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Variability of silver fir (*Abies alba* Mill.) cones – variability structure of scale surface area

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Abstract. This study was conducted on a batch of closed silver fir cones from Jawor Forest District and a mixture of scales from the seed extraction facility Grotniki. The scales were divided into three size classes corresponding to the bottom, middle and upper part of the cones and their area was measured with the Multi Scan Base v.18.03 software. Based on the sum of the inner and outer surface area of all scales, we then determined the total area of evaporation from the cones. In addition, the area of protruding scales was measured for differently sized scales from different parts of the cones. Previous studies have shown that the average outer surface of a closed cone, calculated as the sum of protruding scales, accounts for 10% of the outer surface of an open cone. Pictures of both scale surfaces with the internal seed bed and the external protrusions were taken using a scanning electron microscope. We noticed significant differences in dimension and shape of the channels and trichomes on the scale surface. On the inner side of the scales, we found a high diversity of trichomes of different lengths, whilst the outer side contained channels. Presumably, these characteristics affect the rate of water loss from the cones during desiccation and separation of the seed. In-depth knowledge on the evaporative surfaces of fir cones and scale structure will be helpful for optimizing the industrial processes of seed extraction.

Keywords: cone, scale, area of evaporation, scanning electron microscope

1. Introduction

Harvesting silver fir seeds (*Abies alba* Mill.) does not require special equipment, as its cones naturally break down into stem, scales and winged seeds, although this takes time. Kaniewski and Kucewicz described the process of disintegration in 1978, while Kaniewski et al. (1972) described the structure of scales of the fir's vegetative buds. They state that a fir cone loses a great amount of water in its last period of development. This phenomenon is influenced by sclereids, occurring in cones in significant amounts and characterized by hygroscopic properties. The shrinkage of sclereids in the axial part of the cone and in the scales produces the effect of opposing forces, which causes the individual scales to disconnect from the cone stem. In the controlled collection of fir seeds, cones are gathered from the trees before disintegrating, then they are dried and the seeds are extracted. The winged fir seeds are stripped off their wings and cleaned. All stages of fir seed processing are accompanied by special care to prevent damage to the resin vesicles on their surface, as spilled resin contributes to lower seed quality (Tyszkiewicz 1949).

Aniszewska and Błuszkowska (2016) described the external parameters of fir cones, their interdependencies, the number of scales, mass and apparent density of the cones, as well as proposing models to calculate their surface area and volume. In this article, an attempt was made to calculate the size of the surface area of fir scales from which water evaporates. The outer and inner surfaces of fir scales were described based on images made by a scanning electron microscope. The conducted studies contribute to a better understanding of the parameters of fir cones, including seed scales, their structure and the processes occurring in them. As a result, this will allow the industrial seed processing under controlled conditions to be carried out more effectively.

The use of electron microscopy, applied to study fir scales, is a common technique today, serving to determine surface characteristics, chemical composition, subsurface areas and

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nanostructures in the structure of materials (Kelsall et al. 2008; Gilewska 2010). Since the invention of transmission electron microscope, this method has become the main technique allowing materials and structures to be observed at the atomic level (Aleszkiewicz 1998; Barbacki 2007). It is used in materials and metallurgy engineering, among others, in studies of tribological wear, corrosion damage, the quality control of materials (Klimek 2013; Kuźnia et al. 2012; Pashechko et al. 2011), biology and medicine in determining intracellular structures and processes (Kocoń 1986, Krajewski 1992; Bae et al. 2003; Świercz et al. 2014), as well as in zoological research, the identification of chemical substances and investigative techniques.

2. Materials and methods

2.1. Areas of silver fir scales and apophyses

One scale was taken from the basal, distal and middle section of each of the 30 studied silver fir cones obtained from the managed seed stand in the Jawor Forest District (Regional Directorate of State Forests in Wroclaw).

Depending on the location of scales on the cone and their dimensions, three groups were distinguished − small, medium and large scales (Figure 1). Both sides of each scale were digitized, at 3872 × 2592 pixels, and analyzed using the Multi Scan Base v.18.03 program, which, after calibrating to the measurement standard, reported precise results within 0.01 mm. The inner surface of the scale where the seeds are located (Figure 2a) and the outer surface area were measured. The surface area on the outer side where the apophysis is located was also determined (Figure 2b).

