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Cold Sensitivity of Field-Grown *Allocasuarina* and *Casuarina* Tree Species

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Abstract: The sheoak genera, *Allocasuarina and Casuarina*, contain a diverse range of Australian trees and shrubs of both ecological and economic importance. Although they are well known for tolerance to stressors such as drought, salinity and high temperatures, their tolerance to freezing has not been adequately examined. Some data has been published on the cold sensitivity of *Casuarina cunninghamiana* but other *Casuarina* spp. and no *Allocasuarina* sp. have been assessed. This study examined growth and survival of field-established *Allocasuarina* (2 species) and *Casuarina* (4 species) in Central Anatolia, Turkey with winter temperatures dropping to −15°C. Following establishment in May 2020, the accessions tested (100 plants in total) mostly grew well during summer and autumn in this semi-arid climate with mildly alkaline soil, but the shoots of all plants were killed by the extreme cold of winter, with only seven plants producing weak root sucking in the summer of 2021, which all subsequently died. Although both genera appear to be cold sensitive, the results indicate the merit for wider evaluation of sheoaks in other contexts with harsh environments but not in regions exposed to periods (even if relatively short) of subzero temperatures.

Keywords: Abiotic stress; Casuarinaceae; Cold tolerance; Frost damage; Sheoaks.

INTRODUCTION

Sheoaks comprise a diverse group of shrubs and trees in the Casuarinaceae, mostly in the genera *Allocasuarina* and *Casuarina*. Members of these genera are known for hardiness across a wide range of environmental stressors including aridity, infertility, fire, salinity, high temperatures and strong winds (Turnbull 1997; Riley 2021). Their ecological importance is clear with some 66 species spread across the majority of the Australian continent, and other species across the archipelagos to the north and one species with a natural range extending through to the Indian subcontinent (Wilson and Johnson 1989). The tree species, *Casuarina cunninghamiana*, *C. equisetifolia* and *C. glauca*, have been introduced to many countries across the globe for forestry, agroecosystem and amenity purposes (Potgieter et al. 2014). Mostly this has been to humid and subhumid tropical, subtropical and Mediterranean climate zones, with these species increasingly considered invasive in the warmer, more humid contexts such as Florida, USA (Wheeler et al. 2010).

Much of the Australian flora is endemic and the continent, although having some areas with annual snowfall, temperatures do not normally fall below about −8°C (Riley and Saygı 2021a). Therefore, although the tolerance of Australian flora to many environmental stressors is well known, there has been limited assessment of tolerance to extreme cold. Even within the thermal range experienced within Australia, Bush et al. (2018) concluded that many species have potential thermal limits beyond that evidenced by their native range. This analysis only included records (native and cultivated) from within Australia, so cannot be used to predict tolerance to extreme cold but does support the notion that this is possible. Over half of the species analysed were found to tolerate colder temperatures than predicted by their native ranges.

In the case of the Australian sheoaks, their hardiness is well known, but their tolerance to subzero temperatures and potential for use in regions that consistently experience such temperatures has not been extensively studied. In the field, there is a single report of frost damage to *C. cunninghamiana* in plantations in California, USA (Merwin et al. 1995). There have also been studies on the effect of freezing on seed release in a range of species (Riley and Saygı 2021a) and containerised sapling survival with *C. cunninghamiana* (Riley and Saygı 2021b). Therefore, for this study, plants of 10 accessions including six species of *Allocasuarina* (2 species) and *Casuarina* (4 species) were established in the field in Niğde in Central Anatolia, Turkey and their growth and survival monitored over 1.5 years, using the local species, *Elaeagnus angustifolia* (F. Elaeagnaceae), as a comparator.)

MATERIALS AND METHODS

Plant sources and establishment

Accessions of *Allocasuarina* and *Casuarina* (Table 1) trees were mostly obtained from Australia through collecting mature infructescences from natural or planted specimens and collecting samara after natural release following air drying of these infructescences for 1 to 2 weeks. One accession of *C. cunninghamiana* was obtained from Antalya, Turkey and *E. angustifolia,* as a local comparator, from Niğde, Turkey (Table 1). Seedlings were either established directly in the greenhouse or by germination in a growth room with subsequent transfer to the greenhouse. The seedlings were grown in square plastic pots (115 × 183 mm) in a mixture of local soil and peat for at least 1 year (as described by Riley and Saygı 2021b) before transplanting to the field in mid-May 2020.

