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Casuarina species in Algeria: Reviewing identity, distribution and symbiotic status

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Photos 1.

Utilization of *Casuarina* trees in Algeria, *C. cunninghamiana* tree at the North region of Algeria (campus of Houari Boumedienne university, USTHB, district of Algiers). Photo K. Belaid.

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RÉSUMÉ

Espèces de *Casuarina* en Algérie : revisiter l'identité, la distribution et le statut symbiotique

Malgré l'importance prise par les *Casuarina* en Algérie depuis leur introduction au début du 19^e siècle, le nombre d'espèces aujourd'hui présentes dans le pays, leur identité, leur distribution ainsi que leur statut symbiotique sont encore largement méconnus. Une étude menée sur 2 304 arbres dans 96 peuplements répartis dans toute l'Algérie indique qu'ils sont représentés exclusivement par cinq espèces du genre *Casuarina* : *Casuarina cristata* Miq., *C. cunninghamiana* Miq., *C. cunninghamiana* Miq., *C. equisetifolia* L., *C. glauca* Sieber ex Spreng. et *C. junghuhniana* Miq. Deux espèces, *C. cunninghamiana* et *C. glauca*, sont largement prédominantes et différemment réparties, la première étant plus fréquente dans les zones relativement humides et tempérées du pays tandis que la seconde est présente dans les régions les plus arides et les plus chaudes. La production de plants de *C. cunninghamiana* et *C. glauca* dans 12 pépinières situées dans le nord et le sud du pays indique qu'ils présentent de faibles taux de mycorrhization et de nodulation spontanée. Cela suggère qu'il est nécessaire d'inoculer les jeunes plants avec des souches sélectionnées de champignons mycorrhiziens et/ou de *Frankia* au sein des pépinières afin d'améliorer leur installation durable sur le terrain. Cette étude constitue le premier inventaire de la répartition géographique des *Casuarina* en Algérie et peut conduire à une meilleure exploitation des arbres de ce genre largement utilisé en Algérie.

Mots-clés : *Casuarina*, diversité, distribution, mycorrhizes, *Frankia*, nodulation, Algérie.

ABSTRACT

Casuarina species in Algeria: Reviewing their identity, distribution and symbiotic status

Since their introduction at the beginning of the 19th century, *Casuarina* species have become important components of the Algerian landscape, yet the numbers of species present in Algeria and their identity, distribution, and symbiotic status are still not well understood. A survey conducted on 2,304 trees in 96 *Casuarina* stands across Algeria indicates that they are represented exclusively by five species: *Casuarina cristata* Miq., *C. cunninghamiana* Miq., *C. cunninghamiana* Miq., *C. equisetifolia* L., *C. glauca* Sieber ex Spreng. and *C. junghuhniana* Miq. Two species, *C. cunninghamiana* and *C. glauca*, were the most common – the former occurring more often in the relatively humid and temperate areas of the country while the latter is most frequent in the drier and warmer areas. Production of seedlings of *C. cunninghamiana* and *C. glauca* in 12 nurseries resulted in low rates of spontaneous mycorrhizal infection frequency and nodulation, suggesting that inoculation of seedlings with selected mycorrhizal fungi and/or *Frankia* strains in the nursery would be necessary to improve their establishment in the field. This study is the first survey of the geographical distribution of *Casuarina* species in Algeria and should lead to more efficient utilization of this widely used tree genus in Algeria.

Keywords: *Casuarina* trees, diversity, distribution, mycorrhizae, *Frankia*, nodulation, Algeria.

RESUMEN

Especies de *Casuarina* en Argelia: estudio de su identidad, distribución y estatus simbiótico

A pesar de la importancia de *la Casuarina* en Argelia desde su introducción a principios del siglo XIX, el número de especies actualmente presentes en el país, su identidad, su distribución, así como su estatus simbiótico son todavía muy desconocidos. Un estudio realizado a 2 304 árboles en 96 rodales de toda Argelia indica que están representados exclusivamente por 5 especies del género *Casuarina*: *Casuarina cristata* Miq., *C. cunninghamiana* Miq., *C. equisetifolia* L., *C. glauca* Sieber ex Spreng. y *C. junghuhniana* Miq. Dos especies, *C. cunninghamiana* y *C. glauca*, predominan ampliamente y se distribuyen de forma diferente, siendo la primera más común en las zonas relativamente húmedas y templadas del país, mientras que la segunda está presente en las regiones más áridas y cálidas. La producción de plantas de *C. cunninghamiana* y *C. glauca* en 12 viveros del norte y el sur del país indica que tienen bajas tasas de micorrización y nodulación espontánea. Esto sugiere que es necesario inocular las plántulas con cepas seleccionadas de hongos micorrícicos y/o de *Frankia* en el vivero para mejorar su establecimiento duradero en el terreno. Este estudio constituye el primer inventario de la distribución geográfica de *la Casuarina* en Argelia y puede conducir a una mejor explotación de los árboles de este género, ampliamente utilizados en Argelia.

Palabras clave: *Casuarina*, diversidad, distribución, micorrizas, *Frankia*, nodulación, Argelia.

Introduction

Casuarina trees are native to Australia, Southeast Asia, and the Pacific Islands (Wheeler *et al.*, 2011). Due to their economic and ecological importance, they have been introduced to over 100 countries around the world with three species (*C. cunninghamiana*, *C. equisetifolia* and *C. glauca*) becoming invasive in many parts of their introduced range (Potgieter *et al.*, 2014).

The Casuarinaceae family consists of approximately 90 species (though this number is still widely debated in the literature) across four genera: *Allocasuarina*, *Casuarina*, *Ceuthostoma* and *Gymnostoma* (Johnson and Wilson, 1993; Christenhusz and Byng, 2016). The *Casuarina* genus comprises around 14 species (19 taxa) (Potgieter *et al.*, 2014). They are evergreen, monoecious, or dioecious shrubs or tall trees that resemble conifers by their growth habit and shape, needle-like leaves, and cone-shaped woody fruits. Despite this resemblance which made them considered in the past as gymnosperms, they are a group of dicotyledonous angiosperms (Eudicots) classed in the order Fagales and the Rosids group as defined by APG IV (APG, 2016).

Casuarinas are fast-growing, resilient trees that tolerate harsh habitats and many soil types including some of the most inhospitable (NRC, 1984). They have a fair resistance to heat, drought, salinity, wind, and pests and produce good quality timber of high energy value (Benge, 1982; Dawson, 2008). These adaptations are enhanced by their ability to form multi-beneficial mutualistic symbiosis with mycorrhizal fungi and the actinobacteria *Frankia* leading to the formation of nitrogen-fixing root nodules. Thus, these remarkable properties make them pioneer species that are capable of colonizing and enriching poor soils and barren sites, thereby playing a particularly important role in ecological succession (Wall, 2000; Duponnois *et al.*, 2003). Commercially important in reforestation, carbon sequestration, agroforestry, and land rehabilitation programs, casuarinas are now common features in many landscapes around the world (Zhong *et al.*, 2010; Potgieter *et al.*, 2014).

Casuarinas were introduced to Algeria in the first half of the 19th century (around 1835). Four species of *Casuarina* (*C. cunninghamiana*, *C. equisetifolia*, *C. glauca* and *C. junghuhniana*) and four species of *Allocasuarina* (*A. littoralis*, *A. paludosa*, *A. torulosa* and *A. verticillata*) were introduced to Algeria during the French colonization (GGA, 1850, 1865; Trottier, 1872). Since then, they have been continuously propagated at a large scale (Toth, 1965; Houmani, 1997) and have today become a fundamental part of the woody flora in all bioclimatic zones of Algeria (Toth, 1965). They are extensively planted for ornamental purposes along roadsides and as windbreaks, fences or shelterbelts protecting against wind, erosion, sand shifting and rapid drying of irrigated fields, particularly in the southern part of the country (Toth, 1965; Houmani, 1997; Gherbi *et al.*, 2011; Makhlouf *et al.*, 2012). They are also used for reforestation, land reclamation of barren or polluted areas and to fight against desertification (Gherbi *et al.*, 2011; Maity and Pawlowski, 2021). Their branchlets with teeth-like leaves are

used as fodder and organic matter for soil improvement, and their wood is used for firewood, charcoal production or timber (Bensaid, 1995). In addition, they are a source of food, habitat, refuges, and nest material for many species of insects, birds, and small mammals and thus support and even increase the biodiversity in the areas where they are established (Tarai and Doumandji, 2009).

