

THE POLLEN MORPHOLOGY OF *CITRUS LIMON* CV. "VERNA" FROM THE MURCIA REGION, S.E. SPAIN

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RESUMEN

Morfología del polen de *Citrus limon* cv. "Verna" de la Región de Murcia

Se han examinado ciento sesenta muestras de granos de polen de *Citrus limon* cv. "Verna", usando microscopio de luz y microscopio electrónico de barrido. Los granos son subprolato-prolato, isopolares y de tamaño medio-largo. La **exina** es foveolada tendiendo a reticulada y son tectados con un tectum **microperforado**. Según la vista meridional, los granos son siempre elipsoidales, mientras que según la vista polar, son ángulo-perturados con tres tipos de área polar: tetracolporados, pentacolporados o hexacolporados. Se hace notar la falta de correlación entre la longitud de los granos y la longitud de los **colpos**. Se discute la posibilidad de que esta circunstancia pueda ser una característica interesante para distinguir las diferentes especies de *Citrus* y entre los cultivares de *C. limon*.

Palabras clave: Granos de polen, *Citrus limon*, *Rutaceae*.

ABSTRACT

One hundred and sixty pollen grains from a **sample** taken from *Citrus limon* cv. "Verna" **have been examined** using **both** light and scanning electron microscopy. The grains are subprolate-prolato, **medium-large**, isopolar and radiosymmetric. The exine is semi-tectate, foveolate tending to reticulate. **In** meridional view the grains are ellipsoidal, while in polar view they are **angul-aperturate**, either **rarely**, tetracolporate, **pentacolporate** or, hexacolporate. **A** lack of **correlation** is noted, between the larger grains and the grains **with** the longer colpi. The possibility is discussed that this could be a valuable character in distinguishing between different species of *Citrus* and **also** between the **cultivars** of *C. limon*.

Key words: Pollen **grain**, *Citrus limon*, *Rutaceae*.

INTRODUCTION

The genus *Citrus* (Rutaceae) comprises around 60 species, most of which are cultivated

in the tropical and warm "Citrus belts" which girdle the world, especially the Mediterranean region, southern USA, Mexico, South Africa and Australia (HEYWOOD 1985).

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Of the four varieties of *Citrus limon* established by GONZÁLEZ SICILIA (1968), variety "Vema" is the most widely grown in the region of Murcia, accounting for almost 90% of production.

Bearing in mind the problems presented by palynological studies of cultivated species and that the only references to the pollen of *Citrus limon* are found in the regional studies of ALBERT POLO (1972), and BARTH (1980, 1983) we have attempted, using biometric data obtained from microscopic observation, to identify the most important characteristics of the pollen: symmetry, shape, size, polar and equatorial outlines, apertures and wall stratification of the exine.

MATERIAL AND METHODS

The samples examined were taken from material collected in the field in mid afternoon, when the flowers were in FELIPE and BAGLIOLINI'S (1979) f state, coinciding with the onset of another dehiscence; the best stage for obtaining mature but as yet unreleased pollen. Removal of the pollen from the anthers follows methods described by of BAÑO BREIS (1984).

The samples studied, the pollen collection and all other data are kept in the Plant Physiology Department of the Biology Faculty, University of Murcia, where they can be consulted.

The pollen was first acetolyzed (ERDTMAN, 1969) and then stained with 5% methylene blue, 5% safranin and Guegen Liquid (BAÑO BREIS, 1990) and mounted glycerol jelly. Observations and measurements were made with a Nikon Optiphot-2, optical microscope. The conversion factor to standard measurements defined by the ratio C/25 (C being the size of a grain in conditions in which *Corylus avellana* has a mean size of 25.4 μm) is 1.5 for acetolyzed pollen recently mounted in glycerol jelly, according to FAEGRI & IVERSEN (1975).

The descriptions follow to the terminology of ERDTMAN (1969) for shape, ornamentation and apertures, and that of FAEGRI & IVERSEN (1975) for exine structure.

Using optical microscopy measurements of the following characteristics were taken from 160 grains: polar axis (P), equatorial diameter (E), side of the polar polygon (t), diameter be-

tween colpal apices in polar view (Plate 1C), length of the colpia (c), thickness of the exine (ex) and the thickness of the costae. P/E and t/E ratios were calculated and, where possible the area of the endoapertures. From SEM photomicrographs a count of foveolae was made.

