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ORIGINAL PAPER

Variability of cone parameters and scale morphology in the black alder (*Alnus glutinosa* L.) in the context of seed extraction

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Abstract

In this paper, the results of research on the variability of black alder cones (*Alnus glutinosa* L.) ware presented. The research was carried out for two, significantly different, batches of cones. Basic size parameters and mass were measured. The shape of the cone was determined, and it was described with the fourth-degree polynomial. The surface area and volume of the cone were calculated using the forming curve and formulas for solids: barrel and cylinder. The parameters of cones—shape surface area and volume—were analyzed. It was found that for alder cones (from the researched origins), the average volumes calculated from the barrel formula are 1701 mm^3 and 1162 mm^3, and the areas calculated from the cylinder formula are 807 mm^2 and 597 mm^2. The structure of the inner and outer sides of scales was examined using a scanning electron microscope. Using the MultiScanBase v. 18.03 program, the elements of husk structure that could affect the efficiency of seeds extraction were measured. The results of the research can be used to program the process of seeds extraction from alder cones in commercial installations.

Keywords Cone · Scale · Surface area · Volume · Electron microscope

Introduction

The alder (*Alnus*) naturally occurs in European forests, but small clusters of these trees may be also found in harsh environmental conditions in northwestern Africa, northern Turkey, the Caucasus, and some valleys of the Atlas Mountains (Claessens et al. 2010). Three alder species are widespread in Europe: two trees (black and grey alder) and one shrub (green alder). According to Murat (2002), *Alnus* trees may be planted as part of silvicultural treatments and phytomelioration, mostly in pine stands, as well as in the process of reconversion of degraded soils. Due to their constant demand for nutrients and a rapid growth rate, *Alnus* species are used as pioneer plants for the stabilization of river banks (Meyer et al. 2014) and the reclamation of wetland sites, many of which have dried up in recent years (Beatty et al. 2015). Of the three species mentioned above, the most highly valued one is the black alder as it can fix nitrogen from the atmosphere with the process of symbiosis with *Frankia* bacteria (Bond et al. 1954). Both aqueous and alcohol extracts from the seeds, bark, and leaves of *Alnus glutinosa* L. exhibit strong antibacterial activity (Abedini et al. 2016).

Black alder seeds may be collected already from 20-year-old trees, as long as they grow in open land with ample insolation. In dense stands, seeds appear later, at approximately 30 years. Individual alders may produce seeds annually, but more typically every 3 years (Suszka et al. 2000).

Black alder cones are picked manually from standing or lying (felled) trees and then stored in a well-ventilated area. In enclosed spaces, alder cones release seeds within 2–3 weeks. However, due to high requirements and an emphasis on repeatability, it should be noted that such conditions do not afford full control over seed quality. Thus, the process of cone opening should be explored in detail to determine the optimum temperature and humidity parameters with a view to obtaining the highest quality seeds.

The available literature provides information about black alder stands and their growth patterns(Aguinagalde et al. 2005; Lorenc-Plucińska et al. 2013; Vacek et al. 2016; Socha

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and Ochał 2017), individual trees, wood structure(Yaman 2009; Han et al. 2015), as well as the quality (Kaliniewicz et al. 2018), weight, size, and storage of seeds (Załęski 1995; Aniśko et al. 2006; Bodył 2007; Gosling et al. 2009; Załęski et al. 2009; Kaliniewicz and Trojanowski 2011; Plitta et al. 2013). There is not much information in the available literature regarding seed extraction using kilns or extraction chambers to accelerate scale opening and seed release. According to Murat (2002), black alder cones should be kept in a kiln at 27–38 °C for 6–10 h. Suszka et al. (2000) have reported the extraction process to last 20–25 days at 20 °C and only 1–2 days at 40–45 °C. In practice, the process parameters temperature and humidity and drying time are often set intuitively. After opening, the cones are placed in a rotating drum to obtain seeds. The final moisture content of the seeds after cleaning should be reduced to 3.5% (Aniśko et al. 2006) for storage purposes (Tylkowski 2014). Acquisition of 1 kg of seeds requires the collection and scaling of 15–20 kg of alder cones.