Although *Casuarina* trees are widespread over the country, they rarely disperse naturally and are propagated almost exclusively by the transplantation of individuals produced in nurseries from seed germination or to a lesser extent by cuttings (Houmani, 1997). As a result, few naturalized *Casuarina* populations occur in the country, which might be explained by the more adverse climatic conditions in Algeria compared to their native range (Potgieter *et al.*, 2014).

Despite their considerable importance and increasing application in extensive rehabilitation programs of Saharan lands, the number, identity, importance, and distribution of *Casuarina* species present in Algeria remain largely unexplored.

In this work, we established a comprehensive inventory and distribution of the members of *Casuarina* trees throughout Algerian rangelands. We also assessed the occurrence and frequency of the mycorrhization, and the nodulation of *Casuarina* species cultivated in nurseries across the country. Indeed, since propagation of these trees is done exclusively from nursery stock, the mycorrhization and nodulation of seedlings at the nursery level are crucial both for the successful establishment of the species after transfer into plantation areas and for taking full advantage of their ability to fix nitrogen and to improve soil fertility. This work constitutes the first survey on this family of plants in Algeria and provides valuable data for their utilization in the future.

Materials and Methods

Survey of *Casuarina* trees in Algerian rangelands

To identify the *Casuarina* species present in Algeria, we analyzed plant material collected during the fall of 2014 and 2015 between September and November from 2,304 trees growing in 96 stands of more than 100 individuals of similar size and age (approximately 10-15 meters and 10-30 years), distributed among all the 48 administrative districts of Algeria (figure 1 and table I for GPS coordinates). From each stand, we sampled three branches bearing cones filled with seeds from 24 randomly selected trees. The Random Number Generator v.1.4 software (2XDSOFT) was used to randomly select individuals in each stand. The collected materials were put in large paper envelopes and taken to the laboratory for examination. For each sampled branch, seven measurements were performed on the selected characters (branch length, article length, teeth number and length, stalk length, cone length and diameter). During our prospection, we did not observe *Casuarina* trees that were monoecious with male and female flowers borne on the same individual. All the examined trees were dioecious with male and female flowers on separated individuals.

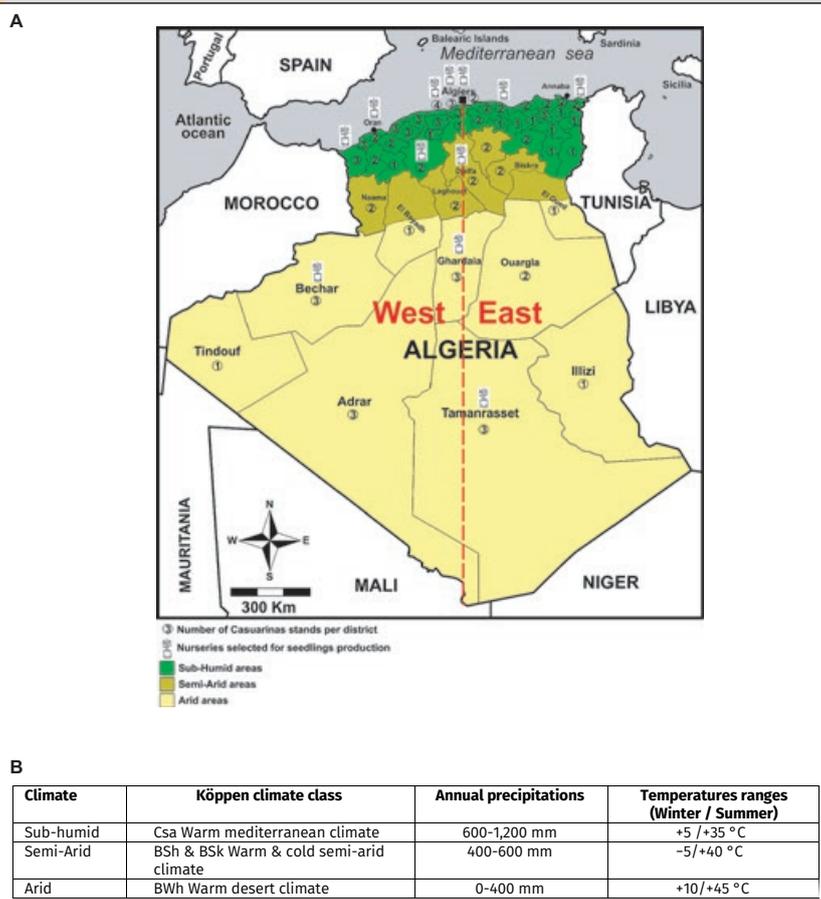


Figure 1.

A. Geographical situation of the *Casuarina* stands and nurseries selected to perform the study. Numbers surrounded by circles represent the number of stands examined for each of the 48 administrative districts of the country. B. Main characteristics of the 3 major climatic zones of Algeria (source: <https://fr.climate-data.org/afrique/algerie-164/>).

Casuarina identification

Casuarina species were identified by their morphometric traits according to the determination key of Wilson and Johnson (1989). Thereby, different traits were considered: the number of sheath teeth (true leaves), the length of branchlets and internodes for the vegetative part, the sizes of inflorescences, cones, and seeds for the floral organs. Macroscopic measurements (length of branchlets, internodes, peduncles, and cones as well as the diameter of cones) were made by using a precision ruler. Microscopic observations and measurements (number and length of teeth by whorls) were carried out under a binocular magnifier (Leica EZ4HD) or a microscope (Premiere MIS-6000T) using (when necessary) a microscope micrometer slide (OPTIKA M-005). However, it should be noted that 0.7% of the individuals (17 of the 2,304 individuals) collected could not be clearly identified on the basis of these morphometric traits, so that they were excluded from the analysis.

Symbiotic status of *Casuarina* grown in nurseries

The occurrence of mycorrhizal and actinorhizal symbioses amongst *C. cunninghamiana* and *C. glauca*, which were found to be the most abundant species in Algeria (see Results), was studied. For this purpose, 12-month-old seedlings of each studied species were produced in 12 selected nurseries from the North and the South of Algeria which regularly produce *Casuarina* seedlings for the local market (figure 1). The seedlings were produced from seeds collected on accurately identified trees growing either in Bâinem arboretum (district of Algiers) for *C. cunninghamiana* or in the district of Tamanrasset for *C. glauca*. For each nursery, plants were analyzed for both mycorrhization and nodulation (24 individuals/species). Germination for young trees production was carried out in March 2015 and symbiotic status were examined in March 2016. The seeds were surface-sterilized by immersion in 5% calcium hypochlorite solution (bleach) for 10 min, rinsed with sterile distilled water and sown on a mixture of nursery soil and thoroughly washed river black sand (2/1: v/v) in perforated polyethylene bags (15 x 25 cm) until they reached 12-month-old.

