

L. ANTONICELLI¹, M.L. RUELLO², R.I. MONSALVE³, R. GONZÁLEZ³, G. FAVA²,
F. BONIFAZI¹

Assessment of airborne soy-hull allergen (Gly m 1) in the Port of Ancona, Italy

¹Department of Internal Medicine, Immuno-Allergic and Respiratory Diseases, Ospedali Riuniti, Ancona, Italy

²Department FIMET, Università Politecnica delle Marche, Ancona, Italy

³ALK-Abelló, R & D, Madrid, Spain

KEY WORDS

Soybean, Gly m 1, PM₁₀,
Ancona, epidemic asthma

SUMMARY

Background: Epidemic asthma outbreaks are potentially a very high-risk medical situation in seaport towns where large volumes of soybean are loaded and unloaded. Airborne allergen assessment plays a pivotal role in evaluating the resulting environmental pollution. **Objective:** The aim of this study was to measure the airborne Gly m 1 allergen level in the seaport of Ancona in order to assess the soybean-specific allergenic risk for the city. **Methods:** Allergen and PM₁₀ were evaluated at progressive distances from the port area. Allergen analysis was performed by monoclonal antibody-based immunoassay on the sampled filters. Daily meteorological data were obtained from the local meteorological station. For estimating the assimilative capacity of the atmosphere, an approach based on dispersive ventilation coefficient was tried. **Results:** The allergen concentrations detected were low (range=0.4–171 ng/m³). A decreasing gradient of the airborne allergen from the unloading area (22.1±41.2 ng/m³) to the control area (0.6±0.7 ng/m³) was detected. The concentration of the airborne Gly m 1 was not coupled with the presence of the soy-carrying ships in the port. A statistically significant relationship between airborne allergen, PM₁₀ and local meteorological parameters quantifies the association with the atmospheric condition. **Conclusion:** Airborne Gly m 1 is part of the atmospheric dust of Ancona. The low level of this allergen seems consistent with the absence of asthma epidemic outbreak.

Introduction

The Gly m 1 allergen, arising from soybean hull, can trigger an outbreak of epidemic asthma in seaport towns (1-3).

The outbreak of epidemic asthma poses a serious medical threat due to the large number of potential simultaneous cases and the severity of the attacks, which can be fatal (4).

Despite the large number of seaports in the world which load and unload soybeans, allergic asthma crises are poorly reported [Barcelona (Spain), Naples (Italy), and New Orleans (USA)] This may be due to the low likelihood of

the simultaneous combination of high levels of the airborne soybean allergen in the atmosphere and favourable meteorological conditions.

An alternative explanation is the underevaluation of soybean-induced asthma because of its misunderstanding in an at-risk population.

One study specifically designed to evaluate this hypothesis documents a small but significant relationship between the airborne presence of soybean dust and minor asthma crises, even in cities, where adverse health effects attributable to soybean bulk cargo handling were not reported (5).

In view of this background, the seaport city of Ancona (Italy) is an interesting examination site. The port is situated near the city centre, and an oil seed processing plant, working with unloaded soybean cargoes is located in the same area. The unloaded seeds are stored in special silos near the plant prior to processing.

In the past, when the same oil processing mill was also producing castor oil, minor asthma epidemics occurred, which especially affected the plant workers and the residents of the surrounding vicinities (6, 7). State-of-the-art technology to control dust emissions was installed, in order to reduce the health risk within the population. Moreover, in 1990 the unloading of castor beans was discontinued.

The average tonnage of soybean which has been unloaded in Ancona over the last 15 amounts to ~200.000 tonnes/year.

In the last 15 years, six patients with positive skin prick tests for soybean hull were detected in our Allergy Unit. Five of the six patients had been admitted to accident and emergency for a severe asthma attack (data not published). However, there is no record of an asthma epidemic.

Several explanations could account for the apparent small impact of soybean asthma in the city.

- ♥ The installation of efficient air pollution control technology, which makes the presence of an industrial soybean processing plant compatible with the health and safety regulations of its urban area (8, 9).

Moreover, based on the estimates carried out in Barcelona, (~1000 soybean allergic asthmatics out of ~1,700,000 exposed inhabitants) (1), it may be assumed that small number of these patients live in Ancona (~100,000 inhabitants), hence accounting favouring the misunderstanding of soybean induced asthma crisis.

