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Morphological variability of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) cones in the context of seed extraction

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Abstract

In the paper generating curves given by fourth-degree polynomials were used to model the shape of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) cones from the Polish Forest Districts of Kołaczyce (one batch) and Opole (two batches), and to calculate the surface area and volume of individual cones. However, it was not possible to construct generalized equations for the surface area and volume of Douglas fir cones due to the high variability of empirical coefficients. The surface area and volume of the cones were also calculated from their length and diameter based on formulas for a cylinder and a barrel corrected by constants k_1 and k_2. The mean surface area of closed Douglas fir cones determined for the first, second, and third batch using the generating function was 4,348.4 mm^2, 3,857.0 mm^2, and 2,844.7 mm^2, and the volume was 27,212.4 mm^3, 21,012.9 mm^3, and 12,844.4 mm^3, respectively. The corresponding values calculated from the geometric formulas for solids were 4,332.0 mm^2, 3,838.0 mm^2, and 2,862.9 mm^2 for the surface area and 27,366.0 mm^3, 20,648.9 mm^3, and 13,375.3 mm^3 for the volume. The evaporation area of open cones was found to be five times greater than that of closed cones, with the difference being statistically significant. The outer and inner surfaces of scales taken from the middle segment of Douglas fir cones were photographed using a Quanta 200 scanning microscope (FEIC). The characteristic elements of scale morphology were evaluated by means of *MultiScan Base* software package. The outer and inner surfaces of Douglas fir scales were found to differ in some important ways, similarly as it has been reported in the literature for the Scots pine, silver fir, European larch, and black alder. The outer surface of scales is formed by thick-walled cells with marked protrusions, while the inner surface reveals cells with thin, frayed walls in the region adjacent to the seeds and wings. Knowledge of the geometry of Douglas fir cones and the morphology of their scales may be helpful in optimizing seed extraction parameters for those cones.

Keywords: seed extraction, model, shape curve, surface area, volume, scanning electron microscope

Introduction

Poland's managed forests feature more than thirty introduced tree species (Bellon et al. 1977), including nine broadleaved and twenty-two coniferous species (Białobok and Chylarecki 1965). They can be grouped into two types of trees: those that were brought deliberately for production purposes, and those that were originally planted on non-forest sites for their ornamental merits, but then spontaneously spread to forests (Bellon et al. 1977). Most of the introduced tree species in Poland originate from North America (McDowell et al. 2000) and Asia, and some from Europe (Szymanowski 1959, Białobok and Chylarecki 1965, Bellon et al. 1977, Feliksik and Wilczynski 2003, St Clair et al. 2005), e.g. Italy (Marziliano et al. 2015, Ravaioli et al. 2019, Marchi and Cocozza 2021), Germany (Marziliano et al. 2015, Bindewald et al. 2021), and Poland (Giedrowicz et al. 2020).

The major tree species introduced in Poland for production purposes include the red oak, Douglas fir, and eastern white pine (Weymouth pine). The introduction of the Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) to Polish forests was motivated by the higher productivity of that species (Piszczek et al., 2012). The best Douglas fir stands in Poland are located in the provinces of Greater Poland, Pomerania, and Silesia. According to provenance studies conducted in 1952–1965 (Piszczek et al. 2012), young Douglas firs tend to be killed by frost east of the Vistula River.

Since 1966, countries that have conducted research on Douglas fir cultivation under the authority of the International Union of Forest Research Organisations (IUFRO) have reported that it is a good alternative to native species in terms of high productivity, especially at a time of climate change (Chylarecki 2004, Viewegh et al. 2014). In Europe, the Douglas fir is the only tree meeting the requirements for introduced species, and so it is increasingly cultivated in the managed stands of countries pursuing modern silvicultural practices. The species is documented to have been present in Poland for almost 200 years. However, due to ecological (Meinartowicz and Lewandowski 1994) and social protests, the introduction of alien plant and animal species, the Forest Act of September 28, 1991, imposed some legal limitations on the cultivation of the Douglas fir, with specific rules laid out in the Decision no. 53 of the Director-General of the State Forests, effective as of January 1, 2013. By 2003, the Silvicultural Guidelines recommended planting the Douglas fir at a rate of 2% to 10% of the overall tree species composition on fresh mixed broadleaved, fresh broadleaved, fresh montane, and mixed/coniferous montane forest sites (Sagan 2014).

The total area of Douglas fir stands in Poland is approx. 5,000 ha; the tree is predominantly used as a plantation species rather than in monospecific stands. Importantly, it exhibits the greatest productivity among the forest species cultivated in Poland, reaching 1000 m^3 ha^{-1} at 100 years (Chylarecki 2004). For the sake of comparison, the volume of larch stands of that age amounts to 500–880 m^3 ha^{-1} (Chylarecki 2004), while that of silver fir stands is 470–725 m^3 ha^{-1} (Sagan, 2014). Thus, bearing in mind the high stand productivity of the Douglas fir, the authors analyzed the external parameters of its cones with a view to optimizing seed extraction.