Mycorrhizal symbiosis

Root systems of seedlings were examined with the naked eye and under a binocular magnifier for the presence of a Hartig net or a hyphal mantle indicating ectomycorrhizal infection (Duponnois *et al.*, 2003). After removing the nodules, the roots of each seedling were excised and stored in ethanol 70° until use. Afterwards, the presence of endomycorrhizal infections was checked and assessed using the method of Phillips and Hayman (1970). For each seedling, five thin roots were randomly chosen, cut into 1 cm segments, and then cleared by soaking in KOH (10%) at 90 °C for one hour followed by soaking in H₂O₂ (9%) for 30 min. Root segments were thoroughly rinsed with distilled water, soaked in HCl (1%) for five minutes and stained with Trypan blue (0.05% Trypan blue in lactophenol (phenol/lactic acid v/v) for 20 min at 90 °C. After removing dye excess by distilled water, segments were incubated in lactophenol for 24 h to enhance their discoloration. To check for the presence/occurrence of dark blue-coloured fungal structures (hyphae, arbuscules, vesicles and spores), 10 stained segments per root (50 root fragments/seedling) were mounted between a microscopic slide and coverslip in a drop of a glycerol solution (20%) and examined under an optical microscope. Endomycorrhizal colonization was scored when fungal structures were found in at least one root segment. The intensity of the mycorrhization (I%) was assessed by counting the number of colonized fragments on the total of roots examined following the

Table I.
 GPS coordinates of the 96 stands from which samples were collected.

| Locality | Latitude | Longitude | Elevation |
|--------------------------------------|---------------|--------------|-----------|
| Adrar - Sali | 26°57'46,44"N | 0°01'45,14"O | 217 |
| Adrar - Sbaa | 28°13'25,09"N | 0°10'17,27"O | 263 |
| Adrar - Tamantit | 27°44'39,84"N | 0°15'45,45"O | 244 |
| Ain Defla - Ain Defla | 36°15'11,73"N | 2°25'39,01"E | 248 |
| Ain Defla - El Attaf | 36°13'30,07"N | 1°41'12,39"E | 165 |
| Ain Defla - Khemis Miliana | 36°15'00,81"N | 2°13'42,08"E | 282 |
| Ain Temouchent - Ain Tolba | 35°15'51,73"N | 1°16'00,02"O | 205 |
| Ain Temouchent - El Malah | 35°23'47,47"N | 1°04'15,51"O | 65 |
| Ain Temouchent - Terga | 35°25'23,72"N | 1°09'38,53"O | 51 |
| Algiers - Bainem (INRF) | 36°48'14,30"N | 2°57'28,03"E | 177 |
| Algiers - Ben Aknoun (Zoo parc) | 36°45'04,36"N | 3°00'56,94"E | 253 |
| Algiers - USTHB | 36°42'40,65"N | 3°42'04,47"E | 14 |
| Algiers - Zeralda (F. des planteurs) | 36°42'40,24"N | 2°51'30,20"E | 63 |
| Annaba - Ben Mhidi | 36°45'14,57"N | 7°55'17,67"E | 7 |
| Annaba - Chabbia | 36°51'04,52"N | 7°42'49,90"E | 6 |
| Batna - Bousalh | 35°12'31,73"N | 6°17'47,86"E | 1064 |
| Batna - Tidjdad | 35°15'52,15"N | 6°05'29,13"E | 1225 |
| Bechar - Abadla | 31°00'34,75"N | 2°44'27,09"O | 584 |
| Bechar - Kenadsa | 31°33'54,47"N | 2°22'25,29"O | 763 |
| Bechar - Ouakda | 31°39'31,74"N | 2°10'29,98"O | 808 |
| Bejaia - Djebira | 36°42'05,72"N | 5°04'14,44"E | 36 |
| Bejaia - Oued Ghir | 36°42'15,22"N | 4°59'01,21"E | 15 |
| Biskra - Doucen | 34°37'48,01"N | 4°58'47,53"E | 223 |
| Biskra - Ouled Djellal | 34°24'37,07"N | 5°04'53,49"E | 183 |
| Blida - Boufarik | 36°34'31,15"N | 2°57'10,57"E | 48 |
| Blida - Meftah (Zayane) | 36°38'03,74"N | 3°16'22,69"E | 82 |
| Blida - Sidi Moussa | 36°36'09,85"N | 3°06'51,13"E | 54 |
| Bordj Bou Arreridj - Medjana | 36°08'12,67"N | 4°41'10,20"E | 1061 |
| Bordj Mokhtar city | 21°19'41,44"N | 0°58'19,64"E | 400 |
| Bouira - Ain Bessem | 36°20'49,95"N | 3°42'22,99"E | 738 |
| Bouira - Said Abid | 36°21'08,02"N | 3°50'36,97"E | 525 |
| Boumerdès - Dellys | 36°54'23,65"N | 3°53'36,67"E | 335 |
| Boumerdès - Tadmempt | 36°54'07,56"N | 3°51'43,34"E | 32 |
| Boumerdès - Zaouia | 36°53'00,49"N | 3°58'42,94"E | 193 |
| Chlef - Boukadir | 36°04'02,80"N | 1°08'17,31"E | 83 |
| Chlef - Oued Fodda | 36°11'22,87"N | 1°31'27,35"E | 162 |
| Djelfa - Ain Oussara | 35°28'42,35"N | 2°53'48,23"E | 663 |
| Djelfa - Hassi Bahbah | 35°05'08,49"N | 2°59'36,33"E | 905 |
| El Bayadh - El Abiodh Sidi Cheikh | 32°51'01,71"N | 0°29'50,01"E | 889 |
| El Oued - Hazoua | 33°45'20,47"N | 7°38'45,19"E | 35 |
| El Tarf - Ain Al Assel | 36°46'15,07"N | 8°22'05,53"E | 30 |
| El Tarf - Boutaldja | 36°48'00,58"N | 8°12'06,75"E | 12 |
| Ghardaia - El Meniaa | 30°38'49,33"N | 2°59'18,83"E | 421 |
| Ghardaia - Hassi El Fahl | 31°36'49,52"N | 3°40'58,91"E | 375 |
| Ghardaia - Zelfana | 32°25'48,11"N | 4°13'40,17"E | 364 |
| Illizi City | 26°28'53,06"N | 8°27'34,00"E | 556 |
| Jijel - Airport | 36°48'00,43"N | 5°52'38,64"E | 5 |
| Jijel - Tamila | 36°47'40,74"N | 5°51'49,66"E | 8 |

Table I (following).