According to the present authors, the current state of knowledge on black alder seed extraction is insufficient, which motivated the study. In order to appropriately design the drying process, the physical parameters of the processed material should be examined, as well as the relationships between them. Thus, the objective of the work was to determine the basic size parameters of black alder cones, including their shape, surface area, volume, as well as the structure of the inner and outer sides of their "pseudo"-scales, which are necessary for developing seed extraction programs under controlled temperature and humidity conditions.

Methodology

Provenance of black alder cones

The study involved closed black alder cones collected in the Maskulińskie Forest District and obtained from a seed extraction facility in Ruciane Nida. The first cone lot was from the Ol40 region with an area of 7.79 ha, registered number MP/3/41009/05. The other lot was of the same provenance, but the cones were collected from a single clone (no. 5534). The cones were harvested in October 2016 and placed in cold storage at 2 °C until measurement.

Size parameters: shape, volume, surface area, and weight

The study material encompassed 100 cones selected at random from two lots (50 from each). Every cone was marked prior to measurements.

In the first step, each cone was weighed with an accuracy of 0.1 mg on a WPA40/160/C/1electronic balance (Radwag, Radom, Poland) designated for precision laboratory weighing. Next, the actual volume of black alder cones (V_{act}) was measured using a 10-mL NS10/19 graduated cylinder (Alchem, Poland) filled with distilled water, with an accuracy of 0.2 mL.

Cones were photographed with a Nikon D3000 (Nikon, Tokyo, Japan) camera with AF-S DX NIKKOR 18–105 mm f/3.5–5.6G ED VR lens. Marked cones were placed on table next to the manual caliper (Modeco MN 85-001, Poland) which was a measurement standard, used later for scaling. Pictures of cones were made in the resolution of 3888 × 2126 pixels, saved on computer hard disk in JPG and RAW format. They were made on a white background with one camera position. The distance between the lens and the cones was 350 mm. The length of the cone (h) from the top to the base, the maximum thickness (D), and the current diameter with the interval of 0.1 cm (d_x) were measured in the cone pictures using the MultiScanBase v. 18.03 program (Computer Scanning System, Warsaw, Poland). The measuring points were determined manually after image scaling and application of grid with mesh size of 1 mm × 1 mm. No additional morphological transformations of the image were used, images were not filtered, and the option "automatically" was chosen.

These measurements were used to calculate the surface area of the examined cones. Each cone was treated as a solid of revolution, and a generating curve for the surface of the solid was defined. The point of origin of the coordinate system was adopted as the distance between the cross section and the base of the cone (Gawart and Mikłaszewicz 2000; Aniszewska 2012).

The cross-section (x_j) and radius (r_j) coordinates for each cone were used to approximate an equation defining the generating curve for the cone surface. Accuracy was evaluated by minimizing differences between squared function values and squared actual values x^2.

$$x^2 = \sum_{j=1}^{n} (y_{ij} - r_j)^2 \tag{1}$$

where y_{ij}—value of ith function at jth cone cross section, r_j—radius at jth cross section, n—number of cone cross sections (Gawart and Mikłaszewicz 2000)

Given that the shape function $y = f(x)$ is continuous and nonnegative for the entire cone height (h), the surface area (S_{cal}) was calculated using the following formula:

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

where dL is the differential of the generating curve.

For the sake of comparison, cone surface area was also calculated by means of another formula treating cones as cylinder-like solids (S_c).

$$S_c = \pi \cdot D \cdot h \tag{3}$$

Cone volume (V_{cal}) was calculated using the following formula:

$$V_{cal} = \pi \int_0^h y^2 \, dx \tag{4}$$

For comparison, volume was computed using a formula treating cones as barrels (V_b) with elliptical generating curves:

$$V_b = \frac{1}{12} \cdot \pi \cdot \left(2 \cdot D^2 + d^2\right) \tag{5}$$

where D—midplane diameter (largest diameter of the cone), d—diameter of the base.

In this formula, d was the sum of the base (d_I) and tip (d_{II}) of the cone divided by two.