Douglas fir cones mature within one season and fall from the tree intact (Tomanek and Witkowska-Żuk 2008). They are cylindrical in shape, with a length of 8 cm and a diameter of 3 cm (Kozakiewicz and Wieruszewski 2005). On the other hand, Tomanek (Tomanek and Witkowska-Żuk 2008) has described them as brown ovate or oblong-ovate pendulous cones that are 7 to 10 cm long. Their characteristic feature is the presence of tridentate bracts extending over the thin seed scales (Sorensen and Campbell 1981). Douglas fir cones ripen in September or October; in dry and warm weather they quickly open and release seeds. Cone harvest time is determined based on maturation markers, such as the colour of the cone, its scales, and seeds, as well as seed separation from the scales (Maciejowski 1950).

A review of the literature on seed extraction from Douglas fir cones showed that the available data are insufficient to automate the process. Seed extraction research involving other coniferous species also lacks information about cone variability. The analysis of reports concerning the silver fir and European larch indicate that it is necessary to determine the surface area and volume of closed and open cones, as well as accurately describe cone morphology to evaluate the cone parameters relevant for seed extraction.

Thus, the objective of the study was to elucidate the characteristics of Douglas fir cones (*Pseudotsuga menziesii* (Mirb.) Franco), including length, diameter, weight, shape, surface area and volume when closed and open, as well as variability in scale morphology and surface area. Those measurements can be then used to calculate the evaporation area of open cones during the seed extraction process.

Materials and methods

Provenance of the studied material

The studied Douglas fir cones were obtained from the Odrzykoń Forest Unit (30 cones) in the Kołaczyce Forest District (batch MAT-1), Krosno State Forests Regional Directorate (GPS: 49°44’ N, 21°46’ E) and from the Lipowa Forest Unit (51 cones) in the Opole Forest District, Katowice State Forests Regional Directorate (GPS: 50°41’ N, 17°41’ E). The cones from the Opole Forest District came from two areas and were designated as batch MAT-2 (17 cones) and batch MAT-3 (34 cones).

Research apparatus and measurements

Cone weight was determined with an accuracy of 0.001 g using a WPS 210 S moisture analyzer (Radwag, Radom, Poland). Each cone was photographed against a unique number and a 150 × 0.05 mm manual caliper (Modeco MN 85-001, Poland) using a D3000 Nikon camera (Nikon, Tokyo, Japan) with a Nikkor AF-S DX 18–105 mm f/3.5–5.6G ED VR lens. The caliper provided a scaling reference for the image processing step. The images had a resolution of 3888 × 2126 pixels and were saved as .jpg files. The distance between the photographed material and the lens was 350 mm. The images were processed using MultiScan Base v. 18.03 software package (Computer Scanning System, Warsaw, Poland) to determine cone length (h) from the base to the tip with an accuracy of 0.1 mm and cone diameter every 1 mm (d_x), with the maximum diameter designated as d_{max}. Measurement points were indicated manually after the images had been scaled and a 1 × 1 mm grid was imposed on them. The images were not subjected to any morphological transformations or filtered (an automatic option was selected).

The measured cone diameters were used to calculate the radius for plotting the generating curve given by equation y. Equation y was approximated to define the generating curve for the cone surface. The surface area of cones (S_{obl}) was found using the following formula (Aniszewska 2007):

$$S_{obl} = 2 \cdot \pi \int_a^b y dL = 2 \cdot \pi \int_0^h y \sqrt{1 + \left(\frac{dy}{dx}\right)^2}\, dx \qquad (1)$$

where dL is the differential of the cone generating curve, y.

For the sake of comparison, the surface area of cones was also calculated from a formula for a cylinder:

$$S_w = \pi \cdot d_{max} \cdot h \qquad (2)$$

Subsequently, the generating curve equation was used to compute cone volume (V_{obl}) (Aniszewska 2007):

$$V_{obl} = \pi \int_0^h y^2 \, dx \quad (3)$$

For comparison, Douglas fir cone volume was also calculated using a formula for barrel volume (V_b):

$$V_b = \frac{1}{12} \cdot \pi \cdot h(2 \cdot d_{max}{}^2 + d^2) \quad (4)$$

$$d = \frac{d_1 + d_2}{2} \quad (5)$$

where: h is the cone length, d_{max} is the maximum diameter, d is the mean diameter calculated from the diameters of the Douglas fir cone base (d_1) and tip (d_2).

To increase accuracy, empirical coefficients k_1 and k_2 were used in equations (2) and (4), with the obtained values designated as S_1 and V_1. In the next step of analysis, the actual surface area obtained from the generating curve S_{obl} was compared with the surface area from the formula for a cylinder (S_w) and the actual volume obtained from the generating curve V_{obl} was compared with the volume from the formula for a barrel (V_b). The solids were fitted to the models based on the smallest difference between the results for surface area and volume.

Scales were counted in every studied cone. For two randomly selected cones from two provenances, three scales were removed from each: the base, middle segment, and tip. The scales were photographed, and the acquired images were used to measure their length, width, surface area, and circumference. Further, the obtained results were used to compute the evaporation area of open cones.