Table 1. Details for *Allocasuarina* and *Casuarina* accessions assessed and the local comparator, *Elaeagnus angustifolia*

Species	Collection details	Clusters (plants)	Code	Accession
Allocasuarina littoralis	3.II.2019, Canberra, ACT, Australia, 35°16' 31.8” S,149°6'23.90” E, 636 masl, single tree, planted, garden, collected by IT Riley	1(5)	Ali	AP29
A. verticillata	26.I.2019, Linden Park, SA, Australia, 34°56’22.8” S, 138°38’34.3” E, 91 masl, planted, garden, collected by IT Riley	3(18)	Ave1-3	AP22
	3.II.2019, Bald Hill, Stanwell Park, NSW, Australia, 34° 13’ 27.9" S, 150° 59’ 43.4” E, 135 masl, natural stand, collected by IT Riley	1(4)	Ave4	AP32
	X.2018, American River, Kangaroo Island, SA, Australia, 35°46’42” S, 137°46’22” E, 45 masl, plantation, collected by KA Davies	1(4)	Ave5	AP19
Casuarina cristata	IX.2018, Gunnedah, NSW, Australia, 30°59’53.5” S, 150°15’29.9” E, 366 masl, natural stand, collected by KA Davies	1(5)	Ccr1	AP18
	27.I.2019, Urrbrae, SA, Australia, 34°58’03.7” S, 138°37’55.0” E, 108 masl, single tree, planted garden, collected by IT Riley	1(4)	Ccr2	AP23
C. cunninghamiana subsp. *cunninghamiana*	27.X.2018, Antalya, Turkey, 36°53’05.6” N, 30°40’54.1” E, 42 masl, single tree, planted, garden, collected by IT Riley	5(30)	Ccu1-5	AP05
	VI.2018, Kogarah Bay, NSW, Australia 33°58’58” S, 151°07’14” E, 5 masl, single tree, planted, collected by MT Riley	1(6)	Ccu6	AP04
C. glauca	20.I.2019, Torrens Park, SA, Australia, 34°58’44.8” S, 138°36’42.0” E, 84 masl, single tree, planted, garden, collected by IT Riley	1(4)	Cgl	AP21
C. obesa	27.I.2019, Urrbrae, SA, Australia, 34°58’03.7” S, 138° 37’ 55.0” E, 108 masl, single tree, planted, garden, collected by IT Riley	1(3)	Cob	AP24
Elaeagnus angustifolia	29.XI. 2019, Niğde, Turkey, 37°54’54.0" N, 34°36’11.4" E, 1177 masl, single tree, planted, roadside, collected by IT Riley, NF Tutar, K Jawneh, EA Olowu	3(18)	Ean	Local

The plants were established on the campus of Niğde Omer Halisdemir University in randomly arranged clusters (at least 3 m apart) across a small (25 × 30 m), freshly-cultivated field (37°56’38" N, 34°37’48" E) that had been an unfertilised, weedy fallow for more than 10 years. Each cluster was established in circular furrows (1.2 m diameter by about 200 mm deep) to facilitate watering during the establishment phase and capture of natural perception after establishment, with the outer furrow bank higher than the natural ground level and the inner bank at ground level (Figure 1). Up to six plants (minimum 3; Table 1) were established in each cluster depending on the available planting stock (1-3 replicate clusters per accession) to give 19 clusters and 100 plants in total. Analysis of soil from this site was reported by Riley et al. (2021).

Immediately after transplanting each cluster was irrigated with 12 L of water followed by three bi-weekly irrigations at the same rate, and then bi-weekly irrigations of 28 L, with the final irrigation on 8 June. In addition, 62 and 28 mm of rain fell in May and June during the establishment period. No further irrigation was applied and rainfall for the remainder of the growing season was negligible (1-1.2 mm/month from July to November). No fertiliser was applied to the clusters at any stage, except for one application of Fe-EDTA (at the recommended dilution) to particularly chlorotic *Allocasuarina littoralis* effecting some moderate, but not complete, remediation. Weeds within the clusters were removed by hand in early June, and twice in July. Regrowth of annual weeds and other wild species were not controlled elsewhere in the field.

Figure 1. Planted cluster of *Casuarina cunninghamiana*. Up to six trees were transplanted in a 1.2-m circular furrow in a freshly-cultivated field in Niğde, Central Anatolia, Turkey.

Assessment of plant growth and survival

Assessment of the number and height of surviving plants were made monthly from the first week of June through to November 2020. From December the temperatures were too low for any further growth and frost damage had become evident (minimum daily temperature for December to May, months with subzero temperatures are shown in Figure 2) Plants that survived the winter to regrow the next year were assessed in October and November 2021.