Structural studies of black alder scales

Structural studies of the inner and outer surfaces of black alder scales were carried out at the Analytical Center of the Warsaw University of Life Sciences. Selected parts of scales from the middle region of the cones were examined using an FEI Quanta 200 ESEM scanning electron microscope with an EDS EDAX analyzer (ThermoFisher Scientific, USA). The scales were placed on a tray that was inserted in the microscope sample chamber. After closing the chamber, the air was evacuated. Subsequently, each sample was photographed at 50×, 500×, and 1000× magnifications. Images were recorded in.tiff format at 2048 × 1886 pixels and a resolution of 190 dpi.

Statistical analysis

Cone size parameters, weight, surface area, and volume were statistically analyzed using Statistica 13 software (Dell Inc. 2016). Normality of distribution of the variables was evaluated by means of the Shapiro–Wilk test. Selected parameters were compared using ANOVA. All analyses were performed at a significance level of 0.05.

Results

Size parameters: shape, volume, surface area, and weight

The height of cones from the first lot ranged from 13.40 to 26.70 mm (with a mean $M = 19.24$ mm), while their diameter ranged from 10.30 to 16.00 mm ($M = 12.61$ mm). In the second lot, cone height was 13.90–21.50 mm ($M = 16.53$ mm), with a diameter of 9.60–13.10 mm ($M = 11.44$ mm) (Table 1).

Table 1 Descriptive statistics for the size parameters of cones from two lots

Lot	Data	Mean	SD	Min	Max	Range	Coefficient of variation
1	Height h_1 (mm)	19.24	3.87	13.40	26.70	13.30	20.12
	Diameter D_1 (mm)	12.61	1.63	10.30	16.00	5.70	12.93
	Diameter d_1 (mm)	2.21	0.84	0.70	4.50	3.80	38.01
	Weight m_1 (g)	0.298	0.135	0.155	0.749	0.593	45.269
	Volume, V_{cal1} (mm^3)	1770.72	849.00	789.79	3945.28	3155.49	47.95
	Volume V_{b1} (mm^3)	1701.14	799.27	760.57	3639.70	2879.13	46.98
	Actual volume V_{act1} (mm^3)	1600.00	600.00	800.00	2800.00	2000.00	41.44
	Surface area S_{cal1} (mm^2)	682.73	325.45	358.78	1561.97	1203.19	47.67
	Surface area S_{c1} (mm^2)	806.87	302.46	433.60	1733.20	1299.60	37.49
2	Height h_2, mm	16.53	1.68	13.90	21.50	7.60	10.18
	Diameter D_2 (mm)	11.44	0.76	9.60	13.10	3.50	6.66
	Diameter d_2 (mm)	1.75	0.36	1.30	2.65	1.35	20.72
	Weight m_2 (g)	0.26	0.05	0.16	0.39	0.22	19.18
	Volume V_{cal2} (mm^3)	1228.65	348.97	630.36	1913.06	1282.70	28.40
	Volume V_{b2} (mm^3)	1161.58	253.40	701.24	1951.10	1249.86	21.82
	Actual volume V_{act2} (mm^3)	1100.00	280.00	800.00	1800.00	1000.00	24.60
	Surface area S_{cal2} (mm^2)	492.39	119.12	315.68	918.95	603.27	24.19
	Surface area S_{c2} (mm^2)	596.85	94.44	434.29	884.83	450.54	15.82

Analysis of relationships between the basic size parameters revealed that height and diameter were significantly correlated for the first lot of cones (0.866): a 1-mm increase in height corresponded to a 0.36-mm increase in diameter. These parameters were also significantly correlated in the second lot (0.701), at a ratio of 1–0.32 mm.

Cones from the first lot were characterized by weight (Table 1), with the extreme values being 0.156 g and 0.749 g ($M = 0.298$ g). The weight of cones from the second lot ranged from 0.162 to 0.387 g ($M = 0.260$ g).

