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http://dx.doi.org/10.1071/BT13150

Ladd, P.G., Midgley, J.J. and Nield, A.P. (2013) Serotiny in southern hemisphere conifers. Australian Journal of Botany, 61 (6). pp. 486-496.

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Serotiny in southern hemisphere conifers

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Running heading - Serotiny in southern hemisphere conifers

Summary text – Protecting seeds in woody containers is a common feature in southern hemisphere woody plants but the cones of southern conifers have been little studied. Cones occur in *Callitris* – Australia and New Caledonia and *Widdringtonia* - South Africa and vary greatly in size, whether they stay closed for some years on the plant (serotiny) and how well they protect the seeds from the heat of a fire. Examining the environmental features that are associated with whether cones stay closed or not helps understand the reproductive and ecological behaviour of the trees.

Abstract

Serotiny is a widespread,trait in angiosperms in the southern hemisphere, however it is less common in conifers and has been little examined in the only two genera of southern conifers (*Callitris* and *Widdringtonia*) that have serotinous cones. There is variation across the family in the size of cones, the amount of seed contained and the time over which the cones stay closed on the plant. Cones from most of the species were collected in the field and various morphometric measurements made including cone wet and dry weight, the number of seeds contained and their likely viability. Cones from a selection of species with different cone sizes were heated to increasing temperatures to investigate the ability of cones to protect the contained seeds from heat. In comparison to the flowering plants, serotiny has developed comparatively recently in southern conifers (in the last 10 - 20 Million years). In *Widdringtonia* serotiny is relatively weak but in *Callitris* varies from strong to non-existent. Cone size and fertile seed production across the two genera varies and the the number of fertile seeds produced is positively related to the size of the cone. In some species there are sterile seed-like bodies. These may have developed to confuse seed predators so fertile seeds have a better chance of survival. Larger (heavier) cones are more effective in protecting the contained seeds from the heat of fires than are smaller ones. There is no simple relationship between the cone size and type of environment occupied by the species. In regions where fire is unlikely, predictable but mild or completely unpredictable the species tend to be non-serotinous. In

temperate regions where hot fires are likely to have been a selective agent the species tend to be more strongly serotinous, although fire is not essential to open the cones. The community and environment in which a species has evolved is likely to have influenced the development of the degree of serotiny for each species and this may still be a variable property between populations of some species depending on the fire regime of the area in which they grow.

Introduction

In many species reproductive propagules are produced in cones (gymnosperms) or infructescences (angiosperms). In a proportion of these, the structures are serotinous (Lamont 1991; Lamont and Enright 2000) and ensure that reproductive propagules are retained in the canopy of the plant to be released in the event of a disturbance that kills or removes the foliage from the plant, such as fire or drought. In most cases propagules are released when the cone/infructescence dries, however in some pines (Radeloff *et al*. 2004), some *Hesperocyparis* (Milich *et al*. 2012) and some southern hemisphere angiosperms (eg. banksia) heat is necessary to open the cones/follicles (Lamont 1991; Clarke *et al*. 2010). Serotiny is most widespread in the southern hemisphere, particularly in South Africa and Australia with over half of serotinous species in the south west of Australia (Lamont and Enright 2000). Serotiny is, to a large extent, phylogenetically constrained with most serotinous species in four angiosperm families (Bruniaceae, Casuarinaceae, Myrtaceae, Proteaceae). Virtually the only serotinous taxa in the northern hemisphere are some pines, and a number of species in the Cupressaceae (Lamont *et al*. 1991). There has been a large number of studies on serotiny in the pines and the Proteaceae but very little examination of the trait in the Cupressaceae. In particular, while it is known that the southern cypress group has species with serotinous cones there has been virtually no examination of how this trait is distributed in the southern hemisphere members of the family, the details of seed production or the degree to which it influences seed survival during a disturbance.

The advantages of canopy seed storage have been summarized a number of times (Lamont *et al* 1991; Lamont and Enright 2000). Lamont *et al*. (1991) evaluated nine hypotheses concerning the advantages and the more important ones include maximizing seed availability, ensuring an optimal seed bed for the shed seeds, satiating post- dispersal seed predators and maximizing protection of seeds from the heat of fire. In addition serotiny may enhance seed protection from predispersal seed predators (Groom and Lamont 1997), though Midgley (2000) questioned the value of increased degree of serotiny in making species more resilient in resisting predispersal seed predation. Very few species are completely serotinous, with most releasing seeds/fruits when the infructesence/cone ages and the water connection to the parent plant is broken. However in a small subset of serotinous species cones or follicles will only open when burnt – these are pyriscent (Lamont 1991). The degree of serotiny in different species has been the subject of considerable discussion. Deterministic models relating fire return interval to degree of serotiny always predict complete serotiny despite the fact that this is very rare in extant species. It was only when stochasticity around the mean fire interval was introduced that the more realistic result of incomplete serotiny was produced (Enright *et al*. 1998; Lamont and Enright 2000). Lamont and Enright (2000) proposed four factors that could produce intermediate serotiny. If fire interval exceeded the life span of a non sprouter species the species would become extinct unless seeds could be released without a disturbance. Fluctuating fire interval would favour intermediate serotiny as would the case where interdisturbance establishment was often possible. Finally it is likely there is a trade off between the costs of seed production versus the seed container, in which case intermediate serotiny is favoured if the fire intervals oscillate between the time taken to accrue an optimal seedbank and intervals shorter than this time.

In the conifers the trait of serotinous cones is disjunct, with the Cupressaceae only distantly related to the pine group. Farjon (2005) in his extensive monograph recognized 30 genera in the Cupressaceae. Of these, 19 (treating *Cupressus* in its broad sense) occur in the northern hemisphere and only two have serotinous cones. In the southern hemisphere there are 11 genera (recognizing *Actinostrobus* as separate from *Callitris*) and three of these have species with serotinous cones.

The two other southern hemisphere conifer families have species that are almost all restricted to mesic environments. The cones of the Araucariaceae are all large, but not serotinous while the Podocarpaceae do not form woody cones. Although all the Cupressaceae have a cone-like female structure, in most species seeds are shed when they are mature. In the southern hemisphere the genera without large cones tend to occur in very mesic areas. *Austrocedrus,* in South America, is one of the few with at least part of its range in a mediterranean-type climate but it lacks a large cone. *Neocallitropsis* on subtropical New Caledonia has a very insignificant 'cone' of several bracts that gape and release the seeds as the bracts dry out. In phylogenetic analyses of the "callitroid" clade *Neocallitropsis* is considered basal to *Callitris* (Piggin and Bruhl 2010) and the most closely related species to this genus are *Callitris sulcata* and *C. neocaledonica* that do have recognizable cones though they are the smallest in the genus.

