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Publisher: Taylor & Francis

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Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

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Available online: 05 Nov 2010

To cite this article: Sofia Pérez-Atanes, Maria Del Carmen Seijo-Coello & Jose Méndez-Alvarez (2001): Contribution to the study of fungal spores in honeys of Galicia (NW Spain), *Grana*, 40:4-5, 217-222

To link to this article: <http://dx.doi.org/10.1080/001731301317223240>

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Contribution to the study of fungal spores in honeys of Galicia (NW Spain)

SOFIA PÉREZ-ATANES, MARIA DEL CARMEN SEIJO-COELLO and JOSE MÉNDEZ-ALVAREZ

Pérez-Atanes, S., Seijo-Coello, M.d.C. & Méndez-Alvarez, J. 2001. Contribution to the study of fungal spores in honeys of Galicia (NW Spain). – Grana 40 (4–5): 217–222. ISSN 0017-3134.

We analysed 30 samples of Galician honey with the aim of quantifying and identifying the fungal spores contained in them. Using an optical microscope, we could identify 40 different types, among them *Cladosporium*, *Penicillium*/*Aspergillus* and the Basidiospores, which were identified in more than 80% of the samples. We calculated the relationship between fungal spores and amount of pollen grains in the samples. The maximum values were obtained in samples 21 (more than 208/100 grains of pollen) and 5 (153 spores/100 grains of pollen).

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(Manuscript received 12 February 2001; accepted 12 September 2001)

Melissopalynology, the study of the microscopic elements present in honey, has as its main objective recognition of the pollen types that honey contains, so that its botanical and its geographic provenience can be recognized. The large number of microscopic elements in honeys vary in abundance and, to our knowledge, have never been the subject of detailed study.

Although fungal spores have been mentioned in studies since the middle of the 20th century (Fehlmann 1911, Gontarski 1951, Maurizio 1953, Louveaux 1968), they have never been quantified or identified.

Fungal spores enter the honey in different ways. Some are airborne (Palmas & Consentino 1989, Von Wahl & Kersten 1991, Sabino & Caretta 1992, Simsekli et al. 1999, Infante et al. 2000a, b, Méndez 2000) and are thus deposited on the flowers, on the beehive, on the honey extraction material or on any other element in the vicinity of the honey. Another important way of spores getting into the honey is through the fungi that develop in vegetables. They thus appear in the nectar and eventually in the beehive. A third way is through the presence of honeydew in the beehive.

Both airborne spores and those from fungi that grow on vegetables can remain attached to the honeydew secreted by insects from different taxa. Once taken by the bee, this honeydew gets into the nectar and forms what in Spain has been commercially called “forest honey”. It is possible that honeydew could be the origins of honey, and that this is honeydew honey. The count of elements such as the fungal spores in relation to the number of pollen grains in the honey has been used as a microscopic indicator of the origin of the honey.

The literature indicates that the origin of the fungal spores present in honey can be atmospheric pollution, as is the case with *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Botrytis cinerea* and *Helminthosporium*. These spores can also belong to the parasitic fungi of the honey, as in the

case of *Pericystis alvei*; to fungi associated with the bees (*Ascosphaera alvei*) or with plant pathogenic fungi (Uredinales and Peronosporaceae). The Basidiomycetes, Uredinales and Ustilaginales are parasites that can be found on the leaves and green parts of wild plants and also on the anthers of flowers, so they are frequently found in honeys from honeydew, but also in those from nectar (Barth 1989). There are fungi associated with the honeydew that grow on these sugary juices and that come from spores transported in the wind.

This work, complementary to the microscopical analysis, is a preliminary contribution to the knowledge on fungal spores in Galician honeys.

MATERIAL AND METHODS

In this study, we carried out an analysis of the fungal spores present in 30 honey samples from Galicia (Table I), which is northwest in the Iberian Peninsula. For the identification and counting of the different fungal spores present in the honey, we used the methodology as proposed by Louveaux et al. (1978).

The study starts with 30 well-homogenized grams of honey dissolved in distilled water at 45°C. This solution is distributed between two test tubes and centrifuged at 3,500 r.p.m. for 10 minutes. Once centrifugation is over, sample decantation takes place and 20 ml of sample is poured onto a slide.