The shape of black alder cones was best described by a fourth-degree polynomial as its correlation coefficient (R) was higher than that for second- and third-degree polynomials, and only insignificantly lower than that for a fifth-degree polynomial. Sample shapes of a large cone (no. 2.39) and a small cone (no. 2.14) from the second lot are given in Fig. 1.

The proposed general equation for the generating curve of cone shape is as follows:

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

where x ranges from 0 to h, with y being the cone radius.

Furthermore, the relationship between the examined cone parameters (height and diameter) and the coefficients of the shape equation (A, B, C, D, E) was examined. Despite the high variability of the coefficients, in the first lot, significant correlations were found for all of them, and in particular between A, C, E and cone height, and between B, D and cone diameter. In the second lot, significant correlations were also found between coefficients A, C, E and cone height, while B and D did not reveal any correlations. Correlation equations and correlation coefficients for both lots are presented in Table 2.

Coefficients A, B, C, D, and E for both cone lots were subjected to ANOVA, which showed that the effects were significant depending on p, where $p < 0.0500$. According to ANOVA, coefficients B ($F = 4.5151$, $p = 0.0376$), C ($F = 7.0285$, $p = 0.01017$), D ($F = 10.3675$, $p = 0.0020$), and E ($F = 33.0702$, $p = 0.0001$) for the two lots were significantly different, in contrast to coefficient A, for which $p > 0.0500$.

Table 2 Correlation equations and correlation coefficients between the coefficients of cone shape generating curves for the first and second lot sand black alder cone height and diameter

Coefficient	Regression equation		Correlation coefficient	
	Lot 1	Lot 2	Lot 1	Lot 2
A	0.0001 h − 0.0028	0.0001 h − 0.0028	0.8541	0.6300
B	− 0.0054 D + 0.0915	–	0.6693	–
C	0.0256 h − 0.8049	0.0200 h − 0.7093	0.8193	0.4543
D	− 0.0949 D + 3.1556	–	0.4362	–
E	0.0638 h + 0.2778	0.0716 h − 0.2006	0.6724	0.3888

The cone surface areas and volumes calculated from Formulas 2 and 4 are given in Table 1. The mean surface area was 6.82 (± SD 3.25) cm^2 for the first lot and 4.92 (± SD 1.19) cm^2 for the second lot, with the mean volume being 1.77 (SD ± 0.85) cm^3 and 1.23 (SD ± 0.35) cm^3 for the first and second lots, respectively.

The surface areas calculated from the formula for a cylinder (3) and volumes computed from the formula for a barrel (5) are presented in Table 1. The mean surface area was 8.06 (± SD 3.02) cm^2 for the first lot and 5.96 (± SD 0.94) cm^2 for the second lot, with the mean volume being 1.70 (SD ± 0.80) cm^3 and 1.16 (SD ± 0.25) cm^3 for the first and second lots, respectively.

When comparing the values of the actual volume V_{act}, for both batches, they have a lower value than the volume calculated from the forming curve V_{cal} by an average of about 0.170 cm^3 and 0.130 cm^3, and lower than V_b by 0.100 cm^3 and 0.061 cm^3, respectively (Table 1). A comparison of surface area computed for the two lots using the curve S_{cal} shows that the results were on average lower relative to the values obtained for S_c by 0.124 cm^2 and 0.104 cm^2. Therefore, to arrive at cone volume and surface area based on height (h) and diameters (D and d), the volume calculated from the formula for a barrel (V_b) should be multiplied by 0.77 and 0.92 for the first and second lots, respectively, while

