

## The aerobiological results from the 1994 cruise of the *Urania* (cnr) on the Adriatic. I. Pollen and spore counts on the Mediterranean sea as compared to mainland Italia

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### Abstract

In July 1994, we were able to collect airborne fungal spores and pollen grains over the Adriatic Sea from the upper deck of the Oceanographic Ship *Urania* (CNR). The biological particles were collected using a modified Lanzoni VPPS 1000 sampler (operating at a flux of 10 LPM), on glycerine-gelatine coated microscopic slides. Not only were the airborne concentrations of different organisms estimated, their viability was also tested with a 1% TTC solution. Particles were collected for 60 min (i.e. a volume of 600 liters of air sampled) at every 2 h from 0600–2100 h. Up to 689 pollen grains/m<sup>3</sup> and an impressive 48 990 spores/m<sup>3</sup> were collected daily. Forty-two fungal taxa were identified and the most abundant spores collected were *Cladosporium* (82.6%), *Smuts* (4.8%), *Ascospores* (2.8%), *Basidiospores* (2.1%) and *Alternaria* (1.7%). 20 pollen taxa were identified, and the dominant pollen were *Urticaceae* (57.9%), *Graminaceae* (20.7%), *Fagaceae* (2.4%), *Plantaginaceae* (1.4%), *Pinaceae* (1.3%) and *Eucalyptus* (1.1%). The most abundant captures were done at 0800 and 1000 h (17.8 and 16.7% respectively) and at 1400 and 1600 h (13.2 and 13.8% respectively). Pollen viability per species ranged from 0 to 100%, but for the most abundant taxa, it ranged from 3.8 to 75%, and averaged 27.7%. Maximum viability was found at 0800 and 1200 h. Pollen concentrations were of the same order of magnitude as the ones found on the mainland (Brindisi, Chieti, Matera). However, its specificity was evident. Future work should therefore look more at the pollen transport process which should account for this different assemblage of pollen.

**Keywords:** Pollen; Spore; Adriatic; Sea; Viability; Italy

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### 1. Introduction

Airborne particles are well known travellers over the Mediterranean sea (see for example Tomadin et al., 1989 who have collected dust at the seawater-air interface). However, pollen grains and fungal spores, which are abundant over the mainland, where their sources are located, have rarely been studied in depth. In addition, they have two fundamental characteristics which warrant further investigation: (1) they are generally of a higher size diameter than other particles, and

(2) they can give us access to another realm: the viability of airborne particles. Moreover, the extensive airborne pollen monitoring networks, available all over Western Europe, would allow us to do a comparative study between mainland (source) pollen concentrations and those found at sea (dispersed).

At the Seawater-Air interface, viable and biological particles can be collected free from any local contribution, and samples are all of long range dispersal origin (*sensu lato*). In the present study, we had no control whatsoever over the ship journey or direction. Therefore, it was not possible to achieve a study of specific long range transport system, such as from the Sahara to southern Europe. Even with these constraints taken

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into account, the systematic sampling of the air on the ship upper deck has allowed us to have a clear picture of the overall biological air content over the Mediterranean sea. The specific aim of this study was to reach conclusions as to the potential of the aerobiological content at the seawater-air interface for future studies and to compare the pollen counts obtained with the one found at existing aerobiological stations on the Mainland.

## 2. Methods

In July 1994, we had the opportunity of collecting airborne biological particles on board of the *Urania*, an oceanographic ship from the CNR. From July 14 to July 25, the *Urania* has cruised the whole width of the Adriatic sea, between 42° and 39° latitude North, beginning at Ancona, and ending at Ravenna, with a last pass alongside the eastern coast of Italy, up to 43°N (Fig. 1).

Because we had no pre-conceived idea of the concentration of biological particles over the Adriatic in summer, two different sampling protocols were used. One of this protocol also allowed the estimation of the viability of the collected pollen grains, while the second allowed us to collect overnight.

