

Seed Dispersal by Frugivores and Germination Success of the Invasive Alien Shrub *Pyracantha Angustifolia* in the Eastern Free State Province, South Africa

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Abstract

Invasive alien plants can use animal-plant interactions to increase their invasiveness. This study investigated the role of frugivorous birds in seed dispersal and germination of the alien plant *Pyracantha angustifolia* (Rosaceae) in South African high elevation grasslands. We monitored which bird species fed on the fruit of the invasive *P. angustifolia* in some farms in the Eastern Free State Province using camera-traps and direct surveillance. Nine bird species visited *P. angustifolia* shrubs to perch or feed on fruits, but only one bird (Speckled Mousebird) fed on the fruits during timed observations. To assess the effect of ingestion by avian frugivores on *P. angustifolia* germination, *P. angustifolia* fruits were fed to captive Cape White-eyes (*Zosterops virens*), Dark-capped Bulbuls (*Pycnonotus tricolor*), Purple-crested Turacos (*Gallirex porphyreolophus*), Red-winged Starlings (*Onychognathus morio*) and Speckled Mousebirds (*Colius striatus*). Seeds collected from bird excreta, whole fruits, and depulped fruits were grown under greenhouse conditions and germination rates recorded. All captive bird species, except for Cape White-eyes, ingested the seeds; Cape White-eyes only fed on fruit pulp that they had manually removed. Bird species with relatively larger body mass had longer seed retention times compared with the smaller bird species. Germination success of both depulped and ingested *P. angustifolia* seeds was high (> 80%) and that of whole fruits low (7%). Ingestion by the four avian frugivore species did not affect germination rate and success; instead, the birds facilitate the spread and germination of seeds by removing the fruit pulp and spreading the seed away from the parent shrubs.

Introduction

An important mutualistic relationship exists between frugivorous birds and fleshy-fruited plants (Fricke et al. 2019). This interaction allows fleshy-fruited plants to disperse their propagules away from the source plant (Tabassum and Leishman 2018). In return, frugivorous birds digest the fruit flesh as an energy reward (Tiebel et al. 2018). In addition, frugivores play an important role in improving germination of the dispersed seeds (Dlamini et al. 2018). Such enhancement is believed to result from the fruit flesh and skin being removed and the breaking of seed dormancy by the digestive juices of the frugivores' gut (Cimi and Campbell 2017). Several studies show no effect on germination while others show lower germination rate post-ingestion by birds (Mora and Smith-Ramirez 2016). Enhancement of seed germination by ingestion is not guaranteed since some birds' digestive conditions are not suitable for some seeds (Valido and Nogales 1994).

Dispersal of fleshy fruited plants is enhanced by the spreading of seeds by frugivores and sometimes enhancing germination (Chama et al. 2013). Some invasive alien plants (IAPs) can use this interaction to help them spread into new environments (Fricke et al. 2019) and allow the seedlings to access new niches and avoid resource competition with the source plant. For example, in South Africa, germination of the invasive Brazilian pepper tree *Schinus terebinthifolius* Raddi (Anacardiaceae) was reported to have been improved by bird ingestion (Dlamini et al. 2018). The seeds of the invasive shrub *Pyracantha angustifolia* (Franch.) C.K.Schneid (Rosaceae) (yellow firethorn) are dispersed mainly by avian frugivorous species in South Africa (Chari et al. 2020). It would be therefore expected that after avian

ingestion, the germination rate of the seeds would be improved. Alternatively, pulp removal might be the factor enhancing germination; or both pulp removal and avian ingestion acting together (Thibault et al. 2018).

Pyracantha angustifolia invasion leads to exclusion of other plants through formation of dense thickets. Its canopy changes the microenvironmental conditions that are necessary for some native plants' growth (Giantomasi et al. 2008). In southern Africa, *P. angustifolia* has been reported to replace native vegetation and pastures (grass), contaminate seedbanks and block access by humans and livestock due to thorns (Wells et al. 1976; Csurhes et al. 2016). Understanding the plant-animal interactions could improve our understanding on how IAPs spread and therefore assist in the management of IAPs (Fricke et al. 2019). In this study, we determined the role of avian frugivores in enhancing the spread of *P. angustifolia* in the high elevation grasslands of South Africa. We achieved this by field observations and determining the ingestion effect of various frugivorous bird species on seed germination. Germination rate and success were compared among non-ingested fruits, ingested fruits and manually depulped seeds. It was predicted that seeds in intact whole fruits have lower germination success compared to seeds from manually depulped and those that were ingested by birds.