RESULTS

Growth

Nearly all plants survived transfer to field and shoots of most accessions grew after establishment (Figure 3). Of the sheoak species, most growth was seen in *Casuarina obesa*, followed by *C. glauca*, *C. cunninghamiana* and *A. verticillata* in that order. The former two, doubling in height over the season with growth rates of 2.3 and 1.6 mm/day, respectively. In contrast, with *Casuarina cristata*, growth was only seen for a couple of plants in one cluster (one growing to 180% of its original height), therefore mean growth was not significant. *Allocasuarina littoralis* did not grow and was chlorotic. This chlorosis was mostly ameliorated by the application chelated iron but this did not result in growth (only one cluster of this accession was planted because during propagation most seedlings suffered severe iron deficiency and died). *Elaeagnus angustifolia* produced considerable new foliage but shoots did not increase in height after transplanting to the field.

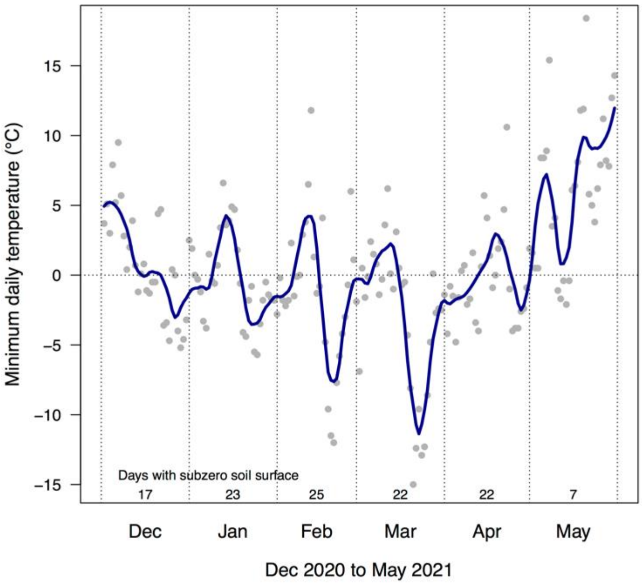

Figure 2. Minimum daily temperatures in at the closest meteorological station (Niğde Meteoroloji Ölçü Sahası, 37°57'30" N 34°40'47" E) to the experimental site. Date covers the months during the course of the experiment that had subzero temperatures (as indicated).

Survival

From mid-November 2020, the sheoaks suffered considerable frost damage and by mid-winter, most foliage was dead and many plants were bent over with weakened stems, particularly after snowfall. No regrowth was expected in 2021, however, during summer a proportion of the sheoaks produced suckers from their root systems. The tallest suckers were seen with *C. cunninghamiana*, although spindly, the mean height reached 425 mm (range 290 to 675 mm) by early November 2021with 5 of the original 34 plants (15%) producing suckers. One plant each of *A. littoralis* (20%) and *C. glauca* (25%) also produced suckers, 195 and 335 mm in height, respectively. By late-November 2021, with the onset of frosts, the shoots of all suckering plants were apparently dead. As expected, *E. angustifolia* recovered (17 of 18 plants) from its winter dormancy. However, no new shoot elongation was recorded under the effects of no supplementary irrigation, no fertilisation and weed competition; the recorded mean height in November 2021 was 595 mm, less than the final mean height of 640 mm in 2020.

DISCUSSION

Following establishment, the accessions of *Casuarina obesa*, *C. glauca*, *C. cunninghamiana* and *A. verticillata* all grew well under local conditions with infertile, mildly alkaline soils, and low humidity and growing-season rainfall. However, with the extreme cold of the winter and early winter (Figure 2), most of these and the other sheoak species in both genera (*Allocasuarina* and *Casuarina*) failed to survive. The few plants that did recover to some degree in the second summer, regrew as suckers from belowground, so all the exposed shoots of all plants had

