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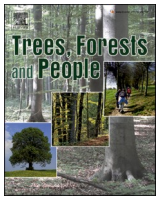

Hiding in plain sight: Reinvasion risk from retained seed after dispersal events in introduced conifers

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ABSTRACT

Non-native conifers are the most successful tree-weeds in the Southern Hemisphere, reproducing annually through seed-bearing cones. Rather than shedding cones, some conifer species retain cones on the tree indefinitely (e.g. *Pinus contorta*), meaning cones persist on the tree even after mortality via common foliar applied herbicide-based control methods. We investigated whether open cones could present a reinvasion risk by retaining seeds after their primary dispersal event and disseminating them over time. We found that 19 % of open cones retained viable seed after opening, with an average of 3.5 viable seeds (ranging from 1-39) per cone. The oldest cone retaining viable seed was 15 years old. The majority of seeds had separated from their wing 6 years after the initial cone opening, however intact wings were recovered up to 11 years post cone opening. These results suggest that cones can still contribute to propagule pressure even after their main dispersal event, potentially leading to the reinvasion of sites years after control operations. However, as the majority of seeds detach from their wings only a few years after cone opening their dispersal ability is hampered and reinvasion would likely occur close to the parent tree.

Introduction

Invasive alien conifers are globally widespread weeds, with significant invasions present in Argentina, Chile, South Africa, Australia, and New Zealand, among other regions (Richardson and Rejmánek 2004). Invasive conifers cause significant economic and environmental detriment by outcompeting native plant species, increasing wildfire risk, altering waterflow regimes, occupying productive land, and supporting invasive mammal populations (Dickie et al. 2022; Sapere Research Group 2022; Carlin et al. 2024a). Many conifers introduced into New Zealand reproduce annually, producing tens of thousands of wind-dispersed seeds, which can travel over many kilometres (Ledgard 2001; Wyse and Hulme 2021, 2022; Vander Wall 2023).

Typical control methods for conifer infestations in New Zealand depend on the extent and stage of the invasion, with ground-based methods preferred for young or scattered conifers, and broad-scale aerially applied herbicide for dense infestations (NWCCP 2023). Alternative methods such as ground- or aerially-based individual herbicide application are also used to target large individual adult trees scattered across the landscape. Due to the difficulty and expense of felling dense infestations of adult trees, most established invasions are controlled by chemical methods, and result in large forests of dead, standing trees. The typical herbicides used affect the roots or foliage of living trees and can hamper new germinants by persisting in the environment for up to 2 years (Rolando et al. 2023; Rolando et al. 2024), however have little, if any, known effects on dormant seeds.

Recent work has identified that large, viable seed banks can persist in New Zealand through control operations as serotinous cones remain sealed until an environmental trigger causes them to open (Critchfield 1980; Koch 1996). This is similar to the fire-adapted Mediterranean pines (e.g., *Pinus halepensis*) which maintain a significant percentage of the yearly cone crop (40-80 %) closed, creating a persistent canopy seed bank. Cones of up to 20 years of age contained a considerable fraction of germinable seeds (Daskalakou and Thanos 1996). Conversely, late flowering and absence of serotinous cones in *P. nigra, P. sylvestris* and *P. uncinata* indicate that their natural forest did not evolve under

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frequent crown fires (Tapias et al. 2001; Tapias et al. 2004).

Despite considerable attention on the role of serotinous cone banks, no-one has thus far considered the role that non-serotinous cones may play in reinvasion beyond their initial dispersal event. While many conifer species shed the majority of cones annually after opening (e.g. *Pseudotsuga menziesii* (Mirb.) Franco), others, such as *Pinus contorta* Douglas ex Loudon, retain their cones indefinitely despite seed release occurring in the year of cone maturity for non-serotinous cones (Critchfield 1980; Miller and Ecroyd 1986; Koch 1996). Seed release can also occur gradually over time, with cones of *P. torreyana* Parry ex. Carr taking multiple years to open fully (McMaster and Zedler 1981). As such, it seems reasonable that non-serotinous *P. contorta* cones persisting on the tree could continue disseminating seed even after opening. This is concerning in herbicide-treated conifer stands due to the accelerated degradation of cones and branches on dead trees over time and reduced understory cover following treatment.