Preparation of pollen for scanning electron microscopy (SEM) also follows methods described by BAÑO BREIS (1984). Subsequent examination was carried out using an ISI "super mini" SEM.

RESULTS

The pollen is radiosymmetric and isopolar. In meridional view the grains are always ellipsoidal while in polar view is either tetra-, penta- and hexagonal, according to the number of colpia.

More than half the grains examined (50.6%) are tetracolporate (Plate 1A); pentacolporate grains are also abundant (48.1%) (Plate 1B) while hexacolporate are very scarce (1.2%) (Plate 1C) WODEHOUSE (1935) and SÁEZ (1978). These findings do not agree with those of TERRADILLOS & LAMAS (1988) who reports tricolporate for *Citrus limon*. No tricolporate grains were found in our samples studied.

The endoapertures are elongate, nomotreme and pantotreme, externally swollen in the equatorial zone and smoothly rounded in the polar zone (Plate 1D). Internally the endexine is thickened around the endoapertures, c.1 and 1.5 μm to form costae. Endoaperture does not show "vestibular chambers".

The pollens in polar area are microperforated tectate. The overall wall thickness is 2 μm and the thickness of the infratectum is 1-1.5 μm (Plate 1E). The exine mesocolpial of these pollen grains is foveolate tending to reticulate, the foveolae having a lumen of 2-2.5 μm .

SEMs show colporus in non acetolyzed pollen grains: meridional view (Plate 2A and 2B) grains not expanded and polar view (Plate 2C) grains expanded. The unruptured endoaperture membrane can be seen as a swelling in the equatorial zone prior to exit of the pollen tube (Plate 2D). Mesocolpia measure 300-500 μm^2 and have 100-500 foveolae; the apocolpia measure 200-800 μm^2 , with 30-100 foveolae and

