

Airborne Cupressaceae pollen in Southern Italy

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The allergenic airborne pollen of Cupressaceae was surveyed in the area of Bari (Southern Italy) over an 8-year period (1982–1989). A Burkard 7-day recording volumetric spore trap was used for the aerobiologic investigation. The results showed a notable annual pollinic presence of airborne Cupressaceae pollen, the largest amount recorded in winter and early spring. Remarkable fluctuations of the Cupressaceae pollen counts in a 2-year cycle were also observed. The features of the main pollen season are described. In particular, its duration was found to be on average 45 days.

A study of Cupressaceae trees distribution indicated that the most widespread genus was Cupressus, represented in particular by the species Cupressus arizonica Green and Cupressus Sempervirens L. The employment of these trees for reforestation and garden-use has increased considerably in the last 40 years.

These findings can also be of interest in other countries where Cupressaceae allergy has been reported, particularly in the Mediterranean basin and in North America.

INTRODUCTION

Airborne pollens often act as inhalant allergens natural-source related. When released by the sources in sufficient amounts, allergenic pollens may evoke allergic responses in sensitive patients leading to pollinosis.¹

The airborne pollens of the Cupressaceae family have been recognized as a cause of respiratory allergy for many years in the Mediterranean area, specifically in Israel,^{2,3} in Southern France,^{4,5} and in Italy.⁶ Cupressaceae pollinosis has also been investigated in extra-Mediterranean areas with a Mediterranean climate, such as Australia,⁷ California,⁸ and South Africa.⁹ The mountain cedar (*Juniperus sabinoides*), a member of Cupressaceae family, is reported to be a major allergen source in central Texas, New Mexico, and Northern Mexico.^{1,10–12} In Japan the most important pollen-related allergy is due to a member of the allied family of Taxodiaceae,

the Japanese cedar (*Cryptomeria japonica*).^{13–15}

Cupressaceae pollens have been studied in detail from a biochemical point of view.^{7,16} Immunologic and epidemiologic studies on Cupressaceae pollinosis have also been carried out.^{7,8,11,12,17} Nevertheless, little is known about release, dispersal, permanence in the atmosphere, and final deposition of these pollens. All these aerobiologic features are of great importance in relation to the comprehension of many epidemiologic aspects of pollen-related diseases. Further, pollinic data are useful to allergists in the diagnosis and therapy of allergic diseases as well as to patients in the self-management of their own illness.

The present study forms part of a wider project regarding Cupressaceae allergy in the area of Bari (Southern Italy) and stems from the need to clarify the aerobiologic features of this pollinosis. In the area of Bari, Cupressaceae pollinosis occurs during the winter months in the form of conjunctivitis variably associated with asthma and/or rhinitis (6; Caiaffa et al, unpublished observations). The occurrence of symptoms is strictly related to the presence of airborne Cupressaceae pollens.

These pollens were investigated in Bari over an 8-year period from

1982 to 1989. This paper deals with the annual airborne pollen sums, the fluctuations from year to year, the main pollen season, and the daily counts of airborne Cupressaceae pollens. In order to gather information about the species mainly responsible for the pollen presence, data regarding the distribution of Cupressaceae species in the area of Bari were also collected and presented in this paper.

Bari (41°7' N, 16°25' E), the capital of Apulia, is on the southeastern corner of Italy (Fig 1). It is situated on the coast, 0 to 20 m above (the Adriatic) sea level and is an industrialized, densely populated town with 371,000 inhabitants.¹⁸ In the inner town of Bari the vegetation is in the form of avenue trees and gardens and is characterized by evergreen trees and perennial plants. Bari is surrounded by an intensively cultivated suburban area rich in vegetation, mainly olive groves and vineyards. A high proportion of Cupressaceae are also present, particularly in a woody area 30 km south of the town. Further, Cupressaceae trees are also used for breakwind hedges to protect cultivated fields.

The climate is Mediterranean, typical of the areas situated in the western margin of the continents between the latitudes 30° and 40°. It is characterized by warm dry

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Figure 1. Map showing the location of Bari, Italy.

summers (July mean temperature +24.7°C) and mild winters (January mean temperature +8.7°C). The annual mean temperature is +16.4°C.²⁰ The annual mean precipitation is 537.6 mm with a winter maximum in December.¹⁸ The prevailing winds are generally northwesterly. In winter, they are more frequently westerly.

MATERIALS AND METHODS

Sampling

Since 1981 continuous atmospheric pollen sampling has been conducted in Bari. The data reported in this paper relate to the period from January 1982 to December 1989.

