

Samara water absorption and floatation in sheoaks, *Casuarina* and *Allocasuarina* (Casuarinaceae)

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Abstract - Diaspores (samaras) of the sheoaks, *Casuarina* and *Allocasuarina*, have a mesocarp composed of hydrophilic fibres that, in *Allocasuarina*, rapidly expand on wetting, capturing significant quantities of water. The ecological function of this water capturing has been the subject of speculation but not quantification or experimentation. Therefore, the rate and quantity of water absorbed, and the floatation properties of samaras of accessions of *Casuarina* and *Allocasuarina* were assessed. *Casuarina* absorbed water slowly (~48 h) with median absorption of 90% (by weight) whereas *Allocasuarina* absorbed water rapidly (<2 min) with median absorption of 240%. The process was reversible and increasing in *Allocasuarina*, with median absorption reaching 400% over three to five wetting/drying cycles. The floating half-life of *Casuarina* and *Allocasuarina* samaras overlapped, 17-70 and 29-57 h, respectively, so *Casuarina* samaras were not shown to be better adapted to hydrochory. Based on five accessions of each genus, it does not appear that water-capturing of sheoak samaras is directly related to habitat aridity, nor to potential hydrochory. Therefore, the ecological implications of differential water-capturing of sheoak samaras remains to be determined.

Key words: *Allocasuarina*, *Casuarina*, diaspore, floatation, hydrochory, samara, water absorption.

Riassunto - Assorbimento di acqua e galleggiamento delle samare in *Casuarina* e *Allocasuarina* (Casuarinaceae).

Le diaspore (samare) di *Casuarina* e *Allocasuarina*, hanno un mesocarpo composto da fibre idrofile che, in *Allocasuarina*, si espandono rapidamente con l'idratazione, captando quantità significative di acqua. La funzione ecologica della cattura dell'acqua è stata oggetto di speculazioni ma non di quantificazione o sperimentazione. Per questo motivo, sono stati valutati il tasso di assorbimento, la quantità di acqua assorbita e le proprietà di galleggiamento delle samare di campioni di *Casuarina* e *Allocasuarina*. *Casuarina* ha assorbito l'acqua lentamente (~48 h) con un assorbimento mediano del 90% (in peso) mentre *Allocasuarina* ha assorbito l'acqua rapidamente (<2 min) con un assorbimento mediano del 240%. Il processo era reversi-

bile e crescente in *Allocasuarina*, con un assorbimento mediano che raggiungeva il 400% dopo tre o cinque cicli di idratazione/asciugatura. L'emivita di galleggiamento delle samare di *Casuarina* e *Allocasuarina* è sovrapponibile, con 17-70 h e 29-57 h rispettivamente; pertanto, le samare di *Casuarina* non si sono dimostrate più adatte all'idrocoria rispetto a quelle di *Allocasuarina*. Valutando cinque campioni per ogni genere, non appare che la cattura dell'acqua delle samare di *Casuarina* sia direttamente collegata all'aridità dell'habitat, né all'idrocoria potenziale. Pertanto, le implicazioni ecologiche dell'assorbimento differenziale dell'acqua nelle samare di *Casuarina* e *Allocasuarina* restano ancora da chiarire.

Parole chiave: *Allocasuarina*, assorbimento dell'acqua, *Casuarina*, diaspore, galleggiamento, idrocoria, samara.

INTRODUCTION

Seeds of sheoaks (Casuarinaceae) are enclosed within samaras (winged achene) with mesocarps consisting of spiral, water-absorbing fibres (Ladd, 1988; Hwang & Conran, 1991). Likewise, mucilaginous diaspores (myxodiaspory) that capture and hold water around the seed are found in many plants and have been extensively studied (Western, 2011; Yang *et al.*, 2012). Yang *et al.* (2012) noted that, at the time of their review, myxodiaspory was known in 110 families across 37 angiosperm orders having ecological functions in seed maturation, dispersal, viability, dormancy and germination as well as in seedling growth. No species in the order Fabales (which includes the Casuarinaceae) was listed by Yang *et al.* (2012) as myxodiasporous. Although the water-capturing of samaras in the Casuarinaceae is not considered mucilaginous (Ladd, 1988), it is likely that this provides one or more of the ecological functions that seed mucilage does in other families.

The ecological functions suggested for the water-capturing fibres of sheoak samaras include: (1) enhanced seedling establishment through improved soil contact and anchorage that aids root penetration (Mott & Groves, 1981), (2) enhanced germination in water-limited contexts (Turnbull & Martensz, 1982), (3) provision of a moister microenvironment around the seed (Ladd, 1988), and (4) detachment of the samara wing reducing secondary wind or water dispersal (Hwang & Conran, 1991). However, there have been no ecological or experimental studies to specifically examine these suggestions. Removal of seed from samaras collected from a *Casuarina equisetifolia*

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× *C. glauca* hybrid enhanced germination on agar (91% germination of separated seed within 1 week versus about 1 and 13% within 1 and 8 weeks, respectively, for whole samara) (Shen *et al.*, 2009). If this response was also to occur for seed versus samaras in a natural context, then the samaras must be providing significant ecological advantage to compensate for any potential inhibitory effects.

The exocarp of *Allocasuarina* is dark, thin and often fragmented with the underlying mesocarp visible whereas in *Casuarina* the exocarp is pale with no obvious fragmentation (Ladd, 1988; Fig. 1). On wetting, the samaras of *Casuarina* do not swell and are considered to capture less water than those of *Allocasuarina* (Ladd, 1988), although this has not been quantitatively assessed. Many *Allocasuarina* spp. are found in climatic zones, including Mediterranean climate zones, that impose significant heat and water stress on plants. In contrast, only three *Casuarina* spp., *C. cristata*, *C. obesa* and *C. pauper*, are found in the relatively arid environments (Riley, 2021). In Australia, *Casuarina cunninghamiana*, *C. equisetifolia*, *C. glauca* and *C. obesa* are found in estuarine, lacustrine, marine, palustrine and/or riparian habitats where secondary dispersal of seed in water (hydrochory) can be an ecological important process (Woolfrey & Ladd, 2001). In such contexts, if relatively less water is absorbed by samaras and the rate of absorption is slow, this might facilitate samara dispersal by water. The rapid and greater absorption of water in *Allocasuarina* (Ladd, 1988) is consistent with the adaptation to seasonally arid environments where surface moisture needed for germination can be highly transient (Turnbull & Martensz, 1982). However, Ladd (1988) considered that this rapid water absorption might occur only once, and that this adaptation might not facilitate rewetting after drying.