Here we investigated: i) whether seeds were retained in open cones, ii) whether these seeds were disseminated over time, and iii) whether these seeds were viable, posing a risk of reinvasion. We utilised *Pinus contorta* as a case study due to its wide distribution as an invasive conifer across the southern hemisphere, broad ecological impacts, and its lengthy cone retention ability. We anticipated that the number of seeds contained within cones would be low and correlate negatively with cone age, suggesting that as cones age, they gradually release seed as they degrade or are agitated.

Methods

Site selection

Four sites representing different latitudes, elevations, and underlying environments were sampled across the South Island of New Zealand from December 2023–March 2024: Mid Dome (Southland), Lake Pukaki (Canterbury), Craigieburn (Canterbury), and the Clarence Valley (Canterbury; Table S1). Two distinct populations were sampled at each site, separated by at least 5 km, except at Mid Dome where only one suitable population was located and sampled.

Field collection

We selected live *P. contorta* trees with a large age range of cones. A minimum of two suitable branches were identified per whorl, with at least two different whorls sampled per tree. Where possible, we favoured branches in opposing orientations to account for variation in prevailing winds, or other disturbances that could affect seed retention, between sides of the tree. We collected cones directly into labelled paper bags prior to biometric measurements, and avoided any agitation of cones that could cause seeds to be dislodged and lost. We collected all available cones for each age cohort from each branch, with a minimum target of four cones per age cohort where possible. Cone age was determined based on the node position along the branch, with each successive full node representing an additional year of growth (Redmond et al. 2016). We considered cones part of the current season's cohort as one year old, however field collections mostly occurred prior to their opening (late--February–April in New Zealand) hence one year old cones (sampled in the current season) were excluded from our collections and data analysis.

Biometric measurements were taken of each tree, including height, orthogonal crown measurements, local competition (assessed as: very high, high, medium, or low), and branch orientation of all sampled branches. Trees were felled, then aged with ring counts, and coordinates recorded using a Garmin GPS (Model: GPSMAP 66i). Selected trees were a minimum distance of 30m from other selected trees to reduce the likelihood of sampling direct siblings. Selected trees were primarily isolated, or with few trees directly surrounding them, as large trees in high density stands tend to lose their lower branches over time, making it difficult to access suitable branches.

Sample processing

Individual cone mass and length were recorded; however, we note that older cones are more degraded therefore may appear shorter and lighter than younger cones. Bract counts were not conducted as they were adversely affected by cone degradation. Cones were carefully dismantled from base to tip by systematically peeling cone bracts away from the base. Following this protocol, bracts can be removed without seeds becoming trapped. All seeds were collected from each cone, and assigned to either the "lower", "middle", or "upper" portion of the cone (Fig. S1). Undeveloped seeds and seedless wings were counted, and fully developed filled seed were also viability tested using 1 % tetrazolium chloride solution (Patil and Dadlani 2014). For a random subset of cones we recorded whether the wings of each seed were fully intact which facilitates their dispersal ability.

Statistical analysis

Elevation, slope, and aspect information were downloaded for each tree using the *elevatr* package (Hollister 2023). To produce a variable measuring overall exposure, we subtracted branch orientation from aspect and inverted the scale. Exposure values close to the extremes (0^o and 360^o) therefore represent branches on the uphill side of the tree, and values close to 180^o represent branches on the downhill side.

Differences between cone ages were assessed using generalised linear mixed models (GLMMs). We used GLMMs with a Poisson distribution to predict: 1) the number of developed seed, and 2) the number of viable seed present in open cones as a function of cone age. We used GLMMs with a Binomal distribution to predict: 3) the proportion of wings that remained attached to developed seeds, and 4) the proportion of cones containing viable seed in open cones as a function of cone age. All models initially included cone age, exposure, their interaction, and site as fixed effects, and tree identity as a random effect. To account for overdispersion, an observation level random effect was included in all models except for model (3). Interaction effects were removed from model (2) to ensure model convergence.

