basis of the shape of the signals, since 3α-H is reported to have a larger $W_{1/2}$ value than 6β-H in compound **2a**[9]. Thus, it is apparent that the phosphate group is attached to the C-3 hydroxy group in both cases. Based on the data described above[10], the structures of **1** and **2** were established to be 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**) and bombycosterol 3-phosphate (**2**), respectively. Additional evidence for the structures was obtained by ^{31}P-NMR in which a signal either at 0.92 ppm (compound **1**) or 1.50 ppm (compound **2**), relative to phosphoric acid, was detected. Further, these signals were sharpened to some extent by the selective proton (3-H) decoupling experiments.

The four other ecdysteroid conjugates eluted in the order as shown in figure 2 were identified as 22-phosphates of 20-hydroxyecdysone (peak A), ecdysone (peak B), 2-deoxy-20-hydroxyecdysone (peak C), and 2-deoxyecdysone (peak D) in the same manner as described above. It should be noted that in the ovaries of *B. mori* the ecdysteroids which have a 22-hydroxyl group are substituted at this position to form conjugates, whereas those lacking a 22-hydroxyl group are substituted at the 3-position.

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1 Present address: Iwaki Mei-Sei University, Iwaki New Town, Iwaki, Fukushima 970, Japan.
2 Hetru, C., Luu, B., and Hoffmann, J. A., Meth. Enzymol. *111* (1985) 411; Rees, H. H., and Isaac, R. E., Meth. Enzymol. *111* (1985) 377.
3 Ohnishi, E., Mizuno, T., Chatani, F., Ikekawa, N., and Sakurai, S., Science *197* (1977) 66.
4 Ikekawa, N., Ikeda, T., Mizuno, T., Ohnishi, E., and Sakurai, S., J. chem. Soc. chem. Commun. *1980*, 448.
5 Ohnishi, E., Mizuno, T., Ikekawa, N., and Ikeda, T., Insect Biochem. *11* (1981) 155.
6 Fujimoto, Y., Miyasaka, S., Ikeda, T., Ikekawa, N., Ohnishi, E., Mizuno, T., and Watanabe, K., J. chem. Soc. chem. Commun. *1985*, 10.
7 Ohnishi, E., Zool. Sci. *3* (1986) 401.
8 Isaac, E., Rose, M. E., Rees, H. H., and Goodwin, T. W., Biochem. J. *213* (1983) 533.
9 Fujimoto, Y., Yamada, T., and Ikekawa, N., Chem. pharm. Bull. *33* (1985) 3129.
10 Details of the procedures of their isolation and characterization of these conjugates will be published elsewhere.

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Airborne pollen content in the atmosphere of central Italy (1982–1986)

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Summary. This report describes qualitatively and quantitatively the content of pollen in the atmosphere of central Italy during the five years 1982–1986. Total production in this period showed fluctuations depending on the flowering seasons of the anemophilous taxa. The season of maximum pollen concentration was from April to July, with a prevalence of arboreal pollen in the first months, and of pollen from herbaceous plants in the last months of the year. During the five years of research more than 81 different types of pollen grains were recorded and identified. In both the cities investigated Curpessaceae/Taxaceae, Fagaceae, Oleaceae, Gramineae and Urticaceae were responsible for the greatest amounts of pollen.
Key words. Aerobiology; pollen census; central Italy.

Studies of the pollen content in the atmosphere of different areas have been carried out by researchers in Europe[1–9] and in Italy[10,11]. Surveys of atmospheric pollen grains in two cities of central Italy, Ascoli Piceno and Perugia, have been carried out since 1981 and since 1982, respectively[12–20]. Since the seasonal patterns for all taxa are characteristic, we describe them in terms of annual mean pollen concentrations. Only those taxa with relatively high concentrations were included. In this paper we report the pattern of pollen-production by 12 selected taxa.

Materials and methods. The pollen was collected by 7-day recording volumetric traps[21,22]. The pollen concentration in the atmosphere is expressed in terms of the number of pollen grains per cubic meter of air (p/m^3). The methods used are described in a previous paper[12].