| Locality | Latitude | Longitude | Elevation |
|-------------------------------|------------------|-----------------|-----------|
| Laghouat - Al Assafia | 33° 49' 53,98" N | 2° 58' 52,08" E | 750 |
| Laghouat - El Hadjeb Tadjmout | 33° 56' 50,75" N | 2° 38' 09,92" E | 972 |
| Mascara - Froha | 35° 17' 47,72" N | 0° 07' 46,36" E | 469 |
| Mascara - Ghriss | 35° 15' 11,04" N | 0° 10' 02,47" E | 495 |
| Mascara - Tizi | 35° 19' 11,04" N | 0° 04' 38,94" E | 455 |
| Mila - Sidi Mèrouane | 36° 30' 51,94" N | 6° 15' 45,62" E | 342 |
| Mila City | 36° 27' 17,57" N | 6° 16' 32,43" E | 431 |
| Mostaganem - Hassi Mameche | 35° 51' 42,91" N | 0° 04' 46,06" E | 137 |
| Mostaganem - Mazagran | 35° 54' 07,58" N | 0° 05' 17,64" E | 131 |
| Msila - Bou Saada | 35° 15' 27,76" N | 4° 13' 36,32" E | 501 |
| Msila - Ouled Mahdi | 35° 34' 07,26" N | 4° 31' 28,74" E | 407 |
| Naama - Ain Sefra | 32° 44' 32,15" N | 0° 35' 36,33" O | 1081 |
| Naama City | 33° 16' 13,89" N | 0° 18' 24,61" O | 1171 |
| Oran - Es senia | 35° 37' 21,52" N | 0° 40' 19,46" O | 90 |
| Oran - Messerghine | 35° 37' 02,82" N | 0° 44' 31,56" O | 125 |
| Ouargla - Haoud El Hamra | 31° 53' 28,69" N | 5° 28' 24,17" E | 153 |
| Ouargla - Hassi Ben Abdelah | 32° 11' 55,52" N | 5° 42' 46,37" E | 155 |
| Relizane - Belassel Bouzegza | 35° 50' 21,34" N | 0° 29' 24,11" E | 67 |
| Relizane - Chaif El Hassene | 35° 40' 58,85" N | 0° 33' 50,04" E | 90 |
| Relizane - Matmar | 35° 43' 45,72" N | 0° 28' 29,28" E | 69 |
| Saida - Ain El Hadjar | 34° 45' 13,68" N | 0° 08' 40,22" E | 1025 |
| Sidi Bel Abbès - Airport | 35° 10' 29,28" N | 0° 35' 40,20" O | 481 |
| Sidi Bel Abbès - Sidi Brahim | 35° 16' 01,99" N | 0° 34' 26,26" O | 433 |
| Sidi Bel Abbès - Tilmouni | 35° 10' 36,43" N | 0° 31' 26,75" O | 510 |
| Sétif - Fermatou | 36° 13' 33,67" N | 5° 23' 46,88" E | 1048 |
| Skikda - Hamadi Krouma | 36° 50' 55,24" N | 6° 54' 15,73" E | 30 |
| Skikda - Larbi Ben Mhidi | 36° 52' 45,26" N | 7° 00' 11,20" E | 88 |
| Tamanrasset - Djanet | 24° 33' 29,96" N | 9° 28' 58,16" E | 1041 |
| Tamanrasset - Idles | 23° 49' 38,29" N | 5° 55' 53,70" E | 1390 |
| Tamanrasset City | 22° 46' 56,36" N | 5° 33' 50,64" E | 1396 |
| Tebessa - El Ma Labiodh | 35° 17' 30,68" N | 8° 11' 26,13" E | 1103 |
| Tebessa - Oum Ali | 35° 00' 32,96" N | 8° 15' 15,11" E | 916 |
| Tiaret - Frenda | 35° 03' 14,05" N | 1° 01' 26,86" E | 837 |
| Tiaret - Sougueur | 35° 10' 50,02" N | 1° 28' 44,64" E | 1150 |
| Tindouf - Aéroport | 27° 41' 49,43" N | 8° 09' 19,68" E | 438 |
| Tipaza ChercHELL | 36° 36' 13,86" N | 2° 13' 01,61" E | 148 |
| Tipaza Hadjout | 36° 32' 09,01" N | 2° 25' 39,01" E | 79 |
| Tipaza Sidi Amar | 36° 32' 52,99" N | 2° 18' 42,34" E | 54 |
| Tissemsilt - City | 35° 35' 18,05" N | 1° 49' 01,65" E | 937 |
| Tizi Ouzou - AzzeFoun | 36° 53' 53,90" N | 4° 25' 27,24" E | 79 |
| Tizi Ouzou - M'letta | 36° 53' 22,71" N | 4° 22' 10,47" E | 24 |
| Tizi Ouzou - Tigzirt | 36° 54' 05,29" N | 4° 09' 06,29" E | 76 |
| Tlemcen - Hennaya | 34° 58' 08,84" N | 1° 22' 14,12" O | 365 |
| Tlemcen - Maghnia | 35° 50' 38,57" N | 1° 42' 54,59" O | 371 |
| Tlemcen - Taffessera | 35° 40' 34,89" N | 1° 26' 30,32" O | 807 |
| Tougourt - El Arfiane | 33° 39' 14,96" N | 5° 58' 48,54" E | 24 |
| Tougourt - El Mansoura | 33° 26' 13,07" N | 5° 58' 12,26" E | 53 |
| Tougourt - Moggar | 33° 13' 19,47" N | 6° 04' 56,40" E | 61 |

Statistical analyses

method described by Trouvelot *et al.* (1986). This method is based on the range of values from n0 to n5 where n indicates the number of root fragments scored and rating corresponds to: 0 = without colonization of the root segment; 1 = less than 1% is colonized or colonization trace; 2 = less than 10%; 3 = 11 to 50%; 4 = 51 to 90%; 5 = more than 90%. From this, the intensity of mycorrhization is calculated according to the following formula: $I\% = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1) / N$, where N is the total of observed root fragments.

Actinorhizal symbiosis

From each of the 12 nurseries investigated, 24 randomly selected plants of *C. cunninghamiana* or *C. glauca* were harvested after 12 months of growth and examined in the laboratory. Root systems of the seedlings were carefully removed from the polyethylene bags, washed thoroughly under a stream of tap water to remove soil and then eye examined for the presence of actinorhizal nodules. Nodules of each seedling were excised, and their dry weights were determined after desiccation at 80 °C until constant weight (1 week).

Canonical Discriminant Analysis (CDA) was performed using the free software Tanagra (Rakotomalala, 2005). CDA is a multivariate statistical method that can highlight differences among groups of individuals (or treatments) and help to better understand of the relationships among the variables measured (branch length, article length, teeth number and length, stalk length, cone length and diameter) within those groups (Cruz-Castillo *et al.*, 1994). We used this method to determine which variables discriminate between two or more naturally occurring groups. Statistical analyses were performed using the free software Past3 (Hammer *et al.*, 2001). Data storage, basic statistics and graphs were performed using Lotus Approach (Lotus Development Corporation, Cambridge, USA).

Data presented in tables II and III were subjected to one-way analyses of variance (ANOVA) and Fisher's Least Significant Difference (LSD) tests to evaluate significant differences for variables at 5% level while the Pearson's χ^2 test was used to assess the homogeneity of proportions in figures 3 and 4 using R Studio v. 2.13 (R Development Core Team, 2016).

Table II.

Endomycorrhizal symbiosis: Percentage of mycorrhized seedlings and mycorrhizal intensity in root tissues (I%) among 12-month-old seedlings of *Casuarina cunninghamiana* and *C. glauca* produced in 12 nurseries located in the North and the South of Algeria. Data of mycorrhizal intensity (I%) were subjected to one-way analyses of variance (ANOVA) and Fisher's Least Significant Difference (LSD) tests at $p = 0.05$ threshold. Mean values \pm SD ($n = 24$) in each column followed by a different letter are significantly different.

| Nursery localization | <i>Casuarina cunninghamiana</i> | | <i>Casuarina glauca</i> | |
|---------------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|
| | % of mycorrhized seedlings | Mycorrhizal intensity (I %) | % of mycorrhized seedlings | Mycorrhizal intensity (I %) |
| Algiers - Bainem | 100.0 | 83.1 \pm 2.3 ^a | 100.0 | 83.4 \pm 3.5 ^a |
| Algiers - Ben Aknoun | 100.0 | 79.4 \pm 3.3 ^a | 100.0 | 72.6 \pm 5.4 ^a |
| Béjaïa | 100.0 | 64.8 \pm 4.5 ^a | 83.3 | 60.8 \pm 6.6 ^a |
| El Kala | 100.0 | 84.2 \pm 3.5 ^a | 100.0 | 80.8 \pm 5.5 ^a |
| Oran | 70.8 | 58.2 \pm 7.1 ^a | 100.0 | 58.5 \pm 9.7 ^a |
| Tlemcen | 100.0 | 69.8 \pm 1.8 ^a | 100.0 | 80.0 \pm 6.5 ^b |
| Mean - Northern nurseries | 95.1 \pm 4.9 ^a | 73.2 \pm 4.3 ^a | 97.2 \pm 2.8 ^a | 72.7 \pm 4.4 ^a |
| Adrar | 79.2 | 33.8 \pm 5.5 ^a | 91.7 | 37.0 \pm 5.2 ^a |
| Biskra | 79.2 | 29.4 \pm 6.9 ^a | 91.7 | 32.2 \pm 8.8 ^a |
| Djanet | 70.8 | 35.2 \pm 5.0 ^a | 87.5 | 37.5 \pm 7.1 ^a |
| El Golea | 83.3 | 41.2 \pm 9.3 ^a | 100.0 | 44.0 \pm 5.3 ^a |
| Tamanrasset | 79.2 | 33.3 \pm 5.2 ^a | 87.5 | 28.7 \pm 7.2 ^a |
| Tindouf | 75.0 | 22.9 \pm 8.3 ^a | 83.3 | 24.6 \pm 9.1 ^a |
| Mean - Southern nurseries | 77.8 \pm 1.8 ^a | 32.6 \pm 2.5 ^a | 90.3 \pm 2.3 ^b | 34.0 \pm 2.8 ^a |
| Overall mean value | 86.5 \pm 3.6 | 52.9 \pm 6.5 | 93.8 \pm 2.0 | 53.4 \pm 6.3 |

Table III.

