

The role of frugivorous birds in fruit removal and seed germination of the invasive alien *Cotoneaster franchetii* in central Argentina

MARÍA C. DÍAZ VÉLEZ,^{1*}  ALICIA N. SÉRSIC,¹ ANNA TRAVESET² AND VALERIA PAIARO¹

¹*Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Universidad Nacional de Córdoba, Av. Vélez Sársfield 1611, C.C. 495, 5000 Córdoba, Argentina (Email: celestemcbiol@gmail.com); and* ²*Instituto Mediterráneo de Estudios Avanzados (CSIC-UIB), Mallorca, Balearic Islands, Spain*

Abstract Many invasive plant species have fleshy fruits that are eaten by native frugivorous birds which disperse their seeds and may facilitate their germination, playing an important role in plant invasion success. The fleshy-fruited shrub *Cotoneaster franchetii* (Rosaceae) is an important invasive alien in the mountainous regions of central Argentina. To determine the role of avian frugivorous in fruit removal of this species, we conducted a frugivore exclusion experiment including bagged and unbagged branches in 75 plants of *C. franchetii*. At the end of the dispersal period, we compared the percentage of missing fruits (removed by birds + naturally dropped) in unbagged branches with the percentage of naturally dropped fruits in bagged branches. To assess whether any mechanism acting on seeds during their passage through bird guts (de-inhibition by pulp removal and/or seed scarification) affects seed germination of this species, we compared percentage and speed of germination among seeds obtained from faeces of the native frugivorous *Turdus nigriceps nigriceps*, from manually de-pulped fruits, and from intact fruits. The percentage of missing fruits per shrub in unbagged branches was significantly higher than the percentage of naturally dropped fruits in bagged branches, suggesting that frugivorous birds play an important role in fruit removal of *C. franchetii* in the study area. Seeds from bird faeces and from manually de-pulped fruits germinated in higher percentage and faster than seeds from intact fruits. Germination percentage and speed of seeds from manually de-pulped fruits were significantly higher than those of gut-passed seeds. These results indicate that *T. nigriceps nigriceps* increases and accelerates seed germination of *C. franchetii* through pulp removal, but not through seed scarification. Overall, our findings indicate that native frugivorous birds facilitate the dispersal and germination success of *C. franchetii*, likely playing an important role in its invasion throughout the mountainous region of central Argentina.

Key words: de-inhibition by pulp removal, fleshy-fruited shrub, gut-passage, seed scarification, *Turdus nigriceps nigriceps*.

INTRODUCTION

Alien invasive species are one of the most critical ecological problems of the modern times, because their proliferation affects biodiversity conservation and ecosystems functioning (Daehler *et al.* 2016). Introduced plants face several barriers to colonizing a new environment, and many of them rely on mutualistic interactions with local species (Richardson *et al.* 2000; Traveset & Richardson 2014). Plants with animal-dispersed fruits seem to overcome the barriers that limit their spread into new habitats more easily than other invasive plants (Buckley *et al.* 2006;

Muñoz & Ackerman 2013; Traveset & Richardson 2014). Thus, the management of invasive fleshy-fruited plants is a critical challenge for biodiversity conservation (Buckley *et al.* 2006; Jordaan *et al.* 2011; Muñoz & Ackerman 2013). In this context, a better understanding about the role of generalist frugivores in invasive plant dispersal is crucial for conservation decision making (Meisenburg & Fox 2002; Gosper *et al.* 2005; Tassin *et al.* 2007).

Among seed dispersal animals, fruit-eating birds are one of the most significant groups in the dynamics of biotic seed dispersal in the landscape (Dennis *et al.* 2007). In fact, over 40% of invasive tree species and over 60% of invasive shrubs, including the most detrimental invasive plant species, are dispersed by generalist birds (Richardson & Rejmánek 2011; Traveset & Richardson 2014). Although fleshy-fruited aliens may rapidly establish loose mutualisms with

*Corresponding author.

Accepted for publication January 2018.

MDV and VP have contributed equally to this study and should be considered as co-first authors.

native frugivorous birds that consume their fruits in the new environment, such consumption may not necessarily result in successful dispersal (Mandon-Dalger *et al.* 2004; Combs *et al.* 2011). This is because different functional groups of frugivorous birds may or may not contribute to seed dispersal with respect to the way they handle/consume the fruit; i.e. *legitimate seed dispersers* swallow the whole fruit and defaecate or regurgitate intact seeds, *pulp consumers* peck the fruit to obtain pulp pieces and may damage the embryo, and *seed predators* feed on the seed contents and always damage the embryo (Jordano *et al.* 2007; see Jordano & Schupp 2000 for classification). The prevalence of legitimate dispersers in the frugivorous assemblage would be a key factor on the spread stage, increasing the invasion success of an alien fleshy-fruited plant. Thus, determining the relative contribution of this functional group of birds to fruit removal, as well as the amount of removed fruits, which is positively related to the population density of plant species (Lonsdale 1999; Lichstein *et al.* 2004), is crucial to evaluate the progress of an eventual invasion by fleshy-fruited aliens.

Legitimate seed dispersers birds play a key role in seed dispersal and establishment, not only by dispersing seeds away from parent plants, but also by altering germination patterns via two processes occurring during seed' gut-passage: de-inhibition and/or scarification (Samuels & Levey 2005; Robertson *et al.* 2006; Traveset *et al.* 2007; Renison *et al.* 2010; Wilson & Downs 2012; Thabethe *et al.* 2015; Díaz Vélez *et al.* 2017). Firstly, germination patterns may be altered by a de-inhibition process occurring by fruit pulp removal during gut-passage. The fruit pulp may contain compounds that can reduce germination success by altering the seeds' microenvironment (e.g. osmotic pressure and light regime) or by more directly blocking or inhibiting biochemical pathways of germination if left in contact with seeds (Samuels & Levey 2005; Traveset *et al.* 2007). Secondly, seed germination may be affected by scarification occurring when mechanical and/or chemical action of the digestive tract alters the seed coat or endocarp (Samuels & Levey 2005; Robertson *et al.* 2006; Traveset *et al.* 2007, 2008).