The meteorological data in Ascoli Piceno was recorded by the SIAP Bologna S 2000 meteorological station. In Perugia it was kindly supplied by the Institute of Ecology of the Faculty of Agricultural Science, Perugia University. The meteorological data were plotted as a climatic diagram. Daily pollen concentrations were also noted and were reported on a 5-day mean basis.

Identification of the pollen grains was based on a comparison with reference slides of the Palynotheca of the Plant Biology Department, Perugia University, and photographs which appear in Hyde and Adams[23], Faegri and Iversen[24] and Erdtman[25,26]. The nomenclature of the families, genera and species may be found in Tutin et al.[27].

Pollens of Chenopodiaceae and Amaranthaceae, in addition to those of Cupressaceae and Taxaceae, and Typhaceae and Sparganiaceae, are referred to as Cheno-Amaranthaceae, Cupressaceae/Taxaceae and Typhaceae/Sparganiaceae respectively (table), since they all produce pollen during the same period and are difficult to distinguish, owing to their similar morphology, to the limitations of the methods available, and to the fact that the material is fresh and fixed on the slide[17].

Study area

Perugia (43° 63′ N; 12° 23′ E; 493 m above sea level) lies on a hill and overlooks the middle valley of the river Tevere between Lake Trasimeno and the Tyrrhenian versant of the Marches-Umbrian Apennines. The relative humidity always reaches high values with an average of 70% and the average

Mean abundance of pollen of each family as percentage of total pollen in the atmosphere of central Italy 1982–1986

Trees and shrubs		Herbaceous plants	
Cupressaceae/Taxaceae	24.128	Urticaceae	15.214
Fagaceae	17.179	Gramineae	13.309
Oleaceae	10.19	Euphorbiaceae	2.16
Corylaceae	4.369	Compositae	1.562
Pinaceae	2.056	Cheno/Amaranthaceae	1.41
Salicaceae	1.66	Plantaginaceae	1.24
Platanaceae	0.712	Polygonaceae	0.351
Betulaceae	0.697	Umbelliferae	0.349
Moraceae	0.432	Liliaceae	0.344
Ulmaceae	0.356	Papaveraceae	0.288
Vitaceae	0.295	Cruciferae	0.158
Ericaceae	0.211	Cannabaceae	0.129
Caprifoliaceae	0.197	Rubiaceae	0.126
Juglandaceae	0.142	Leguminosae	0.084
Aceraceae	0.034	Ranunculaceae	0.077
Myrtaceae	0.031	Cyperaceae	0.068
Anacardiaceae	0.029	Typhaceae/Sparganiaceae	0.036
Simaroubaceae	0.022	Rosaceae	0.032
Tiliaceae	0.022	Boraginaceae	0.022
Araliaceae	0.021	Capparidaceae	0.012
Taxodiaceae	0.015	Caryophyllaceae	0.004
Hippocastanaceae	0.014	Juncaceae	0.003
Palmae	0.014	Labiatae	0.001
Buxaceae	0.01	Potamogetonaceae	0.0005
Aquifoliaceae	0.007		
Lauraceae	0.001		
Elaeagnaceae	0.0003		

annual rainfall is 900 mm; the average summer and winter temperatures are 23 °C and 4 °C respectively with an annual average of 12.9 °C. The prevailing winds in Perugia come from the North-Eastern and South-Western quadrants: the North-Eastern winds prevail in autumn-winter, while the South-Western winds in spring-summer. The climate of Perugia is sub-continental.

Here the pollen trap was positioned approximately 20 m from the ground on the roof of the Faculty of Agricultural Science, Perugia Univeristy.

Ascoli Piceno (42° 51′ N; 13° 34′ E; 136 m above sea level) lies in the valley of the river Tronto about 25 km from the coast of the middle basin of the Adriatic sea. The relative humidity during the year is about 70% and the average annual rainfall, 700 mm; the average summer and winter temperatures are 24 °C and 6 °C respectively, with an annual average of 14.9 °C. The prevailing winds in the area come from the South-Western and North-Eastern quadrants. The climate of Ascoli Piceno may be considered typically sub-mediterranean.

The trap was positioned approximately 10 m from the ground, on the roof of the Technic State Institute in the center of the city.