The origin and function of serotiny has been the subject of considerable discussion in relation to its relevance to helping a species survive a disturbance. The selective force of fire in serotiny evolution has been often raised but opinion varies from one end of the spectrum where serotiny is not a trait evolved to help a species cope with fire (Bradshaw *et al*. 2011) to the other end where it is considered a key aspect of evolution of the reproductive structure to aid the species in regeneration after fire (Bond *et al*. 2004; Keeley *et al*. 2011). In addition the timing of the origin of serotiny has also been discussed with evidence put forward for serotiny in the northern hemisphere pines originating 89 Ma ago (He *et al*. 2012), in *Banksia* 60.8 My ago (He *et al.* 2011) and for flammable biomes in Australia either 25 My ago (Crisp *et al*. 2004) or revised to earlier - 60-62 My ago (Crisp *et al*. 2011). The southern Cupressaceae can be fitted to the geological time scale by noting that the oldest definite fossil evidence of *Callitris* in Australia is of 30 – 32 Ma old *C. leaensis* from

Tasmanian sediments (Paull and Hill 2010). However this species was unlikely to have a serotinous cone. This fossil was used by Crisp *et al.* (2011) to date the divergence of *Callitris* from its sister group *Widdringtonia* in their scheme. Crisp *et al.* (2011) assigned the crown of *Callitris* an age of 16.2 Ma and noted that it was one of the few conifer groups to successfully radiate into arid habitats in Australia. However this radiation was much later than that of angiosperm groups they had examined in their earlier paper (Crisp *et al*. 2004).

In this study we address the variation in cone size and serotiny in the southern hemisphere cone-bearing Cupressaceae, particularly in relation to fire. While we expect that cone size will correlate with seed attributes, that larger cones will better protect the seeds from heat and there will be a relationship between serotiny and the bioclimatic location of different species our null hypotheses are -

1. Cone size is unrelated to seed number, size and viability
2. Cone size has no relationship to seed survival when cones are heated
3. Degree of serotiny has no relationship to environmental conditions where species grow.

While there has been considerable discussion of serotiny in other groups in the southern hemisphere floras, we see this article as a first step towards a better consideration of the details of serotiny in the Cupressaceae.

Materials and methods

Cones of the conifer species were collected in the field (locations listed in Table 1 and Supplementary materials). Usually at least 20 cones from five haphazardly selected trees in a population were kept cool in plastic bags until fresh weight was determined. In some instances sampling of cones was unavoidably limited (such as due to the occurrence of fires in rare New Caledonian species). Cones were then allowed to air dry until they opened and released seeds. Seeds were counted and viable seeds were determined by a cut test to expose the white moist tissue of a healthy embryo. Open cones were oven dried at 70^0 C to constant weight.

Heat treatment of cones was on freshly collected cones that in some cases were kept in a plastic bag in a cool room until the heating could be completed. Cones were allowed to equilibrate to room temperature (approximately 20^0 C) before heating. Cones were then heated to set temperatures in a muffle furnace for 2 minutes. Two minutes was selected as a consistent time to allow a judgment of the protection afforded by a cone to the contained seeds and was not intended to mimic any time - temperature combination produced by a particular fire regime. This time had also been used in a study of *Banksia* infructescences (Enright and Lamont 1989). Seeds released from the cones after heating were sown in sand in a glasshouse where temperatures were maintained at about 20^0 C during the day (cooler at night).

Widdringtonia seeds were moistened and placed in a 10^0 C chamber for 3 days prior to planting in sand. Only fertile seeds were used and because of the difficulty of determining fertile seeds from external appearance, seeds were selected on the mean weight of seeds that had previously been confirmed as viable by dissection.

We compared the mean proportion of successful germinants within each treatment overall for each species using a one-way analysis of variance (ANOVA). Where an overall significant difference was detected, we conducted multiple pair-wise comparisons using Tukey's HSD. We also examined the relationship between cone width and the maximum temperature at which germination was substantially inhibited via linear regression. All analyses, including checking of ANOVA and regression assumptions, were conducted in R (R Core Team 2012) – no data transformations were necessary.

The similarity in many ecological traits between *Callitris, Widdringtonia* and *Pinus* invites comparison. Notional ecological strategies of *Callitris* and *Widdringtonia* were matched with those devised by Keeley and Zedler (1998) for *Pinus* to produce a diagram relating *Callitris and Widdringtonia* species to environmental axes of fire return interval and site productivity. Fire return interval was derived in part from Murphy et al. (2013) while productivity is an amalgamation of general soil type and rainfall distribution in areas where the different species grow.

Results

There is a wide range of cone sizes in *Callitris* (Fig. 1). Cones of the three South African species of *Widdringtonia* are similar in size to each other but are larger than cones in most species of *Callitris*. There is an over thirty-fold difference in dry weight of cones between the heaviest and the lightest. The mean dimensions of a cone of *C. sulcata* from New Caledonia are only 8.9 X 9.7 mm and 0.21g while a *C. preissii* cone from the coastal zone in south west of Western Australia has a mean dry weight of 6.7g and dimensions of 25.8 X 30.0 mm. There is a (predictable) positive correlation between the cone dry weight and the cone length ($r = 0.74$, $p < 0.001$) and cone width ($r = 0.91$, $p < 0.001$). The cones of all species open when they dry out after being removed from the plant (none requires heating to open) and fresh cones contain appreciable amounts of water ranging from 31 – 63% indicating that while closed they maintain a connection to the plant's xylem stream.

An assessment of how much effort a plant puts into reproduction can be gauged by how much biomass it puts into the reproductive structure in relation to the amount of seed produced. Number of seeds per cone is variable throughout the African and Australian species. There is a non significant positive relationship between cone dry weight and number of seeds. However there is a significant positive relationship between cone dry weight and fertile seed number ($r = 0.58$, $p < 0.001$) and with total ($r = 0.68$, $p < 0.001$) and fertile seed weight ($r = 0.50$, $p < 0.007$). Production of viable seed was generally low and particularly low in *C. macleayana, C. sulcata* and the resprouter species (*A. acuminatus, W. nodiflora*, Fig. 1), In the case of the first two species this is probably in part due to the fact the cones were collected from a single tree cultivated in a garden (*C. macleayana*) and from a sole adult survivor of an extensive fire in New Caledonia (*C. sulcata*). These two plants are likely to have been severely pollen limited with only self pollen being available. The highest proportions of fertile seeds were found in *A. arenarius, A. pyramidalis* and *W. schwartzii*. In *C. macleayana* the maximum fertile seed production can only be 12 seeds (two on each bract) and in *C. roei* and *C. drummondii* there can only be 6 fertile seeds. In these three species there are also sterile packing seed-like bodies in the cones but these seem never to form fertile seeds (see Fig. 1). Thus in *C. roei* and *C. drummondii* the realisable fertile seed proportion is much higher than for most other species (Fig. 2) although the number of fertile seeds will often be much lower. *Widdringtonia cedarbergensis* has the largest (heaviest) seed (0.09 g) of all the species, being almost three times the weight of a seed from a species with the next heaviest seed. The seed of this species is also unusual in that it is not winged and looks more like a nut than the winged samara types of the other species. The smallest seed is in *C. drummondii* (0.003 g). All *Actinostrobus* have quite large seeds (0.023 – 0.032 g) with narrow wings. In *A. pyramidalis* and *A. arenarius* the seeds have a large, noticeable gland between each wing (Fig. 1). This contains a pungent oil smelling strongly of “conifer” and is not found on the seeds of any other species.