Methods

Study area

Field data were collected on farms around Clarens (28°32'8"S; 28°25'2"E) and Fouriesburg (28°41'13"S; 28°13'59"E) in the eastern Free State Province, South Africa. This area was selected for the study mainly due to its large *P. angustifolia* invasions in diverse habitats, accessible for research. The study area is typified by private farmland with some patches of natural grassland, rocky outcrops, and sandstone cliffs deeply incised by rivers (Mucina and Rutherford 2006). The area is surrounded by agro-industrial activities to the north, east and west; these include croplands, fallow agricultural fields that are no longer arable and some cattle ranching (Mucina and Rutherford 2006). Two field sites were chosen for observations. The first study field (Clarens) was used for camera traps and the second site (Fouriesburg) for timed observations.

Study species

Pyracantha angustifolia is native to south-western China (Cuizhi and Spongengberg 2003). *Pyracantha angustifolia* has been widely planted and has subsequently become naturalised in many countries including the high elevation, grassland interior of South Africa (Chari et al. 2020). In South Africa, *P. angustifolia* produces white flowers (5 mm in diameter) in October to December and orange-red or orange-yellow berries (5–8 mm across) in large numbers (Chari et al. 2020). In invaded sites, *P. angustifolia* can change habitat to dense monocultures (Daehler 1998). Five *Pyracantha* species have been introduced to South Africa as ornamental plants, with *P. angustifolia* being the most widespread among *Pyracantha* species (Moffet 2018). As indicated by Alien and Invasive Species Regulations of the National Environmental Management: Biodiversity Act, (South African Department of Environmental

Affairs. 2014), all species are included as 1b Category invasive alien plants species (Department of Environmental Affairs 2014). Plants under category 1b must be controlled.

Field observations

We used camera-traps and opportunistic observations to determine which vertebrate species fed on *P. angustifolia* fruits during their fruiting season (July – September 2018). Remote camera-traps (Bushnell Trophy Cam HD Max-Colour LCD, Bushnell Outdoor Products, Overland Park, KS, USA) were used following methods by Zoeller et al. (2016) to identify vertebrate plant visitors, and the timing and frequency of their visits to feed on fruits. Three Bushnell remote camera-traps were mounted on tripods and aimed at fruiting branches of focal plants. Plants bigger in size (at least 4m high) were selected for observations. Each plant was at least 30m away from the next plant. Each camera-trap was placed between 25 cm to one metre away from the. Camera-trap storage capacity and battery strength were checked at least once a week and data downloaded. Camera-traps were put in the field for ten weeks (11 July – 23 August 2018 and 03–24 October 2018). The sum of all hours that all cameras were active totalled 1 826 hours. The remote camera-traps were triggered by heat or motion and would take photographs and videos. When triggered, the camera-traps filmed the visitors' activities for 60 seconds, and could be re-triggered after a 10 second interval. From the videos, the potential seed dispersers were identified using bird field guide books (Hockey et al. 2005; Newman 2013). The number, species and behaviour of visitors, and duration of foraging bouts were noted.

We conducted direct observations to complement the data captured by the camera-traps. Opportunistic observations were conducted in Clarens. Frugivore visitors to *P. angustifolia* were identified using bird field guide books (Hockey et al. 2005; Newman 2013) and their behaviour observed. Only birds observed feeding on *P. angustifolia* fruits were noted in our opportunistic observations.

Timed observations of avian frugivores were conducted at Wynford Holiday Farm (28°41'13"S; 28°13'59"E), located between Fouriesburg town and the Lesotho border. This former grassland area has been transformed into a dense monoculture of *P. angustifolia* (L. Adams, pers. obs.). Observation periods spanned six hours a day, consisting of a three hour period of peak bird activity after sunrise (07:00–10:00) and a three hour period of peak activity before sunset (14:00–17:00) over three days (total of 54 h) in June 2019. We chose three mature fruiting shrubs for observations, observed birds using binoculars and, if possible, took photographs of visiting birds. For each observation, we noted foraging bird arrival and departure time, and behaviour (feeding or resting).

Bird feeding trials

Fresh, ripe *P. angustifolia* fruits were collected from 20 shrubs from the Clarens area (28°32'69"S; 28°25'20"E) during the fruiting season (August 2019). Branches with fruits were collected to keep them as fresh as possible. Fruits were removed from branches by hand and placed in plastic bags at the Animal House at the University of KwaZulu-Natal (UKZN), Pietermaritzburg campus, KwaZulu-Natal Province, South Africa. Fruits from all shrubs were pooled, in order to standardise any differences associated with

local seed origin as well as incorporating genetic variability (Tecco et al. 2016). After collection, fruits were placed in a refrigerator, and the feeding trials started a day after collection.