The vegetation around Perugia and Ascoli Piceno

In the area of Perugia the sub-mountain vegetation is characterized by arboreal communities principally represented by *Ostrya carpinifolia*, Scop., *Fraxinus ornus* L., *Acer* spp., *Castanea sativa* Miller, *Populus tremula* L.; *Quercus* spp. (*Quercus ilex* L. in some Southern versants) and by herbaceous communities (grass-pasture) with a clear predominance of Gramineae, Compositae and Umbelliferae. Here rare woods of *Fagus sylvatica* L. are present. In the hilly uncultivated regions, woods of *Quercus pubescens* Willd. and *Quercus ilex* L. are predominant, while in the agricultural areas, olive trees and vines are grown on a large scale. The plains near the town are rich in cereals, tobacco and sunflowers. The tree vegetation of river banks is characterized by *Salix* spp., *Populus* spp., *Alnus glutinosa* (L.) Gaertner, *Ulmus minor* Miller. The most widespread ruderal weeds belong to Gramineae, Urticaceae and Compositae[19].

In the area of Ascoli Piceno four phyto-climatic regions can be distinguished from East to West and from sea level to the top of Mount Vettore (2478 m), the highest peak in the area under examination and also in all the Marches-Umbrian Apennines. According to Ballelli et al.[28] these regions can be distinguished as follows:

1) a coastal and littoral region, with a mediterranean climate from 0 to 200 m above sea level;
2) an internal hilly region with a sub-mediterranean climate, from 200 to 1000 m;
3) a mountain region with an oceanic axeric-temperate climate from 1000 to 1800 m above sea level;
4) a high mountain or sub-alpine region with an oceanic axeric-cold climate over 1800 m.

Within these regions the vegetation includes arboreal and shrub communities characterized principally by the families Fagaceae, Oleaceae, Corylaceae and Salicaceae, and herbaceous communities (pasture, grass-pasture, uncultivated) with a clear predominance of Gramineae and Compositae and ruderal weeds including the family Urticaceae. The city of Ascoli Piceno lies in a mainly agricultural area where cereals, vegetables, olive trees and vines are grown on a large scale[19].

Results and discussion. Figure 1 shows the climatic diagram plotted with the average of mean temperature and rainfall data in the five-year (1982–1986) period, in central Italy (Perugia and Ascoli Piceno). The climatic diagram reveals a

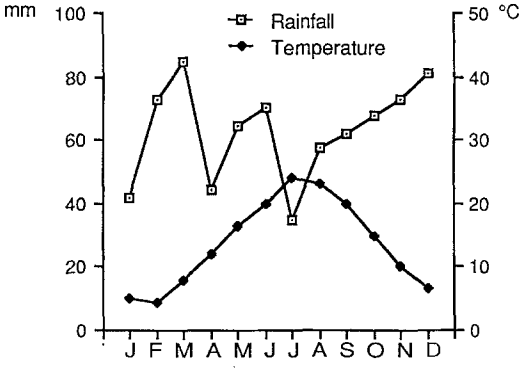

Figure 1. Climatic diagram: mean of the five-year period 1982–1986 in central Italy (Perugia and Ascoli Piceno).

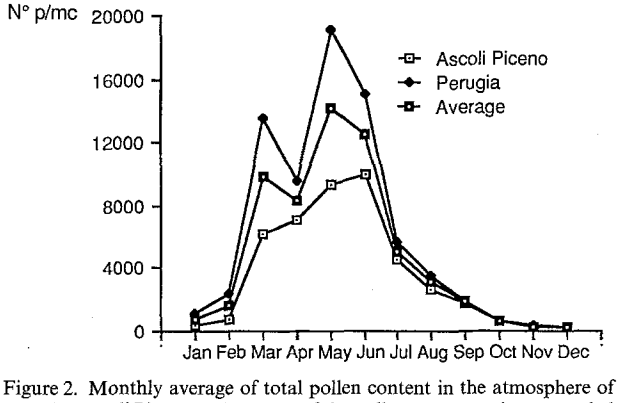

Figure 2. Monthly average of total pollen content in the atmosphere of Perugia, Ascoli Piceno and average of the pollen concentrations recorded in the two monitoring stations: mean for the five-year period 1982–1986.

dry period in July and two wet periods in February–March and in November–December, in accordance with the climatic diagrams of the last decade in central Italy[29].