Plotting the character of serotiny onto the strict consensus phylogram for *Callitris* and related genera of Piggin and Bruhl (2010, Fig 3) shows that the character has been developed and lost a number of times. The basal New Caledonian species are not serotinous and have very small cones. Of the next two to diverge (species with primary ranges in Queensland) *C. macleayana* is serotinous with a large cone while *C. baileyi* is not. The *Actinostrobus* species diverge next in a monophyletic clade and are all serotinous but with light weight cones. In the subsequent clades only one species (*C. endlicheri*) of five is generally not serotinous in clade Y. In clade Z the three central (arid) Australian and tropical species are not serotinous while the other four southern species are, mostly with rather large cones.

In the cone heating trials there was a clear indication that larger cones protected the seeds from heat better than smaller cones (Figs 4, 5). The cones of two *Actinostrobus* species tested showed similar insulating capacity and failed to protect the seeds beyond 300^{0} C. However *A. pyramidalis* showed significantly decreased germination from control levels at 200^{0} C. *A. acuminatus* had overall low germinability but showed decreased success beyond 200^{0} C and none at 300 ^{0}C. For the larger-coned species such as *C. macleayana* and *C. preissii* the cones protected the seeds until 600^{0} C. For *C. preissii* germination was unaccountably significantly decreased at 400^{0} but increased again for 500 and 600^{0} C but there was no germination after 600^{0}. *Callitris roei* showed no significant decrease in germination from control levels up to 250^{0} but produced no germinants at 300^{0}. *Widdringtonia* cones are amongst the largest of the cones in the Cupressaceae and provided good protection of seeds up to 500^{0}. At 600^{0} significantly fewer seeds survived in both species than at 500^{0} and the slightly larger cones of *W. nodiflora* proved a little more protective at 700^{0} than for *W.*

schwartzii. The poor germination of *W. nodiflora* control seeds is likely due to a poorer quality seed lot rather than any real treatment difference.

The cone characteristics of each species are just one part of the adaptive strategy related to the success and survival of the species in the community in which it grows. *Callitris* and *Widdringtonia* ecological strategies can be approximately matched with those devised by Keeley and Zedler (1998) for *Pinus* (Fig. 6). Site productivity is difficult to quantify but will be a combination of soil moisture and nutrient status. In general, *Callitris* and *Widdringtonia* grow in nutrient poor soils but in some cases where soils may be relatively fertile low rainfall may mean the integration of the two factors results in low site productivity. Species in areas with long or short fire return intervals tend to be non serotinous. Sites of intermediate fire return interval and intermediate site productivity tend to be occupied by serotinous species.

Discussion

Seed production

There is an allometric relationship between increasing cone size and a greater number of fertile seeds thus not supporting our first hypothesis. However fertile seed production is still very variable. All species are wind pollinated. As noted above the very low fertile seed number for *C. macleayana* and *C. sulcata* in this study is likely due to their isolation from other conspecific plants but does demonstrate that seeds can be produced from selfing. There has been little specific study of the possibility of masting in the group but it is common in other conifers (Keeley and Zedler 1998; Kelly and Sork 2002) and has been suggested for *C. intratropica* (Trauernicht *et al*. 2012) and *C. glaucophylla* (Thompson and Eldridge 2005). Mast flowering/coning has been considered to be advantageous for wind pollinated species as this helps to over come pollen limitation of seed set (Kelly and Sork 2002). *Callitris glaucophylla, C. endlicheri* and *C. intratropica* tend to occur in groves (Read 1995; Cohn *et al*. 2011; Lunt *et al*. 2011;Trauernicht *et al*. 2012) so mast male coning would serve to produce a very high pollen concentration to enhance pollen receipt by ovules. In *C. intratropica* denser stands tend to have higher seed germinability (Lawes *et al*. 2012). However in this present study *C. glaucophylla* and *C. intratropica* had some of the lowest proportions of fertile seed. Producing infertile seeds has been advocated as a way of enhancing plant fitness via predator confusion – the sacrificial sibling hypothesis (Ghazoul and Satake 2009). In the group of species studied here *C. macleayana, C. drummondii* and *C. roei* particularly demonstrate support for this idea. All three species can produce only a set number of fertile seeds while still producing extra seed-like bodies that could help to dissuade both pre and post dispersal seed predators by providing a low return on foraging effort. Seed production varies between populations. Total and fertile seed numbers in this sample of *C. verrucosa* are approximately twice that for populations from central NSW (Bradstock and Cohn 2002). The sprouter *W. nodiflora* has a much smaller proportion of fertile seeds than its congeneric seeder species, and despite similar number of seeds in the two populations sampled here the proportion of fertile seeds in one population was approximately half that of the other. Keeley *et al*. (1998) found recruitment of *W. nodiflora* was highly correlated with stem density of parent trees but was still very variable between different sites and this is likely to be related to variability in fertile seed production.

Cones and heat protection of seed

There is no support for our second hypothesis and, as might be expected, larger cones insulate the seeds against the heat of a fire better than smaller cones and this is similar to results for a wide range of both conifers (eg, (Habrouk *et al*. 1999; Reyes and Casal 2002) and angiosperms (Judd and Ashton 1991; Bradstock *et al*. 2006). Fire intensity and duration in the canopy of plants will vary depending on the environmental conditions and fuel availability. Even in very severe crown fires in tall eucalypt forests (where regeneration of the dominant species is dependent on severe fire) some seed survives to produce new seedlings, despite the fact that the small capsule of species like *Eucalyptus regnans* provided little effective insulation against temperatures as mild as 250^{0} for 20 seconds (Judd and Ashton 1991). *Widdringtonia nodiflora* cones protect seeds better at the highest temperature than do *W. schwartzii* and this may be related to the structure of the vegetation in which the species grow. Communities with *W. nodiflora* are better able to carry a fire than is the vegetation where the other species grows. In *Hesperocyparis* species heat was needed to open the cones of some species but no cones protected the seeds above 400^{0} C when this was applied for more than 2 minutes. Unfortunately there was no indication of cone size of the different species in that study (Milich *et al.* 2012). In *Pinus,* seeds from cones heated to 400^{0} for 2 minutes has less than 5% germination for *P. nigra and P. sylvestris* but for *P. halepensis* 75% of seeds germinated from the same temperature and time treatment (Habrouk *et al.* 1999).

