Vitis 33, 105-108 (1994)

Scanning electron microscopy study of pollen morphology in seedless grape (*Vitis vinifera* L.) cultivars

by

V. Roytchev, D. Terziisky, Dochka Dimova and S. Karageorgiev

Agricultural University, Plovdiv, Bulgaria

S u m m a r y : A comparative scanning electron microscopy study and biometric analysis on pollen of 27 Bulgarian and foreign seedless grape cultivars have been performed. Both the degree of similarity and the specific differences in exine ultrastructural organisation and the aperture complex were established. In all investigated cultivars, pollen grains were from elongated elliptical to oval-spheroidal, tricolporate in shape. The exine surface is in low relief, with perforations and crest undulations in the mesocolpiums. It was concluded that the type of exine sculpturing, the length of polar and equatorial axes and colp could be used as additional microstructural parameters in cultivar identification of seedless grapes.

K e y w o r d s : pollen, morphology, electron microscopy, seedless grape, identification.

Introduction

The scanning electron microscopy (SEM) studies on the exine ultrastructure and pollen aperture complex in grapevines (*Vitis vinifera*) involve a small part of the existing cultivars (Kozma 1963; Kozma and Scheuringne 1968; Tompane Kasirzkaja and Kozma 1978, 1981; Tompane Kasirzkaja 1982, 1984, 1985, 1988) performed palynological investigations of seeded grape cultivars from subgenus Euvitis. Lombardo *et al.* (1976, 1978); Carraro *et al.* (1979); Cargnello *et al.* (1980) and Ahmedullah (1983) presented SEM studies and micrographs of pollen from seeded vine cultivars. A similar investigation of pollen grains from several seeded *V. vinifera* cultivars and two forms (male and female) of *V. silvestris* Gmel. was reported by Linder and Linskens (1978). Lombardo *et al.* (1983) established a relationship between the clone fertility in cv. Picolit and the type of pollen. Haritonashvili *et al.* (1989) reported some specific peculiarities in the pollen microrelief of local seeded Georgian vine cultivars. So far, no SEM studies of pollen from seedless grape cultivars were made.

Aim of the present study was to obtain information on the ultrastructure of exine surface apertures, as well as to perform a comparative biometric analysis of the elements of the pollen aperture complex in seedless grape cultivars.

Material and methods

The exine ultrastructure in 27 seedless grape cultivars was described through SEM observations. The pollen under investigation was gathered in the period 1990 - 1993 from fully developed aments. Specimens were prepared according to the technique for observation of air-dried pollen (Terziisky and Karageorgiev 1989). The aperture complex elements for 100 normal pollen grains were measured directly on the screen of the electron microscope and mathematically treated by the Duncan Test (Clerg *et al.* 1962).

