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Development of the seed-cones in *Taxus canadensis* in culture (Tsytsin Main Botanical Garden RAS, Moscow, Russia)

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ABSTRACT

The development of the seed-cones of *Taxus canadensis* was studied. Aril is multi-layered. The seed coat is multi-layered, its structure changes during development. Initially, it consists of 33–35 layers of cells, and by the time of maturation they are reduced to 10 layers. A marker sign of changes of seed-cones structure is the appearance of an aril from under the scales. At this time, there is an active thickening of the cuticle of the epidermic cells of the seed, sclerenchymatization of zone I of mesotesta cells and obliteration of zone II, differentiation of cells in the chalaza area. The nucellus is reduced, the endosperm and embryo grow, and oil droplets appear in the cells of the endosperm. A week later, even greater differentiation of cells in the chalaza area starts and a rapid growth of the aril is observed during the week.

Keywords: yew, seed-cone, development, aril, seed, morphology-anatomical structure

РЕЗЮМЕ

Трусов Н.А. Развитие фруктификаций у *Taxus canadensis* в культуре (Главный ботанический сад им. Н.Н. Цицина РАН, Москва, Россия). Исследовано развитие фруктификаций у *Taxus canadensis*. Ариллус многослойный. Семенная кожура многослойная, ее строение меняется в процессе развития. Изначально она состоит из 33–35 слоев клеток, а к моменту созревания редуцируется до 10 слоев. Маркерным признаком изменений строения фруктификаций является появление ариллуса из-под чешуй. В это время активно идет утолщение кутикулы клеток эпидермы семени, склеренхиматизация зоны I клеток мезотесты и облитерирование зоны II, дифференциация клеток в области халазы, редуцируется нуцеллус, разрастаются эндосперм и зародыш, в клетках эндосперма появляются жировые включения. Через неделю, в течение недели, происходит еще большая дифференциация клеток в области халазы и наблюдается стремительное разрастание ариллуса.

Ключевые слова: тис, фруктификация, развитие, ариллус, семя, морфолого-анатомическое строение

The yew family (Taxaceae Gray) for a long time attracted the attention of researchers. Their seed-cones are especially interesting. The most studied is the genus *Taxus* L., which includes 9 species and 2 interspecies hybrids (Germplasm Resources Information Network). The structure of mature seeds and their fleshy appendages of some yew species have been well investigated. There are fragmentary studies concerning the development of seed-cones, but most of them are either devoted to the morphology of seed-cones, or aimed at studying the anatomical structure of the shoot system adjacent to the ovule and the ovules. (Dupler 1920, DiFazio 1996, Spjut 2010). A comprehensive study of the development of seeds and their fleshy appendages has not been carried out. This study is also relevant due to the fact that the causes of the appearance of fleshy structures in Gymnosperm cones have not yet been established and are of interest to modern researchers (Nigris et al. 2021). At this time, as a result of studying the development of dry fruits, the seeds of which have arils, the functions of arils, and reasons for the appearance of arils and their fixation in the process of evolution were proposed (Trusov 2016, 2021).

Yew ovules are unitegmal. In mature seeds, exotesta consists of the epidermis and hypodermis, a mesotesta of sclerenchymal cells, and an endotesta of parenchymal flattened thin-walled cells (Bobrov 1997). According to Dupler (1920), the epidermis of immature seeds include 5 zones: 1) epidermis of large papilliform cells with a thick cuticle; 2) hypodermis of large thick-walled cells with brownish-red content; 3) subhypodermal layer of small radially elongated cells; 4) multi-layered zone of small cells of irregular shape, closer to the next zone, longitudinally elongated; in this zone, large secretory cells and 2 vascular bundles were described; 5) inner epidermis of elongated thick-walled cells with dark-colored contents. Dupler also pointed out that all cells, except for the cells of the epidermis and hypodermis, subsequently become sclerenchymatous.

The fleshy structure around the yew seed is called aril. The origin of this structure has been discussed previously by scientists. Dupler (1920) considered it a fleshy layer of 3-layered seed coat. At the end of the 20th century, the prevailing point of view on the nature of this structure was its funicular origin, and, as a consequence, the legitimacy of designating it as an aril (Shi & Wang 1989, Bobrov 1997, Melikyan & Bobrov 1997). Morphology-anatomical studies of recent years, including teratological seed-cones of *Pseudotaxus chienii* (W.C. Cheng) W.C. Cheng (Dörken et al. 2019), have shown

that the fleshy structures in *Pseudotaxus* W.C. Cheng and *Taxus* are fused, strongly swollen and fleshy scales. The discovery of stomata in their epidermis (Dupler 1920, Dörken et al. 2019) also indirectly confirms this. Modern molecular genetic studies prove that the fleshy structure surrounding the *Taxus* seed arils *de novo*, the B-sister gene responsible for the formation of the fleshy structure in *Ginkgo biloba* L. is not involved in its growth (Lovisetto et al. 2013). At the same time, it was shown that a number of MADS-box genes responsible for the development of structures in Angiosperms involved in the growth of the fleshy structure in *T. baccata* L., especially their expression is noticeable at late stages of seed development (Lovisetto et al. 2012). Earlier, a number of authors homologized the seed scales of conifers, arils of Taxaceae, Cephalotaxaceae Neger, *Phyllocladus* Rich. ex Mirb. and epimathiums of Podocarpaceae Endl. (Sinnott 1913, Florin 1954, Ehrendorfer et al. 1971, Muravyova & Borkhwardt 1978, Morvan 1983, Meijen 1987, Page 1990 – cited by Melikyan & Bobrov 1997). Some confusion is introduced by the work of Spjut (1994), in which he considers the fleshy appendages around the yew seed to be a metamorphosed megasporophyll, but at the same time attributes the fructification of yew to a separate type of "fruit" – "arillocarpium". Homologization is discussed but not accepted by Melikyan & Bobrov (1997). The same article also notes that if the fleshy structure surrounding the seeds of Taxaceae, Cephalotaxaceae, *Phyllocladus* is nevertheless homologous to the seed scales, it is incorrect to call it "aril". Indeed, by definition, the aril is an appendage of the funicle (Planchon 1845, Serrato-Valentii et al. 1991, Melikyan & Devyatov 2001), or, if we consider the aril, more broadly, appendages of the funicle and / or seed coat (Baillon 1876, Pijl 1982, Boesenwinkel & Bouman 1984). Thus, to base up modern morphology-anatomical and molecular-genetic data, to name the fleshy appendage around the yew seed as aril, is strictly speaking incorrect. Due to the fact that angiosperm carpels are megasporophylls, the appendage around yew seeds should rather be compared with a berry-type fruit or with an elaiosome, a fleshy appendage on fruits (Melikyan & Devyatov 2001). At least between the fleshy appendage of the yew and the fleshy fruit of the berry, there is a parallelism. The mechanism associated with the expression of certain genes of the MADS-box and capable of previously creating a fleshy diaspore in Gymnosperms also function in Angiosperms. At the same time, the term "aril" is firmly entrenched for this structure. Probably, for the appendage around the yew seeds, it is still necessary to leave the term "aril", but with the assumption that it is a Gymnosperm aril or an aril of scaly origin, not homologous to an Angiosperm aril. In the future, the terminology should be revised to take into account the term "epimatium" – an additional outer shell of representatives of the Podocarpaceae and related families (Melikyan & Devyatov 2001).