Cones and seed predation

There has been little research on seed predation in *Callitris* or *Widdringtonia* to be able to assess whether cone size is related to predator dissuasion. A highly speculative account of conifer cone size through geological time advocated that an increase in size is correlated with increases in animal predators of seeds (Leslie 2011). Cockatoos will descend on *Callitris* soon after a fire and eat the seeds from the opening cones (Ladd pers observation). However there are no specific records of the birds attacking closed cones. Small wasps have been recorded parasitizing *C. glaucophylla* cones (Lacey 1973 in Thompson and Eldridge 2005) and in the present study only *C endlicheri* had some cones damaged by invertebrate predation (a small wasp). Baboons, hydraxes and rodents are apparently seed predators of *W. cedarbergensis* (Andrag 1977 in Thomas 1995) but their influence on recruitment has not been assessed.

Serotiny in relation to species distribution

The majority of serotinous southern hemisphere Cupressaceae occur in relatively dry temperate latitudes but the group seems to have been derived from wet forest ancestors that were not serotinous. The oldest fossil *Callitris* are dated at early Oligocene (morphologically similar to *C. sulcata*) but the crown group of the genus is considered to be much younger, being dated at from 8.4 – 19 My depending on the phylogenetic method used (Crisp and Cook 2011). Crisp and Cook (2011) noted that *Callitris* is one of the few gymnosperm lineages that seems to have adapted to the aridification of Australia but the radiation of the group is much later than several angiosperm lineages that have been examined. Although conifer fossil cones from the Cretaceous in America have been assigned to *Widdringtonia* the earliest fossil record in South Africa is based on wood from the Knysna formation (Phillips 1927) that has been dated at post Miocene (Carr *et al*. 2010). While this record for *Widdringtonia* is of a similar age to those for *Callitris* from the southern hemisphere use of wood for identification of a genus of conifer is not really very reliable.

Tropical and arid areas provide little selective impetus for serotiny to develop. Recruitment opportunities can occur in the tropics either at any time during the year (e.g. New Caledonia) or in the regular wet season. In the arid zone of Australia wet events of sufficient magnitude to enable recruitment are normally associated with summer rather than winter rainfall coming from tropical low pressure systems that penetrate deeply into the continent. This timing coincides with seed fall in *C. glaucophylla* (Cohn *et al*. 2011) in central south eastern Australia. In arid Australia the reliability of occasional establishment opportunities outweighs the extreme unreliability of fire as an establishment impetus due to low fuel loads and the even less reliable coincidence of a fire being followed by sufficient rain to enable seedling growth.

Although the characteristic of serotiny is assigned as a specific state in recent phylogenetic studies of the evolution of fire related characters (He *et al*. 2011; 2012) the character seems to be somewhat labile in many plant species (e.g. Cowling and Lamont 1987; Bond *et al.* 2004). As with serotinous pines (Gaulthier *et al*. 1996; Nathan *et al*. 1999; Salvatore *et al*. 2007) there is likely to be variation in degree of serotiny depending on the location and the type of fire regime in widespread *Callitris* species. *C. glaucophylla* is generally not serotinous but does retain seeds for more than a year in south eastern Victoria (Clayton-Greene and Ashton 1990) and *C. endlicheri* seems to be serotinous in the south (Lunt *et al*. 2011) but not in the central north of NSW (Cohn *et al*. 2011). In *Cupressus* (*Hesperocyparis*) there are similar examples e.g. tecate cypress in southern California and Mexico (Gouvenian and Delgarilo 2012) and in *P. halepensis* in Israel (Goubitz *et al*. 2004). The interaction of fire frequency and severity in particular regions is likely to be a critical component influencing the degree of serotiny in different species (Ne'eman *et al*. 2004).

There is certainly a relationship between cone size and being serotinous. The species with the smallest cones are not serotinous. However the species with the largest cones (*C. preissii*) and the former *Actinostrobus* species (smaller serotinous cones) both grow in basically the same climatic zone for some of their ranges.

It is likely that there is a trade off between cone size and number of cones that can be produced. *A. pyramidalis* can produce an average of 43.1 ± 5.2 cones per plant in 8 years (on plants of mean stem diameter 1.3cm, Ladd unpublished) while *C. verrucosa* plants (with much larger cones) of over 40 years old had less than 20 closed cones per plant (Bradstock and Cohn 2002). In addition a high variability of fire return time is likely to favour an increase in seed production rather than seed protection (Tonnabel *et al*. 2012). Fire return times in swampy heathlands where *A. pyramidalis* grows may be less regular than in coastal or arid inland sites where species such as *C. verrucosa* and *C. preissii* grow and favour more but less protective cones in *Actinostrobus*.

The cone characteristics of each species are just one part of the adaptive strategy related to the success and survival of the species in the community in which it grows. *Callitris* and *Widdringtonia* ecological strategies can be approximately matched with those devised by Keeley and Zedler (1998) for *Pinus* (Fig. 6). In subtropical New Caledonia fire is unlikely to have been a strong selective force until about 3000 years ago when humans arrived. There are no serotinous species on New Caledonia and *Callitris sulcata* has a small, nonserotinous cone and seedling characteristics (long juvenile period when plants have widely spread, relatively large leaves) that indicate it establishes in competitive situations. At least in the past the species would have occupied sites subject to very rare stand replacing fires. Now the species occupies river valleys that are to some extent fire refuges and provide establishment sites on unstable steep slopes. In pines, species with this strategy have some tolerance of closed canopy conditions and can exploit opportunities provided by other disturbances (Keeley and Zedler 1998) as would be appropriate for *C. sulcata*. *C. macleayana* on the other hand occurs on the edge of closed forests (Ash 1983) and would be subject to infrequent but severe stand replacing fires – the large cone would be effective in protecting the seeds in severe fires. Similarly to *C. sulcata* the juvenile period is long and the juvenile leaves are more widely spreading than on most other *Callitris* species as seedlings would need to compete with fast growing early successional angiosperms after stand replacing fires.

Callitris glaucophylla from arid central Australia fits into the "no predictable fire, low productivity" category. Seedling establishment is rare and trees grow slowly. Prior *et al*. (2011) found that there had been a severe recruitment deficit in the arid zone populations of *Callitris* (*C. glaucophylla)* that they examined in a wide ranging study of the *C. columellaris* complex. Fire, when it does occur may remove many adults, contributing to the patchy distribution of stands in these areas. Seedling establishment is normally dependent on above average rainfall episodes (Read 1995; Cohn *et al*. 2011). In the Pilliga forest the trees occur in patches. Within these patches fire damage is less than in intervening areas under moderate to low fire severity but the patches cannot ameliorate fire damage under intense fire conditions (Cohn *et al*. 2011). A similar situation is found in the Northern Territory where groves of *C. intratropica* are able to exclude low intensity fires due to a different understorey composition and fine fuel load than in areas away from the dense stands (Trauernicht *et al*. 2012).