The measurements allowed us to calculate the evaporation surface area of the cone in an open ($S_{p.o.}$) and closed ($S_{p.z.}$) state. In order to compare the evaporation surface area in a closed cone with the calculated surface area (S_{obl}), according to the polynomial of the fourth degree (Aniszewska, Błuszkowska 2016, Table 3), an indicator of concordance was introduced:

$$k_1 = \frac{S_{obl}}{S_{p.z.}} \qquad (1)$$

The relationship of the evaporation surface area of an open cone to its length was analyzed.

2.2. Characteristics of the structure of the outer and inner surfaces of silver fir scales

Each of the collected scales was cut into three parts, which made it possible to distinguish between: the basal part attaching it to the stem, the middle part and apical part. Then, the marked scale pieces were placed in the Quanta 200 scanning microscope chamber and the image of the selected part of the scale was magnified 50 and 500 times. After defining the location of the image on the scale, it was saved in lossless TIFF compression format, which allows for subsequent image processing. The Multi Scan Base v.18.03 program was used to analyze the scanning microscope images, measuring the length and diameter of the trichomes.

This paper presents only the description of the structure of seed scale surfaces. Each seed scale has an inner (where the seed is located) and an outer side with a visible apophysis.

The Statistica (StatSoft Inc. 2011) program was used to analyze the studied parameters. All analyses were performed at a significance level of α = 0.05.

3. Results

3.1. The evaporation surface area of open and closed silver fir cones

The evaporation surface area of an open silver fir cone is the sum of the outer and inner surfaces of its scales. Table 1 provides descriptive statistics on the measurement results for both surfaces of small, medium and large scales, as well as for the apophyses.

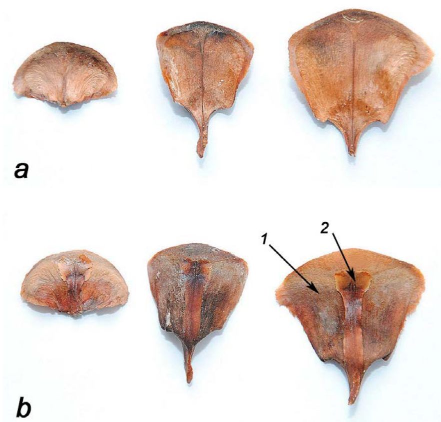

Figure 1. View scales silver fir from the basal part (small), distal part (average) and middle part (large) cones: a – page inner scales, b – page outer scales: 1 – cone scale, 2 – bract (*fot. A. Gendek*)

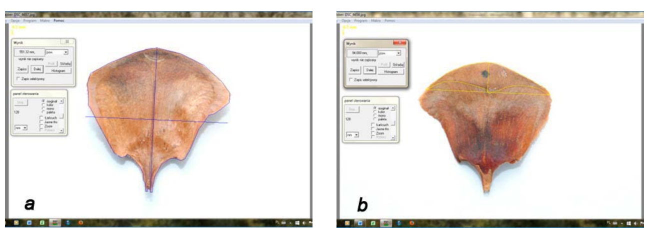

Figure 2. The contour and surface measurement of silver fir scales: a – total, b – apophysis on the outer surface

Table 1. Basic statistical description of surface scales and apophysis of silver fir cones

Data	Field area [mm^2]					
	scales			apophysis		
	small	average	large	small	average	large
Mean	192.1	356.0	453.3	50.8	77.7	97.6
Standard deviation	28.0	22.6	47.7	2.2	2.0	3.8
Min	120.4	304.4	379.7	46.7	72.0	87.3
Max	243.9	400.4	552.3	55.9	83.1	105.6

The average surface area of a single small silver fir cone scale was about 192 (± 10.46) mm^2, and the large scale was 453 (± 17.81) mm^2. The average surface area of a small apophysis was 51 (± 0.82) mm^2, while the surface area of a large apophysis oscillated around 98 (± 1.42) mm^2. A significant dependence was confirmed between the surface area for water evaporation of an open cone and cone length. The linear equation (2) shows that an increase in cone length by 1 mm results in an increase in the evaporation surface area of an open cone by 733 mm^2.

$$S_{p.o.}=733.2\,h+18884 \quad R^2=0.7280 \tag{2}$$

where:
h – cone length, mm.

The surface area for water evaporation of a single cone was calculated for the average number of scales in silver fir cones, with n = 185 (Table 2).