The aim of this study was to quantify water absorption by samaras of accessions of a representative selection of *Casuarina* and *Allocasuarina* spp. from Australia in terms of absolute and relative amount, wetting rates and rewetting capacity, as well as to examine the effect of water absorption on duration of flotation.

MATERIALS AND METHODS

Plant materials

The samaras used in this study were obtained for four species each of *Casuarina* and *Allocasuarina* (Tab. 1) with two accessions each of *Casuarina glauca* and *Allocasuarina verticillata* to provide indicators of within species consistency. Two accessions were from a commercial native seed collector, and eight were collected by air drying (≥ 2 weeks) infructescences (cones) taken from the lower branches of single trees. The accessions were stored at room temperature under low ambient humidity in sealed containers.

Samara water absorption

Preliminary checks were made to determine how quickly water was absorbed by the samaras of the two genera and experiments designed accordingly. For *Casuarina* samaras, which absorb water slowly, water absorption

was assessed over 48 h on the assumption that, during that period, minimal water would be absorbed by the seed relative to the whole samara. The experimental units consisted of five intact samaras for *C. cristata* and ~15 mg of samaras for the other *Casuarina* accessions (i.e., about 20 samaras as these were too small to weigh individually with the available equipment; Tab. 2) with five replicates per accession. Samaras were placed on the surface of a water-saturated paper towel in a Petri dish (50 mm) and weighed as a group after 6, 24 and 48 h. For *Allocasuarina*, which absorb water quickly (< 2 min), water absorption was recorded over multiple wetting cycles; five cycles in an initial experiment and 10 cycles in the final experiment (data for the initial experiment is given only in the supplementary information, detailed below, because water absorption did not stabilise within five cycles). Experimental units consisted of five intact samaras with five replicates per accession. Samaras were placed on a water-saturated paper towel in a Petri dish, weighed after 2 min, then left to air dry for 24 h before repeating the process.

Samara floatation

Duration of floatation of samaras was tested by first placing samaras on the surface of 1.5 ml of tap water in 2 ml Eppendorf tubes and mixed by gentle inversion ($\times 3$, to minimise trapped air bubbles affecting wetting, water absorption or rate of sinking). After 12 h at room temperature, each samara was recorded as floating or sunken, and the contents mixed again by inversion. The process was repeated for up to eight observations over 96 h. For the *Casuarina*, each experimental unit consisted of three samaras for *C. cristata* and six samaras for each of the other accessions with three replicates per accession. For *Allocasuarina*, the assessment was initially made as for *C. cristata* but the samaras aggregated and sunk quickly, so the experiment was repeated with a single samara per tube and five replicates per accession.

Statistical analysis

Data were checked for aberrant values through exploratory statistics (Tukey, 1997) and four values were set to missing in the *Casuarina* water absorption dataset (no more than one value per assessment time within a repeated measures series) because outliers can compromise linear regression analysis. Otherwise, all data were included for analysis. Least squares linear regression was applied using R statistical software (R Core Team, 2020) with linear, quadratic, asymptotic and logistic models. A model using the square root of time was applied to the *Casuarina* water absorption data, linear and asymptotic models to the 5-cycle and 10-cycle *Allocasuarina* water absorption data (the former is presented as supplementary information only, as detailed below) and binomial logistic models to the floatation data. For square root of time, the model was $\text{SamaraWt} \sim \text{Time}^{0.5} + 0$ using the R function “lm”. For the nonlinear least squares asymptotic fit, the model was $\text{SamaraWt} \sim \text{SSasym}(\text{Cycle}, \text{Asym}, \text{R0}, \text{lrc})$, where SSasym function is a self-start model to evaluate

Tab. 1 - *Casuarina* and *Allocasuarina* accessions used in the determination of samara water absorption and floatation. / Campioni di *Casuarina* e *Allocasuarina* utilizzati nella determinazione dell'assorbimento di acqua e del galleggiamento.

^AArborPlan numbers are identification numbers of individual trees on the Waite Campus used for arboricultural management purposes. / I numeri ArborPlan sono usati a scopo di gestione arboricola per individuare i singoli alberi del Waite Campus.

Taxon	Accession details	Code
<i>Casuarina</i>		
<i>C. cristata</i> Miq.	9.VII.2019, Waite Campus, Urrbrae, South Australia, 34°58'5.2" S, 138°38'10.7" E, single tree (ArborPlan 00847), planted, garden (as Riley 2020).	Ccr
<i>C. cunninghamiana</i> Miq. subsp. <i>cunninghamiana</i>	9.VII.2019, Waite Campus, Urrbrae, South Australia, 34°58'5.5" S, 138°38'17.2" E, single tree (ArborPlan 00972), planted, windbreak (as Riley 2020).	Ccu
<i>C. glauca</i> Sieber	20.I.2019, Torrens Park, SA, Australia, 34° 58' 44.8" S, 138° 36' 42.0" E, planted, domestic garden.	Cgl1
	25.VI.2019, Waite Campus, Urrbrae, South Australia, 34°58'7.6" S, 138°38' 11.7" E, single tree (ArborPlan 00811), planted, garden (as Riley 2020).	Cgl2
<i>C. obesa</i> Miq.	4.VII.2019, Old Princess Highway, Monarto, South Australia, 35°07'7.3" S, 139°8'0.9" E, single tree, planted, experimental woodlot (as Riley 2020).	Cob
<i>Allocasuarina</i>		
<i>A. acutivalvis</i> (F.Muell.) L.A.S.Johnson	Western Australia, commercially supplied, Seed Shed (WA) Pty Ltd, Donnybrook, WA, Australia.	Aac
<i>A. huegeliana</i> (Miq.) L.A.S.Johnson	Western Australia, commercially supplied, Seed Shed (WA) Pty Ltd, Donnybrook, WA, Australia.	Ahu
<i>A. muelleriana</i> subsp. <i>notocolpica</i> L.A.S.Johnson	25.VII.2019, Playford Highway, Kangaroo Island, South Australia, 35°45'4.4" S, 137°24'11.7" E, several shrubs, natural stand (as Riley 2020).	Amu
<i>A. verticillata</i> (Lam.) L.A.S.Johnson	26.I.2019, Linden Park, SA, Australia, 334°56'22.8" S, 138°38'34.3" E, several trees, planted, garden.	Ave1
	10.VII.2019, Waite Campus, Urrbrae, South Australia, 34°58'16.5" S, 138°38'19.3" E, single tree (ArborPlan ^A 01642), planted, garden (as Riley 2020).	Ave2

the asymptotic regression and its gradient in the R package “lme4” (Bates *et al.*, 2015). In addition to the fitted regressions, water absorption data were also examined as quartile box plots to indicate consistency of variance within each dataset. For floatation data, the R function “glm” was used with a binomial fit and data plotted with a logistic function and with the sigmoid midpoint designated here as the floating half-life (Float₅₀).

