Técnica para captar e identificar los pólenes

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http://www.uab.es/l-analisis-palinologiques/aerobio.htm

Métodos de captación polínica

Métodos basados en los principios de:

- **Deposición gravitacional (no volumétrico)**

  - Portaobjetos
  - Placa de Petri
  - Método Durham
  - Trampas Tauber

volumétricos

- **Impacto por succión**

  - Hirst spore trap (Burkard, Lanzoni)
  - Impacto en cascada (Andersen)

- **Impacto por filtración**

  - Filtros de fibra (Cour) y de membrana

- **Impacto por rotación**

  - Muestreador Rotorod
  - Muestreador ciclónico

- **Filtración en medio líquido**

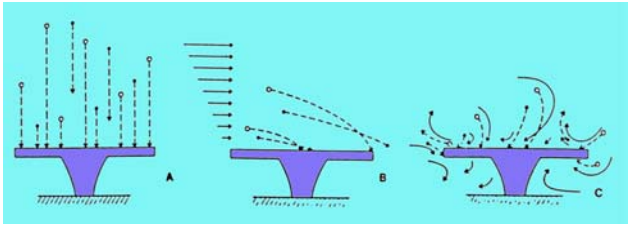
  - AGI (All Glass Impinger)

- **Preciptación electrostática**
- **Precipitación térmica**

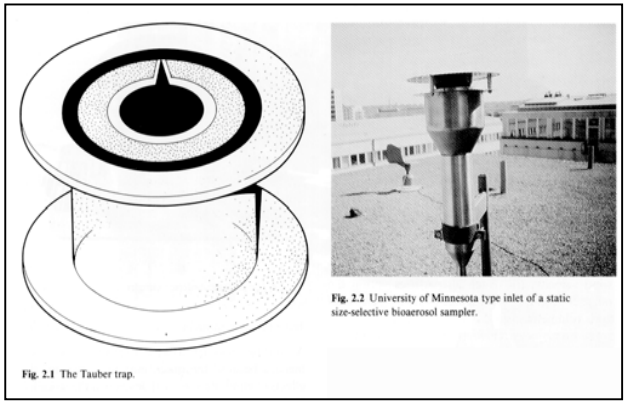
Métodos de captación polínica. Deposición gravitacional

Sistemas no volumétricos

- **Portaobjetos**
- **Placas de Petri**
- **Método Durham**
- **Trampas Tauber**



Captador Durham



Trampas Tauber

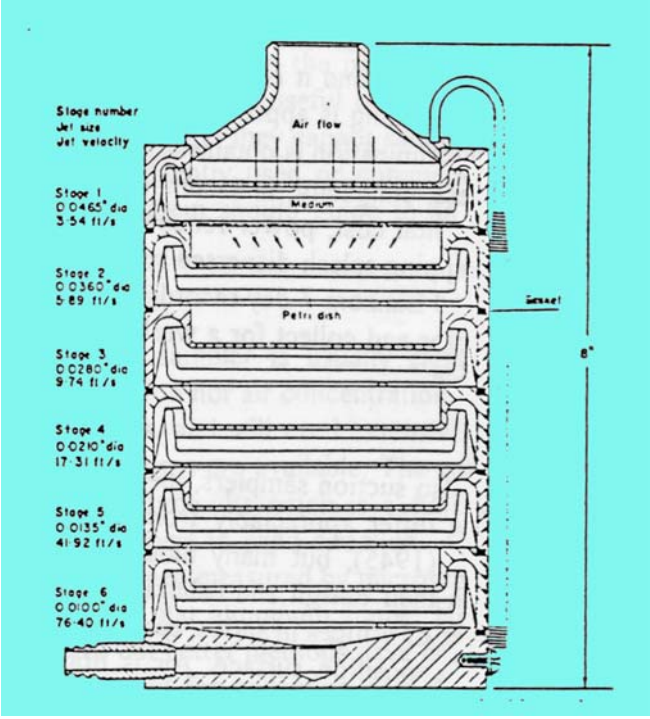
Captador Durham modificado

Catalunya (1983-1988)
¡no gravimétrico!

Métodos de captación polínica. Impacto por succión

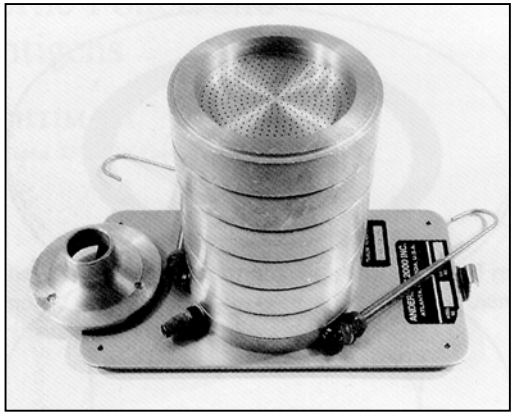
Sistemas volumétricos

- **Hirst spore trap (Burkard, Lanzoni)** → Se presenta detalladamente más adelante
- **Impacto en cascada (Andersen)**

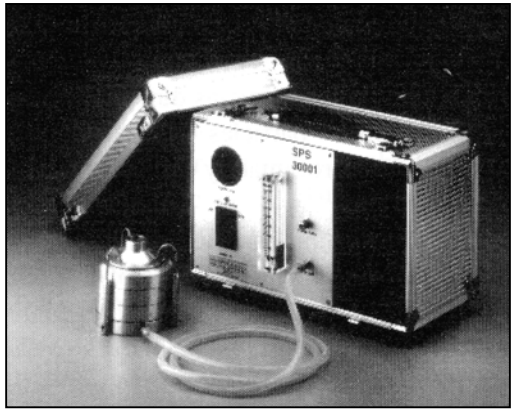


Esquema de muestreador Andersen con seis pisos de recogida de partículas

Captador Andersen



Captador Andersen de Lanzoni

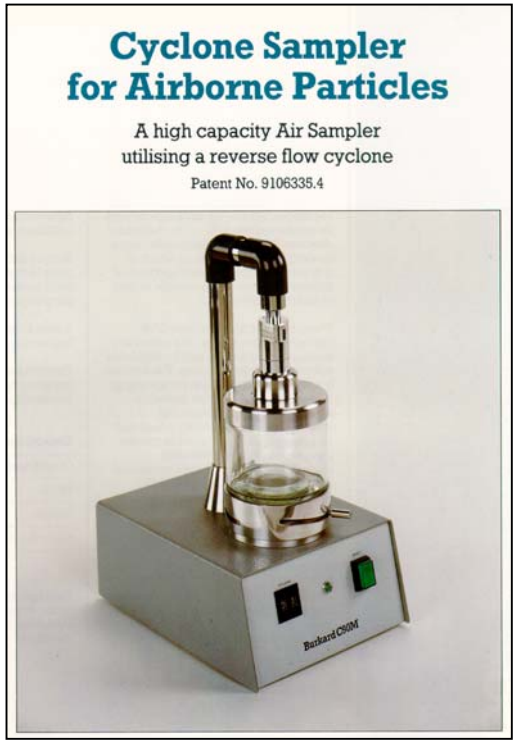


Métodos de captación polínica. Impacto por rotación

Sistemas volumétricos

- **Muestreador Rotorod**
- **Muestreador ciclónico**

Muestreador Rotorod
impacto por rotación mecánica



Muestreador ciclónico de Burkard
impacto por rotación de la masa de aire

Captador de polen y esporas Modelo Hirst

Sistema volumétrico
Impacto por succión

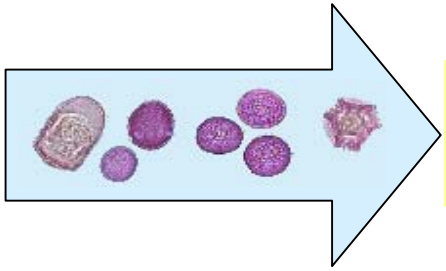
Estandarizado en las redes aerobiológicas europeas

Marcas comerciales

Burkard y **Lanzoni**

Funcionamiento captador Modelo Hirst

Marcas Burkard y Lanzoni



Orificio entrada aire

Sentido de giro del tambor

Tambor

Mecanismo relojería
avance 2mm/hora

Cinta adhesiva alrededor tambor

Cabezal extraible

Cuerda mecanismo relojería

Tambor

Cinta adhesiva alrededor tambor

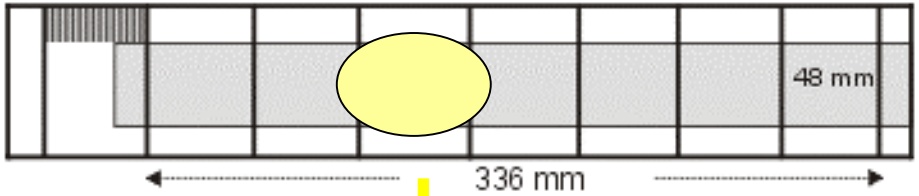
Bomba de vacío

HIRST-Preparación de las muestras, identificación y recuento

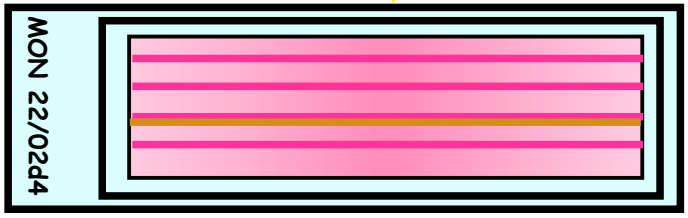
Muestreo semanal, en captadores Hirst

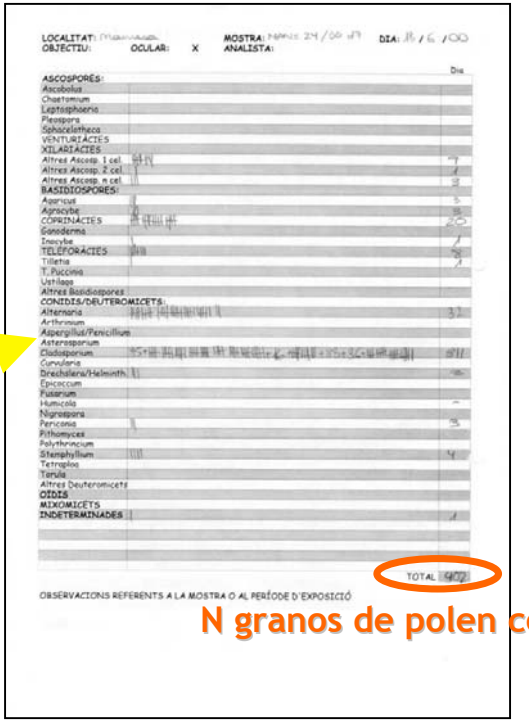
Recuento diario:
Uniformización y estandarización de recuentos

- Número de líneas a contar
- Disposición de líneas a contar
- granos de polen (4 lineas)
- esporas de hongos (1 linea; 2)



Muestra por dia

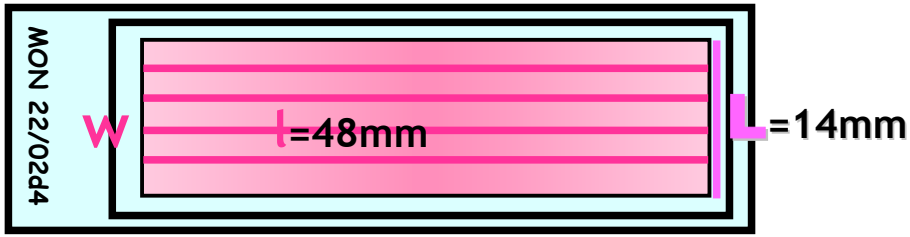




N granos de polen contados

Cálculos en la metodología HIRST

Análisis al microscopio óptico
4 lineas longitudinales



N Número de granos de polen contados en área analizada

Aa Área analizada = 4 (l w) = 4 (48 w) = 192w = 0.000192w m^2

Am Área muestra = l L = 48 mm x 14 mm = 672 mm^2 = 0.000672 m^2

V Volumen de aire analizado por día = 14.4 m^3
10 l/min x (24h/día x 60min/h) x 1/1000 m^3/l

Concentración polínica media diaria
N x (Am/Aa) / 14.4 m^3

Granos de polen por unidad de volumen (P/m^3)

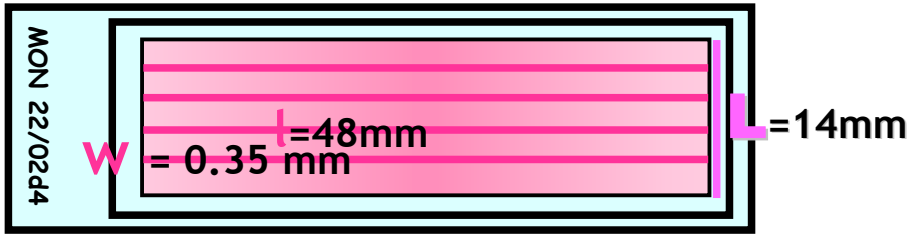
Cálculos en la metodología HIRST
Xarxa Aerobiològica de Catalunya, XAC

Análisis al microscopio óptico

4 lineas longitudinales

objetivo 40X, ocular 15X

w = 0.35 mm



N Número de granos de polen contados en área analizada

Aa Área analizada = 4 (48 x 0.35) = 67.2 mm^2 = 0.0000672 m^2

Am Área muestra = 48 mm x 14 mm = 672 mm^2 = 0.000672 m^2

V Volumen de aire analizado por día = 14.4 m^3

$$N \times (0.000672/0.0000672) / 14.4\ m^3$$

Concentración media diaria de polen

N x 0.7

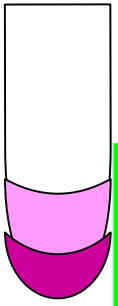
Granos de polen por unidad de volumen (P/m^3)

Muestreador COUR

Sistema volumétrico
Impacto por filtración

Metodología COUR

Protocolo de laboratorio:
H_2SO_4, HCl, HF, KOH…
Acetolisis (Erdtman 1960)



Volúmenes conocidos:

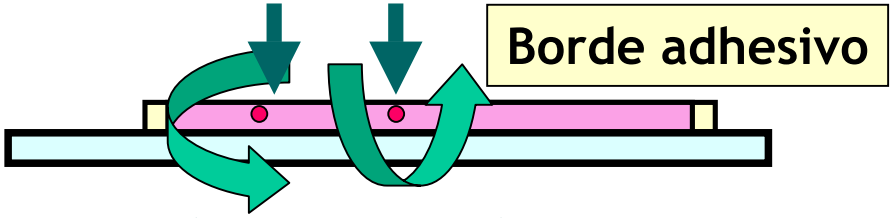
Glicerogelatina

Sedimento polen y esporas

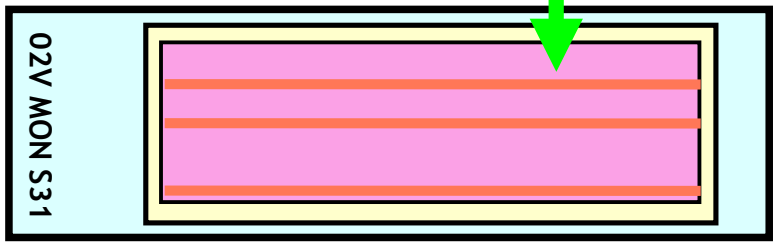
V_t

V_0

50 ul



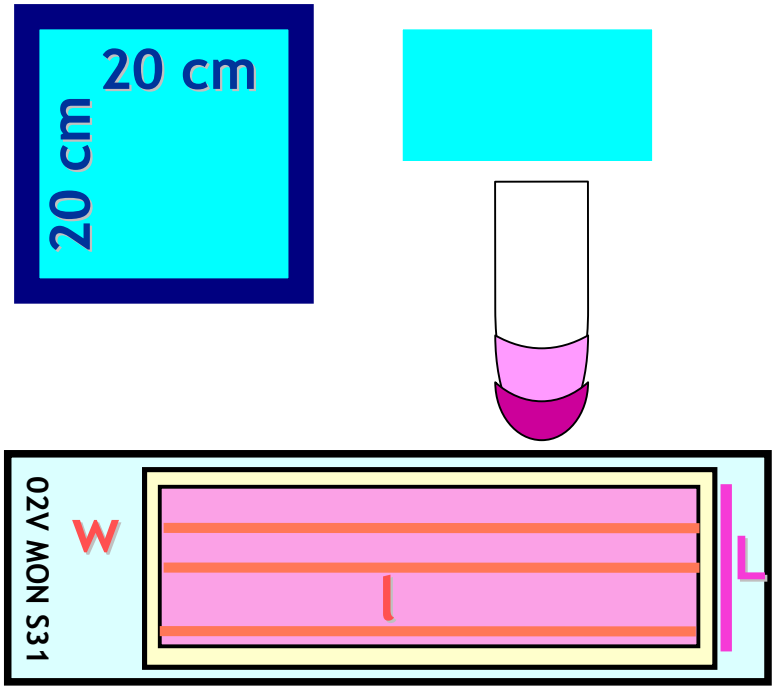
Una ligera presión hace girar los pólenes sobre si mismos



Lectura al microscopio óptico de 3 líneas longitudinales

N Número de granos de polen

Cálculos en la metodología COUR (1)



S Surperfície filtro = 0.02 m^2

V_t Volumen total sedimento

v Volumen muestra = 50 ul

Aa Área analizada = 3 (l w)

Am Área muestra= l L

Número de granos de polen en el área analizada = N

Número de granos de polen en el filtro (semana)

$N \times (V_t / v) \times (Am/Aa) / S$

Granos de polen por unidad de superficie (P/m^2)

Cálculos en la metodología COUR (2)

El anemómetro de recorrido de viento o totalizador mide la longitud de la columna de aire (Rv) que pasa a través del filtro de gasa de 20cmx20cm durante el período de exposición.

Eficiencia (resistencia del filtro) = 1/5 del recorrido de viento.

Concentración polínica media semanal

$$N \times (V_t / v) \times (Am/Aa) \;/\; S \times (Rv/5)$$

Granos de polen por unidad de volumen (P/m³)

Formula XAC

Concentración polínica media semanal

$$P/m^3 = 9.57 \times (N \times V_t) / (n \times S \times Rv)$$

Comparación Cour/Hirst (punto de vista operativo)

		Método Hirst	Método Cour
Electricidad	!!!	Si (Batería)	No ★
Superfície de muestreo		14mm x 2 mm	20 cm x 20 cm ★
Facilidad cambio periódico de muestras		Necesita atención	Fácil ★
Protocolo previo al análisis microscópico		Fácil ★	Necesita protocolo de laboratorio
Observación granos polen	!!!	Buena	Muy buena ★
Observación esporas hongos	!!!	Buena ★	Algunas se pierden
Pérdida de la muestra		Irreparable	Reparable ★
Frecuencia de muestreo	!!!	Diaria (Horaria) ★	Semanal ((Diaria))
Economía			
Coste del captador		Caro	Barato (Anemómetro!!!)
Coste de funcionamiento		Barato ★	Caro

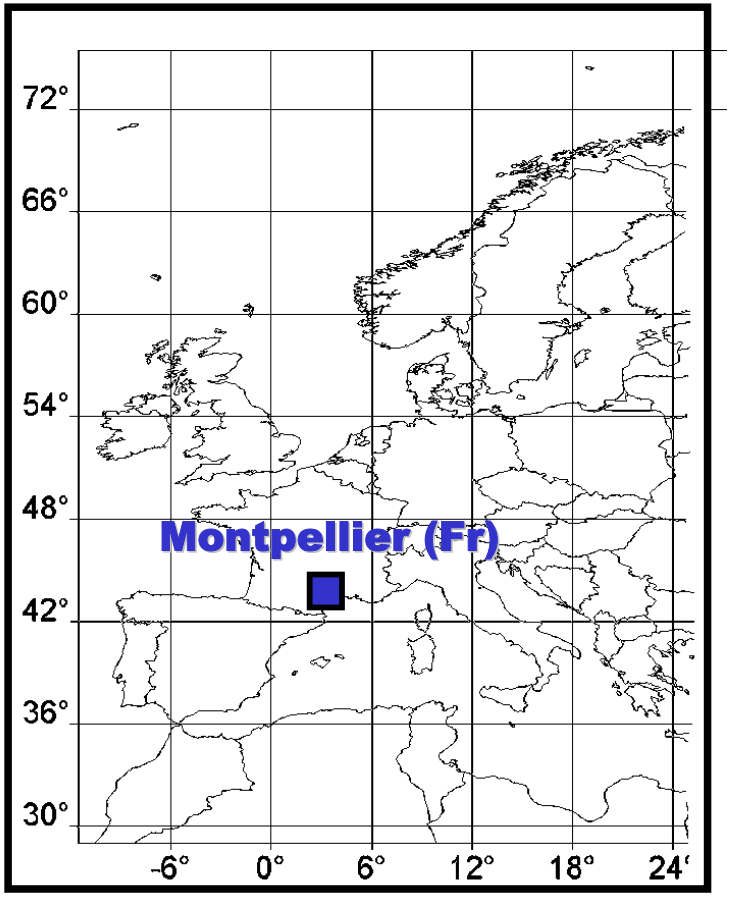
!!! **Aspectos clave** ★ **Opción mejor**

