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Original Contribution

Pollen Loads and Allergic Rhinitis in Darwin, Australia: A Potential Health Outcome of the Grass-Fire Cycle

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Abstract: Although the prevalence of asthma and allergic rhinitis has been increasing in tropical regions, little is known about the allergenicity of pollens from tropical plant families or the importance of ongoing environmental changes. We investigated associations between daily average pollen counts of several tropical plant families and sales of medications for the treatment of allergic rhinitis in Darwin, Australia—a tropical setting in which grass abundance has increased due to increased fire frequencies and the introduction of African pasture grasses. Daily pollen counts with detailed identification of plant species were undertaken in conjunction with a weekly survey of flowering plant species from April 2004 to November 2005. Five pharmacies provided daily sales data of selected medications commonly used to treat allergic rhinitis. We used generalized linear modeling to examine outcomes. All analyses accounted for the potential confounding effects of time trends, holidays, respiratory viral illnesses, meteorological conditions, and air pollution. The peak total pollen count was 94 grains/m^3. Despite the low levels of Poaceae (grass) pollen (maximum daily count, 24 grains/m^3), there was a clear association with daily sales of anti-allergic medications greatest at a lag of 1 day. Sales increased by 5% with an interquartile range rise (3 grain/m^3) in Poaceae pollen (5.07%, 95%CI 1.04%, 9.25%). No associations were observed with pollen from other plant families. Although further testing is required, we suggest that an overlooked aspect of the "grass-fire cycle" that is degrading many tropical landscapes, could be an increase in the prevalence of allergic rhinitis.

Keywords: landscape change, grass-fire cycle, tropics, pollen, allergic rhinitis, medication

INTRODUCTION

Increased human populations have resulted in the clearance of large areas of native vegetation throughout the world. However, changed land management practices such as grazing by stock, introduction of weeds, and fire management can also have far-reaching effects on native vegetation and ecosystem services. A classic example of such indirect effects concerns the "grass-fire" cycle (D'Antonio and Vitousek, 1992). The grass-fire cycle describes a positive feedback where grass biomass increases the likelihood of fire, resulting in increasing dominance of grass to an endpoint where all other plant families are eliminated from the system. Reversing a grass-fire cycle is extraordinarily

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difficult because of substantial changes to microclimate and soil nutrition; the competitive biological environment makes it difficult for non-grass species to reestablish. While the grass-fire cycle has become an exemplar of an ecological feedback that can transform landscapes, attention has focused on the mechanism of the cycle, changes in fire risk, and deleterious biodiversity impacts, with little thought for impacts on human health.

Many tropical countries have recorded an increasing prevalence of atopic diseases, such as allergic rhinitis, and this has been attributed to factors, such as increasing urbanization, that can change exposure to environmental allergens and individual susceptibility to allergic disorders (Beasley et al., 2000; Bjorksten et al., 2008). The use of fire to convert forests to grasslands and agricultural plantations throughout the tropics (Nepstad et al., 2001; Mouillot and Field, 2005; Hansen et al., 2008) is increasingly recognized as negatively affecting human health through air pollution (Lohman et al., 2007). However, an overlooked longer-term effect of such major environmental changes is alterations to the ambient load of airborne pollens. Pollen is a well-established trigger of allergic diseases, however, relatively little has been published about the allergenicity of tropical plant species (Beggs, 2004). A handful of clinical studies from India and Southeast Asia have identified that allergic sensitization in individuals can be triggered by pollen from a range of tropical species (Wang and Chen, 1992; Chew et al., 2000; Singh and Kumar, 2003). Yet, apart from our previous work demonstrating an association between respiratory hospital admissions and Myrtaceae (*Eucalyptus* and *Melaleuca*) pollen in Darwin (Hanigan and Johnston, 2007), we could find no studies that have attempted to quantify population-level impacts of environmental pollen loads from any plant family in tropical settings.