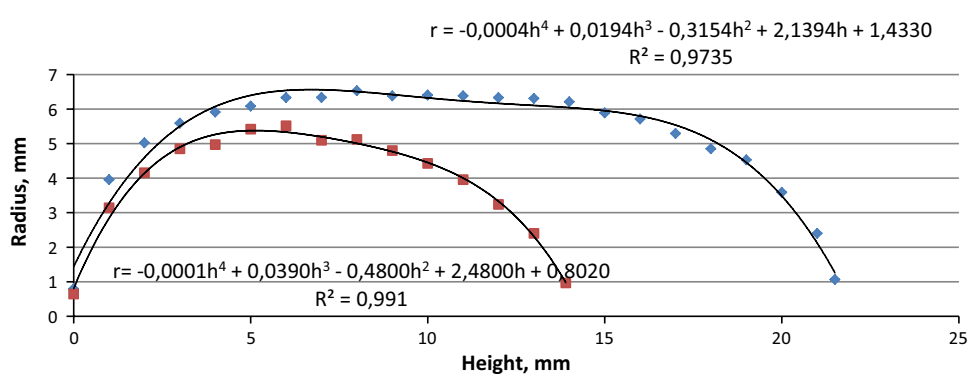

Fig. 1 Sample plots of generating curves for the shapes of larger (no. 2.39—blue points) and smaller (no. 2.14—red points) black alder cones from the second lot and their mathematical equation. (Color figure online)

the surface area calculated from the formula for a cylinder (S_c) should be multiplied by 0.81 for both lots.

Analysis of variance of the measured (actual) and calculated cone parameters showed that except for cone weight ($F = 2.8180$ and $p = 0.0982$), all size parameters differed significantly, with the results being $F = 13.1755$ at $p = 0.0006$ for height, $F = 13.5740$ at $p = 0.0005$ for maximum diameter, $F = 8.0831$ at $p = 0.0060$ for base diameter, and $F = 5.0777$ at $p = 0.0278$ for actual volume. In the case of calculated values, the ANOVA results were as follows $F = 11.1600$ at $p = 0.0014$ for volume calculated from the generating curve, $F = 13.2508$ at $p = 0.0006$ for volume calculated from the barrel formula, $F = 9.6525$ at $p = 0.0029$ for surface area calculated from the generating curve, and $F = 14.0589$ at $p = 0.0004$ for surface area calculated from the cylinder formula.

Characterization of black alder scale structure

Each scale consists of an inner and outer side (Aniszewska and Bluszkowska 2016; Aniszewska et al. 2017a). Figure 2 shows SEM images of the inner side of scales, which is inhomogeneous consisting of elements with different characteristics with longitudinal tissues and some projections. Figure 2a presents an entire scale under 50 × magnification, and Fig. 2c–d, e shows fragments under 500 × and 1000 × magnification. The image in Fig. 2c shows the lower part of a scale with a ribbed structure, with parallel bands of cells adjoining one another. The surface of the bottom part of the scale revealed an irregular pattern of narrow chains of cells.

These narrow cells are not found in fragments from the middle part of the scale, which bears the seeds. In that area, the structure of the scale appears smoother than in the bottom part (near the axis). Between rows of cells, there are round, thin-walled cells forming bundle-like structures (see outlined areas in Fig. 2c), which could be seed attachment points.

Figure 2e shows a fragment of the upper part of the scale, which differs significantly from the middle and bottom ones. It reveals a rough surface made of thick-walled cells with visible lumina. That part of the scale has a layered structure, which resembles that of an apophysis, as in pine cones (Gawart and Mikłaszewicz 2000; Aniszewska 2001; Aniszewska and Bluszkowska 2016).

Figure 3 presents photomicrographs of the outer part of the scale under 50 × magnification: its upper part (3a) and the middle and bottom parts (3b). Close-ups of selected areas were taken (as marked in the figure). As can be seen, the scale consists of several thickened layers, which are fused near the cone axis. Figure 3c presents the bottom part of the scale, which is structurally similar to the inner side of the base of the scale. There are also regularly arranged bands of thick-walled cells forming a uniform tissue.

The middle part also reveals regular bands of cell, with additional transversely oriented thin chains of cells (Fig. 3d). The upper part of the scale ("pseudo-apophysis") is rough and cauliflower-like; it reveals particles of what may be a resinous substance (Fig. 3e). The pseudo-apophysis is more developed and occupies a larger area on the outer side of the scale as compared to its inner side. In alder cones collected from a single clone, the pseudo-apophasis is clearly divided into two parts at the tip of the scale (Fig. 3a), which gives an impression that it is fused in the middle part of the scale.

