Morphological and anatomical investigation of seed cones of *Cupressus glabra* (Cupressaceae): evolutionary aspects

Abstract

Within this study, the morphological and anatomical features of *Cupressus glabra* Sudw. seed cones were investigated, focused on the vascular supply to cone scales and ovules. These investigations should help towards a better understanding of the branching pattern both within seed cones of *C. glabra* and within the primitive cupressaceous seed cones in general. Seed cones of *C. glabra* are strongly reduced and compact structures. They typically lack sterile distal elements, so that all terminal cone scales are usually fertile, and the apex of the cone axis is not visible. The ovules are arranged in up to four axillary rows. Each row, which can be understood as a descending accessory shoot, consists of several ovules. Within a bract/seed scale complex the cone scale and its axillary ovules are each supplied by an own vascular bundle. The vascular bundle strands of the cone scale and of the ovules do not fuse and enter the concentric stem bundle of the cone axis in separate strands. This situation corresponds quite well to the situation of a bract carrying a fertile axillary shoot. It seems that the seed scale was entirely reduced to its ovules, with no further vegetative structures belonging to it were visible. These results suggest the original cupressaceous seed cone was a more open and looser structure, which became strongly reduced to form the highly condensed compact seed cones of the extant *Cupressus* taxa.

Keywords: *Cupressus*, seed cone, morphology, anatomy, vasculature, evolution.

1 Introduction

The reproductive structures of conifers are arranged in compact, unisexual cones. The staminate male ones are called pollen cones; the female, ovuliferous ones, seed cones. Seed cones of extant conifers consist of two types of cone scales, the bract scale and the seed scale, both together are forming the bract/seed scale complex (e.g. Coulter & Chamberlain 1917; Pilger 1926; Florin 1951, 1954; Schweitzer 1963; Stützel & Röwekamp 1999). The seed cones consist species specific of one up to several hundred bract/seed scale complexes. The bract scale, which carries the axillary seed scale, corresponds to a leaf, whereas the seed scale represents a reduced and modified, fertile axillary shoot carrying the ovules. Thus, the coniferous seed cones are compound, polyaxial, inflorescence-like structures (e.g. Schuhmann 1902; Herzfeld 1914; Sporne 1965; Stewart & Rothwell 1993; Escapa et al. 2008; Taylor et al. 2009; Bateman et al. 2011; Rothwell et al. 2011; Rudall et al. 2011; Spencer et al. 2015; Dörken & Rudall 2018). This understanding of the coniferous seed cone structure is based on a concept originating from the morphology of Pinaceae seed cones and from the fossil record. Among extant conifers, the formation of the bract and seed scales as two distinct structures is only visible within Pinaceae and Sciadopityaceae, even at maturity in both families (e.g. Takaso & Tomlinson 1991; Mundry 2000; Dörken & Rudall 2018). Within all other extant coniferous families, the bract/seed scale complex became greatly modified and reduced. However, the evolutionary pathway leading to the compact seed cones of extant conifers is still highly controversial.

Unlike Pinaceae and Sciadopityaceae, only one type of cone scale can be found within cupressaceous seed cones. The bract and seed scales as two distinct structures are not visible, neither at pollination time nor at maturity. Among modern Cupressaceae *s.str.* (Cupressoideae and Callitroideae), all vegetative parts of the seed scale have been entirely reduced so that only the ovules remain, which can be understood as representing the only visible structures of the seed scale. Thus, the visible cone scale in Cupressaceae *s.str.* is represented exclusively by the bract scale (e.g. Page 1990; Farjon 2007, 2010; Gadek et al. 2000; Dörken 2012a, 2012b; Groth et al. 2011; Jagel &
Dörken 2014, 2015a, 2015b; Dörken & Jagel 2017; Dörken & Rudall 2018), which carries the ovules always in an axillary position and never on its adaxial surface (Jagel & Dörken 2015a, 2015b). In their early ontogenetic stages a weakly developed, axillary bulge-like primordium is developed, forming the ovules. This could be interpreted as representing the rudimentary seed scale. In most of the derived Cupressaceae s.str., the ovules are inserted in one or more successively developed rows in the axil of a cone scale. Within a row the single ovules develop centrifugally, whereas each row develops centripetally (Jagel & Dörken 2015a, 2015b). Each row of these axillary ovules is interpreted as an accessory descending fertile short shoot (e.g. Jagel 2001a, 2001b; Dörken 2012b; Jagel & Dörken 2015a, 2015b; Dörken et al. 2017) lacking any visible, sterile vegetative parts of the shoot axis. Thus, the seed scale can be understood as entirely reduced to its ovules.