In this article, we use the tropical city of Darwin, Australia to explore how an increased grass-fire cycle may affect the quality of life of people. Darwin is of prime interest because of a current grass-fire cycle involving both native and nonnative species. Recent surveys have shown that the eucalypt savannas around Darwin that are frequently burnt have much higher fuel loads than comparable forests under Aboriginal fire management (Bowman et al., 2007b; Elliott et al., 2009). Compounding the problem is the intentional introduction of a giant African grass *Andropogon gayensis* which is rapidly transforming the eucalypt savannas into a highly fire-prone monoculture. Concurrent with the increase in grass fuels has been a deterioration of air quality (Bowman et al., 2007a) due to increasing savanna fires, and adverse health impacts have been associated with the regional smoke haze (Johnston et al., 2007; Hanigan et al., 2008). Darwin is also an attractive model system for a study of allergenic pollens because of the high diversity of tropical plant families. In contrast to the native flammable savanna surrounding the city, the urban environment is characterized by lush palm gardens and parkland dominated by a wide cross-section of native and introduced rainforest tree species.

We used the aerobiological record to investigate associations between daily plant pollen counts and sales of medications for the treatment of allergic rhinitis, an approach that has been validated for evaluating the impact of allergic rhinitis in community settings (Fuhrman et al., 2007).

Methods

Ethical approval was gained from the Human Research Ethics Committees of the Northern Territory Government's Department of Health and Community Services, the Menzies School of Health Research, and the Charles Darwin University.

Setting

Darwin is a town of approximately 100,000 residents. The climate alternates between a dry season from April to October, and a wet season from November to March. Our study period ran from April 4, 2004 to November 15, 2005, including two dry and one wet season. Seven taxonomic groups account for 90% of the pollen load. In order of frequency, these are: Myrtaceae (*Eucalyptus* and *Melaleuca* trees); Arecaceae (palms); Poaceae (grasses); *Callitris* (cypress pine); Cyperaceae (sedges); *Casuarina* (sheoak); and *Acacia* (wattles), with the remaining 10% composed of approximately 30 different pollen types seen only occasionally and in low numbers (Stevenson et al., 2007). The one major source of outdoor air pollution is vegetation fire smoke during the dry season (Gras et al., 2001).

Exposure Measures

Daily average airborne pollen and fungal spore concentrations (grains/m^3) were measured using a 7-day Burkard volumetric sampler (Burkard-Scientific Ltd., Uxbridge, UK) located on a rooftop approximately 14 m above the ground (20 m above sea level), in the Darwin suburb of Casuarina, close to the main residential areas of the city (Figure 1). There were 58 days in the series with no aeroallergen observations. The majority of the missing days were during the wet season due to equipment failure, possibly caused by the increased air humidity. The values for these days were imputed by taking the average of the previous and subsequent days with observations. A secondary sampler located in a smaller population center 20 km south of the city operated for the first half of the study period. This smaller dataset was used to validate the pollen counts measured at the primary sampler. Pollens were identified using an existing reference collection held in the Department of Archaeology and Natural History at the Australian National University, Canberra, and by a pollen reference collection compiled specifically for this project by regularly collecting flowering material around the monitoring sites.

Other Explanatory Variables

Meteorological data for average daily temperature, average daily relative humidity, and rainfall were provided by the Australian Bureau of Meteorology. Weekly rates of influenza-like illnesses, an important cause of rhinitis, were provided by the Northern Territory Department of Health and Community Services from the Tropical Influenza Surveillance System, a network of sentinel general practitioners. Particulate air pollution less than 10 microns in aerodynamic diameter (PM_{10}) were obtained using a sequential air sampler on the same rooftop (Rupprecht and Patashnick Partisol plus, model 2025, East Greenbush, NY), which provided 24-hour gravimetric measures ($\mu g/m^3$). The data were validated by interlaboratory comparison analyses conducted with the Marine and Atmospheric Research division of Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO).

