ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Detection of the soy allergen Gly m 1 in the air in Maringá-PR

Presença do alérgeno de soja Gly m 1 no ar em Maringá-PR

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ABSTRACT

Introduction: Knowledge of local aerobiology is essential for allergists. Because airborne allergens can sensitize the population and lead to allergic respiratory diseases, they must be routinely monitored for the effects of climate change, pollution, and agroindustry. **Objective:** To verify the airborne presence and concentration of the main soy hull dust allergen (Gly m 1) in Maringá, PR, Brazil and possible associations with climatic factors. Soybeans were selected due to the high prevalence of this crop in this region. To date, only 1 pilot study (conducted by our group) has evaluated this allergen's presence in Brazil. **Methods:** Atmospheric material was collected between March 2017 and March 2018 in 24- or 48-hour intervals, totaling 70 samples, of which 10 were excluded due to technical problems. The samples were tested for Gly m 1 using enzyme-linked immunosorbent assay, and all samples showed detectable levels of the allergen. **Results:** The median concentration of Gly m 1 was 4.89 ng/m^3, with values ranging from 0.66 ng/m^3 to 1826.1 ng/m^3. Of the 60 samples, 23% showed values > 90 ng/m^3, with June 2017 and March 2018 having the highest concentrations. There was a positive correlation between Gly m 1 concentration and maximum, mean, and minimum temperatures, relative humidity, wind, and insolation. **Conclusion:** The data show that the population is constantly exposed to the Gly m 1 allergen, sometimes at high levels, which may lead to sensitization and symptoms.

Keywords: Allergens, soy, air samples, ELISA.

RESUMO

Introdução: O conhecimento da aerobiologia local é fundamental para o alergista. Os aeroalérgenos são capazes de sensibilizar e levar ao desenvolvimento de doenças respiratórias alérgicas, portanto devem ser monitorados rotineiramente, tendo em vista possíveis mudanças locais conforme alterações climáticas, poluição e atividades agroindustriais. **Objetivo:** Verificar a presença e concentração do alérgeno principal da poeira da casca da soja (Gly m 1) na atmosfera da cidade de Maringá-PR e possíveis associações aos fatores climáticos. A escolha da soja deve-se a alta prevalência desta cultura no Brasil e nesta região do país. Até o presente momento, há apenas um estudo piloto feito por este mesmo grupo avaliando a presença deste alérgeno no Brasil. **Métodos:** Foram realizadas coletas de material atmosférico, durante o período de março de 2017 a março de 2018, durante 24 ou 48 horas distribuídas no decorrer do período, totalizando 70 amostras, das quais 10 foram excluídas por problemas técnicos de coleta. As amostras foram avaliadas pelo método ELISA (*Enzyme linked immunosorbent assay*) para Gly m 1, sendo que todas as amostras apresentaram níveis detectáveis do alérgeno. **Resultados:** A mediana de concentração de Gly m 1 foi de 4,89 ng/m^3. Os valores encontrados variaram de 0,66 ng/m^3 a 1826,1 ng/m^3. Das 60 amostras analisadas, 23% delas apresentaram valores superiores a 90 ng/m^3, sendo os meses de junho/2017 e março/2018 com concentrações mais elevadas. Houve correlação positiva das concentrações de Gly m 1 com as temperaturas máxima, média e mínima, umidade relativa, vento e insolação. **Conclusão:** Os dados evidenciam exposições constantes da população ao alérgeno do Gly m 1, por vezes em níveis elevados possivelmente capazes de gerar sensibilização e sintomas.

Descritores: Alérgenos, soja, amostras de ar, ELISA.