The sampler was a Burkard 7-day volumetric sporetrap, Hirst-type.²¹ It was placed on the roof of the University of Bari's Department of Clinical Immunology and Allergology, 20 m above street level. The Department is located in the center of the town, in a densely populated zone. The sampling airflow rate was 10 L/min. Pollens were caught on a 24-mm wide transparent tape coat by a thin film of silicon. This tape was mounted on a cylinder rotating

at a speed of 2 mm per hour. A complete rotation of the cylinder took seven days. Weekly tape strips were cut into seven pieces, each 48 mm in length. Each piece corresponded to one day sampling. They were then mounted and stained in the following mixture: gelatin : glycerin : H₂O : phenol : basic fuchsin (7.2 : 48.4 : 43 : 1.4 : 0.0001; wt/wt/wt/wt/wt), and examined microscopically. Four longitudinal bands were scanned at a magnification of ×250. Daily pollen concentrations in grains per cubic meter of air were obtained by multiplying the number of pollen grains counted by a conversion factor (CF), determined as follows²²:

$$CF = \frac{A(dt)}{D \times 48 \times 4 \times 14.4}$$

where A(dt) is the area of the daily deposition trace corresponding to a rectangle the length of which is 48 mm (two mm per hour per 24 hours), while the height is 14 mm (the size of the orifice of a Hirst-type sampler being 14 × two mm). The area of the deposition trace is 48 × 14 = 672 mm². D is the di-

ameter of the microscopic field measured for any single microscopic settings. D is also the height of the rectangular bands actually scanned. 48 is the length in mm of the bands. 4 is the number of bands scanned and 14.4 is the volume of air in m³ sucked in 24 hours if the suction rate of ten liters per minute is applied. Therefore

$$CF = \frac{0.243}{D}$$

The identification was performed in accordance with the literature² and pollen specimens derived from individual Cupressaceae trees identified either as *Cupressus arizonensis* Green or as *Cupressus sempervirens* L.

Pollen Data Presentation

The data regarding the annual counts were obtained by the addition of the daily counts. These annual sums referred to the period from 1 January to 31 December of each year studied. Any unavailable data, due to mechanical breakdown of the trap, were calculated as the average from the day before and the day after the breakdown. The main pollen season, according to Lejoly-Gabriel,²⁵ started from the day when the sum of the daily pollen grain catches had reached 5% of the total amount, provided that count on this particular day contributed at least 1% to the total. The period ended on the last day with count being more than 1% of the total and preceded by two days with a combined total of at least 3%. The definition of the high season was found to suit the pollination of Cupressaceae since more than 85% of the annual counts could be encompassed. Furthermore, this definition has been frequently applied.²⁶

Cupressaceae Distribution Data

Information about the presence and cultivation of Cupressaceae in the area studied was collected from: (1) Bureau "Gestione Foreste Regionali," Regione Puglia, which controlled the reforestation of the region in the years studied;

(2) the three largest nurseries in Bari, namely: Luisi Vivai, Garden Center Natura; Grandi Vivai Fratelli De Grecis, Piante Ornamentali e Fruttifere; Grandi Vivai Amoruso-Manzari;
(3) Institute Orto Botanico of the University of Bari.

RESULTS

Pollen Counts

Remarkable amounts of airborne Cupressaceae pollens were recorded in the years between 1982 and 1989 as shown by the annual sums (Fig 2) and by the highest daily counts (Table 1).

Evident fluctuations from year to year were observed. A definite tendency towards a 2-year cycle was noted (Fig 2 and Table 1).

The main pollen season extended from the end of January to March. From the analysis of the data relevant to the years studied, the beginning of the main season occurred in a period of 20 days between the end of January and the first days of February (Table 1). The duration was on average 45 days, ranging from a minimum of 25 days in 1989 to a maximum of 61 days in 1987. The end was always found in March. It was not possible to define the begin-

ning and the end of the main pollen season in the years 1982 and 1986.

During the other months of the year these pollen grains were irregularly observed and when observations were made they were always in low amounts.

A typical pollen season is illustrated in Figure 3. Its trend applies to all the years studied. In 1985, the pollen counts increased during January reaching high values in a few days. The main pollen season extended itself into February and March. At the end of March the counts decreased and by mid-April the pollination could be considered as ended. A clear polyphasic trend can also be noted from the same figures.

The highest daily counts and the dates thereof were also evaluated (Table 1). The highest value, 1,615 grains per cubic meter of air, was registered on 11 February 1983. Furthermore, a typical feature of the Cupressaceae pollen season in Bari was the marked changeability from day to day, as indicated by the daily counts of 1985 and 1988 (Fig 4). From a qualitative point of view, Cupressaceae pollen grains observed in our sampling showed a uniformity of pollen morphology²⁷, but dimensions varying from 15 to 30 μ in diameter.