The inner and outer surfaces of scales collected from the middle segment of the cones were photographed using a FEI Quanta 200 scanning electron microscope (FEI 2006) at magnifications of 500× and 1000×, in triplicate or quadruplicate. The images revealed some characteristic regions on individual scales and enabled comparisons between the scales of Douglas fir cones from two different provenances.

Statistical analysis

Length, width (diameter), and weight measurements of the cones, whole scales, and their parts, as well as surface area and volume calculations (both those based on generating curves and formulas for solids) were processed in Statistica 13.3 (TIBCO 2017) software package. Differences between batches were checked using Tukey's test (also known as the honestly significant difference test, HSD) for unequal sample sizes. Analysis also included basic statistical parameters, such as means, minimum and maximum values, standard deviations, ranges, and coefficients of variability. Analyses were conducted at a statistical significance level of $\alpha = 0.05$. Box-and-whiskers plots were made for all calculated surface areas (S_{obl}, S_w, S_1) and volumes (V_{obl}, V_b, V_1).

To examine relationships between the length, maximum diameter, and weight of each cone, critical r values from statistical tables (Bruchwald 1997) were compared with the R values obtained from the dot plots made for these relationships.

Results

Cone measurements and relationships between them

Tukey's test revealed significant statistical differences (Table 1) between the three studied Douglas fir cone batches from two provenances. Table 1 shows mean cone dimensions for each batch: length (h), maximum diameter (d_{max}), base diameter (d_1), tip diameter (d_2), and weight (m).

Table 1. Cone dimensions in mm and weight in g

Variable	Mean	Min	Max	Range	SD	CV
			MAT-1			
h (mm)	62.3 [a]	51.6	83.7	32.1	7.7	12.3
d_{max} (mm)	28.9 [a]	24.8	33.5	8.7	2.2	7.5
d_1 (mm)	11.0 [a]	7.5	14.9	7.4	1.5	13.9
d_2 (mm)	6.3 [a]	3.1	12.2	9.1	2.6	41.4
m (g)	9.70 [a]	6.19	19.05	12.86	3.13	32.27
			MAT-2			
h (mm)	60.2 [a]	50.0	76.2	26.1	6.4	10.6
d_{max} (mm)	24.6 [b]	21.7	27.8	6.2	1.5	5.9
d_1 (mm)	8.4 [b]	6.2	13.3	7.1	1.6	18.7
d_2 (mm)	6.1 [a]	1.5	10.8	9.4	2.9	46.8
m (g)	8.82 [a]	5.96	13.21	7.25	1.96	22.26
			MAT-3			
h (mm)	50.4 [b]	39.3	60.2	20.9	4.8	9.6
d_{max} (mm)	22.5 [c]	19.4	26.4	6.9	1.8	7.8
d_1 (mm)	8.5 [b]	5.6	11.7	6.1	1.6	18.8
d_2 (mm)	6.9 [a]	1.9	14.8	12.9	3.2	46.0
m (g)	6.03 [b]	4.00	9.13	5.13	1.32	21.81

Notes: SD stands for standard deviation, CV stands for coefficient of variation, [a, b, c] denote groups of homogeneous parameters depending on the origin of the cones. Different letters denote significant differences between features at $p < 0.05$.

As can be seen from Table 1, cones from the Kołaczyce Forest District were the longest (mean length of 62.3 ± 7.7 mm) and had the greatest maximum diameter (mean d_{max} = 33.5 mm). In turn, the smallest mean length and maximum diameter were found for cones from the Opole Forest District (MAT-3), 50.4 ± 4.8 mm and 22.5 ± 1.8 mm, respectively.

In terms of relationships between cone measurements, the highest coefficient of determination R^2 between maximum cone diameter and length was found for the Opole batch MAT-2 ($R^2 = 0.71$) and the lowest one for the Kołaczyce batch MAT-1 ($R^2 = 0.51$).

As concerns the relationship between length and weight, the highest R^2 was obtained for the Kołaczyce cones ($R^2 = 0.90$) and the lowest one for the Opole batch MAT-2 ($R^2 = 0.73$). A 1.0 mm increment in length translated into an average increase in diameter and weight of 0.24 mm and 0.30 g, respectively.

Shape, volume, and surface area of closed cones

Figure 1 presents plots of generating curves for smaller and larger cones from the three tested cone batches. A high coefficient of determination R^2, amounting to almost 1.0, indicated that cone shape was best described by a fourth-degree polynomial with the general formula:

$$y = A \cdot x^4 + B \cdot x^3 + C \cdot x^2 + D \cdot x + E \quad (6)$$

where x adopts values from 0 to h and A, B, C, D, E are coefficients, which minimum, maximum, and mean values, standard deviations, ranges, and coefficients of variation are given in Table 2.