Bird species chosen to be used for feeding and germination trials were informed by the field observations. Bird species used in the germination study included five Dark-capped Bulbuls (*Pycnonotus tricolor*), two Purple-crested Turacos (*Gallirex porphyreolophus*), three Speckled Mousebirds (*Colius striatus*), five Red-winged Starlings (*Onychognathus morio*) and nine Cape White-eyes (*Zosterops virens*). All bird species had been observed feeding on *P. angustifolia* fruits in the field except for Purple-crested Turacos. Purple-crested Turacos and Red-winged Starlings were regarded as relatively large birds as their mean body mass was 285 and 140 g respectively (Newman 2013). In comparison, Speckled Mousebirds, Dark-capped Bulbuls and Cape White-eyes were regarded as relatively small birds weighing on average 55, 39 and nine grams respectively (Newman 2013). Purple-crested Turacos do not naturally occur in the study area, but we used them as a surrogate species for other larger bird species that consume the fruits in the study area; for example, the African Olive Pigeon (*Columba arquatrix*) (L. Adams, pers. obs).

Captive birds used in our study were kept in outside aviaries (1 x 2.12 x 2.66 m) at the UKZN Animal house. They were then moved and acclimatised to experimental indoor cages in a constant environment room with 12L: 12D photoperiod at $25 \pm 1^\circ\text{C}$ (Thabethe et al. 2015) for five days before the commencement of feeding trials. They were fed a maintenance diet daily, including apples, pears, bananas, and oranges, together with a bird protein supplement of AviPlus Softbill/Mynah pellets (Aviproducts, Durban, RSA).

Fruits were fed to birds, and the germination success of defecated seeds investigated. Feeding trials were conducted for three days after the acclimatisation period. Birds were given their supplementary food incorporated with five *P. angustifolia* fruits. Supplementary food was removed from the cages at 17h00 as the birds do not feed at night and would be hungry in the following morning for the feeding trials (Dlamini et al. 2018). Feeding trials began at 08h00 where birds were provided with *P. angustifolia* fruits to feed on. Water was provided to birds *ad libitum*.

Seed retention time

To inform on potential seed dispersal distances, seed retention data were collected to predict the effect of seed dispersal distances. The seed retention time was determined for each bird species to investigate how long seeds took to pass through the birds' gut. This measure was to help predict the spatial implications of seed dispersed by birds. The seed retention time was recorded as the time from when the bird consumed the first fruit/seed until the first seed was defecated by the bird.

Germination trials

Different germination treatments were used in order to compare the germination success and rate. Treatments included seeds ingested by birds, manually depulped seeds where the fruit wall was removed by hand, and intact fruits. The birds were only observed during feeding until sufficient numbers of seeds

were gathered (at least 50) for planting (typically 4h). We placed newspaper under the bird cages to trap all seeds excreted by birds. All seeds were collected and kept in paper bags before planting. Seeds collected from bird excreta were planted in potting soil in seedling trays (265 × 180 × 75 mm) and kept in a greenhouse at 26 °C maximum temperature (Jordaan et al. 2011). The sown seeds were watered daily and were considered to have germinated when the radicle appeared above the soil (Dlamini et al. 2018). After being counted, such seedlings were removed from the seedling tray to avoid recounts (Dlamini et al. 2018).

The treatment using depulped seeds emulated a condition where the fruit wall was removed by other factors but not birds' ingestion. This treatment excluded the digestive juices or abrasion in the frugivores' gut as factors that might trigger germination. A total number of 200 seeds were planted from 40 manually depulped fruits as per above.

Un-ingested intact fruits were sown in the same conditions as the other two treatments to serve as a standard of comparison (control) for the experiment. This treatment emulated the natural environment where seeds fall directly to the soil without being eaten by animals, buried under the soil or litter while intact. One hundred fruits (n = 100) were planted in four separate planting trays, totalling approximately 500 seeds (average of 5 seeds per fruit). Seedling emergence was recorded until the 58th day for all three treatments.

Data analyses

We used GraphPad Prism 5 statistical software (Motulsky 1999) to analyse data for the study. We calculated mean cumulative seedling percentages for ingested, manually depulped and whole fruits. Time taken for seedlings to emerge in each treatment were compared using analysis of variance (ANOVA) (Thabethe et al. 2015). Mean seedling emergence times for seeds ingested by different bird species, manually depulped, and whole fruits (control) were compared using post-hoc Tukey tests for multiple comparisons. Seedling emergence time referred to the time it takes for a single seedling to emerge on top of the soil. The significance level for this analysis was set at $\alpha = 0.05$. We conducted a two-tailed Chi-square test at 95% confidence interval to compare the proportion of seeds that germinated from each treatment. Data normality was tested using a D'Agostino and Pearson omnibus normality test (D'agostino et al 1990).