Cupressaceae Distribution Data

Large numbers of Cupressaceae trees have been planted in the area of Bari during the last forty years. Data were collected to identify the main genera and species of Cupressaceae present. *Cupressus* was the most widespread genus. *Thuja*, *Juniperus* and *x Cupressocyparis* were represented but in smaller quantities.

According to the Bureau "Gestione Foreste Demaniali Regionali," Regione Puglia, during the period from 1982 to 1989, approximately 250,000 *Cupressus arizonica* Green were planted on public land for reforestation purposes, and 150,000 on private land. Many thousands of

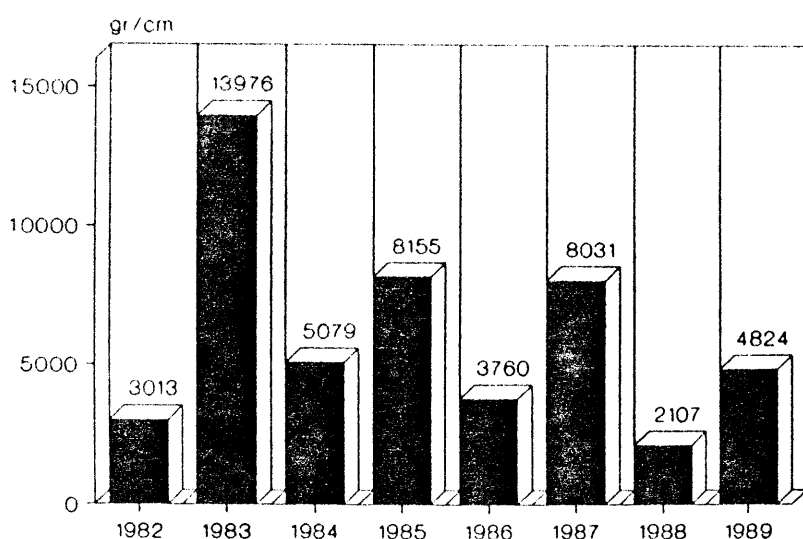


Figure 2. Airborne Cupressaceae pollen, in Bari, from years 1982 to 1989. Annual sums expressed as grains of pollen per m³ of air (gr/cm).

Table 1. Airborne Cupressaceae Pollen in Bari, from Years 1982 to 1989*

Year	Peak Counts		Season		
	Grains/m ³	Date	Onset	End	Duration
1982	ID†	ID	ID	ID	ID
1983	1,615	11 Feb	29 Jan	23 Mar	54
1984	454	22 Jan	20 Jan	3 Mar	44
1985	711	3 Mar	2 Feb	29 Mar	56
1986	266	31 Jan	ID	ID	ID
1987	515	19 Feb	27 Jan	28 Mar	61
1988	294	13 Mar	15 Feb	14 Mar	29
1989	368	27 Feb	9 Feb	5 Mar	25

* The highest daily count expressed in grains of pollen per m³ of air and the date thereof. The date of the beginning, the date of the end and the duration in days of the main pollen season are also shown.

† ID: incomplete data. Analyses of 1982 and 1986 were judged to be partially unreliable due to the high rate of missing daily observations.

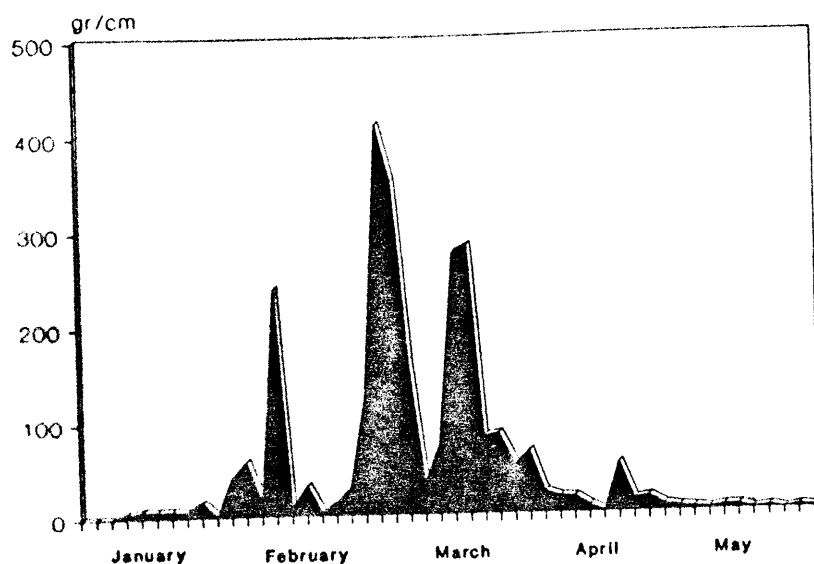


Figure 3. Cupressaceae pollen counts in Bari, 1985. Three day mean values. Data expressed as grains of pollen per m^3 of air (gr/cm).