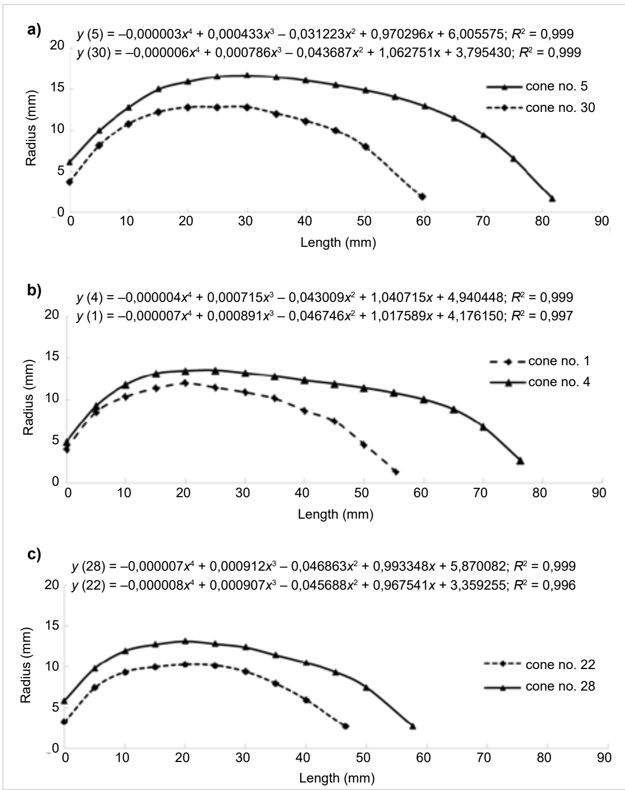

Figure 1. Plots of generating curves and their equations: a) cones no. 5 and 30 from the Kołaczyce Forest District, batch MAT-1, b) cones no. 1 and 4 from the Opole Forest District, batch MAT-2, c) cones no. 22 and 28 from the Opole Forest District, batch MAT-3

The *post-hoc* Tukey HSD test indicated the absence of statistically significant differences for coefficients A ($p = 0.11$) and D ($p = 0.07$) between the three batches and for coefficients C ($p = 0.40$) and E ($p = 0.99$) between the two batches from Opole (MAT-2 and MAT-1). A significant difference was found for coefficient B ($p = 0.01$) between cones from Kołaczyce MAT-1 and Opole MAT-2.

Knowing the coefficients of equation (6) and using formulas (1) and (3), surface area (S_{obl}) and volume (V_{obl}) were computed for all the studied cones. In addition, mean cone volume and surface area were calculated from the geometric formulas (2) and (4); all those results are given in Table 3. Mean S_{obl} and V_{obl} were 4,348.4 mm^2 and 27,212.4 mm^3 for the Kołaczyce cones, 3,857.0 mm^2 and 21,012.9 mm^3 for the Opole MAT-2 cones, and 2,844.7 mm^2 and 12,844.4 mm^3 for the Opole MAT-3 cones, respectively.

As can be seen from Table 3, the cones may be approximated as barrel-shaped solids, since the V_b results were the closest to the actual V_{obl} values. In a preliminary study, calculations were also made using formulas for a cylinder and a geometric cone, with additional correction coefficients k_1 and k_2 introduced to improve the accuracy of the resulting V_1 and S_1 values. The correction coefficients were fitted separately for each batch of cones due to the significant differences ($p < 0.01$) between them revealed by the Tukey test.

The volume correction coefficient k_2 amounted to 0.94 for the MAT-1 batch, 1.02 for the MAT-2 batch, and 0.93 for the MAT-3 batch, while the corresponding values of the surface area correction coefficient k_1 were 0.72, 0.82, and 0.80.

The ANOVA tests for the S_{obl} and S_1 surface areas (MAT-1 – $F_{(1, 58)} = 0.006$, $p = 0.94$; MAT-2 – $F_{(1, 32)} = 0.005$, $p = 0.94$; MAT-3 – $F_{(1, 66)} = 0.023$, $p = 0.88$) as well as V_{obl} and V_1 volumes (MAT-1 – $F_{(1, 58)} = 0.006$, $p = 0.93$;

Table 2. Descriptive statistics for equation coefficients *A*–*E* modelling the shape of Douglas fir cones from the Kołaczyce batch MAT-1 and Opole batches MAT-2 and MAT-3

Variable	Mean	Min	Max	Range	SD	CV
			MAT-1			
A	–0.000003 [a]	–0.000011	0.000004	0.000015	0.000003	–115.648
B	0.000404 [a]	–0.000244	0.001153	0.001397	0.000350	86.474
C	–0.029487 [a]	–0.053868	–0.007100	0.046768	0.012377	–41.976
D	0.883263 [a]	0.578146	1.222174	0.644028	0.149727	16.952
E	5.570633 [a]	3.795430	7.651854	3.856424	0.764487	13.724
			MAT-2			
A	–0.000005 [a]	–0.000011	–0.000001	0.000010	0.000003	–57.240
B	0.000734 [b]	0.000281	0.001255	0.000974	0.000298	40.529
C	–0.041944 [b]	–0.059807	–0.023021	0.036786	0.010407	–24.812
D	0.987529 [a]	0.632571	1.149873	0.517302	0.133211	13.489
E	4.295164 [b]	3.148592	6.685300	3.536708	0.751945	17.507
			MAT-3			
A	–0.000004 [a]	–0.000010	0.000004	0.000014	0.000004	–86.786
B	0.000602 [ab]	–0.000176	0.001117	0.001293	0.000348	57.811
C	–0.036808 [b]	–0.052799	–0.007722	0.045077	0.011301	–30.703
D	0.876119 [a]	0.509262	1.152364	0.643102	0.146432	16.714
E	4.286165 [b]	2.899277	5.870082	2.970805	0.781631	18.236

Note: [a, b] denote groups of homogeneous coefficients depending on the type of material. Different letters denote significant differences between features at $p < 0.05$.