Atmospheric pollen survey: a metrological study between Hirst and Cour sampling methods

Farrera I.[1], M. Calleja [1], J. Belmonte [2], T. Almeras [1] & I. Plaisant [3]

[1] Unité de Palynologie. École Nationale Supérieure Agronomique Montpellier (ENSAM). France
[2] Botany Unit. Autonomous University of Barcelona. Cerdanyola del Vallès. Spain
[3] DRASS Languedoc Roussillon. France

Study design



Comparison Cour-Hirst

From 31/12/01 to 05/08/02

31 weeks

Repeatability Cour and Hirst

From 24/06/02 to 05/08/02

6 weeks

H_1 C_1 H_2 C_2

H Hirst sampler **C** Cour sampler

Same experimental conditions

Farrera et al. 2003

Taxon	Percentage period w1 - w31 HIRST	COUR
ABIES		0,000
ACACIA	0,004	**0,049**
ACER	**1,159**	0,796
AESCULUS	**0,048**	0,036
AILANTHUS	0,002	**0,008**
ALNUS	**0,394**	0,239
AMBROSIA	**0,002**	0,001
ARTEMISIA	0,004	**0,006**
BETULA	**0,989**	0,521
BORAGINACEAE	**0,020**	0,006
BUDLEJA	Not det.	0,002
BUXUS	0,400	**0,456**
CANNABACEAE	0,009	0,011
CARPINUS	0,077	**0,193**
CARYOPHYLLACEAE	0,000	0,001
CASTANEA	**2,224**	2,062
CEDRUS	0,003	0,002
CELTIS	0,217	**0,296**
CENTAUREA	0,001	0,001
CHENOPODIACEAE/AMARANT.	**0,160**	0,107
CISTACEAE	**0,005**	0,001
CONVOLVULUS		0,000
ASTERACEAE ECHINATE	**0,023**	0,015
ASTERACEAE FENESTRATE	0,017	**0,029**
CORIARIA	0,006	0,005
CORYLUS	**0,625**	0,307
CRASSULACEAE	**0,004**	0,000
BRASSICACEAE	0,027	**0,037**
CUCURBITACEAE		0,001
CUPRESSACEAE	38,468	**44,956**
CYPERACEAE	0,053	0,056
DIOSPYROS	Not det.	0,003
DIPSACACEAE	0,001	0,001
ELAEAGNUS	**0,002**	0,001
EPHEDRA		0,001
ERICACEAE TOTAL	**0,113**	0,080
EUCALYPTUS (MYRTACEAE)	0,009	0,010
EUPHORBIA	0,002	0,002
FAGUS	**0,152**	0,126
FRAXINUS	**6,166**	4,541
GINKGO	Not det.	0,036
POACEAE CEREAL	0,008	**0,022**
POACEAE WILD	**2,355**	1,672
HEDERA	0,002	0,001
HELIANTHUS	0,000	**0,002**
HYPERICUM		0,002
ILEX	**0,007**	0,001
JUGLANS	**0,027**	0,018
JUNCUS	**0,016**	Acetolysis!!!
LAMIACEAE	0,007	0,003
LAGERSTROMIA		0,000
LARIX	Non det.	0,004
LIGUSTRUM	0,029	**0,085**

Taxon	Percentage period w1 - w31 HIRST	COUR
LIPPIA		0,000
LIQUIDAMBAR	0,010	0,009
MELIA		0,001
MERCURIALIS	**0,049**	0,024
MONOCOTYLEDONEAE HERBS	0,005	**0,024**
MORACEAE	**3,968**	2,479
OLEA	0,633	0,608
APIACEAE	0,016	**0,036**
OSTRYA	0,001	To be revised
OXALIS		0,000
PALM TREES	**0,017**	0,000
PAPAVERACEAE	0,040	0,036
PAPILIONACEAE	0,004	**0,119**
PARTHENIUM	Not det.	0,007
PHILLYREA	0,024	**0,081**
PICEA	0,016	0,060
PINUS	9,748	**11,111**
PISTACIA	0,187	0,190
PLANTAGO	**0,681**	0,558
PLATANUS	**7,187**	6,726
POLYGONUM		0,002
POPULUS	2,004	**2,698**
PRIMULACEAE	Not det.	0,001
POTERIUM	0,006	0,006
PUNICA		0,002
QUERCUS TOTAL	**18,045**	15,081
RANUNCULACEAE	0,016	**0,037**
RESEDA	0,002	0,001
RHAMNUS	0,016	0,015
RHEUM	Not det.	0,001
RHUS	Schinus?	0,005
RICINUS		0,000
ROSACEAE lign.	0,002	**0,209**
RUBIACEAE	0,005	**0,019**
RUMEX	**0,178**	0,145
SALIX	0,279	**0,348**
SAMBUCUS	0,010	**0,050**
SCHINUS	0,001	Rhus?
SCROFULARIACEAE	0,000	**0,016**
SOLANACEAE	0,000	0,001
TAMARIX	0,064	**0,114**
TAXODIUM	Cupressaceae	0,003
TAXUS	Cupressaceae	0,514
TILIA	0,018	**0,038**
THIMELACEAE	0,000	0,000
TSUGA	Not det.	0,001
TYPHA	0,017	0,018
ULMUS	0,225	0,238
URTICACEAE	**2,450**	1,321
VIBURNUM	0,002	**0,037**
VITIS	0,136	0,155
XANTHIUM	0,000	0,000
ZIZIPHUS	Non det.	0,001
BROKEN -UNKNOWN	**0,095**	0,046

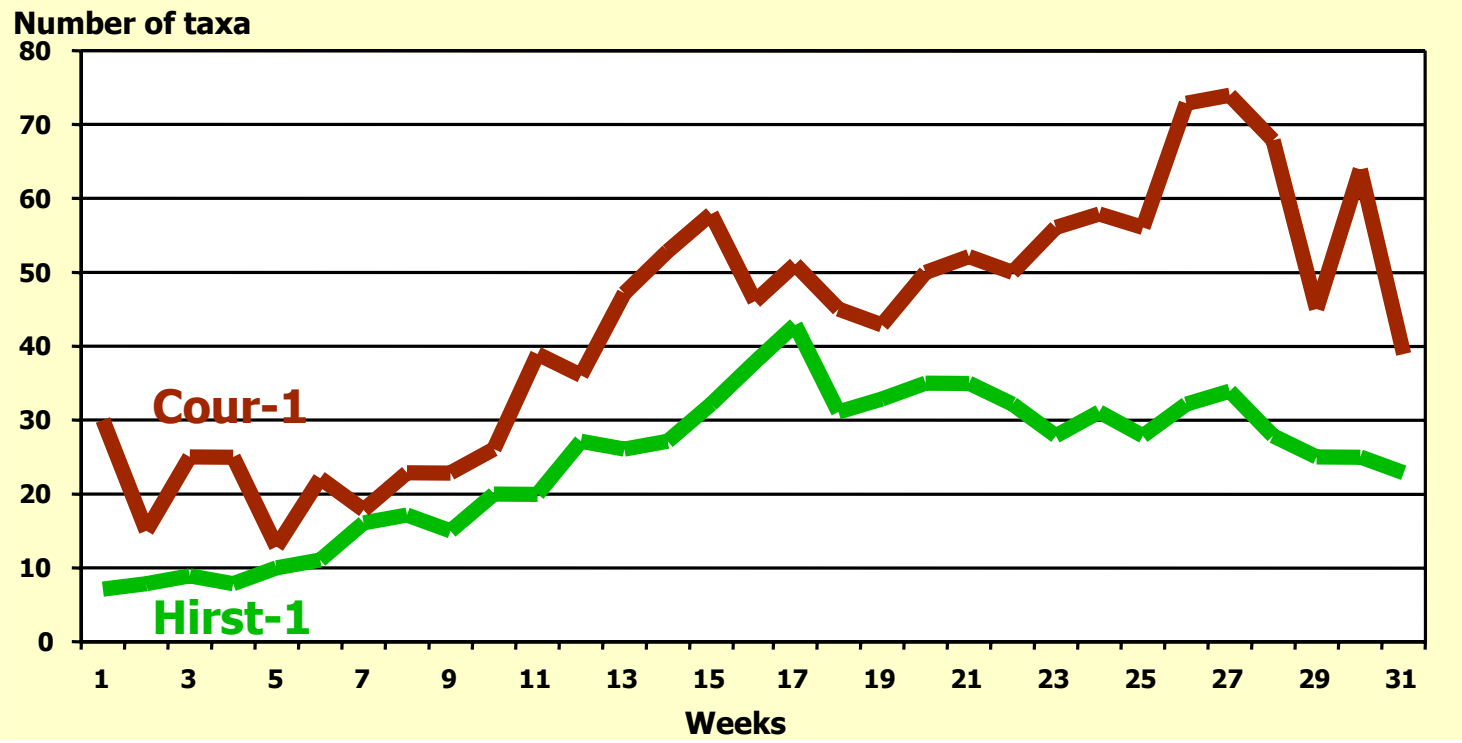
Número de táxones polínicos

Hirst-1 84

Cour-1 112

Farrera et al. 2003

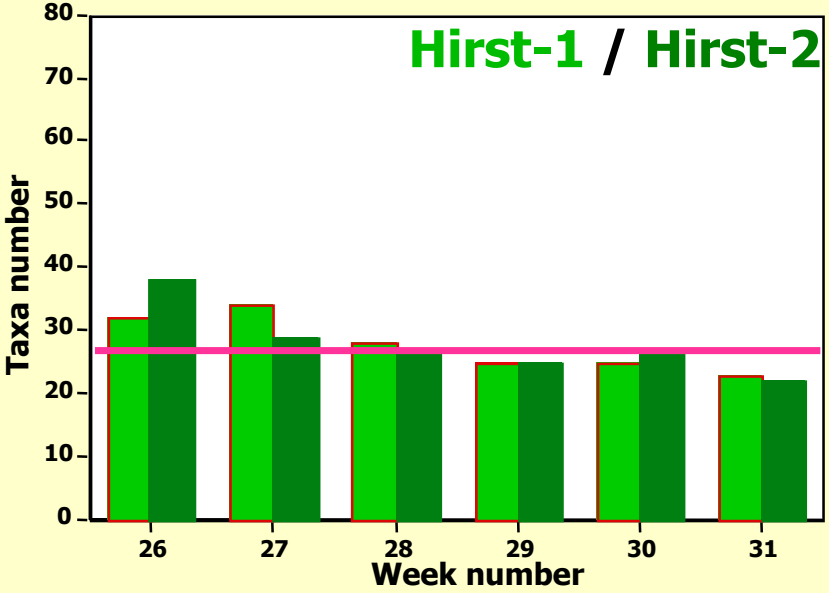
Comparación de la riqueza taxonómica



El método Cour muestra mayor riqueza taxonómica

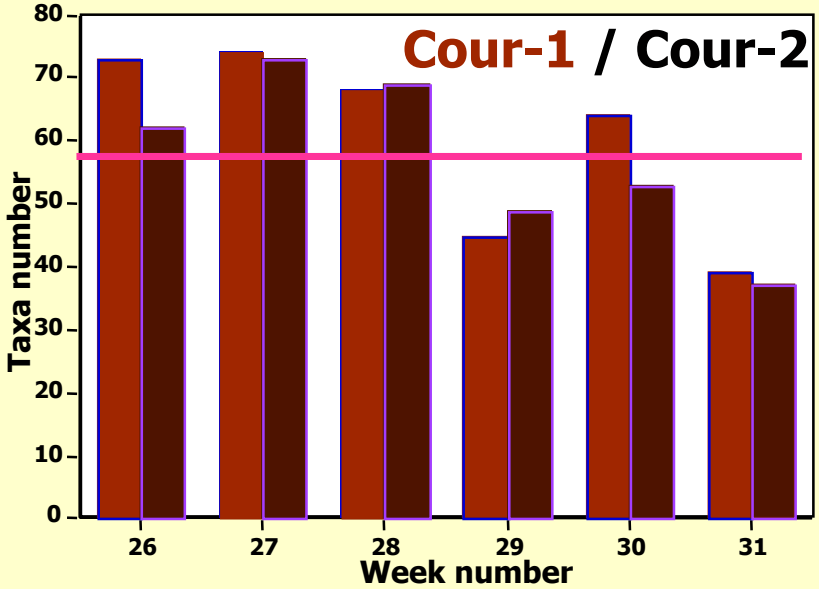
Comparación de la riqueza taxonómica

Método Hirst



Mean = 27
Mean of the weekly differences = 8%

Método Cour

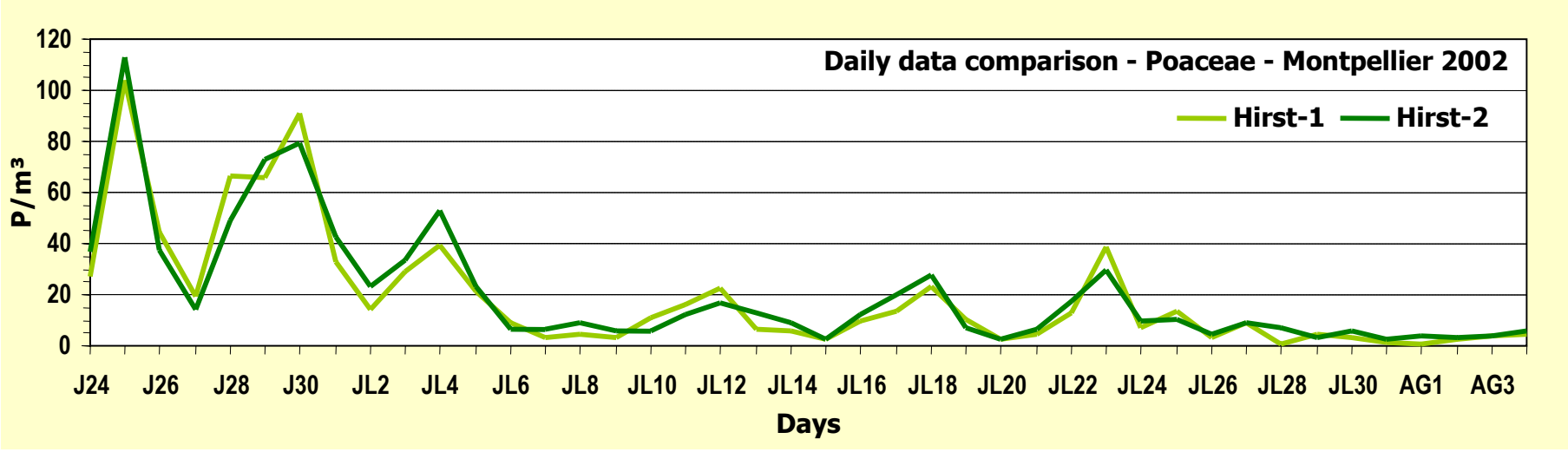


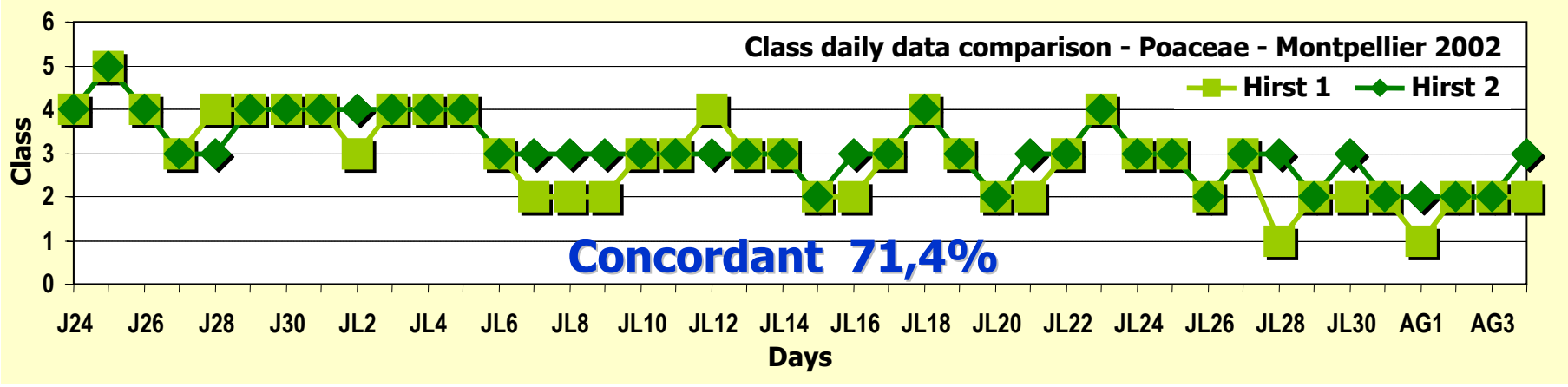
Mean = 58
Mean of the weekly differences = 8%

Ambos métodos, Hirst y Cour son repetibles

Farrera et al. 2003

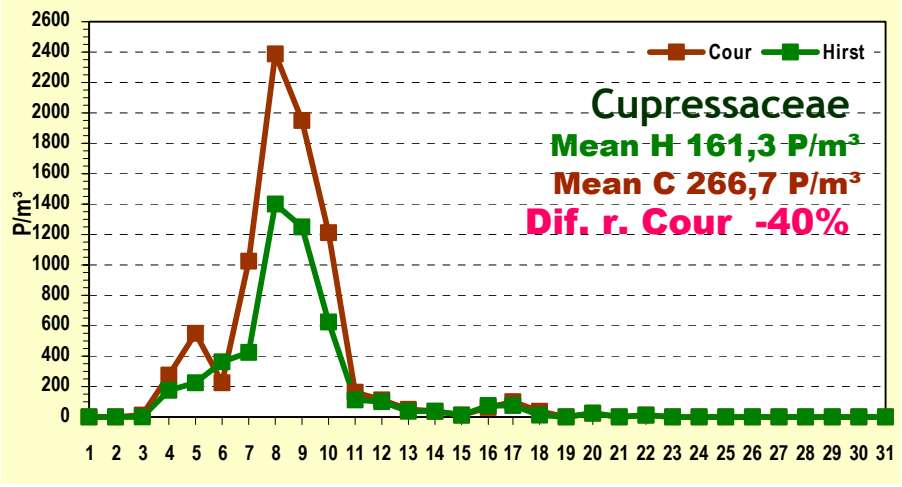
Comparación de las concentraciones polínicas Ejemplo con series diarias método Hirst