Discussion

The studied black alder cones generally fell within the height range reported by other researchers, that is, 1.5–2.5 cm (Suszka et al. 2000). The surface area and volume of individual closed alder cones may be accurately calculated from their height and diameter using a fourth-degree polynomial. However, the proposed model cannot be generalized to other cones as the averaged equation coefficients (A, B, C, D, E) led to significantly overestimated values. The other proposed methods, that is, surface area calculation from a cylinder model and volume calculation from a barrel model, are more useful. They can be readily applied to cones from other lots by defining multipliers. (In the case of the studied cone lots, they amounted to 0.77 and 0.92 for volume and 0.81 for surface area.) The results obtained from these formulas did not differ significantly from actual cone size measurements.

Similar studies have been performed for coniferous species, including the silver fir, Scots pine, and Norway spruce. In those cases, surface area and volume were calculated using the formula for a geometric cone, in addition to a cylinder and a barrel (Gawart and Mikłaszewicz 2000; Aniszewska 2001; Aniszewska and Bluszkowska 2016).

The surface area and volume determined based on the measured size parameters may be helpful in describing changes in moisture content, drying rate, as well as heat and mass transfer, which may be used in designing and programming heat-assisted seed extraction processes in kilns, under commercial conditions, to obtain high-quality seeds for planting. In Poland, the germination capacity of black alder seeds ranges from 35 to 66% (Suszka et al. 2000), and in Ireland from 51 to 56% (De Atrip and O'Reilly 2007).

The described scale structure revealed certain characteristic elements on the inner (concave) side of the scale: bundles of cells which might affect seed release during extraction. The methodology used in this work was previously applied to examine the scales of Scots pine (Aniszewska 2012), European larch (Aniszewska et al. 2017b), and silver fir (Aniszewska et al. 2017a), as well as larch seeds and wings (Aniszewska 2014). It was noted that the outer side of scales differs significantly from the inner side, on which the seeds