According to the literature, the yew's arils are laid in the form of a ring under the scales approximately at the time of pollination of the ovule. In the early stages of development, it is a greenish saucer-shaped structure that grows slowly. After the seed coat is hard, the aril grows quickly and becomes cupped and red in color (Dupler 1920, Bierhorst 1971, DiFazio 1996). Aril consists of an epidermis and a multi-layered parenchyma. Epidermal cells are small, pigmented. The parenchyma cells are large, thin-walled, extended radially and obliquely upward (Dupler 1920). The stomata are longitudinally oriented in the epidermis (Dupler 1920). It is noted that the state of the aril can be an indicator of seed viability; seeds with an underdeveloped aril have low germination (Heit 1969, DiFazio 1996). At the same time, uneven ripening of fructification was found in yews, in particular, in *T. brevifolia* Nutt. (DiFazio 1996). Due to the bright coloration of arils, yew seeds are spread by birds and mammals (mice, squirrels, chipmunks, badgers, foxes) (Suszka 1975, Smal & Fairley 1980, Tittensor 1980, Howe & Westley 1986, DiFazio 1996). Arils of yews are traditionally eaten in different countries. In Indian traditional medicine, they are used as a tonic, gastric and expectorant (Kuzmina & Fokina 1996).

The aim of this research was to study the development of yew's seed-cones using the example of *T. canadensis* Marshall. The main attention was paid to the morphology-anatomical changes occurring in the seeds and arils, and their relationship.

MATERIAL AND METHODS

The morphology-anatomical structure of the seed-cones of *T. canadensis* in development was studied. The seed-cones were collected in the arboretum of the Tsytsin Main Botanical Garden of Russian Academy of Sciences with a weekly interval from the third decade of May to mid-July (8 terms). The material was fixed in 70 % ethanol. The seed-cones sections of different orientations were performed manually using a razor blade. The water and glycerin preparations made from them were studied. A Biolam microscope was used in the work. Photos were taken a Canon EOS 650 D.

RESULTS

Term I. Third decade of May (Fig. 1A)
The seed's size is about 5.2 × 3.7 mm. The scales cover the seed by 3/4. Aril is a small collar-like enlargement in the chalaza area, not visible from under the scales (Figs 2A–A2).

Aril is multi-layered (Fig. 2A3). The epidermis on a longitudinal section is represented by tabular cells, 0.0175–0.025 × 0.0175–0.025 mm. Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchyma cells on a longitudinal section are polygonal, 0.015–0.025 × 0.015–0.025 mm. The intercellular spaces are absent, the cells are densely packed.

The seed coat consists of 33–35 cell layers (Fig. 2A4). Exotesta is two-layer, represented by epidermis and hypodermis. Epidermal cells are tabular, with a convex outer cell wall. The cell walls are thickened; about 0.0125 × 0.0125 mm, the contents of the cells are bright. The hypodermis is single-layered, from cells slightly radially elongated or tabular on a cross section, with thin cell walls and bright content, about 0.0188 × 0.0125–0.0188 mm. The intercellular spaces are imperceptible. Mesotesta is represented by two zones. Zone of the cells with dark content (zone I) from 8 layers. The cells are densely arranged, round, densely plasmatic, thin-walled, about 0.00375 × 0.00375 mm, not elongated along the seed axis. The zone of the cells with bright content (zone II) from 20–22 layers of thin-walled polygonal cells, 0.005–0.01 × 0.005–0.01 mm. The cells of two layers adjacent to the endotesta with dark contents, small, about 0.0025 × 0.0025 mm. In this zone, closer to the zone I, 2

Figure 1 Development of the *Taxus canadensis* seed-cones. A – term I; B – term II; C – term III; D – term IV; E – term V; F – term VI; G – term VII; H – term VIII (explanations are in the text)

vascular bundles are located. Endotesta from one layer of tabular cells, about 0.004 × 0.004 mm.

Nucellus multi-layered, from 3 zones. Outside there are 4–5 layers of dark-colored small rounded cells, about 0.005 × 0.005 mm, outwardly similar to the two inner layers of the mesotesta. The main thickness of nucellus is 10–12 layers of polygonal cells with light content and thickened cell walls, about 0.0087 × 0.0125 mm. To the inside of them is a layer of radially flattened cells with thickened yellowish cell walls, about 0.005 × 0.0125 mm (Fig. 2A4).

Endosperm. On the periphery, 1 layer of densely plasmic rounded cells is about 0.004 × 0.004 mm. The cells of the remaining layers are polygonal 0.006–0.012 × 0.006–0.012 mm. In the center, densely plasmic, rounded cells, about 0.006 × 0.006 mm. All endosperm cells with visible nuclei (Fig. 2A4).

Term II. The beginning of June (Fig. 1B)

The seed's size is about 5.4 × 3.9 mm. The scales cover the seed by 2/3. Aril is a small collar-like enlargement in the chalaza area, not visible from under the scales (Figs 2B–B2).

Aril is multi-layered (Fig. 2B3). The epidermis on a longitudinal section is represented by tabular cells, 0.0175–0.025 × 0.0175–0.025 mm. Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchyma cells on a longitudinal section are polygonal, 0.015–0.025 × 0.015–0.025 mm. The intercellular spaces are absent, the cells are densely packed.

