Carlin et al. *New Zealand Plant Protection 77* (2024) 1-7
https://doi.org/10.30843/nzpp.2024.77.11779
Published: 27/02/2024

RESEARCH NOTE

Conifer samara structure diverges across the height of the tree crown

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(Original submission received 25 October 2023; accepted in revised form 11 February 2024)

Summary Samara morphology, including weight, size, and wing-to-seed ratios, is an important precursor to seed dispersal, and therefore a primary driver in large-scale alien conifer invasions. Prior studies have not reported morphological differences between samaras of different cones within a tree possibly because cone position at differing crown heights has not been examined. This preliminary study investigated whether cones from different crown heights of three lodgepole pine (*Pinus contorta* Douglas) trees differ in the morphological characteristics of their samaras. Samaras from the lower tree crown were 17% heavier on average than those from the upper crown, without any significant differences in wing loading. Cones in the upper crown produced more seeds than in the lower crown, although this was inconsistent across the small sample size. These results suggest the effects on primary seed-dispersal are negligible, but further research is needed to determine the effect on secondary seed-dispersal. Larger seeds from the lower crown are better adapted to survive in a competitive environment near other trees, while cones in the upper crown may produce more, but smaller, seeds which could infer a bet-hedging strategy when dispersing into heterogeneous environments. These results suggest canopy-height should be considered when accounting for inter-cone variation in conifers.

Keywords *Pinaceae*; anemochory; introduced species; weed; segment-anything model

INTRODUCTION

Exotic conifer species have been widely planted in Aotearoa New Zealand over the last 120 years but an unforeseen consequence has been their impacts as invasive weeds, known as “wilding conifers”. In considering which species are more likely to spread from plantings (shelterbelts, woodlots, plantations), much focus has been placed on long-distance dispersal ability. In the context of seed dispersal and germination of conifer species, research effort to date has concentrated on variation between samaras within a cone, with minor variation noted among cones (Wyse et al. 2019; Wyse & Hulme 2021a). Such studies have simply considered inter-cone variation randomly, without considering how this varies at different crown heights within a tree.

According to the “competition-colonisation trade-off” theory (Wyse & Hulme 2022), it is reasonable to assume that seed morphology can and would vary across the crown of a tree. Samaras from cones near the top of the tree have a greater opportunity for long distance dispersal due to a longer fall-time and greater exposure to winds to carry them further, helping them spread out and avoid competition with other seedlings or the parent tree. Samaras from cones near the bottom of the tree are more likely to fall close to the parent tree and other samaras, and therefore selection may favour heavier seeds with more resources to facilitate rapid germination and growth to outcompete competitors (Zentsch 1961; Coomes & Grubb 2003). Diversifying reproductive strategies by selecting for increased dispersal ability in the upper crown and increased seed resource investment in the lower crown could therefore increase the reproductive success of a tree. Moreover, variation either in cone structure or the number of seeds produced per cone at different crown heights, could also infer a bet-hedging strategy to increase reproductive success in a heterogeneous environment (Herrera 2017; Wyse et al. 2019).

Seed terminal velocity and seed weight are traits considered to strongly influence long-distance dispersal (Greene & Johnson 1993; Caplat et al. 2012; Wyse et al. 2019;

Liang et al. 2020; Wyse & Hulme 2021a, b; Lynch 2023). These traits are affected by morphological characteristics of the samara, so if they vary significantly across the height of the tree, then the potential for conifer seed dispersal may be currently underestimated. This omission would be particularly exemplified if cones examined in prior studies were all collected from a similar crown height. As differences in samara traits are a criteria used by the wilding tree risk calculator, a policy tool to evaluate the potential risk of wilding tree spread before conifer plantations can be established (Paul 2015), accurate information on dispersal ability is pivotal to the sustainable management of tree plantings and prevention of wilding tree spread.

This study investigated how samara morphology, specifically seed and wing areas, length, width, weight, and derivative metrics, varied at different crown heights and consider how this may impact overall dispersal ability. We hypothesised that samaras from cones at the top of trees will possess traits that aid in long-distance dispersal, whereas samaras from cones at the bottom of trees will be adapted to succeed in a competitive environment. *Pinus contorta* Douglas cones were used because this species is the most vigorously spreading wilding conifer in Aotearoa New Zealand (Ledgard 2001). The use of a novel artificial intelligence (AI) image recognition tool (segment-anything-model; SAM) for accurate, rapid, partitioning of samara components was also trialled.