Table 3. Basic statistics for the surface area and volume of Douglas fir cones

Parameter	Mean	Min	Max	Range	SD	CV
MAT-1						
S_{obl} (mm^2)	4348.4 [a]	3176.3	6589.6	3413.3	758.1	17.4
S_w (mm^2)	5700.0 [a]	4152.0	8803.6	4651.6	1092.0	19.2
S_1 (mm^2)	4332.0 [a]	3155.5	6690.7	3535.2	829.9	19.2
V_{obl} (mm^3)	27212.4 [a]	17490.9	44927.2	27436.3	6932.4	25.5
V_b (mm^3)	29112.7 [a]	18469.8	52324.8	33855.0	7911.6	27.2
V_1 (mm^3)	27366.0 [a]	17361.6	49185.3	31823.7	7436.9	27.2
MAT-2						
S_{obl} (mm^2)	3857.0 [a]	2777.3	6444.2	3666.8	911.0	23.6
S_w (mm^2)	4680.5 [b]	3574.1	6470.9	2896.8	758.1	16.2
S_1 (mm^2)	3838.0 [a]	2930.8	5306.1	2576.3	621.6	16.2
V_{obl} (mm^3)	21012.9 [b]	12694.1	44538.6	31844.5	7705.1	36.7
V_b (mm^3)	20244.0 [b]	13910.7	30313.1	16402.4	4475.3	22.1
V_1 (mm^3)	20648.9 [b]	14188.9	30919.4	16730.5	4564.8	22.1
MAT-3						
S_{obl} (mm^2)	2844.7 [b]	2031.5	3916.1	1884.6	521.6	18.3
S_w (mm^2)	3578.6 [c]	2532.7	4776.1	2243.4	591.4	16.5
S_1 (mm^2)	2862.9 [b]	2026.2	3820.9	1794.7	473.1	16.5
V_{obl} (mm^3)	12844.4 [c]	2241.1	20696.1	18455.0	3698.1	28.8
V_b (mm^3)	14382.1 [c]	9363.1	22080.7	12717.5	3472.9	24.1
V_1 (mm^3)	13375.3 [c]	8707.7	20535.1	11827.4	3229.8	24.2

Note: [a, b, c] denote groups of homogeneous parameters depending on the origin of the cones. Different letters denote significant differences between features at $p < 0.05$.

MAT-2 – $F_{(1, 32)} = 0.028$, $p = 0.87$; MAT-3 – $F_{(1, 66)} = 0.004$, $p = 0.95$) did not reveal any significant differences between those pairs of values, which indicates that the surface area and volume of Douglas fir cones can be accurately evaluated based on cone length and diameter using geometric formulas for solids.

Number of scales and surface area of open cones

Data about the mean number of scales per cone (Table 4) and their mean inner and outer surface areas were used to compute the overall evaporation area of open Douglas fir cones.

The Kołaczyce cones had on average of 41 scales, the Opole MAT-2 cones 37 scales, and the Opole MAT-3 cones 48 scales. The MAT-3 batch was characterized by the smallest cones, but with the greatest number of scales per cone.

The mean surface area of scales (including the inner and outer surfaces) was 820 mm^2 for the MAT-1 batch (large cones), 587 mm^2 for the MAT-2 batch (large cones), and 404 mm^2 for the MAT-3 batch (small cones). As compared to the surface areas computed from formula (1), it was found that the mean evaporation area increased sevenfold for the large MAT-1 cones, fivefold for the large MAT-2 cones, and sixfold for the small MAT-3 cones.

Table 4. Number of scales in cones from the MAT-1, MAT-2 and MAT-2 batches

Batch	Mean	Min	Max	Range	SD	CV
MAT-1	41 [a]	32	66	34	7	18
MAT-2	37 [a]	31	55	24	4	11
MAT-3	48 [b]	36	59	23	7	14

Note: [a, b, c] denote characters denoting significant differences between features at $p < 0.05$.

Scale morphology

Figure 2 presents the bottom part of the inner surface of scales collected from Opole MAT-2 and Kołaczyce cones (MAT-1). Scale fragments between the seeds are shown in Figures 2b, d, while scale fragments in direct contact with the seeds are given in Figures 2c, e. The former region of the scale is characterized by protrusions with rounded ends, while the latter is devoid of such protrusions; instead, it reveals long chains of cells.

A comparison of MAT-2 and MAT-1 provenances indicates that the scales of Opole cones have fewer protrusions (Figure 2b) and fewer cells arranged in chains (Figure 2c) as compared to the scales of the Kołaczyce cones (Figures 2d, e).