Results

Field observations

We observed four bird species and two domestic animal species feeding on *P. angustifolia* seeds/fruits. Bird species observed included African Red-eyed Bulbuls (*Pycnonotus nigricans*), Speckled Mousebirds, African Pied Starlings (*Lamprotornis bicolor*), Crested Barbets (*Trachyphonus vaillantii*); and domestic animals included goats and horses from camera traps. All livestock observed feeding on *P. angustifolia* did not feed on the vegetative material of adult plants nor seedlings (L. Adams, pers. obs.).

We conducted timed observations to determine visitation frequencies and visitor behaviour (feeding/perching) on *P. angustifolia* fruiting branches over three days (total of 54 h) in June 2019. Cape Bunting (*Emberiza capensis*), Cape Rock Thrush (*Monticola rupestris*), Cape White-eyes, Speckled Mousebirds, Dark-Capped Bulbuls, Dusky Flycatchers (*Muscicapa adusta*), Fiscal Shrikes (*Lanius collaris*), Familiar Chats (*Cercomela familiaris*) and African Red-eyed Bulbuls were the nine bird species observed visiting the *P. angustifolia* shrubs (Fig. 1). Only one species, the Speckled Mousebird, fed on *P. angustifolia* fruit; the remainder of the species used it as a perch site only.

The number of seeds collected from the feeding trials was 443 seeds from the Red-winged Starlings, 298 from Purple-crested Turacos, 133 from Dark-capped Bulbuls and 44 from Speckled Mousebirds. Cape White-eyes did not ingest any seeds.

Seed retention times

Sample size for seed retention times did not pass the D'Agostino and Pearson omnibus normality test as the samples were too small, thus a non-parametric Kruskal-Wallis test was used to assess differences in retention times..

Birds with larger body mass had longer seed retention times (i.e. Purple-crested Turacos and Red-winged Starlings: mean \pm SD of 25 ± 1 and 29 ± 1 min, respectively). Smaller birds generally had shorter seed retention times with Dark-capped Bulbuls and Speckled Mousebirds having mean retention times of 22 ± 2 and 11 ± 6 min, respectively.

Germination times

The D'Agostino and Pearson omnibus normality test revealed that the data were not normally distributed ($P < 0.05$), therefore a non-parametric Kruskal-Wallis test with Dunn's Multiple Comparison *post-hoc* test was used for statistical analysis comparing germination rates between treatments. We found an overall significant difference ($P < 0.01$) in germination rate between the three germination treatments. There was a slower rate of germination in seeds planted as whole fruits than the manually depulped seeds and those ingested by birds (Fig. 2). Mean germination times (mean \pm SD) for all seeds ingested by birds, manually depulped, and whole fruits were 31 ± 6 , 32 ± 6 and 38 ± 7 days respectively. There were no significant differences in the seedling emergence time between the manually depulped and ingested seeds ($P > 0.05$), but there were significant differences between ingested and manually depulped treatments when compared with whole fruits ($P < 0.05$). Seeds planted as whole fruits took a longer time to germinate compared with depulped and ingested seeds ($P < 0.05$).

Seeds defecated by the smaller bird species (Speckled Mousebirds and Dark-capped Bulbuls) germinated faster (approximately 4 days earlier) compared with the larger bird species (Purple-crested Turacos and Red-winged Starlings). However, there were no significant differences in germination rates among bird species (Purple-crested Turacos vs Red-winged Starlings and Dark-capped Bulbuls vs Speckled Mousebirds) ($P > 0.05$).

Germination success

When comparing the cumulative percentage of seeds that germinated from each treatment with number of days it took for the seed to germinate, we found significant differences in the days for germination between the treatments ($X^2 = 1041$, $df = 5$, $P < 0.01$). Germination success of both depulped and ingested seeds was high (Purple-crested Turacos = 94.0%, Red-winged Starlings = 81.9%, Speckled Mousebirds = 88.6%, Dark-capped Bulbuls = 94.7%, depulped = 99.5%) compared with whole fruits (7.6%), with almost all depulped and ingested seeds germinating after 40 days (Fig. 3).

Discussion

From our observations and remote camera-trap data, results support that birds are important potential seed dispersers (Díaz Vélez et al. 2018, Vergara-Tabares et al. 2018). This showed that bird species are effective seed dispersers of *P. angustifolia*. This was further confirmed when we assessed the whole process from ingestion of the fruit by an avian frugivore until the seed germinated into a seedling.