Outcome Measures

We monitored pharmacy sales of treatments for allergic rhinitis as a community-wide indicator of the prevalence of allergic rhinitis. These medications are readily available as they do not require a prescription from a medical practitioner to obtain, and most pharmacies are open every day of the week. On the advice of the local branch of Australia's Pharmacy Guild (an organization of private pharmacists), the six largest pharmacies that collectively service most of the town were invited to participate in the study. These were located in major shopping centers throughout the residential areas of Darwin (Figure 1). We monitored daily sales of five products, chosen after consultation with sales staff as those most frequently recommended to customers for the treatment of allergic rhinitis. These were fexofenadine 120 mg tablets (an antihistamine), loratadine 5 mg with pseudoephedrine 120 mg tablets (a combination of

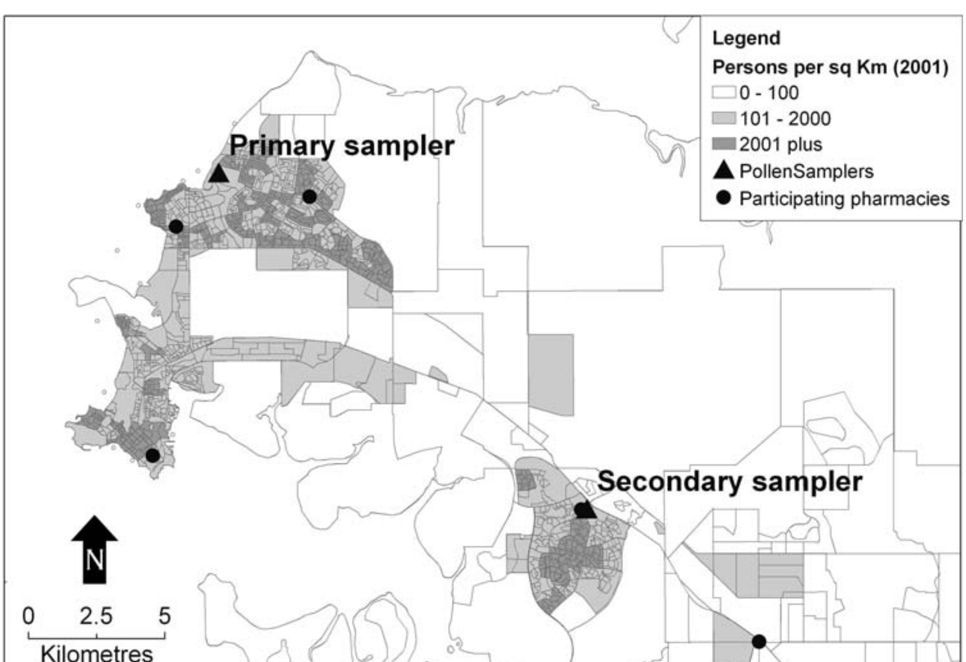

Figure 1. Location of the participating pharmacies, pollen samplers, and population density of Darwin.

antihistamine and decongestant), and three nasal steroid sprays: beclomethasone 50 µg, budenoside 32 µg, and budenoside 64 µg.

Statistical Modeling

We separately examined the association of same-day pollen concentrations and lags up to 3 days with the daily rate of sales per pharmacy for all medications combined. As there is uncertainty regarding the shape of the concentration-response functions and the possibility of threshold levels of aeroallergens and allergic disease outcomes (Anderson et al., 1998; Stieb et al., 2000; Dales et al., 2004), we used a two-stage approach to our analysis. First, we used generalized additive models (GAM) with penalized cubic regression splines to assess the shape of the concentration-response curves at each lag (Wood, 2004). These results were used to determine whether linear or categorical modeling would be used in the second stage of the analysis.

We then used generalized linear models (GLM) to quantify these effects using over-dispersed Poisson models with parametric natural cubic splines for smoothed functions of time and meteorological data. We used a sensitivity analysis similar to Dominici and colleagues to select the optimal amount of temporal smoothing that minimized confounding bias (Dominici et al., 2004). For linear models, the effect estimates are represented as the percentage change in relative risk associated with an interquartile range (IQR) rise in aeroallergen concentration, and 95% confidence intervals (CI) are included. Non-linear relationships were quantified using ordinal variables created from the distributions of each variable, so that the days with values between the minimum and the 50th centile were the reference category and were compared with those lying between the 50th and 75th centiles, the 75th and 90th centiles, and the 90th to the maximum value (Tobias et al., 2004; Atkinson et al., 2006).