Figure 2 shows the monthly average of total pollen content recorded in the atmosphere in central Italy during the years studied. In the two monitoring stations we observed different patterns of pollen-production. In Perugia the levels of pollen concentration in the atmosphere, during the March–June period, were always higher than those registered in Ascoli Piceno owing to the greater incidence of arboreal pollen.

81 pollen types belonging to 54 families were identified in the atmosphere of the studied area (table).

In Perugia pollen of arboreal taxa accounts for the highest percentage (71%); this is due to the vegetation and geographical location of the town. Owing to its high position, Perugia is an easy target for winds from any direction.

In Ascoli Piceno there is a prevalence of arboreal pollen (55%), but also pollen of herbs is of noteworthy importance (45%). This is due to the prevailing winds which come from the North-East, which corresponds to the Adriatic and to the widest part of the River Tronto basin. In this area the absence of woods, the large number of towns and widespread agriculture encourages the growth of herbaceous plants on the walls and on the waste-lands, the pollen of which are responsible for a notable increase in airborne pollen.

On average, in the studied area, arboreal plants account for the highest percentage (63%); among these Cupressaceae/Taxaceae, Fagaceae, Oleaceae and Corylaceae were responsible for the greater pollen amounts.

The percentage (37%) of herbaceous plants whose pollen can be attributed primarily to Urticaceae and Gramineae is lower.

Among the other taxa Pinaceae, Euphorbiaceae, Salicaceae, Compositae, Cheno-Amaranthaceae and Plantaginaceae are significant.

Figures 3, 4 and 5 show, in chronological order, the seasonal trends of the pollen concentration for 12 taxa which are found in greatest quantities (abundance percentage > 1% of the total pollen) in the atmosphere of the studied area. The pollen of these taxa accounts for 94.5% of all observations and they are descriptive of the anemophilous flora in central Italy.

Euphorbiaceae: the pollen is recorded in the atmosphere in limited amounts and is attributable only to *Mercurialis annua* L. The highest concentrations of pollen in the air were recorded at the end of July – beginning of August.

Cupressaceae/Taxaceae: this is a predominant component among airborne pollens in central Italy; the most important species of this family are *Cupressus sempervirens* L. and *C. arizonica* E. L. Greene, while *Juniperus communis* L. pollen is recorded with lower levels.

Corylaceae: *Corylus avellana* L. pollen is present in the atmosphere during the winter and *Carpinus betulus* L. and *Ostrya carpinifolia* Scop. in the spring.

Salicaceae: *Populus* spp. and *Salix* spp., which are particularly widespread along the river and lake banks, are the pollen sources of this family.

Fagaceae: this family is one of the most important aerospora components in our territory; it is represented in April–June mainly by *Quercus* spp. and lesser by *Fagus sylvatica* L. *Castanea sativa* Miller is present in June–July.

Pinaceae: we recorded two principal periods of pollination: the first in April–June with *Pinus* spp. pollen and the second in September–November with *Cedrus* spp.

Gramineae: the most abundant species of wild grasses in central Italy are: *Alopecurus pratensis* L., *Dactylis glomerata* L., *Agropyron repens* (L.) Beauv., *Bromus erectus* Huds., *Hordeum murinum* L., *Lolium perenne* L., *Phleum pratense* L., *Poa trivialis* L., *P. pratensis* L., *Avena fatua* L., *Cynodon dactylon* (L.) Pers., *Arundo donax* L., *Ampelodesmos mauritanicus* (Poiret) Dur. et Sch. *Paspalum paspaloides* (Michx.) Scribner.