The punctual estimation of the airborne biological particles was done using a modified Lanzoni VPPS 1000 sampler, operating at a flux of 10 LPM for 1 h, on a glycerine-gelatine coated microscopic slide. Particles were collected at every 2 h from 0600 to 2100 h, and the sampler head was positioned very near (less than 2 cm) from the upper deck side, directly in the coming wind, so that we always collected long range particles transported passively along with air masses. This protocol has given us samples of 600 l, which correspond to a precision of 1.66 particles per cubic meter. We collected 8 samples a day for 12 consecutive days. Taking into account the discarded samples, we had a total sampling size of 93.

Not only were the airborne concentrations of different organisms estimated, their viability was also tested. The sampled dust band of 2 × 14 mm on the microscopic slide was bathed in a 1% TTC (2,3,5 — Triphenyl tetrazolium chloride) solution (10% TTC in 60% Saccharose) added to the samples before being covered with a cover slip. This test was done in the field immediately (less than five min) after sampling. TTC is a biological stain that is used to detect respiration (= viability) of cells. Therefore a viable pollen takes a red staining, while a dead pollen remains yellow.

The second protocol involved a High Volume (HI-VOL) sampler made by the CNR. This sampler collects particles over an horizontal filter of

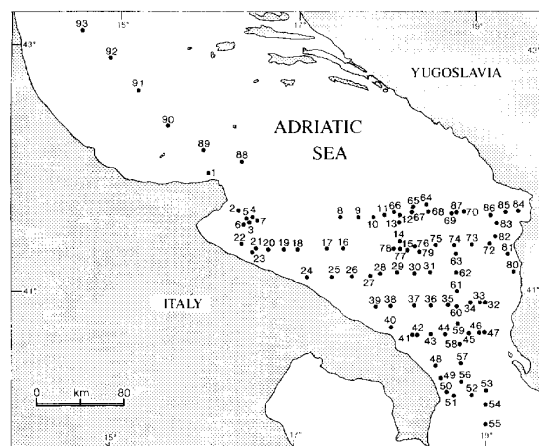


Fig. 1. Sampling sites location.

polycarbonate (Nucleopore) of 15 cm in diameter. The sampler was operated at a mean flux of 50 m<sup>3</sup> per h. The precise flux (varying from 50.5 to 51.4 m<sup>3</sup> per h) was checked every 12 h. The sampler was fixed over the upper deck and the filter was located approximately at 1.5 meters from the deck floor. Filters were protected from precipitations by a conic head. Half day samples were taken (0600–1800 h; then 1800–0600 h).

Therefore samples of 600 m<sup>3</sup>, which correspond to a precision of 0.0016 particles per cubic meter, were collected. Because of operational difficulties with the electrical circuits of our sampler, only a total sampling size of 13 was achieved.

Half filters, solubilized in 50 ml of chloroform were centrifuged. The supernatant was discharged and the precipitate was deposited over 3 microscopic slides and mounted with glycerine/gelatine. Since all the particles on these 3 slides needed to be counted, only pollen concentrations was estimated with this protocol.

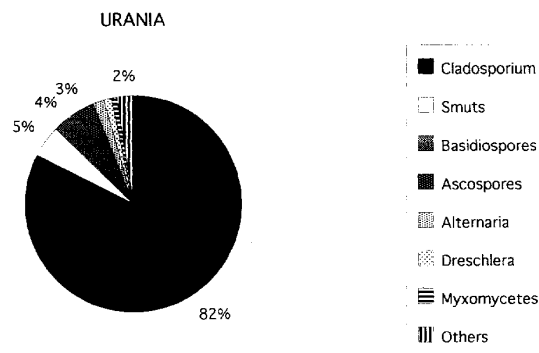


Fig. 2. Fungal spore dominance found on the *Urania*.