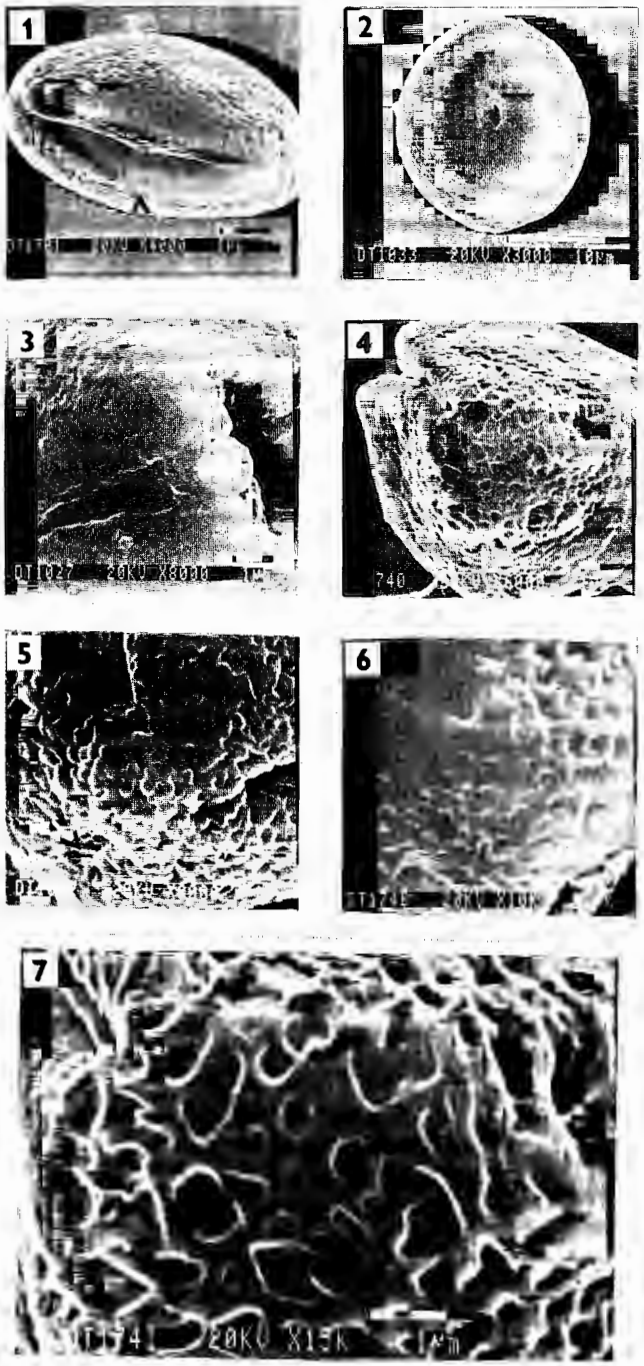
The investigation was carried out at the Electron Microscopy Laboratory of the Agricultural University in Plovdiv with JEM 1200 EX electron microscope equipped with a scanning image device. The pollen grains were covered with carbon in a vacuum evaporator JEE-4x. Pictures of at least 10 different pollen grains were taken for each of the tested seedless grape cultivars.

Results and discussion

Although the pollen grains of all investigated seedless grape cultivars are characterized with some specificities in their exine ultrastructural organization, it was found a good resemblance in the exine elements and the aperture complex. The pollen shape is from elongated elliptical to ovalspheroidal (Figs. 1 and 2). The number of colps in all pollen specimens is three. A well formed, usually rounded and differently protruded pore is clearly seen in the middle part of each colp (Fig. 3). According to Erdtman's classification (1952), the pollen grains of the tested seedless vine cultivars are tricolporate (Fig. 4). The colps in the equatorial zone are usually wide and deep, while those in the polar zone are more shallow and narrow, differing in length, with no demarcating edge. The bottom of colps is uneven with microgranular structure, covered with small and big swellings (Figs. 4 and 5). The exine ultrastructure in the mesocolpium is in low relief, from undulating to perforated reticulate, stratified with irregular, different in shape and size lumens and figures, with coves and dots. This ornamentation is typical for the pollen of all investigated cultivars (Figs. 1-5). The apocolpiums are differently projected, domed, with irregular undulating sculptures, perforations, pits and protrusions (Figs. 4-7).

Correspondence to: Prof. DSC D. Terziisky, Department of Botany, Agricultural University, Mendeleerstr. 12, BG-4000 Plovdiv, Bulgaria

To establish whether the dimensions of the aperture complex in pollen grains could be used as additional indices in cultivar identification, a mathematical treatment of the biometrical data was made by the Duncan Test. This test for multiple comparison enables the differential determination of the statistical significance in the different variants as depending on their place in the complex, since the increase in the distance between the compared quantities leads to increase in the correcting value to the least essential difference (L.E.D.), too (DIMOVA *et al.* 1990).



Figs. 1-7: Shape, size and ultrasculpture of exine and aperture complex.