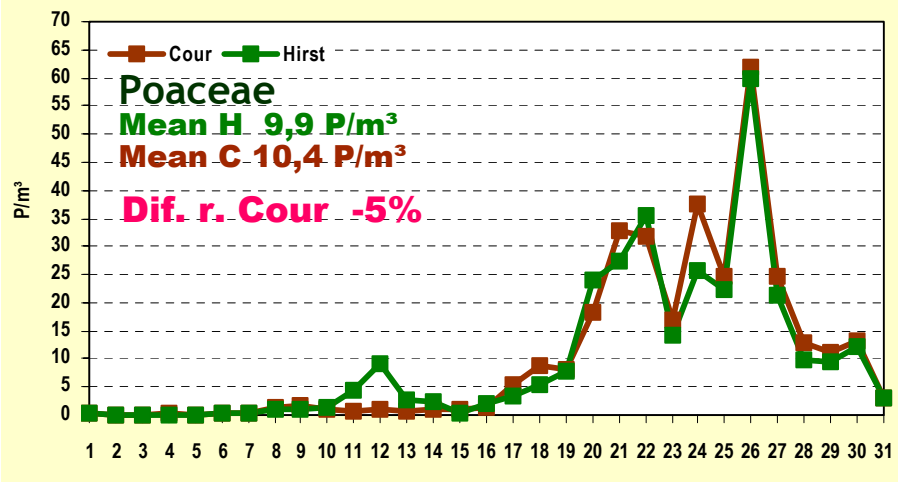


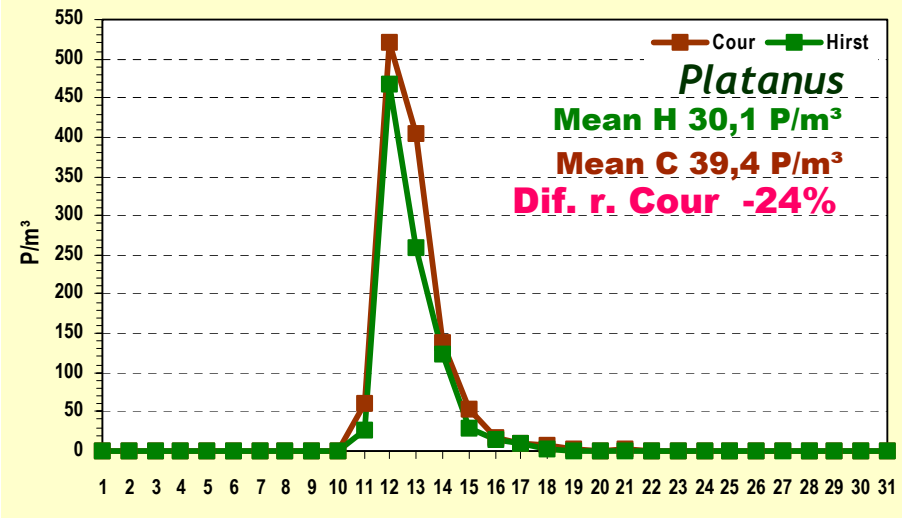


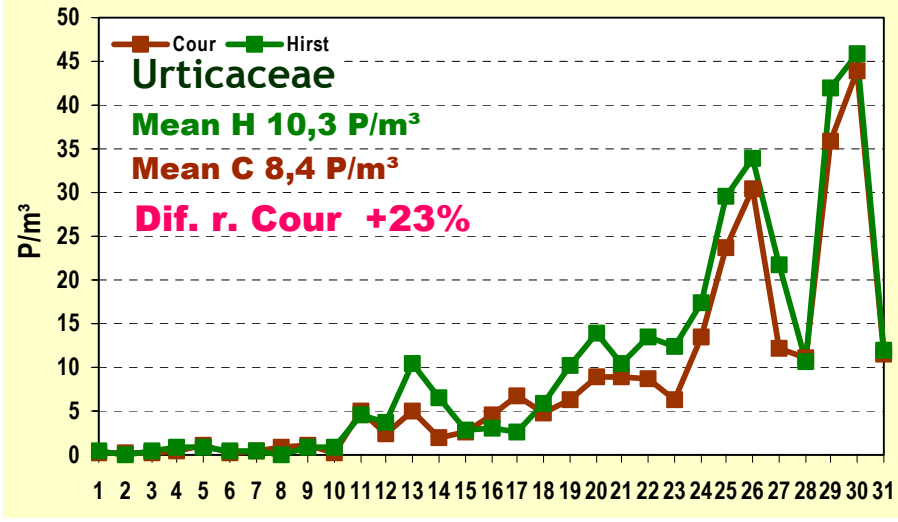
Farrera et al. 2003

Comparación de concentraciones polínicas
Series de datos Hirst/Cour – ejemplos



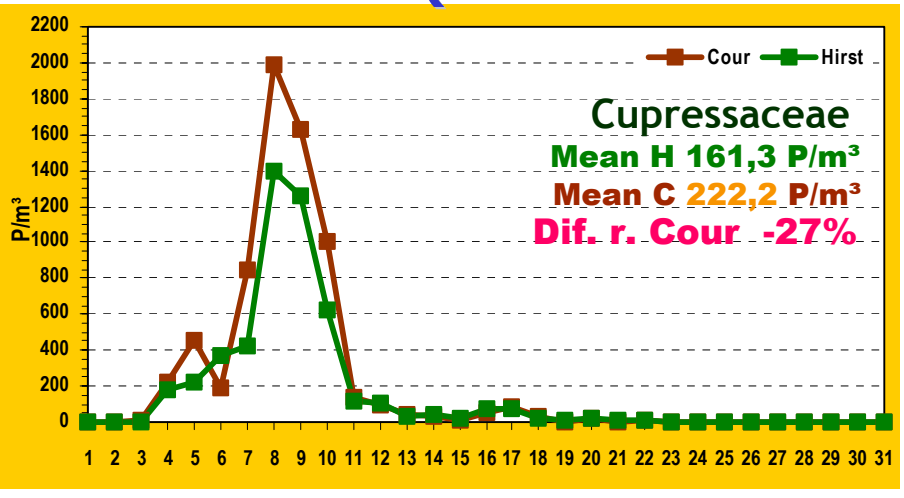


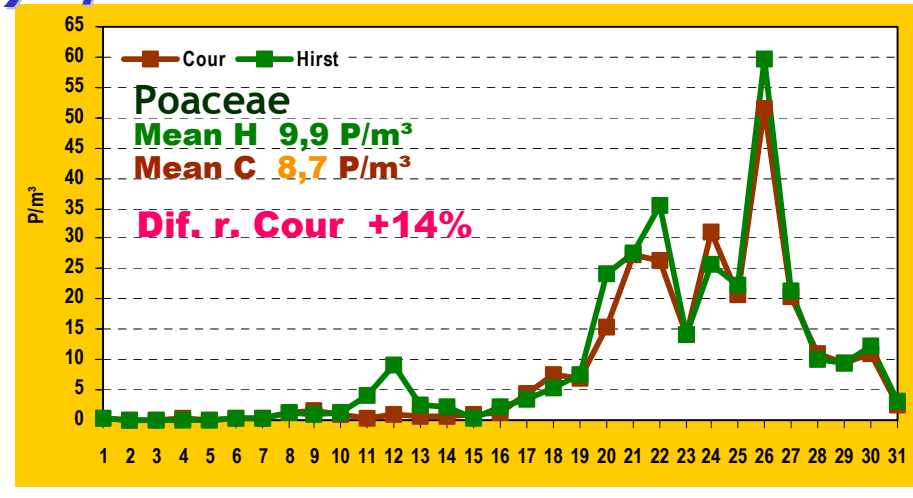


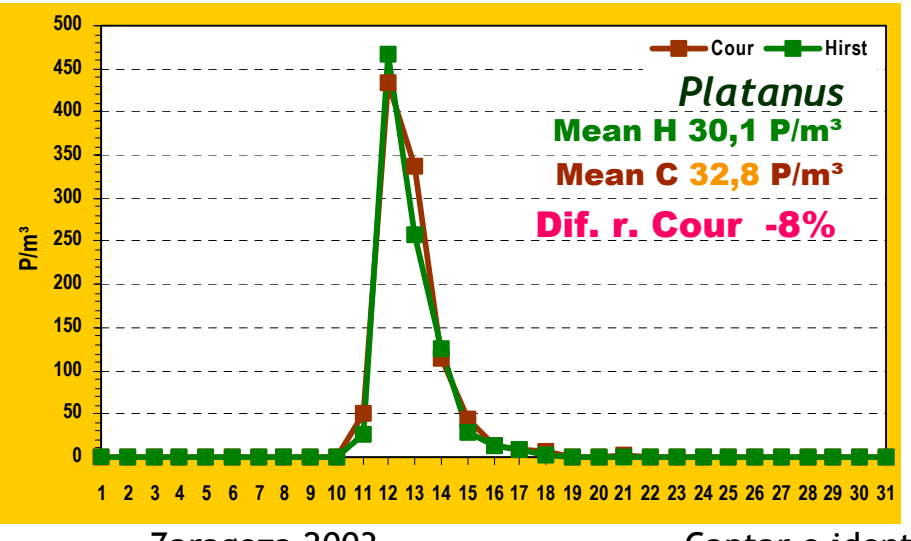


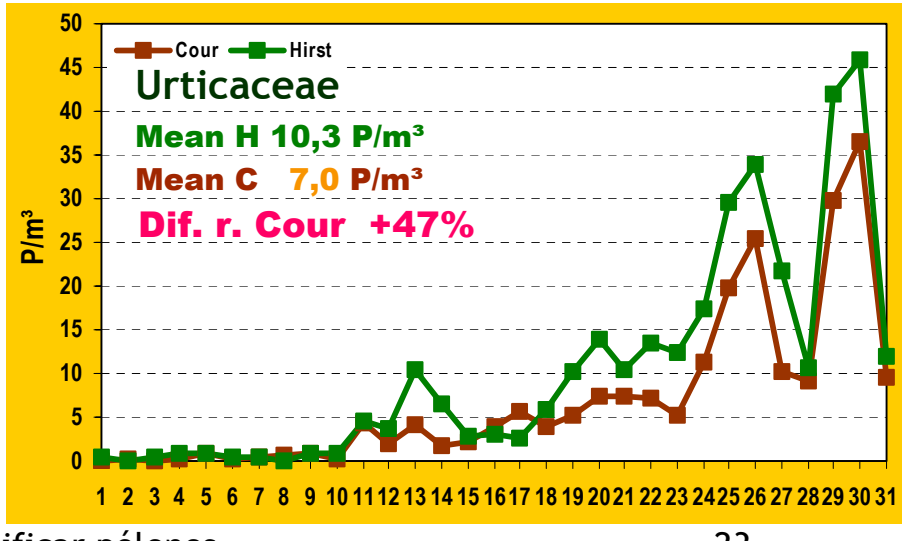
Farrera et al. 2003

Comparación de concentraciones polínicas
Series de datos Hirst/Cour – ejemplos
Eficiencia (resistencia filtro) 1/6 del recorrido de viento

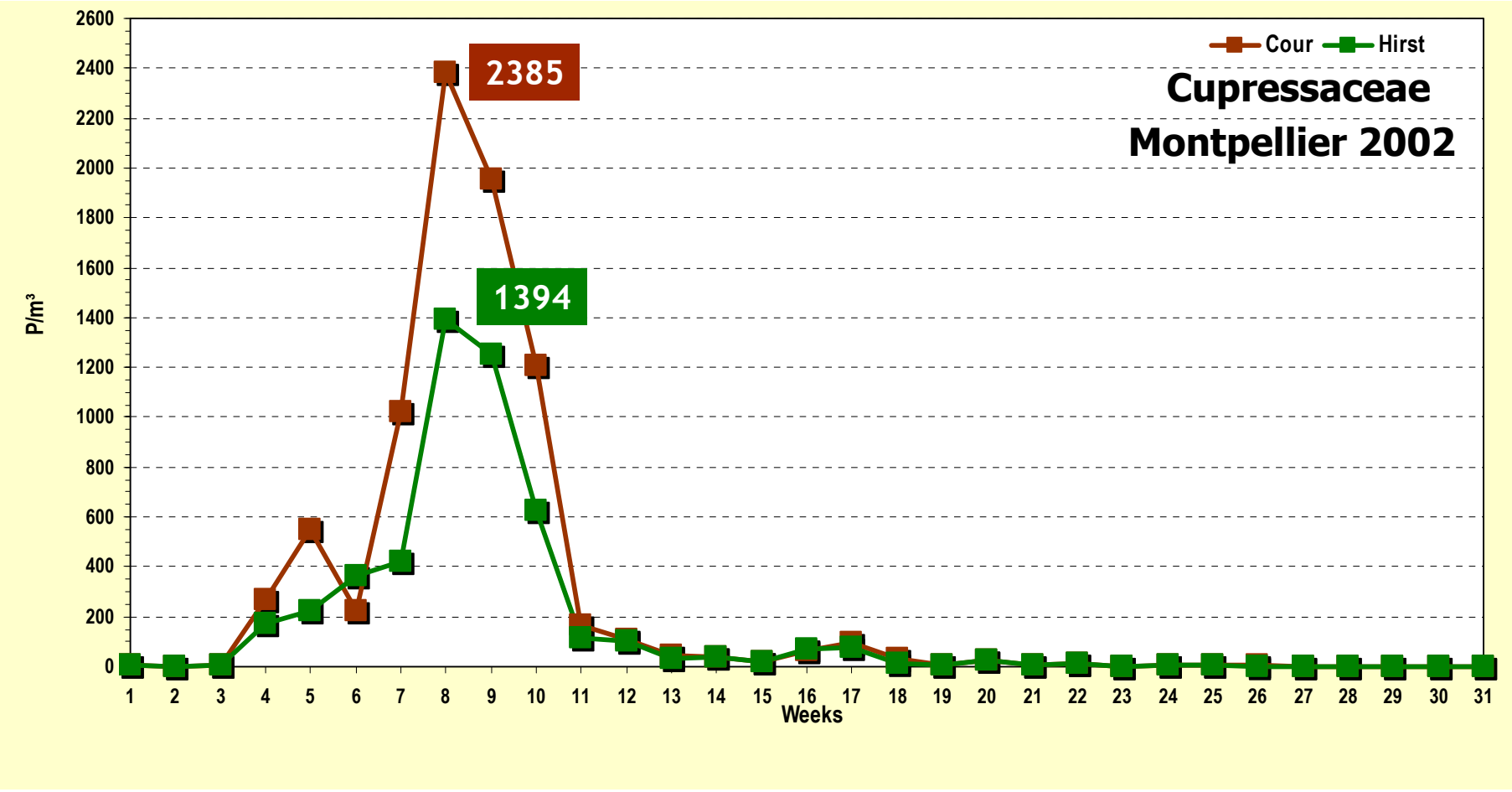






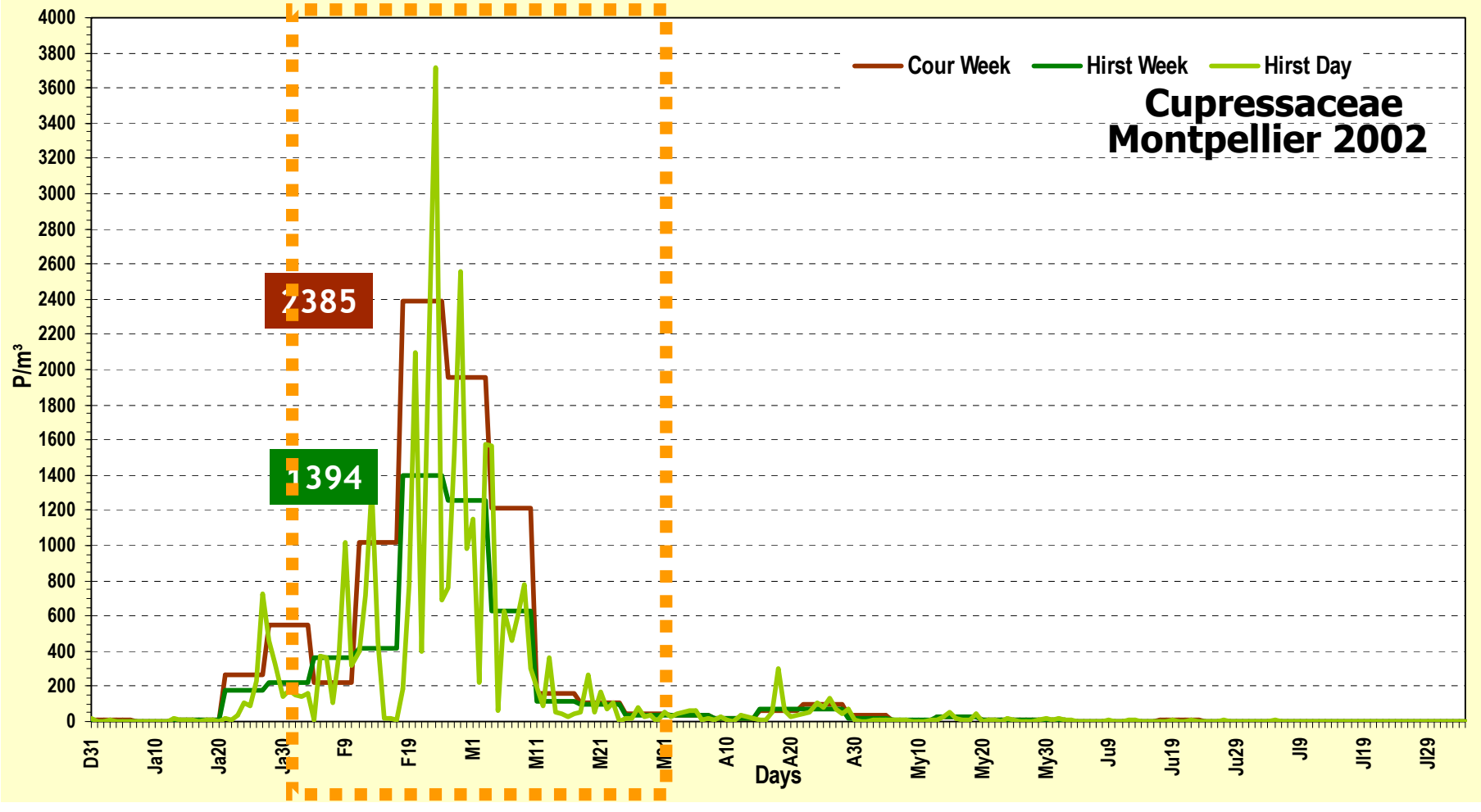


Series Hirst/Cour– otras consideraciones

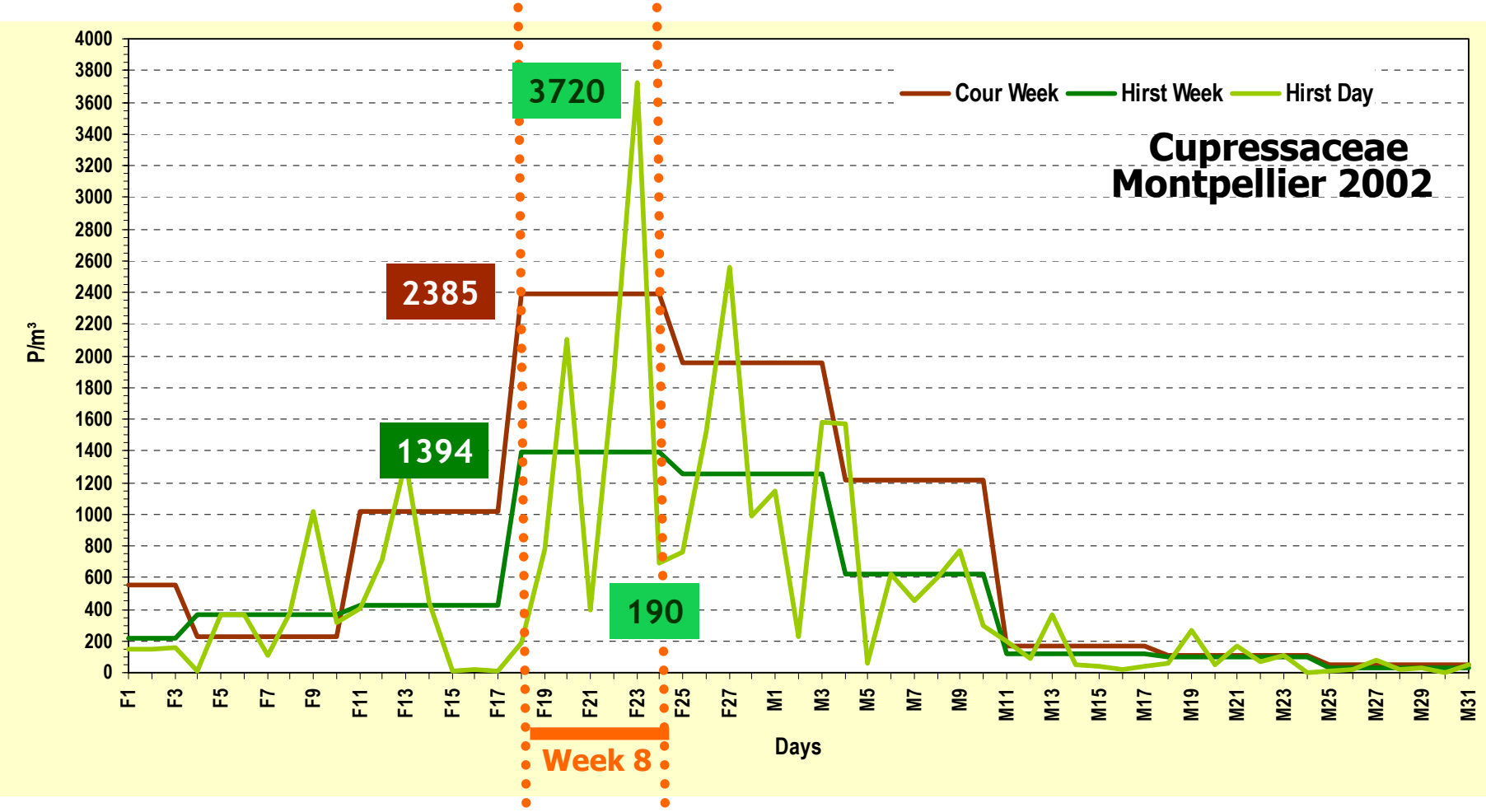


Farrera et al. 2003

Series Hirst/Cour– otras consideraciones



Series Hirst/Cour– otras consideraciones

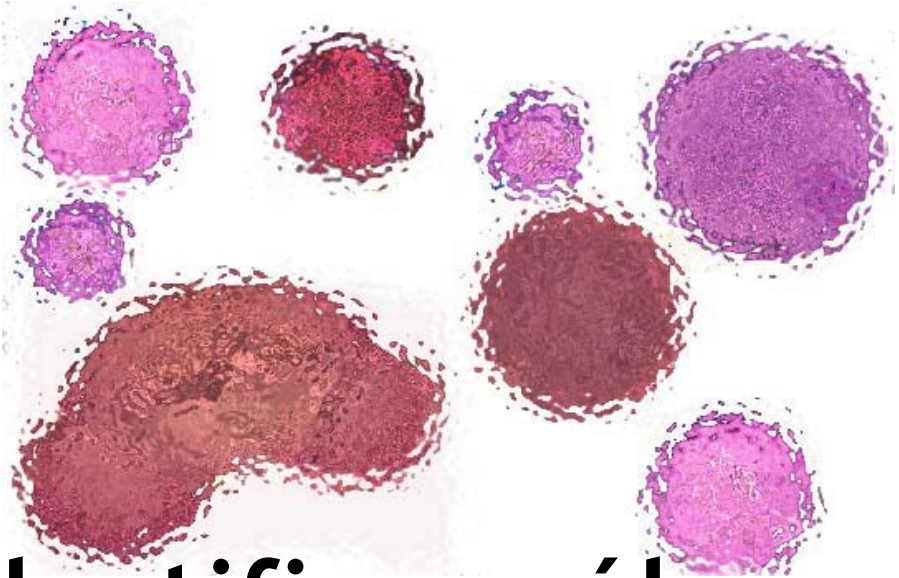


Conclusiones

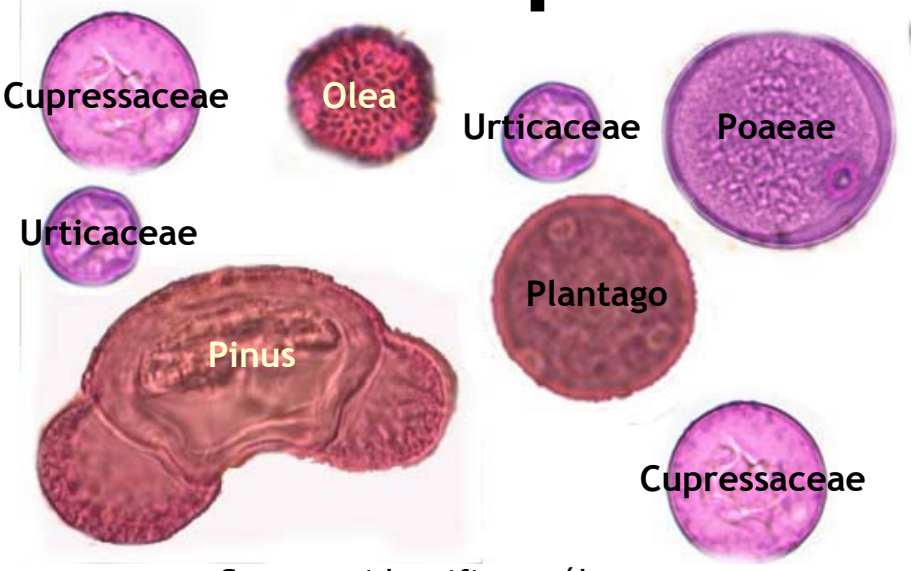
Se debe continuar investigando en aspectos metrológicos (por ejemplo por tipo polínico)