Assuming the theoretical premise that a fir cone consists solely of small scales or large scales, the theoretical evaporation surface area resulting from the measurements of inner and outer scale surfaces will be in the range of 71069.9 to 167732.1 mm^2. In reality, fir cones have scales of varying sizes, so after a detailed analysis of the size of scales and their types (small, medium and large) for the tested provenance and size of the cones (Aniszewska, Błuszkowska 2016, Table 1), we calculated that an average cone with 185 scales had about 20% of small scales, 25% of average scales and 55% of large scales, that is, 37, 46 and 102 scales respectively. Thus, the surface area for water evaporation of an open cone was approximately 139336.7 (± 5728.2) mm^2 (Table 2).

Assuming that the evaporation surface area of a closed silver fir cone is the surface area of its visible apophyses, then on average, the area of a cone of 185 small, medium and large scales is 9403.6 mm^2, 14374.5 mm^2 and 18048.6 mm^2 respectively. An average cone had about 20% of small scales, 25% of medium scales, and 55% of large scales, so the evaporation surface area of the cone was approximately 15395.5 (±632.92) mm^2 (Table 2). The evaporation surface area of a closed cone ($S_{p.z.}$) was compared to the calculated surface area using the function of the polynomial of the fourth degree (S_{obl}), which was 15698.0 mm^2 (Aniszewska, Błuszkowska 2016, Table 3). The correlation between these values is shown by the anon average high k_1 indicator of 1.02 (±0.074) (Table 2).

Comparing the evaporation surface area of an open and closed cone allowed us to determine the surface area of the apophyses, relative to the area of all of the scales in the fir cone, which amounted to 11.0%.

3.2. Description of the surface structure (epidermis) of inner and outer sides of silver fir scales

Each silver fir cone has two types of scales: seed and bract (cover) scales, with the former being larger and usually containing two triangular seeds with wings. The bract scale touches the outer side of the seed scale, is lingulate and bent towards the base of the cone.

Figure 3 shows an image of the upper section of the inner side of a fir scale made with a scanning microscope at magnifications of (a) 50x and (b) 500x. Many trichomes (hairs) of different lengths and diameters are seen on the surface of the scale. The trichomes with the smallest external dimensions are located at the very edge of the scale. Particles are visible between the trichomes, most probably resinous.

In Figure 4b, the middle section of the scale was found to have a greater number of trichomes per one square millimetre compared to its upper part (Figure 3b). The trichomes in the middle section have higher external parameters than the trichomes at the edge of the scale. The mean length of these trichomes was 0.103 (± 0.016) mm, while the diameter at the thickest point was 0.034 (± 0.0033) mm (Table 3).

The analysis of the basal (lower) part of the inner side of the scale showed either no trichomes or a negligible number. This part of the scale's inner side is characterized by a smooth and glossy surface with few resin particles (Figure 6).

The structure of the fir scale's outer surface varies markedly from the inner side. Figure 7 shows the apical section of the outer side of the scale with the apophysis. The apophysis is usually irregularly shaped with a relatively smooth surfa-

Table 2. Number of scales and surface evaporation from the opened and closed cones

Cones	Scales				Surface evaporation of water from the cones		Coefficient
	total	small	average	large	opened	closed	k_1
	pcs.				$\times 10^3$ mm^2		-
1	187	37	47	103	140.89	15.57	0.79
2	184	37	46	101	138.63	15.32	1.10
3	188	38	47	103	141.65	15.65	0.91
4	216	43	54	119	162.74	17.98	1.45
5	199	40	50	109	149.94	16.57	1.04
6	182	36	46	100	137.13	15.15	0.89
7	157	31	39	86	118.29	13.07	0.85
8	202	40	51	111	152.20	16.82	1.08
9	155	31	39	85	116.78	12.90	0.89
10	162	32	41	89	122.06	13.49	0.92
11	190	38	48	105	143.15	15.82	1.22
12	192	38	48	106	144.66	15.98	1.20
13	202	40	51	111	152.20	16.82	1.12
14	197	39	49	108	148.43	16.40	0.91
15	186	37	47	102	140.14	15.48	1.22
16	152	30	38	84	114.52	12.65	1.07
17	196	39	49	108	147.68	16.32	1.21
18	198	40	50	109	149.18	16.48	1.13
19	197	39	49	108	148.43	16.40	0.88
20	206	41	52	113	155.21	17.15	0.93
21	179	36	45	98	134.87	14.90	1.36
22	177	35	44	97	133.36	14.74	1.22
23	192	38	48	106	144.66	15.98	1.00
24	176	35	44	97	132.61	14.65	1.03
25	174	35	44	96	131.10	14.49	1.23
26	125	25	31	69	94.18	10.41	0.90
27	171	34	43	94	128.84	14.24	0.82
28	208	42	52	114	156.72	17.32	0.88
29	179	36	45	98	134.87	14.90	0.59
30	219	44	55	120	165.00	18.23	0.66
Mean	184.9	37.0	46.2	101.7	139.34	15.39	1.02
Standard deviation	20.4	4.1	5.1	11.2	15.34	1.69	0.20
Min	125.0	25.0	31.3	68.8	94.18	10.41	0.59
Max	219.0	43.8	54.8	120.5	165.00	18.23	1.45