The aim of this study was to measure the airborne levels of Gly m 1 in order to be able to accurately assess the magnitude of the soybean-specific allergenic health risk for the city.

Materials and methods

Sampling site and investigation strategy

Three sampling sites were chosen at gradually increasing distances from the area where soybean cargoes are unloaded and processed (Figure 1). The first sampling site (P) was located outside the soybean manufacturing plant within the port area. Samples were collected either during

unloading or processing for oil and flour. Samplings were performed at the flow rate of 10 L min^{-1} , with a 50% aerodynamic cut-off of $10 \mu\text{m}$. (Analitica Strumenti, mod. Air Cube, cod. 600/A30001P, Italy). Teflon filters were used as a sample impact surface (PTFE w PTFE Support, 47 mm diameter, $2.0 \mu\text{m}$ pore size, Omega Specialty Instrument Co., USA). A second sampling unit was placed at the exit of the port area (H), at a distance of about one kilometre from the unloading jetty (Wedding 10 micron inlet, Critical Flow High Volume Sampler, U.S. Patent 4461183, Wedding & Associates, Inc.). A third sampling unit (U) was placed on the roof of the University building about five kilometres from the port and 200 meters above sea level (PM_{10} reference method n° RFPS-1287-063, Andersen Sampler Inc. General Meta Work Inc., USA). High volume samplers were used at each of the sites (flow rate $1.13 \text{ m}^3 \text{ min}^{-1}$, glass fiber filter 203 mm x 254 mm, Grade G653, Whatman International Ltd. Maidstone, England).

Data for the unloading ships were provided by the Port Authority. The port of Ancona only has one dock where soybeans are unloaded and the analyses were conducted according to the unloading schedule.

The samples were collected on 50 different days over a 15-month period, from July 2004 to October 2005

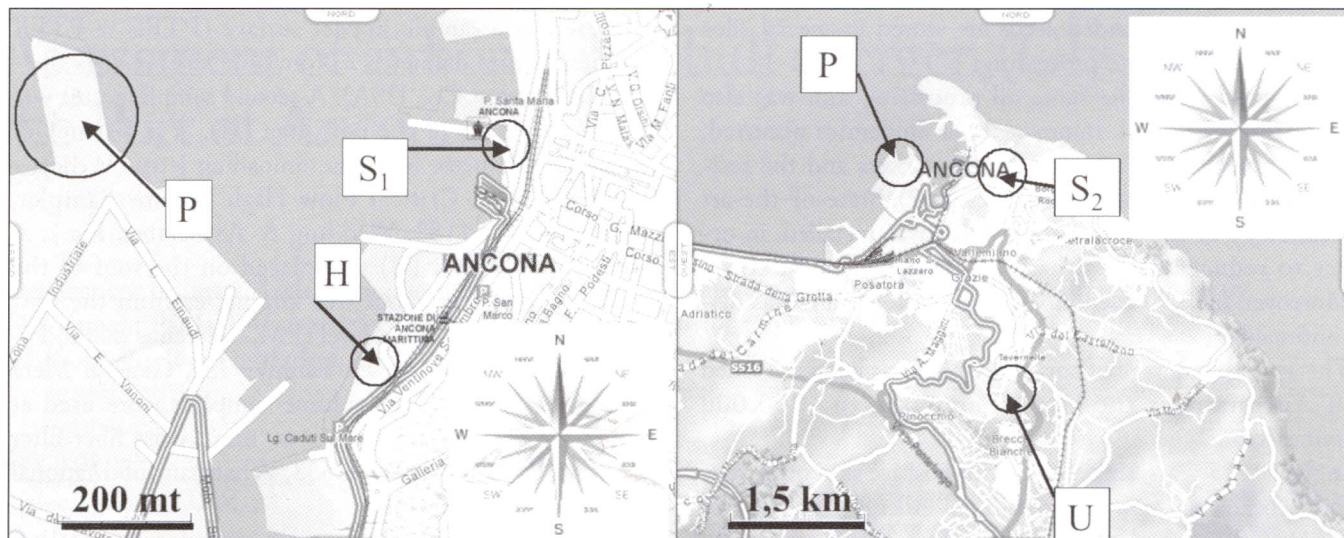
Daily meteorological data (temperature, relative humidity, and wind speed) were obtained from the local meteorological station.