Actinorrhizal symbiosis: Percentage of nodulated seedlings, mean number of nodules per seedling (nodules/seedling) and nodule dry weight per seedling (NDW mg/seedling) among 12-month-old seedlings of *Casuarina cunninghamiana* and *C. glauca* produced in 12 nurseries located in the North and the South of Algeria. Data were subjected to one-way analyses of variance (ANOVA) and Fisher's Least Significant Difference (LSD) tests at $p = 0.05$ threshold for all variables. Mean values \pm SD (n=24) in each column followed by a different letter are significantly different.

| Nursery localization | <i>Casuarina cunninghamiana</i> | | | <i>Casuarina glauca</i> | | |
|-------------------------|---------------------------------|----------------------------|-------------------------------|-----------------------------|----------------------------|-------------------------------|
| | % of nodulated seedlings | Nodule no./ seedling | NDW mg/ seedling | % of nodulated seedlings | Nodule no./ seedling | NDW mg/ seedling |
| Algiers Baïnem | 70.8 | 6.5 \pm 1.0 ^a | 532.7 \pm 81.8 ^a | 54.2 | 4.5 \pm 0.8 ^a | 329.7 \pm 74.5 ^b |
| Algiers Ben Aknoun | 37.5 | 1.8 \pm 0.6 ^a | 124.4 \pm 35.7 ^a | 41.7 | 3.0 \pm 0.8 ^b | 141.7 \pm 38.4 ^b |
| Béjaïa | 50.0 | 2.9 \pm 0.6 ^a | 243.0 \pm 54.9 ^a | 37.5 | 2.6 \pm 0.8 ^a | 202.3 \pm 64.3 ^a |
| El Kala | 54.2 | 7.0 \pm 1.4 ^a | 278.1 \pm 56.2 ^a | 29.2 | 3.0 \pm 1.1 ^b | 156.7 \pm 56.7 ^a |
| Oran | 33.3 | 2.0 \pm 0.6 ^a | 97.2 \pm 30.2 ^a | 45.8 | 2.8 \pm 0.7 ^b | 100.8 \pm 26.0 ^a |
| Tlemcen | 33.3 | 1.3 \pm 0.5 ^a | 33.9 \pm 13.9 ^a | 37.5 | 2.0 \pm 0.6 ^b | 71.2 \pm 21.5 ^b |
| Mean-Northern nurseries | 46.5 \pm 6.0 ^a | 3.6 \pm 1.0 ^a | 218.1 \pm 73.2 ^a | 41.0 \pm 3.5 ^a | 3.0 \pm 0.3 ^b | 167.1 \pm 37.4 ^b |
| Adrar | 29.2 | 1.1 \pm 0.4 ^a | 16.9 \pm 6.3 ^a | 25.0 | 0.5 \pm 0.2 ^b | 12.8 \pm 5.4 ^a |
| Biskra | 20.8 | 1.0 \pm 0.4 ^a | 16.2 \pm 7.1 ^a | 25.0 | 0.6 \pm 0.2 ^a | 8.57 \pm 3.4 ^b |
| Djanet | 0.0 | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a | 0.0 | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a |
| El Goléa | 0.0 | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a | 0.0 | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a |
| Tamanrasset | 0.0 | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a | 0.0 | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a |
| Tindouf | 16.7 | 0.5 \pm 0.2 ^a | 6.5 \pm 3.3 ^a | 20.8 | 0.3 \pm 0.2 ^a | 6.9 \pm 3.1 ^a |
| Mean-Southern nurseries | 11.1 \pm 5.2 ^a | 0.4 \pm 0.2 ^a | 6.6 \pm 3.3 ^a | 11.8 \pm 5.3 ^a | 0.2 \pm 0.1 ^b | 4.71 \pm 2.2 ^a |
| Overall mean value | 28.8 \pm 6.5 | 2.0 \pm 0.7 | 112.4 \pm 47.3 | 26.4 \pm 5.3 | 1.6 \pm 0.4 | 85.9 \pm 30.3 |

Results

Casuarina species in Algerian rangelands

Surveys carried out all over Algeria to select sufficiently large and representative tree stands allowed us to observe members of both genera *Casuarina* and *Allocasuarina*. *Casuarina* species are distinguished by their dull and grey to yellow-brown winged seed (samara) body while *Allocasuarina* species are easily recognizable by their shiny red-brown to black samara body.

In all the prospected locations, the examined stands consisted exclusively of representatives of the genus *Casuarina* which are widespread across all Algerian rangelands and bioclimatic areas (figure 1, photos 2). In contrast, only one species of *Allocasuarina* (likely *A. verticillata* (Lam) L.M.S. Johnson), was recorded in a unique site at the arboretum of Baïnem in Algiers indicating that species of this genus are seemingly rare in Algeria.

Casuarina genus is represented by 5 species in Algeria

Casuarina plant material was collected from 2,304 randomly selected trees present in 96 stands (24 individuals per stand) located in the 48 administrative districts of the

country (figure 1). Analyses based on different morphometric traits of the vegetative parts (sheath teeth, length of branchlets and internodes) and of floral organs (size of cones and seeds) allowed us to identify five species: *Casuarina cristata* Miq., *C. cunninghamiana* Miq., *C. equisetifolia* L., *C. glauca* Sieber ex Spreng. and *C. junghuhniana* Miq. (figure 2).

The results of Canonical Discriminant Analysis (CDA) revealed that branch (BR-L) and article (ART-L) lengths were the best discriminating traits since they allow a satisfactory grouping of the individuals from the same species into rather well delineated and cohesive clouds. Nevertheless, except for *C. cunninghamiana*, all the other groups showed some individuals that were scattered far from the centre of their corresponding cloud (figure 2).

Analysis of the 2,304 plants indicated that the composition of the *Casuarina* species present in the 96 stands was not homogeneous (χ^2 test, $p < 0.001$) since more than half (55.2%) included individuals belonging to at least two species (figure 3) and nearly 25% of the stands contained individuals belonging to three species or more. On the other hand, 44.8% of the stands were monospecific (figure 3) and were dominated by either *C. cunninghamiana* or *C. glauca* (figure 2, photos 2).

Species of *Casuarina* that occur in the 96 examined stands are unevenly represented. The proportion

of each species as well as their distribution in the stands were not homogeneous (χ^2 test, $p < 0.001$) (figure 4). Two species, *C. cunninghamiana* (1,101 individuals) (photos 3) and *C. glauca* (1,070 individuals) (photos 4), were largely predominant accounting together for nearly 95% of all 2,304 trees examined (figure 4). The two species are also the most widespread since they were found respectively in 68 and 64 of the 96 stands investigated representing 70.8% and 66.7% of the cases, respectively (figure 4). The three other *Casuarina* species, *C. cristata* (12 individuals), *C. equisetifolia* (42 individuals) and *C. junghuhniana* (62 individuals) are much less frequent and represent altogether only 5.0% of sampled trees (116 out of 2,304). They were found in 4, 16 and 18 stands respectively representing 4.2%, 16.7% and 18.7% of the 96 stands (figure 4). These species can thus be considered as minor in Algeria. In addition, 17 individuals representing 0.7% of the total plants could not be identified (figure 4).