Explanatory variables were chosen a priori and included in all models. These are listed in Table 1. All analyses were conducted using the statistical software package R version 2.6.1 (R Foundation for Statistical Computing, 2006).

Table 1. Explanatory variables included in all models

Flu	Influenza epidemics (weeks during which influenza-like illness exceeded the 90th centile) modeled as a categorical variable
Time	A smooth function of time in days (represented by a spline with 10 degrees of freedom [df])
PM_{10}	Average same day PM_{10} (a linear term)
Temp	Average daily temperature (with 6 df)
Rolling av temp	Rolling averages of daily average temperatures at lags 1, 2, and 3 days (6 df)
Relative humidity	Relative humidity (with 3 df)
Rolling av humidity	Rolling averages of relative humidity (with 3 df)
DOW	Day of the week
Holidays	Public holidays
Rain	Days with rainfall greater than 5 mm
Change in pharmacies	An indicator variable to account for changes in the participating pharmacies

Results

Environmental Exposures

Pollen grain counts and other environmental exposures during the study period are shown in Table 2. Pollen loads were validated against those from the secondary sampler. For the first 12 months of the study, these showed similar temporal distribution and loads of each taxa with the following exceptions: the primary sampler has a more urban location, and has the highest values of Arecaceae (palms) and *Casuarina* pollen, reflecting the proximity of the sample location to suburban gardens (that commonly feature palms) and the coast (where native *Casuarina* sp predominates); while the secondary site is in closer proximity to the regional savanna landscape and had slightly higher counts for Poaceae, Cyperaceae, and the native conifer *Callitris* (Stevenson et al., 2007). Some large *Callitris* plantations were established on the outskirts of Darwin in the 1960s.

The peak total pollen count was 94 grains/m^3, while the peak Poaceae count was just 24 grains/m^3. Poaceae pollen had a distinctive seasonal pattern with peaks each April that coincided with the flowering of *Sarga intrans,* the predominant grass that is native to the region. The introduced grass species *Andropogon gayensis* generally flowers later than the native species and, in 2004, the flowering of this species coincided with a second Poaceae pollen peak during June (Rossiter-Rachor, 2008). Daily medication sales, total pollen count, and counts from the three pre-

Table 2. Summary statistics for exposure variables

Variables (common names)	Units	Proportion (%)	Daily mean	SD	50th centile	75th centile	90th centile	Max
Total pollen	(grains/m^3)	100	15.4	11.7	13.0	20.5	31.1	94.0
Myrtaceae (*Eucalyptus* and *Melaleuca*)	(grains/m^3)	31.9	4.9	7.3	2.7	5.9	11.9	78.9
Arecaceae (palms)	(grains/m^3)	25.2	3.9	4.0	2.7	5.9	9.2	27.0
Poaceae (grasses)	(grains/m^3)	17.8	2.7	4.4	0.5	3.1	9.2	24.3
Callitris (cypress pine)	(grains/m^3)	5.7	0.9	2.2	0.0	0.5	2.2	23.2
Cyperaceae (sedges)	(grains/m^3)	5.4	0.8	1.8	0.0	0.5	2.7	13.5
Casuarina (sheoak)	(grains/m^3)	3.1	0.5	2.0	0.0	0.5	0.8	32.7
Acacia (wattle)	(grains/m^3)	2.7	0.4	0.8	0.0	0.5	1.6	5.4
Total fungal spores	(spores/m^3)	100	1348.2	939.7	1158.0	1804.0	2488.9	5703.7
Influenza rates	(per 1000 consults)	—	13.3	11.4	10.5	18.3	25.7	61.9
Average daily temperature	(°C)	—	27.7	2.3	28.2	29.3	30.3	32.2
Average daily relative humidity	(%)	—	67.2	11.4	68.9	73.9	79.1	92.1
PM_{10}	(μg/m^3)	—	17.5	7.3	16.7	22.0	27.6	68.7