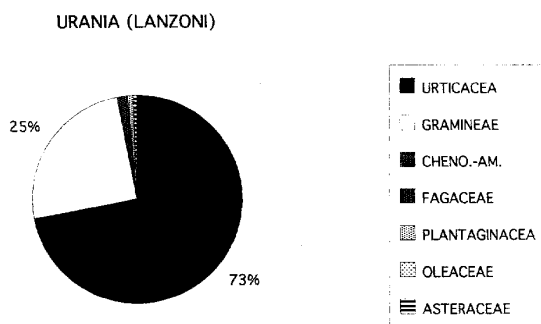


Fig. 3. Pollen taxa dominance found on the Urania (Lanzoni sampler).

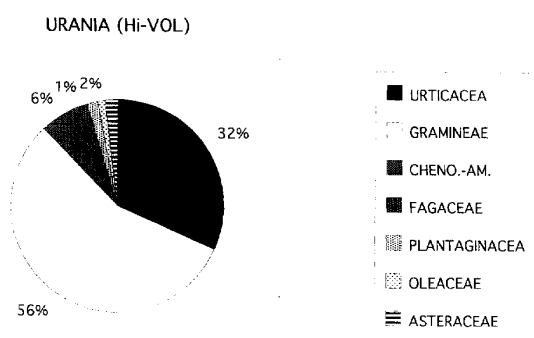


Fig. 4. Pollen taxa dominance found on the Urania (Hi-Vol sampler).

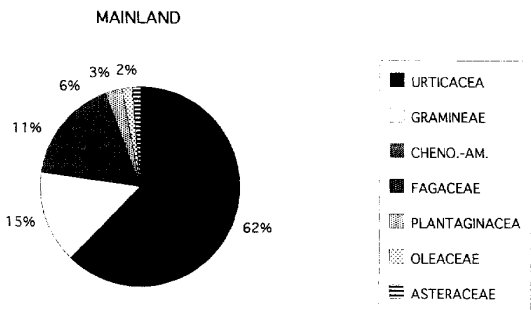


Fig. 5. Pollen taxa dominance found on the Italian Mainland (AIA).

### 3. Results

#### 3.1. Taxonomical diversity

Up to 689 pollen grains/m<sup>3</sup> and an impressive 48 990 spores/m<sup>3</sup> were collected daily. Forty two fungal taxa were identified and the most abundant spores collected were *Cladosporium* (82.6%), *Smuts* (4.8%), *Ascospores* (2.8%), *Basidiospores* (2.1%) and *Alternaria* (1.7%) (Fig. 2). This pattern totally reflects the cosmopolitan fungal spore flora found all over the world.

On the Lanzoni samples, 20 pollen taxa were identified, and the dominant pollen were Urticaceae (57.9%), Gramineae (20.7%), Fagaceae (2.4%), Plan-

Table 1

Listwise Pearson's correlation coefficients

| Compared set     | Coefficient | Significance |
|------------------|-------------|--------------|
| Brindisi/Matera  | 0.97        | ***          |
| Brindisi/Lanzoni | 0.95        | ***          |
| Matera/Brindisi  | 0.90        | ***          |
| Lanzoni/Chieti   | 0.88        | ***          |
| Brindisi/Chieti  | 0.77        | *            |
| Hi-Vol/Chieti    | 0.76        | *            |
| Hi-Vol/Lanzoni   | 0.65        | N.S.         |

\*\*\*,  $P = 0.001$ ; \*,  $P = 0.05$ ; N.S., non-significant.

taginaceae (1.4%), Pinaceae (1.3%) and *Eucalyptus* (1.1%) (Fig. 3). On the Hi-Vol samplers, the larger precision (by a factor of 1000) allowed us to collect more taxa ( $n = 34$ ). However, even if the pattern of dominance was different, the most important taxa list was more or less the same, with Gramineae (36.2%), Urticaceae (20.5%), Chenopodiaceae/Amaranthus (3.9%), Plantaginaceae (1.2%), Asteraceae (1.1%), Fagaceae (0.9%) and Oleaceae (0.7%) (Fig. 4).