Identification of the fungal spores was carried out with a Nikon Optiphot-2 microscope at 100× to facilitate observation. Among others, the following literature was used: Ellis (1971), Ellis (1976), Grant Smith (1990), Muntahola (1998) and Haines et al. (1999). Once recognized, the fungal spores were classified by type.

RESULTS AND DISCUSSION

The 40 types of spores found in this study are listed in Table II. The “Others” group includes all spores that were not identified. The maximum values of each type and the percentage presence in the set of samples are also given.

Cladosporium, *Penicillium*/*Aspergillus* and the basidio-

Table I. Types of honeys studied and data.

Sample	Site of origin	Date of extration	Honey type
1	Neda – A Coruña	July 1999	<i>Eucalyptus</i>
2	As Ponte – A Coruña	July 1999	Polyfloral
3	A Capela – A Coruña	August 1999	Polyfloral
4	Valdoviño – A Coruña	July 1999	<i>Eucalyptus</i>
5	Ponteceso – A Coruña	July 1999	Polyfloral
6	Ferrol – A Coruña	July 1999	Polyfloral
7	Xermade – Lugo	August 1999	Polyfloral
8	Sarria – Lugo	August 1999	Polyfloral
9	O Corgo – Lugo	August 1999	<i>Rubus</i>
10	As Nogais – Lugo	September 1999	Polyfloral
11	Quiroga – Lugo	August 1999	<i>Erica</i>
12	Láncara – Lugo	August 1999	<i>Rubus</i>
13	Samos – Lugo	August 1999	<i>Castanea</i>
14	Fonsagrada –Lugo	September 1999	Polyfloral
15	Navia de Suarna – Lugo	September 1999	<i>Castanea</i>
16	Celanova – Ourense	September 1999	<i>Rubus</i>
17	Allariz – Ourense	August 1999	<i>Rubus</i>
18	Cartelle – Ourense	August 1999	Polyfloral
19	Os Peares – Ourense	August 1999	Polyfloral
20	Lobios – Ourense	September 1999	<i>Erica</i>
21	A Peroxa – Ourense	September 1999	<i>Castanea</i>
22	Entrimo – Ourense	September 1999	Polyfloral
23	Castro Caldelas – Ourense	September 1999	Polyfloral
24	Viana do Bolo – Ourense	September 1999	Polyfloral
25	A Mezquita – Ourense	August 1999	<i>Castanea</i>
26	Rubiá – Ourense	August 1999	<i>Castanea</i>
27	A Rua – Ourense	August 1999	<i>Rubus</i>
28	Poio – Pontevedra	July 1999	<i>Eucalyptus</i>
29	Sandías – Ourense	August 1999	Polyfloral
30	Viana do Bolo – Ourense	September 1999	<i>Castanea</i>

spores are present in more than 75% of the analysed honey samples. It is noteworthy that *Cladosporium* is present in 97% of the samples, and reaches values of 18,733 spores/10 g of honey. Other highly represented types are basidiospores, present in 87% of the samples, and *Penicillium/Aspergillus*, present in 83%. *Leptosphaeria*, *Venturia*, Uredinales, Myxomycetes, *Alternaria*, *Coprinus* and *Drechslera* can be found in 50–75% of the samples. The first three are represented in 73% of the honeys, while Myxomycetes and *Alternaria* have been determined in 63% and 60%, respectively, although it is important to note the 2160 spores/10 g of honey reached by the Myxomycetes-type. The remaining types identified in the samples represented less than 45%. The counts of spore types/10 g of honey are given in Table III.

The *Agrocybe*-type has its highest values in sample 4 with 53 spores/10 g of honey, while sample 6 presents 47. It is important to note the 33 spores in sample 17 and the 27 in sample 21.

The *Acrodactis*-type can only be found in sample 10, with 27 spores/10 g of honey.

The *Alternaria*-type has been found in 18 of the analysed samples. It reaches significant values in samples 21 and 13, where we counted 87 and 73 spores/10 g honey, respectively, though they do not reach 1% of the total in either case. However, in sample 29, 13 of the analysed spores belong to this type and represent 9.5% of the total.

The *Amphisphaeria*-type has only been found in samples

8, 16 and 21. In 8 and 16 we counted 7 spores/10 g of honey, while in 21 we counted 60.

The *Asperisporium*-type is present only in sample 19, where 7 spores/10 g of honey were counted.