MATERIALS AND METHODS

Tree selection

Three 8–9 year old coning *Pinus contorta* trees were selected in the Mackenzie District, Canterbury, near Lake Pukaki in March 2023. All selected trees possessed a full crown and were considered lone, with no significant competition despite being part of a wider invasion into an exotic grassland. Tree height, age, diameter at breast height, and crown width were measured before the trees were felled (Supplemental Table S1). While these trees do not represent the maximum height of *P. contorta*, they are representative of a typical invasion stage prior to control measures. All trees possessed more than six whorls, meaning trees could be partitioned into at least three sections with at least two whorls per section.

Cone collection

Ripe, closed, cones were collected and processed in a similar manner to that described by Wyse and Hulme (2021b). All cones on branches of the top and bottom two whorls were collected along with a random sample of cones from the remaining whorls, which were considered mid-height. Epicormic cones were observed but none assessed. However, it is noted that epicormic cones can express different traits than cones from branches, and so would need to be assessed separately (McGinley et al. 1990).

Seed processing

Cone length was measured for each collected cone, before placing each cone into a separate paper bag in a drying oven at 30°C for 12 hours to facilitate cone opening. All cones opened at 30°C and hence were considered non-serotinous (Lotan 1976). All seeds were extracted from a random subset of cones from each tree and crown position (Supplemental Table S1), first by agitating, then by peeling away all cone scales and removing seeds with tweezers if necessary. Seeds were counted and categorised as either fully developed or undeveloped (Figure 1A). Ten developed samaras were randomly selected and individually photographed under a microscope alongside a 5-mm red circle before weighing (Figure 1B & 1C).

Key regions of interest of each samara were segmented from the photographs using a computer vision annotation tool (CVAT.ai corporation 2022) within Facebook AI Research's segment-anything-model (SAM) developed by Kirillov et al. (2023), running on an NVIDIA graphics processing unit for fast model-assisted annotation. By using SAM inside CVAT, the annotator marks positive points belonging to the samara section of interest and has the option of clicking on negative (unrelated) points to correct the model where necessary. Automated segmentations are quickly produced by the model and can be iteratively refined until visually satisfied with the accuracy of the segmentation. This process typically takes a few seconds for each image. An image mask was exported that contained different samara sections represented by different colours for use in image processing. To measure seed and wing orthogonals, minimum boundary rectangles were applied to each wing and seed segment in *Python* software version 3.11 (Python Software Foundation 2023). The boxes were then rotated with the longest length considered to be the wing length. The original minimum bounding boxes for wing length also incorporated seed length due to the presence of some "wing" pixels surrounding the seed (Figure 1C), therefore "true" wing length was calculated by subtracting seed length from the original wing length measurements. Seed area and wing area were automatically calculated as a count of pixels in each segmented section. This technology was not empirically validated against traditional measurement techniques for samaras with regard to human error, but all visual inspections demonstrated accurate partitioning of samara components.

Statistical analyses

Wing loading (seed mass / wing area) was calculated as it represents one of the most important determinants of both primary and secondary dispersal and is proportional to terminal velocity (Norberg 1973; Green 1980; Liang et al. 2020; Wyse & Hulme 2022). Similar to the method of McGinley et al. (1990), wing loading was calculated here using seed mass rather than samara mass, to avoid minor inconsistencies if wings became damaged during processing even though it is noted that the wing contributes a negligible amount to overall samara mass.

Linear mixed effects models were used to assess whether crown height had any effect on either seed weight, wing loading, seed area to wing area ratios, or wing length. Crown height position was used as a fixed effect, and cone length, and cone ID nested within tree ID were included as random effects. Linear models using crown height, tree ID, and