Los métodos Hirst y Cour han demostrado:
- **ser metrológicamente aceptables**
- **cuantificar el contenido polínico atmosférico de forma bastante parecida**

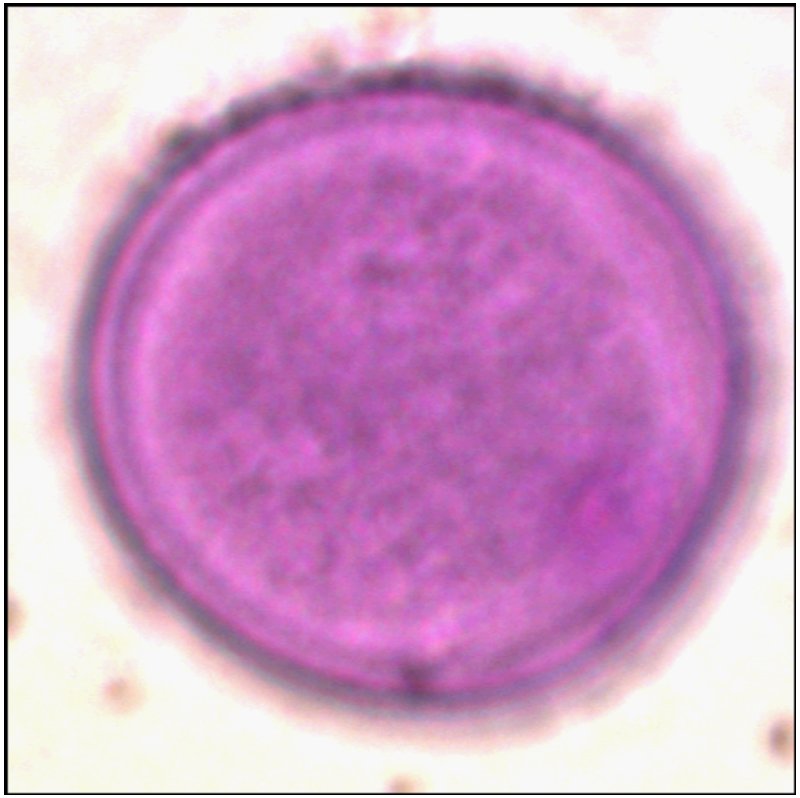
La elección del método de muestreo debe basarse en los objetivos que se persiguen.



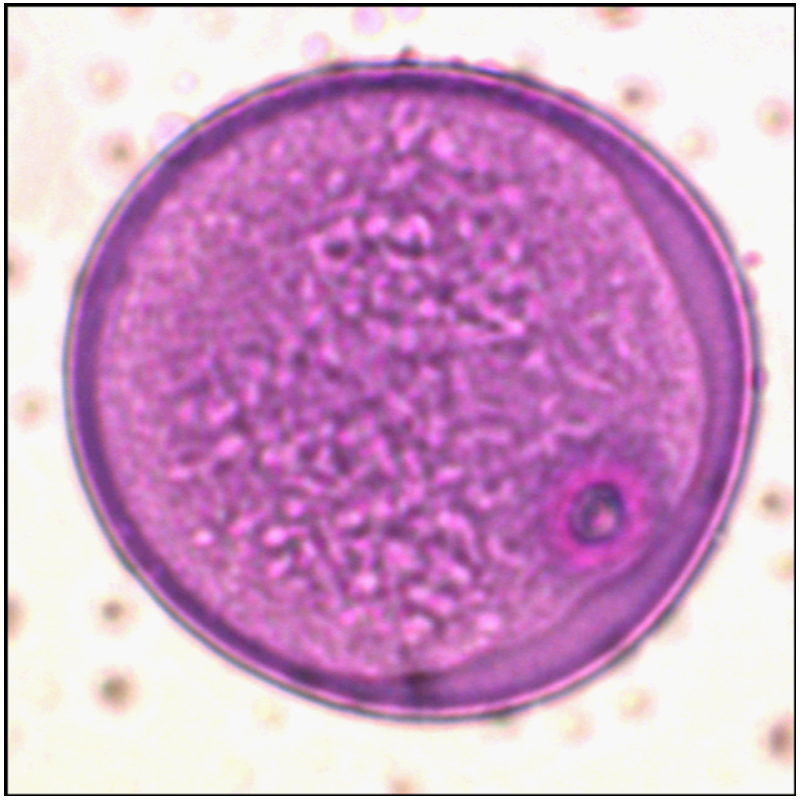
Identificar pólenes



El polen visto al microscopio óptico

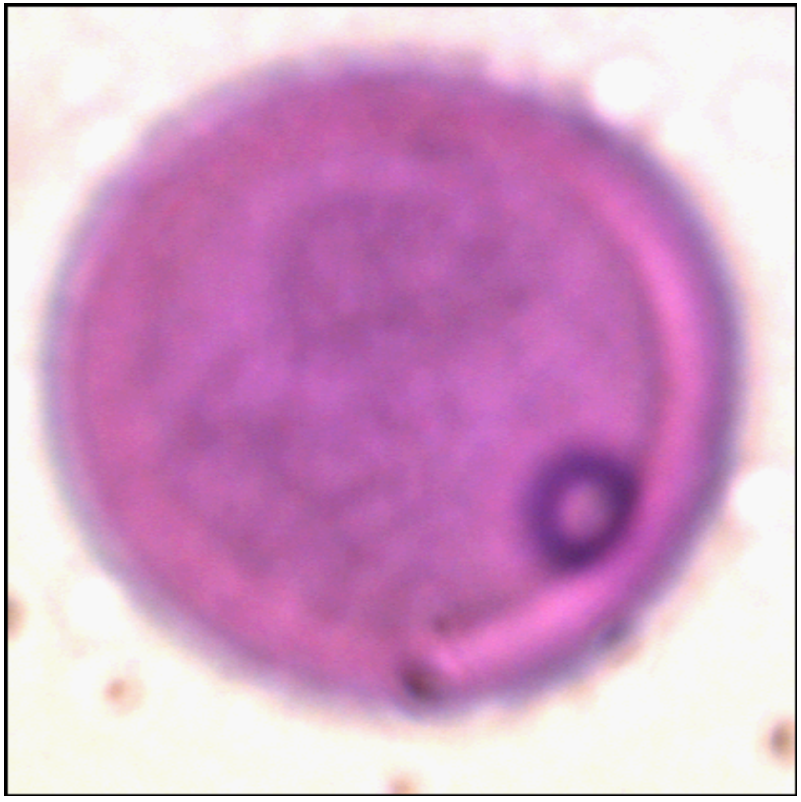


El polen visto al microscopio óptico

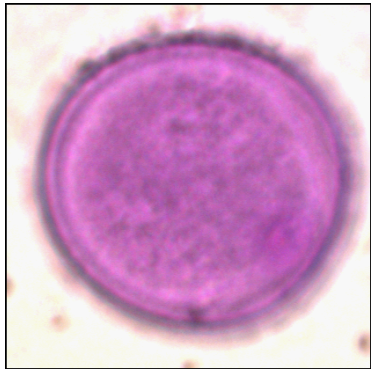
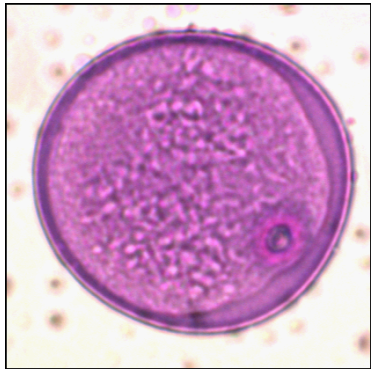


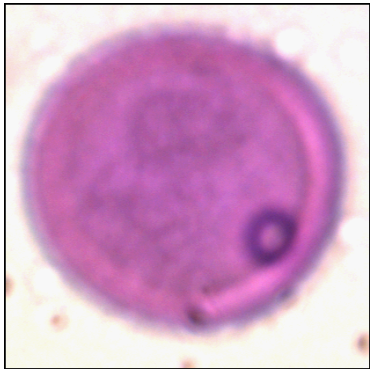
El polen visto al microscopio óptico

El polen visto al microscopio óptico



El polen visto al microscopio óptico

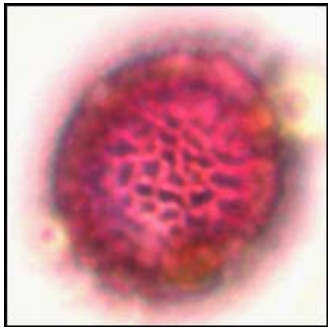
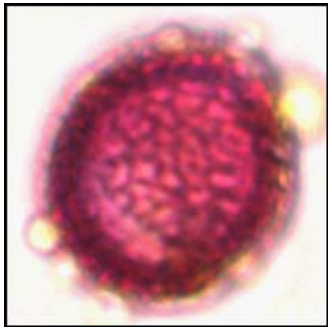
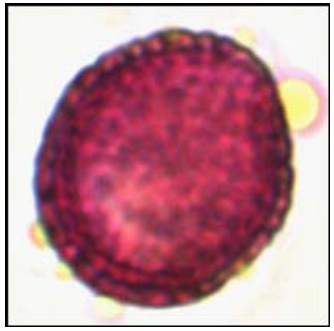
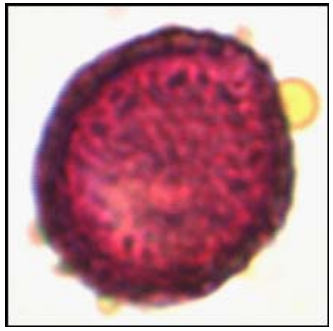
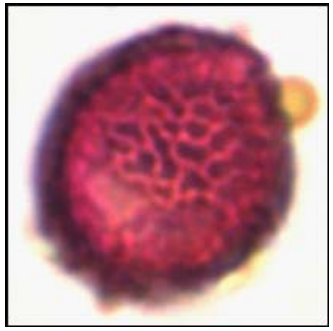





El polen visto al microscopio electrónico de barrido

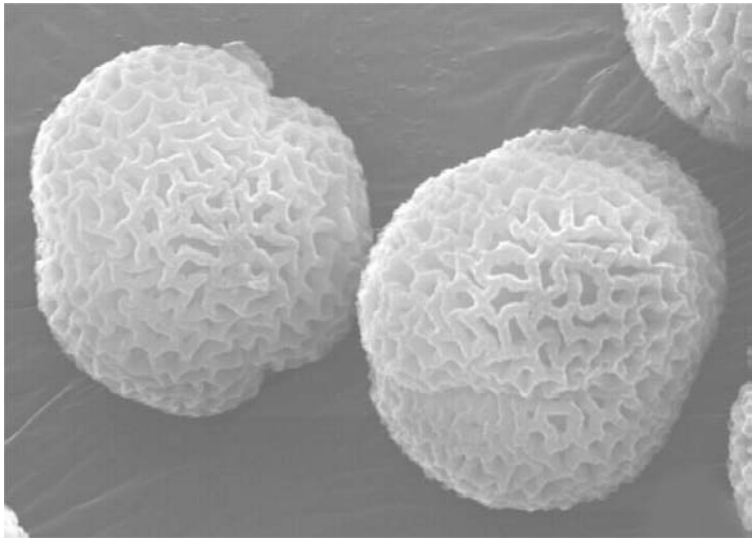
Polen de *Dactylis glomerata*
Poáceas o Gramíneas

El polen visto al microscopio óptico

Superficie Corte óptico Fondo

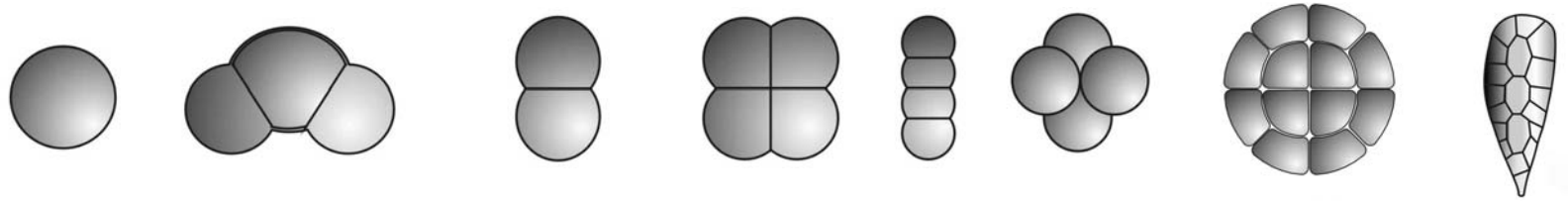
El polen visto al microscopio electrónico de barrido



Polen de *Olea europaea*
Oleáceas

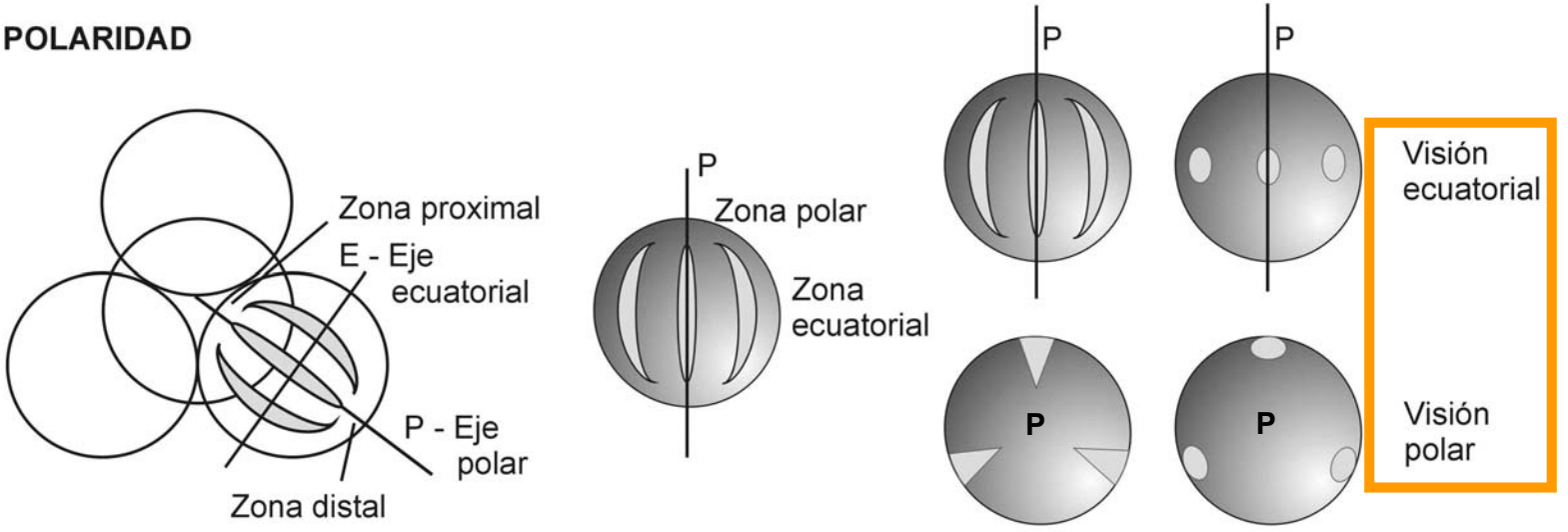
¿Como es el polen?

Forma del grano de polen

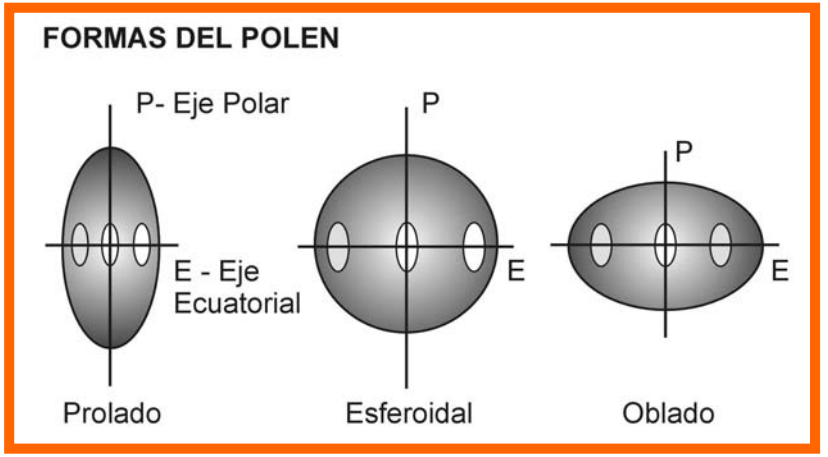


simple vesiculado díada tétrada políadas

Polaridad del grano de polen



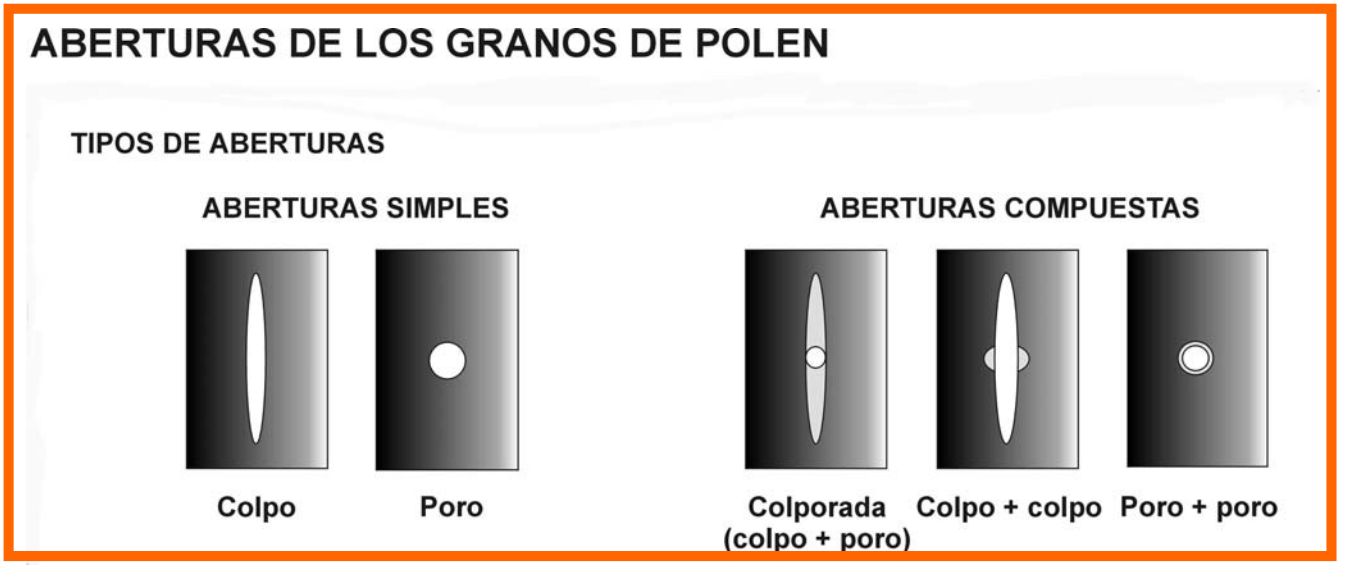
¿Como es el polen?