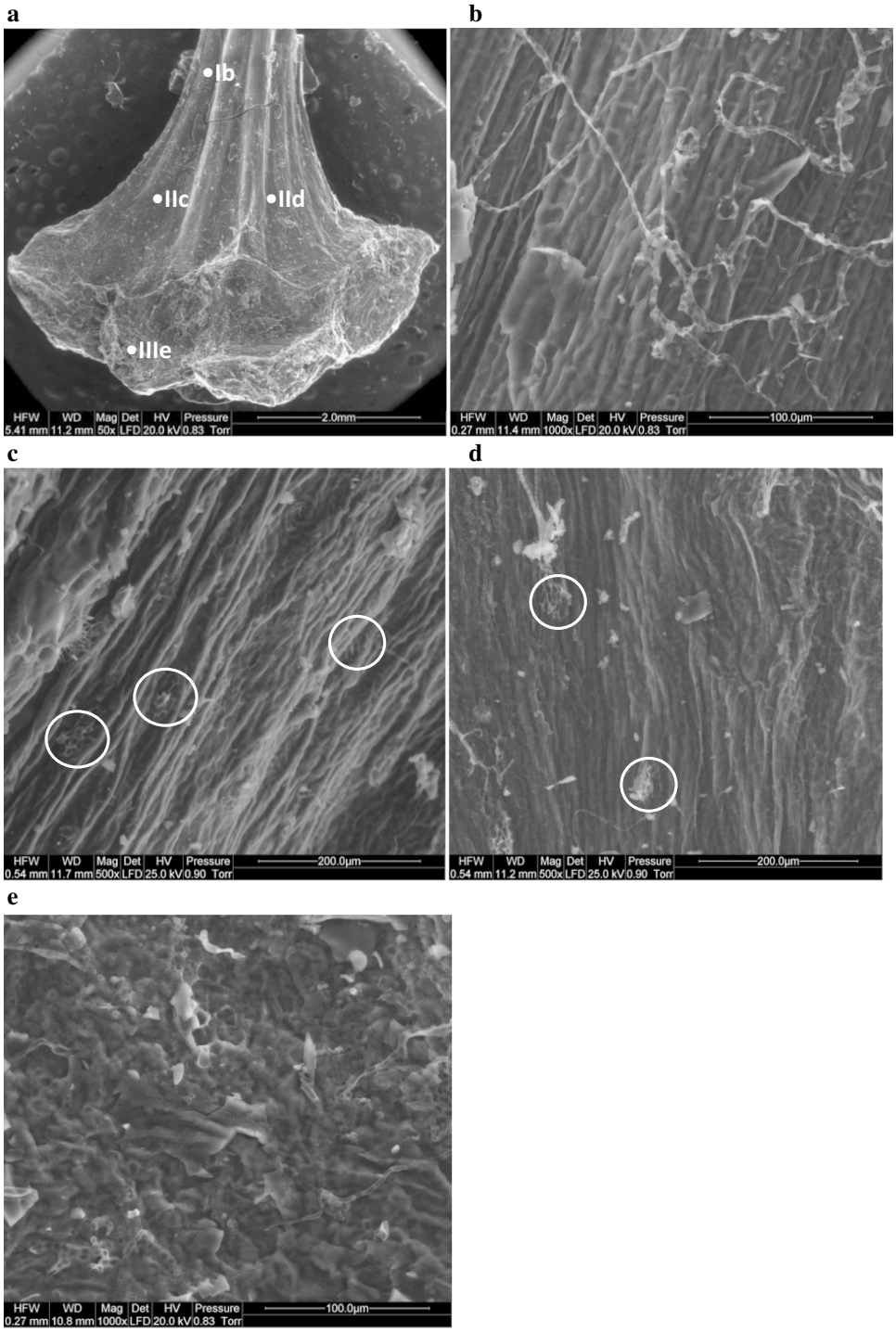

Fig. 2 Inner side of black alder scale: **a** entire scale under ×50 magnification, **b** bottom part of the scale (×1000), **c**, **d** middle part of the scale (×500), **e** upper part of the scale (×1000), I—bottom (at the axis), II—middle, III—top

are borne. The outer side is typically made of evenly sized thick-walled cells, throughout its length. The upper part of pine and fir scales features an apophysis, which is absent in the larch and spruce. On the inner side of the scale, two distinct parts may be observed: one corresponding to the original location of the wings, and the other one outside that area. The former consists of frayed cells, probably formed in the process of detaching the wing and seed from this part of the scale. In other species, this part of scales exhibits cells arranged in chains (Scots pine), small processes or thick-walled cells (in silver fir and Norway spruce). Finally, in the black alder, the inner part of the scale outside the wing area is characterized by regularly arranged cells, similarly to the outer side of the scale.