All models were performed using R version 4.3.1 (R Core Team 2023) and the *lme4* package (Bates et al. 2015). Models were tested for overdispersion and zero-inflation, and had their residuals checked via the *DHARMa* package (Hartig 2022). Significance of effects was determined with the *emmeans* (Lenth 2023), *multcomp* (Hothorn et al. 2008), and *report* (Makowski D et al. 2023) packages. Figures were generated using the *ggplot2* package (Wickham 2016).

Results

A total of 22 live trees were sampled: 9 from Craigieburn, 4 from Mid Dome, 7 from Clarence Valley, and 4 from Lake Pukaki. A total of 625 cones were dissected ranging from 2–19 years in age (Table 1), including 68 cones that had their wing integrity assessed. The oldest cone containing viable seed was 15 years old (Fig. 1A), and the oldest cone containing developed seed was 17 years old (Fig. S2). Overall, 28 % of cones contained developed seed, and 19 % contained viable seed.

Model (1) predicting the number of viable seed per cone had substantial explanatory power although most variation was explained by cone or tree identity ($R^2_{\text{marginal}} = 0.18$; $R^2_{\text{conditional}} = 0.72$). As expected, the number of retained viable seeds decreased with cone age ($\beta_2 = -0.09$, 95 % CI [-0.17, -0.01], $p = 0.025$; Fig. 1B). Interestingly cones from Mid Dome had significantly greater numbers of viable seeds than at other sites ($\beta_2 = 1.97$, 95 % CI [-0.85, -3.09], $p < 0.001$). No other effects were significant (Table S2).

Model (2) predicting the number of developed seeds per cone had substantial explanatory power although most variation was explained by cone or tree identity ($R^2_{\text{marginal}} = 0.07$; $R^2_{\text{conditional}} = 0.72$). The

Table 1
Number of open *Pinus contorta* cones dissected of each cone age cohort across four study sites.

	Cone Age (Years)																		
Site	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
Craigieburn	85	18	26	7	7	5	2	1	-	-	-	-	-	-	-	-	-	-	151
Clarence Valley	46	21	24	17	14	5	5	11	3	3	2	-	-	-	-	-	-	-	151
Lake Pukaki	16	17	16	16	16	16	16	15	12	13	10	8	8	9	6	4	2	1	201
Mid Dome	6	9	17	20	24	17	7	8	7	4	2	-	1	-	-	-	-	-	122
Total	153	65	83	60	61	43	30	35	22	20	14	8	9	9	6	4	2	1	625

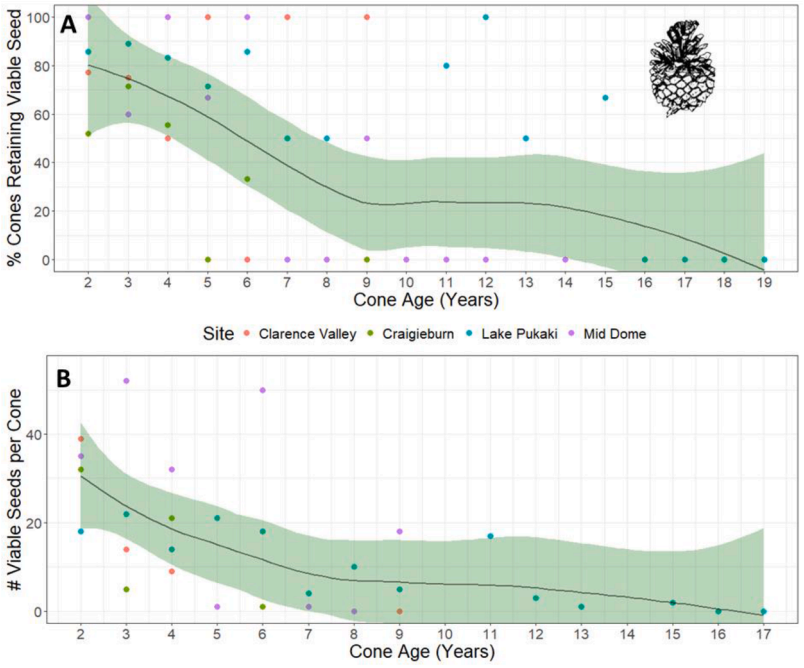

Fig. 1. A) The percentage of open *Pinus contorta* cones that retained viable seed ($n = 119$) out of cones that contained fully developed seed ($n = 174$), separated by age and site. Inset sketch shows an open *P. contorta* cone. B) The number of viable seeds found retained in open cones. Points represent individual cones that contained fully developed seed ($n = 174$). Polynomial lines (black) and 95 % confidence intervals (green) were calculated using the loess method. A total of 625 cones were dissected.