In the urban area, the town council continuously monitors levels of particulate matter (PM_{10} , and $\text{PM}_{2.5}$). The monitoring network includes three permanent stations. Figure 1 shows the locations of two of these stations, one of which is in the port and the other in the city (S1, S2). A third station (S3) is located in a rural area distant from industrial and traffic exhaust emissions. The recordings between January 2004 and December 2005 at these three sites were analysed. These sites are exposures to varying degrees to port (S1) and urban sources (S2) of pollution, as well as peripheral environmental conditions (S3).

Consistent with the most common measured atmospheric parameters, the Weibull distribution proved to be the most appropriate method for calculating the airborne concentration of the allergen at any one site. Two theoretical distributions, log-normal and Weibull, were used to fit all the collected data of the measured PM_{10} and Gly m 1. The distributional parameters were estimated by the maximum-likelihood method for the theoretical distributions.

To estimate the assimilative capacity of the atmosphere, a dispersive-ventilation-coefficient-based approach was

Figure 1 - Sampling sites location: P = soybean manufacturing plant; H = exit of the harbour area; U = University building. In the maps are shown also the location of two permanent stations of the monitoring network: S1 and S2 respectively industrial (harbour) and traffic (urban) monitoring station.



adopted. Ventilation coefficient is the product of atmosphere mixing height and the average wind speed for the winter and summer seasons (10, 11).

Evaluation methods

Prior to the use of samplers, the filters were conditioned for 24 hours and then weighed (analytical balance KERN 770, Germany). After sampling, the filters were removed from the sampling heads and placed in sealed individual containers, reconditioned, and reweighed for PM_{10} mass evaluation. After weighing, the filters were kept dry and dark storage until treatment for Gly m 1 analysis (on the Teflon filter from the low-rate sampler and on five 37 mm dishes cut, using a steel punch, from each 203 mm x 254 mm glass fiber filter surface).

Allergen Gly m 1 evaluation was performed (within four weeks from sampling) following a procedure described elsewhere (12). Filter sections were extracted at 1% wt/vol in PBS-BSA-T (Phosphate-Buffered Saline PBS, containing 1% Bovine Serum Albumin BSA and 0.1% Tween®20), in an orbital shaker overnight at 4°C. The solution was then recovered by centrifugation, filtered through a 0.22 µm filter (Sartorius, Göttingen, Germany), and stored frozen in aliquots at -20°C until the sandwich-ELISA was carried out. This two-site ELISA was carried out as described (12). Briefly, 96-well microtiter plates (EIA/RIA plates, Costar),

coated with a specific anti-Gly m 1 monoclonal antibody (6G1) and blocked with the corresponding blocking buffer (PBS-BSA-T) were used to capture the allergen. The wells were washed and sequentially incubated with samples and references, biotin-labeled anti-Gly m 1 mAb 1G10, and streptavidin/peroxidase conjugate (Amersham, UK). Samples, controls and reagents were conveniently diluted in PBS-BSA-T, and all the incubations were carried out for 1 h at room temperature with intermediate washes between successive steps. Finally, the wells were incubated for 20 min at room temperature in the dark with a solution of Orthophenylenediamine (OPD, DAKO products), and the colour reaction was stopped by adding 2M HCl. The optical density was then read at 490 nm with a 650-nm reference filter. Samples were assayed in duplicate at several twofold dilutions. The Gly m 1 content of samples, in mass units, was obtained by interpolating in a standard curve from serial twofold dilutions of purified Gly m 1 (1:1 mixture of the two isoforms), the protein concentration of which had been determined by an amino-acid composition analysis.

Results

A total of 26 air filter samples were analysed. A summary of the local meteorological conditions during the sampling, as well as that of the PM_{10} and Gly m 1 concentrations is presented in Table 1. Gly m 1 was detected in 18 filter samples,

although not in the remaining 8 samples. Notably, the concentration of the airborne Gly m 1 was not always coupled with the presence of the soy-carrying ships in the port.