Supplementary materials

Supplementary materials including additional figures (Figs. S1-S11, with Figs. S7-S9 for the initial experiment on *Allocasuarina* mentioned above), video and raw data are made available at doi:10.4081/nhs.2022.541.

RESULTS

As representative examples of both genera, Fig. 1 shows a dry samara of *C. cristata*, and a dry and wet samara of *A. verticillata* (wet and dry samaras of the other *Allocasuarina* accessions are shown in Fig. S1). On exposure to water, *Casuarina* samaras did not undergo any distinct visual change (consequently, only the photograph of the dry samara is included), whereas *Allocasuarina* became rapidly swollen and changed in appearance (Fig. 1, Fig. S1 and as detailed below).

Samara water absorption

Casuarina samaras absorbed water slowly up to the final assessment at 48 h (Fig. 2a, Fig. S2). During this time, the visual appearance of the *Casuarina* samaras remained largely unchanged. The water absorption regressions (Fig. 2a,c and Figs. S2 and S3) gave the best linear response to the square root of time compared to fits with untransformed time or the logarithm of time. However, with four time points it was not possible to determine an equilibrium absorption. Also, with the assessment made at room temperature, extended assessment would have included an increasing proportion of water absorbed by the germinating embryo, so might not represent the samara water absorption. *C. cristata* absorbed considerably more water than the other *Casuarina* spp. (Fig. 2a) because its samaras are over five times heavier (Tab. 2). However, the proportion of water relative to samara weight was consistent among the accessions (Fig. 2c), ranging from 85 to 111% with a median of 90% (Tab. 2), with *C. cristata* at the lower end of the range.

Water absorption by *Allocasuarina* samaras was distinctly different from *Casuarina* samaras in morphological response, rate and quantity. The shiny dark samara surface, from the commencement of water absorption, rapidly and progressively became swollen and undulating with the exocarp separating into strands of darker fibres interspersed across a grey, gel-like surface (as exemplified in *A. verticil-*

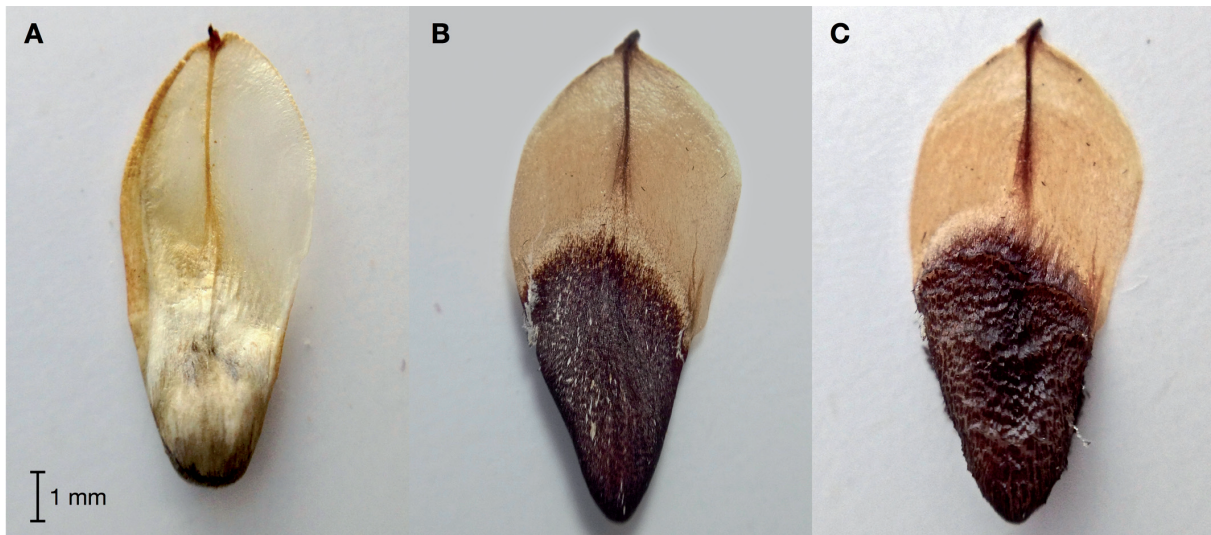


Fig. 1 - Dry samara of *Casuarina cristata* (A) and dry and wet samara of *Allocasuarina verticillata* (B, C). The wet samara (C) was photographed after being placed on a droplet of water ~2 min. A wet samara of *C. cristata* was not included as there was no distinct visual change associated with wetting. / Samara secca di *Casuarina cristata* (A) e samara secca e bagnata di *Allocasuarina verticillata* (B, C). La samara bagnata (C) è stata fotografata dopo essere stata posta su una goccia d'acqua per ~2 min. Una samara bagnata di *C. cristata* non è stata inclusa perché non mostrava un distinto cambiamento visivo associato all'idratazione.

lata in Fig. 1 and Supplementary Video 1, which shows full water absorption in less than a minute, contrasting with the response of *C. cristata* in Supplementary Video 2, which showed no visible water absorption over the same time period). This process took less than 2 min in all accessions when samaras were placed on a droplet of water. Therefore, the data collected for *Allocasuarina* were not just for a single water absorption cycle (as for *Casuarina*), but over 10 wetting cycles interspersed with air drying for 24 h.