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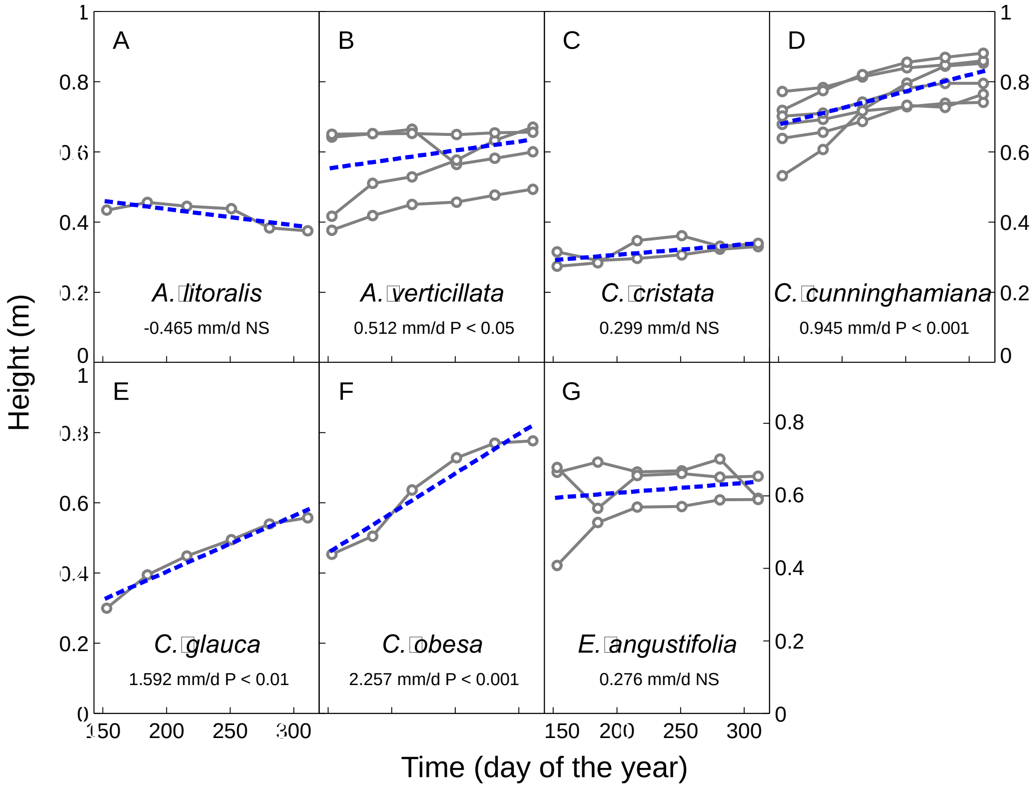

Figure 3. Growth of two *Allocasuarina* (A-B) and four *Casuarina* (C-F) species and the local comparator species, *Elaeagnus angustifolia* (G). The trees were grown in the field in Niğde, Central Anatolia, Turkey. The first height assessments were made 1 June (day 153, within 2 weeks of transplanting the nursery stock to the field) and the last on 6 November (day 111) in 2021. The initial heights vary due to different growth rates and periods during preparation of the nursery stock. Lines with circles are means of planting clusters of 3 to 6 plants each and the dotted line is the least squares regression across all clusters within species.

been killed. Therefore, the key finding of this study was that some species of sheoak have potential for high growth rates under harsh conditions as in Turkey but are not tolerant of the extreme cold of Central Anatolia.

At the study location in Niğde, the soil does not normally freeze in winter, so the death of the shoots of the sheoaks was consistent with the results of Riley and Saygı (2021b) but the recovery of some plants from belowground indicates partial root survival. However, this recovery was weak and unlikely to result in the persistence of these species. Freezing damage to the stems and foliage of the sheoaks was severe with the loss of structural integrity, evidence by stem bending, which was also seen in the earlier work. Of the species tested, *Allocasuarina verticillata* have the most robust foliage (0.7-1.5 mm diameter cf. 0.4-0.7 mm in C. *cunninghamiana;* Wilson and Johnson 1989) but this provided no protection from freezing damage. The Casuarinaceae evolved before the breaking up of the supercontinent Gondwana but although being widespread they failed to survive the subsequent periods of glaciation in the Northern Hemisphere, and thus becoming restricted to the current distribution largely in the milder Oceania (Johnson and Wilson 1989; Simonet et al. 1999). The biographical history of the family could potentially have been driven to a substantial degree by their lack of freezing tolerance. Also, growing of sheoaks in contexts where unanticipated extreme cold can occur even under a global warming trend (Gu et al. 2008; Schattenberg 2021) comes with significant risks that should not be ignored.

CONCLUSION

This study has confirmed the early work with container-grown *C. cunninghamiana* that demonstrated its cold sensitive (Riley and Saygı 2021b) and extended this to other *Allocasuarina* and *Casuarina* accessions in a field context. Notwithstanding this, the growth performance of the accessions tested in this work show that there is clear merit in accessing sheoaks more widely in Turkey in climate zones, e.g., along the Mediterranean and Aegean coasts, where extreme and/or extend subzero temperatures are unlikely to occur. In addition, this work highlights that a wider range of Australia flora should be evaluated in different climatic zones and, in particular, to document cold sensitivity which is largely unknown from the native ranges of this flora.

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Conflict of Interest

The authors declare no conflicts of interest.

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