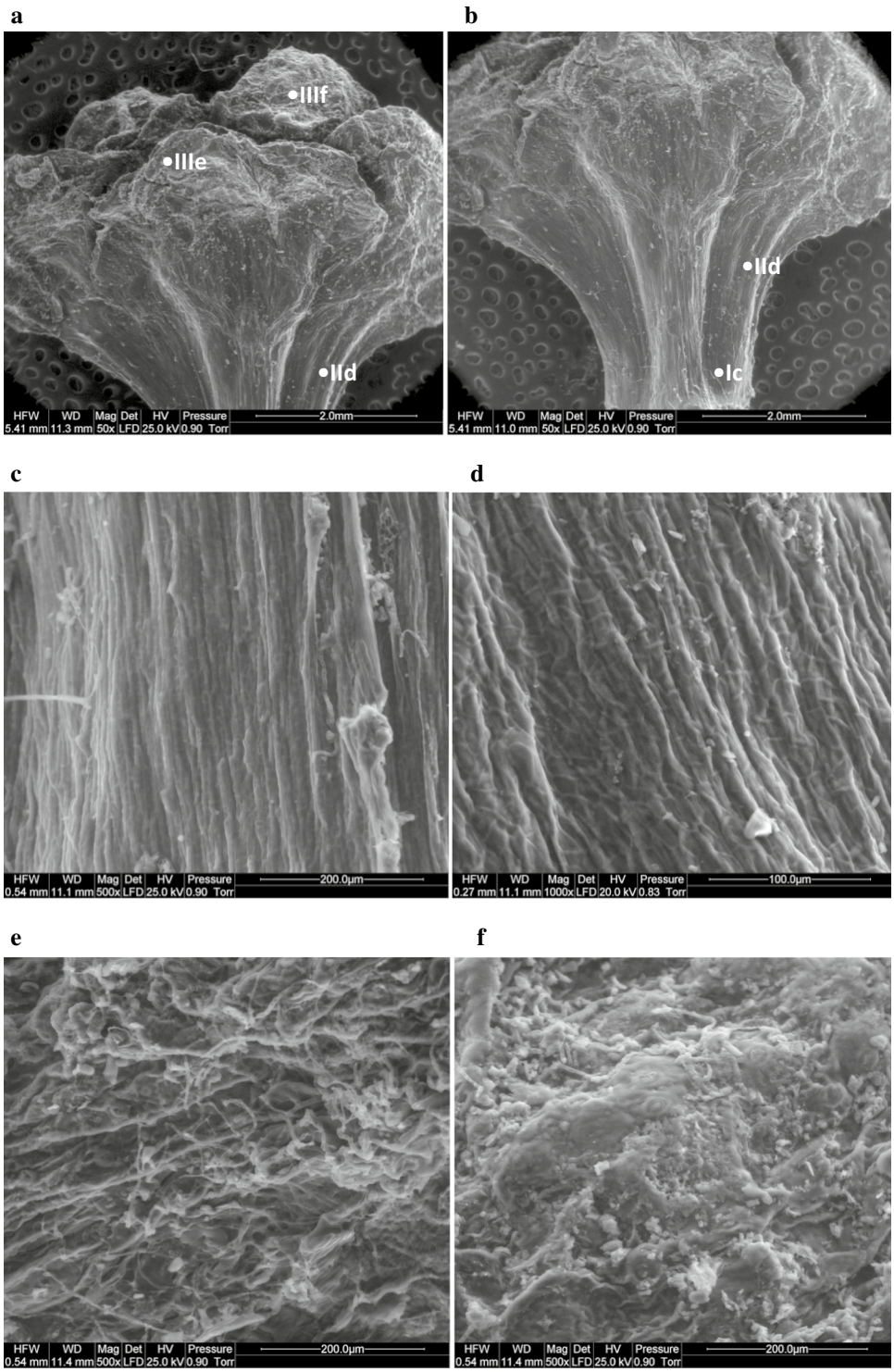

Fig. 3 Outer side of black alder scale: **a**, **b** entire scale under ×50 magnification, **c** bottom part of the scale (×500), **d** middle part of the scale (×1000), **e**, **f** upper part of the scale (×500), I—bottom (at the axis), II—middle, III—top

The presented work describing black alder cones may be treated as a pilot study. The results should be corroborated by research involving a larger number of alder cones of different provenances and collected in different years.

Conclusions

1. Cones from the first batch were larger and had a larger mass compared to cones from the second party. The length was 14% larger, thickness 9% larger, and mass 13% greater.

2. The shape of black alder cones is sufficiently well described by a fourth-degree polynomial curve. However, due to substantial differences between the averaged values of the polynomial and the actual cone measurements, this function cannot be used for the calculation of the volume and surface area of other cones, despite the existence of a significant correlation between cone height and diameter.
3. The volume and surface area of black alder cones may be calculated using formulas for a barrel and a cylinder, respectively. The values calculated from the former should be multiplied by 0.77 and 0.92 for the first and second lots, respectively, while the values obtained from the latter should be multiplied by 0.81 for both lots. Some small deviations in these coefficients should be expected for other lots of alder cones.
4. Examination of the outer and inner sides of black alder scales from two cone lots led to the identification of their characteristic morphological elements with potential implications for cone opening and seed extraction; these include bundles of thin-walled cells on the inner side of the scale (where the seeds are found) and thick-walled cells forming parallel bands of tissues. Resin-like droplets were found on the scales, and especially on the pseudo-apophysis, similarly as in conifers.

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