Non-consumption of *P. angustifolia* seeds by Cape White-eyes indicated that relatively small bird species probably do not consume entire *P. angustifolia* fruits. Cape White-eyes were similarly unable to consume larger fruits of another IAP, Indian laurel (*Litsea glutinosa* (Lour.) C.B. Rob., Lauraceae) (Dlamini et al. 2018). The same behaviour of feeding on only the fruit wall when presented to the Cape White-eyes was observed in studies by Dlamini et al. (2018) as they also ate *L. glutinosa*'s pulp. This then excluded Cape White-eyes as potential long-distance seed dispersers of *P. angustifolia* as they did not ingest the seeds. However, eating the fruit pulp would still assist in germination when these depulped seeds fell to the ground.

Retention time appeared to affect the rate of germination of ingested *P. angustifolia* seeds, although not significantly. Smaller bird species, Dark-capped Bulbuls and Speckled Mousebirds, had shorter seed retention with faster rates of germination than larger bird species. Purple-crested Turacos and Red-winged Starlings had longer seed retention and slower rates of seed germination. This showed that these relatively larger bird species would likely deposit *P. angustifolia* seeds at further distances away from source plants compared with smaller bird species with shorter retention times. However, the smaller bird species would deposit more seeds in a shorter time. Thus, the temporal aspect of dispersal was more important in the smaller bird species than the spatial aspect of dispersal in relatively larger bird species. These data suggest that small birds contribute more to the rate of *P. angustifolia* spread as they would deposit more seed per time, and seeds would germinate faster. However, many other factors might be involved (e.g. feeding rates which is based on visitation rates influenced by local abundances and frugivorous bird feeding preferences). Larger bird species are expected to contribute more to the area of spread than the rate of spread as they feed on more *P. angustifolia* seeds and have longer seed retention times. Retention times did not affect *P. angustifolia* germination success since all ingested seeds had high germination success together with the manually depulped seeds.

Seed retention times were contrary to the results found by Dlamini et al. (2018) as Speckled Mousebirds had significantly higher retention time (25 min) compared with Dark-capped Bulbuls (13 min) when they fed on *L. glutinosa*. In the present study Speckled Mousebirds had slightly lower retention times (11 min) than Dark-capped Bulbuls (22 min). Jordaan et al. (2011) found similar results to those of Dlamini et al. (2018) when feeding Speckled Mousebirds and Dark-capped Bulbuls with fruits from IAPs *Solanum mauritianum* Scop. (Solanaceae), *Psidium guajava* L. (Myrtaceae), and *Lantana camara* L. (Verbenaceae). Such results might be influenced by the handling (how the bird feed on the fruit) of fruits by the latter birds' digestive systems, indicating that a Speckled Mousebird's digestive system digests the fruit wall of *P. angustifolia* better than the fruit wall of *L. glutinosa*. In contrast, Dark-capped Bulbuls' digestive system handled *L. glutinosa* seeds relatively well.

Another factor that might have affected the results might be that Speckled Mousebirds "squashed" *P. angustifolia* fruits with their feet before feeding on fruit pulp and seeds (L. Adams, pers. obs.). Seeds may have been ingested by mistake because fruit pulp might have been what the Speckled Mousebirds aimed for when feeding on fruits.

As there were no differences between the germination rates of manually depulped and bird ingested seeds, it showed that birds' ingestion did not inhibit or enhance germination but rather facilitated germination by mechanically removing the fruit wall. Similar results have been reported for germination of *S. mauritianum*, *Cinnamomum camphora* (L.) J.Presl (Lauraceae), *L. camara* and *P. guajava* after being ingested by Red-winged Starlings, Speckled Mousebirds, and Dark-capped Bulbuls (Jordaan et al. 2011). Although ingestion in this study did not affect the rate of germination, birds do help with the dispersal of the seed away from the source plant and therefore reduce competition between seedlings, provided that the seed reaches a favourable site for germination (Dellafiore et al. 2019).

The low rate of germination observed in seeds planted as whole fruits was an expected result. The slower rate of seedling emergence from intact fruits has been reported by numerous studies (Jordaan et al. 2011; Thabethe et al. 2015; Mokotjomela et al. 2016; Dlamini et al. 2018). The fruit wall of fallen fruits must be decomposed by soil microorganisms first before the seedling emerges. This typically takes longer than when the fruit wall is removed by a bird as it takes less than an hour for a bird to remove the fruit wall, but it takes days for the soil microorganisms to digest the fruit wall (Holmes 1989). This implies that seeds fallen as whole fruits have lower germination success and rates than those dispersed by birds. Such a phenomenon shows that avian frugivore species remain important seed dispersers of *P. angustifolia*. Our study's results conformed with those presented in many studies whereby seeds ingested by birds germinate significantly faster than those in intact fruits (Jordaan et al. 2011; Thabethe et al. 2015; Mokotjomela et al. 2016; Díaz Vélez et al. 2018)