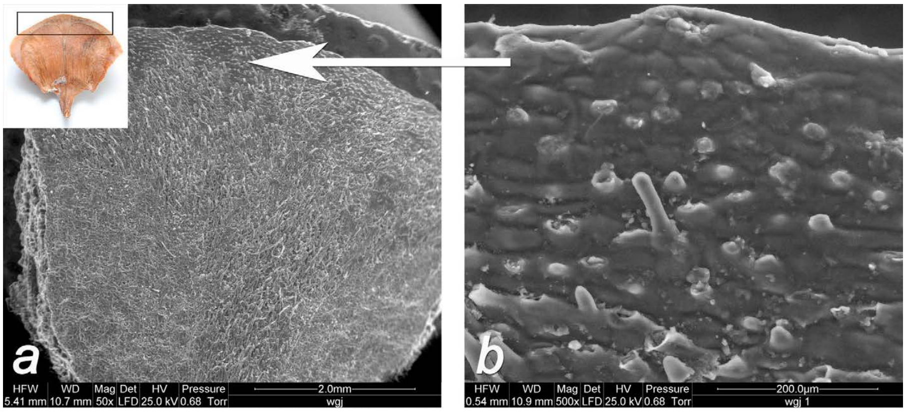

Figure 3. The upper part of the scales from the inside of the (a) 50x and (b) 500x (Stadnik, Śliwińska 2015)

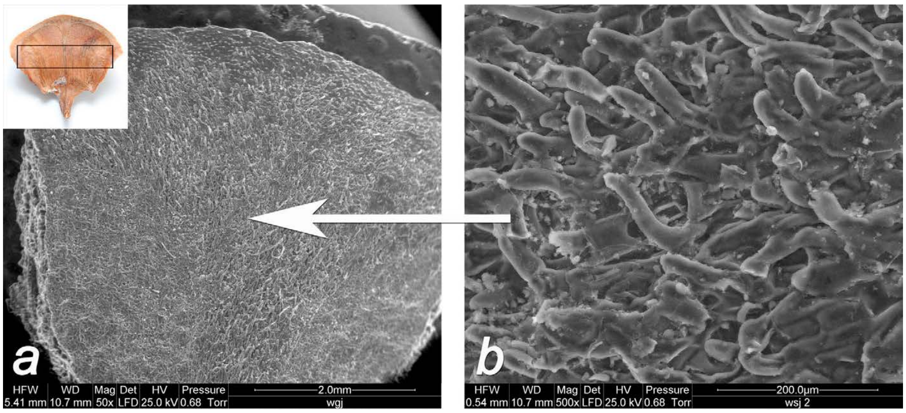

Figure 4. Central portion of the scales from the inside of the (a) 50x and (b) 500x (Stadnik, Śliwińska 2015)

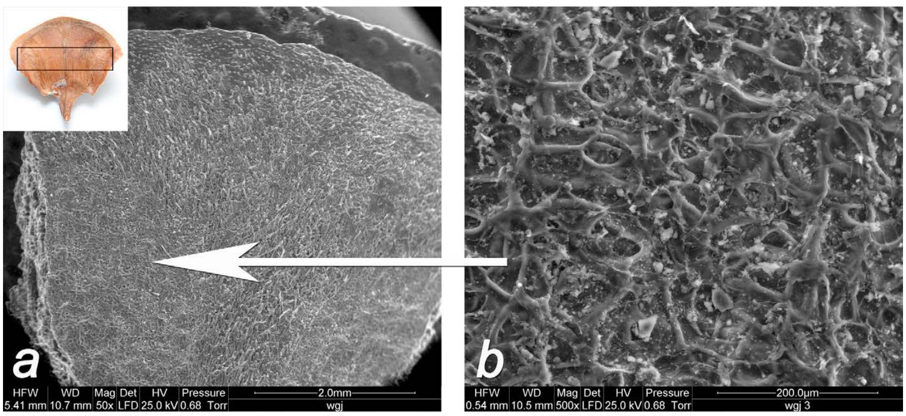

Figure 5. Central portion of the scales from the inside (under the wing) of the (a) 50x and (b) 500x (Stadnik, Śliwińska 2015)