Airborne Gly m 1 levels ranged between 0.4 and 171 ng m⁻³, which were mostly <10 ng m⁻³. Since the level of the allergen generally represents the average value for 2 days, a dilution effect may occur. However, the low level of allergenic pollution is consistent with the low level of other environmental pollutants (benzene, carbon monoxide, nitrogen dioxide) as measured by the continuous monitoring stations S1 and S2, respectively located in the port

and city centre (data not shown). At both these sites the health risk threshold values were respected (13, 14).

A decreasing gradient of the airborne allergen from the unloading area was recorded even though statistical significance was not achieved. The highest airborne concentrations of Gly m 1 were found very close to the ship during the unloading in the port (sample P5, Table 1). Quantifiable levels of Gly m 1 were found in 9 out of 11 samples of the port's atmosphere; in the samples obtained at the university site (5 km from the port) lower levels of Gly m 1 were found in 9 out of 14 samples. The mean airborne al-

Table 1 – Air conditions during sampling periods and particulate matter concentrations

Sample location	Sampling date	load/unload	Average Temp [°C]	Aver. RH [%]	Aver. WD	PM ₁₀ [ug m ⁻³]	Gly m 1 [ng m ⁻³]	*Gly m 1 [ng mg ⁻¹]
H1	Jul 12-13, 2004	no	22	60	240	36	11,2	308
H2	Jul 19-21, 2004	no	22	60	208	98	n.d.	n.d.
U1						16	0,5	28
H3	Jul 24-26, 2004	yes	23	68	Var.	72	12	168
U2						34	0,7	20
U3	Aug 17-20 2004	yes	27	58	Var.	11	n.d.	n.d.
U4	Sep 9-10, 2004	yes	20	56	202,5	11	n.d.	n.d.
H5	Sep 14-16, 2004	no	23	77	Var.	76	n.d.	n.d.
U5						10	n.d.	n.d.
H6	Oct 26-28, 2004	yes	19	80	Var.	118	2,1	18
U6						23	1,3	55
H7	Jan 12-14, 2005	no	5	86	Var.	213	141	661
U7						51	n.d.	n.d.
U8	Jan 20-21, 2005	no	7	70	Var.	16	n.d.	n.d.
H9	Jan 22-24, 2005	no	5	77	Var.	104	3,0	28
U9	Jan 22-24, 2005	no				25	0,4	17
H12	Apr 10-12, 2005	no	11	79	67,5	6	22,5	3821
U12	Apr 12-13, 2005	no	11	78	Var.	30	0,9	29
U10	Apr 18-19, 2005	no	12	68	225	14	n.d.	n.d.
P5	May 10, 2005	no	18	81	Var.	50	171	3457
U11	May 10-12, 2005	no	14	77	Var.	7	0,7	103
H11	May 12-15, 2005	no	15	69	Var.	63	4,5	72
U11a	May 12-16, 2005	no	16	68	Var.	14	0,7	54
H13	Oct 12-14, 2005	no	15	75	Var.	108	41	377
U13						28	2,8	100
H14	Oct 17-21, 2005	no	13	75	Var.	34	6,3	184
* concentration of Gly m 1 on total particulate matter						n.d.=not detected if <1 [ng cm ⁻³]		
RH = Relative Humidity; WD = Wind Direction;						H = Harbor; U = University; P = Plant		

lergenic level in the port area was $22,1 \pm 41,3 \text{ ng m}^{-3}$ while in the university area it was $0,6 \pm 0,8 \text{ ng m}^{-3}$ ($p = 0.07$).

The median value of the airborne allergenic level was 12-fold higher in the port ($6,3 \text{ ng m}^{-3}$) than in the university area ($0,5 \text{ ng m}^{-3}$). The difference between the median, the mean and its standard deviations of the allergen levels, at the two sites suggests the presence of scattered airborne allergenic peaks in the port area.

Furthermore, the scattered presence in the filters of high levels of Gly m 1 [$>100 \text{ ng mg}^{-1}$ of suspended particulate matter (PM_{10})] was more common in the dust collected in the port than at the university site (6 times and 2 times greater respectively) (Table 1).

Ventilation coefficient and inversion height during winter (December) and summer (July) in Ancona are shown in Figure 2. The maximum value of the ventilation coefficient was recorded as about $2300 \text{ m}^2\text{s}^{-1}$ in the month of

July and $1200 \text{ m}^2\text{s}^{-1}$ in the month of December.