Cumulative percentage germination of *P. angustifolia* was high in both ingested and depulped seeds compared with whole fruits. This showed that germination success was not affected by ingestion as in studies by Lafleur et al. (2009) where European Starlings (*Sturnus vulgaris*) increased germination success of *Elaeagnus umbellate* Thunb. (Elaeagnaceae) and *Celastrus orbiculatus* Thunb. (Celastraceae)

compared with manually depulped seeds. Similar results were observed when *Solanum mauritianum*, *Cinnamomum camphora*, *Psidium guajava*, and *Morus alba* L., were fed to Knysna (*Tauraco corythaix*) and Purple-crested Turacos in which germination success was unaffected (Thabethe et al. 2015). Contrary results were reported when Castro et al. (2016) found that germination success of the glossy privet tree *Ligustrum lucidum* W.T. Aiton. (Oleaceae) was reduced after ingestion by Austral Thrushes (*Turdus falcklandii*).

In contrast, Mora and Smith-Ramirez (2016) found the IAPs *Aristotelia chilensis* (Molina) Stuntz. (Elaeocarpaceae) had increased germination success post-ingestion by frugivorous Austral Thrushes, while they did not affect the germination success of IAPs *Rubus ulmifolius* Schott. (Rosaceae) and *Ugni molinae* Turcz. (Myrtaceae). Dellafiore et al. (2019) found that *Pyracantha atalantoides* (Hance) Stapf (Rosaceae) germination was lower for bird-ingested seeds than manually depulped seeds. This might be because the seeds were collected from birds' excreta in the field, while in our study, the seeds were collected immediately after defaecation and in controlled conditions, while still fresh. Seeds collected from excreta in the field might have already been exposed to harsh environmental conditions such as sunlight.

Such contrasts show that seeds of different plant species respond differently to ingestion by different bird species and, therefore, affect germination. Findings presented in our study highlight the importance of frugivorous bird species in facilitating the spread of IAPs (Castro et al. 2016). Factors that might have affected the results are highlighted in a review by Traveset (1998), including size and thickness of the seed coat, differences in gut passage times, diets of birds, and seed age.

Although absolute numbers of bird species feeding on *P. angustifolia* (and ingesting seed) at the study site is low, those birds that do ingest seed are important catalysts for the species dispersal (by removing the pulp) and ease of establishment away from parent plants. The implication is that there should be a directly proportional relationship between natural presence of indigenous frugivores and *P. angustifolia* invasion rate. For example, the eastern Free State is not rich in frugivores, but other grasslands close to frugivore-rich forests are, thus dispersal potential increases for sites where there is close proximity of such species. Of course, this does not take the population numbers of frugivores into account. A high population size of one (widespread) species might facilitate invasion more than smaller populations of several (more localised) species.

Declarations

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Author contributions

LDA, GDM and SS conceived the study; LDA, VT and AR collected the data; LDA analysed the data. All the authors led the writing of the manuscript and contributed substantially to revisions.

Data availability statement

The data sets analysed during the study will be available in Zenodo repository [<https://doi.org/10.5281/zenodo.4534703>].

Compliance with ethical standards

Ethical clearance (Protocol reference number: 020/15/Animal) was obtained for the use of aviary birds in the study from the University of KwaZulu-Natal.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