However, among the basal taxa belonging to the Cupressaceae s.l. (the members of the former Taxodiaceae family), the bract and seed scale are completely fused to each other and form a common type of cone scale, which carries the ovules on its adaxial surface. However, within this fusion product, it is still unclear which exact portion of the cone scale belongs to the bract and which part belongs to the seed scale. Within some Cupressaceae s.l., in the earliest ontogenetic stages weakly developed tongue-like structures on the upper surface of the cone scales are developed. They can be interpreted as vegetative parts of the seed scale as is the case in e.g. Cunninghamamia and Athrotaxis. This indicates that in basal Cupressaceae the cone scale represents a fusion product of both bract and seed scale (Schulz & Stützel 2007; Jagel & Dörken 2014; Dörken & Rudall 2018).

In this study the morphology and anatomy of Cupressus glabra Sudw. seed cones were investigated. The study is focused on the formation of the vasculature within the seed cone and should help to answer the question if additional to the ovules further, however, sterile structures, can be found, which could be interpreted as belonging to the seed scale. The results should help towards a better understanding of the original branching pattern within Cupressus seed cones.

2 Material and Methods

Material

Cupressus glabra (Cupressaceae s.str.) is a monoecious, evergreen tree species, native to southern USA and northern Mexico. The investigated seed cones were collected by one of the authors (VMD) at pollination time on 12.04.2010-04-12 from a tree cultivated in the Botanic Garden of the Ruhr-University, Bochum (Germany).

Methods

Freshly collected material was immediately fixed in FAA (100 ml FAA = 90 ml 70% ethanol + 5 ml acetic acid + 5 ml formaldehyde solution 37%), thereupon being stored in 70% ethanol. The anatomy of the cones was studied from serial sections using the classical paraffin technique and subsequent astra blue/safranin staining (Gerlach 1984). Microphotography was accomplished with a digital microscope (Keyence VHX 500F) equipped with a high-precision VH mounting stand with X-Y stage and bright-field illumination (Keyence VH-S5).

3 Results

3.1 Morphology of wild-type seed cone

Seed cones are inserted terminally on short lateral shoots. They are in a more or less plagiotropic to upright position at pollination time (Fig. 1A) and at maturity as well. The seed cones consist of only a single type of cone scales (Fig. 1B). At pollination time the seed cones are about 4 mm in length and about 3 mm in width. The ovules do not exceed the cone scales. The seed cones consist of (2-)3-4 pairs of decussate fertile cone scales (Fig. 1B). Below the fertile cone scales two pairs of sterile decussate transitional leaves are developed (Fig. 3C). Sterile distal cone scales are usually absent. Sometimes the rudimentary apex of the cone axis is still visible as a small, central columella. Typically, the apex of the cone axis is completely used up, while the distal pair of scales is developed (Fig. 1E).
Fig. 1: *Cupressus glabra*, seed cone morphology.

**A-C**: At pollination time; **D-F**: At maturity; **A**: Position of seed cones within the branching system; **B**: Top view of a seed cone with several pollination drops; **C**: Fusion of pollination drops of adjacent ovules (arrow); **D**: Open seed cone in lateral view, the tip of the cone scale visible as a distinct dorsal prickle; **E**: Open seed cone in top view, with a weakly developed central columella; **F**: Open seed cone from below.
Within each cone scale, the ovules are inserted in up to four rows (Fig. 1C). Within a row several ovules are developed. The ovules are always inserted axillary and never on the surface of the cone scale. Within a seed cone the second and third pair of cone scales in the middle part of the cone develops the highest number of ovules (Fig. 1C). Due to a lack of space within the seed cone the ovules are always inserted closely to each other. As a result, the ovules of the distal cone scales accumulate all together on a convex plain at the tip of the cone (Fig. 1B).