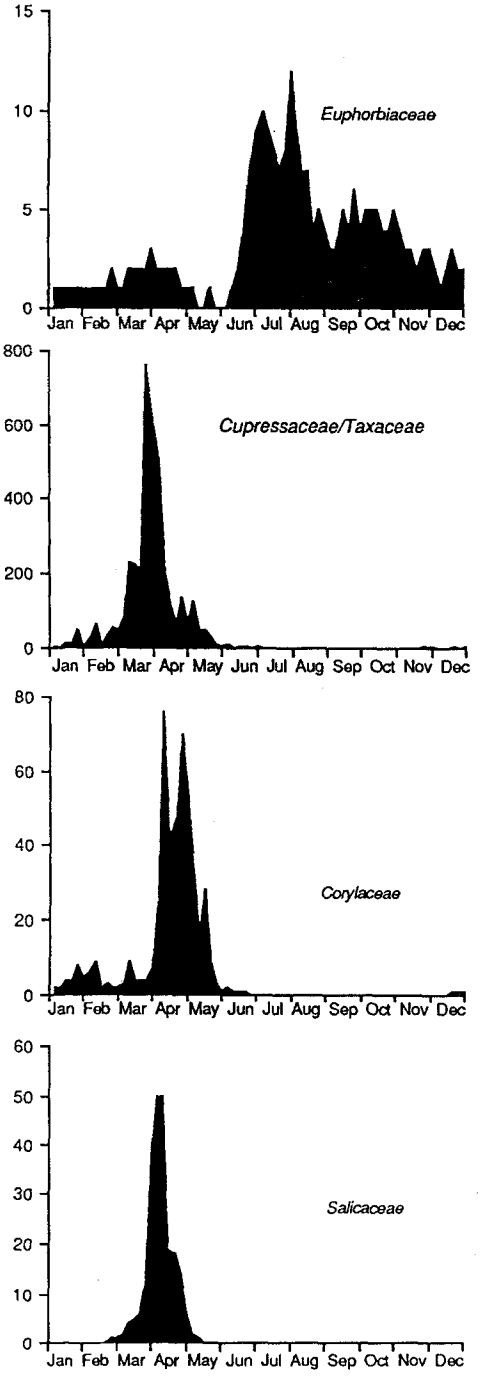

Figure 3. Five-day means of daily pollen counts: means of the pollen concentrations recorded in Perugia and Ascoli Piceno during the five years 1982–1986.

Oleaceae: the onset of pollination is in May with *Fraxinus ornus* L. followed in June by *Olea europaea* L. which reaches very high values. In July–August a low concentration of *Ligustrum* spp. pollens is present.

Urticaceae: this is the first component among herbaceous taxa and it is especially represented by *Parietaria* spp. and with lower percentage by *Urtica* spp.

Plantaginaceae: *Plantago lanceolata* L., *P. major* L. and *P. media* L. are the species responsible for the pollen of this family.