1: Elliptical shape of pollen grain and general view of exine sculpture and mesocolpium, cv. Kondarev-10. Arrow shows lack of colp edge (x 4000). 2: Oval shape of pollen grain, cv. Korintsko white (x 3000). 3: General view of an equatorial pore, cv. Russalka (x 8000). 4: General view of a pollen grain, cv. Tarnau, in the apocolpium. The perforations and the three meridional colps are seen (x 6000). 5: View of apocolpium, cv. Seedless White at higher magnification. Arrow shows ultrasculptural ornamentation in the bottom of colps (x 8000). 6: Part of the apocolpium and mesocolpium zones, cv. Hibrid 720-19. Unequal perforations of the exine are seen (x 10,000). 7: Detail of the ultrastructural ornamentation of apocolpium cv. Tarnau (x 15,000).

The results from the comparative analysis for all investigated parameters in the cultivars are given in Tab. 1.

The pollen of cv. Hibrid V-6 has the greatest size of the polar axis (27.03 µm). Together with cv. Kishmish Moldavski they belong to the same group, the difference between them being insignificant. Cvs Vita, Nishava, Russalka, and Kolarovets form a second group. All the rest cultivars have lower values of this parameter, the degree of significance being shown in the next column. The smallest polar axis was found for the pollen of cvs Korintsko White and Red seedless.

Hibrid V-6 showed the highest mean value (18.21 µm) for the equatorial axis, too. Red Seedless belongs to the same group (17.69 µm) irrespective of its last position with respect to the polar axis.

At the comparison of the pollen colp lenghts, the cvs Hibrid V-6 and Kishmish Moldavski showed again the highest mean values - 24.13 and 24.04 µm, respectively, and cv. Red Seedless - the lowest (16.19 µm).

With respect ot the mesocolpium, cvs Hibrid V-6, Kishmish Moldavski and Red Seedless showed again the highest mean values which put them in the same group according to this parameter. A correlation among these parameters is very probable to exist in these cultivars.

The data analysis for the apocolpium dimensions has shown that only cv. Kishmish Moldavski has kept its position in the order established (Tab. 2). This cultivar had the highest significant value of the apocolpium size if compared to the other cultivars belonging to the first group. The rest of the cultivars are ambiguous in terms of the different parameters and are related to different arbitrary groups.

The mean values for the width of the pollen colp in the various cultivars are rather different. According to this parameter, all cultivars are included in 3 groups of significance. The outlined cvs Hibrid V-6, Kishmish Moldavski, and Red Seedless have shown significantly lower values of this prameter, irrespective of their being in the same group of significance.

The arrangement of culivars according to the depth of colp is rather different, much like that with the colp width. Therefore, the cultivars are included in the same group of significance, the differences between them being statistically insignificant.

In addition to the comparative analysis, the degree of variability of the parameters (5 %) has been determined for each of the investigated cultivars. It shows which of the parameters is more variable and pliable to the influence of external factors, and which is more steadily genetically determined. The results (Tab. 3) show that the parameter polar axis is most variable in cv. Russalka. By following the data obtained, it is seen that the variability in the parameters polar axis, equatorial axis, mesocolpium and length of colp is in the admissible limits, up to 10 %

Table 1

Comparative analysis on the elements of the pollen aperture complex (μm) by the Duncan Test