TAMAÑO DEL POLEN

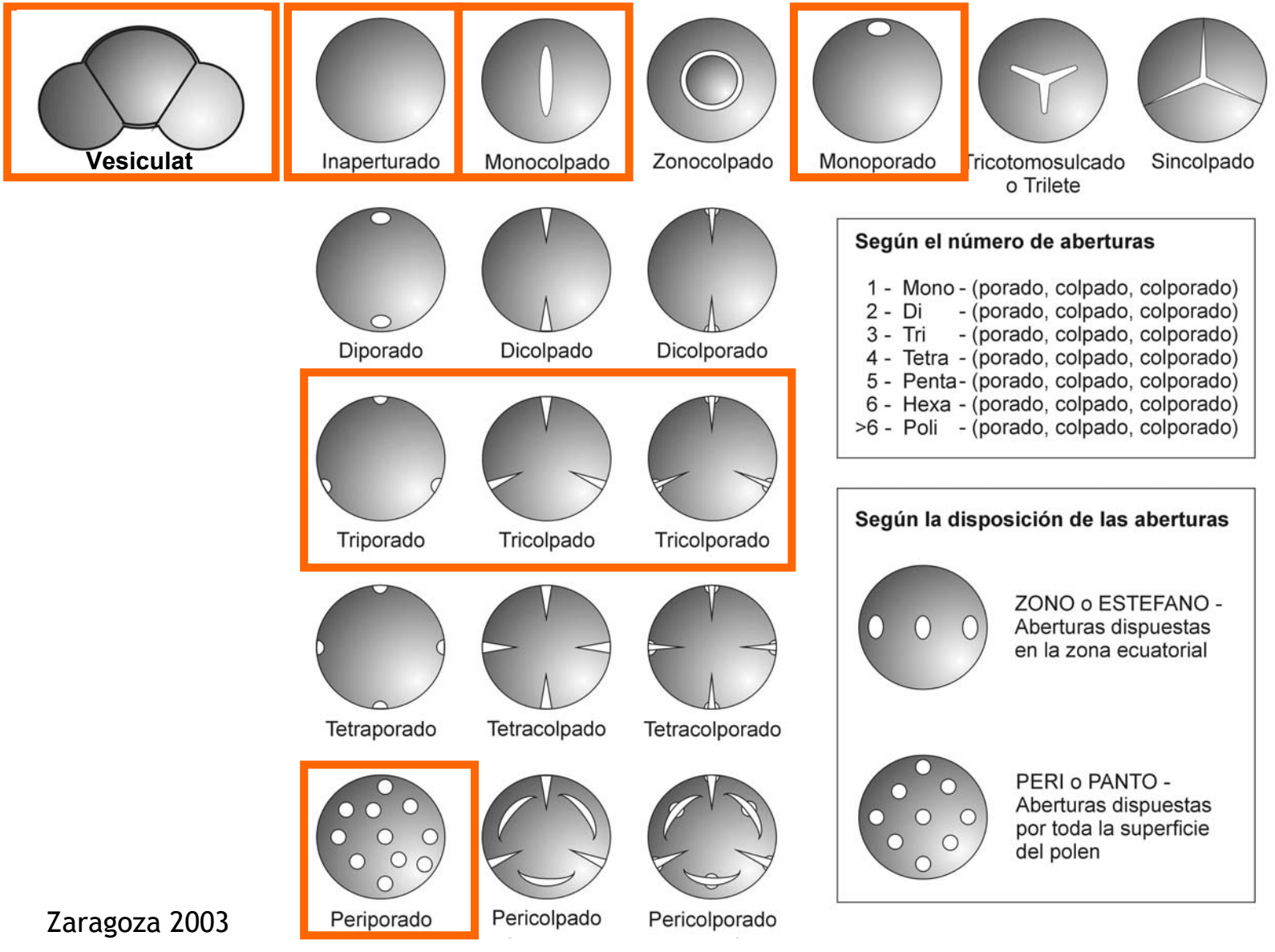
En función de la longitud del eje más largo:

< 10 µm	-	Muy pequeño
10 - 25 µm	-	Pequeño
25 - 50 µm	-	Mediano
50 - 100 µm	-	Grande
100 - 200 µm	-	Muy grande
> 200 µm	-	Gigante



Belmonte y Roure (2002) Menarini

TIPOS POLÍNICOS SEGÚN EL NÚMERO Y DISPOSICIÓN DE LAS ABERTURAS



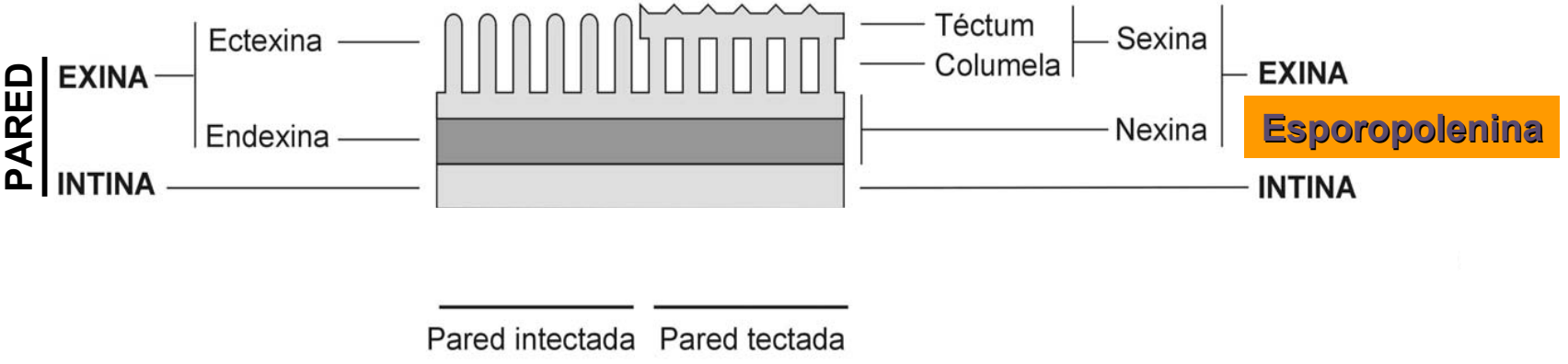
Zaragoza 2003

Belmonte y Roure (2002) Menarini

Una característica del polen: la pared

LA PARED DEL GRANO DE POLEN

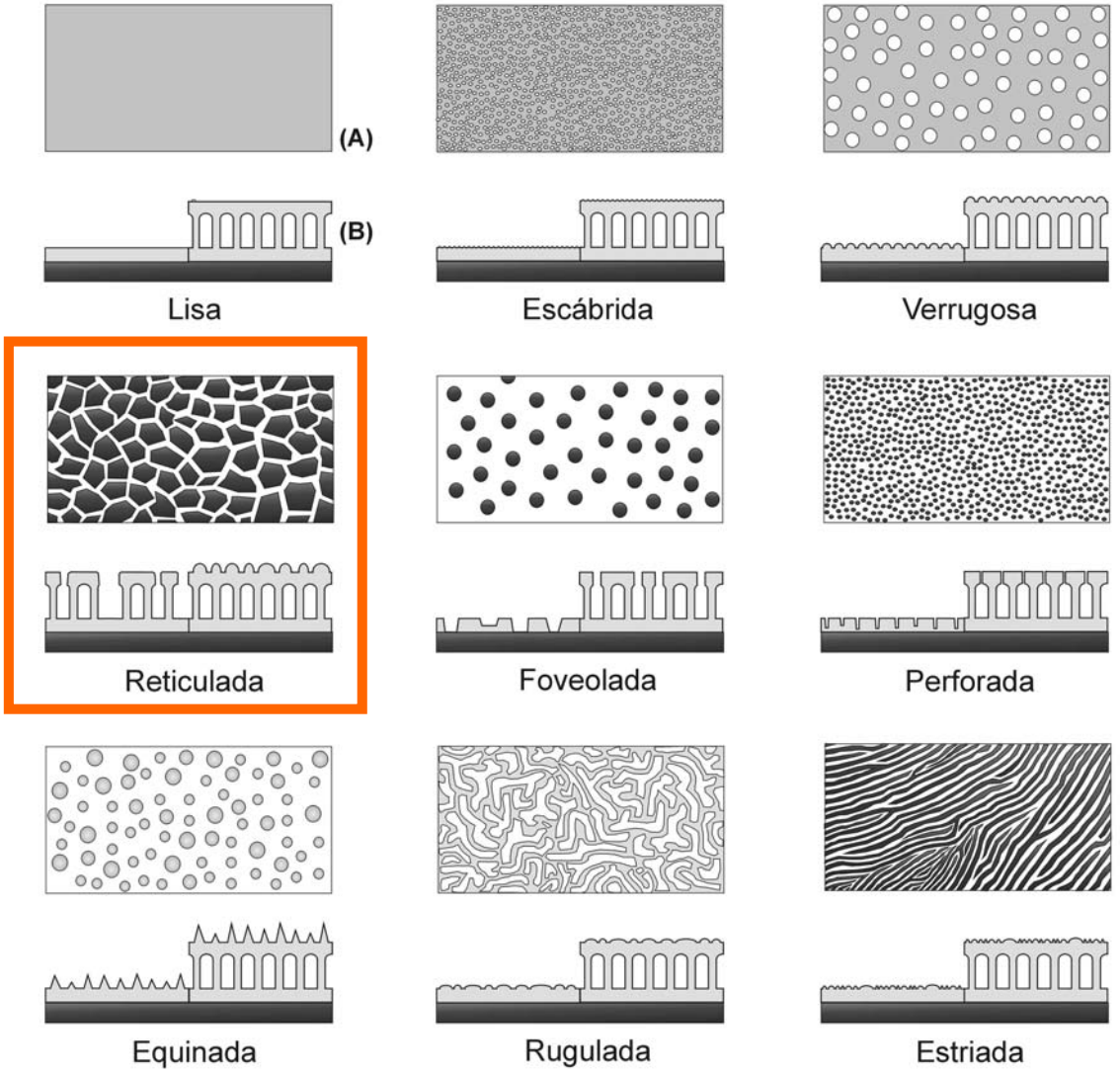
ESTRUCTURA DE LA ESPORODERMIS (PARED POLÍNICA)



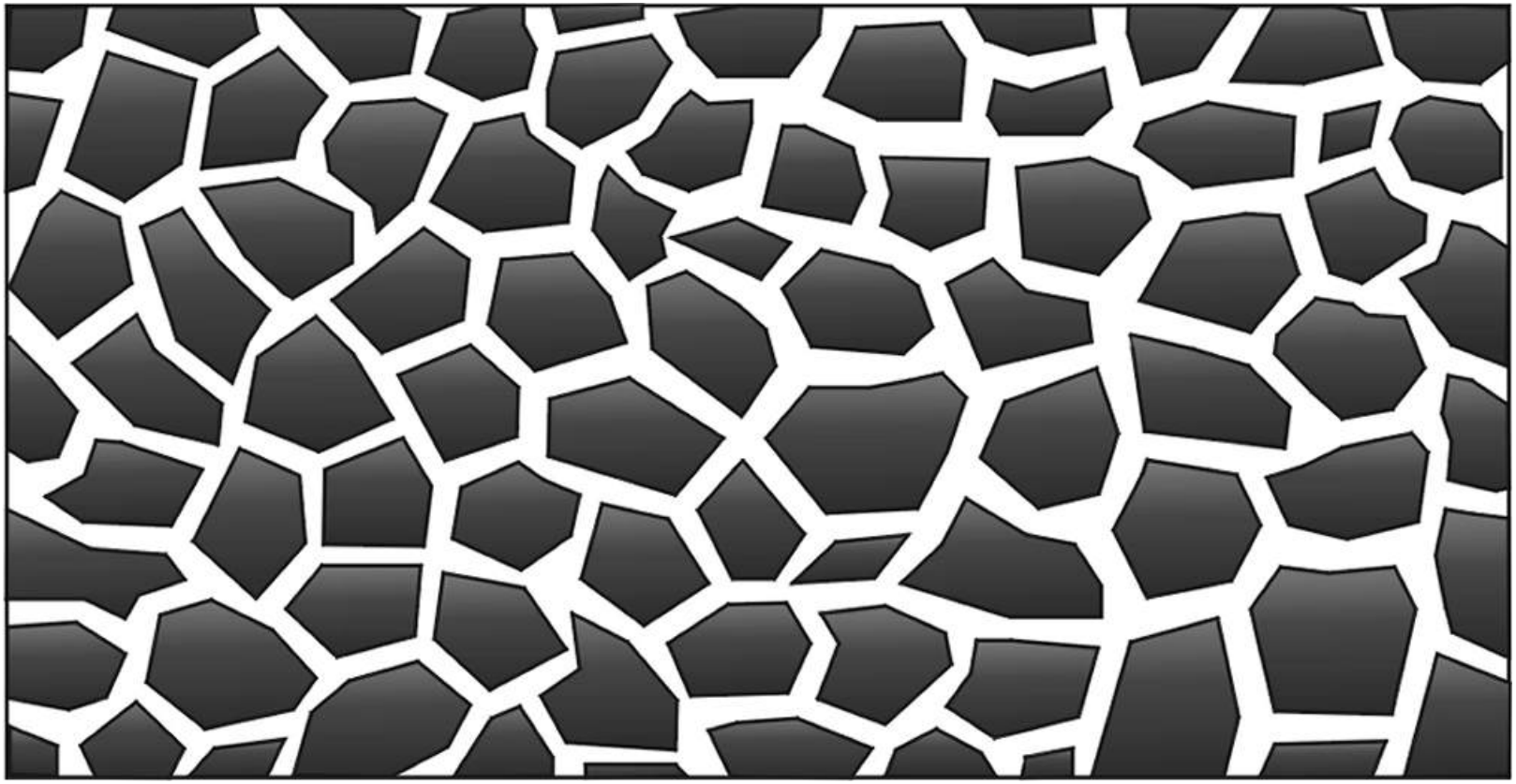
Germinación Fosilización Acetolisis

Belmonte y Roure (2002) Menarini

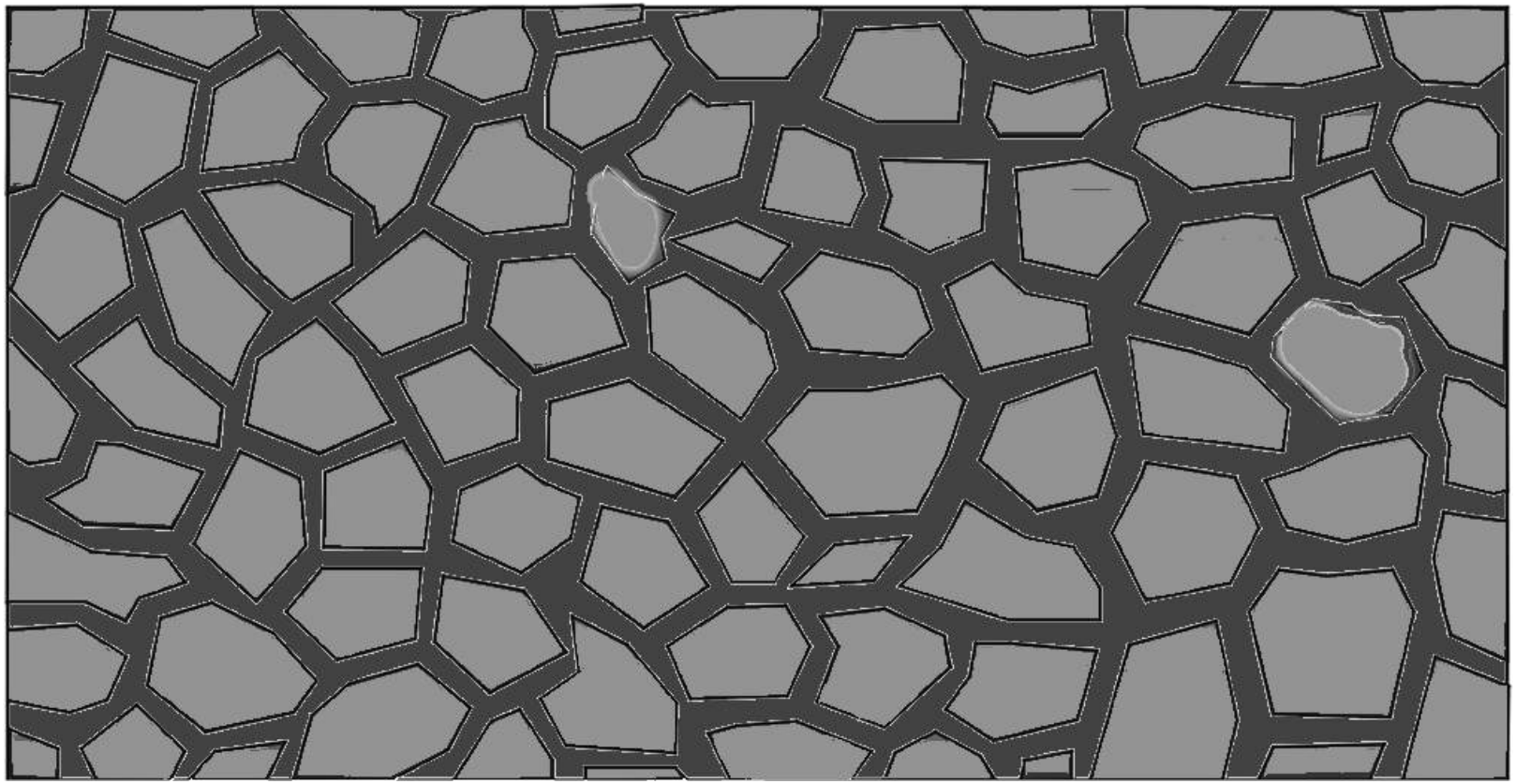
ORNAMENTACIÓN DE LA EXINA.
(A) VISIÓN DE LA SUPERFICIE. (B) SECCIÓN TRANSVERSAL



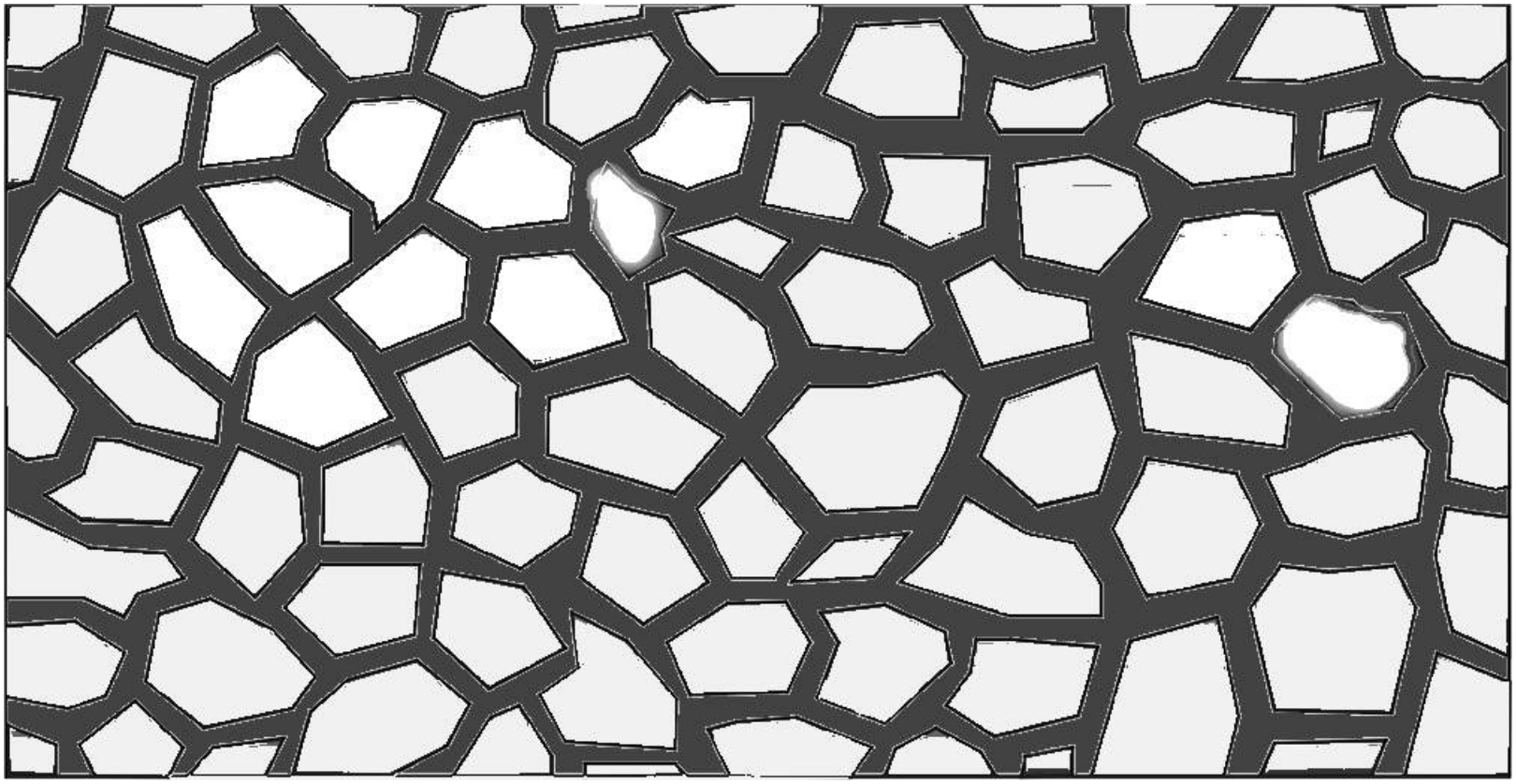
Interpretar una ornamentación reticulada