Relevance for germination of the different mechanisms acting on seeds during their passage through bird guts varies among alien plant species. In some fleshy-fruited aliens, the importance of the scarification effect on seed germination has been emphasized (Mandon-Dalger *et al.* 2004; Linnebjerg *et al.* 2009; Amodeo *et al.* 2017). However, in others only pulp removal by frugivorous birds would be sufficient to increase seed germination, with no evidence of a scarification effect (Panetta & McKee 1997; Mandon-Dalger *et al.* 2004; Linnebjerg *et al.* 2009; Jordaan *et al.* 2011; Thabethe *et al.* 2015). On the other hand, studies on alien species rarely consider the

effect of dispersal agents on fruit removal and seed germination simultaneously (*but see* Ferreras & Galetto 2010), which is fundamental to understand the importance of plant–animal interactions in the population dynamics and spread of invasive alien species.

Cotoneaster franchetii Bois. (Rosaceae) is a fleshy-fruited shrub that constitutes an invasive plant in several countries (Alston & Richardson 2006; Richardson & Rejmánek 2011; Rejmánek & Richardson 2013), including Argentina (Giorgis & Tecco 2014), where it widespread in different habitats from the central mountains (Giorgis *et al.* 2011a). Neither apomixis nor vegetative reproduction appears to play an important part in the reproduction of *C. franchetii* in this region (Lett *et al.* 2015), whereas ornithocorous dispersal has been suggested to play a key role in its spread and naturalization (Gurvich *et al.* 2005; Giorgis & Tecco 2014). However, the extent to which the abundant quantities of fleshy fruits of this invasive species are consumed by native birds in the mountainous region of central Argentina and their effect on seed germination are still unclear.

In this study, we assessed (1) whether native legitimate disperser birds are important consumers of the fleshy fruits of *C. franchetii*, and (2) whether seed germination (speed and percentage) of this alien species is improved by the de-inhibition and/or scarification of seeds during their passage through native bird guts. To evaluate the mechanism(s) responsible for changes in germination patterns following gut-passage, i.e. to distinguish between the de-inhibition and scarification effects, we assessed germination of both manually de-pulped (M) and gut-passed (G) seeds in addition to intact fruits (F) (Samuels & Levey 2005). If $F < M = G$, then birds enhance germination only by separating seeds from fruit pulp. If $F = M < G$, then mechanical/chemical action of the gut is important, but there is no effect of pulp removal. If $F < M < G$, then gut-passage may enhance germination both by removing seeds from fruit, and by altering the seed coat or endocarp. If $F = M = G$, then there are no de-inhibition or scarification effects on seed germination.

METHODS

Study area

The study was conducted in a site located at 1451 m elevation in the Córdoba mountains, central Argentina (31°37'32.2"S, 64°40' 14.7" W). This is a conservation-relevant region because of its water provisioning services (Cingolani *et al.* 2015) and numerous endemic plants (Giorgis *et al.* 2011b). Macroclimate in the region is typically montane, i.e. temperate and wet. Mean annual temperature at the study elevation is 11.6°C, whereas mean

annual precipitation is 887 mm and concentrates mainly during the warmer season (70% of the rain falls from November to March; Cabido *et al.* 1997). Soils are generally residual derived from granite alteration and alternate with 30–60% rock outcrops.

The natural vegetation of the study area consists of a mosaic of tall tussock grasslands, grazing lawns, outcrop communities, *Polylepis australis* woodland and eroded areas with exposed rock surfaces (Cingolani *et al.* 2004, 2008; Giorgis *et al.* 2017). The alien shrub *Cotoneaster franchetii* (Rosaceae, Maloideae) spreads markedly in the region (Tecco 2006; Giorgis *et al.* 2011a,b; Paiaro *et al.* 2011), forming a dense population in the study site.

Study species

Cotoneaster franchetii is native to China and was introduced in South America and other continents as an ornamental garden plant due to its attractive fruits, but it later spread from gardens and has since become naturalized or invasive (Richardson & Rejmánek 2011). It is an evergreen, multi-stemmed, 1 m to 2 m tall shrub with pendent twigs, which are likely to be attractive for perching birds. Its flowers are small and pink, arranged in inflorescences of 5–15 axilar flowers on short spurs. Fruits, produced synchronously in large numbers per shrub, are slightly elongated (6 × 9 mm in diameter, Sérsic *et al.* 2015), bright red when ripe, and contain two to three internal seeds (V. Paiaro & M.C. Díaz-Vélez, pers. obs., 2013). In central Argentina, flowering occurs from October to January (Sérsic *et al.* 2015), and mature fruits are available from March (late summer) to September (late winter) (Tecco *et al.* 2006), contrasting with most fleshy-fruited native species (Gurvich *et al.* 2005). Fruits of *C. franchetii* are consumed and dispersed by birds; two bird species, which differ in the foraging behaviour and immediate consequences for seed dispersion, have been observed feeding on them at the study site (M.C. Díaz-Vélez, pers. obs., 2012). While *Turdus nigricaps nigricaps* (slaty thrush) swallows the whole fruit and defecates the seeds, behaving as a legitimate seed disperser, *Zonotrichia capensis* (rufous-collared sparrow) is a seed predator that feeds on the seed contents and leaves the fruit pulp attached to the pedicel. In addition, according to Ordano (1996), *T. nigricaps nigricaps* is the only legitimate seed disperser bird present at high elevations of central Argentina.