At pollination time the cone scales are spreading widely, so that the necks of the micropyles and the pollination drops are freely exposed to the airflow (Figs 1B & 1C). A fusion of pollination drops of adjacent ovules is common and occurs between neighboured ovules within the same row, but also between ovules from different rows. This fusion allows the formation of a significantly larger pollination drop and increases the success in pollen capture (Fig. 1C). The cone scales are strongly covered with a thick cuticle, enabling the large pollination drops to lean on its surface which allows a further increase of the pollination drop size (Fig. 1C).

After successful pollination, the seed cones close rapidly by a strong ventral swelling of the cone scales. The ventral bulge is formed by a strongly swollen tissue rich in water, which finally is so strongly developed that the cones get closed rapidly after pollination and the ovules can develop well protected within the cone. Due to this ventral swelling the tip of the cone scale is shifted onto the back of the cone scale, visible as a short backward dorsal umbo (Figs 1D to 1F). In addition, resin secretions from the cone scales help to seal the maturing cones. At maturity, the cone dries out and the cone scales shrink (Figs 1D to 1F), so that the winged seeds are freely exposed and can be dispersed from the opened cone by the wind. Once opened, the seed cone cannot close again.

3.2 Anatomy of wild type seed cones

The cone axis shows a concentric stem bundle with inner xylem and peripheral phloem (Fig. 2B). The xylem and phloem are not separated from each other by any cambium. In the centre of the cone axis a large section of pith is developed (Fig. 2B). Collateral leaf traces in an opposite arrangement leave the concentric stem bundle and enter the cone scales in basal parts (Figs 3A & 3B), where they branch several times dichotomously (Fig. 3B). Each of the vascular strands ends blindly in the tissue of the cone scale. They do not fuse (Fig. 3D). Within the fertile cone scales, an additional bundle strand is developed supplying the ovules (Figs 2C, 3D & 3F). However, not all ovules have a vascular supply. Only the lowest row located directly on the cone scale shows a vascular supply, which is lacking in the distal rows. However, within the lowest row only the outer ovules, which are those developed first and are the oldest ones within the row, have a vascular supply in the form of a distinct strand of vascular bundle (Fig. 3E). The inner ovules within this row lack a direct connection to a vascular bundle and are supplied by a cell-to-cell transport. Thus, within a fertile bract/seed scale complex, at least two vascular bundles are developed (Fig. 3), which enter the concentric stem bundle of the seed cone in separate strands (Figs 2C & 3D). Fusion of the vascular bundles of the cone scale and the ovules does not occur.

3.3 Anatomy of teratological seed cone

In addition to the wild type of seed cones with decussate cone scales, some teratological seed cones show cone scales in trimerous whorls were found (Fig. 2D). Here an abrupt change in the phyllostaxis from decussate vegetative scale leaves (Fig. 2A) to the trimerous arranged cone scales took place (Fig. 2D). All other features are similar to the normal type of seed cones.
Fig 2: *Cupressus glabra*, anatomy of seed cones at pollination time, I.

A: Sterile leaves below the fertile cone scales in a decussate arrangement at the shoot axis; B: Detail of the central cone axis showing a concentric vascular stem bundle with inner xylem and peripheral phloem and a large central pith; C: Cone scales and ovules supplied by their own vascular bundle; D: Trimerous arranged cone scales, each supplied by a collateral vascular bundle; the vascular bundles of the cone scales come out of the concentric stem bundle in more or less the same plane (arrows).

A = axis, CS = cone scale, CSB = concentric stem bundle, O = ovule, P = phloem, TL = transitional leaf, VB = vascular bundle, VBCS = vascular bundle of the cone scale, VBO = vascular bundle of the ovules, VL = vegetative leaf, X = xylem.
**Fig. 3: Cupressus glabra**, anatomy of seed cones at pollination time, II.