ce where resin particles are noticeable. The structure at the bottom of the apophysis under study differs from the middle and upper scale sections in its lack of resin particles.

Figure 8 shows the middle section of the outer side of the scale. The structure of the scales here is rough, with long, cylindrical channels placed in a chain-like manner, with few resin particles between them. This middle section of the silver fir scale has a similar structure to the inner side of the scale under the seed wing (Figure 5). It is also partially in contact with the surface of the bract scales.

Figure 9 presents the basal section of the outer side of the scale under study, just next to the cone's axis, where the seed scale touches the bract. The scale's basal surface differs structurally from the central and upper sections. The part next to the axis has fewer numbers of trichomes or none at all and a rough surface.

Table 3. The length and width of trichome on the surface of the scale

Trichome	Length	Width
	[mm]	
1	0.0814	0.0363
2	0.0880	0.0299
3	0.0871	0.0300
4	0.0667	0.0187
5	0.0805	0.0287
6	0.0952	0.0485
7	0.0910	0.0364
8	0.0786	0.0317
9	0.0890	0.0361
10	0.0479	0.0196
11	0.0563	0.0317
12	0.0425	0.0308
13	0.0564	0.0202
14	0.0737	0.0295
15	0.0769	0.0410
16	0.0688	0.0325
17	0.0764	0.0285
18	0.1320	0.0422
19	0.1727	0.0442
20	0.1161	0.0278
21	0.1938	0.0440
22	0.1354	0.0454
23	0.1271	0.0235
24	0.0933	0.0324
25	0.0810	0.0292
26	0.1502	0.0357
27	0.1315	0.0466
28	0.2299	0.0569
29	0.1227	0.0418
30	0.1570	0.0335
31	0.0980	0.0278
Mean	0.1031	0.0342
Standard deviation	0.0436	0.0089

4. Discussion

The cone parameters of Scots pine (Tyszkiewicz 1949; Staszkiewicz 1968; Załęski 1995; Aniszewska 2012), Norway spruce (Barzdajn 1996; Kulej, Skrzyszewska 1996; Buraczyk 2009) or European larch (Bałut 1969; Hall 1985; Antosiewicz 1970; Orlova et al. 2014), such as length, thickness and mass, as well as the size of scales, seeds and wings, are described in Polish and foreign literature. These features of silver fir cones are not well known. One of the few publications describing the morphological parameters listed above is Tracz and Barzdajn (2007), in which the authors provide cone length and diameter, scale and seed wing length and width, and the seed length of fir trees growing in the Sudetes Mountains. For example, the average length of the seed scale is 21.63 mm, with a 27.03 mm width at its thickest point, whereas according to Tracz and Barzdajn (2007), this was 27.9 mm and 27.1 mm respectively. For comparison, the dimensions of a spruce scale from the middle part of the cone are: length – 36 mm and width at the thickest point – 22 mm (Mikłaszewicz 2000).

The authors of this publication measured the surface of individual scales from the basal, distal and middle parts of the cones. They were respectively 192, 356 and 453 mm^2. For comparison, the surface area of a spruce scale taken from the centre of a cone is 494 mm^2, according to Mikłaszewicz (2000). By knowing the surface area of individual scales and their numbers in a cone, it is possible to calculate the evaporation surface area of the whole cone. The surface area of water evaporation of an open fir cone, calculated as the sum of the surface area of seed scales of different sizes, is almost 90 times greater than that of a closed cone, calculated as the sum of the surface area of only the apophysis. According to Mikłaszewicz (2000), this value for an average Norway spruce cone is 93-fold, and according to Gawart (2000) − 84 times more for Scots pine cones. This means that for an average spruce cone, the total area will be about fourteen times greater after it opens (Aniszewska 2001), and for a pine cone − six times greater than the area of a closed cone.