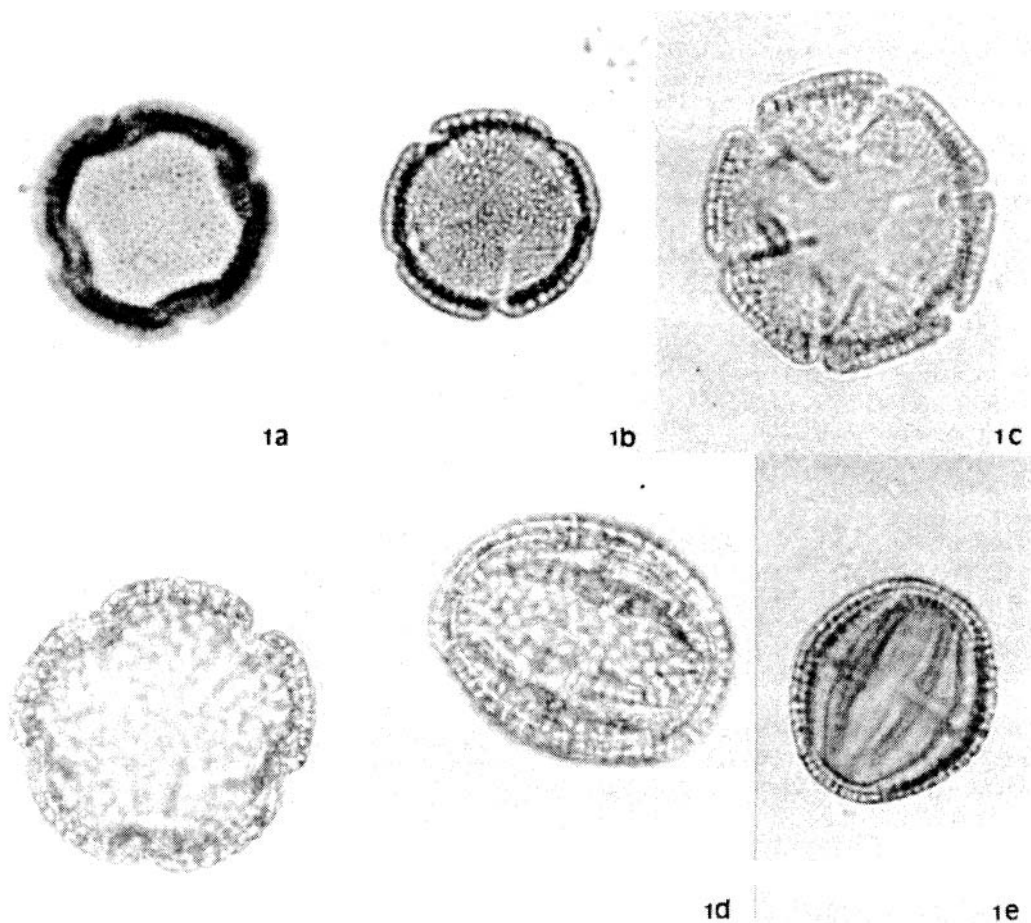


PLATE 1 (LM). 1A. Tetracolporate pollen grain of *Citrus limon* cv. "Verna", polar view, stained with Guegen liquid (**x400**). 1B. Pentacolporate pollen grain of *Citrus limon* cv. "Verna", polar view, stained with Guegen liquid (**x400**). 1C. Hexacolporate pollen grain from *Citrus limon* cv. "Verna", polar view, without stain (**x400**). 1D. Pentacolporate pollen grain from *Citrus limon* cv. "Verna" polar (left) and meridional (right) views, without stain (**x400**). 1E. Pollen grain from *Citrus limon* cv. "Verna", meridional view without stain (**x400**). Note endoapertures in equatorial zone.

FOTOGRAFÍA 1. 1A. Grano de polen de *Citrus limon* cv. "Verna". tetracolporado en vista polar, teñido con líquido de Guegen (**x400**). 1B. Grano de polen de *Citrus limon* cv. "Verna", pentacolporado en vista polar, teñido con líquido de Guegen (**x400**). 1C. Grano de polen de *Citrus limon* cv. "Verna", Hexacolporado en vista polar, sin teñir. (**x400**). 1D. Grano de polen de *Citrus limon* cv. "Verna", pentacolporado en vista polar (izquierda) y meridional (derecha), sin teñir. (**x400**). 1E. Grano de polen de *Citrus limon* cv. "Verna", en vista meridional sin teñir (**x400**). Notar las endoaperturas en la zona ecuatorial.

the area of the hexagonally shaped apocolpium may be as much as $1000 \mu\text{m}^2$ (see Table I).

TABLE I. Biometric data of pollen grains from *Citrus limon* L. cv. "Vema", using optical microscopy and SEM (*).

Datos biométricos de los granos de polen de *Citrus limon* cv. "Vema", usando microscopio óptico y SEM

	Range μm	Mean μm	Desvia- tion μm	Area μm^2	Foveolae N (*)
P	27.5-42.5	35.10	2.97		
E	22.5-37.5	30.31	3.18		
P/E	1.0-1.55	1.16	0.14		
t	10.0-22.5	17.79	3.42		
t/E	1.0-0.26	0.59	0.13		
ex	1.5-2.50	2.00	0.15		
c	20.0-32.5	30.12	2.16		
M				300-500	50-100
A				200-800	30-100

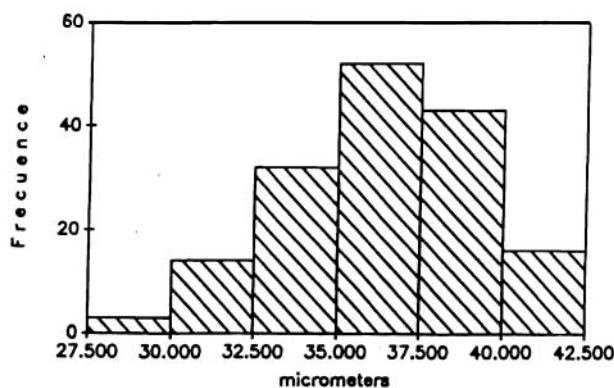
P: polar axis; E: equatorial axis; t: side of the polar polygon; ex: thickness of the exine; c: length of the colpi; M: mesocolpium; A: apocolpium. (N° of grains measured = 160).
*(SEM): scanning electron microscopy

From the biometric data of Table I the following results have been established: The P and E values are tabulated in histograms (Diags. I

and II). Both P value and E value are similar to Gauss curves. The mean values of $P=35.10 \mu\text{m}$ and $E=30.31 \mu\text{m}$ and their respective frequencies (Diags. I and II) qualifies the grains as medium-large in accordance with the nomenclature of PLÁ DALMAU (1961). The mean value of the ratio $t/E = 0.59$ qualifies the grains as having a large polar area, according to the classification of HYDE and ADAMS (1958). The mean length of the colpi $C = 30.12 \mu\text{m}$ in comparison with the polar length of the grains shows the pollen to be of the long colpare type. When these values are tabulated in five frequency ranges, the greatest percentage lies in the range $30-32.5 \mu\text{m}$ (Diag. III) a fact which will be discussed below.