POLAR AXIS				EQUATORIAL AXIS				LENGTH OF THE COLP			
No	x̄	Rp 5%	Rp 1%	No	x̄	Rp 5%	Rp 1%	No	x̄	Rp 5%	Rp 1%
1	27.03	a	a	1	18.21	a	a	1	21.13	a	a
2	26.16	ab	ab	6	17.74	ab	ab	2	24.04	a	ab
3	25.69	bc	abc	27	17.69	ab	ab	6	23.04	ab	abc
4	25.32	bcd	abcd	11	17.35	ab	ab	3	22.49	ab	abcd
5	24.95	bcde	bcde	4	17.27	bc	ab	9	22.23	abc	abcde
6	24.82	bcdef	bcde	22	16.26	cd	bc	10	22.10	abcd	abcdef
7	24.67	cdefg	bcde	7	15.85	de	cd	15	21.37	bcde	abcdef
8	24.64	cdefg	bcde	10	15.78	de	cd	13	21.33	bcde	abcdef
9	24.36	cdefg	bcdef	3	15.54	def	cde	14	21.30	bcde	abcdef
10	24.35	cdefg	bcdef	2	15.39	def	cde	4	21.26	bcde	abcdef
11	23.89	defgh	cdefg	12	15.34	def	cde	8	21.17	bcde	abcdefg
12	23.74	efgh	defg	8	15.32	def	cde	12	21.02	bcde	abcdefg
13	23.39	fghi	efgh	13	15.23	defg	cdef	11	20.98	bcdef	bcdefgh
14	23.30	ghi	efgh	15	15.16	defg	cdef	16	20.60	bcdef	cdefgh
15	23.30	ghi	efghi	16	15.07	defg	cdef	7	19.90	cdefg	cdefghi
16	22.76	hik	fghi	19	14.97	efg	cdef	18	19.76	cdefg	cdefghi
17	22.15	ikl	ghik	25	14.88	efg	cdef	22	19.70	defg	defghi
18	21.80	kl	hik	18	14.64	efgh	defg	24	19.67	defg	defghi
19	21.77	kl	hik	14	14.37	fghi	defgh	20	19.60	defgh	defghi
20	21.67	klm	hik	9	14.33	fghi	defgh	21	19.30	defgh	defghik
21	21.47	klm	ik	17	14.03	ghi	efgh	23	19.01	efgh	efghik
22	21.38	klm	ik	21	13.69	hik	fghi	27	18.52	fgh	fghik
23	20.88	lm	k	24	13.28	ikl	ghi	19	17.90	gh	ghik
24	20.87	lm	k	23	12.87	kl	hi	25	17.83	gh	hik
25	20.31	m	k	26	12.53	l	i	5	16.98	h	ik
26	19.47			20	12.08			26	16.43		
27	19.41			5	12.00			27	16.19		

CULTIVARS:
1. Hybrid V-6; 2. Kishmish moldovski; 3. Vita; 4. Nishava; 5. Russalka; 6. Kolarovets; 7. Kondarev 10; 8. Rushaki; 9. Delight; 10. Jangier; 11. Kara Sultani; 12. Russalka 5-A; 13.Flame seedless; 14. Hybrid 720-19; 15. Slavjanka; 16. Gigant; 17. Tarnau; 18. Focha seedless; 19.Seedless white (Sultanina); 20. Superior seedless; 21. Early Superior seedless; 22. Nimrang x Sultanina; 23. King ruby; 24. Kishmish Tjurkmenski; 25. Korint seedless; 26. Korintsko white; 27. Red seedless.

Table 2

Comparative analysis on the elements of the pollen aperture complex (μm) by the Duncan Test. No = cultivars, see Tab. 1