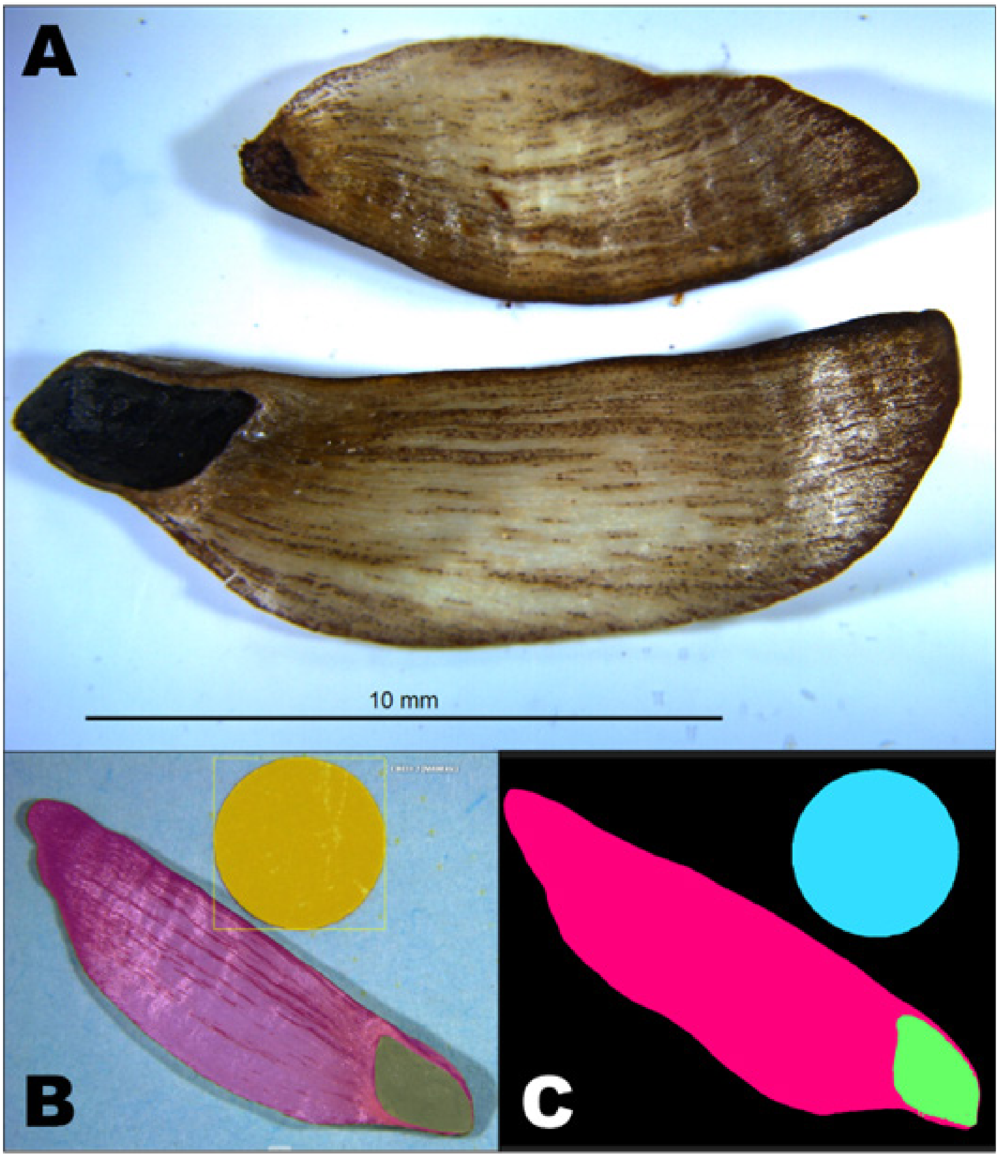

Figure 1 A) Example of fully developed samara (bottom) and undeveloped, non-viable samara (top). B) Example of segment-anything model (SAM) demonstrating differentiation of different samara components. Seed (green), wing (pink), 5 mm circle for known size comparison (yellow). C) Example of resulting masks from SAM. Seed and wing areas were calculated based on pixel counts, and seed and wing lengths using minimum boundary boxes, reducing human error in measurements (Koeshidayatullah 2023). Please note that different samaras are shown in A, B, and C panels.

their interaction as predictor variables were used to assess whether cone length, cone orthogonal measurements, or the number of developed seed varied in cones at different crown heights. Pairwise post-hoc tests with the Tukey-method identified significant differences between comparisons. All analyses were conducted in *R* version 4.0.5 (R Core Team 2023) using the *lme4* (Bates et al. 2015), *emmeans* (Lenth 2023), and *multcomp* (Hothorn et al. 2008) packages.