If we compare these long range data to the pollen collected on the mainland with similar samplers (Lanzoni VPPS 2000) by the Italian Aerobiology Association (AIA) Pollen Network, at Brindisi, Chieti and Matera, the averaged data (Fig. 5) also gives us a dominance of Urticaceae (40.4%), Gramineae (9.7%), Chenopodiaceae/Amaranthus (6.7%), Fagaceae (4.3%), Plantaginaceae (1.7%), Oleaceae and Asteraceae (0.8%). However, it must be noted that at these stations, only the 16 more important taxa are routinely identified.

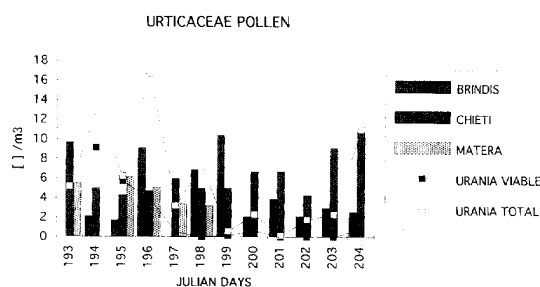


Fig. 6. Urticaceae daily pollen concentrations.

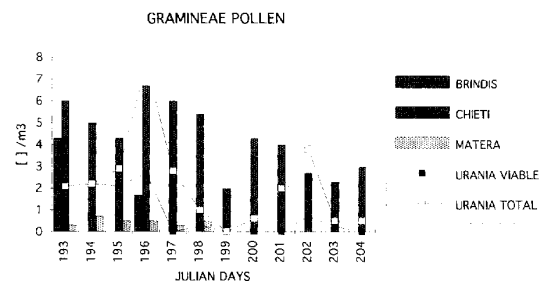


Fig. 7. Gramineae daily pollen concentrations.

Table 2  
Bilateral paired t test between Sea and Mainland

| Paired set        | Gramineae |        | Urticaceae |        |
|-------------------|-----------|--------|------------|--------|
|                   | t value   | signi. | t value    | signi. |
| Brindisi/Urania V | −0.74     | N.S.   | 1.35       | N.S.   |
| Urania V/Chieti   | −8.88     | ***    | 3.74       | **     |
| Urania V/Matera   | 2.26      | N.S.   | 0.60       | N.S.   |
| Urania V/Urania T | −3.13     | **     | −3.39      | **     |
| Brindisi/Urania T | −2.76     | *      | −1.29      | N.S.   |
| Chieti/Urania T   | 4.24      | ***    | 0.39       | N.S.   |
| Matera/Urania T   | −2.77     | *      | −1.91      | N.S.   |

\*\*\*,  $P = 0.001$ ; \*\*,  $P = 0.01$ ; \*,  $P = 0.05$ ; N.S., non-significant, V, viable; T, total.

A comparison of these different data set, using a listwise correlation coefficient gives us the  $r$ 's (in decreasing order) of Table 1.

### 3.2. Day to day variations

Figs. 6 and 7 show respectively Urticaceae and Gramineae pollen concentrations and viability variations, at all mainland stations and from the Lanzoni Urania samples. If pollen concentrations were generally lower on the Urania, this was not the case for all stations. For Gramineae, the decreasing order of mean daily concentration was: Chieti ( $4.3/\text{m}^3$ ) > Urania-total ( $2.2/\text{m}^3$ ) > Urania-viable ( $0.8/\text{m}^3$ ) > Brindisi ( $0.5/\text{m}^3$ ) > Matera ( $0.4/\text{m}^3$ ). For Urticaceae, the order was almost the same, the biggest exception being for viable pollen on the Urania: Chieti ( $6.4/\text{m}^3$ ) > Urania total ( $5.8/\text{m}^3$ ) > Matera ( $4.3/\text{m}^3$ ) > Brindisi ( $3.6/\text{m}^3$ ) > Urania-viable ( $1.8/\text{m}^3$ ).