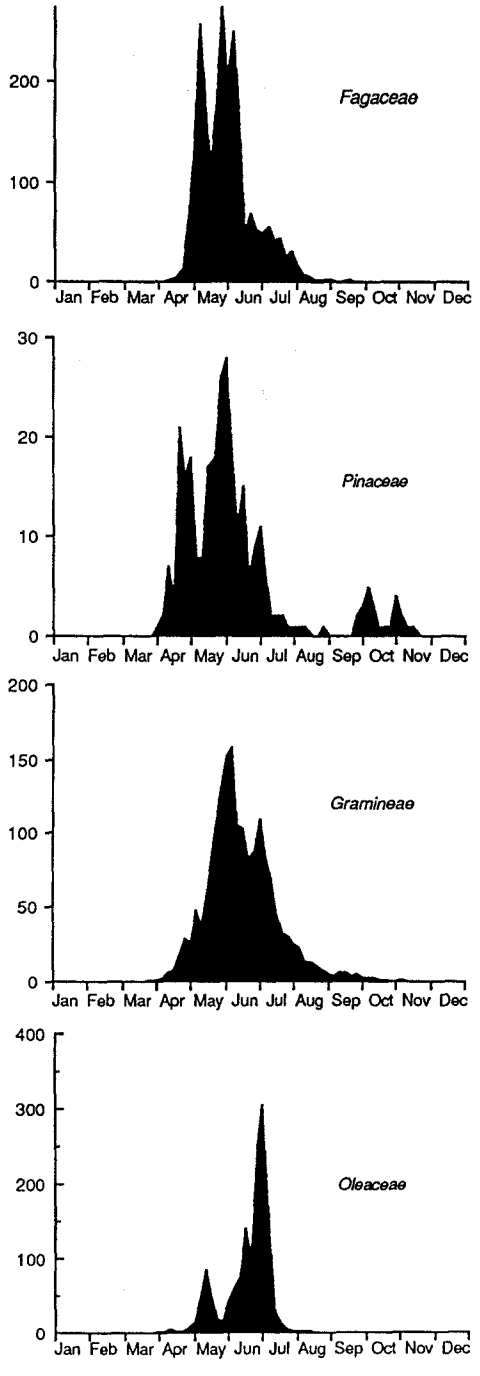

Figure 4. Five-day means of daily pollen counts: means of the pollen concentrations recorded in Perugia and Ascoli Piceno during the five years 1982–1986.

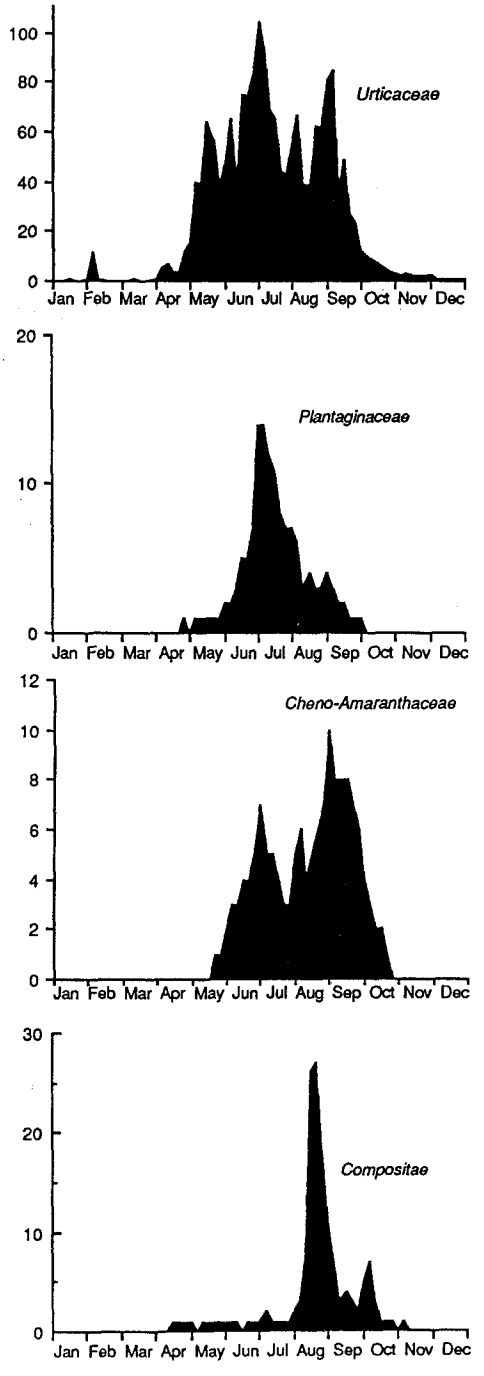

Figure 5. Five-day means of daily pollen counts: means of the pollen concentrations recorded in Perugia and Ascoli Piceno during the five years 1982–1986.

Cheno-Amaranthaceae: *Amaranthus retroflexus* L. and *Chenopodium album* L. are the most representative species of these families.

Compositae: the pollination of this family begins in April with the pollen of Compositae, Liguliflorae and Tubuliflorae. The latter reaches high levels of concentration with *Artemisia vulgaris* L. in August and with *A. verlotorum* Lamotte in September–October.

1 Leuschner, R. M., Verh. naturf. Ges. Basel *84* (1974) 521.
2 Fuckerieder, K., Berichte 9/76 1976, Umweltbundesamt.
3 Stix, E., Ferretti, M. L., and Leuschner, R. M., Ber. dt. bot. Ges. *90* (1977) 541.
4 Morrow Brown, H., and Jackson, F. A., Clin. Allerg. *8* (1978) 611.
5 Michel, F. B., Seignalet, Ch., and Cour, P., in: Les Pollinoses. Fisons Ed., Ecully, France 1979.
6 Spieksma, F. Th. M., Charpin, H., Nolard, N., and Stix, E., Clin. Allerg. *10* (1980) 319.