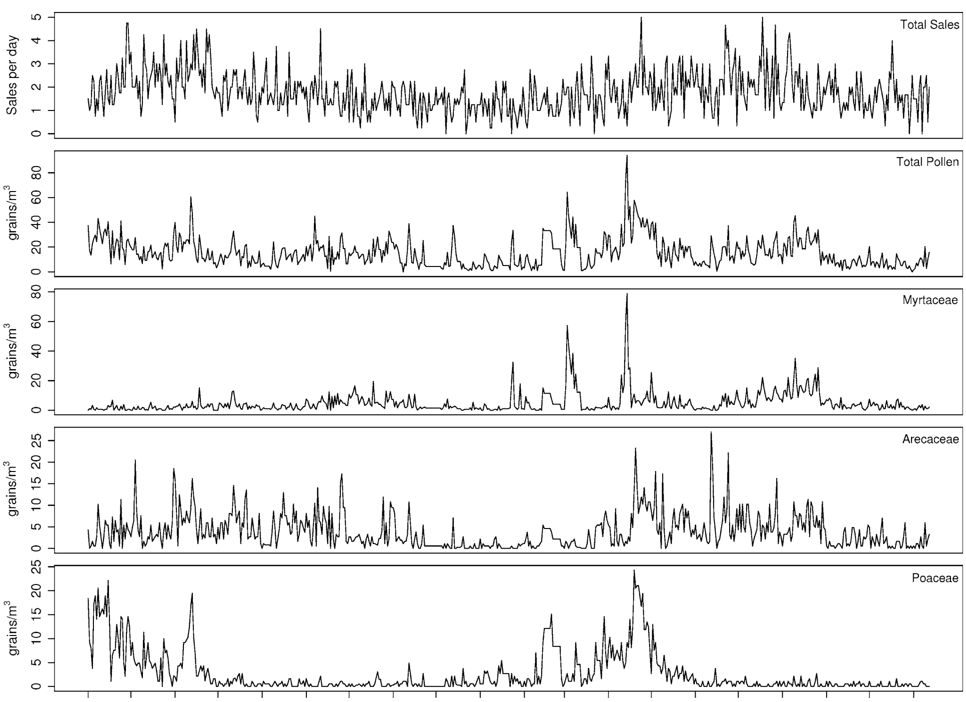

Figure 2. Daily medication sales per pharmacy, daily total pollen count, and daily counts of pollen from the three predominant taxa: Myrtaceae (*Eucalyptus* and *Melaleuca*), Arecaceae (palms), and Poaceae (grasses); Darwin, April 2004–November 2005.

dominant plant taxa, Myrtaceae, Arecaceae, and Poaceae, are shown in Figure 2.

Pharmacy Sales

Five of the six pharmacies approached, contributed data to this study. Three contributed data for the entire study period, while one participated for the first year only and another joined the study for the second year. Results for all pharmacies are presented here. We repeated all analyses only including the three pharmacies that participated for the entire study period with no appreciable difference in the results (available on request). The mean daily rate of sales of all products ranged from 0 to 5 per pharmacy per day. Of the products that we monitored, fexofenadine was the most frequently sold (40.9%), followed by nasal ste-

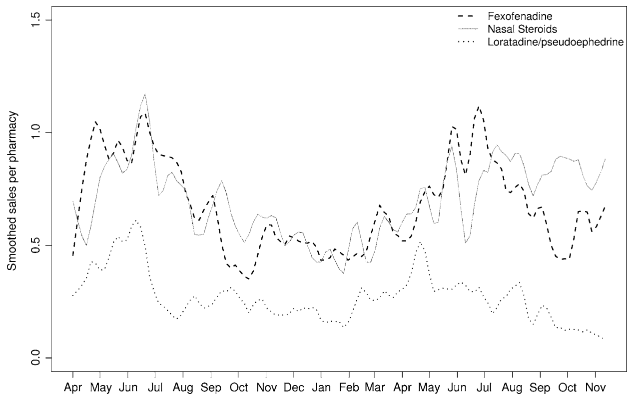

Figure 3. Smoothed time trends of medication sales by product type; Darwin, April 2004–November 2005.

roids (40.6%), followed by loratidine/psuedoephedrine combination (18.5%). There was similar pattern of sales for these three products through time, although from June 2005, sales of loratidine/pseudoephedrine fell, while sales of nasal steroids rose, coinciding with increased local regulation of products containing pseudoephedrine (Figure 3).