Fruit removal

Before fruit ripening, on March 2012, we chose 75 individuals of *C. franchetii* which were at least 5 m away from each other at the study site. Six branches on each plant were marked with plastic ribbon and other two branches were marked and enclosed with voile-bags to exclude frugivorous birds and provide a control for naturally dropped fruits. On each bagged and unbagged branch, we recorded the initial number of fruits (Fi) and the number of remaining fruits (Fr) by the end of the fruiting season, on August 2012. The number of naturally dropped fruits (FDB) and the number of missing fruits (removed + naturally dropped, FMB) for bagged and unbagged branches, respectively,

were estimated as the difference between (Fi) and (Fr) at each branch. Given that bagged branches prevent the access of frugivores that normally remove fruits, this method possibly generates an overestimation of the fruits dropped. However, we found this approach as one of the most realistic ways to estimate both the number of dropped and removed fruits.

The percentage of missing fruits per plant (%FMP) was calculated using the relationship between the total number of missing fruits and the total number of initial fruits per plant, considering the six unbagged branches per plant:

$$\%FMP = \left[\frac{\sum (FMB_{1-6})}{\sum (Fi_{1-6})} \right] \times 100$$

The percentage of naturally dropped fruits per plant (%FDP) was calculated using the relationship between the total number of dropped fruits and the total number of initial fruits per plant, considering the two bagged branches per plant:

$$\%FDP = \left[\frac{\sum (FDB_{1-2})}{\sum (Fi_{1-2})} \right] \times 100$$

In order to determine whether fruit removal by legitimate seed disperser birds is important for *C. franchetii*, we conducted a generalized linear model (GLM) with binomial error structure, comparing the percentage of *C. franchetii* fruits that were missing per plant (%FMP) with the percentage of naturally dropped fruits per plant (%FDP). This analysis was performed in the R3.3.3 statistical package (R Development Core Team 2017).

Germination experiment

Following Samuels and Levey (2005), three seed treatments were selected to determine the mechanisms acting in bird gut-passed seeds:

- Gut-passed seeds (G): include seeds that were defecated by birds. During the winter of 2012, *C. franchetii* seeds were obtained from *T. nigricaps nigricaps* faeces at the study site.
- Intact fruits (F): mature fruits from 10 individual plants were gathered and mixed to avoid fruit-maternity effects and to randomize seed selection. The fruits were stored in paper bags until the beginning of the germination experiment. The dehydration process that fruits suffer within the paper bags simulates what would happen with undispersed fruits.
- Manually extracted seeds (M): A random sample of gathered fruits was de-pulped manually (pulp and skin were removed) and any pulp remaining on the seeds obtained were washed with water."

Forty two replicates ($n = 42$) of 12 seeds for treatments (G) and (M) and five fruits for treatment (F) were sown in separate transparent plastic recipients (6 cm height, 5 cm diameter, 100 cm³) covered and drilled for oxygenation, containing 2 cm of a sterilized mixture of soil and sand. As *C. franchetii* has a mean of 2.59 ± 0.33 SD seeds per fruit (M.C. Díaz Vélez & V. Paiaro, pers. obs., 2013), we chose to sow five intact fruits per replicate rather than manipulate the fruit to reach the number of 12 seeds in the (F) treatment.

Recipients were housed in a germination chamber and maintained at 15°C, with 12 h light per day and the moisture was controlled and maintained by adding distilled water.

Every 3–5 days, seeds were checked for germination, germinated seeds were counted and removed to reduce possible effect on non-germinated seeds. Germination was defined as the radicle emergence from seed or fruit. Germination was recorded for 11 months (337 days), until there was no germination in any recipient for a period of 2 weeks. At the end of the experiment, fruits of the (F) treatment were opened and non-germinated seeds were counted at each recipient in order to calculate germination percentages.

To test for differences among treatments (i.e. seeds ingested by birds, seeds from de-pulped fruits and intact fruits) in the percentage of germinated seeds at the end of the trial period, we applied a generalized linear model (GLM) with binomial error structure in the R 3.3.3 statistical package (R Development Core Team 2017).

As an estimation of germination speed, we used the Survival Analysis module of Kaplan-Meier available in SPSS 10 to compare cumulative germination percentages among treatments. We used log-rank tests (Mantel-Cox), a non-parametric test recommended for evaluating the effect of categorical predictive variables. In all cases, $P \leq 0.05$ was considered to assess significant differences among treatments.

RESULTS

Fruit removal

The mean percentage of missing fruits in unbagged branches of *C. franchetii* plants was $92 \pm 1\%$ (SE). This percentage was significantly higher than the percentage of naturally dropped fruits from bagged branches ($64 \pm 3\%$; $z = 39.07$; $P < 2 \times 10^{-16}$). Fruits were missed entire, since seedless or partially de-pulped fruits were not recorded on the unbagged branches.

Seed germination

Percentage of seeds germinated at the end of the experiment (Deviance analysis $F = 201.15$;

$P < 0.0001$) and germination speed ($\chi^2 = 18.671$; $P < 0.0001$) significantly differed among treatments. Only 5% of seeds from intact fruits germinated at the end of the trial, while both manually extracted and ingested seeds germinated in a significantly higher percentage (Table 1; Fig. 1). Manually extracted seeds germinated significantly more than gut-passed seeds (Table 1, Fig. 1).