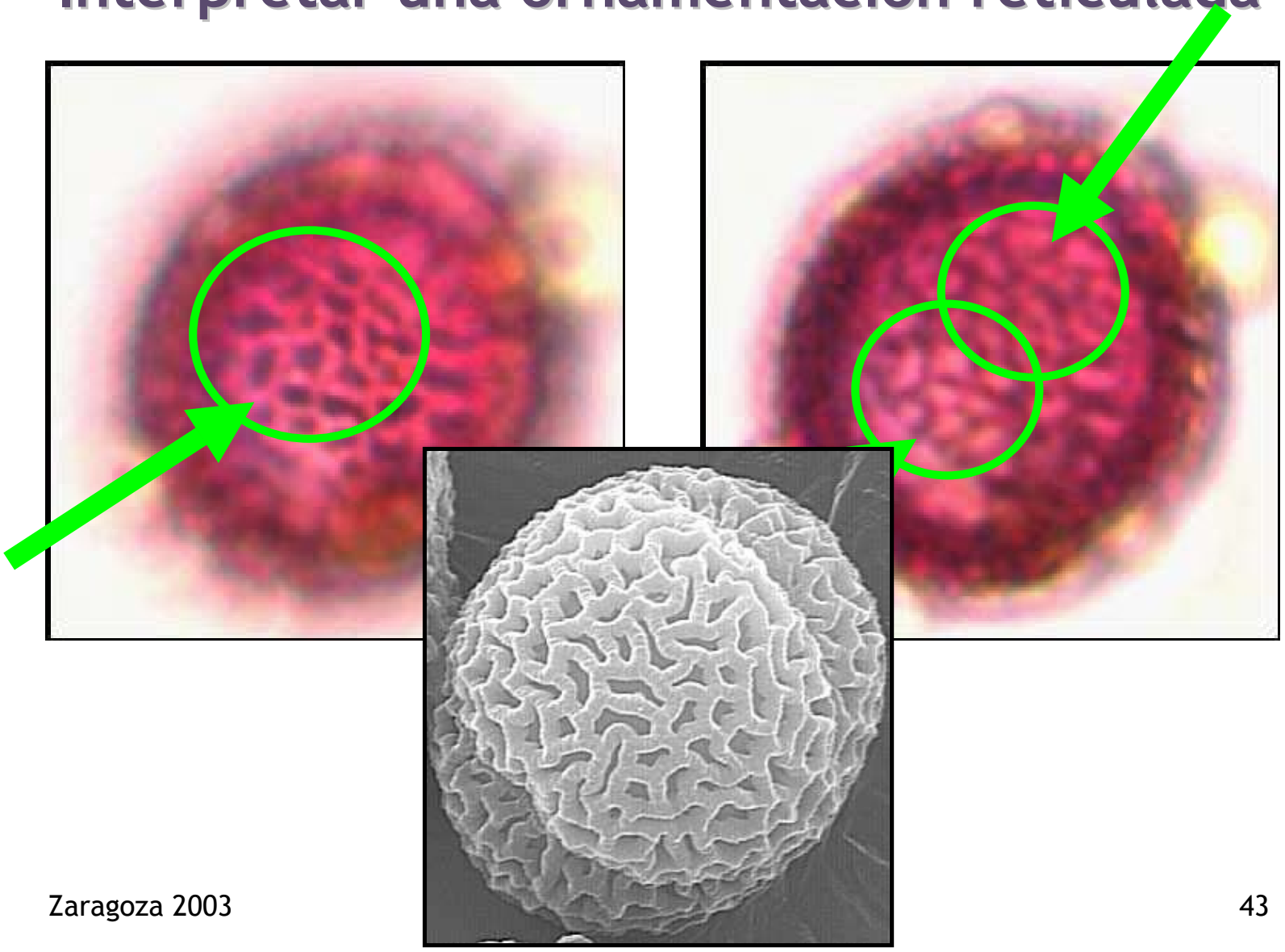
Interpretar una ornamentación reticulada



Interpretar una ornamentación reticulada



Interpretar una ornamentación reticulada



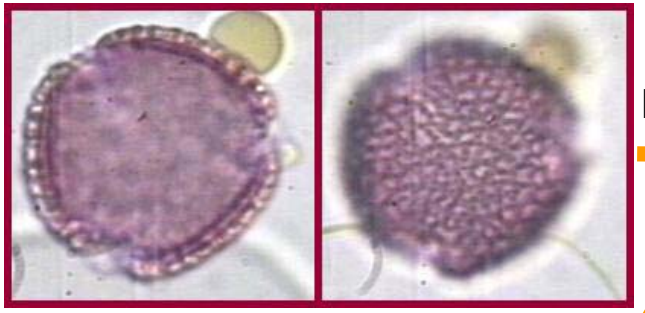
Conclusiones sobre la morfología del polen

Se puede identificar la procedencia floral de los granos de polen, gracias a su combinación de características morfológicas.

La precisión de la identificación varía:

- nivel específico	*Plantago lanceolata*
- nivel grupo especies	*Quercus* tipo perennifolio
- nivel género	*Salix*
- nivel família	Poáceas o Gramíneas
- nivel grupo famílias	Quenopodiáceas/Amarantáceas

¿Es fácil identificar el polen?

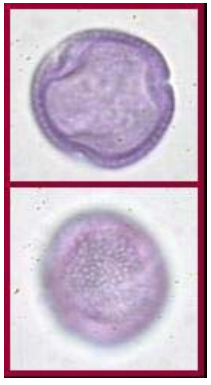


Olea

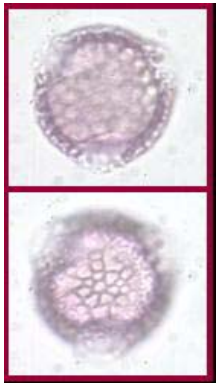
Descripción

tricolporado (tricolporoidado)
Exina de 2-3.5 µm
Tamaño medio
Ornamentación reticulada (1-2 µm)
Periodo de floración Mayo-Junio

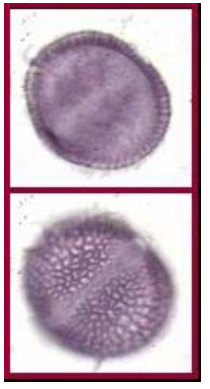
Posible confusión con



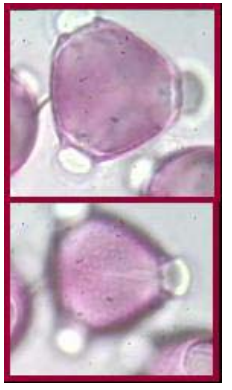
Fraxinus



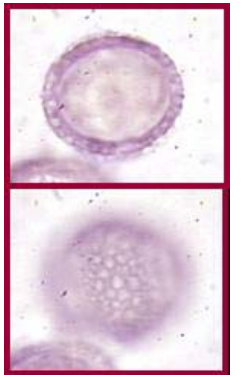
Ligustrum



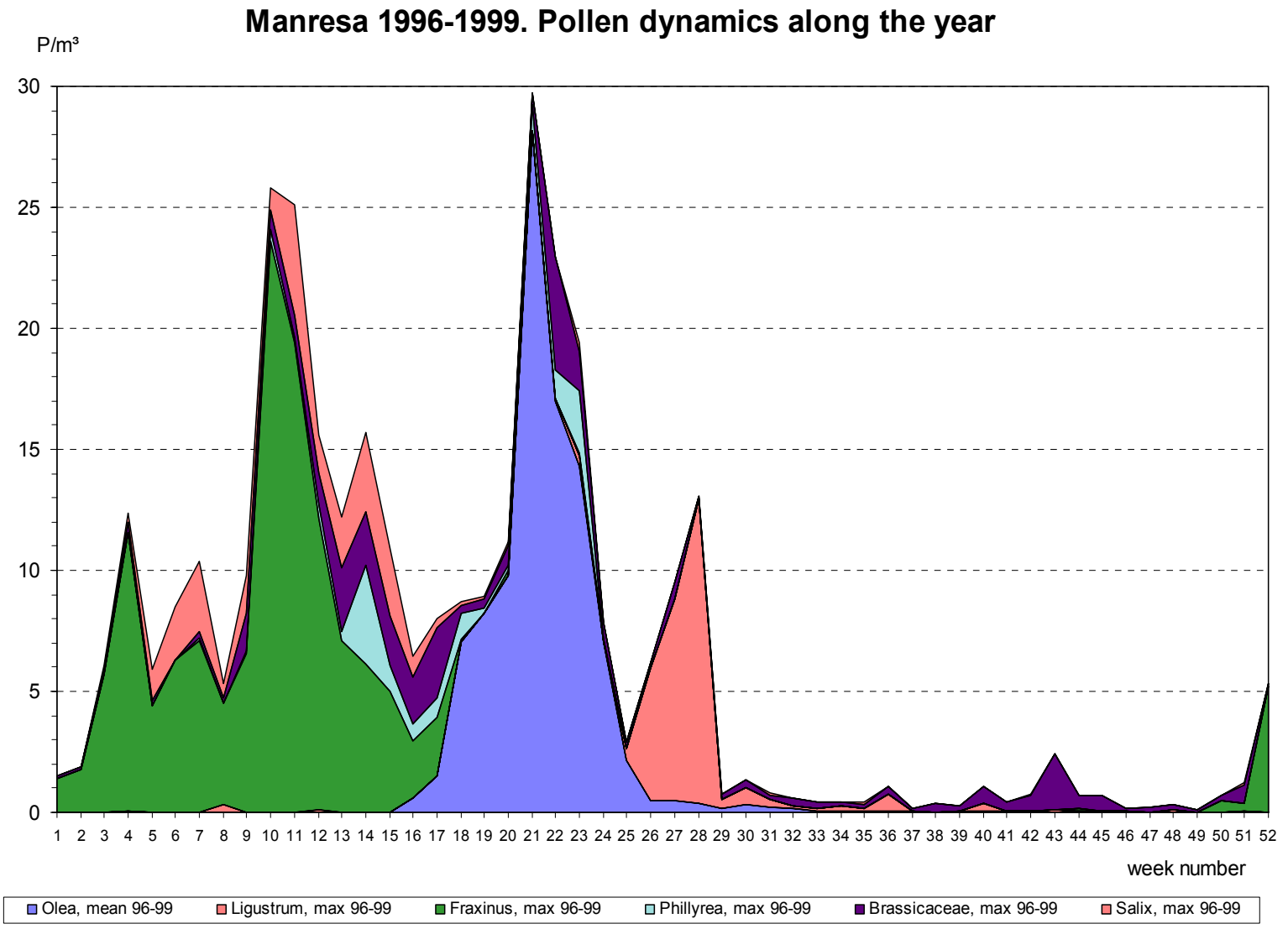
Brassicaceae



Phillyrea



Salix

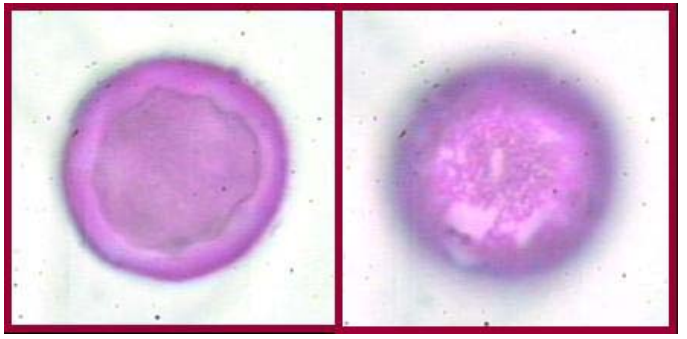

Manresa 1996-1999. Pollen dynamics along the year
P/m³
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
week number
Olea, mean 96-99
Ligustrum, max 96-99
Fraxinus, max 96-99
Phillyrea, max 96-99
Brassicaceae, max 96-99
Salix, max 96-99

Cupressus (Cupresáceas)

Descripción

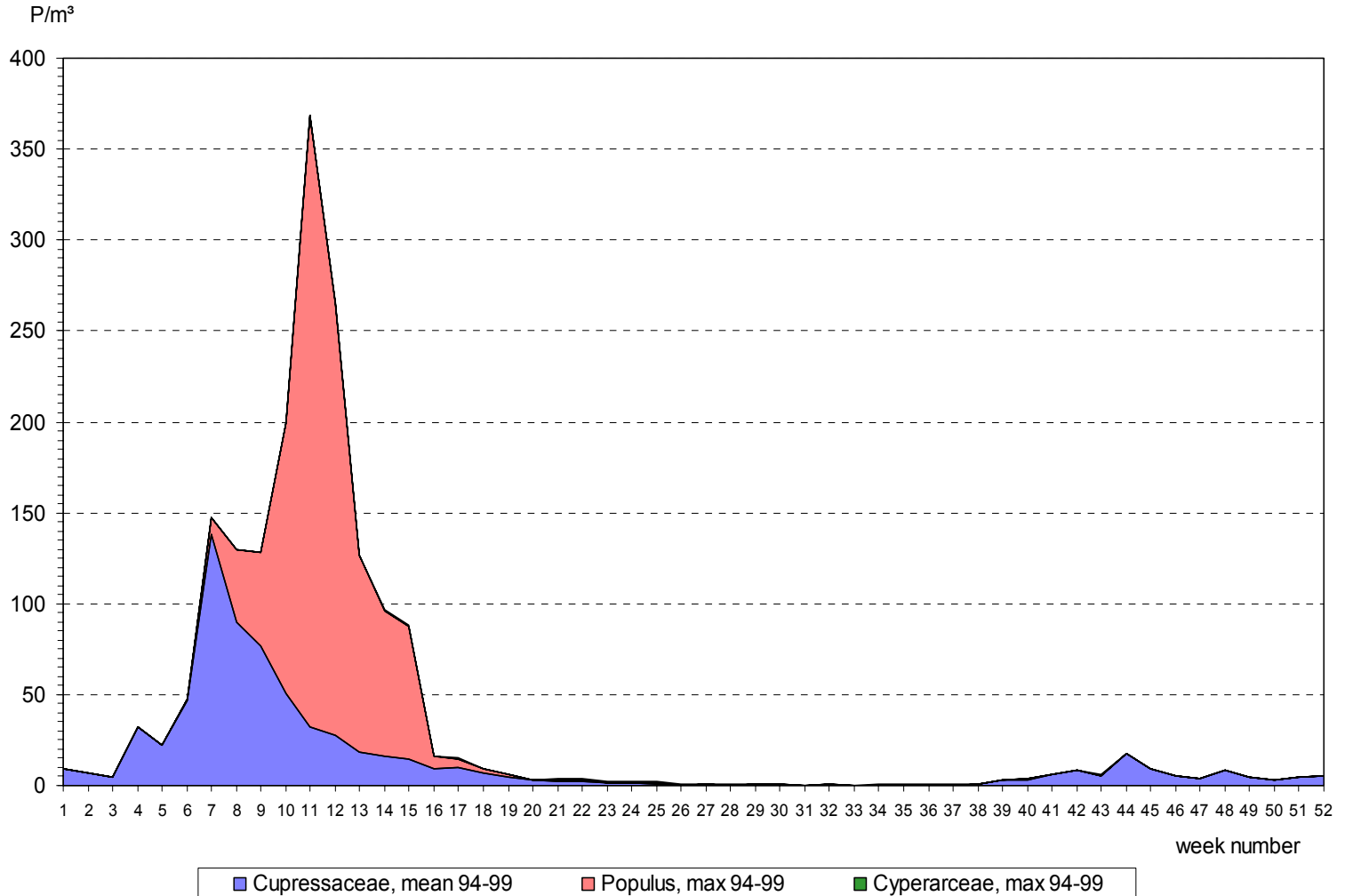
Inaperturado
Exina de 1 µm
Tamaño pequeño a medio
Ornamentación psilada con gránulos
Floración año entero excepto verano

Posible confusión con

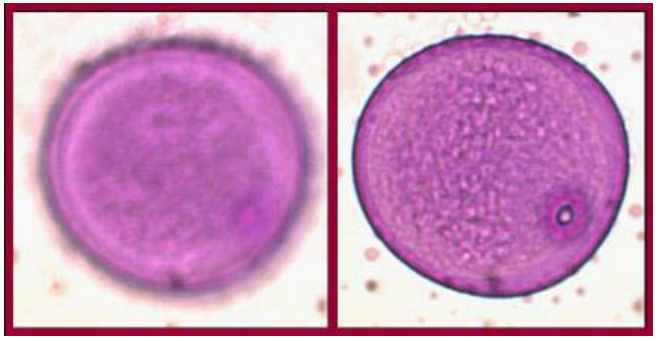


Populus

Bellaterra 1994-1999. Pollen dynamics along the year



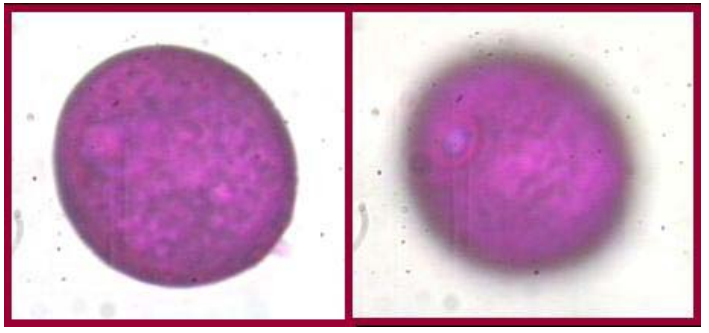
Poáceas o Gramíneas



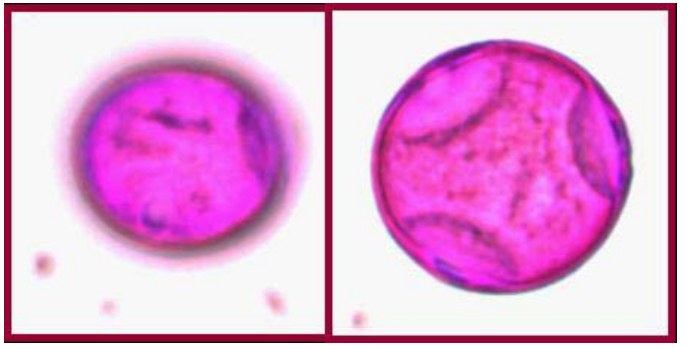
Descripción

Monoaperturado
Exina de 1-1.5 µm.
Tamaño pequeño a grande
Ornamentación granulada
Floración año (primavera-verano)

Posible confusión con

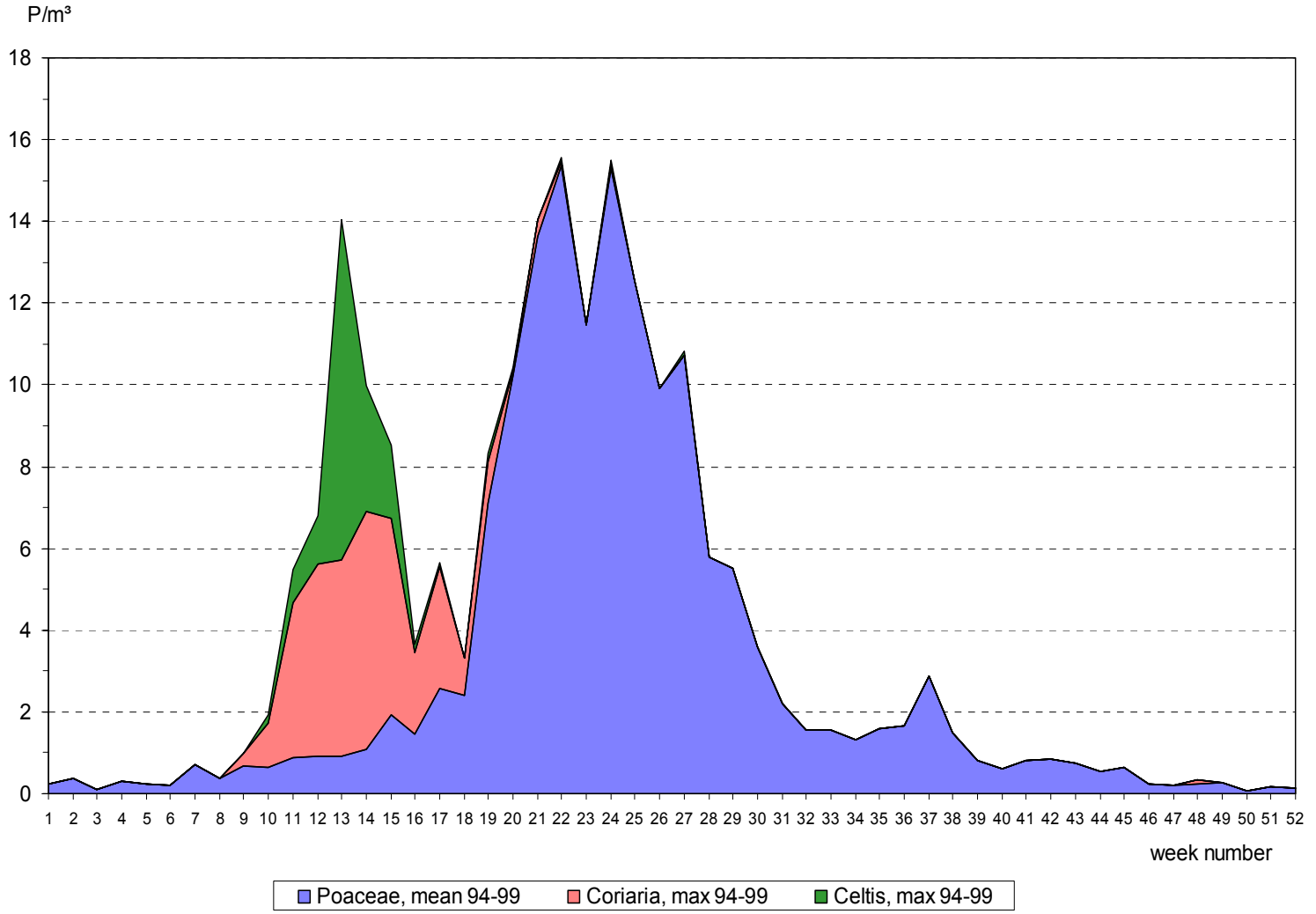


Celtis



Coriaria

Barcelona 1994-1999. Pollen dynamics along the year



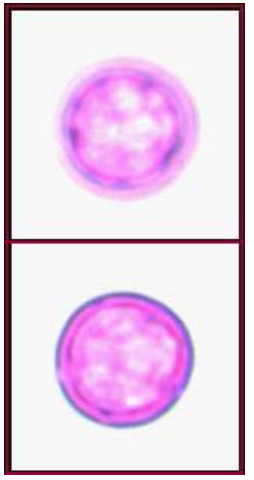
Parietaria (Urticáceas)