Determining the evaporation surface area can be one of the parameters used to model the process of drying fir cones for commercial purposes. Although silver fir cones open easily by themselves, the process can be accelerated by placing cones in drying cabinets or chambers at an appropriate temperature and humidity. However, based on the current knowledge, these parameters cannot be determined unequivocally, although, according to the 'General instructions for collecting and storing genetic resources for the Forest Gene Bank in Kostrzyca' (2007), the temperature should not be higher than 20°C. Optimising seed extraction, while reducing energy expenditures, requires information on the best conditions for its implementation and the development of appropriate algorithms to control this process. The calculated values of the fir cone's evaporation surface area, as in the case of pine and spruce cones, can serve as the input data for programming seed processing for this species. Based on the parameters of

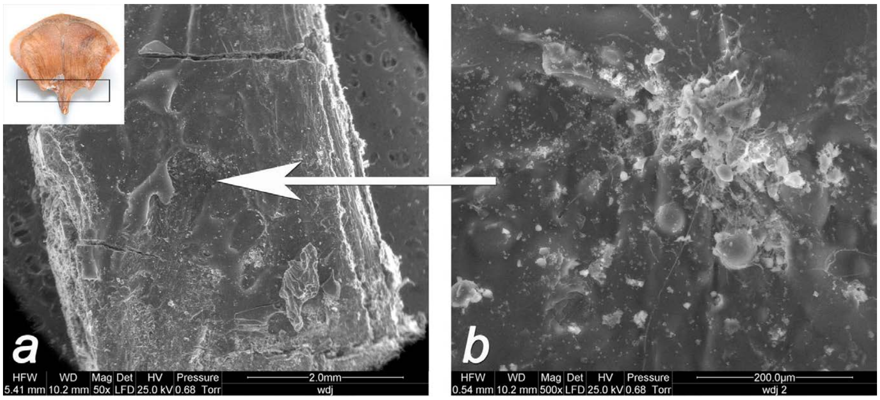

Figure 6. The basal part of the scales from the inside of the (a) 50x and (b) 500x (Stadnik, Śliwińska 2015)

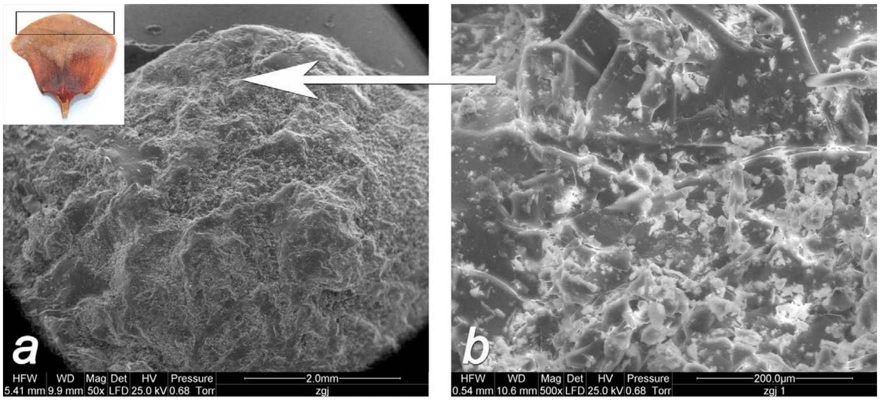

Figure 7. The upper part of the scales from the outside of the (a) 50x and (b) 500x (Stadnik, Śliwińska 2015)

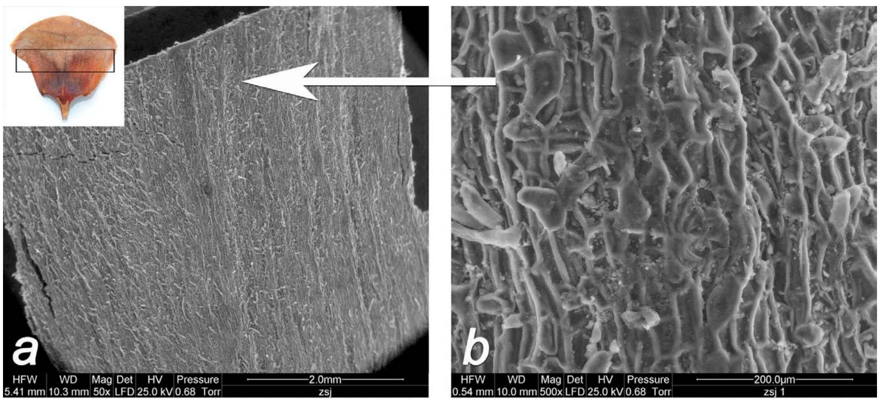

Figure 8. The central part of the scales from the outside of the (a) 50x and (b) 500x (Stadnik, Śliwińska 2015)