Included in the basidiospore-types are all the Basidiomycetes not included in other groups. They were found in 26 of the 30 samples. The highest value was found in sample 21 with 760 spores/10 g of honey; however, despite the high number of spores found in this sample, they represent only 2.7%. Samples 30, 4, 1, 17 and 22 have 373 and 260 spores. The remaining samples do not reach 200 spores/10 g of honey.

The *Botrytis*-type is found in only two of the analysed samples: samples 11 and 27, where 13 and 7 spores, respectively, were counted.

The *Cercospora*-type appears only in sample 22, where 13 spores/10 g of honey were counted.

The *Cerebella*-type reaches maximum values in sample 21, with 160 spores/10 g of honey. In sample 19, we identified 73 spores. The remaining samples have fewer than 20 spores/10 g of honey.

The *Cladosporium*-type reaches maximum values in sample 21, with 18,733 spores/10 g of honey. This spore represents 66.1% in this sample. In sample 19, we identified 8,673 and in sample 22 we found 5,587 spores. Samples 17, 1, 7, 14, 5, 10, 12 and 2 have between 1,000 and 4,687 spores/10 g of honey. As a percentage over the total, we have to note

Table II. Maximum amount (per 10 g of honey) and presence (%) of fungal spore types identified.

Fungal spore types	Max. value	%
<i>Cladosporium</i>	18,733	97
Basidiospores	760	87
<i>Penicillium/Aspergillus</i>	10,933	83
<i>Leptosphaeria</i>	547	73
<i>Venturia</i>	140	73
Uredinales	153	73
Myxomycetes	2,160	63
<i>Alternaria</i>	87	60
<i>Coprinus</i>	53	57
<i>Drechslera</i>	53	57
<i>Agrocybe</i>	53	40
<i>Stemphylium</i>	160	40
<i>Curvularia</i>	40	33
<i>Helminthosporium</i>	40	30
<i>Cerebella</i>	160	27
<i>Exosporiella</i>	60	27
<i>Torula</i>	33	27
<i>Pithomyces</i>	20	23
<i>Chaetomium</i>	13	17
<i>Mytilidion</i>	33	13
<i>Oidium</i>	20	13
<i>Saccobolus</i>	33	13
<i>Spondylocodiella</i>	33	13
<i>Amphisphaeria</i>	60	10
<i>Helicoma</i>	13	10
<i>Paraphaeosphaeria</i>	33	10
<i>Pleospora</i>	20	10
<i>Puccinia</i>	13	10
<i>Botrytis</i>	13	7
<i>Fusarium</i>	7	7
<i>Melanomma</i>	20	7
<i>Periconia</i>	27	7
<i>Acrodactis</i>	27	3
<i>Asperisporium</i>	7	3
<i>Cercospora</i>	13	3
<i>Inocybe</i>	7	3
<i>Massarina</i>	7	3
<i>Neohendersonia</i>	7	3
<i>Polythrincium</i>	7	3
<i>Splanchnonema</i>	7	3
Others	1,187	93

sample 7 with the 91.1% and sample 14 with 81.4%. The remaining samples show less than 1,000 spores/10 g of honey.

The *Coprinus*-type was found in 17 of the analysed samples. Representation is always lower than 2%. Sample 21 has the highest values for this type, i.e. 53 spores/10 g of honey.

The *Curvularia*-type presents its highest levels in samples 10 and 22, with 40 spores/10 g of honey. In the remaining samples it presents less than 20 spores.

The *Chaetomium*-type was found in only 5 of the analysed samples, with a low representation in each of them. Samples 4, 17, 19 and 26 present the highest values for this type – 13 spores/10 g of honey – while in sample 9 there are 7 spores/10 g.

The *Drechslera*-type is highest in sample 29, with 9.6%. Sample 12 shows the highest number of spores/10 g of honey, with 53, which represents 3.2% of the total. Samples 11 and 17 show 33 and 47 spores, respectively. In samples 8, 13, 21, 26, 29 and 30 we counted 13 spores/10 g of honey.

Spores belonging to the *Exosporiella*-type reach values of 60/10 g honey in sample 21. In sample 17 we found 40 spores of this type and in samples 9 and 12 we counted 20 spores each.

The *Fusarium*-type has only been identified in samples 12 and 19 with 7 spores/10 g honey.