The seed coat consists of 33–35 cell layers (Fig. 2B4). Exotesta is two-layer, represented by epidermis and hypodermis. Epidermal cells are elongated radially, with a convex outer cell wall. The cell walls are thickened; about 0.0188 × 0.0125 mm, the contents are bright. The hypodermis is single-layered, from radially elongated or tabular cells on a cross section, with thin cell walls and bright content, 0.0188–0.021 × 0.0088–0.0125 mm. The intercellular spaces are imperceptible. Mesotesta is represented by two zones. Zone of the cells with dark content (zone I) from 8 layers. The cells are densely arranged, round, densely plasmatic, thin-walled, about 0.00375 × 0.00375 mm, not elongated along the seed axis. Zone of the cells with bright content (zone II) of 20–22 layers of thin-walled polygonal cells, 0.005-0.01 × 0.005-0.01 mm. Cells of two layers adjacent to the endotesta with dark contents, small, about 0.0025 × 0.0025 mm. In this zone, closer to the zone of the cells with dark content, 2 vascular bundles are located. Endotesta from one layer of tabular cells, about 0.004 × 0.004 mm.

The structure of nucellus and endosperm did not change.

Term III. End of the first decade of June (Fig. 1C)

The seed's size is about 6.9 × 4.4 mm. The scales cover the seed by 1/3. Aril is a small collar-like enlargement in the chalaza region, not visible, located flush with the scales (Figs 2C–C2).

Aril is multi-layered (Fig. 2C3). The epidermis on a longitudinal section is represented by tabular cells, 0.0188–0.025 × 0.0175-0.025 mm. Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchyma cells on a longitudinal section are polygonal, 0.0188–0.025 × 0.0188–0.025 mm. The intercellular spaces are absent, the cells are densely packed.

The seed coat consists of 33–35 cell layers (Fig. 2C4). Exotesta is two-layer, represented by epidermis and hypodermis. The cells of the epidermis are elongated radially, with a convex outer cell wall with a cuticle. The cell walls are thickened, 0.0188–0.025 × 0.0125–0.015 mm, the contents are bright. The hypodermis is single-layered, from radially elongated or tabular cells on a cross section, with thin cell walls and bright content, 0.0188–0.021 × 0.0088–0.0125 mm. The intercellular spaces are imperceptible. Mesotesta is represented by two zones. Zone of the cells with dark content (zone I) from 8 layers of cells. The cells are densely arranged, round, densely plasmatic, thin-walled, about 0.00375 × 0.00375 mm, not elongated along the seed axis. Outer layer of cells slightly elongated radially, about 0.00625 × 0.00375 mm. Zone of the cells with bright content (zone II) of 20–22 layers of thin-walled polygonal cells, 0.005–0.01 × 0.005–0.01 mm. Cells of two layers adjacent to the endotesta with dark contents, small, about 0.0025 × 0.0025 mm. In this zone, closer to the zone of the cells with dark content, 2 vascular bundles are located. Endotesta from one layer of tabular cells, about 0.004 × 0.004 mm.

The structure of nucellus and endosperm did not change.

Term IV. Mid June (Fig. 1D)

The seed's size is about 7.1 × 4.7 mm. The scales cover the seed by 1/3. Aril protrudes slightly from the scales (Figs 2D–D2).