RESULTS and DISCUSSION

Thirty-four cones were assessed, with 10 randomly chosen fully developed, intact samaras assessed per cone, totalling 340 samaras. Developed seeds at the bottom of the tree weighed on average 17% more than those at the top (top $\bar{x}$ = 3.90 mg ± 0.09 mg SE; mid-height $\bar{x}$ = 4.22 mg ± 0.06 mg SE; bottom $\bar{x}$ = 4.57 mg ± 0.01 mg SE). Average seed mass was significantly different between samaras at the top or bottom of the tree ($t(29) = 3.14$, $p = 0.01$), yet neither of these averages were significantly different from that of mid-height samaras ($t(29) = 3.11$, $p = 0.40$; $t(29) = 2.05$, $p = 0.12$; Figure 2A). This finding suggests that seed mass is likely negatively correlated with crown height, rather than simply differentiating at the height extremes. Initial analysis suggested that cones in the upper crown may produce greater numbers of developed seed (Supplemental Figure S1), however this effect was inconsistent across the small sample of three trees (Supplemental Figure S2) and therefore requires further evidence before conclusions can be drawn. No evidence was found that cone length or orthogonal measurements consistently varied with crown height (Supplemental Figures S3 & S4).

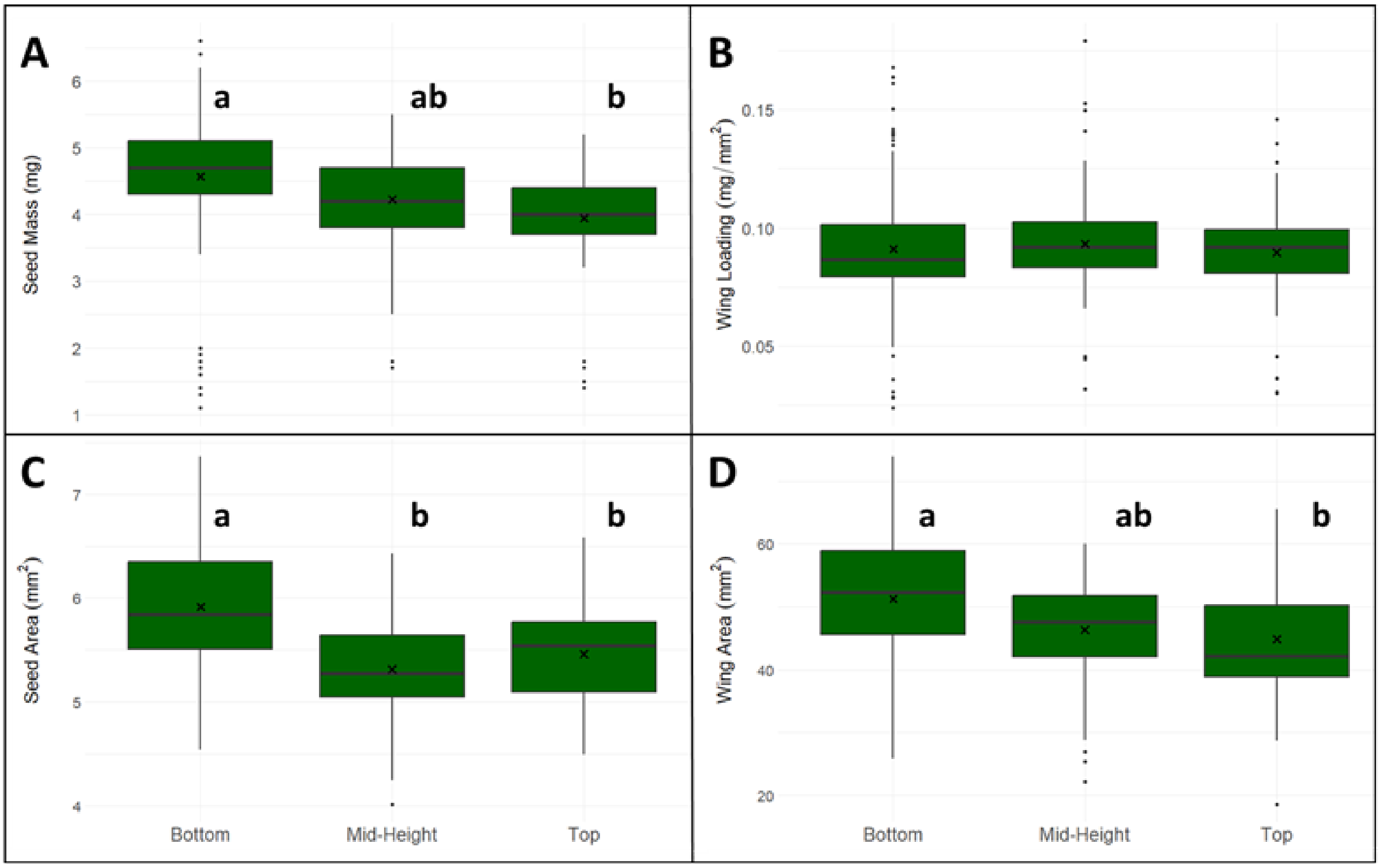

Figure 2 Box plots indicating differences in samaras at different crown heights from cones of three *Pinus contorta* trees with respect to: A) seed mass; B) samara wing loading; C) seed area; and D) wing area. Black crosses indicate mean values. Matching lowercase letters above boxes indicate non-significant differences between groups, determined by Tukey tests for pairwise mean comparisons. If no letters are present, no groups are significantly different from one another. In panel A, low seed mass outliers represent unfilled seed; however, the results remain consistent regardless of whether or not they are included.