A comparison of these mean values, using a bilateral paired t test (Table 2) shows that for Gramineae, Brindisi/Urania viable and Matera/Urania viable comparisons were non significant, while all Urania-total

Table 3  
Pairwise Pearson's correlation coefficients

| Pairwise set          | Gramineae |        | Urticaceae |        |
|-----------------------|-----------|--------|------------|--------|
|                       | r         | signi. | r          | signi. |
| Urania V/<br>Urania T | 0.72      | ***    | 0.65       | *      |
| Matera/Urania<br>V    | 0.58      | **     | 0.84       | *      |
| Chieti/Urania T       | 0.58      | **     | −0.04      | N.S.   |
| Brindisi/Urania<br>T  | 0.30      | N.S.   | 0.18       | N.S.   |
| Chieti/uranian V      | 0.49      | *      | −0.35      | N.S.   |
| Brindisi/Chieti       | 0.51      | **     | −0.37      | N.S.   |
| Brindisi/Matera       | −0.50     | N.S.   | −0.17      | N.S.   |
| Matera/Chieti         | −0.44     | N.S.   | −0.17      | N.S.   |

\*\*\*,  $P = 0.001$ ; \*\*,  $P = 0.01$ ; \*,  $P = 0.05$ ; N.S., non-significant; V, viable; T, total.

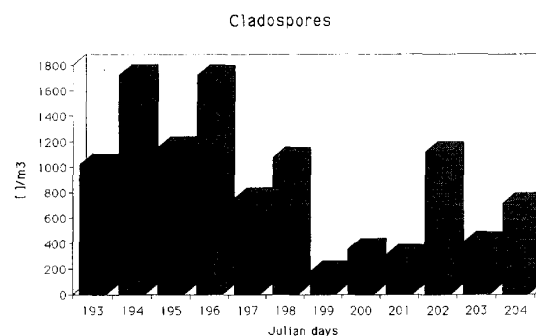


Fig. 8. *Cladosporium* daily concentrations.



Fig. 9. Basidiospores daily concentrations.

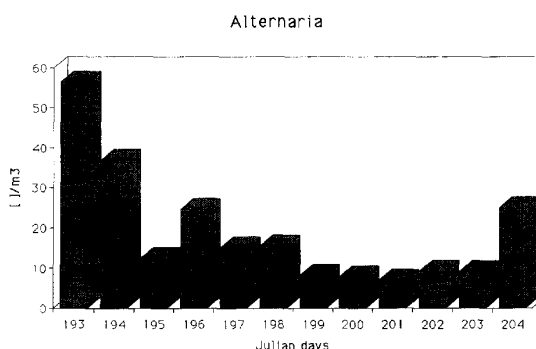


Fig. 10. *Alternaria* spores daily concentrations.

comparisons were significant. For Urticaceae, all comparisons were non-significant, with the exception of Chieti/Urania-viable.

Looking at the overall Pollen curves, and not only at the central tendencies parameters, we can see (Table 3), again Using a Pairwise  $r$  correlation coefficient, that many significant correlations can be found between the mainland and the Urania Pollen concentrations. If the highest correlation was found between Urania Total and Urania viable for the Gramineae, this was not the case for the Urticaceae, where Urania Viable Pollen was more correlated to Matera concentrations.

It is quite visible on Figs. 6 and 7 that pollen viability pattern variations mimic the one found for total concentrations. However, peaks are more pronounced in

the viability curves, as can be seen on Julian dates 196 and 202 for Gramineae and Julian dates 194, 200 and 204 for Urticaceae. For this last taxa, there were also some increases of viability at the same time as there was a decreasing trend in concentrations.