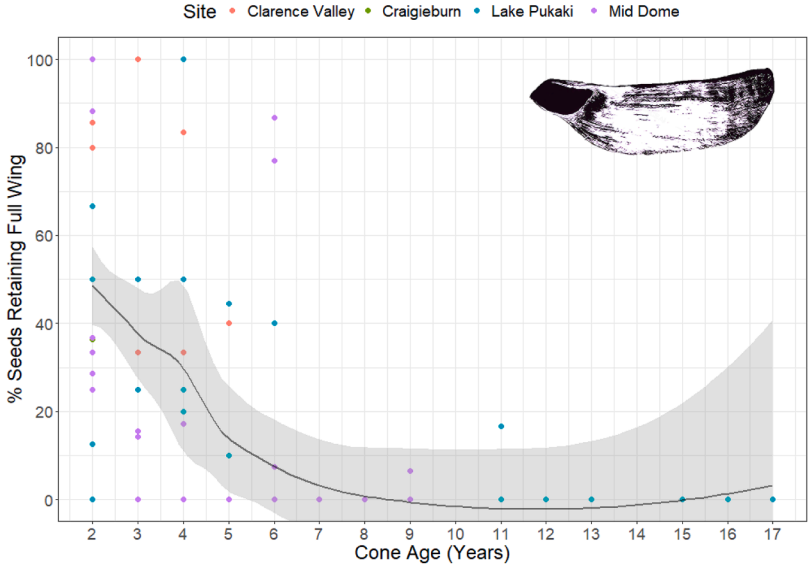

Fig. 2. The percentage of *Pinus contorta* seeds that retained a full wing over time, rather than having the seed separate from the wing. Inset shows a sketch on a fully intact seed with wing. Points represent individual cones ($n = 68$). The polynomial line (black) and 95 % confidence intervals (grey) were calculated using the *loess* method.

number of retained developed seeds decreased with cone age (β_2 = -0.20, 95 % CI [-0.28, -0.11], $p < 0.001$). No other effects were significant (Table S2).

Model (3) predicting the proportion of wings that remained attached to developed seeds had moderate explanatory power largely attributable to the fixed effects ($R^2_{marginal} = 0.43$; $R^2_{conditional} = 0.56$). Cone age significantly and negatively affected the proportion of intact wings (β_2 = -0.46, 95 % CI [-0.62, -0.30], $p < 0.001$). No other effects were significant (Table S2).

Model (4) predicting the proportion of viable seeds per cone had low explanatory power ($R^2_{marginal} = 0.09$; $R^2_{conditional} = 0.22$). Overall seed viability per cone decreased with cone age (β_2 = -0.18, 95 % CI [-0.27, -0.10], $p < 0.001$). Cones from Mid Dome and Lake Pukaki had a higher proportion of viable seeds than at other sites (MD: $\beta_2 = 0.86$, 95 % CI [0.02, 1.71], $p = 0.025$; LP: $\beta_2 = 1.61$, 95 % CI [0.71, 2.51], $p < 0.001$; Fig. S2). No other effects were significant (Table S2).

Discussion

Our results demonstrate that open cones of invasive *Pinus contorta* still present a viable seed source for populations to reinvade up to 15 years after their initial opening (Fig. 1B). However, the long-distance dispersal potential of seeds decreases as cones age due to the deterioration of wings, ultimately removing the primary dispersal capability of seeds older than 5 years (Fig. 2). Operationally, this has implications for the management of conifer infestations as delayed seed release could allow populations to reestablish after control operations, especially as residual herbicide effects wane after two years (Rolando et al. 2023).