This study observed no differences in wing loading of samaras between different crown heights ($t(29) = -0.68$, $p = 0.78$; $t(29) = 1.07$, $p = 0.54$; $t(29) = 1.698$, $p = 0.22$), suggesting any differences in terminal velocity of falling samaras would be minor (Figure 2B). This finding is in line with Wyse and Hulme (2021b) and McGinley et al. (1990) who found no evidence for an intraspecific seed mass-dispersal trade off in *P. contorta*. In light of this, and the results of Lynch (2023) who showed fall speed was related to wing loading in *P. contorta,* the results of this study suggest that differences in dispersal ability would be minor between samaras at different crown heights. While other studies have demonstrated that lighter seeds can benefit from both primary (Greene & Johnson 1993; Lynch 2023) and secondary (Groom 2010; Liang et al. 2020) wind dispersal beyond the effect of wing loading, it is currently unclear whether these effects could meaningfully affect dispersal ability between samaras from different crown heights.

Data generated using AI image recognition in the current study indicate that both wing area and seed area are significantly greater for samaras produced at the bottom of the tree. While wings are larger, this is unlikely to relate to an increased dispersal potential when combined with the increased seed mass, which is reflected in the lack of differences observed in wing loading. It is also possible that seed mass decreases more rapidly than seed area with regard to crown height (Figures 2A & 2C). Assuming the density of seed contents is consistent, this would suggest that seeds from the mid- and lower-crown are thicker, which would provide an explanation for why these seeds weigh more without displaying a greater two-dimensional seed area in the mid-crown. Samara thickness has been shown to affect dispersal ability in other systems with twisted samara structures (Planchuelo et al. 2017), but this is unlikely to meaningfully affect dispersal ability in conifers due to their relatively simple samara morphology.

The results of this study demonstrate that inter-cone variation in samara morphology exists in *P. contorta* and is readily detectable even with small sample sizes of relatively young trees. However, how this variation in samara morphology across the height of the tree affects tree fitness is currently unclear. The lighter seeds observed in the upper crown are unlikely to be the result of cone morphology constraints, given that this study found no differences in cone structure across different crown heights (Supplemental Figures S3 & S4). If future work with a larger sample size demonstrates that the number of seeds produced is consistently higher in the upper canopy, this could indicate the presence of a bet-hedging strategy (Herrera 2017; Wyse et al. 2019). By reducing resource investment in individual seeds and increasing the overall number produced in the upper crown an individual tree may be able to increase the chances of its offspring finding suitable germinating conditions in a heterogeneous environment. This would also impact estimates of conifer seed production rates unless cones were sampled across the height of the tree. The patterns observed in this study for seed mass and cone morphology are consistent with similar research on *Pinus sylvestris* L. (Zentsch 1961).

Future works investigating intra-tree cone variation should account for crown height, measured on a continuous scale, rather than simply sampling from one part of the tree. The primary limitation of this preliminary study is its small sample size, so additional research is required to generalise these findings across other populations, environments, and conifer species. Future work, with a larger sample, may elucidate differences in the number of seeds produced at different crown heights, which could help explain the results of this study with regards to tree reproductive fitness. Finally, future studies that employ AI image-processing tools for rapid partitioning of ecological materials would benefit from comparisons with traditional techniques to quantify any accuracy increases.