Figs. 8–10 represent fungal spores day to day variation on the Urania. Only *Cladosporium*, Basidiospores and *Alternaria* are presented. Unfortunately no data is available from the mainland, and therefore no comparison analysis could be made, as we have done for major pollen grains. From these graphs, we can see that fungal spores are not as variable, on a day to day basis, as pollen grains. Cycles have generally an amplitude of 2–3 days. Contrary to pollen grains, fungal spores seem to be less influenced by emission phenology and more by transport parameters.

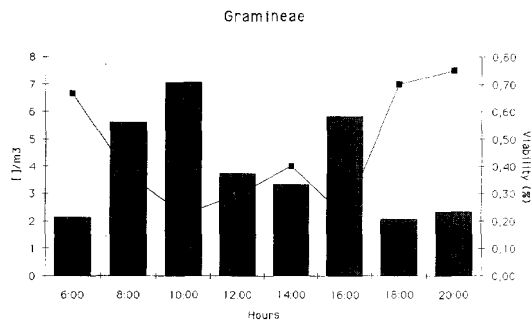


Fig. 11. Gramineae hourly pollen concentrations and viability.

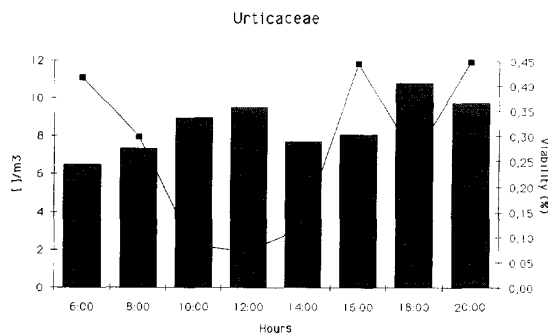


Fig. 12. Urticaceae hourly pollen concentrations and viability.

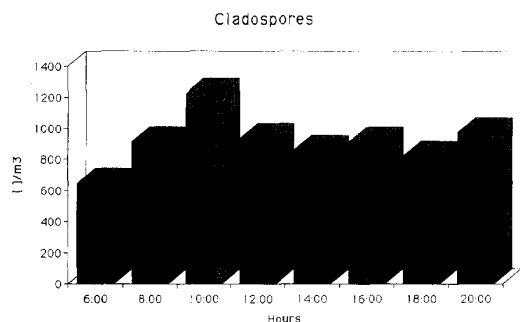


Fig. 13. *Cladosporium* hourly concentrations.

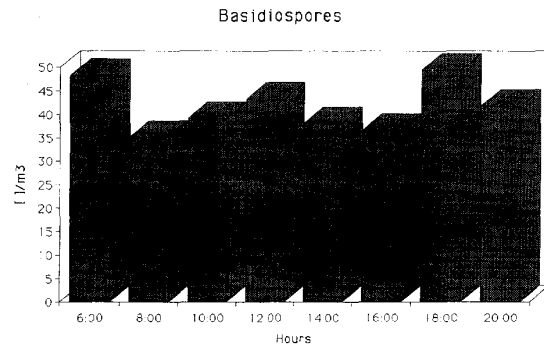


Fig. 14. Basidiospores hourly concentrations.

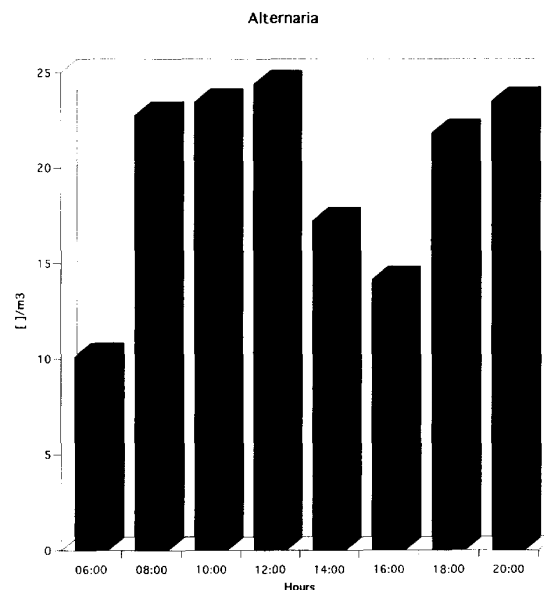


Fig. 15. *Alternaria* spores hourly concentrations.