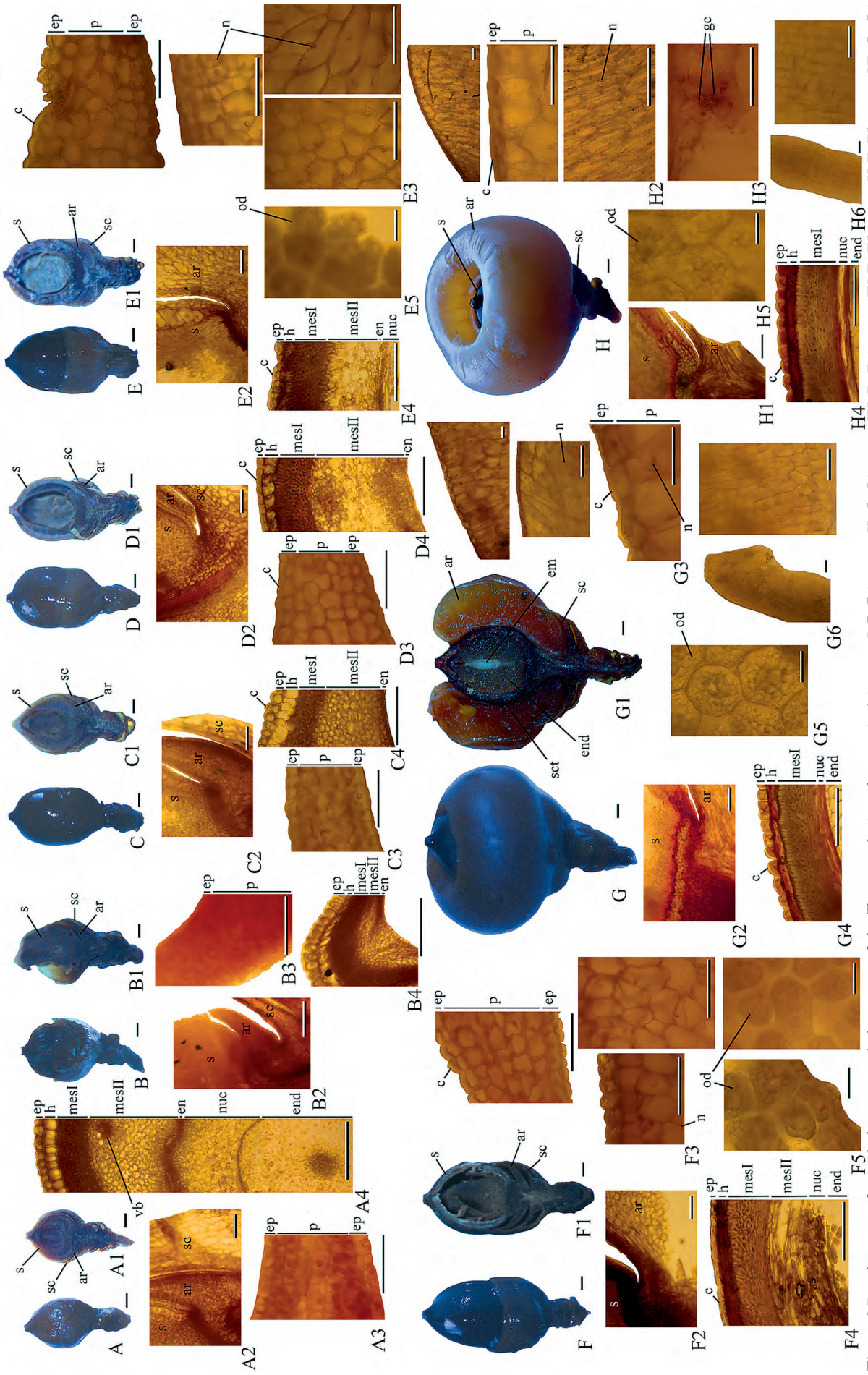

Figure 2 Development of morphology-anatomical structure of the *Taxus canadensis* seed-cones. A–A4 – term I; B–B4 – term II; C–C4 – term III; D–D4 – term IV; E–E5 – term V; F–F5 – term VI; G–G6 – term VII; H–H6 – term VIII. A–H – appearance of seed-cones; A1–G1 – longitudinal section of seed-cones; A2–G2, H1 – attachment of the aril (longitudinal section); A3–G3, H2 – fragments of the structure of the aril (cross section); H3 – epidermis of aril (surface view); A4–H4 – seed coat (cross section); E5–H5 – endosperm (cross section); G6–H6 – embryo (cross section). ar – aril; c – cuticle; em – embryo; en – endoderma; end – endosperm; ep – epidermis; h – hypodermis; mesI – mesoderma I zone; mesII – mesoderma II zone; n – nucleus; nuc – nucellus; od – oil droplet; p – parenchyma; s – seed; sc – scale; sct – seed coat; vb – vascular bundle. Scale bar: A-H, A1'-G1 – 1 mm; H1, A2-H2, A3-H3, A4-H4, E5-H5, G6-H6 – 0.01 mm.

Aril is multi-layered (Fig. 2D3). The epidermis on a longitudinal section is represented by tabular cells with a thick cuticle (0.0088–0.0125 mm thick) extending to half of the radial cell walls. The cell sizes are 0.025–0.031 (with cuticle) × 0.025–0.031 mm. Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchyma cells on a longitudinal section are polygonal, less often round, 0.025–0.0375 × 0.025–0.0375 mm. The intercellular spaces are absent, the cells are densely packed.

The seed coat consists of 33–35 cell layers (Fig. 2D4). Exotesta is two-layer, represented by epidermis and hypodermis. Epidermal cells are tabular, with a convex outer cell wall, with cuticles on the outer and radial walls (0.0025 mm thick). The cell walls are thickened, the size of the cells is about 0.01 × 0.0125 mm, the contents of the cells are bright. The hypodermis is single-layered, from radially elongated or tabular cells on a cross section, with thin cell walls and light contents, 0.015–0.01875 × 0.015–0.01875 mm. The intercellular spaces are imperceptible. Mesotesta is represented by two zones. A zone of cells with thickened cell walls (sclereids) (zone I) of 8 layers. The cells are densely arranged, round, about 0.00625 × 0.00625 mm, not elongated along the seed axis. Outer layer of slightly elongated radially sclerenchymal cells, about 0.0125 × 0.00625 mm. Zone of the cells with bright content (zone II) of 20–22 layers of thin-walled polygonal cells, 0.005–0.0875 × 0.005–0.01 mm. The cells of two layers adjacent to the endotesta with dark contents, small, about 0.0025 × 0.0025 mm. In this zone, closer to the zone of the cells with dark contents, there are two vascular bundles. Endotesta from one layer of tabular cells, about 0.004–0.005 × 0.004–0.005 mm.

The structure of nucellus and endosperm did not change.

Term V. Third decade of June (Fig. 1E)

The seed's size is about 7.8 × 4.7 mm. Scales at the base of the seed. Aril protrudes from the scales, covering the seed by 1/3 (Figs 2E–E2).

Aril is multi-layered (Fig. 2E3). The epidermis on a longitudinal section is represented by tabular cells with a thick cuticle (0.0088–0.0125 mm thick), extending to half of the radial cell walls. The cells sizes are 0.025–0.031 (with cuticle) × 0.025–0.031 mm. From the side of the seed, closer to the chalaza, "at the flexion", there are cells with a convex outer cell wall (most likely due to the presence of a "flexion"). Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchymal cells vary. The cells located closer to the epidermis are polygonal on a longitudinal section, less often round, 0.031–0.05 × 0.031–0.05 mm, with visible nucleuses. The intercellular spaces are absent, the cells are densely packed. In the middle part of the aril, the cells elongated along the seed axis and somewhat at an angle to it, are densely located, 0.0875–0.1375 × 0.0375–0.05 mm, also with visible nuclei.

The seed coat is composed of 23–25 cell layers (Fig. 2E4). Exotesta is two-layer, represented by epidermis and hypodermis. Epidermal cells are tabular, with a convex outer cell wall, with cuticles on the outer and radial walls (0.005 mm thick). The cell walls are thickened, the cells size is 0.0125–0.015 (with cuticle) × 0.0125–0.015 mm, the contents are bright. The hypodermis is single-layered, from radially flattened or tabular cells on a cross section, with thin cell walls and dark contents, 0.0125 × 0.015–0.0188 mm. The intercellular spaces are imperceptible. Mesotesta from two cell zones. A zone of cells with thickened cell walls (sclereids) (zone I) of 8 layers. The cells are densely arranged, round, about 0.0075 × 0.0075 mm, not elongated along the seed axis. Outer layer of slightly elongated radially sclerenchymal cells, about 0.0125 × 0.0075 mm. The zone of the cells with bright content (zone II) of 12–14 layers of thin-walled polygonal cells, of different sizes, 0.005–0.0088 × 0.005–0.01 mm. The cells flatten radially and begin to obliterate. Cells of two layers adjacent to the endotesta with dark contents, small, about 0.0025 × 0.0025 mm. In this zone, closer to the zone of the cells with dark contents, there are two vascular bundles. Endotesta from one layer of tabular cells, 0.004–0.005 × 0.004–0.005 mm. In the chalaza zone, the contents of the cells become dark.

Nucellus (residue) multi-layered, from 2 zones. Outside there are 4–5 layers of dark-colored small rounded cells, about 0.0025 × 0.0025 mm, outwardly similar to the 2 inner layers of the mesotesta. Inside there is a layer of radially flattened cells with thickened yellowish cell walls, about 0.0063 × 0.025 mm.

Endosperm from large polygonal cells, about 0.0125 × 0.015 mm, with small oil droplets.

Term VI. End of June (Fig. 1F)

The seed's size is about 9.4 × 5.0 mm. Scales at the base of the seed. Aril is yellowish, protrudes from the scales, and covers the seed by 1/3 (Figs 2F–F2).