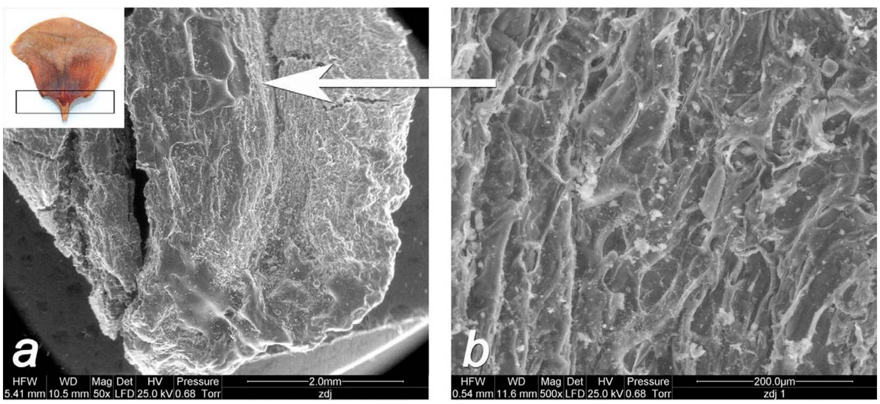

Figure 9. The basal part of the scales from the outside of the (a) 50x and (b) 500x (Stadnik, Śliwińska 2015)

the air used for drying, changing temperature and humidity during the seed extraction process from pine or spruce cones, programs have been developed to control the course of processes in drying chambers, for example, in the commercial seed processing facility in Czarna Bialostocka.

Silver fir cones processed in modern drying chambers could break down into axes and scales in a shorter time without damaging the seed cover as a result of frequent shaking and airing used in the conventional methods of fir seed extraction. In turn, extracting seeds from the cones of Scots pine, Norway spruce and European larch occurs at higher air temperatures, up to 50‒55°C, and according to Tyszkiewicz (1949) even up to 65°C at low air humidity, with no damage to the seeds.

In order to propose new methods of separating the seeds from the scales, one needs to have precise information about the structure of fir scales. The performed electron microscope studies enabled us to identify the elements of the scales, that is, the trichomes and channels that are in contact with the seeds and their wings. Kaniewski and Kucewicz (1978), who described the structure of seed scales and bract scales, concluded that when a cone loses moisture, presumably a significant role is played by the many live hairs (trichomes) occurring on the epidermis of the seed scales. Similar studies of scale surfaces were made of Scots pine cones (Aniszewska 2012), in which no trichomes were found. Instead of trichomes, elliptical pores were found, arranged in rows of chains through which moisture presumably enters and the excess is released. The dimensions of the channels, like the trichomes (hairs) on the inner side of the scale, change when water is evaporated.

The convection drying of cones can help to more effectively separate the seeds from their wings and at the same time counteract the negative effects associated with the need to 'shake' the cones in commercial seed processing methods. This action can lower seed quality by damaging the resin vesicles on its surface.

5. Conclusions

The water evaporation rate from the surface area of an average, closed silver fir cone, calculated as the surface area of the apophysis, was close to the value of the surface area calculated with the polynomial function of fourth degree, with an indicator of concordance of 1.02.

The water evaporation surface area of a closed silver fir cone is 11% of an open cone's evaporation surface area, calculated as the sum of the outer and inner surfaces of the seed scales.

The structure of the inner and outer sides of the silver fir seed scale differs. On the inner side, more trichomes (hairs) are observed, which likely serve to evaporate significant amounts of moisture. The outer side has long, convex cell walls of cylindrical shape.

The apophysis of the silver fir seed scale is smooth, but has an irregular surface, like the basal section next to the axis. It therefore has a different structure than the rest of the scale.

Conflict of interest

The authors declare the lack of potential conflicts of interest.

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Authors' contribution

M.A. – concept, literature review, methodology, measurements, developing the results, conclusions, writing and correcting the text; J.Ś. – measurements, developing the results; A.G. – measurements, developing the results, correcting the text.

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