For the 10 *Allocasuarina* wetting cycles, air drying for 24 h returned the samaras to the initial weight for all accessions with no significant increase or decrease in weight (Fig. S4), providing confidence that the water absorption in each cycle could be validly compared. The five accessions ranged in initial samara weight from 2.3 to 3.0 mg with a median of 2.5 mg (Tab. 2). Within accessions, *A. verticillata* samaras were more variable in weight than others, despite being selected to be largely uniform. In the initial cycle, with the exception of *Allocasuarina huegeliana*, the *Allocasuarina* accessions absorbed two to three times their weight in water (Tab. 2). *A. huegeliana* absorbed about 1.3 times but this was still greater than any of the *Casuarina* accessions. With repeated wetting cycles, the quantity of water increased approaching an equilibrium after three to five cycles (Fig. 2b and Fig. S5). This response was well defined by asymptotic regression for both absolute and proportional water absorption (Fig. 2b,d and Figs. S5 and S6), with the asymptote representing the predicted final water absorption (Tab. 2). All estimated asymptotes were highly significant ($p < 0.001$) indicating potential absorption of mostly around four to five times the initial samara weight, with *A. huegeliana* the lowest at about 2.7 times (Tab. 2). *A. huegeliana* had the smallest samaras of the *Allocasuarina* accessions and took up the least water both in absolute and proportional terms, which contrasted with *C. cristata*, which had the largest

samaras of the *Casuarina* accessions but the lowest water absorption. Consistent with the *A. huegeliana* response, the differences in *Allocasuarina* samara water absorption in absolute terms were also reflected in proportional terms (Fig. 2b,d and Figs. S5 and S6). A clear difference was for *A. verticillata* (accession Ave2), which despite having the heavier samaras took up proportional less water than accession Ave1. Notably, the variation in *A. verticillata* data was greatly reduced when expressed on a proportional basis showing that this variation was due to samara size rather than their water absorption properties.

Samara floatation

The floatation results for both genera are given in Fig. 3. Of the *Casuarina* accessions, *C. cristata* samaras were the first to sink with a Float_{50} of 17 h, half that of any other accession. Samaras of three of the other accessions sank at a similar rate ($\text{Float}_{50} \sim 36$ h), but samaras of *C. glauca* (accession Cgl2) remained floating for twice as long (Float_{50} 70 h). *C. cristata* samaras having a distinctly different response is not unexpected given they are many times larger than all other *Casuarina* samara, but the response of accession Cgl2 samaras was less expected, as these were the heaviest of the non-*C. cristata* accessions and took up the most water (Tab. 2). However, accession Cgl2 had the most variable estimate of Float_{50} , so it is not possible to make a firm conclusion about this difference other than that variation between accessions of the same species will occur due to various attributes of individual samples. For the *Allocasuarina* accessions, Float_{50} ranged from 29 to 57 h, so none sank as quickly as *C. cristata* or as slowly as *C. glauca* (accession Cgl2). *Allocasuarina acutivalvis* sank most rapidly and *Allocasuarina muelleriana* most slowly, with the two *A. verticillata* accessions differing. However, given the standard errors in the Float_{50} estimates, it is