Aril is multi-layered (Fig. 2F3). The epidermis on a longitudinal section is represented by tabular cells with a thick cuticle (0.0088–0.0125 mm thick) extending to half of the radial cell walls. Cell sizes – 0.031–0.0375 (with cuticle) × 0.025–0.0375 mm. From the side of the seed, closer to the chalaza, "at the flexion", there are cells with a convex outer cell wall (most likely due to the presence of a "flexion"). Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchymal cells vary. Closer to the epidermis on the longitudinal section, polygonal, less often round, 0.0375–0.05 × 0.0375–0.05 mm, with visible nuclei. The intercellular spaces are absent, the cells are densely packed. In the middle part of the aril, the cells stretch along the seed axis and somewhat at an angle to it, are densely located, 0.0875–0.1375 × 0.0375–0.05 mm, with visible nuclei.

The seed coat is composed of 20–22 cell layers (Fig. 2F4). Exotesta is two-layer, represented by epidermis and hypodermis. Epidermal cells are strongly flattened radially, with a slightly convex outer cell wall, with cuticles on the outer and radial walls (about 0.005 mm thick). The cell walls are thickened, the size of the cells is about 0.0125 (with cuticle) × 0.01875–0.03125 mm, the contents are bright. The hypodermis is single-layered, of cells radially flattened on the cross section, with thin cell walls and dark contents, 0.0125–0.0188 × 0.025–0.0313 mm. The intercellular spaces are imperceptible. Mesotesta from two cell zones. A zone of cells with thickened cell walls (sclereids) (zone I) of 8 layers. The cells are densely arranged. The cells of the 4 middle layers are polygonal, about 0.00875 × 0.00875 mm, not elongated along the seed axis. The cells of the underlying layers are flattened radially. Outer layer of slightly elongated radially sclerenchymal cells, about 0.0125 × 0.0085 mm. A zone of cells with a bright content (zone II) of 10–12 layers of thin-walled, radially flattened cells of different sizes, up to 0.00875 × 0.0375 mm. Some of the cells are obliterated. Cavities were found in places. Cells of two layers with dark contents adjacent to the endotesta were not found, obliterated. Endotesta is not allocated.

Nucellus (residue) multi-layered, from 2 zones. Outside there are 4–5 layers of dark-colored small rounded cells, about 0.0025 × 0.0025 mm. To the inside is a layer of radially flattened cells with thickened yellowish cell walls, about 0.00625 × 0.025–0.0375 mm (Fig. 2F4).

Endosperm from large polygonal cells, about 0.0125 × 0.01875 mm, with small oil droplets (fig. 2F5).

Term VII. The first ten days of July (Fig. 1G)

The seed's size is about 7.5 × 5.8 mm. Scales at the base of the seed. Aril is eumorphous. It surrounds the seed from the sides, exceeds it in height, but from above it stays open. The thickness of the aril is about 3.8 mm (Figs 2G–G2).

Aril is multi-layered (Fig. 2G3). The epidermis on a longitudinal section is represented by radially flattened cells with a thick cuticle (0.0088–0.0125 mm thick) extending to half of the radial cell walls. Cell sizes about 0.0375 (with cuticle) × 0.05–0.0625 mm. Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchymal cells elongate perpendicular to the seed axis and somewhat at an angle to it, 0.23–0.27 × 0.1–0.17 mm, with visible nuclei. The intercellular spaces are absent, the cells are densely packed.

The seed coat is composed of 10 cell layers about 0.5 mm thick (Fig. 2G4). Exotesta is two-layer, represented by epidermis and hypodermis. Epidermal cells are strongly radially flattened, with a slightly convex outer cell wall, with cuticles on the outer and radial walls (0.005 mm thick). The cell walls are thickened, 0.0125–0.0188 (with cuticle) × 0.0188–-0.0313 mm, the contents are bright. The hypodermis is single-layered, of cells radially flattened on a cross section, with thin cell walls and dark contents, 0.0125–0.0188 × 0.0375–0.05 mm. The intercellular spaces are imperceptible. Mesotesta from one remaining cell zone. A zone of cells with thickened cell walls (sclereids) (zone I) of 8 layers. The cells are densely arranged, the cells of the 4 middle layers are polygonal, up to 0.0125 × 0.0125 mm in size, not elongated along the seed axis. The cells of the underlying layers are flattened radially. Outer layer of slightly elongated radially sclerenchymal cells, about 0.0125 × 0.0085 mm. The zone of the cells with bright content (zone II) is obliterated. In the zone of chalaza, cells differentiate into a layer of cells with dark contents and several layers of rounded bright cells, possibly sclerenchymatous.

Nucellus (residue) from one layer of radially flattened cells with thickened yellowish-orange cell walls, about 0.0088 × 0.056 mm (Fig. 2G4).

Endosperm from large polygonal cells, up to 0.0225 × 0.02 mm, with small oil droplets (Fig. 2G5).

The embryo is vertical, about 3.8 × 0.08 mm. The cells are polygonal, thin-walled, with bright contents, elongated along the axis of the embryo. The size of the cells is 0.06–0.095 × 0.025–0.0375 mm (Fig. 2G6).

Term VIII. Mid-July (Fig. 1H)

The seed's size is about 7.5 × 5.8 mm. Scales at the base of the seed. Aril is eumorphous. It surrounds the seed from the sides, exceeds the seed in height, but from above it stays open. The thickness of the aril is about 3.8 mm (Figs 2H–H1).

Aril is multi-layered (Fig. 2H2). The epidermis on a longitudinal section is represented by radially flattened cells with a thick cuticle (0.0088–0.0125 mm thick) extending to half of the radial cell walls. Cell sizes about 0.0375 (with cuticle) × 0.05–0.0625 mm. Between the two layers of the epidermis, there is a multi-layered parenchyma that makes up most of the aril. Parenchymal cells are elongated perpendicular to the seed axis and somewhat at an angle to it, 0.23–0.27 × 0.1–0.17 mm, with visible nuclei. The intercellular spaces are absent, the cells are densely packed. Aril has rare stomata. Guard cells about 0.0375 × 0.015 mm. The stomatal apparatus is anomocytic (Fig. 2H3).

The seed coat is composed of 10 cell layers (Fig. 2H4). Thickness – 0.5 mm. Exotesta is two-layer, represented by epidermis and hypodermis. Epidermal cells are strongly flattened radially, with a slightly convex outer cell wall, with cuticles on the outer and radial walls (about 0.005 mm thick), the cell walls are thickened. Cell sizes are 0.0125–0.0188 (with cuticle) × 0.0188–0.0313 mm, the contents are bright. The hypodermis is single-layered, of cells radially flattened on a cross section, with thin cell walls and dark contents, 0.0125–0.0188 × 0.0375–0.05 mm. The intercellular spaces are imperceptible. Mesotesta from one remaining cell zone. A zone of cells with thickened cell walls (sclereids) (zone I) of 8 layers. The cells are densely arranged, the cells of the 4 middle layers are polygonal, up to 0.0125 × 0.0125 mm in size, not elongated along the seed axis. The cells of the underlying layers are flattened radially. The outer layer is composed of slightly radially elongated sclerenchymal cells, about 0.0125 × 0.0085 mm in size.