The *Helicoma*-type was found in 3 of the 30 analysed samples, reaching its highest levels in samples 26 and 27 with 13 spores/10 g of honey.

The *Helminthosporium*-type reaches its highest level in sample 13, representing 2.4% of the total. Sample 9 shows 27 spores/10 g of honey, and the rest less than 15.

The *Inocybe*-type was found only in sample 2 (7 spores/10 g of honey).

The *Leptosphaeria*-type was found in 22 of the 30 analysed samples, reaching maximum value in sample 21, where 547 spores were counted, representing 1.9% of the spores found in this sample. Sample 19 presents 407, and sample 17 shows 153. In the remaining samples the values are lower than 40 spores/10 g of honey.

The *Massarina*-type has been identified only in sample 21 with 7 spores/10 g of honey.

The *Melanomma*-type was found only in samples 19 and 16, where it reaches values of 20 and 16 spores/10 g of honey, respectively.

The *Mytilidion*-type reaches its maximum value in sample 10, with 33 spores/10 g of honey. In sample 26 it shows 13 spores, while in samples 12 and 30 it presents 7 spores. The percentage representation is always low.

The Myxomycete-type reaches its maximum value in sample 1 with 2,160 spores. But it is also important to note the values reached in samples 4 and 13, namely 553 and 340 spores/10 g of honey. In samples 2, 6 and 21 these values are close to 200 spores. In the remaining samples where it has been identified the number of spores was lower.

The *Neohendersonia*-type was identified only in sample 19.

The *Oidium*-type was identified in 4 of the analysed samples but with a low percentage in all of them. Thus, sample 26 containing 20 spores/10 g of honey, presents the highest level, representing only 3% of the spores that were found.

The *Paraphaeosphaeria*-type was identified in only 3 of the analysed samples; highest in sample 17 with 33 spores/10 g of honey.

The *Penicillium/Aspergillus*-type has its maximum representation in sample 1, with 10,933 spores/10 g of honey, which is 67.1% of the spores found in this sample. Sample 21 presents 6,667 spores, representing 23.5%. In samples 4 and 5 we identified 5,233 spores, corresponding to 67.3% and 81.8%, respectively. In sample 19 the *Penicillium/Aspergillus*-type represents 31.5% (4,460 spores/10 g of honey). Excepting samples 2 and 17, with 1,367 and 2,100 spores/10 g of honey, respectively, all the other analysed samples have lower than 600 spores/10 g of honey.

The *Periconia*-type was found in sample 21, with 27 spores/10 g of honey, and in sample 19, with 13 spores.

The *Pithomyces*-type was identified in 7 of the analysed samples. Sample 11 shows the maximum value (20 spores), while 24 shows 13 spores.

The *Pleospora*-type reaches its maximum value in sample

Table III. Number of each fungal spore type identified/10 g of honey.