This makes it possible, therefore, besides the spatial distribution, to assume a temporal distribution of environmental pollution.

The Weibull distribution was used to fit the measured values. Table 2 shows the PM_{10} and Gly m 1 shape and scale factors for the H and U sampling locations, Table 2 includes S1, S2, S3 town council monitoring locations.

The results of fitting a multiple linear regression model for the port (H) data are given by the equation:

$$1) \text{ Gly m 1} [\text{ng m}^{-3}] = 0.44 * \text{PM}_{10} [\mu\text{g m}^{-3}] - 3.23 * T [^{\circ}\text{C}] + 0.56 * \text{RH} [\%] \quad (R^2 = 88.4\%)$$

While selecting University (U) data it becomes:

$$2) \text{ Gly m 1} [\text{ng m}^{-3}] = 0.002 * \text{PM}_{10} [\mu\text{g m}^{-3}] + 0.007 * T [^{\circ}\text{C}] + 0.005 * \text{RH} [\%] \quad (R^2 = 71.7\%)$$

On the whole, in Ancona a significant mean seasonal decrease of the PM_{10} between autumn /winter levels ($72 \mu\text{g m}^{-3}$) to the spring/summer levels ($34.2 \mu\text{g m}^{-3}$) was documented ($p=0.03$). This result did not correlate with the allergenic pollution: the overall mean airborne Gly m 1 concentrations were 19.7 ng m^{-3} in autumn/winter and 14.0 ng m^{-3} spring/summer. This result is consistent with the different sources of the two pollutants.

Indeed, the similar dilution effect of weather related factors yielded different results in the two sites. In the university area a homogeneous decrease of the very limited degree of allergenic pollution was documented. In the port area the differences between the median, mean $\pm$ standard deviations levels of airborne Gly m 1 suggest different conditions. The mean Gly m 1 pollution fell four times (from 38.6 ± 59.4 to $8.36 \pm 8.6 \text{ ng m}^{-3}$) while no effect on the median Gly m 1 levels (from 6.3 to 7.8 ng m^{-3}) was documented (Figure 3).

This suggests the role of weather conditions more on the scattered peaks of allergen dispersion than on basic level of allergenic pollution.

Figure 2 - Diurnal variation of ventilation coefficient and atmospheric inversion height in Ancona. hs = inversion height summer; hw = inversion height winter; vcs = ventilation coefficient summer; vcw = ventilation coefficient winter

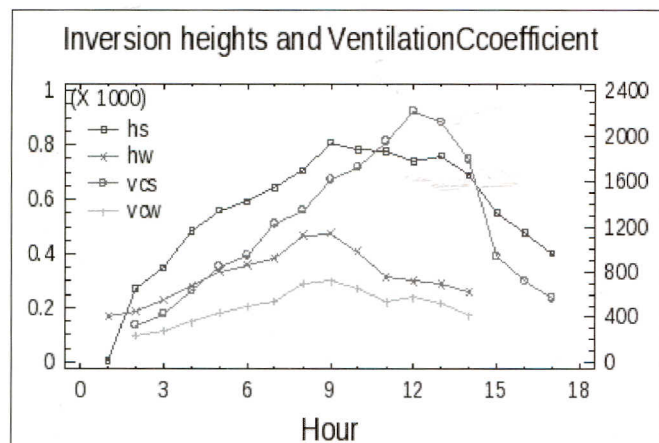
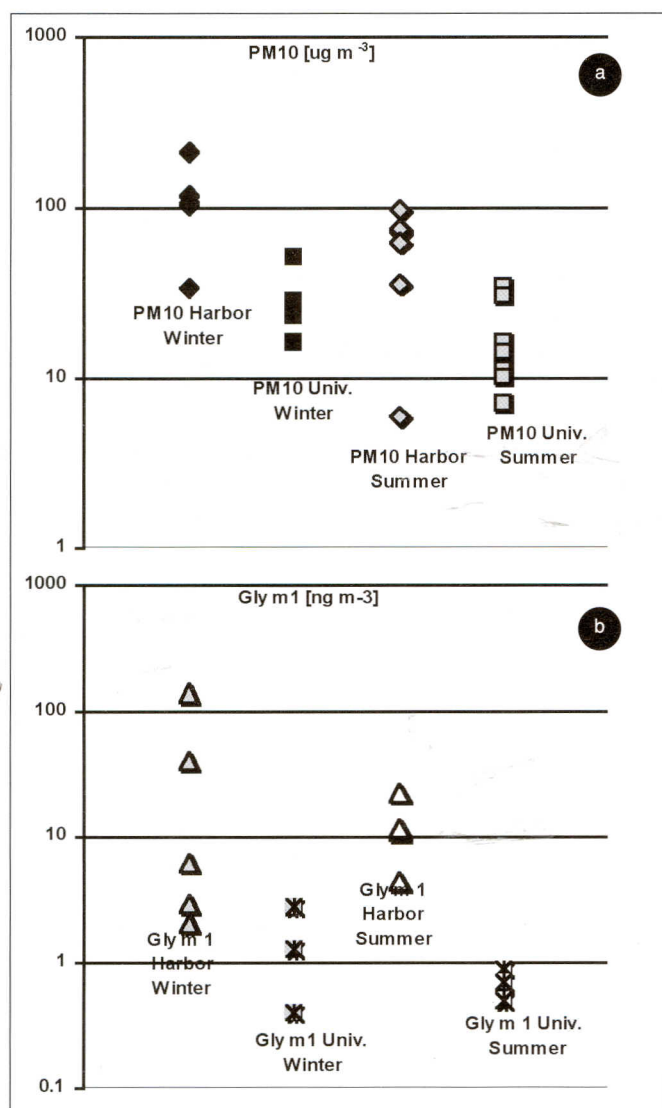