**A,C-E:** Longitudinal sections; **B & F:** Cross sections; **A:** The fertile bract/seed scale complex supplied with a collateral vascular bundle which branches several times (arrows); **B:** Dichotomous branching pattern of vascular bundles supplying the cone scale; **C:** Overview of a whole seed cone; **D-F:** A distinct separate vascular bundle supplying the ovules of the lowest row located directly at the cone scale; within this row only the outermost ovules are connected to the vascular bundle, the innermost ovules are supplied by a cell-to-cell transport. CS = cone scale, CSB = concentric stem bundle, I = integument, N = nucellus, O = ovules, TL = transitional leaves, VBO = vascular bundle of the ovule, VBCS = vascular bundle of the cone scale.
4 Discussion

4.1 Seed cone morphology and anatomy

Conifer seed cones are characterized by the presence of the bract/seed scale complex. The bract scale represents a leaf carrying axillary a fertile short shoot, the seed scale, which produces the ovules (e.g. Schuhmann 1902; Herzfeld 1914; Sporne 1965; Stewart & Rothwell 1993; Escapa et al. 2008; Taylor et al. 2009; Bateman et al. 2011; Rothwell et al. 2011; Rudall et al. 2011; Spencer et al. 2015; Dörken & Rudall 2018). However, this bract scale/seed scale complex became variably modified within the different coniferous groups so that in some taxa bract and seed scale are fused to each other e.g. within Araucariaceae or Cupressaceae s.l., while in other groups e.g. Cupressaceae s.str. the seed scale is strongly reduced, and the visible cone scale represents the bract scale (e.g. Page 1990; Farjon 2007, 2010; Gadek et al. 2000; Dörken 2012a, 2012b; Groth et al. 2011; Jagel & Dörken 2014, 2015a, 2015b; Dörken & Jagel 2017; Dörken & Rudall 2018). In previous studies on seed cones ontogeny it is suggested that among Cupressaceae s.str. the seed scale was continuously reduced to their ovules (Jagel & Dörken 2015a, 2015b). Only within basal species of Cupressaceae s.l. such as in the subfamilies Cunninghamioideae and Athrotaxioideae, tongue like structures below the ovules are visible until pollination time. These structures could be interpreted as vegetative parts of the seed scale. Within other basal Cupressaceae s.l. such as the subfamilies Taiwaniaioideae, Sequoiioideae and Taxodiioideae, the seed scale is fused to the upper surface of the bract scale, thus within these subfamilies the ovules are inserted on the upper surface of the cone scale (Jagel 2001a; Jagel & Dörken 2014; Schulz & Stützel 2007; Dörken et al. 2017; Dörken & Rudall 2018). In modern Cupressaceae s.str., subfamilies Cupressioideae and Callitrioideae, ovules are never inserted on the upper surface of the cone scale but always axillary. Compared to the basal Cupressaceae s.l. within the modern Cupressaceae s.str. the number of ovules per cone scale is strongly increased so that within Cupressus or Callitris up to four rows of ovules per cone scale are developed (Jagel & Dörken 2015a, 2015b). For the modern Cupressaceae s.str. it is suggested that the seed scales are completely reduced to their ovules and each row of ovules can be regarded as a descending accessory short shoot (e.g. Jagel 2001a, 2001b; Dörken 2012b; Jagel & Dörken 2014; Schulz & Stützel 2007; Dörken et al. 2017; Dörken & Rudall 2018). In addition to the increase in the number of ovules per cone scale a second major evolutionary trend within Cupressaceae is the dislocation of the fertile zone into apical parts of the seed cone so that sterile apical elements as can be found frequently in the basal taxa of Cupressaceae s.l. are always absent, maybe as an important improvement within the pollination mechanism to increase the success in pollen capture (Dörken & Jagel 2014). In basal Cupressaceae s.l., only the cone scales in the middle of the seed cones are fertile while the basal and apical ones are sterile, so that in most basal taxa a sterile terminal piece is formed consisting of the tip of the cone axis and several sterile, incompletely separated cone scales (Jagel & Dörken 2015a). Among Cupressus, as one of the most modern and derived cupressaceous taxa, no sterile apical elements are developed, except in rare cases the apex of the cone axis is visible as a small central columella. Only among Cupressus species such as C. vietnamensis and C. nootkatensis, which are regarded as the most primitive extant species among the genus, the tip of the cone axis is frequently visible in the form of a distinct central columella, in more derived Cupressus species, however, only occasionally. Only in few derived Cupressus species, a distal pair of sterile and fused cone scales are found frequently as in e.g. C. sempervirens.