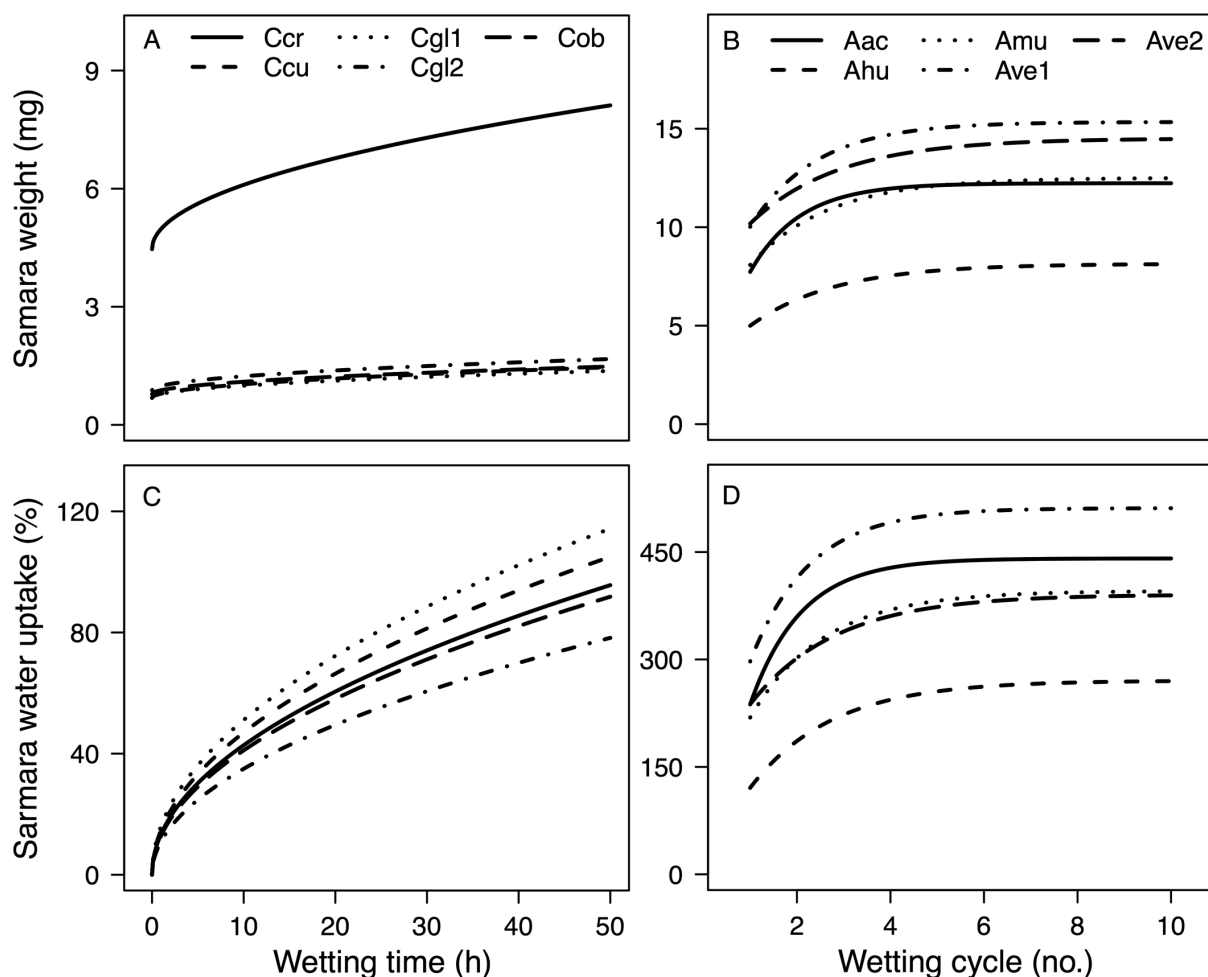


Fig. 2 - Absorption of water, increasing weight (A) and proportion of water (C), by samaras of five accessions of *Casuarina* over 48 h: *Casuarina cristata* (Ccr), *C. cunninghamiana* (Ccu), *C. glauca* (Cgl1 and Cgl2) and *C. obesa* (Cob). Regression lines are for a least squares fit of the model, $\text{SamaraWt} \sim \text{Time}^{0.5} + 1$, and in all cases were significant at $p < 0.001$. Final wet weight (B) and proportion of water (D) of samaras of five accessions of *Allocasuarina* after being permitted to absorb water for 2 min and then air dried for 24 h through 10 wetting cycles: *Allocasuarina acutivalvis* (Aac), *A. huegeliana* (Ahu), *A. muelleriana* (Amu), and *A. verticillata* (Ave1 and Ave2). Regression lines are for a nonlinear least squares fit of the model, $\text{SamaraWt} \sim \text{SSasymp}(\text{Cycle}, \text{Asym}, \text{R0}, \text{Irc})$, where *SSasymp* is a self-start model to evaluate the asymptotic regression and its gradient in the R package “lme4” (Bates *et al.*, 2015). In all cases, asymptotes (*Asym*) were significant at $p < 0.001$. / Assorbimento di acqua, aumento di peso (A) e proporzione di acqua (C) in samare di cinque campioni di *Casuarina* in 48 h: *Casuarina cristata* (Ccr), *C. cunninghamiana* (Ccu), *C. glauca* (Cgl1 e Cgl2) e *C. obesa* (Cob). Le linee di regressione sono per un'approssimazione ai minimi quadrati del modello non lineare, $\text{SamaraWt} \sim \text{Time}^{0.5} + 1$, e in tutti i casi erano significativi a $p < 0,001$. Peso umido finale (B) e proporzione di acqua (D) di samara di cinque campioni di *Allocasuarina* dopo avere assorbito acqua per 2 min e poi essere asciugati all'aria per 24 h attraverso 10 cicli di idratazione: *Allocasuarina acutivalvis* (Aac), *A. huegeliana* (Ahu), *A. muelleriana* (Amu) e *A. verticillata* (Ave1 e Ave2). Linee di regressione sono per un'approssimazione ai minimi quadrati del modello non lineare, $\text{SamaraWt} \sim \text{SSasymp}(\text{Cycle}, \text{Asym}, \text{R0}, \text{Irc})$, dove *SSasymp* è un modello di auto-avvio per valutare la regressione asintotica e il suo gradiente nel pacchetto R “lme4” (Bates *et al.*, 2015). In tutti i casi gli asintoti (*Asym*) erano significativi a $p < 0,001$.

not possible to conclude that individual accessions differ, nevertheless, there appears to be the potential for a real difference across the extent of the range flotation times.

DISCUSSION

This study has confirmed, and for the first time quantified, the striking difference in water absorption between *Casuarina* and *Allocasuarina* samaras, which had previously only been described in general or morphological terms (Ladd, 1988; Hwang & Conran, 1991). On initial wetting, the samaras of the *Casuarina* accessions studied will take up between 85 to 110% (median 90%) of their weight

in water in 48 h. In clear contrast, the samaras of the *Allocasuarina* accessions studied will take up between 125 and 300% (median 240%) of their weight in water within 2 mins. Also, with repeated wetting cycles, the water absorption of *Allocasuarina* samaras increased between 270 and 510% (median 400%). However, *C. cristata*, a species adapted to greater aridity than, say *C. cunninghamiana* or *C. glauca*, had samara water absorption (in relative terms) consistent with its genus, so not distinct for its ecological adaptation. In the case of *A. huegeliana*, with a distribution extending from the wetter areas of SW Australia into arid locations of the southern Eremaean botanical province, its samaras had the lowest water absorption both

Tab. 2 - Summary (mean \pm sd, $n = 5$ or as shown) of samara parameters, water absorption (per samara) and floatation for five accession each of *Casuarina* and *Allocasuarina*. In *Casuarina*, water absorption of samaras (uptake) was monitored over 48 h because water absorption was slow. In *Allocasuarina*, water absorption was monitored over 10 wetting cycles because water absorption is rapid (<2 min). Initial absorption is for the first cycle and predicted for a fitted asymptotic regression. Extra_{50} is the number of additional cycles required for 50% of the additional water to be absorbed. Float_{50} is the time for 50% of floating samaras to sink. / Riassunto (media \pm sd, $n = 5$ o come mostrato) dei parametri della samara, dell'assorbimento dell'acqua (per samara) e della flottazione per cinque campioni per genere di *Casuarina* e *Allocasuarina*. In *Casuarina*, l'assorbimento dell'acqua delle samare (uptake) è stato monitorato per 48 ore perché l'assorbimento dell'acqua era lento. In *Allocasuarina*, l'assorbimento dell'acqua è stato monitorato su 10 cicli di idratazione perché l'assorbimento dell'acqua è rapido (<2 min). L'assorbimento iniziale è indicato per il primo ciclo ed è previsto per una regressione asintotica adattata. Extra_{50} è il numero di cicli aggiuntivi richiesti per assorbire il 50% dell'acqua supplementare. Float_{50} è il tempo necessario affinché il 50% delle samare galleggianti affondi.