Widdringtonia schwartzii and W. cedarbergensis occupy an ecological position between the predicable stand replacing fires and the no predictable fires areas of the diagram (Fig. 6). Both are poorly fire tolerant and grow in rocky landscapes with low understorey density and/or in fire shadow valleys (*W. schwartzii*). Both have relatively weak serotiny with generally only one season's cone load present or in *W. schwartzii* a variation in different populations from serotinous to nonserotinous (W. Bond pers com 2012). In deep, sheltered gullies inter-fire recruitment may be relatively successful. Trees also self prune very well, providing little fuel for fires to "ladder" up trees. However fires under severe, hot, windy weather conditions may kill adult plants over extensive areas. Cones are large and provide good seed protection, so as long as fires are infrequent, regeneration will generally be reliable.

Most *Callitris* species occupy sites where predictable stand replacing fires will occur. Most of these species have cones with reasonably thick valves. Pines at similar sites are either basal resprouters or have serotinous cones (Keeley and Zedler 1998). In *Callitris* and *Widdringtonia* there are only two resprouters (*W. nodiflora* and *A. acuminatus*) and all species have serotinous seed banks of variable storage duration. The heathland to low open forest where most of the serotinous species grow tends to have a relatively dense understorey of low shrubs and graminoids making interfire establishment difficult but carriage of fire efficient (Keith *et al*. 2002). Understorey density coupled with a generally winter rainfall maximum in the temperate zone makes it difficult for seedlings to survive through dry summer conditions if there has been no stand clearance. An exception to this is *A. pyramidalis* that grows in ephemeral winter wet swamps in Western Australia. Here open ground may be available due to the limited number of species that seem to be able to cope with waterlogging in winter and extreme drought in summer. In mature stands of *C. pyramidalis* seedlings can establish interfire because the soil moisture conditions are reliably high in winter/spring and cones do release seed after several years (Ladd unpublished data). Cone production in some species (e.g. *C. verrucosa*, Bradstock and Cohn 2002) is variable between years but serotiny will even out the fluctuations. However the unpredictability of reliable rain following fire means that seedling recruitment can be variable and may lead to the patchy distribution of *Callitris* species with this life history strategy.

It can be considered *Callitris intratropica* occupies productive sites (with relatively reliable rainfall in summer) and high fire frequency (from 2 – 8 years, Price and Bowman 1994). High intensity fires are inimical to population survival of *C. intratropica*. However mature trees can survive low intensity fires. Smaller trees are more likely to be killed than large trees (Prior *et al*. 2007) but even some small saplings can survive mild fires, especially if they are patchy (Russell-Smith 2006). It is considered that the distribution and maintenance of *C. intratropica* populations has diminished since the cessation of prehistoric and early historic Aboriginal burning patterns due to the lower incidence of frequent, low intensity fires and less frequent but high intensity fires that kill many saplings and also adult trees (Price and Bowman 1994; Prior *et al*. 2007). In the most fire prone areas of the tropics *C. intratropica* is restricted to rocky fire refuge sites (Prior *et al*. 2011).

It might be considered that the serotinous species should not be found in the same climatic conditions as the non serotinous species. However this is not always the case. Fire is not essential for *Callitris* regeneration as the cones of serotinous species are xeriscent, not pyriscent and there are many examples of abundant regeneration of some species in the absence of fire after wet years (Thompson and Eldridge 2005). *Callitris glaucophylla* may occur with *C. verrucosa* in relatively arid parts of New South Wales (Thompson and Eldridge 2005) on sandy soils and in arid Western Australia *C. glaucophylla* occurs in the same general area as *C. preissii* but the species grow in different plant communities and on different soil types. The vegetation with *C. glaucophylla* is relatively open and with a sparse understorey on rocky or clayey substrates while *C. preissii* occurs in much denser heath vegetation on sand. These different vegetation types would have very different fire regimes with the heaths being much more fire prone. Thus our third hypothesis is not supported and the development of serotiny is a product of the environmental conditions and community in which a species grows. This is a general conclusion that is supported by the variation in degree of serotiny in other taxa such as tecate cypress (Gouvenian and Delgarilo 2012), several *Pinus* species, some *Widdringtonia* and *Callitris* populations and angiosperms (eg, Cowling and Lamont 1987; Whelan *et al.* 1998; Bond *et al*. 2004).

Acknowledgements

PGL thanks the Botany Department at the University of Cape Town for support during preparation of this paper; M. Henwood and M. Lawes collected cones of some species. T. Hall assisted in the field in South Africa.

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Table 1. Species from which cones were sampled, location and sample size

Species	Collection location	No. of cones measured
Callitris (*Actinostrobus*) *acuminata* (Parl.) F.Muell. (1)	30°28′13.99″S, 115°22′10.78″E	23
C. acuminata (2)	31°58′33.56″S, 115°59′36.99″E	20
C. acuminata (3)	29°43′57.77″S, 115°13′55.92″E	20
C. (*Actinostrobus*) *arenaria* (C.A.Gardner) J.E.Piggin & J.J.Bruhl[A]	30°50′30.41″S, 116°47′53.59″E	17
C. arenaria	29°49′48.90″S, 115°14′20.77″E	21
C. (*Actinostrobus*) *pyramidalis* Sweet ex J.E.Piggin & J.J.Bruhl[A]	32°02′56.79″S, 115°55′59.71″E	20
C. pyramidalis	32°02′56.79″S, 115°55′59.71″E	20
C. macleayana (F.Muell.) F. Muell.[A]	Cultivated	16
C. roei (Endl.) F.Muell.	Not recorded	22
C. roei[A]	33°06′11.91″S, 118°45′50.68″E	25
C. drummondii (Parl.) F.Muell.	33°37′57.60″S, 120°09′33.98″E	21
C. tuberculata R.Br. Ex R.T.Baker & H.G.Sm.	31°16′31.14″S, 119°52′03.56″E	22
C. rhomboidea R.Br. Ex Rich.	41°40′51.73″S, 148°16′45.5″E (approximately)	22
C. oblonga Rich.	41°53′44.64″S, 148°15′25.67″E	22
C. muelleri (Parl.) F.Muell.	33°44′50.73″S, 150°22′39.08″E	25
C. gracilis murrayensis (J.Garden) K.D.Hill	35°13′19.21″S, 138°29′38.28″E	12
C. endlicheri (Parl.) F.M.Bailey	NSW	13
C. intratropica R.T.Baker & H.G Sm.	13.10403, 130.79395	25
C. glaucophylla J.Thompson & L.A.S.Johnson	30°28′13.99″S, 115°22′10.78″E	13
C. preissii Miq.1	31°15′21.35″S, 119°27′10.21″E	10
C. preissii 2[A]	32°02′56.79″S, 115°55′59.71″E	23
C. preissii 3	30°59′9.34″S, 121°09′20.08″E	25
C. preissii 4	30°28′54.72″S, 119°33′32.31″E	20
C. verrucosa (A.Cunn. Ex Endl.) F.Muell.	30°59′9.34″S, 121°09′20.08″E	17
C. canescens (Parl.) S.T.Blake (1)	31°22′54.73″S, 118°43′43.98″E	20
C. canescens (2)	31°22′30.75″S, 118°43′08.97″E	10
C. sulcata (Parl.) Schltr.	22°06′27.06″S, 166°30′40.66″E (approximately)	7
W. nodiflora (L.) Endl.	33°58′56.22″S, 18°26′4.10″E	25
W. nodiflora[A]	33°34′16.73″S, 19°08′18.47″E	22
W. cedarbergensis Marsh	32°08′27.01″S, 18°56′17.33″E (approximately)	25
W. schwartzii (Marloth) Mast[A]	33°30′43.67″S, 23°38′28.83″E	23