Germination speed was significantly lower for seeds in intact fruits than for seeds which passed through birds' gut and for those which were manually extracted from fruits (Table 1; Fig. 2). The germination of either the seeds manually extracted from fruits or those from bird faeces was continuous over time until approximately the day 200, after which the cumulative number of germinating seeds stabilized (Fig. 2). By contrast, the germination of seeds of the intact fruits ceased between days 50 and 100 after sowing, when around 5% of seeds had already been germinated (Fig. 2). Manually extracted seeds germinated significantly faster than gut-passed seeds (Table 1; Fig. 2).

DISCUSSION

Fruit removal

The higher percentage of total missing fruits in *C. franchetii* compared with that of naturally dropped fruits suggests an important role of birds in fruit removal from plants of this alien species. This effect might be even stronger considering that naturally dropped fruits might have been overestimated by the method used in this study. The high percentage of missing fruits (92%), most likely removed by birds, found in *C. franchetii* is similar to that found for ornithocorous woody natives (*Celtis ehrenbergiana*, *Lycium cestroides*, *Zanthoxylum coco*, *Passiflora morifolia*, *P. suberosa*, *Lantana grisebachii*; Grilli & Galetto 2009; Ponce *et al.* 2012) at a lower elevation of the central Argentinean mountains. However, the percentage of fruits removed from *C. franchetii* plants is

Table 1. Differences in percentage and speed of germination between pairs of seed treatments (gut-passed seeds, seeds in intact fruits, and seeds from manually de-pulped fruits) in the invasive alien *Cotoneaster franchetii*. A generalized linear model (GLM) with binomial error structure was used for germination proportion and a non-parametric log-rank test (Mantel-Cox) was used for germination speed

	Germination percentage		Germination speed	
	z	P	χ^2	P
Intact fruits (F) vs. gut-passed seeds (G)	-8.53	$< 2 \times 10^{-16}$	81.50	1.75×10^{-19}
Intact fruits (F) vs. manually extracted seeds (M)	11.17	$< 2 \times 10^{-16}$	153.85	2.50×10^{-35}
Manually extracted seeds (M) vs. gut-passed seeds (G)	3.69	2.22×10^{-4}	14.93	1.12×10^{-4}

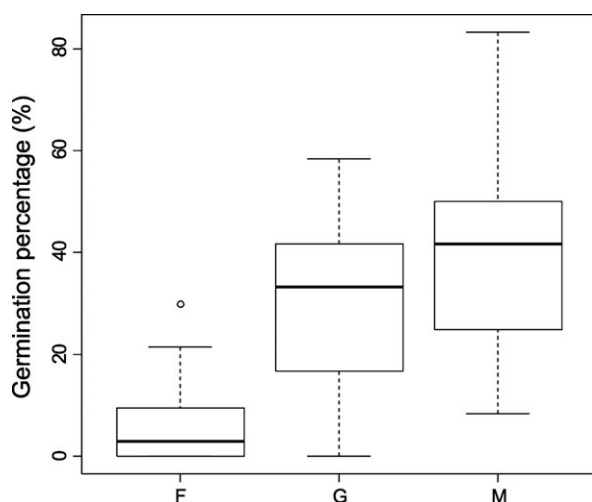


Fig. 1. Germination percentage of *Cotoneaster franchetii* seeds under different germination treatments: seeds in intact fruits (F), seeds obtained from manually de-pulped fruits (M) and gut-passed seeds (G) at the end of the experiment. Boxes are 25 and 75% quartiles, the lines within the boxes indicate the medians, bars show 10 and 90% values, and dots highlight outliers.

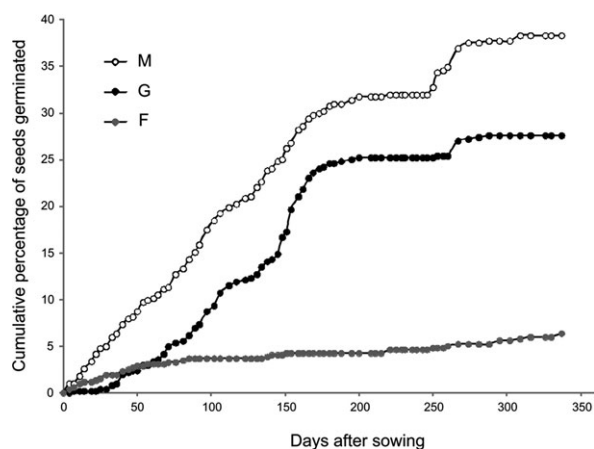


Fig. 2. Mean cumulative germination percentage of *Cotoneaster franchetii* seeds in the three treatments (G: gut-passed seeds, F: seeds in intact fruits, and M: seeds from manually de-pulped fruits) for the duration of the study.

considerably higher than that found for two other invasive aliens of the same region, the cattle dispersed *Gleditsia triacanthos* (Ferrerás & Galetto 2010) and the bird dispersed *Ligustrum lucidum* (Ferrerás *et al.* 2008; Ponce *et al.* 2012). The higher percentage of fruit removal observed for *C. franchetii* compared with that of *L. lucidum* suggests that the landscape context and the frugivorous bird assemblage may condition alien fleshy-fruited removal. In addition, such differences could be associated to differences in fruit abundance or in species population densities.