Accession	Samara length (mm) $n = 10$	Samara/replicate (median)	Samara weight (mg)	Initial water uptake (mg)	Initial water uptake (%)	Predicted water uptake (mg)	Predicted water uptake (%)	Extra water uptake (%)	Extra_{50} (cycles)	Float_{50} (h)
<i>Casuarina</i>										
<i>C. cristata</i>	8.8 \pm 0.5	5	4.20 \pm 0.45	3.56 \pm 0.20	85	-	-	-	-	17
<i>C. cunninghamiana</i>	4.5 \pm 0.5	22	0.71 \pm 0.06	0.78 \pm 0.13	111	-	-	-	-	38
<i>C. glauca</i> (1)	4.3 \pm 0.4	23	0.65 \pm 0.03	0.67 \pm 0.11	104	-	-	-	-	34
<i>C. glauca</i> (2)	4.8 \pm 0.4	16	0.92 \pm 0.04	0.83 \pm 0.11	90	-	-	-	-	70
<i>C. obesa</i>	4.4 \pm 0.5	20	0.78 \pm 0.05	0.67 \pm 0.13	86	-	-	-	-	36
Median	4.5	-	0.78	0.78	90	-	-	-	-	36
<i>Allocasuarina</i>										
<i>A. acutivalvis</i>	10.1 \pm 1.2	5	2.30 \pm 0.17	5.50 \pm 1.22	241	9.93 \pm 0.62	441 \pm 29	200	0.8	29
<i>A. huegeliana</i>	6.3 \pm 0.4	5	2.25 \pm 0.27	2.87 \pm 0.63	126	5.89 \pm 0.80	271 \pm 21	145	1.2	44
<i>A. muelleriana</i>	9.3 \pm 0.7	5	2.53 \pm 0.16	5.83 \pm 0.35	231	9.98 \pm 0.56	396 \pm 25	165	1.1	57
<i>A. verticillata</i> (1)	8.6 \pm 0.7	5	2.58 \pm 0.69	7.52 \pm 1.10	301	12.8 \pm 1.37	511 \pm 37	210	0.9	31
<i>A. verticillata</i> (2)	9.5 \pm 0.6	5	2.96 \pm 0.58	7.24 \pm 2.18	240	11.6 \pm 1.67	391 \pm 22	151	1.3	49
Median	9.3	-	2.53	5.83	240	9.98	396	165	1.1	44

in absolute and relatively terms of the five *Allocasuarina* accessions studied. Therefore, it was not possible to conclude from data collected that greater and more rapid samara water absorption is clearly or consistently associated with adaptation to water-limited environments, as has been found in other taxa such as *Plantago* (Teixeira *et al.*, 2020).

Likewise, a proposition that less and slower samara water absorption is associated with species found near water bodies with the greater likelihood of water-dispersed samaras was not supported by the floatation data. *C. cristata* samaras sank rapidly, which would be consistent with this proposition, but there was no evidence that the samaras of the other *Casuarina* spp. floated any longer than *Allocasuarina* samaras. Indeed, *A. muelleriana* floated for a longer period than most of the *Casuarina* accessions, and this *A. muelleriana* subspecies occurs in shrublands of Kangaroo Island, where water dispersal of samaras is unlikely to be significant, other than in heavy rainfall events mostly over short distances. Sun *et al.* (2012) found that myxodiasporic seeds of annual brassica, *Alyssum minus*, sank quickly compared to seed from which the mucilage had been removed. This relatively recent finding was consistent with observations made a century ago by Murbeck (1919; cited by Ryding, 2001) in his work on desert plants,

but this phenomenon might not occur in the sheoaks. It is possible that the samara wing influences floatation, so assessing *Casuarina* versus *Allocasuarina* samara floatation with and without wing removal would have merit.

Water absorption by samaras of sheoak is mechanistically different from most water-capturing seeds (mucilaginous seeds), which are covered in detail in the reviews of Western (2011) and Yang *et al.* (2012). In sheoaks, the samara mesocarp consists of spiral fibres that were described and illustrated by early botanists (Lindley, 1848; Hooker, 1860; Fig. S10) but have attracted no experimental investigation. Lindley (1848), when discussing seed testa integuments, describes these as “a layer of spiral vessels below the epidermis, very thin and delicate, and extremely minute”. Ladd (1988) and Hwang & Conran (1991) published micrographs of these fibres (Fig. S11), the latter showing the intact, individual spiral (coiled) form. In *Allocasuarina*, these fibres expand on wetting (Fig. 1) presumably taking up water by capillary action (surface tension) rather than through forming a colloidal suspension (Ladd, 1988) as is the case in many mucilaginous seeds, so this raises the question how the water-capturing in sheoak samaras compares quantitatively with mucilaginous seeds. Ferreira *et al.* (2020) report weight gains from 400% to over 8,000%