[A]Species and sample used in the heat trials.

Figure 1. *Callitris* cones and seeds in front of the relevant cone of the species. From left to right – *C. roei* (large seed is fertile and small is an infertile seed-like structure), *C. (Actinostrobus) pyramidalis* (the dark patch on the centre of the seed is an oil gland), *C. macleayana* (large seed is fertile and small is an infertile seed-like structure), *C. preissii*. Scale is in millimeters.

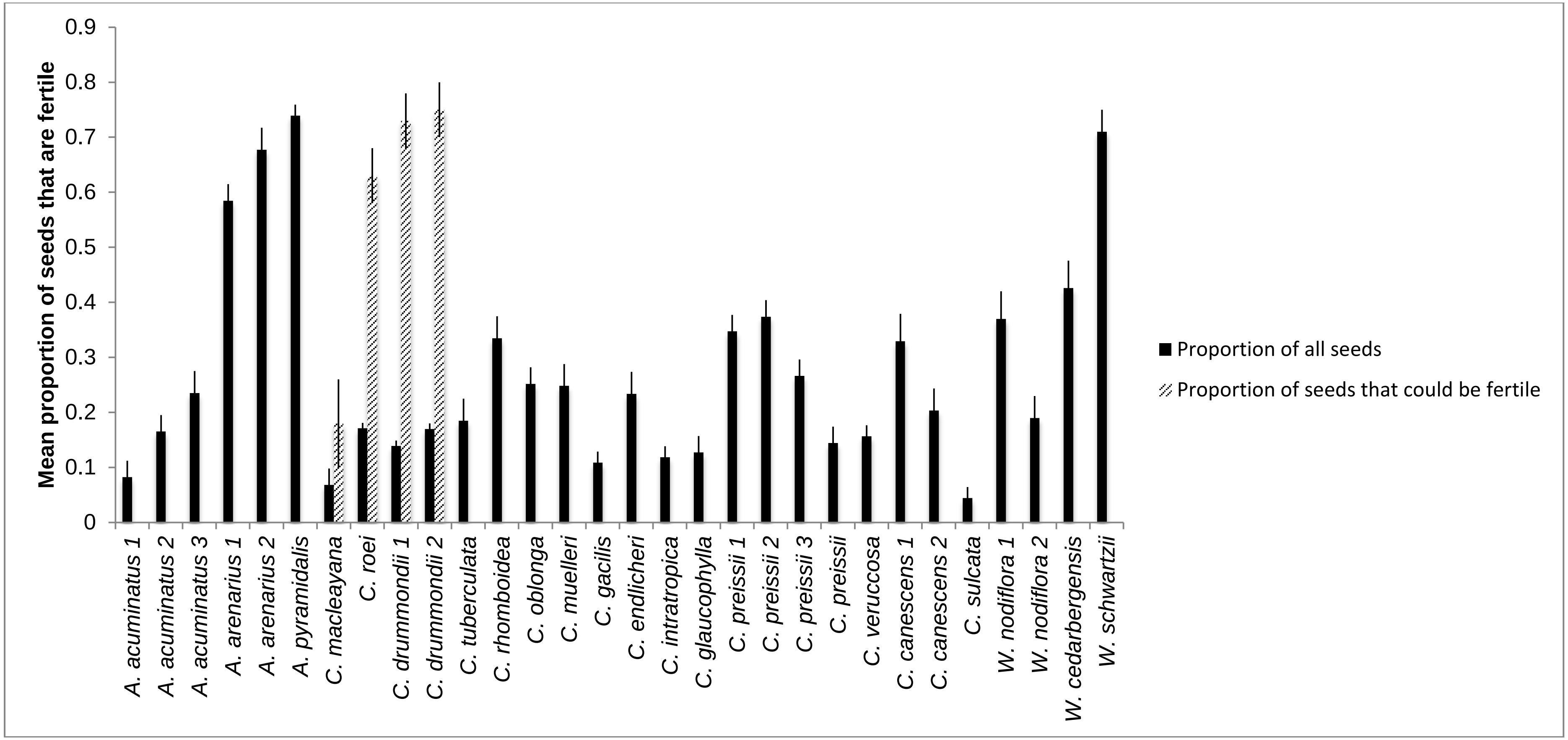

Figure 2. Mean proportion of fertile seeds (± SE) produced by *Actinostrobus, Callitris* and *Widdringtonia* species. Number after species names refer to different populations. The proportion of seeds that could be fertile only applies to *C. macleayana* that can only produce a maximum of 12 fertile seeds and *C. drummondiii* and *C. roei* that can only produce six.