Sample	No. of spore types/10 g of honey	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
<i>Agropyce</i>		13	13	7	53	-	47	-	-	-	-	-	-	7	-	20	-	33	7	-	-	27	-	-	-	7	-	-	-	7	-
<i>Acrodactis</i>		-	-	-	-	-	-	-	-	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Alternaria</i>		-	7	-	-	-	-	7	20	20	7	27	73	13	-	-	-	47	-	53	7	87	13	-	-	7	7	-	7	13	13
<i>Amphisphaeria</i>		-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	7	-	-	-	-	60	-	-	-	-	-	-	-	-	-
<i>Asperisporium</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-
<i>Basidiospores</i>	287	180	93	347	-	87	40	60	33	93	40	13	107	80	33	53	273	20	173	87	760	260	80	107	-	113	60	-	-	-	373
<i>Botrytis</i>		-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-
<i>Cercospora</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-
<i>Cerebella</i>		-	-	-	-	-	-	-	-	20	-	-	13	7	-	-	-	20	-	73	-	160	-	20	-	7	-	-	-	-	-
<i>Cladosporium</i>	2400	1107	640	317	1153	393	1707	327	940	1547	173	1027	467	1600	407	-	4687	687	8673	260	18733	5587	333	40	233	247	333	107	93	473	
<i>Coprinus</i>		-	20	20	-	-	13	-	7	7	7	20	13	13	-	-	7	-	7	27	13	53	7	13	-	7	7	-	7	-	-
<i>Curvularia</i>		-	-	-	-	-	-	7	7	40	-	7	13	13	-	-	-	7	-	7	-	-	40	20	-	-	-	-	-	-	-
<i>Chaetomium</i>		-	-	-	13	-	-	-	7	-	-	-	-	-	-	-	-	13	-	13	-	-	-	-	-	13	-	-	-	-	-
<i>Drechslera</i>	7	7	-	-	-	-	-	13	20	-	33	53	13	-	-	-	-	47	7	20	-	13	7	-	7	13	27	-	13	13	-
<i>Exosporiella</i>		-	-	-	-	-	-	-	20	7	-	20	-	-	-	-	-	40	-	13	-	60	13	7	-	-	-	-	-	-	-
<i>Fusarium</i>		-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-
<i>Helicoma</i>		-	-	-	-	-	-	-	7	27	-	-	7	40	7	-	-	-	-	-	-	-	7	13	-	7	13	-	-	-	-
<i>Helminthosporium</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Inocybe</i>		-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Leptosphaeria</i>	-	27	13	13	-	-	-	7	7	33	13	-	40	20	27	13	27	153	7	407	33	547	27	-	20	-	33	20	-	-	13
<i>Massaria</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	7	-	-	-	-	-	-	-	-	-
<i>Melanomma</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-
<i>Mytilidion</i>		-	-	-	-	-	-	-	-	33	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	7
<i>Myxomycetes</i>	2160	200	53	553	13	227	47	-	-	20	-	40	33	340	13	-	-	13	47	20	-	220	13	60	-	-	53	-	-	-	-
<i>Neohendersonia</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-
<i>Oidium</i>		-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	7	-	-	-	-	-	-	20	7	-	-	-	-
<i>Paraphaeosphaeria</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	33	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium</i>	10933	1367	353	5233	5233	-	40	7	207	180	73	53	220	53	20	553	2100	247	4460	473	6667	587	33	-	-	-	-	47	133	-	67
<i>Periconia</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	27	-	-	-	13	7	-	-	-	-
<i>Pithomyces</i>	7	-	-	-	-	7	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-
<i>Pleospora</i>		-	-	-	7	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-
<i>Polythrincium</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Puccinia</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	13	-	-	7	-	-	-	-	-	-	-
<i>Saccobolus</i>		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	27	-	33	-	-	27	-	-	-	-	-	-
<i>Splanchnonema</i>		-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spondyliocladiella</i>		-	-	-	-	-	-	-	-	-	-	33	-	-	7	7	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-
<i>Stemphylium</i>		-	-	-	7	-	-	-	-	7	-	33	47	-	-	-	-	33	-	40	13	160	13	33	7	-	-	-	-	-	7
<i>Torula</i>		-	-	-	-	-	-	-	-	-	-	27	-	-	13	7	33	-	33	-	-	20	-	-	33	7	-	-	-	-	-
<i>Venturia</i>		-	-	-	-	-	20	20	60	-	27	33	-	60	33	40	73	60	13	33	33	40	107	27	-	140	-	60	-	-	47
<i>Uredinales</i>	-	33	13	47	-	13	-	7	33	33	20	153	33	47	-	7	40	73	20	-	47	47	40	7	-	47	20	-	-	27	-
<i>Others</i>	480	653	433	1187	-	27	13	27	27	60	13	93	173	53	7	-	200	7	47	7	593	140	207	100	60	27	27	67	13	53	-
<i>Total of spores</i>	16287	3620	1627	7777	6400	833	1873	533	1400	2113	474	1666	1647	1967	573	753	7860	1120	14160	967	28320	6894	880	353	480	627	633	326	140	1093	-
<i>Honeydew index</i>	1.38	1.03	0.12	1.01	1.53	0.28	0.07	0.02	0.04	0.31	0.18	0.06	0.14	0.06	0.11	0.2	0.53	0.2	0.31	0.05	2.08	0.55	0.26	0.05	0.14	0.04	0.18	0.05	0.11	0.11	-

20 with 20 spores/10 g of honey. It was also found in samples 4 and 13.

The *Polythrincium*-type was identified only in sample 17, with 7 spores/10 g of honey.

The *Puccinia*-type was found in 3 of the analysed samples. Its maximum value is 13 spores/10 g of honey in samples 15 and 20.

The *Saccobolus*-type presents its maximum value in sample 21, where 33 spores were counted. In samples 19 and 24, 27 spores of this type were also identified. We also found these spores in sample 17.