Nucellus (residue) from one layer of radially flattened cells with thickened yellowish-orange cell walls, about 0.0088 × 0.056 mm (Fig. 2H4).

Endosperm from large polygonal cells, up to 0.0225 × 0.02 mm, with small oil droplets (Fig. 2H5).

The embryo is vertical, about 3.8 × 0.08 mm. The cells are polygonal, thin-walled, with bright contents, elongated along the axis of the embryo. The size of the cells is 0.06–0.095 × 0.025–0.0375 mm (Fig. 2H6).

DISCUSSION

In this studt, at term I, the seed has a length of about 5.2 mm and a width of about 3.7 mm, green, elliptical, slightly flattened from the sides, with a convex micropyle (Figs 1A, 2A–A1). Upon reaching maturity, the length of the seed increases to about 7.5 mm, and the width to 5, 8 mm. The color of the mature seed is brown (Fig. 1H). At the beginning of development, the seed is covered with greenish scales by 3/4, the aril in the form of a small collar-like thickening in the chalaza area, is not visible from under the scales (Fig 2A2). In mid-June (term IV), the size of the seed is about 7.1 × 4.7 mm, while it is covered with scales by 1/3, and the aril is greenish, slightly protruding from the scales (Figs 1D, 2D–D2). From the third decade of June (term V) to the beginning of July (term VI), the aril grows even more, covers the seed by 1/3, its color changes to yellowish (Figs 1F, 2F–F1). In this case, the scales are bent perpendicular to the axis of the seed; they are only at its base. From the first ten days of July (term VII), the aril grows strongly, thickens, looks fully formed (Figs 1G, 2G–G1). It surrounds the seed from the sides, exceeding in height, but from above the seed stay open. The mature aril is about 3.8 mm thick. The growth pattern of the seed and aril corresponds to that described in the literature. *T. canadensis*, as well as *T. brevifolia*, is characterized by uneven ripening of seed-cones.

The seed coat is multi-layered, formed by one integument. Exotesta, mesotesta and endotesta are distinguished. At the first observation term (the third decade of May), the seed coat consists of 33–35 layers of cells and has a thickness of about 0.225 mm (Fig. 2A4), by the time of maturation it is reduced to 10 layers of cells and has a thickness of about 0.125 mm (Fig. 2H4). Exotesta throughout development is represented by two layers of cells: the epidermis and the un-

derlying hypodermis. In the third decade of May (term I), epidermal cells are tabular, with bright cellular content (Fig. 2A4). The size of the cells is about 0.0125 × 0.0125 mm. Already at the first observation term, the cell walls of the epidermis are thickened, the outer cell wall is convex. At the beginning of June (term II), the cells increase in size, elongate radially (Fig. 2B4). At the end of the first decade of June (term III), the cells reach a size of 0.0188–0.025 × 0.0125–0.015 mm, a cuticle appears on the outer cell wall (Fig. 2C4). In mid-June (term IV), the cells again become tabular, their size is about 0.01 × 0.0125 mm (Fig. 2D4). The cuticle thickens to 0.0025 mm and is present on the outer and radial walls. Starting from the end of June (term VI), epidermal cells flatten radially (Fig. 2F4). In a mature seed, they are strongly flattened radially, 0.0125–0.0188 × 0.0188–0.0313 mm (Fig. 2H4). The cell walls are thickened, the outer cell wall is slightly convex, the cuticle is on the outer and radial walls, and its thickness is about 0.005 mm. The hypodermis is single-layered, at the first observation term from slightly radially elongated or tabular cells on a cross section, densely located, with thin cell walls and bright contents. The size of the cells is about 0.0188 × 0.0125–0.0188 mm (Fig. 2A4). From the beginning (term II) to mid-June (term IV), the cells slightly increase in size (0.015–0.0188 × 0.015–0.0188 mm), retaining their outline (Fig. B4). Starting from the third decade of June (term V) and before the maturation of the seed, the cells of the hypodermis flatten radially (about 0.0125 × 0.015-0.0188), their contents acquire a dark color (Fig. 2E4). In a mature seed, the cells of the hypodermis have dimensions of 0.0125–0.0188 × 0.0375–0.05 mm, their cell walls are thin, and the contents are dark-colored (Fig. 2H4). Mesotesta is multi-layered. At the beginning of the development of seed-cone, it is represented by two zones of cells: with dark-colored content (zone I) and with bright content (zone II) (Fig. 2A4). Starting from the third decade of June (term V), the cells of the zone II begin to obliterate (Fig. 2E4); it is absent in the mature seed (Fig. 2H4). At the first observation term, the zone I consists of 8 layers. The cells on a cross section are densely arranged, rounded, densely plasmatic, thin-walled. The cell size is about 0.00375 × 0.0038 mm. In the cross section, the cells are also rounded, not elongated along the seed axis. At the end of the first decade of June (term III), the cells of the outer layer are elongated radially, their size is about 0.0063 × 0.0038 mm (Fig. 2C4). Starting from mid-June (term IV), the cells increase in size 0.0063 × 0.0063 mm (cells of the outer layer – 0.0125 × 0.0063 mm) (Fig. 2D4). The thickening of their cell walls begins, the cells are sclerenchymatized. The cells are densely arranged, the cells of the 4 middle layers are polygonal, about 0.0088 × 0.0088 mm, not elongated along the seed axis. The cells of the underlying layers are flattened radially. Outer layer of slightly elongated radially sclerenchymal cells, about 0.0125 × 0.0085 mm. At the end of June (term VI), the cells of the outer layer are slightly radially elongated (about 0.0125 × 0.0085 mm), the cells of the 4 middle layers are polygonal (about 0.0088 × 0.0088 mm), the cells of the 3 underlying layers flattened radially (Fig. 2F4). In a mature seed, the same structure of this mesotesta zone is preserved. The cells of the 4 inner layers are up to 0.0125 × up to 0.0125 mm. The zone I at the first observation term consists of 20–22 layers. The cells are thin-walled, polygonal, their size is 0.005–0.01 × 0.005–0.01 mm (Fig. 2H4). The cells of the two layers adjacent to the endotesta are different; they are small, about 0.0025 × 0.0025 mm, with dark contents. In the zone II, closer to the zone I, there are 2 vascular bundles on both sides of the seed (Fig. 2A4). In the third decade of June (term V), the number of layers in the zone II is reduced to 12–14 (Fig. 2E4). Most of the cells are thin-walled, polygonal, of different sizes (0.005–0.0088 × 0.005–0.01 mm), flattened radially, and begin to obliterate. At the end of June (term VI), the number of layers is reduced to 10–12, cavities are found in places (Fig. 2F4). The cells are even more radially flattened, their sizes are different, up to 0.0088 × up to 0.0375 mm, and some of the cells are obliterated. Two layers of cells with dark content adjacent to the endotesta were not detected. In the first decade of July (term VII), the zone II is completely obliterated (Fig. 2G4). Endotesta from the beginning of observation (term I) to the third decade of June (term V) from one layer of tabular cells, their dimensions are 0.004–0.005 × 0.004–0.005 mm. In early July (term VI), the endotesta is not detected, it is obliterated (Fig. 2F4). In general, the structure of the seed coat of the studied *T. canadensis* corresponds to the description of the seed coat of the yew. The main difference is the absence of endotesta in the mature seed (according to Dupler (1920) – the internal epidermis). Weekly observations have shown that the elongated cell layer is a derivative of nucellus. In this case, the cells are thick-walled, but do not have dark-colored contents.