7 Van Campo, M., and Quet, L., C.r. Séances Acad. Sci., Sér. 3, *295* (1982) 61.
8 Lejoly-Gabriel, M., and Leuschner, R. M., Grana *22* (1983) 59.
9 Frenguelli, G., Spieksma, F. Th. M., Romano, B., Nikkels, A. H., Mincigrucci, G., Dankaart, W., and Bricchi, E., Proc. 3rd Internat. Conf. Aerobiology (1986) Basel, Eds. R. Leuschner and G. Boehm. Abstracts, p. 62.
10 Caramiello Lomagno, R., Polini, V., Siniscalco, C., Mincigrucci, G., Romano, B., Frenguelli, G., and Bricchi, E., Aerobiologia *1* (1985) 39.
11 Romano, B., Mincigrucci, G., Frenguelli, G., Bricchi, E., Murgia, M., Cresti, M., and De Dominicis, V., Grana *25* (1986) 215.
12 Frenguelle, G., Romano, B., Mincigrucci, G., Paola, G., and Bricchi, E., Ann. Fac. Agr., Univ. Perugia *35* (1981) 389.
13 Frenguelli, G., Mincigrucci, G., Romano, B., and Bricchi, E., New Phytol. *95* (1983) 147.
14 Romano, B., Mincigrucci, G., Frenguelli, G., Bricchi, E., Marcucci, F., Sensi, L., Pierdomenico, R., and Castrica, C., Folia allergol. immun. clin. *31* (1984) 313.
15 Marcucci, F., Sensi, L., Pierdomenico, R., Castrica, C., Bizzarri, G., Romano, B., Mincigrucci, G., Frenguelle, G., and Bricchi, E., Eur. Rev. med. pharm. Sci. *6* (1984) 1.
16 Nardi, G., Demasi, O., Marchegiani, A., Romano, B., Mincigrucci, G., Frenguelle, G., and Bricchi, E., Proc. XI World Congr. Asthmology (1984) Mexico.
17 Mincigrucci, G., Romano, B., Frenguelli, G., and Bricchi, E., G. bot. ital. *119* (1985) 67.
18 Nardi, G., Demasi, O., Marchegiani, A., Pierdomenico, R., Mincigrucci, G., Romano, B., Frenguelli, G., and Bricchi, E., Ann. Allerg. *57* (1986) 193.
19 Mincigrucci, G., Romano, B., Frenguelli, G., and Bricchi, E., Aerobiologia *2* (1986) 51.
20 Romano, B., Mincigrucci, G., Frenguelli, G., and Bricchi, E., Proc. XIV Internat. Bot. Congr. (1987) Berlin (West).
21 Hirst, J. M., Ann. Appl. Biol. *39* (1952) 257.
22 Hirst, J. M., in: Ecological Systems Approaches to Aerobiology. I. Identification of Component Elements and Their Functional Relationship, p. 80. Eds W. S. Benninghoff and R. L. Edmonds. US/IBP Aerobiol. Program Handbook 2. University of Michigan, 1972.
23 Hyde, H. A., and Adams, K. F., Atlas of Airborne Pollen Grains. MacMillan London 1958.
24 Faegri, K., and Iversen, J., Textbook of Pollen Analysis. Munksgaard, Copenhagen, Denmark 1964.
25 Erdtman, G., Pollen Morphology and Plant Taxonomy, Angiosperms. Almqvist & Wiksell, Stockholm 1952.
26 Erdtman, G., Handbook of Palynology. Munksgaard, Copenhagen 1969.
27 Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., and Weeb, D. A., Flora europaea I–V. Cambridge University Press, London 1964–1980.
28 Ballelli S., Biondi, E., Cortini Pedrotti, C., Francalancia, C., Orsomando, E., and Pedrotti, F., Regione Marche, Ancona 1981.
29 Bollettini Comitato Glaciologico Italiano, Tip. Soc. Tor., Torino 1928–55.

0014-4754/88/070625-05$1.50 + 0.20/0