CONCLUSIONS

A novel AI "segment-anything" model approach for rapid, accurate partitioning of samara components was used to assess morphological differences among samaras produced at different crown heights of three *Pinus contorta* trees. This preliminary study found that seeds produced in the upper crown were significantly lighter than those at the bottom, with no difference in wing loading. This finding suggests that intraspecific variation among cones is a negligible contributor to terminal velocity, and by extension, to primary wind dispersal. The smaller seed size shown in the upper crown may help increase secondary dispersal distance, but further work is needed to quantify this. Mixed evidence was found to suggest that cones in the upper crown produce higher numbers of seed which, if verified, could indicate a bet-hedging strategy in a heterogeneous landscape. Differences were detectable even with small sample sizes, and as such additional investigation is required to determine how samara morphology varies across the tree crown for different populations, species, and environmental conditions. The concept of seed-trait variation across crown heights affecting dispersal ability or reproductive fitness has thus-far not been accounted for in spread models or invasiveness assessments. The use of novel AI technology enabled rapid and accurate collection of complex data yet still requires empirical comparisons against traditional measurement techniques for samaras with regard to human error.

ACKNOWLEDGEMENTS

We would like to acknowledge Ryan Vorster and Casey Springford, both of whom assisted with seed processing. We thank Brian Richardson for his thoughtful insight and interest when discussing this work. Finally, we would like to thank Dr Sarah Wyse (Referee), the Section Editor, and Dr Ruth Falshaw (Journal Editor) whose comments greatly improved this work.

DATA ACCESSIBILITY STATEMENT

If accepted for publication, data will be made immediately available at: https://github.com/TomC-93/VLR

Code associated with this research note followed standard analysis pipelines but is available upon reasonable request.

SUPPLEMENTARY DATA

Table S1 Tree measurements and number of assessed cones from each tree position.

Figure S1 Box plots indicating differences in developed seed counts at different crown heights.

Figure S2 Box plots indicating the significant interactions between crown height (intra-tree variation) and tree ID (inter-tree variation) affecting the number of developed seed in cones.

Figure S3 Box plots indicating the significant interactions between crown height (intra-tree variation) and tree ID (inter-tree variation) affecting cone length.

Figure S4 Box plots indicating there are no significant differences in cone orthogonal measurements between different tree crown heights (intra-tree variation).

REFERENCES

Bates D, Maechler M, Bolker B, Walker S 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1-48. https://doi.org/10.18637/jss.v067.i01

Caplat P, Nathan R, Buckley YM 2012. Seed terminal velocity, wind turbulence, and demography drive the spread of an invasive tree in an analytical model. Ecology 93: 368-377. https://doi.org/10.1890/11-0820

Coomes DA, Grubb PJ 2003. Colonization, tolerance, competition and seed-size variation within functional groups. Trends in Ecology & Evolution 18: 283-291. https://doi.org/10.1016/S0169-5347(03)00072-7

Green DS 1980. The terminal velocity and dispersal of spinning samaras. American Journal of Botany 67: 1218-1224. https://doi.org/10.1002/j.1537-2197.1980.tb07754.x

Greene DF, Johnson EA 1993. Seed mass and dispersal capacity in wind-dispersed diaspores. Oikos: 69-74. https://doi.org/10.2307/3545096

Groom P 2010. Implications of terminal velocity and wing loading on Hakea (Proteaceae) seed dispersal. Journal of the Royal Society of Western Australia 93: 175.

Herrera CM 2017. The ecology of subindividual variability in plants: patterns, processes, and prospects. Web Ecology 17: 51-64. https://doi.org/10.5194/we-17-51-2017

Hothorn T, Bretz F, Westfall P 2008. Simultaneous inference in general parametric models. Biometrical Journal 50: 346-363. https://doi.org/10.1002/bimj.200810425

Kirillov A, Mintun E, Ravi N, Mao H, Rolland C, Gustafson L, Xiao T, Whitehead S, Berg AC, Lo W-Y 2023. Segment anything. arXiv preprint arXiv:2304.02643. https://doi.org/10.1109/ICCV51070.2023.00371

Koeshidayatullah A Riding the Wave: One-Touch Automatic Salt Segmentation by Coupling SAM and SegGPT. Paper presented at the ADIPEC, Abu Dhabi, UAE, October 2023. https://doi.org/10.2118/216419-MS

Ledgard N 2001. The spread of lodgepole pine (*Pinus contorta*, Dougl.) in New Zealand. Forest Ecology and Management 141: 43-57. https://doi.org/10.1016/S0378-1127(00)00488-6

Lenth R 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means. Version 1.8.9. CRAN, R package.

Liang W, Liu Z, Liu M, Qin X, Baskin CC, Baskin JM, Xin Z, Wang Z, Su Z, Zhou Q 2020. Wing loading, not terminal velocity, is the best parameter to predict capacity of diaspores for secondary wind dispersal. Journal of Experimental Botany 71: 4298-4307. https://doi.org/10.1093/jxb/eraa170

Lotan JE 1976. Cone serotiny-fire relationships in lodgepole pine. The Bark Beetles, Fuels, and Fire Bibliography: 8.