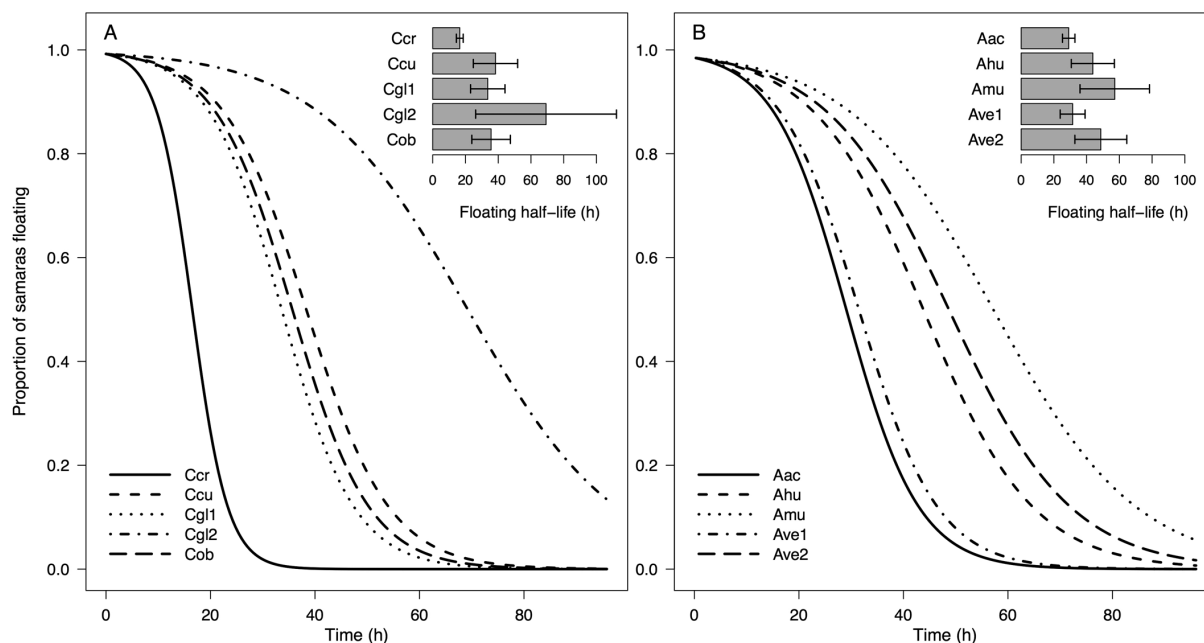


Fig. 3 - Proportion of samaras of five accessions each of *Casuarina* (A) and *Allocasuarina* (B) as a function of time (general linear model binomial fit, plotted with a logistic function) with floating half-life (sigmoid midpoint). Accessions: A, *Casuarina cristata* (Ccr), *C. cunninghamiana* (Ccu), *C. glauca* (Cgl1 and Cgl2, accessions 1 and 2) and *C. obesa* (Cob), and B, *Allocasuarina acutivalvis* (Aac), *A. huegeliana* (Ahu), *A. muelleriana* (Amu) and *A. verticillata* (Ave1 and Ave2, accessions 1 and 2). *Casuarina* samaras were assessed in five replicate groups of five, whereas *Allocasuarina* samaras were assessed individually in five replicates because initial tests indicated wet samaras aggregated and sunk together. Error bars are standard errors of the estimates of the sigmoid midpoints ($n = 5$). / Proporzione di samare di cinque campioni ciascuna di *Casuarina* (A) e *Allocasuarina* (B) in funzione del tempo (adattamento binomiale del modello lineare generalizzato, rappresentato con una funzione logistica) con emivita fluttuante (punto medio della sigmoide). Campioni: A, *Casuarina cristata* (Ccr), *C. cunninghamiana* (Ccu), *C. glauca* (Cgl1 e Cgl2, campioni 1 e 2) e *C. obesa* (Cob), e B, *Allocasuarina acutivalvis* (Aac), *A. huegeliana* (Ahu), *A. muelleriana* (Amu) e *A. verticillata* (Ave1 e Ave2, accessioni 1 e 2). Le samare di *Casuarina* sono state valutate in cinque gruppi di cinque repliche, mentre le samare di *Allocasuarina* sono state valutate individualmente in cinque repliche perché i test iniziali indicavano che le samare bagnate si aggregavano e affondavano insieme. Le barre di errore rappresentano l'errore standard delle stime dei punti medi della sigmoide ($n = 5$).

in mucilaginous seeds of species subfamily Nepetoideae (Lamiaceae) endemic to Portugal and provided evidence of a relationship between habitats and mucilage production. Although, this is only one example, water absorption by sheoak samaras (from less than 100% to around 500%) is not exceptional. However, if samara water absorption were to be calculated on the weight of the true seed, not the whole samara, the proportional water uptake would be considerably higher. The literature appears silent on the weight the true seed of sheoaks. Shen *et al.* (2009) found samaras with filled seed sank quickly in an organic solvent, presumably because true seed density is higher than that of the whole samara. Although these authors removed true seed from samaras by dissection under a microscope, the resultant weight was not reported. Detailed botanical illustrations, such as in Hooker (1860; Fig. S10), show the true seed occupying over two-thirds of the volume of the samara nutlet, so true seed could represent around half the samara weight so water absorption based on true seed weight could be twice the amount given above.

As indicated in the Introduction, there have been a range of ecological functions suggested for the water-capturing properties of *Allocasuarina* samaras. The data presented here cannot be used to support or refute most of these, but for a few these the data have provided some clarity. Ladd's (1988) suggestion that the process is irrever-

sible and non-repeatable was not supported, on the contrary, water-capturing was found to be repeatable with the amount water absorbed increasing over several wetting cycles. Therefore, it is possible that the first wetting cycle could provide adhesion of samaras to the substrate (Mott & Groves, 1981) and subsequent cycles water-capturing that promotes germination. This ability to rewet and absorb more water in *Allocasuarina* samaras is consistent with Ladd's (1988) suggestion that this serves to provide a moister environment of seed germination. However, the suggestions that wing detachment or water dispersal (Hwang & Conran, 1991) might be involved both seem unlikely. Wet *Allocasuarina* samaras were manipulated multiple times in wetting cycle experiment and no wings became detached, and their air-dry weight in each cycle remained stable. Also, there was no clear evidence that the samaras of *Casuarina* (with slower water-capturing) would float (thereby aiding water dispersal) longer than the samaras of *Allocasuarina*. It is more likely that the response of *Allocasuarina* samaras to wetting contributes to adhesion to the substrate to minimise secondary dispersal (atelechory; Ryding, 2001). This is considered an adaptive feature for plant species in arid areas where favourable microsites are more likely to occur in close proximity to the parent plant so secondary dispersal over great distance might not be advantageous (Yang *et al.*, 2012).

The data presented here provide new quantitative information and perspectives on earlier suggestions regarding the ecological function of water-capturing in *Allocasuarina*. Nevertheless, there would be merit in the assessment of a wider range of material. This could include more species, or more accessions within species, for example, material collected across rainfall gradients that extend from low to high rainfall environments. *A muelleriana* in South Australia would provide such an opportunity as it occurs from Mawson Plateau in the north to Pelican Point in the south, from around 250 mm to over 700 mm annual rainfall (Mawson Plateau to Pelican Point; data from Naturemaps, Department for Environment and Water, Government of South Australia and Bureau of Meteorology, Australian Government). Equally, there would be value in assessing variation with members of a localised population. Likewise, experimental examination of the contribution of exocarp fragmentation to the rate of water absorption (Ladd, 1988), and wetting on samara immobilisation and anchorage as mechanism to improve seedling establishment (Mott & Groves, 1981) could be productive.

In summary, this work has provided new data on the comparative rates and amounts of water absorption by *Casuarina* and *Allocasuarina* samaras, with water absorption being substantial and reversible in *Allocasuarina*. However, perhaps in part due to the scale of the study, there was no clear evidence that differences in water absorption between species/genera were related to the aridity of habitat or a factor in diaspore dispersal in/by water. It is recommended that this feature of sheoak samaras receive further experimental attention because its role in the ecology of this unique and largely Australasian plant family remains conjectural.