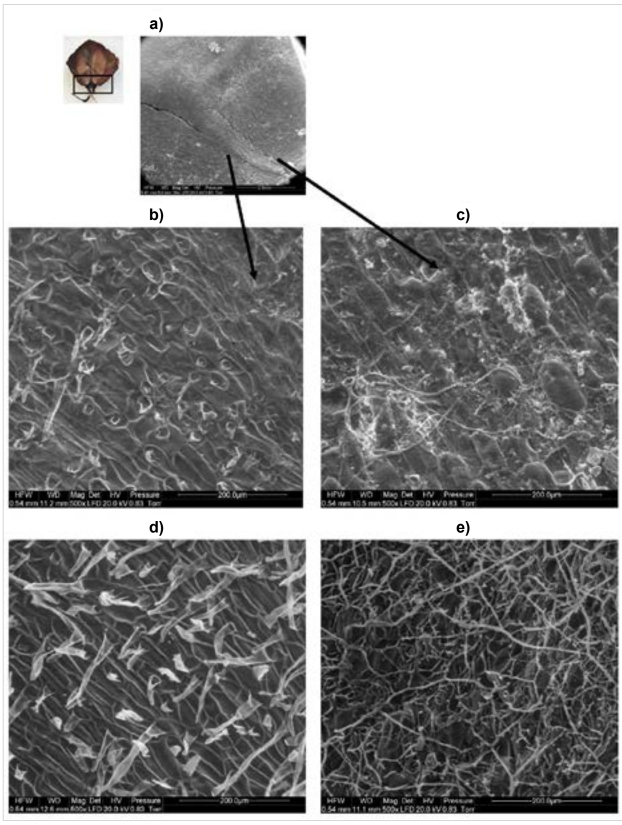

Figure 2. Scanning electron microscope images: a) inner surface of the bottom part of a scale collected from the middle segment of a cone from Opole (MAT-2), 50× magnification; b) morphology of a scale fragment between the seeds, cone from Opole, 500× magnification; c) morphology of a scale fragment in contact with the seeds, cone from Opole, 500× magnification; d) morphology of a scale fragment between the seeds, cone from Kołaczyce, 500× magnification; e) morphology of a scale fragment in contact with the seeds, cone from Kołaczyce, 500× magnification

Figure 3 presents the upper internal surface of a scale from an Opole (MAT-2) cone. Figure 3b shows the morphology of a scale near its upper margin, above the seed wing, while Figure 3c depicts the scale fragment in contact with the wing. A clear boundary between these two regions can be seen in Figure 3d. Outside the region adjoining the seed wing, the scale exhibits numerous protrusions of varying diameters and areas. In turn, the region outside the seed wing is composed of thin-walled cells of diverse shapes. The morphology of that scale fragment does not differ significantly between the Opole and Kołaczyce cone batches.

Figure 4 presents an image of the bottom external part of a scale from an Opole cone. Similarly, as in Figure 2, here scale fragments opposite the seed (4c) and above it (4b) are also included. The seed itself is enclosed by the scale below it. Above the seed, the cells are regular, thick-walled, and roughly rectangular in shape. In turn, the cells in 4c have diverse shapes and are not so regularly arranged.

Figure 5 shows the upper external fragment of a scale from an Opole cone. Figure 5b (cone from Opole) and Figure 5d (cone from Kołaczyce) show the morphology of a scale fragment in contact with the seed wing enclosed by a scale below it. That region of the scale is markedly different from the other ones. It is completely or mostly devoid of typical conical protrusions; instead its surface is covered with small truncated projections. The cells in that region are oval and rather regular in size, resembling small chan-

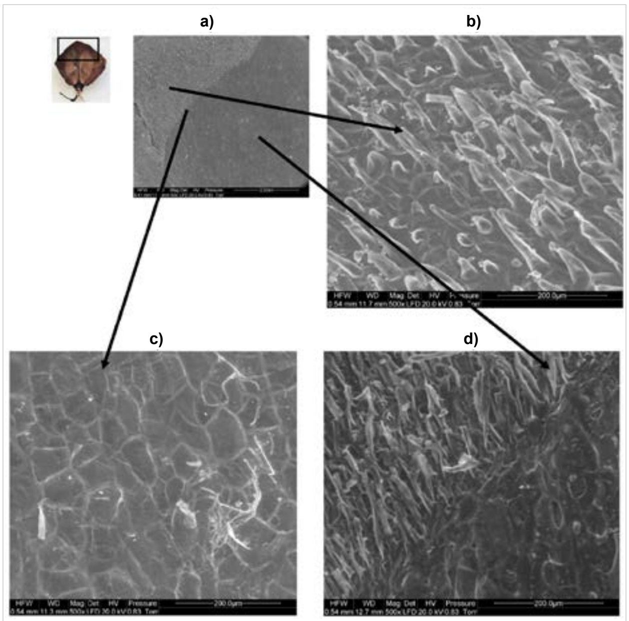

Figure 3. Scanning electron microscope images: a) inner surface of the upper part of a scale collected from the middle segment of a cone from Opole (MAT-2), 50× magnification; b) morphology of a scale fragment above the seed wing, 500× magnification; c) morphology of a scale fragment in contact with the seed wing, 500× magnification; d) morphology of a scale fragment at the boundary of the seed wing, 500× magnification