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Introduction

Changes in the distribution of aeroallergens occur gradually over time and require monitoring in order to identify individuals' environmental exposure, as well as possible new sensitization and triggers of respiratory symptoms. The emergence of molecular allergology has allowed us to identify, with sensitivity and specificity, the sensitization profile of individuals. Likewise, it has broadened our understanding of allergens in each location, their concentrations and potential population exposures.[1,2]

In this context, the main soy hull dust allergen, Gly m 1, began to be investigated, particularly in European countries, because of its potential relationship with asthma outbreaks that had occurred at the seaports of some cities related to the unloading of soybeans into silos.[3-7] With advances in research in the field, allergen dispersion was found to be associated with local climate issues, as well as with population genetic aspects.[8-10] New strategies to avoid the spread of this allergen began to be used in European ports, such as the installation of bag filters in the silos, which led to a significant reduction of Gly m 1 in the atmosphere and prevented new asthma outbreaks.[11]

However, despite the importance of Gly m 1 as an aeroallergen, research has not advanced to investigate the presence of this allergen in areas of potential dispersion. In the last decade, no aerobiological survey of this allergen was conducted in any location in the world, despite the fact that Gly m 1 is a sensitizer both in occupational exposure and indirect exposure.[6,7,10,12] In Brazil, Pinto et al.[12] showed frequent sensitization to soybean in workers and residents of soy-producing areas, but they did not assess allergen concentrations in the atmosphere. In a pilot survey of Gly m 1 conducted in 2016 by our research group, we demonstrated the presence of this allergen in the atmosphere, which motivated the current study.[13]

Methods

Air samples were collected periodically from March 2017 to March 2018 in pure borosilicate glass fiber filters (Pallflex®), 47 mm in diameter, with heat-resistant, moisture-resistant fluorocarbon coating (tetrafluoroethylene, TFE). During air sampling, moisture variations in the air or gases did not cause chemical reactions in the filter.

A total suspended particulate sampler capable of capturing particles of different sizes was used. The sampler was placed in the urban area of Maringá, a city in the state of Paraná, south of Brazil, at 23.41° south latitude, 51.97° west longitude, and 537 m altitude in an external environment at 90 cm above the ground. Seventy samples were collected at 24- or 48-hour intervals. The duration of each collection was based on our pilot study and on previous studies that showed the presence of Gly m 1 in 24-hour samples.[7,9,10,13] Samples that experienced technical problems, such as power outages, excessive rainfall, and inadequate collection time, were excluded. The collection point was located at a distance of 2.5 km, in a straight line, from the major soybean processing cooperative in the region. The collected filters were stored at -20 °C until analysis.

To prepare the samples, the filters were cut into 3 cm^2 pieces and eluted in a solution of NH_4HCO_3 with 1% bovine serum albumin (BSA) and 0.1% Tween 20® (Polysorbate), homogenized in an orbital shaker at 4 °C overnight, for a final volume of 3 mL. After the elution process, the material was centrifuged twice, and the supernatant used for analysis was extracted by enzyme-linked immunosorbent assay (ELISA).

The ELISA was performed with plates presensitized with anti-Gly m 1 monoclonal antibody (ALK-Abello®), as described by Gonzalez et al.[6] This method is highly specific with a rare possibility of cross-reactivity with other allergens. Plates were previously washed, and samples, standard curve, controls and blank filters were added in duplicate. Subsequently, the biotinylated detection antibody (ALK-Abello®) conjugated to streptavidin-peroxidase (STAV-PO, Thermo Fisher-Scientific®) was added to the plates. Finally, 3,3',5,5'-tetramethylbenzidine (TMB) was added to the plates incubated in a dark environment, and the reaction was interrupted with $4NH_2SO_4$. The reading was performed by spectrophotometry at 490 to 650 nm wavelengths, inferring the concentrations.

The following meteorological data were collected daily from March 2017 to March 2018 from the National Institute of Meteorology (INMET) website at the Maringá weather station (WMO: 83767; latitude: -23.4; longitude: -51.91; altitude: 542 m): maximum, minimum, and average compensated temperature; relative humidity (RH); precipitation; wind speed and direction; and insolation.

Results

A total of 70 filters were collected during the 12-month study period (from March 29, 2017, to March

26, 2018). The collections were standardized to occur in 1 or 2 days, with samples collected at an average of 24 hours and 6 minutes or 48 hours and 20 minutes, respectively. The volume of air sampled in each filter was, on average, 1.13 m^3 of air per hour. Filters were collected every month, with a mean and median interval between collections of 5 days. Of the total of 70 filters, 10 were excluded from the analysis due to inappropriate collection.