### 3.3. Hourly variations

As was mentioned in the introduction, viability estimates allow some sort of knowledge over aging. Contrary to day to day variations, diurnal viability pattern is not related to pollen concentrations (Figs. 11 and 12). Pollen viability has a similar pattern for both Urticaceae and Gramineae, and minimum viability was found at the hours of maximum emission (end of morning–mid-day). Therefore, hourly viability is a good indicator of long distance transport, which could be estimated from these data to be a 12-h process. Urticaceae pollen showed hourly variations of low amplitude, while Gramineae showed larger variations, especially because of low concentrations between 1800 and 0600 h.

Fungal spores hourly variations (Figs. 13–15) are quite stable. Again, this is a reflection of amplitudes felt over more than 24 h as was seen in the day to day variations of concentrations.

Table 4  
Mean and paired t test for night and day pollen counts

| Taxa           | mean [ ] day | mean [ ] night | Paired t value | Probability |
|----------------|--------------|----------------|----------------|-------------|
| Fagaceae       | 20.0         | 11.5           | 2.11           | 0.12        |
| Cheno./Am.     | 75.5         | 48.5           | 1.05           | 0.37        |
| Graminaceae    | 750.5        | 385.0          | 2.72           | 0.07        |
| Urticaceae     | 452.5        | 398.5          | 1.44           | 0.24        |
| Plantaginaceae | 28.2         | 16.2           | 1.43           | 0.24        |

The Hi-Vol sampler allowed us to separate day and night concentrations. For major pollen types, even if all taxa showed a larger concentrations by day, none of these differences were significant. This is probably because of a low sampling size ( $n_1$ : 6;  $n_2$ : 5), and overall low airborne concentrations. Indeed, for the most abundant pollen type (Graminaceae), the difference is almost significant ( $P = 0.07$ ) (Table 4).

#### 4. Discussion and conclusion

Taxonomical diversity found on the Urania was significantly correlated to the one found on the mainland, and especially for Lanzoni samplers. This is a good indication that using a Hirst type spore trap over the Mediterranean sea allowed us to follow adequately pollen transport from Italy to the Adriatic.

If maximum pollen concentrations were found on the mainland (at Chieti), the Urania concentrations were often higher than those found elsewhere on the continent, and generally showed no significant differences. Using viable pollen only did bring a significant difference.

Viable pollens are therefore a different sub-set than total pollen load. However, the daily variations of these two sets were significantly correlated, and most Graminaceae pollen variations on the mainland could be forecasted from the Urania data.

Highest load of pollen was received by day, but no precise daily emission pattern was not found in the daily pollen curves. This pattern was found for viability, but with a delay of about 12 h. Daily and hourly

fungal spores variations were insignificant and could not be directly related to daily transport pattern.

On the Urania, maximum pollen concentration was found on July 17th, while minimum pollen load was found on July 20th. Synoptic situations on these dates show that on July 17th, a High pressure zone was found on central Europe and a Low pressure zone was found over the southern coast of the Mediterranean sea and the Atlantic coast of France. On July 20th, a High pressure zone was located over the Atlantic and North sea and a Low zone could be seen all over southern Europe. These contrasting situations allowed or prevented transport.

In conclusion, it does seem that airborne pollen grains and fungal spores are abundant over the Adriatic, and that they mainly form a sub-set of what is emitted from the Italian peninsula. Contrasting abundance between the Adriatic and the continent are a reflection of air masses location, i.e. of the possibility or not of passive transport. Viability estimation is a prerequisite for finding evidence of long range transport in medium range dispersal while pollen assemblages are similar to those found at the nearest coast.

#### References

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