The nucellus in the third decade of May (term I) is multi-layered, consists of 15–18 layers of cells, which can be divided into 3 zones (Fig. 2A4). Outside there are 4–5 layers of small, rounded (about 0.005 × 0.005 mm) cells with dark-colored contents. Outwardly, they are similar to the two inner layers of the mesotesta. The main thickness of nucellus is 10–12 layers of polygonal cells with bright content and thickened cell walls, about 0.0087 × 0.0125 mm. The lower layer of nucellus is made of radially flattened cells with thickened yellowish cell walls, about 0.005 × 0.0125 mm. In the third decade of June (term V), a significant reduction of nucellus is observed (Fig. 2 E4). It has 5–6 layers, 2 zones. The outer 4–5 layers of cells are preserved, and increased in size (about 0.0025 × 0.0025 mm) and the lower layer of cells, their size is about 0.00625 × 0.025 mm. In a mature seed, only the lower layer of cells is preserved, while their size increases about 0.00875 × 0.056 mm (Fig. 2H4).

The endosperm is multi-layered. At the first observation term, one layer of densely plasmic rounded cells, about 0.004 × 0.004 mm in size, stands out on the periphery of the endosperm (Fig. 2A4). The cells of most of the endosperm are polygonal, their size is 0.006–0.012 × 0.006–0.012 mm. In the center of the endosperm there are densely plasmic rounded cells, about 0.006 × 0.006 mm in size. The cells of all layers of the endosperm with visible nuclei. Starting from the third decade of June (term V), the endosperm is not differentiated (Fig. 2E5). The cells are large, polygonal, about 0.0125 × 0.015 mm in size. There are

small oil droplets in the cells. In a mature seed, the size of the endosperm cells increases to 0.0225 × 0.02 mm.

The embryo becomes visible from the beginning of July (term VI). In a mature seed, it is vertical, oblong, about 3.8 × 0.08 mm in size. The cells of the embryo are elongated along the axis of the embryo, polygonal, 0.06–0.095 × 0.025–0.0375 mm, thin-walled, with bright content (Fig. 2H6).

Aril is multi-layered throughout its development. Vascular bundles were not found in it. In the third decade of May (term I), the epidermis is represented by tabular cells measuring 0.0175-0.025 × 0.0175-0.025 mm (Fig. 2A3). Most of the aril is a multi-layered parenchyma located between two layers of the epidermis. Parenchyma cells are polygonal, their size is 0.015–0.025 × 0.015–0.025 mm. The intercellular spaces in the parenchyma are absent, the cells are densely located. Until mid-June (term IV), the aril cells increase in size, a cuticle appears on the outer cell wall of the epidermal cells, gradually thickening (fig. 2D4). In the third decade of June (term V), aril slowly grows (Fig. 2E4). Epidermal cells are tabular, their size is 0.025–0.031 × 0.025–0.031 mm. The cuticle is thick (0.0088–0.0125 mm thick), extending up to half of the radial cell walls. The cells of the parenchyma are different. The cells located closer to the epidermis are polygonal, less often round, densely located, measuring 0.031–0.05 × 0.031–0.05 mm, with visible nuclei. In the middle part of the aril, the cells elongate along the seed axis and somewhat at an angle to it. Their size is 0.0875–0.1375 × 0.0375–0.05 mm. They are located tightly, nucleuses are observed in the cells. At the beginning of July (term VI), aril grows rapidly (Figs 2F1, F3). Mature aril (mid-July) is represented by epidermis and multi-layered parenchyma. Epidermis of radially flattened cells with a thick cuticle (0.0088–0.0125 mm thick) extending to half of the radial cell walls (Fig. 2H2). The size of the cells is about 0.0375 × 0.05–0.0625 mm. Rare stomata are found in the epidermis (Fig. 2H3). The stomatal apparatus is anomocytic. The cells of the parenchyma are elongated perpendicular to the seed axis and somewhat at an angle to it, densely arranged, their size is 0.23–0.27 × 0.1–0.17 mm, nuclei are observed in the cells. The structure of the aril of *T. canadensis* is similar to that described in the literature.

CONCLUSION

Thus, the growth of the seed is observed before it ripening. The structure of the seed coat changes during the development of the seed. The key point of change is the third decade of June. At this time, there is an active thickening of the cuticle of epidermal (exotesta) cells, sclerenchymatization of one zone of the cells of the mesotesta and obliteration of another zone of cells, differentiation of cells in the chalaza area. At the same time, nucellus is reduced, the endosperm and the embryo grow, and small oil droplets appear in the cells of the endosperm. These changes indicate that the supply of nutrients to the seed coat stops, and their outflow to the endosperm and the embryo, as well as the aril, takes place. The protrusion of aril from under the scales can be considered a marker of these changes in the structure of the seed. A week later, at the beginning of July, during the week, a rapid growth of the aril is observed. At this time, there is an even greater differentiation of cells in the chalaza area. It can be assumed that nutrients are still supplied to the seed, but their transport to the seed is stopped or severely restricted. These anatomical studies confirm the fact that seeds with underdeveloped arils are not viable.

LITERATURE CITED

Baillon, M.H. 1876. *De Botanique*. Librairie Hachette et C., Paris, 788 pp.

Bierhorst, D.W. 1971. *Morphology of vascular plants*. Macmillan Co, New York. 560 pp.