MESOCOLPIUM				APOCOLPIUM				WIDTH OF THE COLP			
No	x̄	Rp 5%	Rp 1%	No	x̄	Rp 5%	Rp 1%	No	x̄	Rp 5%	Rp 1%
1	13.06	a	a	2	6.46	a	a	19	1.63	a	a
2	12.85	ab	ab	13	5.57	b	b	5	1.59	a	ab
27	12.69	abc	abc	4	5.48	bc	bc	26	1.54	ab	abc
6	12.56	abcd	abcd	3	5.41	bcd	bcd	25	1.14	b	abc
11	12.16	bcde	abcde	9	5.38	bcd	bcd	27	1.10		bc
10	11.97	bcde	bcdef	18	5.29	bcde	bcd	1	1.09		bc
22	11.86	def	bcdefg	19	5.27	bcdef	bcd	23	1.03		c
17	11.76	efg	cdefg	11	5.22	bcdefg	bcd	18	1.03		c
13	11.72	efg	cdefg	15	4.93	bcdefgh	bcde	12	1.00		c
8	11.65	efgh	defgh	5	4.87	bcdefgh	bcde	6	1.00		c
7	11.61	efgh	defgh	12	4.75	cdefgh	bcdef	16	1.00		c
25	11.57	efgh	defgh	26	4.73	cdefgh	bcdef	4	0.99		c
4	11.50	efghi	efgh	25	4.71	cdefgh	bcdef	7	0.97		
3	11.47	efghi	efgh	7	4.61	defgh	bcdef	22	0.97		
9	11.44	efghi	efgh	14	4.50	efghi	bcdef	11	0.95		
12	11.40	efghi	efgh	27	4.50	efghi	cdef	15	0.94		
18	11.37	efghi	efgh	16	4.47	fghi	cdef	2	0.92		
16	11.09	fghik	fghi	1	4.46	fghi	cdef	14	0.92		
14	11.05	fghik	fghi	21	4.43	ghi	cdef	17	0.91		
21	11.01	ghik	fghi	8	4.42	ghi	cdef	8	0.90		
15	10.82	hikl	ghi	10	4.41	ghi	def	13	0.90		
19	10.69	ikl	hik	17	4.40	ghi	def	10	0.90		
23	10.32	klm	ikl	23	4.38	hi	def	9	0.90		
24	10.11	lmn	ikl	20	4.13	hi	ef	3	0.86		
5	9.75	n	l	22	4.13	hi	ef	21	0.81		
20	9.47			6	3.72	i	f	24	0.78		
26	8.96			24	3.57			20	0.76		

for almost all cultivars. This makes us assume the presence of a definite genetic stability in the dimensions of these parameters. As for the rest of the tested parameters, their variability is rather different. The most variable parameter for cv. Kolarovets is the size of apocolpium (S = 39.96 %); vor Tarnau - the length of colp (S = 47.33 %); for Kishmish Moldavski - the width of colp (S = 81.08 %); and for Red Seedless - the depth of colp (S = 49.92 %).

On the basis of the results obtained from the electron microscopy study of the pollen of 27 seedless grape cvs

Table 3

Values of the main elements of the pollen aperture complex (μm). Cultivars see Tab. 1