Lynch J 2023. The response of wilding pine samaras to perturbation as experienced in turbulent wind conditions. Unpublished Masters thesis, University of Canterbury. https://ir.canterbury.ac.nz/server/api/core/bitstreams/cb0aaf42-2934-40de-ae76-cd2b3918eed9/content

McGinley M, Smith C, Elliott P, Higgins J 1990. Morphological constraints on seed mass in lodgepole pine. Functional Ecology: 183-192. https://doi.org/10.2307/2389337

Norberg RÅ 1973. Autorotation, self-stability, and structure of single-winged fruits and seeds (samaras) with comparative remarks on animal flight. Biological Reviews 48: 561-596. https://doi.org/10.1111/j.1469-185X.1973.tb01569.x

Paul T 2015. Guidelines for the use of the Decision Support System "Calculating Wilding Spread Risk From New Plantings". Rotorua: Scion. https://www.mpi.govt.nz/dmsdocument/51082/direct

Planchuelo G, Catalán P, Delgado JA, Murciano A 2017. Estimating wind dispersal potential in Ailanthus altissima: The need to consider the three-dimensional structure of samaras. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology 151: 316-322. https://doi.org/10.1080/11263504.2016.1174170

Python Software Foundation 2023. Python Language Reference, version 2.7. Amsterdam, Centrum voor Wiskunde en Informatica.

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

Wyse SV, Hulme PE, Holland EP 2019. Partitioning intraspecific variation in seed dispersal potential using a low-cost method for rapid estimation of samara terminal velocity. Methods in Ecology and Evolution 10: 1298-1307. https://doi.org/10.1111/2041-210X.13202

Wyse SV, Hulme PE 2021a. Dispersal potential rather than risk assessment scores predict the spread rate of non-native pines across New Zealand. Journal of Applied Ecology 58: 1981-1992. https://doi.org/10.1111/1365-2664.13947

Wyse SV, Hulme PE 2021b. Limited evidence for a consistent seed mass-dispersal trade-off in wind-dispersed pines. Journal of Ecology 109: 284-293. https://doi.org/10.1111/1365-2745.13477

Wyse SV, Hulme PE 2022. Competition-colonisation trade-offs are found among but not within wind-dispersed *Pinus* species. Functional Ecology 36: 1023-1035. https://doi.org/10.1111/1365-2435.14004

Zentsch W 1961. Über Eigenschaften von Kiefernsaatgut aus verschiedenen Kronenregionen. Forstwissenschaftliches Centralblatt 80: 287-294. https://doi.org/10.1007/BF01821296

SUPPLEMENTARY DATA

Table S1 Tree measurements and number of assessed cones from each tree position. Every cone was collected from the top and bottom two whorls, while a subsample of mid-height cones were collected from each tree. "DBH" stands for diameter at breast height, measured at 1.4m above ground. Orthogonal measurements are measurements of crown width. "Orthogonal x" represents the widest measurement of the tree crown parallel to the ground, and "Orthogonal y" represents the perpendicular measurement from Orthogonal x.

Tree	No. of Cones Assessed at each Canopy Position			Height (m)	Age (Years)	DBH (mm)	Orthogonal x (m)	Orthogonal y (m)
	Bottom	Mid-Height	Top					
1	6	7	3	6.54	9	94	3.4	3.4
2	3	3	3	5.80	8	90	3.3	2.3
3	3	3	3	6.56	9	103	3.6	2.5

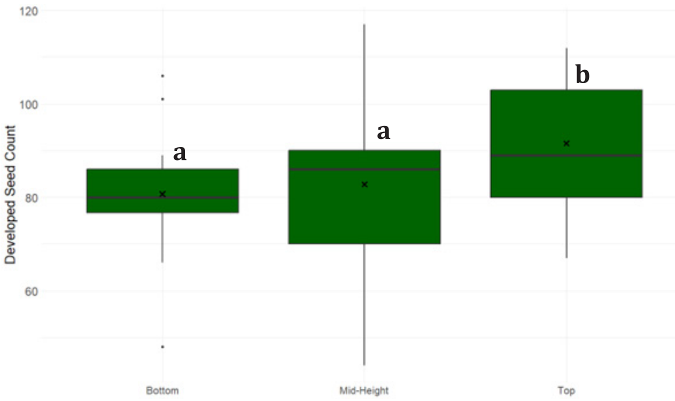

Figure S1 Box plots indicating differences in developed seed counts at different crown heights. Initial inspection appears to show upper crown cones produce more developed seeds than lower crown cones, however this result is highly variable between trees so further evidence is required before drawing conclusions (Figure S2). Matching lowercase letters above boxes indicate non-significant differences between groups.