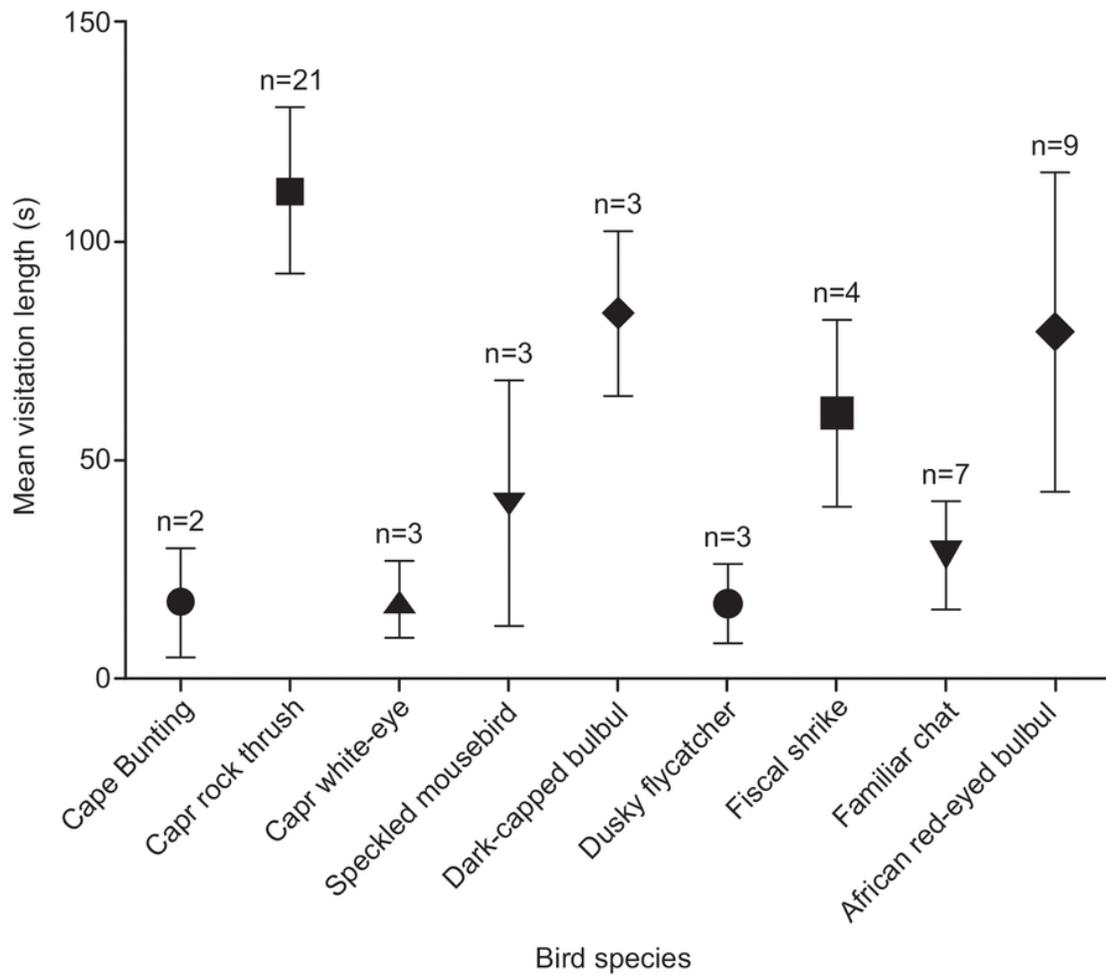


Figure 1

Visitation length (seconds) of different bird species that visited *Pyracantha angustifolia* shrubs during the timed observations in the field at Wynford Holiday Farm (28°41'13"S; 28°13'59"E). Numbers above bars indicates the sample size (n).