Taking into account that *T. nigriceps nigriceps* is the only legitimate seed disperser bird present at high elevations of central Argentina (Ordano 1996), that it appears to be the only frugivorous bird removing entire fruits from *C. franchetii* plants and that a high quantity of faeces of this bird species with seeds of *C. franchetii* was found at the study site (V. Paiaro & M.C. Díaz-Vélez, pers. obs., 2012), it is likely that fruits of this invasive plant were removed and consumed by this frugivorous bird. It is important to notice that this species is also the main fruit consumer of the invasive aliens *Pyracantha angustifolia* and *P. coccinea* (Vergara-Tabares *et al.* 2016) at lower elevations of the mountains of central Argentina, where it also eats fruits of the native shrubs *Condalia montana* (Ponce *et al.* 2012) and *Celtis ehrenbergiana* (Vergara-Tabares *et al.* 2016). Moreover, a species of the same genus, *Turdus merula*, was observed eating fruits of *C. franchetii* in New Zealand (Williams & Karl 1996) and of other *Cotoneaster* species in their native ranges (Crofts & Jefferson 1999; Belkouche & Doumandji 2014). Although most relationships between frugivorous birds and invasive plants involve a generalized dispersal system (Richardson *et al.* 2000; Renne *et al.* 2002; Traveset & Richardson 2014), exceptions exist where plants reliant on one or few biotic dispersal agents become invasive (Gosper *et al.* 2005 and references therein). This last appears to be the case of *C. franchetii*, at least at the study site, where *T. nigriceps nigriceps* would be considered the only seed disperser of this invasive plant. However, taking into account that other birds also consume this species fruits and excrete viable seeds at lower elevations of the Córdoba mountains (Dellafiore & Rosa 2015), the legitimate dispersers of *C. franchetii* may be different depending on the landscape context and bird assemblages.

Seed germination

The relatively low percentage of seed germination found, even after bird gut-passage or manually de-pulped, agrees with findings from previous studies on germination of *C. franchetii* in the mountains of central Argentina (Dellafiore & Rosa 2015; I. Lett, personal communication, 2016). In addition, our results demonstrated that the study species shows a progressive germination curve lasting more than 300 days, supporting the idea that germination rates of *Cotoneaster* species are low or lag (Fryer & Hylm 2009).

That manually extracted and gut-passed seeds germinated more and faster than seeds within intact fruits is indicative of a de-inhibition effect (Robertson *et al.* 2006; Traveset *et al.* 2007). Such effect was supported by the fact that the seeds of the intact fruits treatment that were recovered after the

experiment showed a high germinability after being de-pulped (C. Díaz Vélez & V. Paiaro, unpublished data, 2012). Our findings agree with those found by a previous study in the area, in which manually extracted seeds of *C. franchetii* germinated in a greater proportion than intact fruits (I. Lett, unpublished data, 2015). Similar results were found in *Ligustrum lucidum*, another fleshy-fruited alien invading the mountains of central Argentina (Panetta 2000; A. E. Ferreras, unpublished data, 2005).

On the other hand, that manually extracted seeds of *C. franchetii* germinated more and faster than seeds from *T. nigriceps nigriceps* faeces suggests that seed passage through the gut of this bird species might negatively affect its potential germination, possibly due to an excessive abrasion of seeds or the toxicity and hydrophobic nature of excreta (Reid & Armesto 2011). Alternatively, given that manually extracted seeds were subsequently washed, this might have caused a more efficient removal of the inhibitors present in the pulp than in the birds' guts. The germination decrease and delay found in ingested seeds are uncommon and the mechanism by which they occur remains unknown (Traveset *et al.* 2001; Traveset & Verdú 2002). In fact, our results differ from those found in other alien species in which frugivore gut-passage improved germination with respect to mechanical removal of fruit pulp (Mandon-Dalger *et al.* 2004; Linnebjerg *et al.* 2009) or in which these treatments did not differ in their effects (Panetta & McKee 1997; Mandon-Dalger *et al.* 2004; Linnebjerg *et al.* 2009; Jordaan *et al.* 2011). However, experiments performed with the alien *Ligustrum lucidum* (A. E. Ferreras, unpublished data, 2005) and with the native *Schinus fasciculatus* (Díaz Vélez *et al.* 2017) in the mountains of central Argentina showed a significant lower germinability of seeds from bird's faeces compared with seeds without fleshy pulp (manually removed), in concordance with our results. Our data also partially agree with a recent study, in which seeds of *C. franchetii* obtained from faeces of captive specimens of the granivorous bird *Thraupis bonariensis* had a significantly lower germination percentage than seeds manually extracted from fruits (Dellafore & Rosa 2015). In that case, however, percentage of germination of gut-passed seeds was lower than in our study. This difference indicates that *T. nigriceps nigriceps* and *T. bonariensis* would exert different treatments on seeds of *C. franchetii*, probably due to different retention times (Jordaan *et al.* 2011), diet specializations or foraging methods, since *T. nigriceps nigriceps* swallows entire fruits, whereas *T. bonariensis* mandibulates fruits (D'Avila *et al.* 2010). In concordance with other studies, this finding suggests that effects of seed passage through avian guts are dependent on the interacting bird species (Robertson *et al.* 2006;

Wilson & Downs 2012; Thabethe *et al.* 2015; Amodeo *et al.* 2017).

Despite the apparently negative effect of gut-passage on *C. franchetii* seeds, the 'true control' of the effect of gut-passage on germination used in the present study (i.e. seeds in intact fruits *sensu* Samuels & Levey 2005; Robertson *et al.* 2006) demonstrated that bird gut-passage mainly enhances seed germination. Additionally, our results indicate that release from germination inhibitors through pulp removal would be the mechanism that improves germination of *C. franchetii*, suggesting that pulp removal is sufficient and specialized gut treatment is not required for successful germination in this species.