Casuarina cunninghamiana is more common in the northern and eastern parts of the country, which are the wettest and most temperate zones, while *C. glauca* is more abundant in the South and the West, the driest and warmest areas of the country (figure 5).

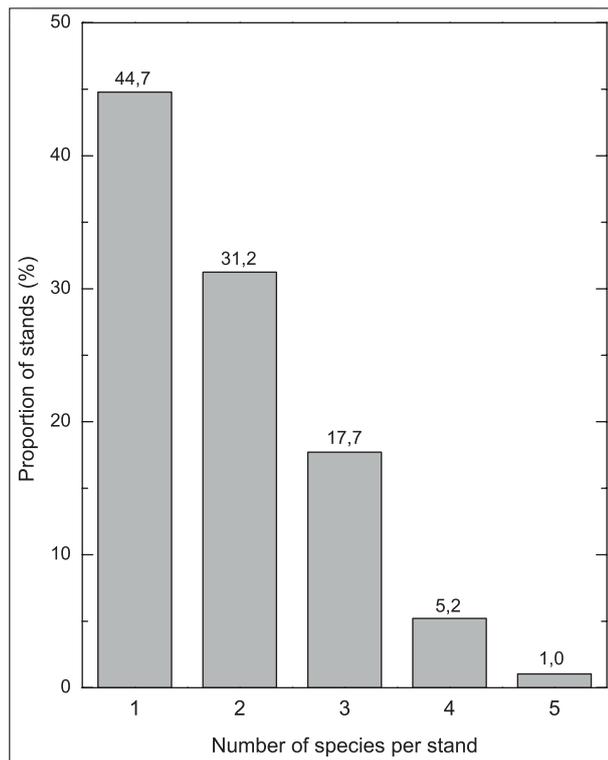


Figure 3. Distribution of the stands studied according to the number of co-occurring *Casuarina* species. The composition in *Casuarina* species present in the 96 stands was not homogeneous (χ^2 test: $\chi^2_{(4)} = 63.58$, $p < 0.001$).

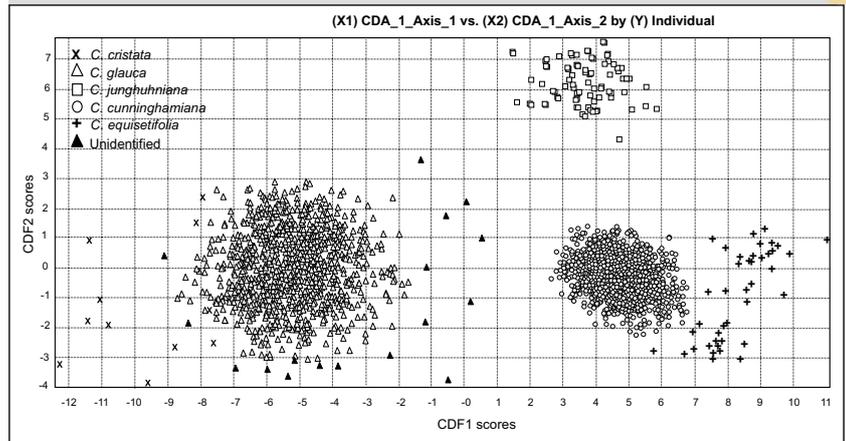


Figure 2. Canonical scores of the first two canonical discriminant functions (CDFs) of the five species of *Casuarina* identified on the basis of their morphometric traits. (X) *C. cristata*, (Δ) *C. glauca*, (□) *C. junghuhniana*, (○) *C. cunninghamiana*, (+) *C. equisetifolia*.



Photos 2. Utilization of *Casuarina* trees in Algeria. A. Roadside delimitation by *C. cunninghamiana* in the coastal zone (El Kaddous, district of Algiers). B. *C. cunninghamiana* tree at the North region of Algeria (campus of Houari Boumedienne university, USTHB, district of Algiers). C. Plantation of *C. glauca* as a windbreak in a palm grove in the South of the country (USTHB experimental field station, El-Golèa, district of Gharðaïa). D. *C. glauca* tree at the South of Algeria (District of Tamanrasset). Photos K. Belaid.

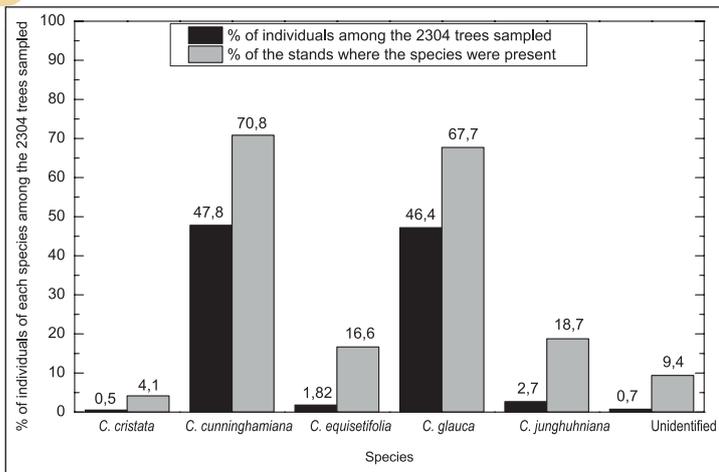


Figure 4.

Distribution of the 2,304 trees analyzed by species and proportion of stands where each species was present (all data are given as % of the total individuals and % of the 96 selected stands). Based on the χ^2 tests, the proportion ($\chi^2_{(11)} = 205$, $p < 0.001$) of each species as well as their distribution ($\chi^2_{(6)} = 3850$, $p < 0.001$) in the stands were not homogeneous.

Symbiotic status of the *C. cunninghamiana* and *C. glauca* seedlings grown in nurseries

We examined whether seedlings of *C. cunninghamiana* and *C. glauca* produced in nurseries in Algeria are naturally mycorrhized and nodulated before their dissemination on the local market. For this, we assessed the prevalence of mycorrhizal and actinorhizal symbioses in 12-month-old seedlings produced in 12 nurseries chosen from different areas of Algeria.

Prevalence of mycorrhization

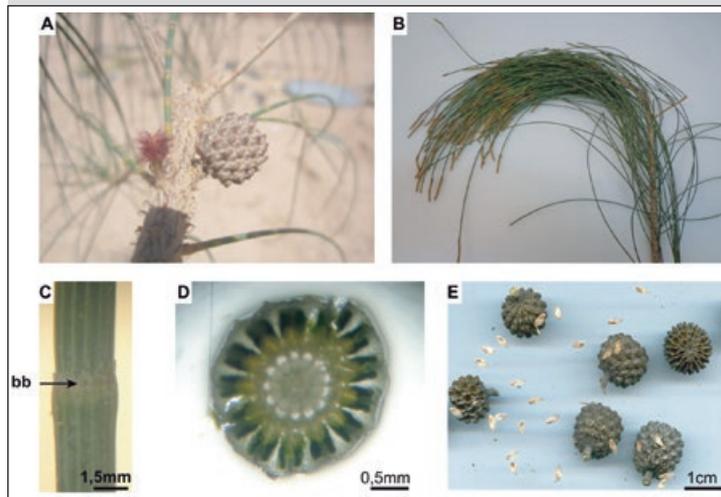
Careful examination of root systems of *C. cunninghamiana* and *C. glauca* plants did not reveal the presence of any ectomycorrhizal structures (Hartig net of hyphal mantle) indicating that these actinorhizal species were unable to form ectomycorrhizal symbiosis in the 12 nurseries from the North and the South of Algeria which regularly produce *Casuarina* seedlings for the local market (figure 1). In contrast, endomycorrhizal structures, mainly in the form of intra- and intercellular hyphae, were observed on most of the root systems of the two *Casuarina* species in all the nurseries included in the analysis. The presence of arbuscules and vesicles was also observed (photos 5A and 5B). The percentage of mycorrhized plants ranged from 70.8% to 100% for *C. cunninghamiana* and from 83.3% to 100% for *C. glauca* (table II). However, seedlings growing in nurseries located in the South were less mycorrhized (table II). These results indicate that both species can interact with endomycorrhizal fungi.