Table 2 - Weibull Shape and Scale factor for Gly m 1 (ng m^{-3}) and PM_{10} ($\mu\text{g m}^{-3}$)

Location	Shape factor Gly m 1	Scale factor Gly m 1	Scale factor PM_{10}	Scale factor PM_{10}
Harbour (H)	0.72	19	1.49	91
University (U)	1.56	4	2.95	25
Permanent Station (S1)	n.a.	n.a.	1.76	53
Permanent Station (S2)	n.a.	n.a.	2.17	58
Permanent Station (S3)	n.a.	n.a.	1.47	24

n.a.- Not available

Figure 3 - Particulate matter (Figure 2a, PM_{10} , $\mu\text{g m}^{-3}$) and allergen (Figure 2b, Gly m 1, ng m^{-3}) concentrations at harbour and University sampling sites in winter and summer season



Discussion

Our results documented the presence of low levels of the Gly m 1 allergen in Ancona's atmospheric environment. The airborne allergen is not always related to the port's soy cargoes and our findings suggest that other sources are involved in emissions of the allergen. The presence of the soybean oil processing plant as well as the transport of soybean seeds from ships to stores and plants might be additional sources, releasing the allergen into the atmosphere via the prevailing breezes.

The scope of this discussion covers the use of Gly m 1 to evaluate soybean dust air pollution and its interplay with meteorological variables.

The assessment of soybean airborne allergens in different working environments has shown that the allergens responsible for soybean allergy can differ, though the specific role of Gly m 1 in asthma epidemics or in occupational asthma in soy processing plants is well documented (15). In Barcelona the Rast-inhibition immunoassay established a strong correlation between the asthma epidemic days and the several variables involved in the emission of the allergen (weather, effectiveness of measures adopted to control the risk, etc.) (1, 8). During the epidemic days the allergen doses ranged between 1600 and 10600 U m^{-3} (1). Before the installation of efficient filters to control emissions the mean airborne allergen level was very high (1089 U m^{-3}); after the installation of filters no differences in mean airborne allergen levels were observed between unloading (67 U m^{-3}) and idle days (63 U m^{-3}) (8).

The most relevant problem of the immunoassay used here is the availability of sera from allergic patients. Two methods were developed to overcome this problem: the Gly m 1 monoclonal antibody-based EIA and the EIA method using rabbit polyclonal antibodies. Both methods were effective and sensitive for the assessment of levels of airborne soybean dust (12, 15, 16).

The monoclonal antibody-based EIA is more specific and sensitive (12) and therefore especially suitable for the purpose of this study. However, the risk of underestimating the true quantity of allergen present does exist due to single epitope-specific identification, which is possibly hidden by other components of the dust mixture (15, 16).