Our study of C. glabra seed cones clearly shows that the ovules, which are developed in up to four rows, are always located axillary and never on the cone scale surface (Figs 2C, 2D, 3C & 3D). Except for the small axillary buldge, no further structures or tissues were found which could be interpreted as representing vegetative parts of the seed scale. Investigation of the vasculature showed that each fertile bract/seed scale complex is supplied by more than one vascular bundle strand (Fig. 3A). There is always one collateral bundle strand supplying the cone scale and a further axillary one supplying the lowest row of ovules (Figs 2C, 3C-F). However, not all ovules within an axillary row have a vascular supply, only the oldest, outer ovules have a vascular connection, the younger central ovules are supplied by a cell-to-cell transport as is also the case for all distal rows.
of ovules (Figs 3E & 3F). The vascular bundle strands supplying the cone scale and the axillary one supplying the ovules do not fuse before entering the concentric stem bundle (Figs 2C, 3D & 3F). This branching pattern of the vasculature within a bract/seed scale complex is quite similar to that occurring in vegetative parts of the crown, as Dörken (2012) showed for Metasequoia and Sequoia. In these taxa in the vegetative part of the crown accessory short shoots are developed in the axil of a long shoot leaf (= bract). The bract and the axillary short shoots have a distinct separate vascular supply; the bract is supplied by a collateral vascular bundle, each accessory shoot with a concentric one. These vascular bundles do not fuse before entering the stem bundle of the long shoot, a type of vasculature which is quite similar to the one found in the fertile bract-seed scale complexes of the investigated C. glabra. This kind of vascular supply that Dörken (2012) described for the vegetative, sterile parts of Metasequoia and Sequoia gives strong support for the idea that each row of ovules within the Cupressus seed cone could be regarded as an accessory ovuliferous short shoot, whose axis, however, became greatly reduced to the ovules, so that finally no visible vegetative structures which could be interpreted as belonging to the seed scale could be found. Thus, the Cupressus seed cone could be regarded as representing a strongly reduced and condensed compound structure, which might have originated from a former more open and loose inflorescence, whose lateral, accessory fertile shoots became greatly reduced to their ovules.

4.2 Phyllotaxis within the cone

Within cupressaceous seed cones different types of cone scale arrangements can be found. Most of the basal Cupressaceae s.l. show helically arranged cone scales, e.g. all species of Cunninghamioideae, Athrotaxioideae, Taiwaniioideae, Taxodioideae and most Sequoioideae (except Metasequoioideae). Within the derived modern Cupressaceae s.str., the situation is much more diverse. Species of the subfamily Callitroidae show a large variability regarding the arrangement of cone scales which can be arranged in decussate pairs or in trimerous or occasionally tetrasterous whorls, whereas cone scales of Cupressioideae are mostly decussate, except many Juniperus species showing cone scales in whorls of three (Krüssmann 1955, 1983; Dallimore & Jackson 1966; Farjon 2005, 2010; Eckenwalder 2009; Jagel & Dörken 2014, 2015a, 2015b). However, Jagel (2001b) described also for Cupressus seed cones consisting exceptionally of trimerous whorls of cone scales. The interpretation of these anomalous trimerous whorls is quite difficult. It may not to be a change in the phyllotaxis from decussate to whorled, but could be caused by an abortion of a basal cone scale, which leads to a dislocation and shift of the subsequent cone scales into a secondary, apparently “whorled” position. Within the investigated anomalous trimerous seed cone it seems that the vascular bundles supplying the cone scales would come out of the stem bundle of the cone axis all in the same plane as it would be common for typical shaped node showing trimerous whorls of leaves. However, it should be considered that if one of the basal cone scales was aborted in its primordial stage there would be no need to develop a vascular bundle for it, a fact that could lead to the impression that the distal vascular bundles supplying the three distal most cone scales are all in the same plane.

5 Conclusions

The investigation of C. glabra seed cones were focused on vasculature supplying the cone scales and the ovules. It was found that the cone scale and the inserted axillary rows of ovules were each supplied by its own vascular bundle strand, which gives strong support for the idea that the several axillary rows of ovules per cone scale can be regarded as descending accessory fertile shoots (= seed scales), which, however, got reduced to their ovules. Except to the vascular bundle strand supplying the lowest row of ovules, no additional visible vegetative structures were found that could be explained as belonging to the seed scale. This finding gives rise to the hypothesis that the former cupressaceous seed cone was a more open and loose branched structure, before it became greatly reduced to the compact structures typical of extant species.
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Bibliography