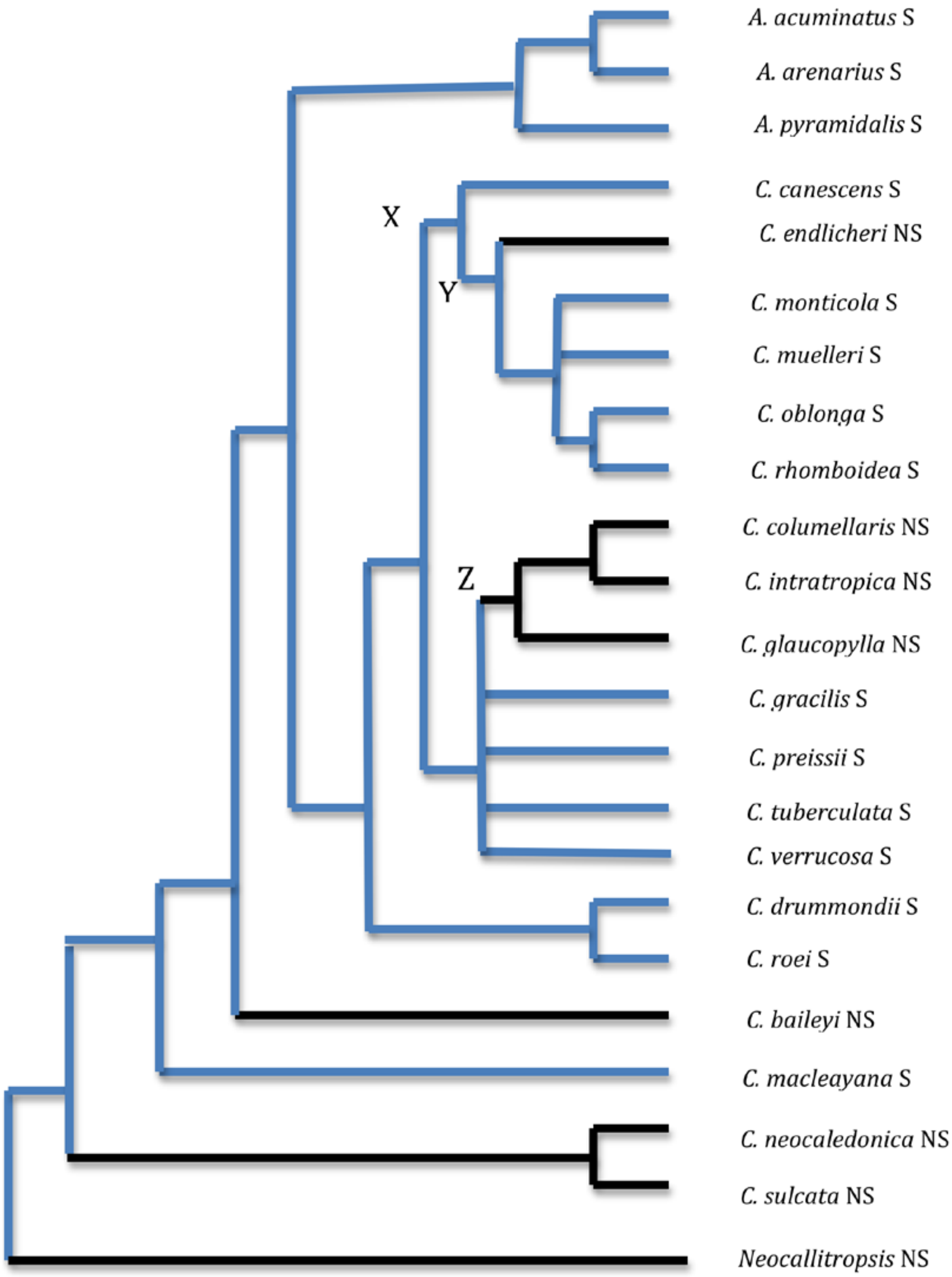

Figure 3. Phylogram of *Actinostrobus* and *Callitris* species modified from Piggin and Bruhl (2010). *C = Callitris, A = Actinostrobus,* X, Y and Z are clades mentioned in the text, S = serotinous, NS and dark lines = Nonserotinous.

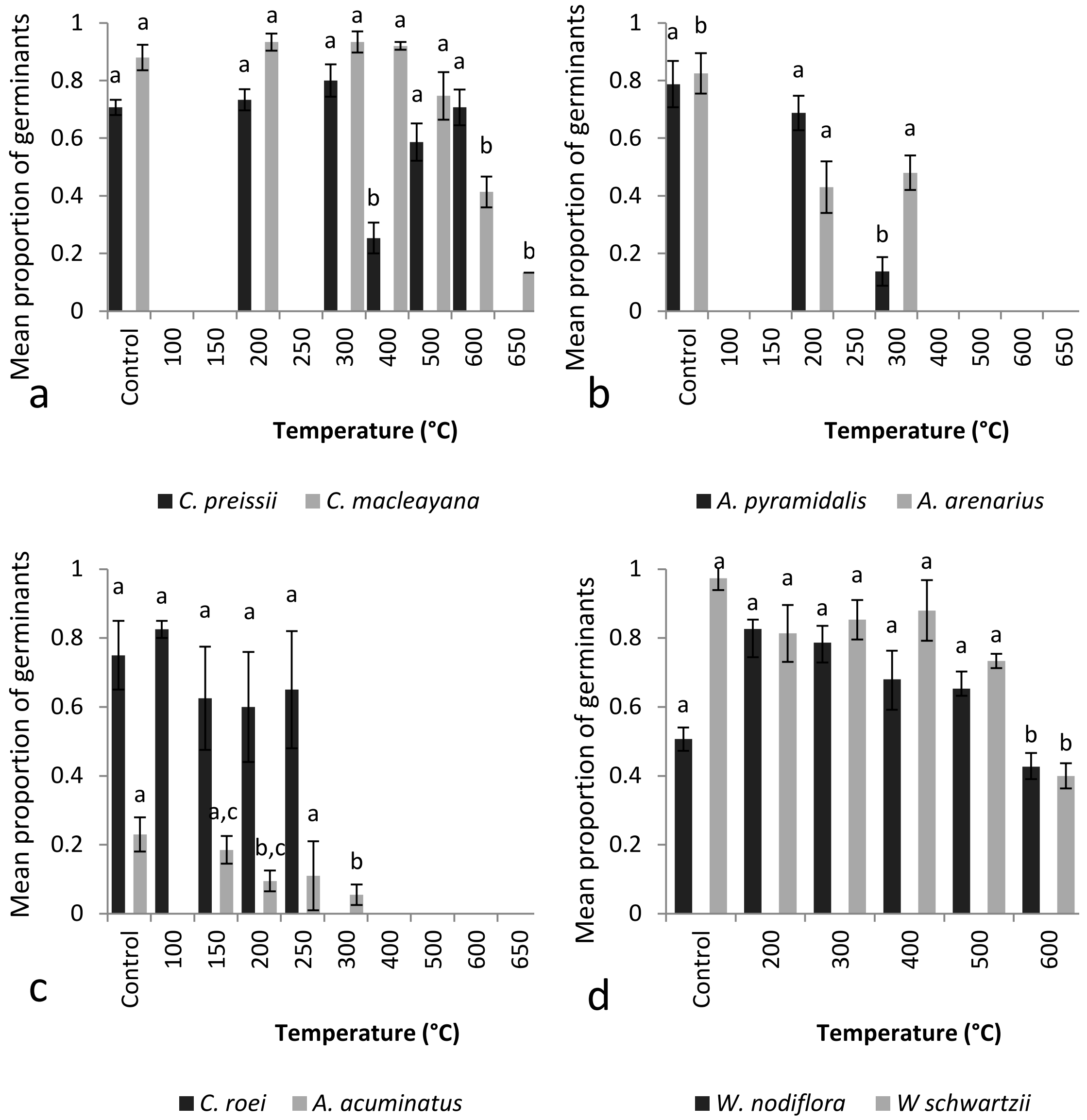

Figure 4. Mean proportion (± SE) of seeds that germinated from heat-treated cones of; (a) *C. preissii* & *C. macleayana*, (b) *A. pyramidalis* & *A. arenarius,* (c) *C. roei* & *A. acuminatus* and, (d) *W. nodiflora* & *W. schwartzii*. Columns with different letters are significantly different.