Descripción

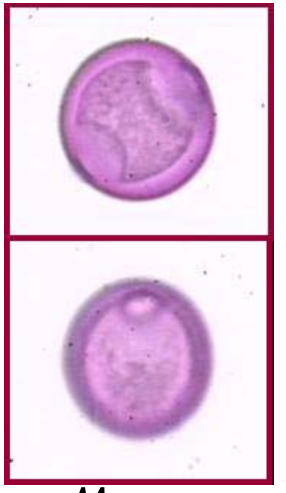
3-zonoporado
Exina de <1 µm
Tamaño pequeño
Ornamentación Psilada
Floración todo el año (Febrero-Junio)

Posible confusión con

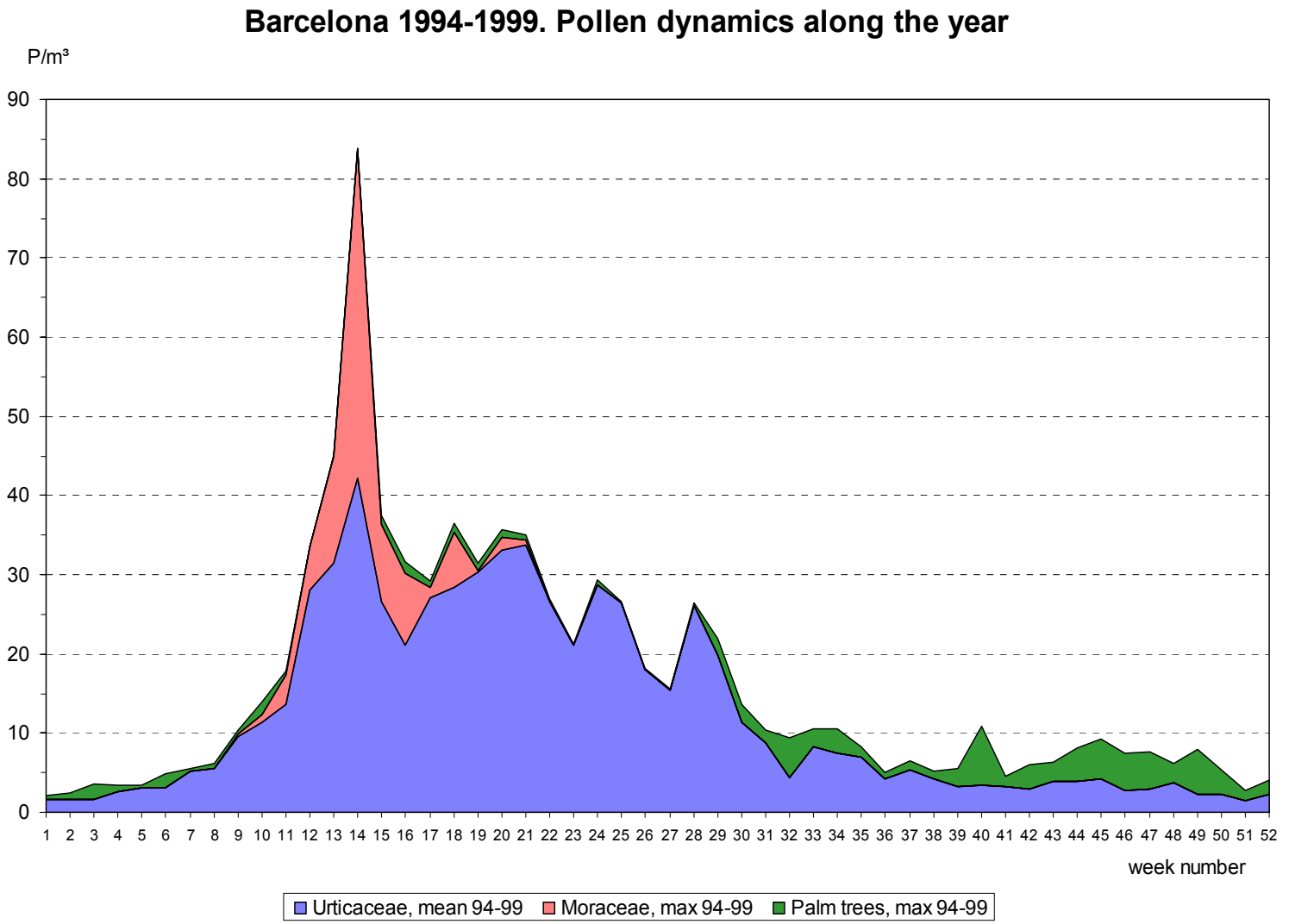
Broussonetia



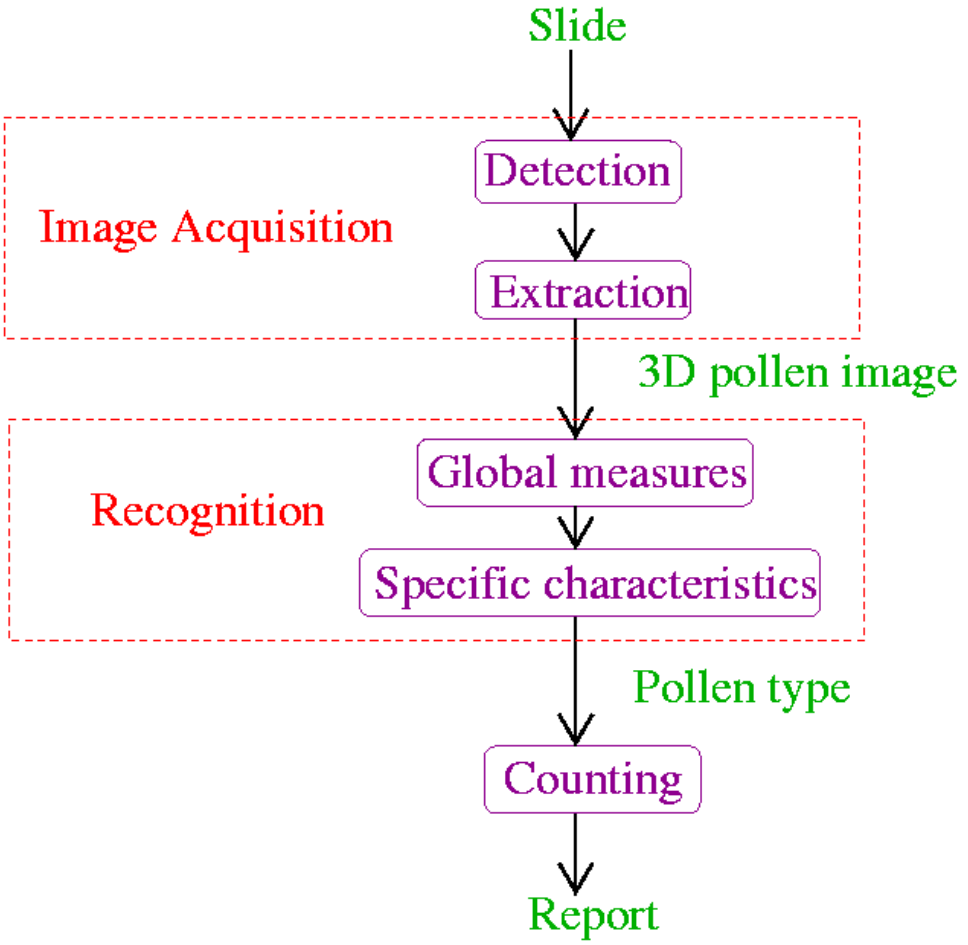
Urtica membranacea



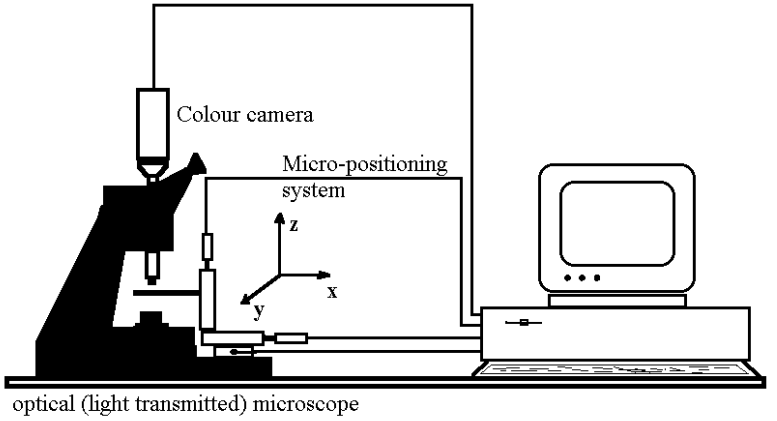
Morus


Barcelona 1994-1999. Pollen dynamics along the year
P/m³
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
week number
Urticaceae, mean 94-99
Moraceae, max 94-99
Palm trees, max 94-99

System Architecture

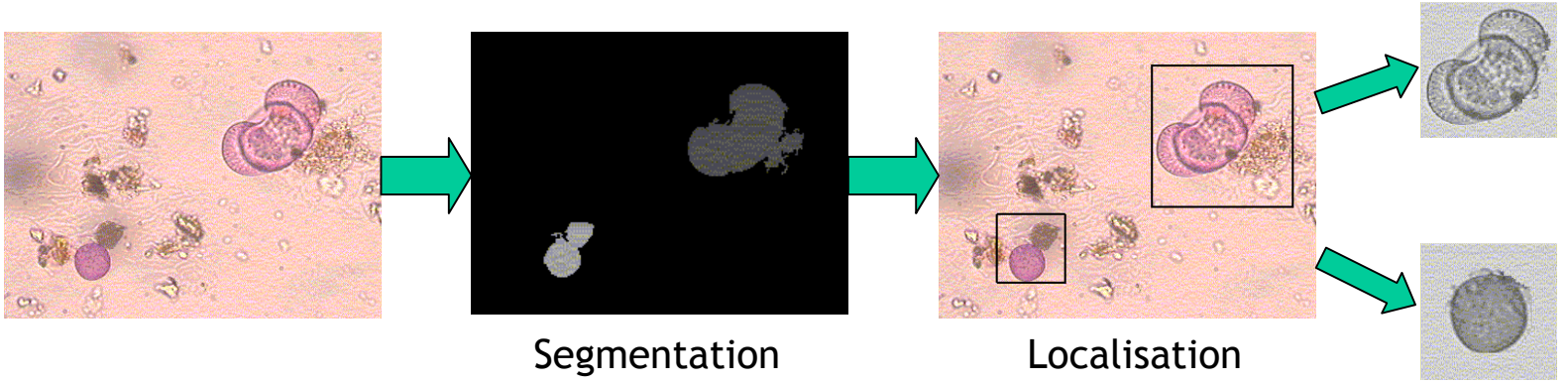


System Hardware



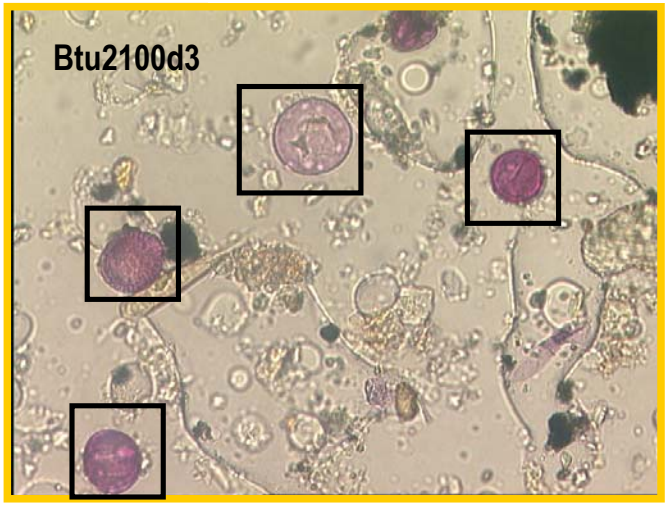
A light microscope driven automatically by a computer

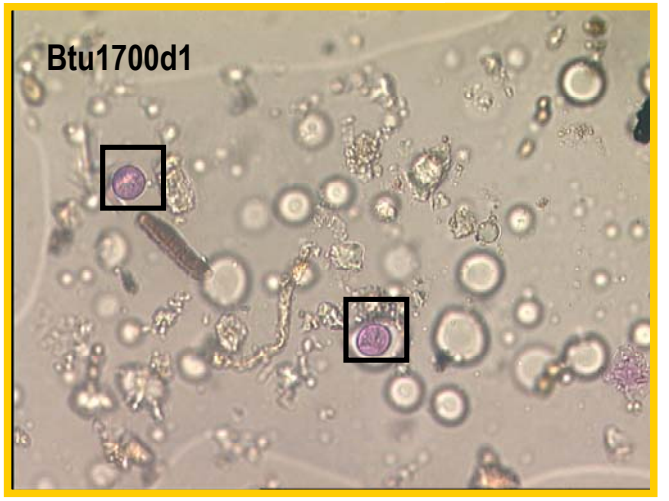
Pollen Grain Detection and Localisation

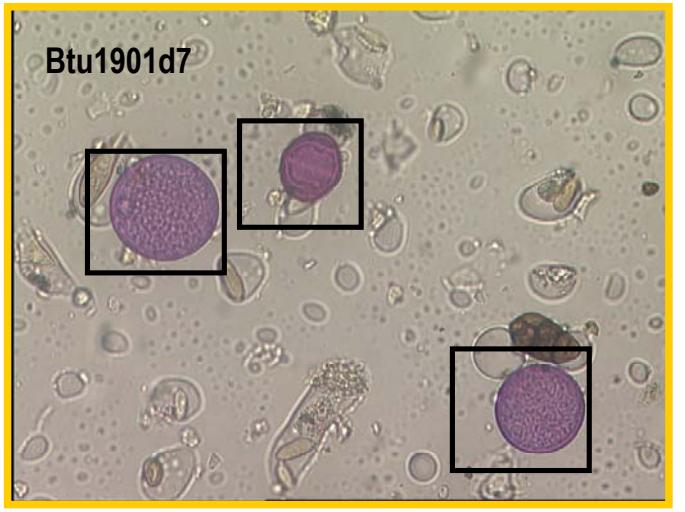


Automatic pollen grain detection

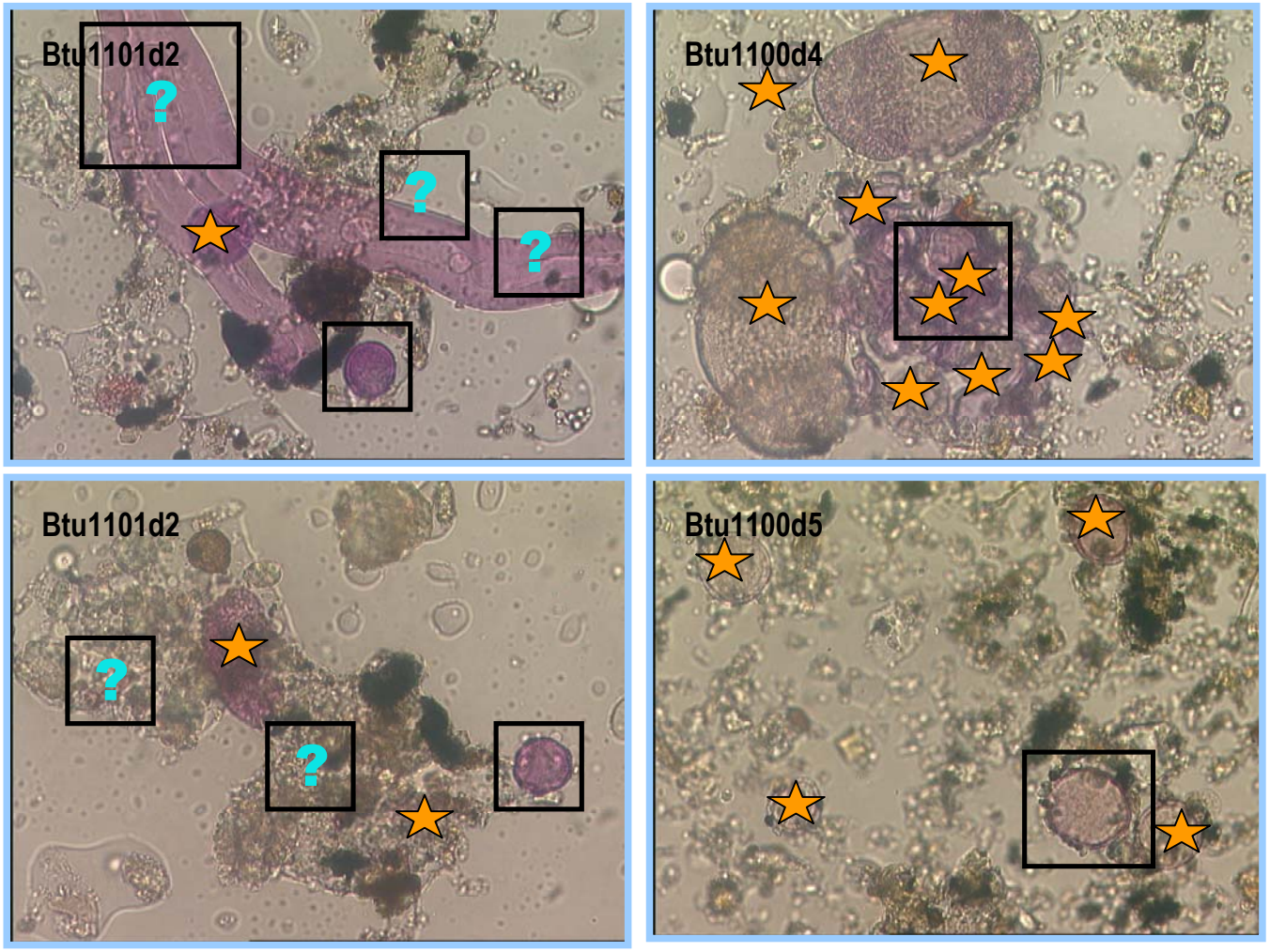
Validation of the LASMEA image acquisition module: Aerobiological slides - "Good slides"







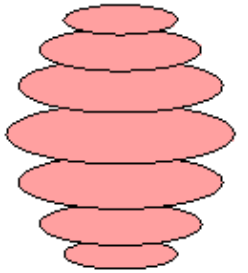
Validation of the LASMEA image acquisition module: Aerobiological slides - “Bad slides”