Implications for the invasion process and future research direction

Cotoneaster franchetii is currently very abundant in the mountainous regions of central Argentina (Giorgis *et al.* 2011a,b), the number of fruits produced by each plant is actually very high, and, as shown in the present study, an important percentage of these fruits is removed from plants and consumed by the native bird *T. nigriceps nigriceps*, which is very frequent in the study region (Ordano 1996) and increases seed germination of this alien plant. Thus, despite the relatively low percentages of seed germination found even after bird gut-passage or manually de-pulped, the total quantity of dispersed seeds of *C. franchetii* that effectively germinate in the mountains of central Argentina is likely very high. A high germinability is presumably necessary to maximize reproductive success and seedling recruitment (Harper 1977), which in the study case could be translated into an enhancement of the invasion process. An acceleration of germination, in contrast, could have either negative or positive consequences for plant populations, depending on climatic conditions of the area at the time when seeds germinate. Given that seeds of *C. franchetii* are dispersed mainly during the dry season in the study area, a faster germination would ensure seedling establishment opportunities at the beginning of the wet period. In addition, the increase in the germination speed of this plant species promoted by *T. nigriceps nigriceps* could also facilitate the invasion by this alien by reducing the risk of death by sibling competition or by seed predation through decreasing the amount of time a seed is available for consumption by granivores (Traveset & Verdú 2002 and references therein).

In summary, our findings suggest that the invasion by *C. franchetii* in mountains of central Argentina is facilitated by the native bird *T. nigriceps nigriceps*. At the same time, by providing fruits in a period of the year of food shortage, the recent invasion by this

fleshy-fruited alien might explain the current year-round presence of this native frugivorous bird in the area, where it was a migrant species in past decades (Vergara-Tabares & Rojas 2016). Where invasive plants comprise part of the diet of native frugivores, there may be a conservation conflict between control of the invasive and the maintenance of the populations of the native frugivore (Buckley *et al.* 2006). In such cases, understanding the nature of the invasive plant–frugivore interaction is essential for determining an appropriate management. In this sense, additional studies on the reproductive biology and ecology of animal–plant interactions considering *C. franchetii* are needed in the Córdoba mountains. In this sense, studies of seed retention time combined with bird movements' patterns will be useful in order to determine seed dispersal distances and the potential colonization of new suitable habitats. This would provide greater predictive capability in relation to seed dispersal patterns and spread of this invasive plant to higher elevations of the study area where it is not present yet. Moreover, exploring whether in natural conditions pulp removal from *C. franchetii* fruits happens through handling by biotic agents other than birds or through the effect of abiotic factors will help to evaluate whether this alien plant might maintain its invasive ability in the absence of frugivorous birds. On the other hand, it is important to determine whether this alien species form persistent seed banks which might germinate once parent plants are cleared. All this information could then be integrated into management programs tending to preserve native plant communities in the mountains of central Argentina.

ACKNOWLEDGEMENTS

We thank Leonardo Ontivero, Eugenia Haro, Gustavo Haro, María Cristina Acosta, Gabriela Ferreiro, Silvina Córdoba and Mauricio Renny for assistance in the field work. This research was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). MCDV is a CONICET fellowship holder, AS and VP are CONICET researchers, AT is a research professor of the Spanish Research Council (CSIC).

REFERENCES

- Alston K. P. & Richardson D. M. (2006) The roles of habitat features, disturbance, and distance from putative source populations in structuring alien plant invasions at the urban/wildland interface on the Cape Peninsula, South Africa. *Biol. Conserv.* **132**, 183–98.
- Amodeo M. R., Vázquez M. B. & Zalba S. M. (2017) Generalist dispersers promote germination of an alien fleshy-fruited tree invading natural grasslands. *PLoS ONE* **12**, e0172423.
- Belkouché S. & Doumandji S. (2014) Distinctive feature of blackbird's trophic diet (*Turdus merula mauritanicus*, Aves) and its seeds scattering role in suburban environment near Algiers. *Int. J. Zool. Res.* **4**, 85–98.
- Buckley Y. M., Anderson S., Catterall C. P. *et al.* (2006) Management of plant invasions mediated by frugivore interactions. *J. Appl. Ecol.* **43**, 848–57.
- Cabido M., Ateca N., Astegiano M. E. & Anton A. M. (1997) Distribution of C3 and C4 grasses along an altitudinal gradient in central Argentina. *J. Biogeogr.* **24**, 197–204.
- Cingolani A. M., Renison D., Zak M. R. & Cabido M. R. (2004) Mapping vegetation in a heterogeneous mountain rangeland using landsat data: an alternative method to define and classify land-cover units. *Remote Sens. Environ.* **92**, 84–97.
- Cingolani A. M., Renison D., Tecco P. A., Gurvich D. E. & Cabido M. (2008) Predicting cover types in a mountain range with long evolutionary grazing history: a GIS approach. *J. Biogeogr.* **35**, 538–51.
- Cingolani A. M., Poca M., Giorgis M. A. *et al.* (2015) Water provisioning services in a seasonally dry subtropical mountain: identifying priority landscapes for conservation. *J. Hydrol.* **525**, 178–87.
- Combs J. K., Reichard S. H., Groom M. J. *et al.* (2011) Invasive competitor and native seed predators contribute to rarity of the narrow endemic *Astragalus sinuatus* Piper. *Ecol. Appl.* **21**, 2498–509.
- Crofts A. & Jefferson R. G. (1999) *The Lowland Grassland Management Handbook*. English Nature/The Wildlife Trusts, Peterborough, UK.
- Daehler C. D., van Kleunen M., Pysek P. & Richardson D. M. (2016) EMAPi 2015: highlighting links between science and management of alien plant invasions. *NeoBiota* **30**, 1–3.
- D'Ávila G., Gomes-Jr A., Canary A. C. & Bugoni L. (2010) The role of avian frugivores on germination and potential seed dispersal of the Brazilian Pepper *Schinus terebinthifolius*. *Biota. Neotrop.* **10**, 45–51.
- Dellafiore C. M. & Rosa M. J. (2015) Efecto de las aves sobre la germinación de *Cotoneaster franchetti* bois (Rosaceae). *Eur. Sci. J.* **33**, 319–26.
- Dennis A. J., Schupp E. W., Green A. J. & Westcott D. A. (2007) *Seed Dispersal: Theory and its Application in a Changing World*. CAB International, Wallingford, UK.
- Díaz Vélez M. C., Ferreras A. E., Silva W. R. & Galetto L. (2017) Does avian gut passage favour seed germination of woody species of the Chaco Serrano woodland in Argentina? *Botany* **95**, 493–501.
- Ferreras A. E. & Galetto L. (2010) From seed production to seedling establishment: Important steps in an invasive process. *Acta Oec.* **36**, 211–18.
- Ferreras A. E., Torres C. & Galetto L. (2008) Fruit removal of an invasive exotic species (*Ligustrum lucidum*) in a fragmented landscape. *J. Arid Environ.* **72**, 1573–80.
- Fryer J. & Hylm B. (2009) *Cotoneasters: A Comprehensive Guide to Shrubs for Flowers, Fruit, and Foliage*. Timber Press, Portland, OR.
- Giorgis M. A. & Tecco P. A. (2014) Invasive alien trees and shrubs in Córdoba Province (Argentina): a contribution to the systematization of global bases. *Bol. Soc. Argent. Bot.* **49**, 581–603.
- Giorgis M. A., Tecco P. A., Cingolani A. M., Renison D., Marcora P. & Paiaro V. (2011a) Factors associated with woody alien species distribution in a newly invaded