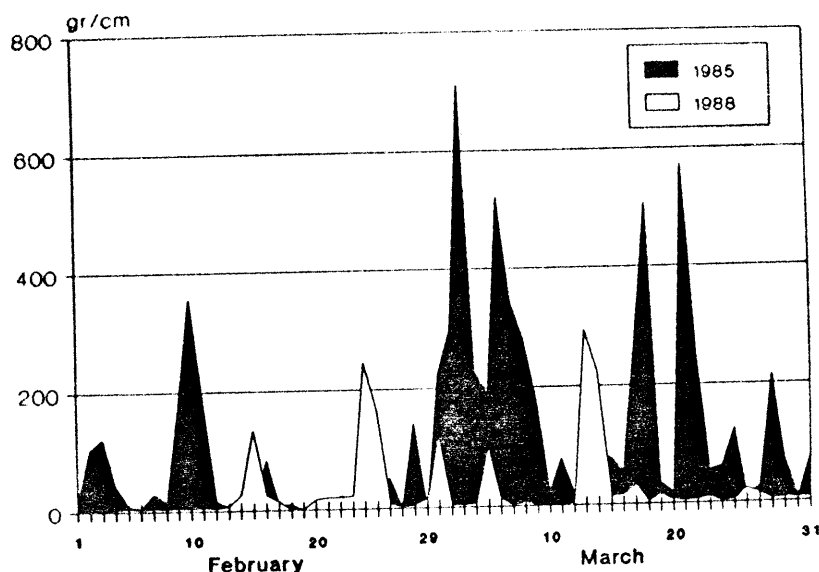


Figure 4. Cupressaceae pollen counts in Bari, in 1985 and 1988. Daily values expressed as grains of pollen per m^3 of air (gr/cm). The years 1985 and 1988 were chosen as representative of a year with relatively high counts and a year with low counts, respectively.

Cupressus sempervirens L. were also planted.

According to the estimate released by the three most important nurseries mentioned above, about 30,000 Cupressaceae of various species were planted for garden use in the urban area of Bari from 1982 to 1986. The species used the most

were *x Cupressocyparis leylandii* Jacks. et Dall., *Cupressus arizonica*, *Cupressus sempervirens* "Pyramidalis," *Cupressus macrocarpa* Hartweg, *Cupressus macrocarpa* Hartweg "Lutescens," and *Chamaecyparis lawsoniana* (Murray) Parl. With the exception of *x Cupressocyparis leylandii*, there has been a

lower employment of Cupressaceae for garden use since 1987. During the last 2 years studied, approximately 3,500 plants of this species were sold making it the most species sold.

DISCUSSION

Knowledge of the airborne pollen composition of the air is basic to the identification of the main respiratory allergies in a defined area.¹ The data presented above showed the considerable importance of the airborne Cupressaceae pollen grains in the area of Bari, where they were responsible for very high pollen counts in the years 1982–1989. Cupressaceae pollens were recorded in the largest amount in late winters and early springs.

Large total pollen counts and winter or late winter and early spring occurrence feature in most of the reports about Cupressaceae pollens relevant to countries situated in the northern hemisphere.^{3,4,10–12} In non-tropical climates, allergenic pollens are usually released in the atmosphere in spring and summer. As a consequence, pollinosis is ordinarily regarded as a spring and summer disease.

Cupressaceae pollens instead, may cause allergic symptoms during winter months. Awareness of this possibility may support physicians in the correct recognition of the cause of such symptoms avoiding their misinterpretation as relevant to viral-related or cold-related conditions.

The Cupressaceae family includes a number of species that grow naturally or are cultivated as ornamental plants in many parts of the world. It is conceivable that the winter occurrence of the pollen season and the relevant allergic symptoms could have caused an underestimation of Cupressaceae allergy in some areas.