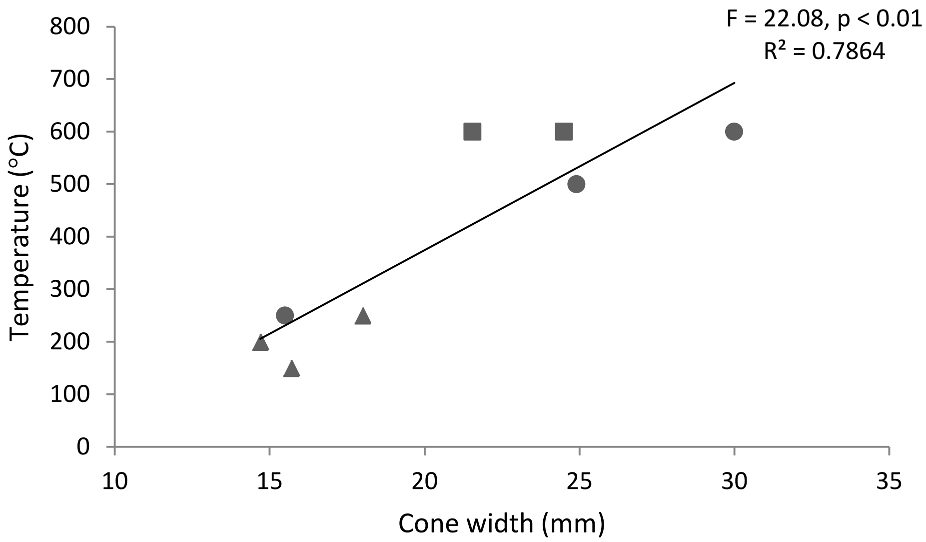

Figure 5. Relationship between cone width (as a surrogate for cone size in general) and the temperature at which germination of seed is severely decreased. Circle = *Callitris* spp., Triangle = *Actinostrobus* spp., Square = *Widdringtonia* spp.

A – No predictable fire , B – unpredictable stand replacing fire, C – Predicatable stand thinning fire, D – Predicatable stand replacing fire.

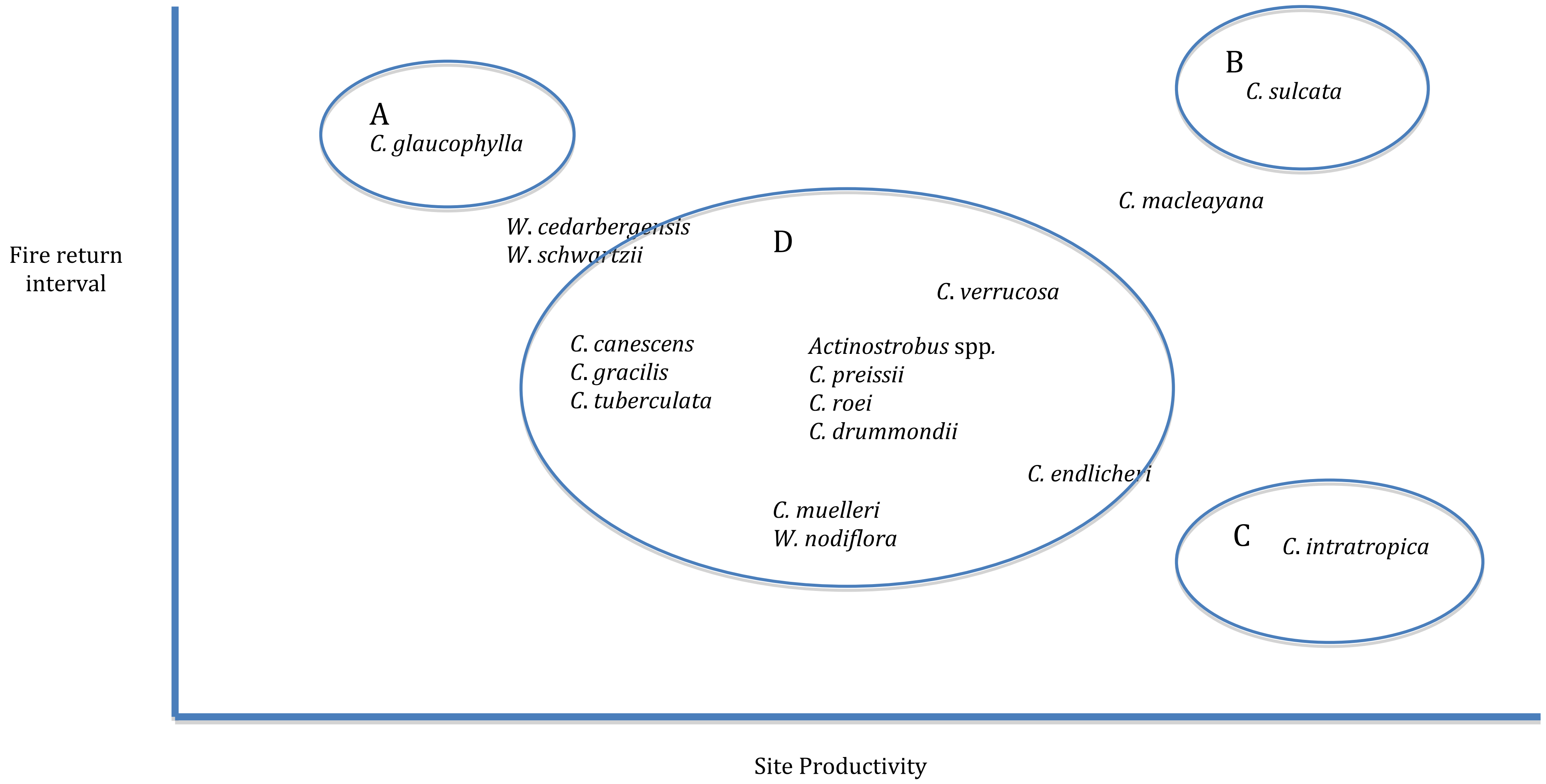

Figure 6. Diagram indicating the ecological position of southern hemisphere cupressaceous species in relation to fire return interval and site productivity. A = no predicable fire; B = unpredictable stand replacing fire; C = predictable stand thinning fire; D = predictable stand replacing fire.