- mountain system of central Argentina. *Biol. Invasions* **13**, 1423–34.
- Georgis M. A., Cingolani A. M., Chiarini F. et al. (2011b) Composición florística del Bosque Chaqueño Serrano de la provincia de Córdoba, Argentina. *Kurtziana* **36**, 9–43.
- Georgis M. A., Cingolani A. M., Gurvich D. E. et al. (2017) Changes in floristic composition and physiognomy are decoupled along elevation gradients in central Argentina. *Appl. Veg. Sci.* **20**, 558–71.
- Gosper C. R., Stansbury C. D. & Vivian-Smith G. (2005) Seed dispersal of fleshy-fruited invasive plants by birds: contributing factors and management options. *Divers. Distrib.* **11**, 549–58.
- Grilli G. & Galetto L. (2009) Remoción de frutos de una especie invasora (*Lantana camara* L.) en el Bosque Chaqueño de Córdoba (Argentina). *Ecol. Aust.* **19**, 149–56.
- Gurvich D. E., Tecco P. A. & Díaz S. (2005) Plant invasions in undisturbed ecosystems: the triggering attribute approach. *J. Veg. Sci.* **16**, 723–8.
- Harper J. L. (1977) *Population Biology of Plant*. Academic Press, London, UK.
- Jordaan L. A., Johnson S. D. & Downs C. T. (2011) The role of avian frugivores in germination of seeds of fleshy-fruited invasive alien plants. *Biol. Invasions* **13**, 1917–30.
- Jordano P. & Schupp E. W. (2000) Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecol. Monogr.* **70**, 591–615.
- Jordano P., García C., Godoy J. A. et al. (2007) Differential contribution of frugivores to complex seed dispersal patterns. *Proc. Natl Acad. Sci. USA* **104**, 3278–328.
- Lett I., Hensen I., Hirsch H. & Renison D. (2015) No differences in genetic diversity of *Cotoneaster franchetii* (Rosaceae) shrubs between native and non-native ranges. *Bol. Soc. Argent. Bot.* **50**, 377–84.
- Lichstein J. W., Grau H. R. & Aragón R. (2004) Recruitment limitation in secondary forests dominated by an exotic tree. *J. Veg. Sci.* **15**, 721–8.
- Linnebjerg J. F., Hansen D. M. & Olesen J. M. (2009) Gut passage effect of the introduced red-whiskered bulbul (*Pycnonotus jocosus*) on germination of invasive plant species in Mauritius. *Aust. Ecol.* **34**, 272–7.
- Lonsdale W. M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology* **80**, 1522–36.
- Mandon-Dalger L., Clergeau P., Tassin J. et al. (2004) Relationships between alien plants and an alien bird species on Reunion Island. *J. Trop. Ecol.* **20**, 635–42.
- Meisenburg M. J. & Fox A. M. (2002) What role do birds play in dispersal of invasive plants. *Wildland Weeds* **2**, 8–14.
- Muñoz M. C. & Ackerman J. D. (2013) Invasive plants and mutualistic interactions between fleshy fruits and frugivorous animals. In: *Invasive Plant Ecology* (eds J. Shibu, P. S. Harminder, R. B. Daizy & K. K. Ravinder) pp. 121–36. CRC Press, Boca Raton, FL.
- Ordano M. (1996) Estudio de una comunidad de aves altoserrana (Córdoba, Argentina) durante un ciclo anual. *Rev. Asoc. Cs. Nat. Lit.* **27**, 83–94.
- Paiano V., Cabido M. & Pucheta E. (2011) Altitudinal distribution of native and alien plant species in roadside communities from central Argentina. *Aust. Ecol.* **36**, 176–84.
- Panetta F. D. (2000) Fates of fruits and seeds of *Ligustrum lucidum* WT Ait. and *L. sinense* Lour. maintained under natural rainfall or irrigation. *Aust. J. Bot.* **48**, 701–6.
- Panetta F. D. & McKee J. (1997) Recruitment of the invasive ornamental, *Schinus terebinthifolius*, is dependent upon frugivores. *Aust. Ecol.* **22**, 432–8.
- Ponce A. M., Grilli G. & Galetto L. (2012) Frugivoría y remoción de frutos ornitócoros en fragmentos del bosque chaqueño de Córdoba (Argentina). *Bosque (Valdivia)* **33**, 33–41.
- R Development Core Team (2017) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reid S. & Armesto J. J. (2011) Avian gut-passage effects on seed germination of shrubland species in Mediterranean central Chile. *Plant Ecol.* **212**, 1–10.
- Rejmánek M. & Richardson D. M. (2013) Trees and shrubs as invasive alien species –2013 update of the global database. *Divers. Distrib.* **19**, 1093–4.
- Renison D., Valladares G. & Martella M. B. (2010) The effect of passage through the gut of the Greater Rhea (*Rhea americana*) on germination of tree seeds: implications for forest restoration. *Emu* **110**, 125–31.
- Renne I. J., Barrow W. C., Randall J., Lori A. & Bridges W. C. (2002) Generalized avian dispersal syndrome contributes to Chinese tallow tree (*Sapium sebiferum*, Euphorbiaceae) invasiveness. *Divers. Distrib.* **8**, 285–95.
- Richardson D. M. & Rejmánek M. (2011) Trees and shrubs as invasive alien species—a global review. *Divers. Dist.* **17**, 788–809.
- Richardson D. M., Allsopp N., D’Antonio C. M. et al. (2000) Plant invasions—the role of mutualisms. *Biol. Rev.* **75**, 65–93.
- Robertson A. W., Trass A., Ladley J. J. & Kelly D. (2006) Assessing the benefits of frugivory for seed germination: the importance of the deinhibition effect. *Funct. Ecol.* **20**, 58–66.
- Samuels I. A. & Levey D. J. (2005) Effects of gut passage on seed germination: do experiments answer the questions they ask? *Funct. Ecol.* **19**, 365–8.
- Sérsic A. N., Cocucci A. A., Acosta M. C. et al. (2015) *Flores del Centro de Argentina II: Nueva Guía Ilustrada Para Conocer 229 Especies*. Instituto Multidisciplinario de Biología Vegetal (IMBIV), Córdoba, Argentina.
- Tassin J., Rivière J. N. & Clergeau P. (2007) Reproductive versus vegetative recruitment of the invasive tree *Schinus terebinthifolius*: implications for restoration on Reunion Island. *Rest. Ecol.* **15**, 412–19.
- Tecco P. A. (2006) Riqueza, abundancia y características morfo-funcionales de las especies nativas y exóticas establecidas bajo distintas condiciones de uso de la tierra en cinco ecosistemas del centro-oeste de Argentina (PhD Thesis). Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Spain.
- Tecco P. A., Gurvich D. E., Díaz S. et al. (2006) Positive interaction between invasive plants: The influence of *Pyracantha angustifolia* on the recruitment of native and exotic woody species. *Aust. Ecol.* **31**, 293–300.
- Thabethe V., Wilson A.-L., Hart L. A. & Downs C. T. (2015) Ingestion by an invasive parakeet species reduces germination success of invasive alien plants relative to ingestion by indigenous turaco species in South Africa. *Biol. Invasions* **17**, 3029–39.
- Traveset A. & Richardson D. M. (2014) Mutualistic interactions and Biological Invasions. *Annu. Rev. Ecol. Evol. Syst.* **45**, 89–113.