Remarkable fluctuations of these pollens in a 2-year cycle were observed. The annual variation in the abundance of Cupressaceae pollens could be explained on the basis of

the alternation of pollen production in a 2-year cycle. This phenomenon has also been observed in the area of Bari for olive pollen. These fruit-trees have a biennial cycle.²⁸ This cycle depends on the competition for nutritional metabolized substances of ripening fruits versus developing gemmae. Biennial pollen rhythms have also been reported for allergenic airborne pollens such as *Betula* (birch)²⁶ and *Quercus* (oak).²⁹ It was hypothesized that the cycle of oak pollen production was an inherent physiologic characteristic of the species related to biotic nutritional rhythms. This hypothesis could also be applied to the 2-year cycle of Cupressaceae.

The duration of the symptomatic season of a given pollinosis is related to the main pollen season of the relevant agent, although the former does not exactly overlap the latter. In our study, the duration of the main pollen season was about 45 days, between the months of February and March. This is a relatively extended period of time. To our knowledge, there are no other reports dealing with this aspect of Cupressaceae pollinosis. The chance of direct comparison is therefore not available. One could, however, speculate that this trend applies to other countries, particularly those situated in the Mediterranean basin.

The polyphasic trend of the pollen season we observed could be related either to meteorologic conditions or to the flowering of Cupressaceae plants at different times. The marked changeability of pollen concentration from day to day appeared to be another of the most important features of the Cupressaceae pollen season. It could be explained by the meteorologic variability of the winter season.

The fluctuations from year to year, the extent of the main pollen season, its polyphasic trend and marked changeability from day to day have important practical consequences. Awareness of these aerobiologic features does help in the

evaluation of the variations in clinical symptoms in the affected subjects, and in the forecasting of seasonal severity of pollinosis.

In Cupressaceae allergy as well as in other pollinosis, knowledge of the airborne pollen season is crucial if all the diagnostic tools are to be applied. This knowledge permits, in particular, the verification of the clinical history and the pollen-symptom correlations.⁶ This knowledge also makes possible the carrying out of a correct *in vivo* diagnosis based on skin prick tests and conjunctival provocation tests.

Collecting data on the distribution of allergenic cultivated species is particularly informative and useful for possible applications. Cultivated plant distribution is highly influenced by human activities. Urbanization, land utilization, and introduction of ornamental plants may substantially change the plant cover of a certain area and consequently, the atmospheric pollen composition within a relatively short time. On the other hand, it is possible to prevent the increase of potentially allergenic pollens by an appropriate selection of the ornamental and noneconomic plants to be introduced.³

Cupressaceae are not native to the South of Italy. In particular, *Cupressus sempervirens*, also called "Italian Cypress," was imported by the Romans from the Middle East. *Cupressus arizonica* was imported in more recent years. At present, they are ideally suited to the climate

of many areas in Italy. From the point of view of the environmental distribution of Cupressaceae in the area studies, the role of changes for reforestation and garden use has been remarkable. In particular, they have been extensively planted in the last 40 years.

These environmental changes due to reforestation and garden use might have played an important epidemiologic role in the area of Bari. The increased use of Cupressaceae that has been observed could have been a reason for an increasing Cupressaceae pollinosis prevalence. Unfortunately, aerobiologic data as well as epidemiologic data are not available from the years before 1982. There is therefore no possibility of proving such an increase.

As stated above, based on proper aerobiologic observations, the diagnosis of Cupressaceae pollinosis is not a particularly challenging one because of the relatively isolated winter pollination of these plants. The occurrence of Cupressaceae pollinosis in Bari relevant to 1988 was 9% in all subjects with pollinosis (Caiaffa et al, unpublished observations). This rate placed Cupressaceae fifth after Graminaceae, *Parietaria*, olive and Compositae as a cause of pollinosis as can be seen in Table 2. Also indicated in the same table are the annual pollen sums relevant to these pollens to enable interpollen comparisons of the relative allergenic strength to be made. Based on these data, one can speculate that Cupressaceae pollens

Table 2. Annual Pollen Sums and Occurrence Rates Relevant to the Five Most Important Allergenic Pollens in Bari in 1988*

	Annual Pollen Sums gr/m ³	Occurrence %
<i>Parietaria</i>	2,255	54.30
Graminaceae	1,669	49.94
Olive	12,012	49.16
Compositae	229	19.22
Cupressaceae	2,107	12.60

* The annual pollen sums are expressed as pollen grains/m³ of air. The occurrence rates represent the percentage of patients sensitive to the considered pollens, out of all the patients seen at our clinic in 1988 and given treatment as a result of suffering from any pollinosis (Caiaffa et al., unpublished observations).

are relatively weaker allergens than other pollens, such as Gramineae or *Parietaria*, commonly found in the area of Bari.

Cupressus arizonica and *Cupressus sempervirens* should be considered the two species most responsible for Cupressaceae pollinosis in the area studied.

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