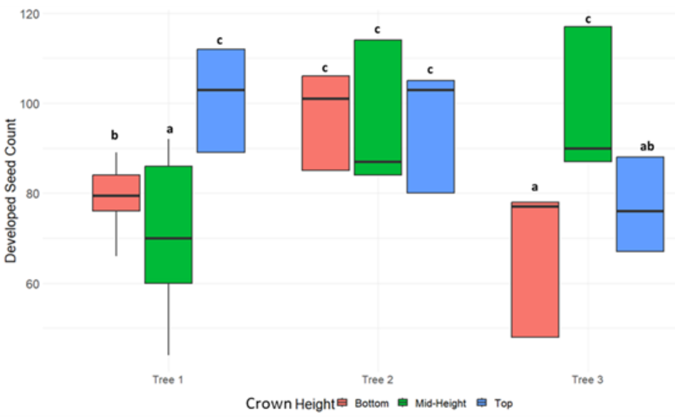

Figure S2 Box plots indicating the significant interactions between crown height (intra-tree variation) and tree ID (inter-tree variation) affecting the number of developed seed in cones. Crown height could be a potential influencer of developed seed counts (Tree 1), but this is inconsistent across assessed trees (Trees 2 & 3). Matching lowercase letters above boxes indicate non-significant differences between groups.

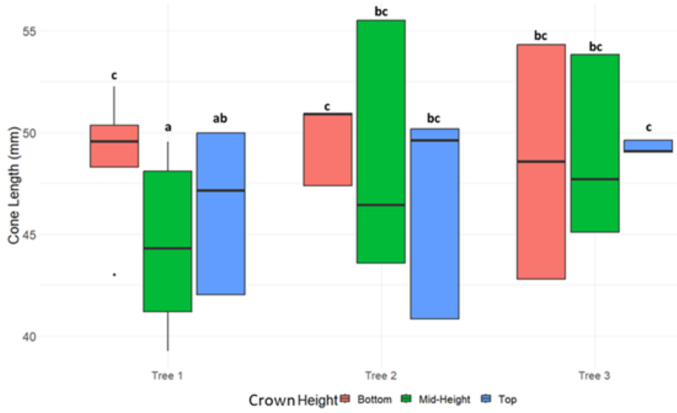

Figure S3 Box plots indicating the significant interactions between crown height (intra-tree variation) and tree ID (inter-tree variation) affecting cone length. No obvious patterns occur suggesting crown height is a major factor affecting cone length. Matching lowercase letters above boxes indicate non-significant differences between groups.

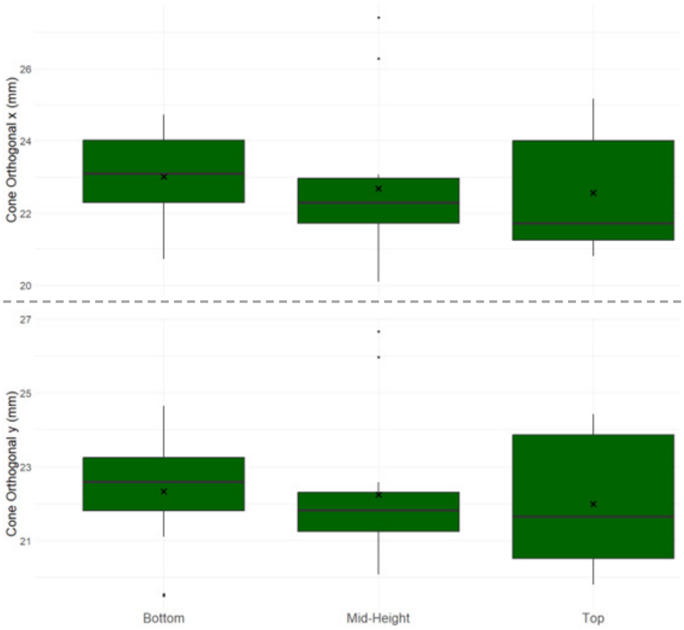

Figure S4 Box plots indicating there are no significant differences in cone orthogonal measurements between different tree crown heights (intra-tree variation).