Besides a few peak values which were detected very close to the ship (171 ng m^{-3}) during unloading, the airborne levels of Gly m 1 in Ancona were low (0.4 to 141 ng m^{-3}). In particular, the airborne levels were in a range lower than those reported in Barcelona, (4-797 ng m^{-3}) (12), and appeared similar to those found in soybean fields in Ontario, Canada (0.13 to 73 ng m^{-3}) during soybean harvesting (17).

It should be pointed out that the period of sampling (most of the filters were sampled for two days) could have thinned down the allergen concentrations.

However, levels of Gly m 1 ranging within 100-200 ng m^{-3} were able to induce asthmatic symptoms in very sensitive soybean allergic patients (8) suggesting that, at least in the port area, this might represent a risk for asthma attacks.

As regards the interplay with meteorological variables, the Gly m 1 shape factors indicate that though the larger

amount of dry sedimentation is near the port area, dispersion of the allergen dispersion may be spreading over a much wider area.

A statistically significant relationship (equations 1 and 2) between Gly m 1, PM₁₀ and local meteorological parameters (temperature, T [°C] and relative humidity, RH [%]) quantifies this association (19, 20).

The two locations, H (near the port) and U (at a distance of five kilometres), both differ with respect to the level of Gly m 1 in the dust samples and the dependence of Gly m 1 airborne concentrations on temperature. This is consistent with the assimilative capacity of the atmosphere which is responsible for the dilution (10, 11). The Atmospheric Environment Services, Canada, has classified that high pollution potential occurs when the afternoon ventilation coefficient is less than 6000 m² s⁻¹ and the mixing height is less than 500 m and mean wind speed does not exceed 4 m s⁻¹ (21, 22). Due to the high wind speed and mixing height of the Ancona area, the ventilation coefficient is higher in summer than it is in winter, but neither of the two seasons satisfy the Canadian Atmospheric Environment Services criteria.

Our data suggest that the atmospheric dispersion of PM₁₀ and Gly m 1 is dependent on the same meteorological conditions (23); therefore and particularly in winter, the meteorological risk of the port and bordering city centre area necessitates state-of-the-art technology to check allergenic emissions thereby preventing the simultaneous presence of high levels of airborne soybean allergens and conducive weather conditions.

This is consistent with the previous finding yielded by the Barcelona study.

Although an association between air stagnation and asthma outbreaks was demonstrated, no outbreak was documented when polluting allergens were absent (1). The high standard of performance of the soybean dust pollution control technology installed in Barcelona, irrespective of the weather is indirect confirmation of this (8).

The clinical significance of the low levels of allergens detected, in particular their ability to induce allergic sensitisation in exposed subjects remains an open question.

In a study performed in Argentina, in a rural district where soybean is produced, 15% of asthmatics presented soybean hull positive skin prick test, and a relationship between levels of exposure and sensitisation to soybean allergens was demonstrated, though airborne allergen levels were not measured (18).

The most significant limitation of the study is the small number of samples collected, which could contribute to

an underestimation of the real threat posed by minor occurrences of atmospheric soybean dispersion, even though no asthma epidemic was reported during the period of airborne allergens assessment.

In conclusion, we demonstrate that airborne Gly m 1 is a component of the atmospheric dust in Ancona. The low levels of this allergen present in the air seems consistent with the inexistence of epidemic asthma.

Acknowledgments

The authors are grateful for the financial support awarded by the Project "La salute va in porto" Legge N° 448/98 art 71- Progetto Area Urbana di Ancona (PAUAN) -Azione Intervento F3 and wish to thank Paul Bowerbank for his help in reviewing the English of the manuscript.