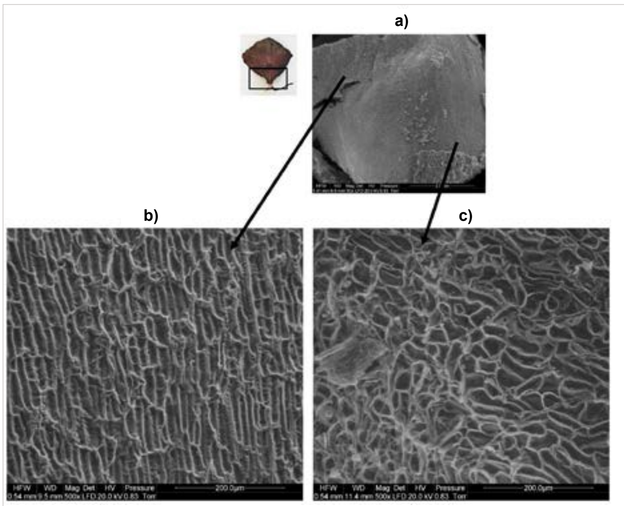

Figure 4. Scanning electron microscope images: a) outer surface of the bottom part of a scale collected from the middle segment of a cone from Opole (MAT-2), 50× magnification; b) morphology of a scale fragment above the seed, 500× magnification; c) morphology of a scale fragment opposite the seed, 500× magnification

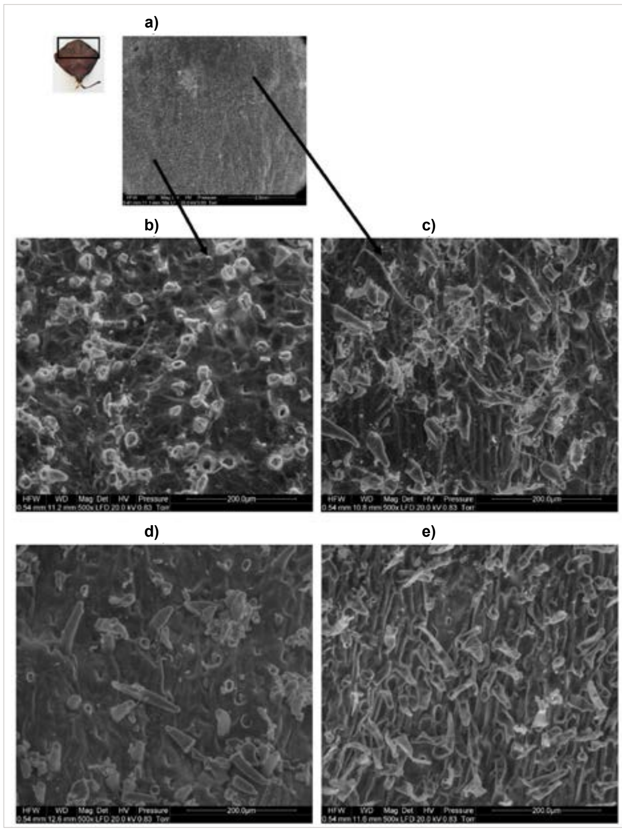

Figure 5. Scanning electron microscope images: a) outer surface of the upper part of a scale collected from the middle segment of a cone from Opole (MAT-2) 50× magnification; b) morphology of a scale fragment in contact with the seed wing, cone from Opole, 500× magnification; c) morphology of a scale fragment near the upper scale margin, cone from Opole, 500× magnification; d) morphology of a scale fragment in contact with the seed wing, cone from Kołaczyce, 500× magnification; e) morphology of a scale fragment near the upper scale margin, cone from Kołaczyce, 500× magnification

nels. Many well-developed protrusions can be seen in the region outside the seed wing (Figure 5c – cone from Opole and Figure 5e – cone from Kołaczyce).

Analysis of the SEM images of different parts of scales did not reveal any significant differences between Douglas fir cones from the two studied provenances, except from the bottom internal fragment of the scale shown in Figure 2.

Discussion

Numerous authors have studied the variability of cones and seeds of both coniferous and broadleaved tree species present in Polish forests, including the Scots pine (Zajączkowski 1949, Staszkiewicz 1968, Białobok et al. 1993, Hauke-Kowalska et al. 2019), Norway spruce (Chmielewski and Tyszkiewicz 1968, Barzdajn 1996, Kulej and Skrzyszewska 1996, Aniszewska 2001, Tomanek and Witkowska-Żuk 2008, Buraczyk, 2009), Silver fir (Barzdajn 1996, Tracz and Barzdajn 2007), European larch (Bałut 1969) and Black alder (Aniszewska et al. 2019). Nevertheless, to the best of our knowledge, there are no studies on the parameters of Douglas fir cones in the context of seed extraction.