The percentage of mycorrhized plants of *C. cunninghamiana* and *C. glauca* in the nurseries was high (from 86,5% to 93,8%) while the mycorrhizal intensity (I%) had an overall mean of about 53% for both species (table II). Furthermore, I% was more intense in nurseries located in the North than those located in the South of the country (table II). However,



Photos 3.

Morphological characters of *Casuarina cunninghamiana*. A. Branches with female flowers; B. Branches with male flowers; C. Detail of a branch under a magnifying glass highlighting the leaves in whorls with small sharp teeth and a brown transverse band (bb); D. Cross section of a branch observed under a light microscope (10 teeth); E. Female cones and seeds. Photos K. Belaid.



Photos 4.

Morphological characters of *Casuarina glauca*. A. Branches with female flowers; B. Branches with male flowers; C. Detail of a branch under a magnifying glass highlighting the leaves in whorls with small sharp teeth and a brown transverse band; D. Cross sections of a branch observed under a light microscope (13 teeth); E. Female cones and seeds. Photos K. Belaid.

in the nurseries in the North as well as in the South, 1% for both *C. glauca* and *C. cunninghamiana* seedlings were not significantly different (table II).

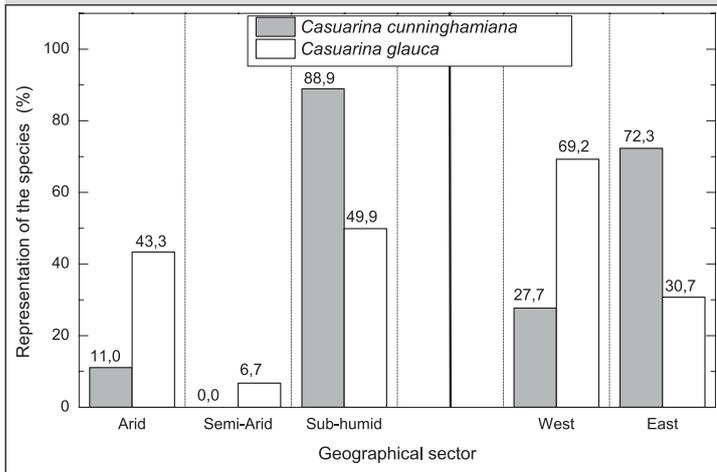
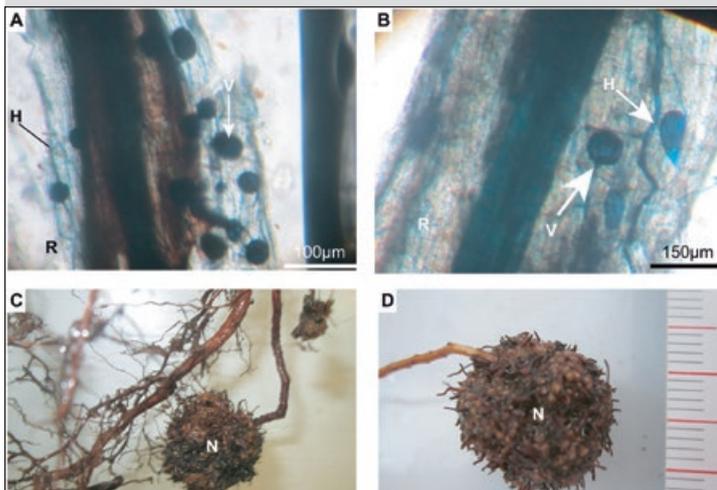


Figure 5. Geographical distribution of the two dominant *Casuarina* species (data are given as % of the total individuals for each geographical zone).



Photos 5. Symbiotic status of *Casuarina cunninghamiana* and *C. glauca*. A. Light microscope photograph of arbuscular mycorrhizal (AM) colonization of the root of *C. cunninghamiana* showing intercellular hyphae in the root cortical tissue and vesicles in the cortical layer; B. Light microscope photograph of arbuscular mycorrhizal colonization of the root of *C. glauca* showing extraradical hyphae of AM attached with the root and vesicles in the cortical layer; C. Root nodules of *C. cunninghamiana*; D. Root nodules of *C. glauca*. H: hyphae; R: root; N: nodule; V: vesicle. Photos K. Belaid.

Prevalence of nodulation

Root systems of *C. cunninghamiana* and *C. glauca* seedlings were examined for the presence of nodules. Results indicate that a low proportion of seedlings produced in the 12 nurseries were nodulated (photos 5C and 5D). The overall mean of nodulation was 28.8% for *C. cunninghamiana* and 26.4% for *C. glauca* with high differences between seedlings growing in northern and southern nurseries (table III). We have observed that seedlings growing in southern nurseries

were much less nodulated (11.1% and 11.8% respectively) than those growing in northern nurseries (46.5% and 50% respectively). In most cases, only a small fraction of seedlings was nodulated except for those growing in the nurseries of Algiers, Béjaia and El Kala located in a wetter zone. In addition, nodulation was absent in three nurseries located in the South (Districts of El Golea, Tamanrasset and Djanet). For both species, the number and dry weight of nodules per seedling were drastically lower in nurseries located in the South than those located in the North of the country. Overall, the two species showed a relatively reduced number of nodules and nodule dry weight per seedling (table III) and had a similar susceptibility to nodulation by *Frankia* in the 12 nurseries.

Discussion

Our survey conducted on 2,304 trees from 96 *Casuarina* stands situated all over Algeria indicates that *Casuarina* trees belong in most cases to the genus *Casuarina*. In Algeria, apart from 12 individuals likely to be *Allocasuarina verticillata* found in a unique site in the arboretum of Baïnem (district of Algiers), we did not find any records of *Allocasuarina* introduction listed in the literature: *A. littoralis*, *A. paludosa* and *A. torulosa* (GGA, 1850, 1865; Trotter, 1872; SGA, 1875; Toth, 1965). Our observations are consistent with those of Maire (1957) who stated that *Allocasuarina* species are very scarce in Algeria with *A. verticillata* (cited as *Casuarina quadrivalvis*) reported as the only species present.

Our investigations throughout the country showed that the genus *Casuarina* is represented by 5 species: *C. cristata*, *C. cunninghamiana*, *C. equisetifolia*, *C. glauca* and *C. junghuhniana*. However, individuals of *C. cunninghamiana* and *C. glauca* are by far the most frequent species in Algeria accounting for nearly 95% of the individuals sampled. These findings contradict previous reports considering *C. equisetifolia* as the most common or the only *Casuarina* species occurring in Algeria (Houmani, 1997). Our results are however partially in line with those of Maire (1957), who reported that only *C. cunninghamiana* and *C. glauca* occur in Algeria and stated that *C. equisetifolia* specimens previously reported from Algeria in the literature were in fact *C. cunninghamiana*, *C. glauca* or *Allocasuarina verticillata* (cited as *Casuarina quadrivalvis*). Such predominance of *C. cunninghamiana* and *C. glauca* is also observed in Morocco, Tunisia, and Egypt (El-Lakany, 1988; Ducousso *et al.*, 2003; Tellal *et al.*, 2008).

