea was increased to 15 g/l and 20 g/l in treatments 16 and 17 respectively. This proved effective. Three months after application, 40% of the leaves of all plants were dry and all plants were still alive. By the tenth month (January 2018), all plants had at least 95% dry leaves and 12 plants were still alive. After a year, all except six plants were 100% dry (Figure 7). The exceptions were two plants in

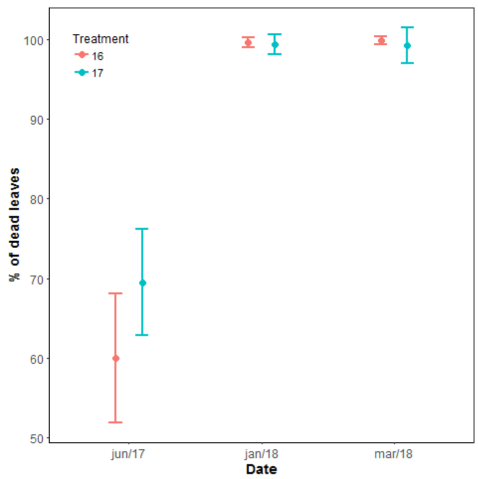

Figure 7. Percentage of dry murtilla leaves resulting from treatments 16 and 17 in Phase 4 of the trials to control murtilla with triclopyr, glyphosate and urea conducted on Cerro Centinela, Robinson Crusoe Island, Juan Fernandez Archipelago, Chile. Treatments were applied in March 2017.

Figure 8. Murtilla shrub partially affected by Phase 2, treatment 10 of the control trials carried out on Cerro Centinela, Robinson Crusoe Island, Juan Fernandez Archipelago, Chile.

ISSN 1758-2067

Figure 9. Completely dry murtilla shrub after Phase 4, treatment 16 of the trials carried out on Cerro Centinela, Robinson Crusoe Island, Juan Fernandez Archipelago, Chile.

treatment 16 and four plants in treatment 17 that were less than 100% dry but more than 90% dry 12 months after application. Twenty-three months after application, in February 2019, all treated shrubs treated were 100% dry (Figure 9). There was little difference in the results of treatments with 15 g/l and 20 g/l urea (Figure 7).

The size of the shrubs did not influence the results. No plants of any size died in Phase 1 of the trials while all plants of all sizes dried up in the last trial. Again, the plants in the control plot were not affected (Figures 10 and 11).

DISCUSSION

This study aimed to determine an effective method for the control of murtilla in the Juan Fernandez Archipelago in Chile. Several of the initial trials did not yield satisfactory results, but an effective treatment was ultimately identified using a solution of 3% triclopyr, 2% glyphosate and 15 g/l urea.

The cut stump method proved impractical and was abandoned for three reasons. First, it took between one and two hours to manually cut and treat the stems of a single shrub (when cut with a chainsaw, the stumps were longer and more difficult to treat); second, it was not possible to be sure that all the stems of the same shrub were cut and treated, especially in areas where there was a continuum of invasion by murtilla and individual plants could not be distinguished; and third because each shrub had many thin, tiny stems growing from the roots, some of 1-2 mm diameter, which were not viable to treat with the cut stump method without contaminating the soil with herbicide mixture, which was not desirable in the National Park. Any tiny stems left untreated continued to grow, compromising the success of this method.

Figure 10. Aerial view of murtilla invasion with dead shrubs among non-treated shrubs and non-target plants after treatments in Phase 3, Cerro Centinela, Robinson Crusoe Island, Juan Fernandez Archipelago, Chile, October 2017.

Figure 11. Completely dry murtilla shrubs in February 2019, 23 months after the treatment in Phase 4 trial on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile, Neighbouring untreated shrubs were not affected.

The Phase 1 and 2 trials showed that the thick cuticle of murtilla leaves prevented herbicide absorption and applying higher herbicide concentrations was not enough to overcome this (Figure 8). Similarly, urea was ineffective until the dosage was increased to 15 g/l. Two years after the application of treatments 16 and 17 (Phase 4) all shrubs treated were completely dry and breaking apart (Figure 11). Treatment 16 (3% triclopyr, 2% glyphosate and 15 g/l urea diluted in water with a dye) was therefore considered an effective control treatment. No side effects were observed in neighbouring non-treated shrubs or other non-target plants.

Given the steepness and isolation of areas invaded by murtilla in the Juan Fernandez Archipelago (Figure 10), it was important to develop a one-time treatment that does not require much follow-up work. A one-time treatment was also an important goal because the climate on the archipelago only allows for control work to be carried out in the summer, between December and March, when it rains less. The average precipitation in summer (January to March) is 42.6 mm, while in winter (July to September) it is 125.2 mm (Dirección Meteorológica de Chile 2019, data for 1961 to 1990).

Priority areas for control in the Juan Fernandez Archipelago are those where invasion by murtilla is in its early stages and native vegetation still predominates. Therefore, on Alexander Selkirk Island, control should begin by eliminating isolated murtilla plants in the high areas of the island. There are several murtilla shrubs along the trails to the Tres Torres sector and La Cuchara that could be eliminated with low effort. On Robinson Crusoe, priority must be given to controlling murtilla in sites where it affects plants in critical risk of extinction, such as the El Camote, Cerro Damajuana, Cordón Atravesado and La Piña sectors, as well as the high mountain areas where endemic species of *Robinsonia* and *Eryngium* occur.