DISCUSSION

The number of colpi in the range $30-32.5 \mu\text{m}$ (Diag. III) is surprising being four times greater than those of the next most frequent length of $27.5-30 \mu\text{m}$. Other lengths are comparatively insignificant. A comparison of Diag. I and III shows no immediate correlation between the two sets of values, although it is generally accepted that a greater grain length —represented by the P axis— corresponds to a greater aperture length (RECUPERO



DIAG. 1. Pollen of *Citrus limon* cv. "Vema". Histogram to show values of polar axis. Mean value: $35.10 \mu\text{m}$. Standard deviation: 2.97572 .

DIAG. 1. Grano de polen de *Citrus limon* cv. Vema. Valores del eje polar: valor medio = $35.10 \mu\text{m}$ Desviación estándar = 2.97572 .

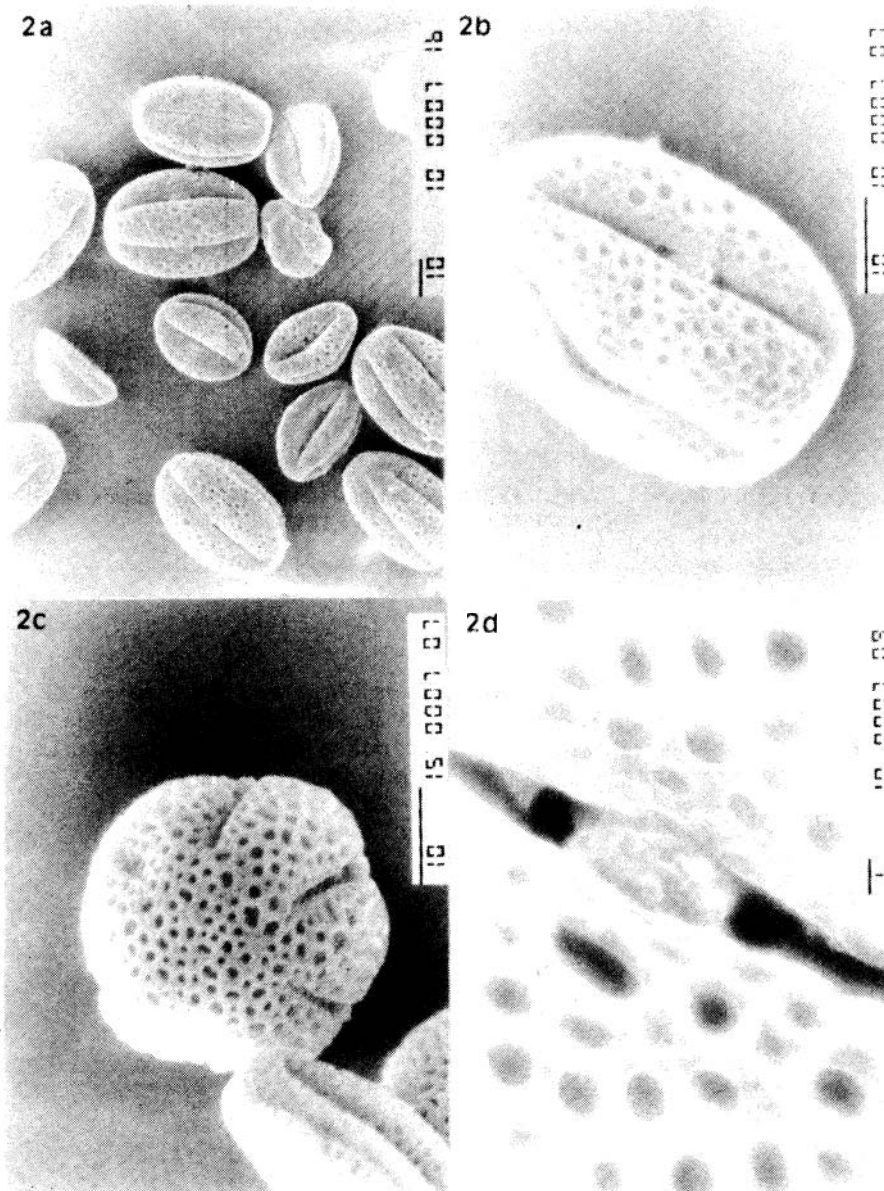
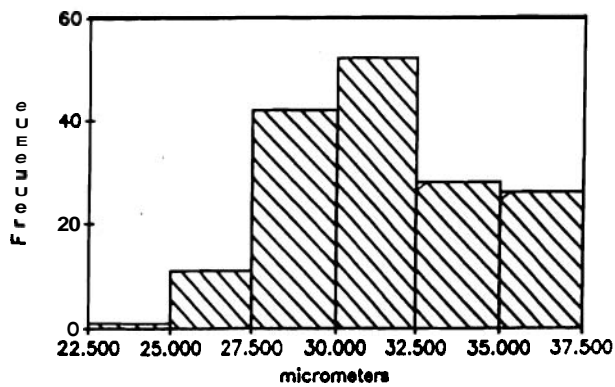