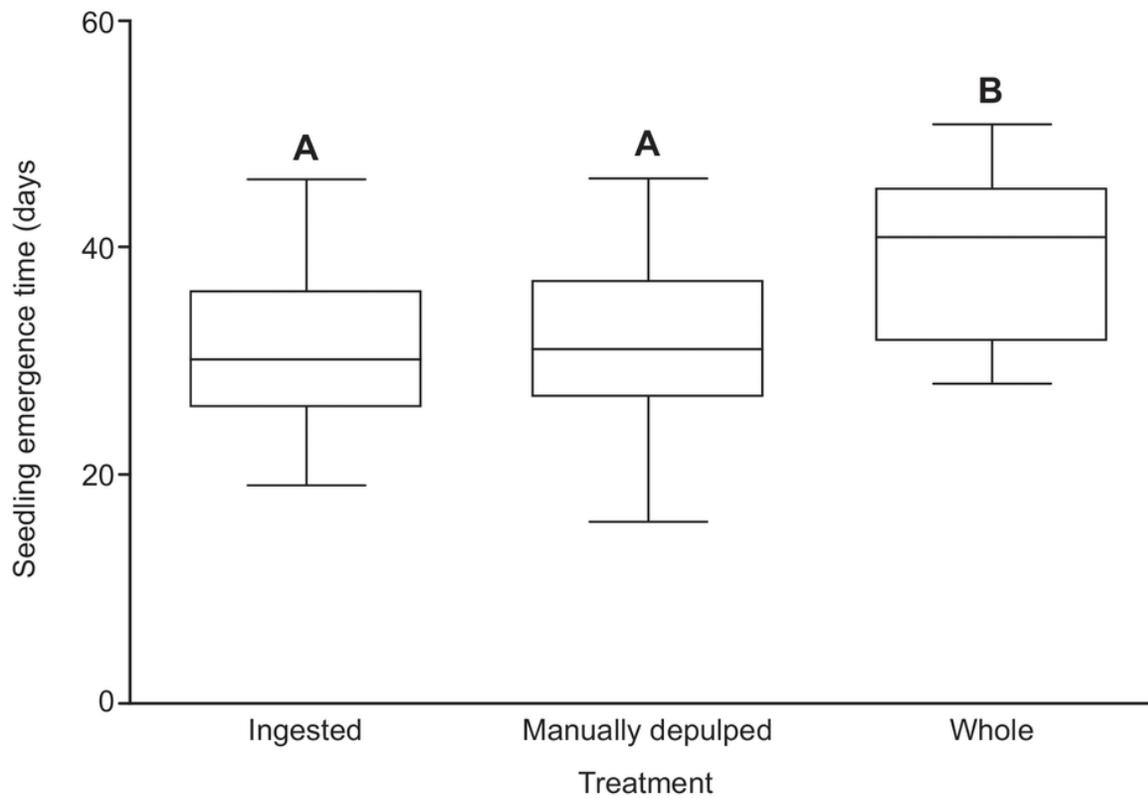


Figure 2

Box and whisker plot (minimum to maximum) of *Pyracantha angustifolia* seedling emergence time in days for three treatments, namely ingested by birds (n = 805 seeds), manually depulped by hand (n = 199 seeds) and whole fruits (n = 38 fruits). Boxes represent mean \pm SE. Different letters on top of boxes indicate statistical significance (H-value = 43.21, $P < 0.05$, $df = 2$) and bars indicate minimum and maximum values.

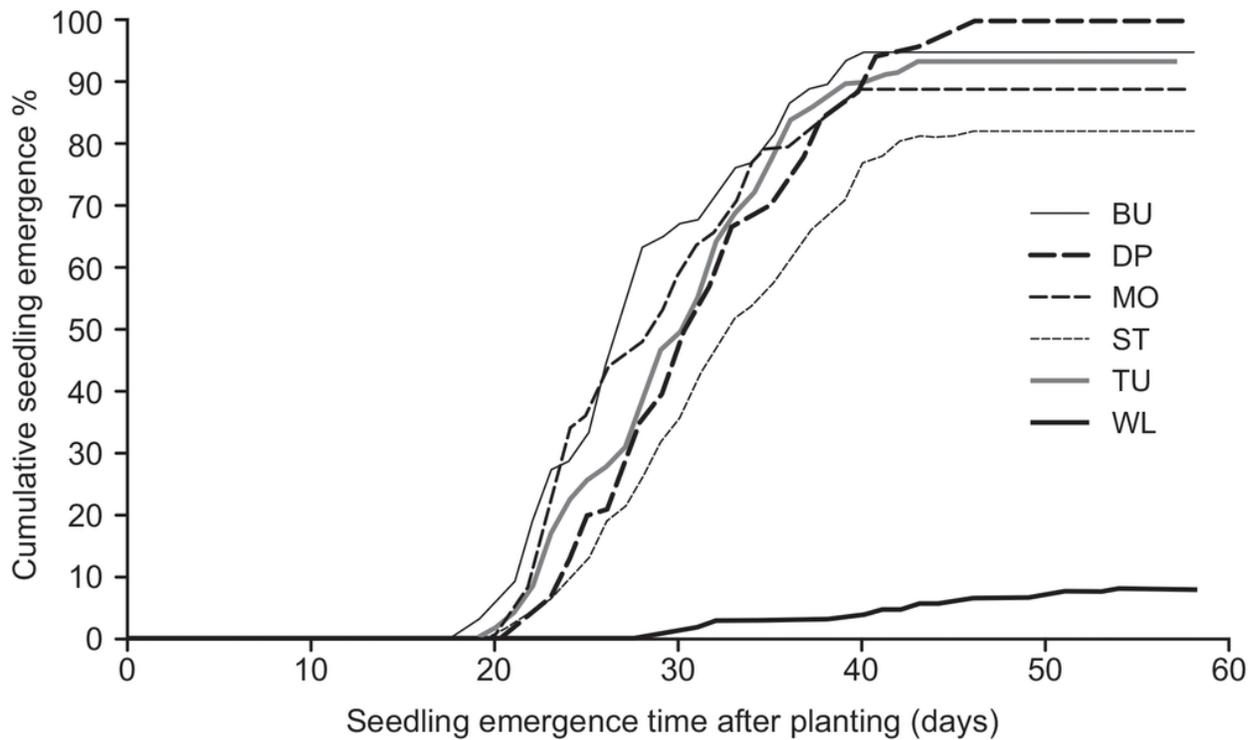


Figure 3

Cumulative *Pyracantha angustifolia* seedling emergence percentage with the number of days after planting for seeds defecated by Purple-crested Turacos (TU), Red-winged Starlings (ST), Speckled Mousebirds (MO) and Dark-capped Bulbuls (BU) together. Seedling emergence from manually depulped (DP) fruits and seeds planted as whole fruits (WL) are also shown. Seedling emergence was recorded for 58 days in total.