The potential for soil erosion following treatment needs to be considered and monitoring to verify potential regeneration of native species is needed. This will support decision making on whether to eliminate strips of murtilla plants to help avoid erosion, or whether eliminating murtilla shrubs on whole slopes at once is feasible. As the shrubs took about a year to die, and longer than this to dry up and drop all leaves, there may be less concern about soil erosion, as there will be time for native plants to develop and replace murtilla. Working in strips to maintain

ISSN 1758-2067

partial cover and prevent erosion is recommended until data on regeneration is available.

ACKNOWLEDGEMENTS

We thank the Corporación Nacional Forestal (CONAF) for support from the Manager and park rangers of the Archipiélago Juan Fernández National Park; Felipe Sáez, in charge of the ecological restoration project on Robinson Crusoe; the GEF Project “Fortalecimiento de los Marcos Nacionales para la Gobernabilidad de las Especies Exóticas Invasoras (EEI) - Proyecto Piloto en el Archipiélago Juan Fernández for funding the work and consultancy; the local Oikonos staff, especially Ariane Claussen, Tomás González, Lake Thelen, Isabel Bastías, Paola González,, Guillermo de Rodt, Sara de Rodt and Pablo Manríquez for support in setting up and monitoring the control trials; Ryan Carle (Oikonos) for reviewing our paper; and María José Faúndez for helping us prepare the location map.

REFERENCES

Bellard C., Cassey P. & Blackburn, T.M. (2016) Alien species as a driver of recent extinctions. *Biology Letters*, **12**, 20150623.

Cavieres A., Mellado F., Tramón S. & Rebolledo S. (2011) *Plan de Conservación Biodiversidad Terrestre. Archipiélago Juan Fernández*. Santiago: Fundación Biodiversa. 51p.

Cuevas J.G. & Van Leersum G. (2001) Project “Conservation, restoration and development of the Juan Fernandez Islands, Chile”. *Revista Chilena de Historia Natural*, **74**, 899-910.

Cuevas J.G., Marticorena A. & Cavieres L.A. (2004) New additions to the introduced flora of the Juan Fernandez Islands: Origin, distribution, life history traits and potential of invasion. *Revista Chilena de Historia Natural*, **77**, 523-538.

Danton P. (2004) *Plantas Silvestres de la Isla Robinson Crusoe. Guía de Reconocimiento*. Viña del Mar, Chile: Corporación Nacional Forestal. 194p.

Danton P., Perrier & Reyes G.M. (2006) Nouveau catalogue de la flore vasculaire de l'archipel Juan Fernández (Chili) Nuevo catálogo de la flora vascular del Archipiélago Juan Fernández (Chile). *Acta Botanica Gallica*, **153**, 399-587.

Díaz-Vega R. (2013) Análisis y modelación de la evolución espacio-temporal de la invasión de Rubus ulmifolius, Aristotelia chilensis y Ugni molinae, en la isla Robinson Crusoe. Tesis, Ingeniería en Recursos Naturales, Facultad de Ciencias Agronómicas, Universidad de Chile. Santiago, Chile.

Dirección Meteorológica de Chile (2019). http://www.meteochile.cl/PortalDMC-web/index.xhtml. (Accessed 5 March, 2019).

Dirnböck T., Greimler J., López P. & Stuessy T.F. (2003) Predicting future threats to the native vegetation of Robinson Crusoe Island, Juan Fernandez Archipelago, Chile. *Conservation Biology* **17**, 1650-1659.

Estades C. (1998) Especie non grata: efectos ecológicos de las especies exóticas. *Ciencia al día*, **2**, 1-12.

Greimler J., Stuessy T.F., Swenson U., Baeza C.M. & Matthei O. (2002) Plant invasions on an oceanic archipelago. *Biological Invasions* **4**, 73-85.

Hoffmann A. (1997) *Flora Silvestre de Chile, Zona Araucana*. Ediciones Fundación Claudio Gay, Santiago de Chile.

Johow F. (1896) *Estudio sobre la Flora de las Islas de Juan Fernández*. Imprenta Cervantes, Santiago de Chile.

Matthei O. (1995) *Manual de las malezas que crecen en Chile*. Alfabeta Impresores, Santiago, Chile.

Mittermeiers R., Myers N., Robles P. & Mittermeiers C.G. (1999) *Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions*. CEMEX Conservation International, Washington D.C., USA.

Myers N., Mittermeier R., Mittermeier C., da Fonseca G. & Kent J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853-858.

Pastenes C., Santa-María E., Infante R. & Franck N. (2003) Domestication of the Chilean guava (*Ugni molinae* Turcz.), a forest understorey shrub, must consider light intensity. *Scientia Horticulturae* **98**, 71-84.

Penneckamp Furniel D. (2018) Flora Vascular Silvestre del Archipiélago Juan Fernández. Primera Edición (versión electrónica). Planeta de Papel Ediciones, Valparaíso, Chile.

Retamales H.A., Scherson R. & Scharaschkin T. (2014) Foliar micromorphology and anatomy of *Ugni molinae* Turcz. (Myrtaceae), with particular reference to schizogenous secretory cavities. *Revista Chilena de Historia Natural* **87**, 7.

Seguel I., Peñaloza E., Gaete N., Montenegro A. & Torres A. (2000) Colecta y caracterización molecular de germoplasma de murta (*Ugni molinae* Turcz.) en Chile. *Agrosur* **28**, 32-41.

Spatz D.R., Zilliacus K.M., Holmes N.D., Butchart S.H.M., Genovesi P., Ceballos G., Tershy B.R. & Croll D.A. (2017) Globally threatened vertebrates on islands with invasive species. *Science Advances*, **3**, e1603080.

ISSN 1758-2067