ELISA concentrations of Gly m 1 (ng/mL) were converted to ng of allergen per m^3 of air, as this is the most commonly used unit of measurement. The median concentration of Gly m 1 was 4.89 ng/m^3. All samples showed the presence of Gly m 1, with concentrations ranging from 0.67 ng/m^3 to 1826.1 ng/m^3 (Figure 1).

The 24- and 48-hour filters showed similar concentrations (p=0.32; Mann-Whitney test), with a median concentration of 4.4 ng/m^3 in the 24-hour filters and of 6.3 ng/m^3 in the 48-hour filters. The analysis of the two groups, excluding outliers, showed a median concentration of 4.03 ng/m^3 in the 24-hour group and 3.09 ng/m^3 in the 48-hour group, once again demonstrating homogeneity between the groups (p=0.157; Mann-Whitney test). The first quartile value of 2.24 and the third quartile value of 91.4 were used to calculate outliers. Outliers were all values above 225.2 ng/m^3, totaling 52 samples.

Regarding the meteorological data on collection days, the average temperature was 22.4 °C, RH was 68.5%, and insolation was 7.7 hours. The data were similar to those of other days of the year when no collection was performed. The same was true for wind speed, with an average of 1.48 m/s on collection days, which is considered light air. As for wind direction, there was a predominance of east and northeast winds on the collection days. It should be noted that the cooperative is located between north and northeast to the collection point.

Gly m 1 concentrations were positively correlated with temperature and RH (Table 1). The same statistical analyses performed excluding outliers showed a positive correlation of Gly m 1 concentrations with temperature, RH, insolation, and wind speed (Table 2).

Discussion

The soybean dust allergen can cause respiratory symptoms as confirmed by bronchial provocation tests.[14,15] However, the impact of the presence of Gly m 1 in the atmosphere remains unknown. Based on data from outbreaks in Barcelona in the 1990s, soybean dust allergenicity is believed to occur in 1 in every 1700 people.[16] Climate factors and particular characteristics of the population are known to

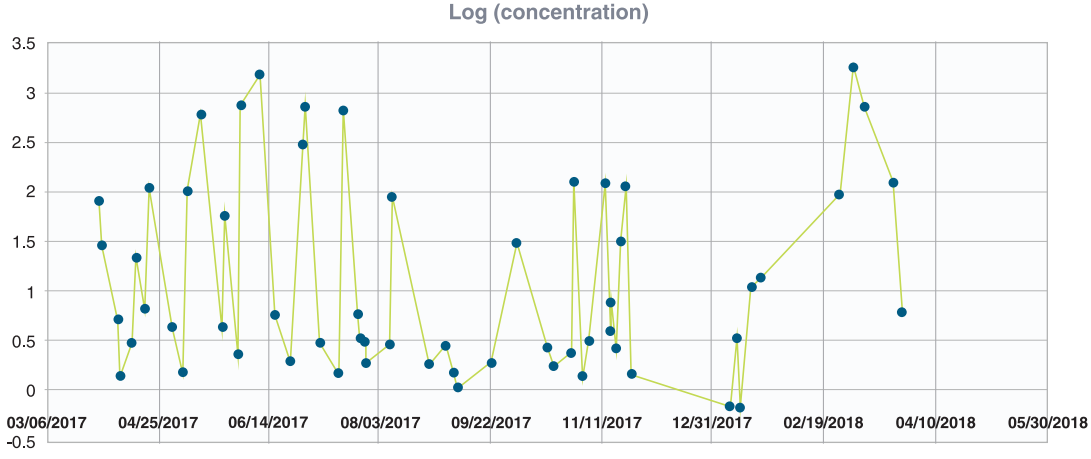

Figure 1
Gly m 1 concentrations on a logarithmic scale (Log^{10}) in the 60 samples

Table 1
Coefficients of correlation (R) and determination (R^2) between Gly m 1 concentrations and meteorological data