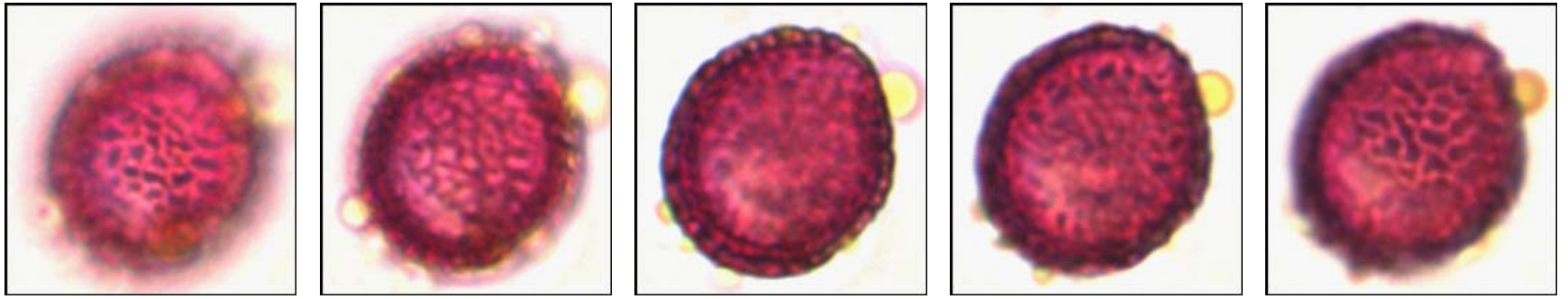


3D pollen grain digitisation

- 3D acquisition of pollen grains
  - set of images at different depths

For each grain
- *100 optical sections*
- *step = 0.5 microns*

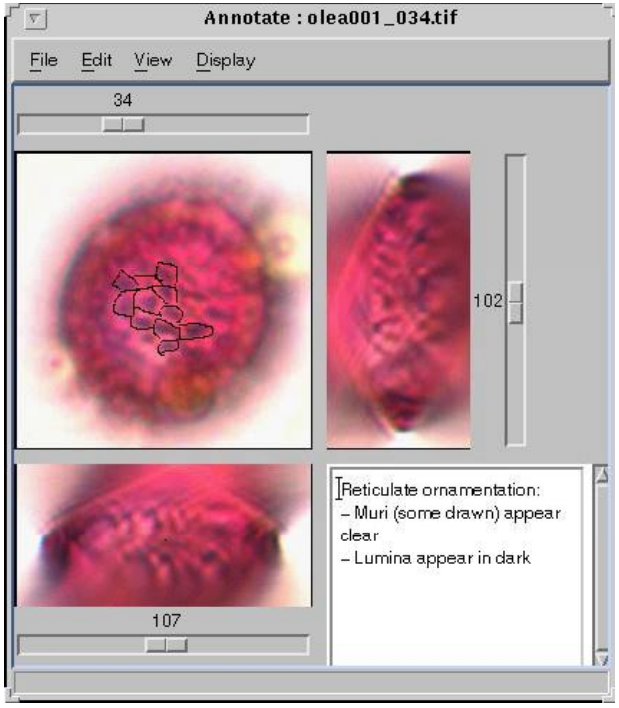


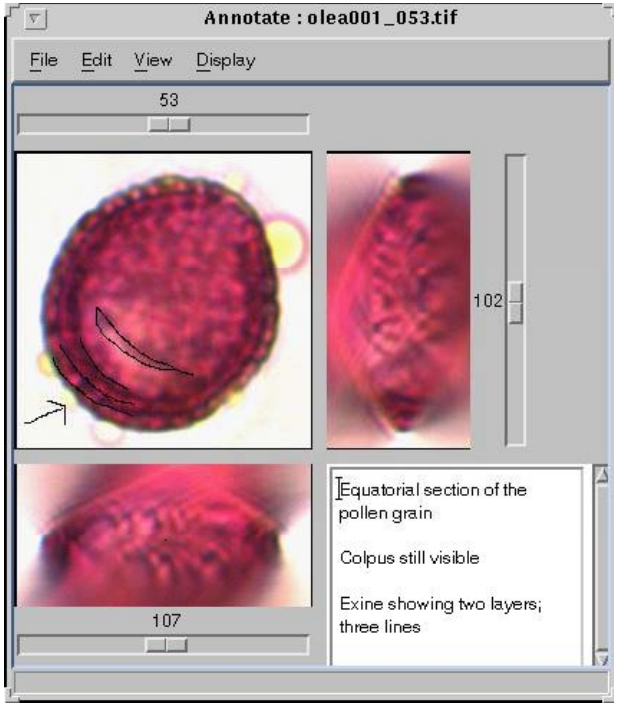


Features may appear on different heights

Palynological knowledge

- The system tries to mimic the palynologists
- Knowledge is necessary to identify pollen grains

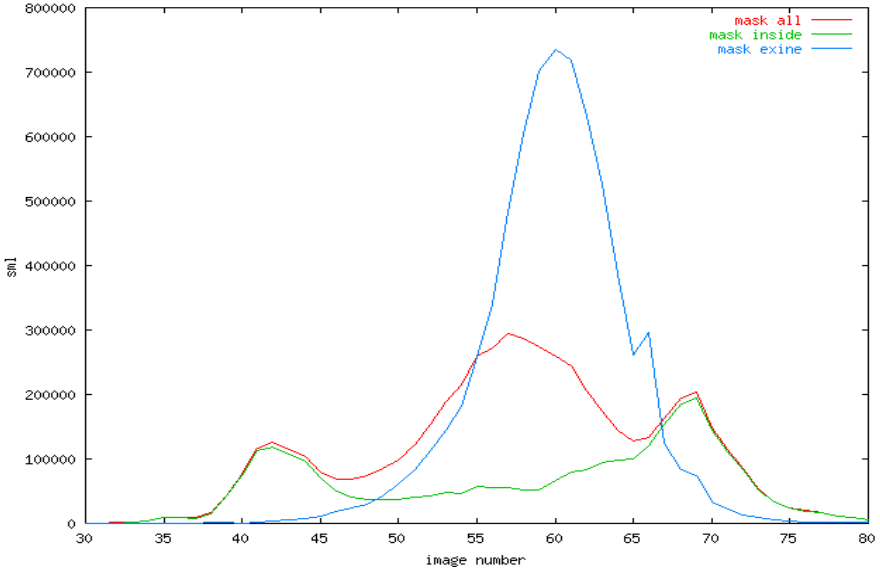


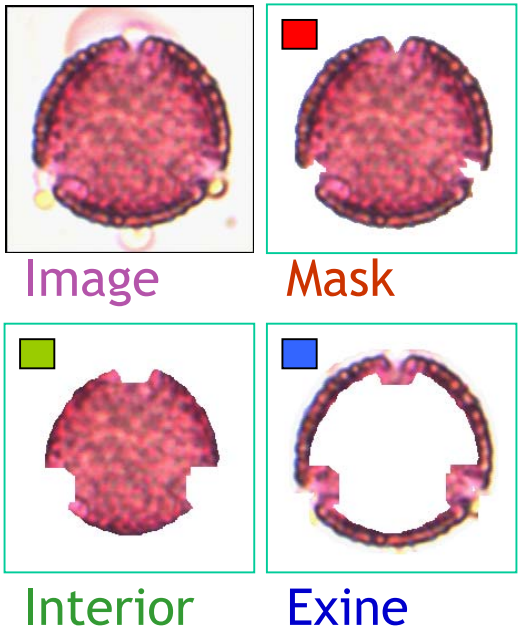


Search in a blurred image sequence

- 3D search in optical slices *(key images - less blurred)*
- 2D search in possible zones *(regions of interest)*

Blur measure (SML) vs image number

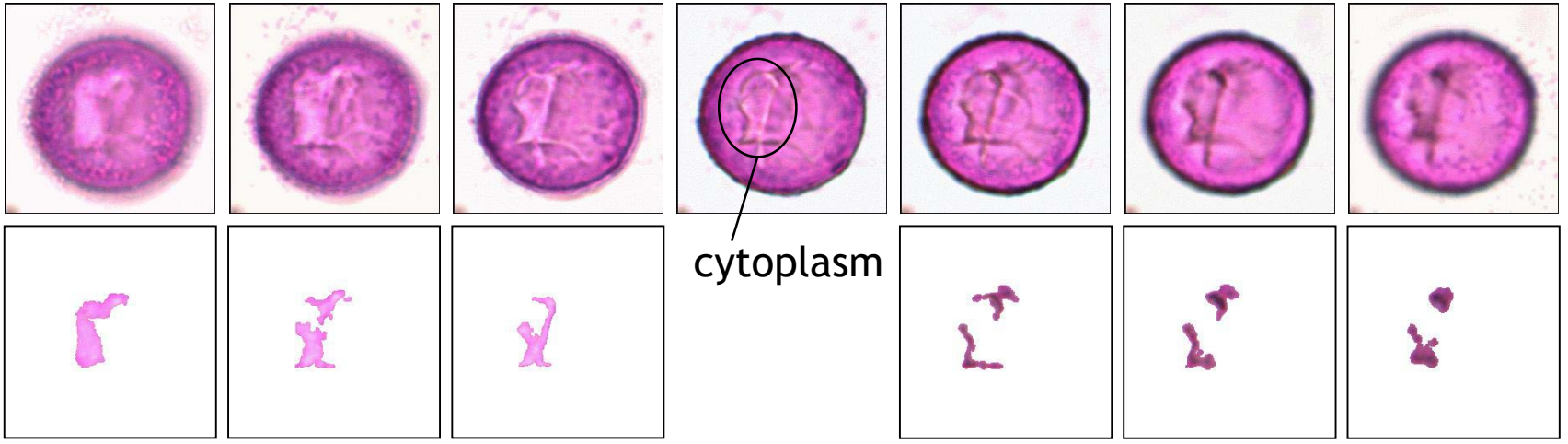




Example: Cupressaceae cytoplasm

Above central image

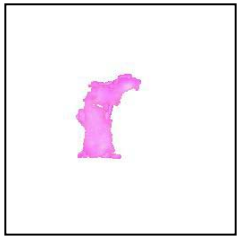
Below central image



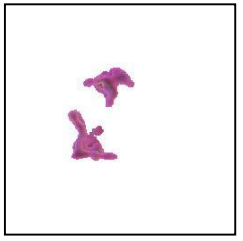
Segmentation of bright regions

Segmentation of dark regions

Sum of bright regions



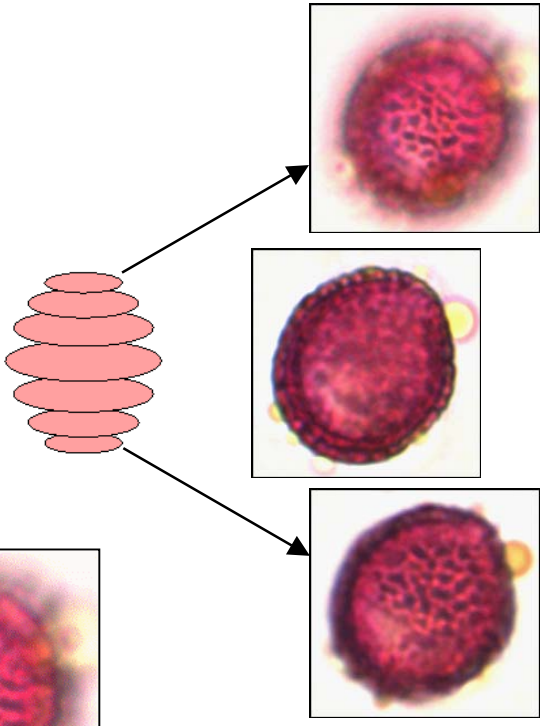
⇔

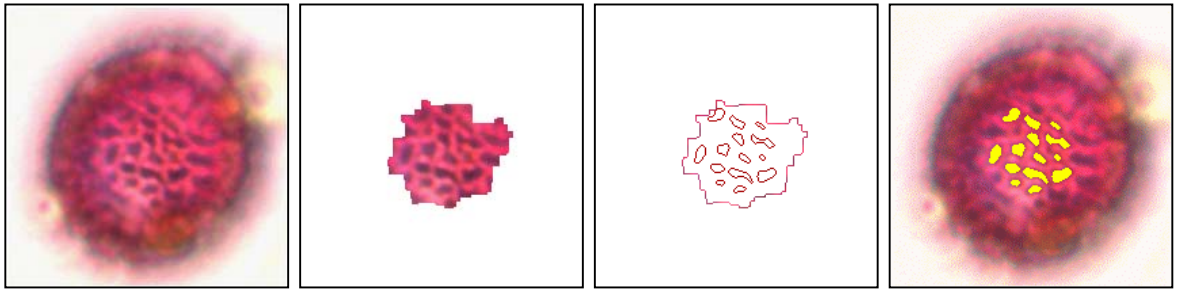


Sum of dark regions

Example: *Olea* reticulum

- Network located on the external surface
- Visible on top and bottom images
- Detection steps:
  - Check if the grain is reticulated
  - Localise the reticulum (3D)
  - Analyse the reticulum





Example: Poaceae Pore

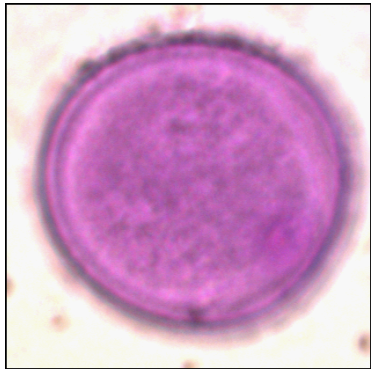


Image 35 /100

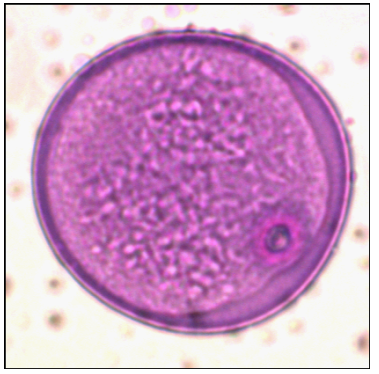


Image 50 / 100

Image 65 / 100

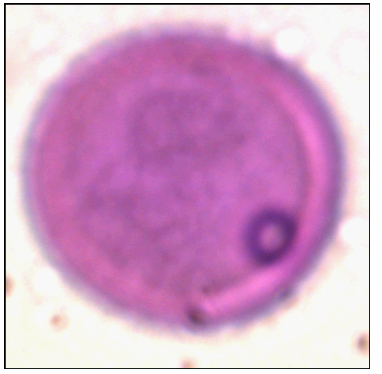
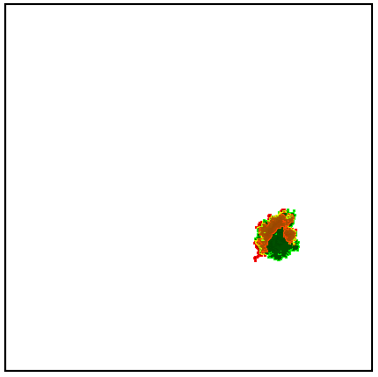
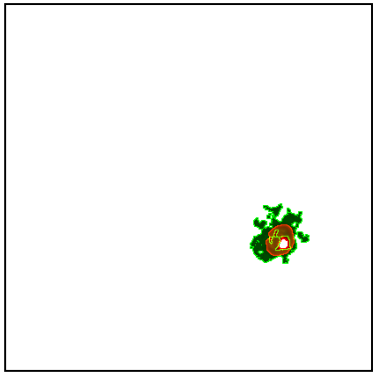
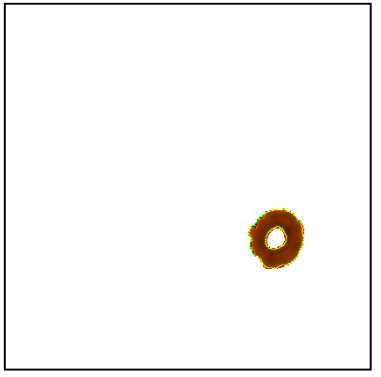
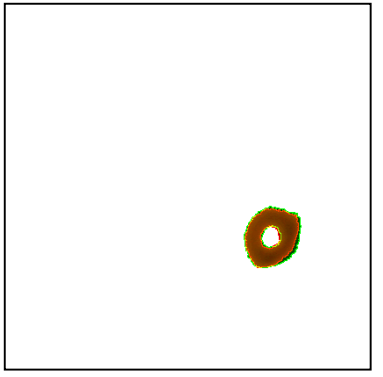


Image 80 / 100









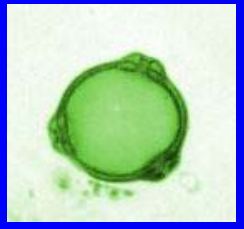
Results

- **Test on a database of 350 pollen grains**
  - Reference images (pollen grains without dust and pollution)
  - Simulation of the sampling date
  - *Leave-one-out* method used for validation
  - Results of recognition
    - Recognition rate between 4 allergenic types + others (5 classes) : **99,7%**
    - Recognition rate between 31 pollen types + others (32 classes) : **77,7%**

- **Test on a new set of images** (different conditions)
  - Low recognition rate between 4 allergenic pollen types (5 classes) : **45 % !!**
  - Problem of calibration and robustness for colour variation
    - Need to improve colour processing (more flexible system)
    - Need to normalise image acquisition conditions

T.E.S.A. Worcester - 2003

Specific detection of pollen by solid phase Cytometry. (Feasibility study)



THIBAUDON M. (RNSA)

RABILLON J. (Institut Pasteur)

DROCOURT J-L. (Chemunex)

RNSA - FRANCE

T.E.S.A. Worcester - 2003

Objective

Specific detection of pollen species in air, for the prevention of allergy, using FITC conjugated antibodies combined with laser scanning cytometry

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AIM :

Feasability study of a new system to:

- Collect the pollen in a soluble filter or on a 10 micron filter.
- Analyse the filter in a few minutes by immunofluorescence scanning.

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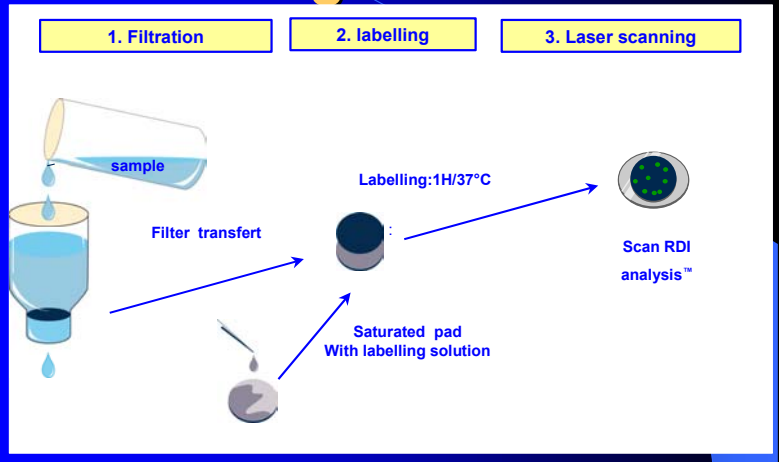
Labelling Procedure



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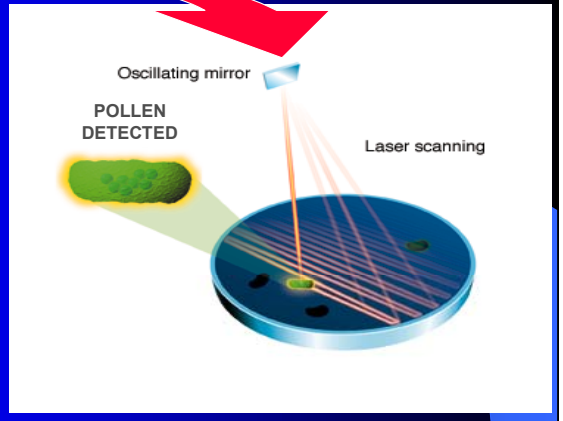
Laser sanning



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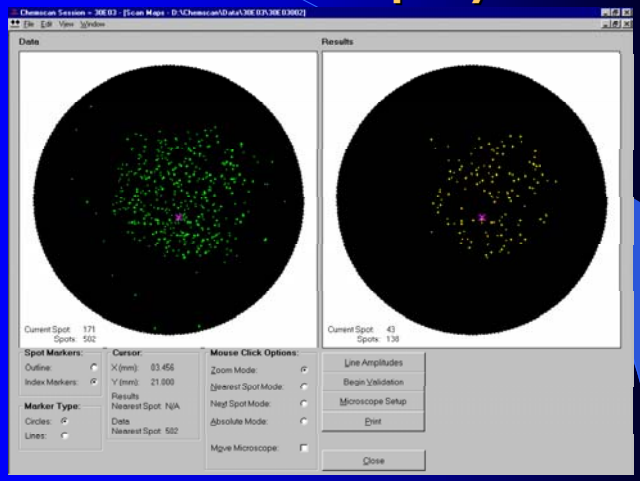
Results display



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T.E.S.A. Worcester - 2003

Pollen detection using FITC conjugated Anti-*parietaria* antibody

POLLEN type	w/o labelling w/o discriminatiion (autofluorescnce)	labelling with FITC conjugated Anti-*parietaria*	
		control w/o antibody with Discrimination RATIO	labelling 1H at 37°C with Discrimination RATIO
Pollen ***parietaria***	147	0	152
Pollen ***dactylis***	165	0	0
Pollen ***betula***	195	1	15

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Pollen detection using FITC conjugated Anti-*dactylis* antibody

POLLEN type	w/o labelling w/o discriminatiion (autofluorescnce)	labelling with FITC conjugated Anti-*dactylis*	
		control w/o antibody with Discrimination RATIO	labelling 1H at 37°C with Discrimination RATIO
Pollen ***dactylis***	165	0	138
Pollen ***betula***	195	1	26

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Conclusions

1) Pollen bloom (all taxa) can be detected in air within minutes.
2) Specific allergenic pollen can be selectively detected using appropriate immunological probes and accurately counted using laser scanning cytometry.

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