PLATE 2 (SEM). **2A.** Pollen grains of *Citrus limon* cv. "Verna", mainly in meridional view. **2B.** Pollen grain of *Citrus limon* cv. "Verna", meridional view showing colpius and endoaperture membranes still intact. **2C.** Hexacolporate pollen grain of *Citrus limon* cv. "Verna", polar view. Detail of foveolae. **2D.** Close up of pollen grain of *Citrus limon* cv. "Verna", detail showing endoaperture membrane.

FOTOGRAFÍA 2. (SEM). **2A.** Grano de polen de *Citrus limon* cv. "Verna" principalmente en vista meridional. **2B.** Grano de polen de *Citrus limon* cv. "Verna". La vista meridional muestra colpios y la endoapertura de la membrana aun intacta. **2C.** Grano de polen de *Citrus limon* cv. "Verna", hexacolporado en vista polar. Detalle del faveolo. **2D.** Grano de polen de *Citrus limon* cv. "Verna". Detalle de la endoapertura de la membrana.

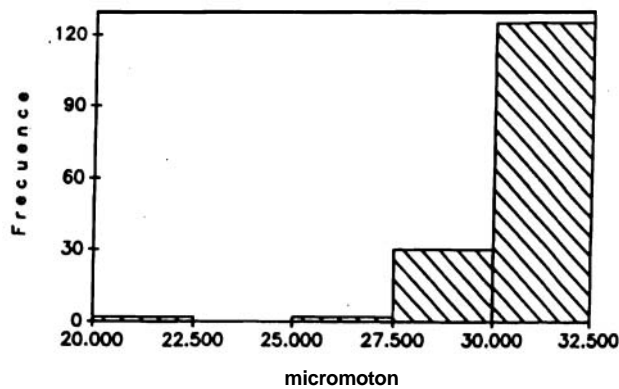


DIAG. 2. Pollen of *Citrus limon* cv. "Verna". Histogram to show values of equatorial axis. Mean value: 31.12 m. Standard deviation: 3.1545.

DIAG. 2. Grano de polen de *Citrus limon* cv. Verna. Valores del eje ecuatorial: valor medio = 31'12 m. Desviación estandard = 3.1545.

& Russo, 1978). If this lack of correlation between colpius length and the length of the polar axis could be confirmed it might be a useful distinguishing characteristic within the

genus *Citrus* and the cultivars of *C. limon* and of possible taxonomic value. Studies of the pollen of other *Citrus* species are currently being undertaken.



DIAG. 3. Pollen of *Citrus limon* cv. "Verna". Histogram to show length of the colpia. Mean value: 30.12 m. Standard deviation: 2.1615.

DIAG. 3. Grano de polen de *Citrus limon* cv. Verna. Longitud de los wlpios: valor medio = 30'12 m. Desviaciónestandard = 2,1615.

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