References

1. Aceves M, Grimalt JO, Sunyer J, Antó JM, Reed CE. Identification of soybean dust as an epidemic asthma agent in urban areas by molecular marker and RAST analysis of aerosols. *J Allergy Clin Immunol* 1991;88:124-34.
2. Antó JM, Sunyer J, Reed CE, et al. Preventing asthma epidemics due to soybeans by dust-control measures. *N Engl J Med* 1993;329:1760-3.
3. Navarro C, Márquez M, Hernando L, Galván F, Zapatero L, Caravaca F. Epidemic asthma in Cartagena, Spain, and its association with soybean sensitivity. *Epidemiology* 1993;4:76-9.
4. Ferrer A, Torres A, Roca J, Sunyer J, Antó JM, Rodríguez-Roisin R. Characteristics of patients with soybean dust-induced acute severe asthma requiring mechanical ventilation. *Eur Respir J* 1990;3:429-33.
5. Ballester F, Soriano JB, Otero I, et al. Asthma visits to emergency rooms and soybean unloading in the harbors of Valencia and A Coruña, Spain. *Am J Epidemiol* 1999;149:315-22.
6. Bonifazi F, Sanguinetti CM. Bronchial asthma in subjects with positive skin reactions to seeds of edible legumes *Minerva Med* 1980;71:235-7.
7. Thorpe SC, Kemeny DM, Panzani R, Lessof MH. Allergy to castor bean. I. Its relationship to sensitization to common inhalant allergens (atopy). *J Allergy Clin Immunol*. 1988;82:62-6.
8. Villalbí JR, Plasencia A, Manzanera R, Armengol R, Antó JM; Collaborative and Technical Support Groups for the study of soybean asthma in Barcelona. Epidemic soybean asthma and public health: new control systems and initial evaluation in Barcelona, 1996-98. *J Epidemiol Community Health*. 2004 58:461-5.
9. Rodrigo MJ, Cruz MJ, García MD, Antó JM, Genover T, Morell F. Epidemic asthma in Barcelona: an evaluation of new strategies for the control of soybean dust emission. *Int Arch Allergy Immunol* 2004; 134:158-64.
10. Manju N, Balakrishnan R, Mani N. Assimilative capacity and pollutant dispersion studies for the industrial zone of Manali, At-

- mospheric Environment 2002; 36 (21):3461-71.
11. Goyal SK, Chalapati Rao CV. Air assimilative capacity-based environment friendly siting of new industries - A case study of Kochi region, India. *Journal of Environmental Management* 2007;84:473-83.
 12. González R, Duffort O, Calabozo B, Barber D, Carreira J, Polo F. Monoclonal antibody-based method to quantify Gly m 1. Its application to assess environmental exposure to soybean dust. *Allergy*. 2000; 55: 59-64.
 13. 2000/69/EC Directive 2000/69/EC of the European Parliament and of the Council of 16 November 2000 relating to limit values for benzene and carbon monoxide in ambient air;
 14. 1999/30/EC Council Directive 1999/30/EC of 22 April 1999 relating to limit values for sulphur dioxide, nitrogen dioxide and oxides of nitrogen, particulate matter and lead in ambient air.
 15. Gómez-Ollés S, Cruz MJ, Bogdanovic J, et al. Assessment of soy aeroallergen levels in different work environments. *Clin Exp Allergy* 2007; 37:1863-72.
 16. Gómez-Ollés S, Cruz MJ, Renström A, Dockes G, Morell F, Rodrigo MJ. An amplified sandwich EIA for the measurement of soy aeroallergens. *Clin Exp Allergy* 2006;36:1176-83.
 17. Gijzen M, González R, Barber D, Polo F. Levels of airborne Gly m 1 in regions of soybean cultivation. *J Allergy Clin Immunol* 2003 Oct;112(4):803-5.
 18. Codina R, Arduzzo L, Lockey RF, Crisci C, Bertoya N. Sensitization to soybean hull allergens in subjects exposed to different levels of soybean dust inhalation in Argentina. *J Allergy Clin Immunol* 2000;1051:570-6.
 19. Taylor JA, Jakeman AJ, Simpson RW. Modeling distributions of air pollutant concentrations-I. Identification of statistical models. *Atmospheric Environment* 1986; 20: 1781-9.
 20. Horowitz J, Barakat S. Statistical analysis of the maximum concentration of an air pollutant: effects of autocorrelation and non-stationarity. *Atmospheric Environment* 1979; 13: 811-8.
 21. StackPole JD. The Air Pollution Potential Forecast Programme, ESSA Tech, Memo WBTM-NMC 43, 1967.
 22. Gross E. The National Air Pollution Forecast Programme, ESSA Technical Memo., WBTM-NMC 47, US Department of Commerce, 1970.
 23. Yu TY, Chang LW. Delineation of air-quality basins utilizing multivariate statistical methods in Taiwan. *Atmospheric Environment* 2001; 35: 3155-66.