	Gly m 1 concentration (Log^{10})			Gly m 1 concentration (Cube root)		
	R	R^2	p	R	R^2	p
Maximum temperature	0.73	0.53	< 0.001	0.72	0.51	< 0.001
Minimum temperature	0.71	0.5	< 0.001	0.7	0.49	< 0.001
Average temperature	0.72	0.51	< 0.001	0.71	0.5	< 0.001
Relative humidity	0.75	0.56	< 0.001	0.75	0.56	< 0.001
Precipitation	0.37	0.14	0.003	0.4	0.16	0.001
Insolation	0.66	0.43	< 0.001	0.64	0.41	< 0.001
Wind speed	0.67	0.45	< 0.001	0.69	0.48	< 0.001
Wind direction	0.32	0.10	0.012	0.32	0.10	0.012
Multivariate [a]	0.79	0.63	NS	0.79	0.62	NS

[a] With all climate variables.
NS = non-significant.

Table 2
Coefficients of correlation (R) and determination (R^2) between Gly m 1 concentrations (without outliers) and meteorological data

	Gly m 1 concentration (Log^{10})			Gly m 1 concentration (Cube root)		
	R	R^2	p	R	R^2	p
Maximum temperature	0.77	0.59	< 0.001	0.86	0.73	< 0.001
Minimum temperature	0.76	0.57	< 0.001	0.85	0.72	< 0.001
Average temperature	0.76	0.58	< 0.001	0.85	0.73	< 0.001
Relative humidity	0.74	0.55	< 0.001	0.83	0.69	< 0.001
Precipitation	0.29	0.08	0.038	0.36	0.13	0.008
Insolation	0.71	0.51	< 0.001	0.79	0.62	< 0.001
Wind speed	0.65	0.42	< 0.001	0.74	0.55	< 0.001
Wind direction	0.22	0.05	0.11	0.28	0.08	0.045
Multivariate [a]	0.82	0.67	0.008 for WS	0.89	0.78	0.010 for WS

[a] With all climate variables.
WS = wind speed.

interfere with this sensitization and certainly with symptom presentation.[10,17,18] Furthermore, soybean hull allergenicity can be affected by heat during grain storage and transport, which can produce new allergen determinants or increase epitope exposure by conformational changes.[19]

Some studies have evaluated ELISA-determined sensitization to Gly m 1 in port populations and produced important results correlating the allergen with asthma outbreaks.[3,20,21] Codina et al.[22] and Pendino et al.[23] evaluated sensitization to soybean hull extract by skin prick test in adults and children, respectively, with asthma. Both studies showed sensitization to soybean hulls even in populations without direct exposure to soybeans. However, all those tested were polysensitized, also showing reactivity to mites, fungi, or pollens.

In Brazil, Pinto et al.[12] identified that symptomatic individuals who lived in rural areas within soybean belts showed 28% of sensitization to soybean by skin tests with total extract, and 5% of them were monosensitized to soybean. Likewise, among residents of the manufacturing area and truck drivers transporting soybeans, sensitization was high (22% for both groups), but not exclusive, as they were also sensitized to storage mites and fungi that colonize the soybeans. Therefore, there is a group of polysensitized atopic individuals in whom soybean can be an additional sensitizer in a context in which mites and fungi play the main role as protagonists of allergic respiratory manifestations.

The Gly m 1 levels detected in the atmosphere are higher near soybean handling sites. Measurements were 12 times higher in a port area than 5 km away from the port.[10] In the current study, the collection point was at an intermediate distance from the cooperative (2.5 km). Despite a relative distance, we showed detectable Gly m 1 levels in all samples collected for 12 months. Soybean hull dust can also be produced during the soybean harvest period and at all stages of soybean processing,[9,10,24] which explains the continuing and not only seasonal presence of Gly m 1 in the atmosphere of Maringá. Although the harvest period occurs between January and March, the cooperative processes soybeans throughout the year.