The *Splanchnonema*-type was found in sample 10, with 7 spores/10 g of honey.

The *Spondylocyadiella*-type reaches its maximum value in sample 12, with 33 spores. It also presents 7 spores/10 g of honey in samples 15, 16 and 20.

The *Stemphylium*-type reaches 160 spores/10 g of honey in sample 21 and values lower than 50 spores/10 g of honey in 11 more samples.

The *Torula*-type has its highest values in samples 17 and 24, with 33 spores/10 g of honey. In sample 12 we counted 27 spores, and in the samples 21 and 15 only 20 and 13 spores, respectively. Samples 16, 25 and 26 present 7 spores of this type.

Spores belonging to the *Venturia*-type were identified in 19 of the 30 samples analysed, reaching the highest level in sample 25 (140 spores/10 g of honey). Other samples containing significant values are 22, 16, 8, 13, 17 and 27. In the remaining samples in which this spore was found, the values are lower than 50 spores/10 g of honey.

The Uredinales-type is frequently found in samples of honey, but as a general rule it does not represent a high percentage of the total spores. Its presence was determined in 22 of the studied samples. Sample 12 presents the highest level, with 153 spores/10 g of honey (9.2% of the total). In sample 18, we counted 73 spores (6.6%). Samples 4, 14, 21, 22 and 26 showed 47 spores. The rest of the samples presented lower values.

Figure 1 shows the number of spores/100 grains of pollen. Sample 21 reflects the highest number of spores, more than 200, followed by samples 5 and 1 with 153 and 138 spores,

respectively. Samples 21 and 5 show a mean value of between 1.5 and 2. In the remaining samples, the value is low or zero. Samples 20 and 28 have 5 spores/100 grains of pollen; samples 9 and 26 with 4 spores/100 grains of pollen and sample 8 with 2 spores are those with the lowest levels.

Some studies on Spanish honeys (Pérez de Zabalza 1989, Jato et al. 1991, Valencia-Barrera 1991, Hidalgo 1993) give reference values as the number of spores/100 grains of pollen, with the aim of determining the honeydew index. Samples 21 and 5 reflect a mean value between 1.5 and 2. In the remaining samples, the value is low or zero. These data indicate that, in the case of samples 1, 2, 4, 5 and 21, the levels of spores/100 grains of pollen counted are higher than those indicated in the mentioned literature. Probably the spores in the honey did not come from the honeydew, which means that establishing a relation between the pollen grains of the honey and other microscopic elements was a mistake, since many of the spores found in the samples can come from the atmosphere in the extraction and canning room and so having nothing to do with the honeydew.

CONCLUSIONS

In this study, 113,395 fungal spores belonging to 40 different types of spores have been counted. A group was determined to include a set of spores which could not be classified. The most abundant spores found in honeys are: the *Cladosporium*-type, *Penicillium/Aspergillus*-type, Myxomycete-type and basidiospores-type. It is important to note that both *Cladosporium* and *Penicillium/Aspergillus* are spores of cosmopolitan fungi that can colonize a large number of habitats and is why they have a wide representation in the atmosphere. Out of the identified types in this work *Aspergillus*, *Penicillium*, *Cladosporium*, *Helminthosporium*, basidiospores and Uredinales have been previously referred to by other authors (Barth 1970, 1989).

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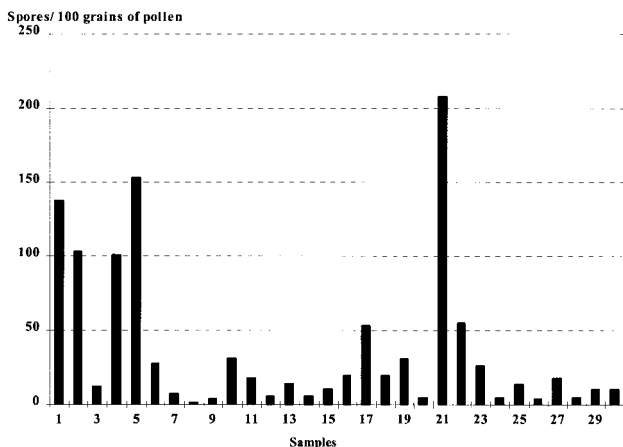


Fig. 1. Number of spores/100 grains of pollen.

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