Furthermore, the two major species of *Casuarina* highlighted by this study showed a different distribution. *Casuarina cunninghamiana* is more common in the North and East which are the wettest and most temperate areas of the country while *C. glauca* is more represented in drier and warmer areas of the South and West (figure 5). Since there is no detailed history of the spread of *Casuarina* species after their introduction and establishment in Algiers, it is difficult to explain the origin of the current distribution of these

two species throughout Algerian rangelands. However, this difference in their distribution can probably be linked to the ecological properties of the two species. Indeed, *C. glauca* species is characterized by its better tolerance to drought, salinity, frost, and high temperatures (El-Lakany and Luard, 1983) and would have been preferred for plantations in the driest and hottest parts of the country. *Casuarina cunningghamiana* is a more temperate species and less adapted to drought, salinity and high temperatures. This explains why *C. cunningghamiana* is preferably planted in coastal and wet areas of Algeria.

Amongst the list of species highlighted by this study, *C. cristata* has yet to be reported in Algeria or any other North-African country. Its presence should be considered with caution since the CDA resulted in a very scarce grouping of the 12 individuals we attributed to this taxon (figure 2). The misidentification of *C. glauca* as *C. cristata* could have occurred as their morphometric traits are remarkably similar (Wilson and Johnson, 1989). This confusion is common and has led many authors to mistakenly state that *C. cristata* occurs in areas in which it was never introduced such as in Florida, Caribbean Islands, and Taiwan (Woodall and Geary, 1985). A similar misidentification could have occurred by Beddiar *et al.* (2015) who describe *C. obesa* as present in the North-East of Algeria, a species for which no introduction history to the country exists.

In our study, the size of *Casuarina* samples (2,304 individuals) was large enough to limit misidentifications. However, one should not exclude the existence of atypical individuals or hybrids which are quite frequent in nature (El-Lakany and Yunes, 1995; Kamalakannan *et al.*, 2006). In their native range of Australia, *Casuarina* species are typically allopatric - gene flow is limited by ecology (i.e., phenology and spatial distribution), rather than anatomical, biochemical or genetic incompatibilities (Woodall and Geary, 1985). Thus, crosses between species of the genus in which pollen is disseminated mainly by wind are possible as populations of *Casuarina* species with synchronous flowering periods overlap. Crossing between members of *Casuarina* genus has been observed in several areas of the world (Woodall and Geary, 1985; Wilson and Johnson, 1989).

Given that almost all *Casuarina* stands present in the country comprise two or more species and that the species present in Algeria all flower nearly simultaneously in early fall (September and October), the overlap of populations of different species may have favoured the appearance of many hybrids. The presence of hybrids in the stands we explored could explain why in almost all groups delimited by CDA, several individuals were ranged far from the centre of their corresponding cloud (figure 2). This could explain why we attributed some individuals to *C. cristata*, a species for which no introduction history in Algeria has been reported. This also could explain why a small number of individuals (17) could not be identified. Genetic markers could be used to confirm our identifications and to detect possible hybrids that can constitute an interesting plant genetic resource.

Regarding the symbiotic status of *C. cunningghamiana* and *C. glauca*, we observed that young individuals growing in each of the 12 nurseries were unable to deve-

lop ectomycorrhizal symbiosis. By contrast, the two species showed susceptibility to endomycorrhizal fungi with a high proportion of individuals that were colonized (table II). Although *Casuarina* species can interact with both ecto- and endomycorrhizal fungi, our observations are in line with previous reports indicating that they are more susceptible to endomycorrhizal fungi. Indeed, studies conducted by Mejstřík and Cudlín (1983), Nouasria and Beddiar (2010) and Beddiar *et al.*, (2015) reported only endomycorrhizae for *C. equisetifolia*, *C. cunningghamiana* and *C. obesa* growing in Algeria. Furthermore, Duponnois *et al.* (2003) showed that under axenic conditions, isolates of the ectomycorrhizal fungi *Pisolithus* and *Scleroderma* were unable to form a Hartig net on *C. glauca* while they could form ectomycorrhizae on *Allocasuarina verticillata*.

Although the endomycorrhization of *Casuarina* seedlings was globally high, the overall mycorrhizal intensity (1%) was lower. This 1% does not exceed 53% overall, which could be attributed to the reduced concentration of fungal inocula in culture substrates of the nurseries and/or to their composition. For instance, a high phosphate content of the substrate can strongly reduce or inhibit mycorrhization (Duponnois *et al.*, 2003).

Concerning the nodulation by the actinobacteria *Frankia*, our investigations indicate that the presence of root nodules on *C. cunningghamiana* and *C. glauca* seedlings was scarce in the 12 nurseries studied. The overall proportion of nodulated seedlings was less than 30% with a reduced number of nodules and nodule dry weight per seedling (table III). These results are interesting as there is no information about the history of the inoculation of these trees in Algeria. It is known that generally *Casuarina* cannot be nodulated outside their natural range due to the absence of compatible *Frankia* strains (Reddell *et al.*, 1986; Simonet *et al.*, 1999). Nodulated plants observed in the different nurseries indicate that the soils contained viable *Frankia* strains able to nodulate the *Casuarina* plantlets. Since the specificity between *Frankia* and their *Casuarina* hosts results from a long co-evolution process between the two symbionts, it is unlikely that native *actinobacteria* have adapted to *Casuarina*, given their relatively recent introduction to the country. The most probable explanation for the presence of these compatible strains is their co-introduction by *Casuarina* plant material (seedlings, cones, seeds, and solid substrates). Once in Algeria, these strains persisted and spread through the country via local exchanges of plant material between central and regional nurseries. The extensive use of *Casuarina* in the country has likely contributed to the spread of actinobacteria (Houmani, 1997).

Nevertheless, overall, the seedlings were scarcely nodulated and showed reduced nodule numbers and nodule dry weights indicating that the symbiosis with *Frankia* was not very effective. This could result from different factors such as the presence of low concentrations of *Frankia* bacteria in the culture substrate in the nurseries (Reddell *et al.*, 1986) or the chemical composition of the culture substrate. A phosphorus deficiency or a high content in nitrogen can also limit the nodulation of the seedlings (Dawson, 2008; Chaia *et al.*, 2010).

Furthermore, in the framework of this study, we could isolate a *Frankia* strain (named B2) from nodules collected from *C. cunninghamiana* seedlings growing in a nursery located at Souk El Tenine (District of Bejaia). The sequencing and the analysis of its genome indicate that the strain B2 is a new Algerian isolate (Belaid *et al.*, 2020).

Conclusion

Since their introduction in Algeria at the beginning of the 19th century, *Casuarina* trees have been rapidly propagated and widely planted for their rapid growth and hardiness and have become an important part of the ligneous flora of the country.

Our analysis revealed that the *Casuarina* trees currently occurring in the country belong to only 5 species with an overwhelming representation (95%) of the 2 species *C. cunninghamiana* and *C. glauca* which displayed distinct distribution. *Casuarina cunninghamiana* is more common in the North and the East while *C. glauca* is more commonly found in the South and the West. In addition, we found that *C. cunninghamiana* and *C. glauca* seedlings produced in nurseries showed low rates of spontaneous mycorrhizal intensity and nodulation. This can have an impact in the successful establishment of the trees in the planting areas where soils are poor or degraded.

Finally, our survey of *Casuarina* in Algeria brings new data and confirms with greater accuracy not only the identity but also the relative abundance and the distribution of a globally important tree genus. Further work is needed to evaluate the presence of putative hybrids (e.g., using genetic markers for the analysis of the morphometric traits), which can have implications for their use as commercially important trees. Similar works are needed in other countries where *Casuarina* have been widely planted, especially in the neighbouring countries in North Africa. This will allow to have an inventory of their distribution and to assess their ecological impact in this region.

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Data access

Data collected as part of the work summarised in the article submitted to *Tropical Timber and Forests* (Excel files) may be used by contacting the corresponding author and citing the article. They are publicly available: <https://drive.google.com/drive/folders/18DGeFPOYfQb68adI4kjudBSOtMjetKSo?usp=sharing>

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