Cultivar	Polar axis		Equatorial axis		Mesocolpium		Apocolpium		Length of the colp		Width of the colp		Depth of the colp	
	x̄	S%	x̄	S%	x̄	S%	x̄	S%	x̄	S%	x̄	S%	x̄	S%
25	20.31	7.33	14.88	7.93	11.58	5.98	4.71	22.63	17.83	12.31	1.91	8.50	1.36	17.20
6	24.32	6.20	17.74	4.92	12.56	2.58	3.73	39.96	23.04	2.47	4.00	39.96	1.32	0.08
11	23.90	3.51	17.35	3.90	12.16	3.00	5.22	6.54	20.98	2.32	1.96	10.79	1.02	11.37
22	21.39	1.36	16.26	2.34	11.86	3.03	4.13	0.19	19.71	5.03	2.01	11.23	1.28	21.57
18	21.80	4.51	14.64	2.81	11.38	5.90	5.29	8.44	19.77	6.99	1.83	5.68	0.99	16.31
4	25.32	3.98	17.28	7.66	11.50	2.83	5.48	4.77	21.26	5.87	1.59	7.40	1.03	18.40
27	19.41	1.55	17.69	7.19	12.69	8.34	4.50	7.32	18.52	9.45	1.11	10.75	0.61	49.92
7	24.68	6.25	15.85	7.03	11.61	6.37	4.61	10.45	19.90	7.18	0.98	0.09	0.62	30.39
8	24.64	4.29	15.32	4.84	11.65	7.31	4.43	2.89	21.17	5.10	0.90	0.08	0.64	13.09
12	23.74	7.82	15.34	7.50	11.40	6.43	4.75	11.13	21.03	4.48	1.01	0.09	0.81	9.05
17	22.15	7.87	14.03	6.80	11.76	5.52	4.40	10.28	16.20	47.33	0.91	0.08	0.83	0.06
14	23.30	3.64	14.38	7.72	11.05	6.48	4.50	11.07	21.30	4.48	0.93	0.06	0.62	0.06
9	24.36	1.93	14.33	1.03	11.44	1.96	5.38	10.47	22.24	5.23	0.91	17.18	0.62	0.07
20	21.67	1.93	12.08	3.16	9.48	7.10	4.13	8.40	19.61	3.12	0.76	0.03	0.51	0.15
23	20.88	1.48	12.88	1.70	10.32	3.69	4.39	9.83	19.02	2.04	1.04	0.07	0.57	0.04
21	21.47	3.53	13.70	4.04	11.01	7.20	4.44	19.53	19.30	1.28	0.82	0.04	0.58	0.06
3	25.69	3.81	15.54	4.72	11.47	4.31	5.41	8.45	22.49	4.50	0.87	0.09	0.61	8.26
5	24.95	10.36	12.00	19.02	9.07	9.35	4.97	6.97	16.98	7.21	1.17	18.51	0.97	32.47
26	19.47	6.47	12.53	3.93	8.94	6.79	3.75	15.14	16.43	10.72	1.67	0.09	1.31	12.57
19	21.78	2.38	14.97	3.97	10.70	3.16	5.27	7.61	17.90	9.61	1.68	15.44	1.42	9.28
15	23.30	1.33	15.16	4.47	10.82	5.54	4.93	14.25	21.37	4.64	0.94	0.08	0.60	0.02
2	26.16	4.16	15.40	1.32	12.85	2.00	6.46	7.20	24.04	4.17	0.92	81.08	0.55	0.04
10	24.35	3.79	15.78	2.78	11.97	7.26	4.42	12.73	22.10	4.40	0.90	14.81	0.72	0.05
16	22.76	2.46	15.07	5.77	10.09	7.18	4.47	9.15	20.55	3.41	1.00	17.98	0.63	0.10
24	20.87	3.76	13.28	6.10	10.11	3.68	3.57	12.77	19.67	1.68	0.79	0.10	0.65	0.02
13	23.39	2.80	15.23	4.26	11.72	2.83	5.57	11.66	21.33	6.79	0.90	14.00	0.64	0.06
1	27.03	3.33	18.22	4.51	13.06	3.63	4.46	15.99	24.13	4.82	1.09	0.03	0.69	0.06

and the biometric investigations of the elements of the pollen aperture complex, the following conclusions could be drawn:

1. The shape of the pollen grains is elliptical-oval. The aperture complex is tricolporate, with equatorially situated pores. The pollen colps are almost of the same type, most often shallowly open, with no demarcating edge and uneven microverrucose structure of the bottom.

2. The exine sculpture in the mesocolpiums is in low relief, with perforations, pits and crest undulations. The shape, depth and size of the surface sculpture formations are specific for the different cultivars. Comparatively of the same type are the zones of apocolpium, too. Some details, such as the projections and the type of sculptural ornamentation are also typical microstructural characters for the different cultivars.

3. According to the degree of variability, the elements of the pollen aperture complex could be divided into two arbitrary groups:a) low variability - polar axis, equatorial axis, meso-colpium, length of colp; b) medium to high variability - apocolpium, width of colp, depth of colp.

4. The dimensions of the elements with low variability - polar axis, equatorial axis, mesocolpium and length of colp - could be used as additional microstructural criteria for cultivar identification in grapevines.