In the current study, we identified some positive correlations when the concentrations were transformed for data analysis (Log ^{10} and cube root). We decided to perform these data transformations because of the wide dispersion of concentration values (ranging from 0.67 ng/m^3 to 1826.1 ng/m^3). Correlation and regression analyses are influenced by the presence of extreme values, and data transformation can reduce the bias effect. Correlations were stronger when outliers were removed. There was a positive correlation between Gly m 1 concentrations (in cube root) and temperature ($R \geq 85\%$), RH ($R = 83\%$), insolation ($R = 79\%$), and wind speed ($R = 74\%$). The univariate linear regression models using again the cube root-transformed concentrations showed $R^2 > 70\%$ for temperature and $R^2 = 69\%$ for RH. Similarly, in a study conducted in the port of Ancona, Italy, Antonicelli et al.[10] observed in the multivariate linear regression model that the Gly m 1 concentrations were influenced by temperature and RH.

High atmospheric pressure, calm winds and wind direction have been potentially associated with greater dispersion of the Gly m 1 allergen.[25,26] In the current study, there was a positive correlation between wind speed and Gly m 1 concentrations. However, during the study period, wind speed was considered calm, ranging from light air to light breeze. This may have been a contributing factor to the continuing spread of the allergen. Wind direction was not correlated with Gly m 1 concentrations, but there was a predominance of winds from the cooperative toward the collection point, which may have favored the daily presence of detectable Gly m 1 levels.

Although there is controversy about the Gly m 1 values capable of triggering symptoms, in the current study, some values were high. Of the 60 samples analyzed, 14 (23%) showed values greater than 90 ng/m^3, with a peak of 1826 ng/m^3 in March 2018. It should be noted that in almost all months at least one measurement was greater than 90 ng/m^3, except for September 2017 and January 2018, which showed the lowest monthly concentrations, ranging from 1.09 to 2.85 ng/m^3 and from 0.66 to 13.9 ng/m^3, respectively. In contrast, the highest Gly m 1 levels were detected in June 2017 and March 2018. In June 2017, values ranged from 1.98 to 1549 ng/m^3 (median 303.44 ng/m^3), and in March 2018, values ranged from 6.35 to 1826 ng/m^3 (median 428 ng/m^3).

The low Gly m 1 levels found in January 2018 can be attributed to precipitation, which was intense this month, with an average of 21 mm on collection days, in line with previous studies.[9] Higher values observed in March 2018 coincided with the soybean harvest period. Gijzen et al.[9] also observed that peak Gly m 1 levels occurred during the harvest month (reaching 73 ng/m^3). The presence of nearby crops and the

pattern of harvest operations may have influenced the Gly m 1 levels found.

Some limitations should be addressed in this study. Samples were collected at a single site in the city and reflect only the situation of that collection point. The sampler was placed at a height of only 90 cm above the ground, which might have negatively influenced the sampling due to nearby objects. Collection days were chosen by convenience. Very rainy days were automatically discarded due to the impossibility of exposing the device to heavy rainfalls. The 48-hour collections, in particular, may have had losses of protein content due to prolonged exposure to high average temperatures. However, the 24- and 48-hour groups showed similar concentrations. In addition, climate data were provided only at specific time points (2 or 3 times a day) and averaged.

The present Gly m 1 findings need to be further explored. The use of ELISA to assess Gly m 1 in individuals with documented sensitization to soybean (by skin test or specific IgE) could determine the actual sensitization in this population, whether it occurs through food, the airway, or both. Another point to be considered and investigated is whether individuals sensitized through the airway could have soybean-related food symptoms and vice versa. Performing nasal, conjunctival or bronchial provocation tests with the soybean allergen may elucidate the true impact of Gly m 1 dispersion in the atmosphere on soybean-sensitized individuals.[27]

Despite the limitations reported, the study was innovative in performing environmental analyses of soybean hull dust allergens for the first time in Brazil. Studies of environmental aeroallergens are essential for increasing allergists' awareness, enabling them to better understand the environment to which their patients are exposed, identifying risks, promoting prevention, and allowing personalized treatment. In terms of public health, awareness of these exposures allows the allergists to collaborate with other professionals (biologists, environmentalists, and environmental engineers), as it strengthens and encourages discussions in the political sphere about climate change, pollution, gas emissions, and all types of aerobiological monitoring.

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