Most remaining viable seeds were in the lower portion of the cone under scales which typically do not open as far as scales in the middle portion (Fig. S1). Over time, a combination of agitation and cone deterioration appears enough for retained seed to release. This effect would likely be exacerbated in herbicide treated stands, where the canopy deteriorates until eventual collapse occurs approximately 10–15 years post-treatment, which could cause additional damage to cones or severe agitations that could knock seeds loose. Given that the level of exposure did not appear to significantly affect the number of seeds dispersed, cones on all sides of the tree seem to experience similar levels of seed dissemination. While we anticipated exposure would affect initial cone opening and seed release, it seems that the length of time cones persist on the tree outweighs any effect of additional agitation on exposed branches.

Wing size and structure are important determinants of invasive conifer seed dispersal (Wyse and Hulme 2022; Vander Wall 2023; Carlin et al. 2024b), and our findings suggest that after ~5 years seeds become detached from their wing structures (Fig. 2). The lack of an intact wing is unlikely to affect the germination success after control operations, as viable seed are still able to germinate where they fall. Given areas where control measures are undertaken are suitable for tree-weed invasion, seeds without wings can be assumed to fall into environments suitable for their establishment yet may still encounter competition from successional understory species. The proportion of seeds that go on to germinate is unclear, and many will be predated before germination (Carlin et al. 2024a).

Notably, our estimates of the number of seeds present in cones may vary between populations or at different crown heights. Cones near the top of the tree appear to possess greater numbers of seeds (Carlin et al. 2024b), however, we deliberately selected branches near the base of the tree as they retain a greater age range of cones. Additionally, cone morphology is known to vary between populations with Lake Pukaki and Mid Dome producing longer and thinner cones than those from the Clarence Valley (Carlin et al. 2025). These differences in cone morphology may be responsible for the greater number of viable retained seed we observed at these sites.

Conclusions

Here, we identified a previously overlooked mechanism for reinvasion by highlighting that roughly one fifth of *Pinus contorta* cones can continue disseminating viable seeds into the environment years after their initial cone opening. The timescale of seed dissemination exceeds any recorded residual herbicide effects from control operations in New Zealand, suggesting that *Pinus contorta* populations have the potential to regenerate even without assistance from outside seed sources. However, dispersal ability is impacted by the deterioration of wing structures after ~5 years, meaning that treated invasion fronts are unlikely to expand until the next generation of invasive conifers establishes. These results are likely generalisable to other coniferous tree-weeds that retain their cones for any considerable time. In New Zealand, this may help explain how infestations of conifers such as *Pinus banksiana, Pinus sylvestris,* and *Pinus muricata* (among others) re-invade after control operations.

Code availability

Code is available upon reasonable request.

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CRediT authorship contribution statement

Thomas F. Carlin: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tyler P. Roberts:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Matthew B. Scott:** Writing – review & editing, Methodology, Investigation. **Ryan S. Vorster:** Writing – review & editing, Methodology, Investigation. **Carol A. Rolando:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Thomas S H Paul:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tfp.2025.100852.

Data availability

All data used in this manuscript are available at: https://github.com/TomC-93/VLR/tree/main/Seed-Retention.

References

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.

Carlin, T.F., Paul, T.S., Dudenhoeffer, J.H., Rolando, C., Novoselov, M., Vorster, R.S., Springford, C.R., Scott, M.B., 2024a. The enemy of my enemy… Exotic mammals present biotic resistance against invasive alien conifers. Biol. Invasions 1–16.

Carlin, T.F., Vautrin, A.J., Paul, T.S.H., Rolando, C., Davidson, S.J., Scott, M.B., 2024b. Conifer samara structure diverges across the height of the tree crown. New Zealand Plant Protection 1–7.

Carlin, T.F., Scott, M.B., Paul, T.S., 2025. Reproductive trait shift in *Pinus contorta* helps explain invasion success in Aotearoa New Zealand. New Zealand J. Ecol. 49, 10.

Critchfield, W.B., 1980. Genetics of Lodgepole Pine. U.S. Department of Agriculture, Forest Service, Virginia.

Daskalakou, E.N., Thanos, C.A., 1996. Aleppo pine (Pinus halepensis) postfire regeneration: the role of canopy and soil seed banks. Int. J. Wildland Fire 6, 59–66.