- Traveset A. & Verdú M. (2002) A meta-analysis of the effect of gut treatment on seed germination. In: *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation* (eds D. J. Levey, W. R. Silva & M. Galetti) pp. 339–50. CAB International, Wallingford, UK.
- Traveset A., Riera N. & Mas R. E. (2001) Passage through bird guts causes interspecific differences in seed germination characteristics. *Funct. Ecol.* **15**, 669–75.
- Traveset A., Robertson A. W. & Rodríguez-Pérez J. (2007) A review on the role of endozoochory in seed germination. In: *Seed Dispersal: Theory and its Application in a Changing World* (eds A. J. Dennis, E. W. Schupp, R. J. Green & D. A. Westcott) pp. 78–103. CAB international, Oxfordshire, UK.
- Traveset A., Rodríguez-Pérez J. & Pias B. (2008) Seed trait changes in dispersers' guts and consequences for germination and seedling growth. *Ecology* **89**, 95–106.
- Vergara-Tabares D. L. & Rojas P. N. (2016) Year-round presence of Slaty Thrush (*Turdus nigriceps nigriceps*) in mountains of central Argentina. *Rev. Bras. Orn.* **24**, 329–34.
- Vergara-Tabares D. L., Badini J. & Peluc S. I. (2016) Fruiting phenology as a “triggering attribute” of invasion process: do invasive species take advantage of seed dispersal service provided by native birds?. *Biol. Invasions* **18**, 677–87.
- Williams P. A. & Karl B. J. (1996) Fleshy fruits of indigenous and adventive plants in the diet of birds in forest remnants, Nelson, New Zealand. *New Zeal. J. Ecol.* **20**, 127–45.
- Wilson A. L. & Downs C. T. (2012) Knysna Turacos (*Tauraco corythaix*) do not improve seed germination of ingested fruit of some indigenous South African tree species. *S. African J. Botany* **78**, 55–62.