The mean cone length of the three statistically different batches studied herein was smaller than that reported in the literature (Kozakiewicz and Wieruszewski 2005, Tomanek and Witkowska-Żuk 2008). Also the mean diameter of the cones from the three batches was lower than elsewhere (Tomanek and Witkowska-Żuk 2008). The mean cone weight amounted to 9.70 g, 8.82 g, and 6.03 g at a moisture content of 28%. The coefficient of determination R^2 between the maximum cone diameter and length was the greatest for the second batch – MAT-2 ($R^2 = 0.71$), while the highest R^2 between cone weight and length was found for the first batch – MAT-1 ($R^2 = 0.90$).

The shape of Douglas fir cones was modelled using a generating curve given by a fourth-degree polynomial. Subsequently, the surface area and volume of closed cones were computed using a differential of that curve. In a preliminary study, those parameters were also calculated from formulas for a cylinder and geometric cone. Similar comparisons of tree cone shapes to geometric solids, such as a cone, barrel, and cylinder, have been previously carried out for the Scots pine, Norway spruce, European larch, Silver fir, and Black alder (Gawart and Mikłaszewicz 2000, Aniszewska 2001, Aniszewska et al. 2017a, 2019). However, the best approximations of Douglas fir cone volume were obtained using a formula for a barrel corrected by coefficient k_2 equal to 0.94, 1.02, and 0.93 for the first (MAT-1), second (MAT-2), and third batches (MAT-3), respectively. The surface area was determined from a formula for a cylinder corrected by coefficient k_1 amounting to 0.76, 0.82, and 0.80, respectively. The obtained results were not significantly different from the actual values of V_{obl} and S_{obl}.

The number of scales per cone in the three studied batches was 41, 37, and 48, respectively. The mean surface area of scales ranged from 404 mm^2 to 820 mm^2, which was greater than that of scales collected from the bottom (192 mm^2), top (356 mm^2), and middle (453 mm^2) parts of silver fir cones. Furthermore, it was smaller than the surface area of scales from the middle segment of Norway spruce cones, which was 494 mm^2.

Knowing the surface area of scales and their number per cone, it was possible to calculate the evaporation area for whole open cones, which was found to be five to seven times greater than that for closed cones. For the sake of comparison, the difference between the evaporation area of closed and open cones reported from other studies was 14-fold for Norway spruce (Mikłaszewicz 2000, Aniszewska 2001) and sixfold for Scots pine (Gawart 2000).

The scale morphology of the two Douglas fir provenances (Kołaczyce and Opole) was examined by the SEM method, which was previously used to study the morphology of Scots pine, silver fir, European larch, and black alder scales (Aniszewska et al. 2017a, 2017b, 2019). Considerable morphological differences were observed between the outer surface of scales and their inner (concave) surface enclosing the seeds. The inner surface revealed protrusions with rounded tips between the seeds, as in the European larch, and long chains of cells in regions which were in contact with the seeds, as in the Scots pine. Those protrusions and chains of cells are structures that probably serve to supply and remove water from the cones. This is consistent with the morphological study of seed and bract scales by Kaniewski and Kucewicz (1978), who reported that the loss of moisture from cones was largely mediated by numerous live trichomes (projections) present on the epidermis of seed scales. The cones from Opole had fewer protrusions and chains of cells as compared to the ones from Kołaczyce. In the cones of both provenances, the inner surface of scales in contact with the seed wings revealed thin-walled cells, while outside the wings numerous protrusions were observed, with the two regions of the scale being separated by a clear boundary (Figure 2d). Similarly to the European larch and black alder, the outer surface of Douglas fir scales does not have apophyses (Aniszewska et al. 2017a). In the present study, the bottom part of the outer scale surface exhibited irregularly shaped cells arranged in an asymmetrical manner. They were present in the region that was in contact with the seed enclosed by the scale below. Above that region of the scale, the cells were regular, thick-walled, and roughly rectangular in shape, as in the silver fir, European larch, and black alder (Aniszewska et al. 2017b, 2019). The upper external surface of scales (under the bracts) exhibited no whole elongated cells, but rather small oval cells resembling little channels. Outside the bract-adhering region, the seed scales revealed numerous well-developed protrusions. A comparison of the various scale parts collected from cones of the two provenances did not show any significant differences except for the bottom part of the concave scale surface.

The presented investigations of Douglas fir cones may be treated as a pilot study. The results should be verified in further research.

Conclusions

The shape of conifer cones can be accurately modelled by a generating curve given by a fourth-degree polynomial, whose coefficients were determined in the present study. The polynomials of curves representing the shapes of individual cones were used to calculate the surface area and volume of Douglas fir cones, which were adopted as their actual values. However, averaged polynomials cannot be applied to determine the surface area and volume of individual cones.

The surface area and volume of Douglas fir cones can be calculated from their length and diameter using formulas for a cylinder and barrel, respectively, corrected by the empirically defined coefficients k_1 and k_2.

A more than fivefold increase in evaporation area was noted for open cones as compared to closed ones, with the result being statistically significant.

Differences in the morphology of the various parts of inner and outer scale surfaces were observed, which may have implications for the seed extraction process.

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