Dickie, I.A., Sprague, R., Green, J., Peltzer, D.A., Orwin, K., Sapsford, S., 2022. Applying ecological research to improve long-term outcomes of wilding conifer management. New Zealand J. Ecol. 46, 1–16.

Hartig, F., 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level /Mixed) Regression Models. R package version 0.4.6.

Hollister, J.W., 2023. elevatr: Access Elevation Data from Various APIs. Version R package version 0.99.0, CRAN.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometr. J. 50, 346–363.

Koch, P., 1996. Lodgepole pine in North Amerca. The Forest Products Society, Madison, Wisconsin, pp. 13–31.

Ledgard, N., 2001. The spread of lodgepole pine (*Pinus contorta*, Dougl.) in New Zealand. Forest Ecol. Manag. 141, 43–57.

Lenth, R., 2023. Emmeans: estimated marginal means, aka least-squares means. Version 1.8.9. CRAN, R package.

Makowski, D., Lüdecke, D., Patil, I., Thériault, R., Ben-Shachar, M., B, W., 2023. Automated Results Reporting as a Practical Tool to Improve Reproducibility and Methodological Best Practices Adoption. CRAN, Vienna.

McMaster, G.S., Zedler, P.H., 1981. Delayed seed dispersal in pinus torreyana (Torrey Pine). Oecologia 51, 62–66.

Miller, J., Ecroyd, C., 1986. Introduced forest trees in New Zealand: recognition, role and seed source. 2. *Pinus contorta* Loud. Contorta pine. Rotorua.

NWCCP, 2023. Aerial Foliar Spray Application (AFSA). Industries MfP, Wellington. https://www.wildingpines.nz/assets/Documents/Wilding-Conifers-Good-Practice-AFSA-May-2023.pdf.

Patil V, Dadlani M 2014. Tetrazolium Test for Seed Viability and Vigour. In: Singh C Ed. A Handbook of Seed Testing. New Delhi, SeedNet India. Pp. 209–241.

R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Redmond, M.D., Weisberg, P.J., Cobb, N.S., Gehring, C.A., Whipple, A.V., Whitham, T.G., 2016. A robust method to determine historical annual cone production among slow-growing conifers. Forest Ecol. Manag. 368, 1–6.

Richardson, D.M., Rejmánek, M., 2004. Conifers as invasive aliens: a global survey and predictive framework. Diversity Distribut. 10, 321–331.

Rolando, C.A., Scott, M.B., Baillie, B.R., Dean, F., Todoroki, C.L., Paul, T.S.H., 2023. Persistence of triclopyr, dicamba, and picloram in the environment following aerial spraying for control of dense pine invasion. Inv. Plant Sci. Manag. 16, 177–190.

Rolando, C.A., Fields, F., Scott, M.B., Sapsford, S., Paul, T.S., 2024. Impacts of herbicides used for control of invasive *Pinus contorta* on the potential for reinvasion and germination of restoration species. Inv. Plant Sci. Manag. 1–10.

Sapere Research Group 2022. Benefits and costs of additional investment in wilding conifer control. Wellington. https://www.mpi.govt.nz/dmsdocument/58519-2022-Benefits-and-costs-of-additional-investment-in-wilding-conifer-control.

Tapias, R., Gil, L., Fuentes-Utrilla, P., Pardos, J.A., 2001. Canopy seed banks in Mediterranean pines of south-eastern Spain: a comparison between Pinus halepensis Mill., P. pinaster Ait., P. nigra Arn. and P. pinea L. J. Ecol. 629–638.

Tapias, R., Climent, J., Pardos, J.A., Gil, L., 2004. Life histories of mediterranean pines. Plant Ecol. 171, 53–68.

Vander Wall, S.B., 2023. Seed dispersal in pines (*Pinus*). Botanical Rev. 89, 275–307.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, 2 ed. Springer, Cham, p. 260.

Wyse, S.V., Hulme, P.E., 2021. Limited evidence for a consistent seed mass-dispersal trade-off in wind-dispersed pines. J. Ecol. 109, 284–293.

Wyse, S.V., Hulme, P.E., 2022. Competition–colonisation trade-offs are found among but not within wind-dispersed *Pinus* species. Funct. Ecol. 36, 1023–1035.