References

AHMEDULLAH, M.; 1983: Pollen morphology of selected *Vitis* cultivars. J. Amér. Soc. Hort. Sci. **108**, 155-160

CARGNELLO, G.; CARRARO, LUISA; LOMBARDO, GIULIANA; GEROLA, F. M.; 1980: Pollen morphology of Picolit grown in different Italian regions. Vitis **19**, 201-206

CARRARO, LUISA; LOMBARDO, GIULIANA; CARGNELLO, G.; GEROLA, F. M.; 1979: Studies on the embryosac and on the stigmatic receptivity of *Vitis* cultivars with different productivity (Picolit giallo and Verduzzo friulano). Vitis **18**, 285-290.

CLERG, E. L.; LEONARD, W. H.; CLARK, A. G.; (EDS.) 1962: Field Crop Technique. Burgess Publishing Company, Minneapolis.

DIMOVA, D.; MOKREVA, T.; SHABAN, N.; 1990: Comparative evaluation of the resolving power of the Duncan anf Fischer criteria. Rast. Nauk. **27** (4), 107-111.

ERDTMAN, G. E.; 1952: Pollen Morphology and Plant Taxonomy. I. Angiosperms. Stockholm, Waltham.

KHARITONASHVILI, L. A.; RAMISHVILI, R. M.; KIPIANI, M. G.; 1989: The study of pollen in different types of the flower *Vitis vinifera* L. by scanning electron microscope. Soobsh. Akad. Nauk Gruz. SSR **136**, 653-656.

KOZMA, P.; 1963: Flower biology as a basis for vine fertility and breeding. Akaemiai Kidao, Budapest.

- -; SCHEURING, J.: 1968: Shape and structure of vine pollen in electron microscope. Kertészeti és Szölészeti Föiskola Közleményei, **32** (2), 5-29.

LINDER, R.; LINSKENS, H. F.; 1978: Le pollen de vigne d'Alsace. In: Génétique et Amélioration de la Vigne. IIe Symp. Intern. Amélior. Vigne, Bordeaux, 14-18 juin, 1977, 75-88.

LOMBARDO, GIULIANA; CARGNELLO, G.; BASSI, MARIA; GEROLA, F. M.; CARRARO, LUISA; 1978: Pollen ultrastructure in different vine cultivars with low productivity. Vitis **17**, 221-225.

- -; CARRARO, LUISA; CARGNELLO, G.; BASSSI, MARIA; 1976: Ultrastructure of pollen of *Vitis vinifera* L., cv. "Picolit giallo" and its behaviour in experiments of self- and cross-pollination. Vitis **15**, 73-81.

- -; - -; - -; GEROLA, F. M.; 1983: Observations on the natural fall of the colyptra in clones of Picolit giallo with high and low productivity. Vitis **22**, 129-136.

TERZIISKY, D.; KARAGEORGIEV, S.; 1989: (Please indicate english title: Rakovodstvo po osnovi na electronnata mikroskopia. Plovdiv, VSI.

TOMPÁNÉ KASIRSZKAJA, A.; 1981: Scanning electron microscopic characteristics of pollen of geographic-ecologic vine variety groups. Kertészeti Egyetem Közleményei **45**, 91-100.

- -; 1982: Surface characteristics of pollen in North-American and East-Asian *Vitis* species. "Lippai János" Emlékülés és Tudemanyos Üllésszak Elöadásai. I. Kötet, 315-326.

- -; 1984: Scanning electron microscopic characteristics of pollen taken from vine intra- and interspecific hybrids. Kulonlenyomat a Kerteszeti Egyetem 1984. evi kozlemenyeibol. Budapest.

- -; 1985: Sculpture and structure of the pollen sporodermis with different grape flower types. Kulonlenyomat a Kerteszeti Egyetem 1985. evi kozlemenyeibol. Budapest.

- -; 1988: The ultrastructure of riped cane. Szölötmersztés és Borászat **10** (2-3), 10-15.

- -; KOZMA, P.; 1978: Study of vine pollen with scanning electron microscope. Kertészeti Egyetem Közleményei **42** (2), 23-39.

Received December 13, 1993