Bobrov, A.V. 1997. *Comparative morphology and anatomy of seeds of representatives of the orders Podocarpales, Cephalotaxales and Taxales (Gymnospermae) in connection with their taxonomy and phylogeny*. Abstract diss. for the degree of Candidate of Biological Sciences, MSU, Moscow, 24 pp. (in Russian). [Бобров А.В. 1997. Сравнительная морфология и анатомия семян представителей порядков Podocarpales, Cephalotaxales и Taxales (Gymnospermae) в связи с их систематикой и филогенией. Автореф. дисс. на соиск. уч. ст. к. б. н. Москва: МГУ. 24 с.].

Boesenwinkel, F.D. & F. Bouman 1984. The seed structure. In: *Embriology of angiosperms* (B.M. Johri, ed.), pp. 567–610, Springer-Verlag, Berlin.

DiFazio, S.P. 1996. *The reproductive ecology of pacific yew (Taxus brevifolia* Nutt.*) under a range of overstory conditions in Western Oregon*. A Thesis submitted to Oregon State University in partial fulfillment of the requirements for the degree of Master of Science. 178 pp.

Dörken, V.M., Nimsch H. & P.J. Rudall 2019. Origin of the Taxaceae aril: evolutionary implications of seed-cone teratologies in *Pseudotaxus chienii*. *Annals of Botany* 123:133–143.

Dupler, A.W. 1920. Ovuliferous structures of *Taxus canadensis*. *Botanical Gazette* 69:492–520.

Germplasm Resources Information Network. *Taxus* L. Available from: http://tn-grin.nat.tn/gringlobal/taxon/taxonomysimple. Last accessed 09.03.2022.

Heit, C.E. 1969. Propagation from seed – part 18: Testing and growing seeds of popular *Taxus* forms. *American Nurseryman* 129(2):10–11, 118–128.

Howe, H.F. & L.C. Westley 1986. Ecology of pollination and seed dispersal. In: *Plant Ecology* (M.I. Crawley, ed.), pp. 185–215. Blackwell Scientific Publications, Oxford, England.

Kuzmina L.V., Fokina G.A. 1996. Genus 1. *Taxus* L. – Yew. In: *Plant resources of Russia and neighboring countries: Part I – Families Lycopodiaceae – Ephedraceae, part II – Supplements to volumes 1–7* (A.L. Budantsev, ed.), pp. 41–43, Mir i semya-95, Saint-Petersburg (in Russian). [Кузьмина Л.В., Фокина Г.А. Род 1. *Taxus* L. – Тисс // Растительные ресурсы России и сопредельных государств: Часть I – Семейства Lycopodiaceae – Ephedraceae, часть II – Дополнения к 1–7 томам / отв. ред. А.Л. Буданцев. Санкт-Петербург: Мир и семья-95. С. 41–43].

Lovisetto, A., F. Guzzo, N. Busatto & G. Casadoro 2013. Gymnosperm B-sister genes may be involved in ovule/seed development and, in some species, in the growth of fleshy fruit-like structures. *Annals of Botany* 112:535–544.

Lovisetto, A., F. Guzzo, A. Tadiello, K. Toffali, A. Favretto & G. Casadoro 2012. Molecular analyses of MADS-box genes trace back to Gymnosperms the invention of fleshy fruits. *Molecular Biology and Evolution* 29(1):409–419.

Melikian, A.P. & A.V. Bobrov 1997. On the structure of outer seed envelopes – aril and epimathium – in the family Podocarpaceae. *Bulleten' Moskovskogo Obshchestva Ispytatelei*

Prirody. Otdel biologicheskii 102(5):46–53 (in Russian with English summary). [Меликян А.П., Бобров А.В. 1997. О строении наружных покровов семян – эпиматия и ариллуса – у представителей семейства Podocarpaceae Endlicher 1847 s. 1. // Бюлл. МОИП. Отд. биол. Т. 102, № 5. С. 46–53].

Melikyan, A.P. & A.G. Devyatov 2001. *Main carpological terms*. KMK, Moscow, 47 pp. (in Russian). [Меликян А.П., Девятов А.Г. 2001. Основные карпологические термины. Москва: КМК. 47 с.].

Nigris, S., G. D'Apice, S. Moschin, R. Ciarle & B. Baldan 2021. Fleshy structures associated with ovule protection and seed dispersal in Gymnosperms: A systematic and evolutionary overview. *Critical Reviews in Plant Sciences* 40(4):285–302.

Planchon, J.E. 1845. Développements et caractères des vrais et des faux arilles. *Annales des science naturelles* 3:275–312.

Serrato-Valentii, G., L. Cornara, P. Modenesii & P. Profumo 1991. The aril of the *Strelitzia reginae* banks seed: structure and histochemistry. *Annals of Botany* 67(6):475–478.

Shi, L. & F.-H. Wang 1989. Development of the megasporangiate shoot of Taxaceae. *Cathaya* 1:83–91.

Smal, C.M. & J.S. Fairley. 1980. Food of wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) in oak and yew woods at Killarney, Ireland. *Journal of Zoology* 191:413–418.

Spjut, R.W. 1994. A systematic treatment of fruit types. *Memoirs of the New York Botanical Garden* 70:1–181.

Spjut, R.W. 2010. *Overview of the genus Taxus (Taxaceae): The species, their classification, and female reproductive morphology*. Available from: http://www.worldbotanical.com/TAXNA.HTM. Last accessed 09.03.2022.

Suszka, B. 1975. Generative and vegetative reproduction. In: *The yew – Taxus baccata L. (Cis pospolity)*. (H. Markewicz, translator), pp. 87–102, National Technical Information Service, Springfield, VA.

Tittensor, R.M. 1980. Ecological history of yew *Taxus baccata* L. in southern England. Biological Conservation 17:243–265.

Trusov, N.A. 2016. Aril morphological nature and its function in some *Aristolochia*, *Asarum*, *Celastrus*, *Euonymus*, *Euphorbia*, *Viola* and *Taxus*. *Turczaninowia* 19(3):106–114. (in Russian with English summary) [Трусов Н.А. 2016. Морфологическая природа и функции ариллусов некоторых представителей родов *Aristolochia*, *Asarum*, *Celastrus*, *Euonymus*, *Euphorbia*, *Viola* и *Taxus* // Turczaninowia. Т. 19, № 3. С. 106–114].

Trusov, N.A. 2021. Arils of dried fruits and their relationship with dissemination. *Contemporary Problems of Ecology* 14(6): 690–700.

van der Pijl, L. 1982. *Principes of dispersal in higter plants*. Springer-Verlag, Berlin, 214 pp.