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REFERENCES

- Bates D., Mächler M., Bolker B. & Walker, S., 2015 – Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67 (1): 1-48. <<https://doi.org/10.18637/jss.v067.i01>>
- Ferreira B., Montesinos D. & Sales F., 2020 – Mucilage in Portuguese Lamiaceae. *Botany Letters*, 167 (4): 430-438. <<https://doi.org/10.1080/23818107.2020.1790035>>
- Hooker J. D., 1860 – Flora Tasmaniae. *Lovell Reeve*, London, UK.
- Hwang Y. & Conran J. G., 1991 – Seedlings of Australian casuarinas. I: germination. *Western Australian Naturalist*, 18 (7): 88-197.
- Ladd P. G., 1988 – The status of Casuarinaceae in Australian forests. In: Australia's Ever Changing Forests. Frawley K. J. & Semple N. M. (Eds.). Proceedings of the First National Conference on Australian Forest History. Canberra, 9-11 May, 1988. *Campbell, ACT, Australia, Department of Geography and Oceanography, Australian Defence Force Academy*: 63-85.
- Lindley J., 1848 – An Introduction to Botany. 4th ed., with corrections and numerous additions. *Longman, Brown, Green & Longmans*, London, UK. <<https://doi.org/10.5962/bhl.title.21458>>
- Mott J. J. & Groves R. H., 1981 – Germination strategies. In: The Biology of Australian Plants. Pate J. S. & McComb A. J. (Eds.). *University of Western Australia Press*, Nedlands, Western Australia: 307-341.
- R Core Team, 2020 – R: a language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. <<https://www.R-project.org/>>

- Riley I. T., 2020 – Infructescence and samara morphometrics and potential mechanism of samara release in *Allocasuarina* and *Casuarina* (Casuarinaceae). *Australian Journal of Botany*, 68 (2): 108-118. <<https://doi.org/10.1071/BT19153>>
- Riley I. T., 2021 – A case for assessing *Allocasuarina* and *Casuarina* spp. for use in agroecosystem improvement in semi-arid areas with a focus on Central Anatolia, Turkey. *Frontiers of Agricultural Science and Engineering*, 8 (4): 568-582. <<https://doi.org/10.15302/J-FASE-2019270>>
- Ryding O., 2001 – Myxocarpy in the *Nepetoideae* (Lamiaceae) with notes on myxodiaspory in general. *Systematics and Geography of Plants*, 71 (2): 503-514. <<https://doi.org/10.2307/3668696>>
- Shen X., Castle W. S. & Gmitter F. G., 2009 – Petroleum ether separation and seedcoat removal enhance seed germination of a *Casuarina equisetifolia* L. x *C. glauca* Sieb. ex Spreng hybrid. *HortScience*, 44 (3): 770-773. <<https://doi.org/10.21273/HORTSCI.44.3.770>>
- Sun Y., Tan D. Y., Baskin C. C. & Baskin J. M., 2012 – Role of mucilage in seed dispersal and germination of the annual ephemeral *Alyssum minus* (Brassicaceae). *Australian Journal of Botany*, 60 (5): 439-449. <<https://doi.org/10.1071/BT11314>>
- Teixeira A., Iannetta P., Binnie K., Valentine T. A. & Toorop P., 2020 – Myxospermous seed-mucilage quantity correlates with environmental gradients indicative of water-deficit stress: *Plantago* species as a model. *Plant and Soil*, 446 (1): 343-356. <<https://doi.org/10.1007/s11104-019-04335-z>>
- Tukey J. W., 1997 – Exploratory Data Analysis. *Addison-Wesley*, Reading, MA, USA.
- Turnbull J. W. & Martensz P. N., 1982 – Aspects of seed collection, storage and germination in Casuarinaceae. *Australian Forest Research*, 12 (4): 281-294.
- Western T. L., 2011 – The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. *Seed Science Research*, 22 (1): 1-25. <<https://doi.org/10.1017/S0960258511000249>>
- Wilson K. L. & Johnson L. A. S., 1989 – Casuarinaceae. In: Flora of Australia. Vol. 3: Hamamelidales to Casuarinales. George A. S. (ed.). *Australian Government Publishing Service*, Canberra, Australia: 100-174.
- Woolfrey A. R. & Ladd P. G., 2001 – Habitat preference and reproductive traits of a major Australian riparian tree species (*Casuarina cunninghamiana*). *Australian Journal of Botany*, 49 (6): 705-715. <<https://doi.org/10.1071/BT01009>>
- Yang X., Baskin J. M., Baskin C. C. & Huang Z., 2012 – More than just a coating: Ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. *Perspectives in Plant Ecology, Evolution and Systematics*, 14 (6): 434-442. <[doi:10.1016/j.ppees.2012.09.002](https://doi.org/10.1016/j.ppees.2012.09.002)>

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Figs. S1-S11. See captions in the PDF file. / Didascalie nel file PDF.

Video 1. Water absorption by a samara of *Allocasuarina verticillata* over 1 min. The shiny dark samara surface, from the point of water absorption, rapidly and progressively becomes swollen and undulating with the exocarp separating into strands of darker fibres interspersed across a grey, gel-like surface. / Assorbimento dell'acqua da parte di una samara di *Allocasuarina verticillata* nel corso di 1 minuto. La superficie lucida e scura della samara, con l'assorbimento d'acqua, diventa rapidamente e progressivamente gonfia e ondulata, con l'esocarpio che si separa in filamenti di fibre più scure sparse su una superficie grigia, simile a gel.

Video 2. Water absorption by a samara of *Casuarina cristata* over 1 min. There is no visible water absorption or change to the samara surface, as distinct contrast to water absorption in *Allocasuarina verticillata* shown in Video 1. / Assorbimento dell'acqua da parte di una samara di *Casuarina cristata* nel corso di 1 minuto. Non c'è nessun assorbimento d'acqua visibile o cambiamento sulla superficie della samara, in netto contrasto con l'assorbimento d'acqua in *Allocasuarina verticillata* mostrato nel Video 1.

Samara water absorption and floatation. Raw data in PDF format. / Dati grezzi in formato PDF.