Association Between Sales and Pollen

We found positive linear associations between Poaceae counts and daily sales of all products. The percent increase in daily sales associated with an IQR rise (3 grains/m^3) was 2.67% (95%CI -1.22%, 6.71%) for same day exposures, 5.07% (95%CI 1.04%, 9.25%) at a lag of 1 day, and 4.07% (95%CI 0.01%, 8.29%) at a lag of 2 days. No associations were observed with fungal spores, the total pollen count, or other individual pollen taxa. No individual product was positively associated with pollen or fungal spore counts (Table 3).

Discussion

This is the first study, to our knowledge, to report an association between grass pollen and a community-wide marker of allergic rhinitis in a tropical setting. The association was linear and of greatest magnitude at a lag of 1 day. No other pollen type was found to be associated with our measure of rhinitis.

Relatively few studies have examined the health impacts of daily changes in ambient aeroallergens. Three studies from locations in France, Canada, and Britain have found strong associations between measures of allergic rhinitis and daily Poaceae pollen counts (Ross et al., 1996; Cakmak et al., 2002; Fuhrman et al., 2007). Indeed, our results for Poaceae were similar to the studies from France where an interquartile rise of grass pollen was also associated with an approximate 5% increase for daily sales of hay fever medications. However, in our study, the Poaceae counts ranged from zero to just 24 grains/m^3, far lower than that described in any of the above studies. For example, the daily mean counts were all greater than 60 grains/m^3, and maximum counts often exceeded 600 grains/m^3. In two studies from the US and Kuwait, weed species dominated the pollen counts, and the daily Poaceae pollen counts remained less than 10 grains/m^3. However, in these settings, associations with allergic rhinitis symptoms or clinic attendances were identified with the dominant weed pollen species, but not with Poaceae (Behbehani et al., 2004; Cashel et al., 2004).

Previously published minimum threshold Poaceae pollen levels for inducing nasal symptoms in sensitized individuals have ranged from 20 to 71 grains/m^3 (Puc and Puc, 2004; Rapiejko et al., 2007). Although thresholds are influenced by environmental factors such as high levels of concomitant air pollution, or by individual factors such as priming of the immune system (Frenz, 2001; Peden, 2001), it was interesting that we found population-level associations at grass pollen concentrations that barely reached currently accepted thresholds. This suggests that the allergenic potential of the grass species dominant in our setting could be different from those studied to date.

The few published studies of clinical thresholds for Poaceae pollen have been conducted in Europe, where grass species with the C3 photosynthetic pathway dominate. Investigations of protein allergens from tropical grasses, or indeed other tropical plant families, are an acknowledged gap in the available literature (Mohapatra et al., 2005; Radauer and Breiteneder, 2006). The majority of allergenic grass species characterized thus far belong to the Pooideae sub-family which has the C3 photosynthetic pathway, yet on a global scale, about one-half of all known grass species belong to a tropical functional group with the C4 photosynthetic pathway (Gaut, 2002). Significantly, many allergens from four C4 species that have been characterized to date have been shown to be distinct from those in the Pooideae sub-family (Andersson and Lidholm, 2003). These include important allergic species *Sorghum halepense* (Johnson grass) and *Cynodon dactylon* (Bermuda grass) that are now present on most continents. Given that the two dominant grasses surrounding Darwin (*Andropogon*

Table 3. Percentage change in relative risk and 95% confidence intervals (CI) for sales of medications for the treatment of allergic rhinitis for an interquartile range change in same day aeroallergen load for linear responses and compared with the reference category for non-linear responses; Darwin, Australia, April 2004–November 2005

Outcome	Response	Reference	Total pollen	Myrtaceae	Arecaceae	Poaceae	*Callitris*	Total sedges	*Casuarina*	*Acacia*
Lag 0 (same day)	Linear	Interquartile range rise				2.67 (−1.22, 6.71)	−1.01 (−2.32, 0.31)	−0.35 (−1.88, 1.21)	0.87 (−0.22, 1.98)	−0.7 (−3.53, 2.22)
	Non-linear[a]	Category 2	−3.51 (−11.95, 5.75)	−0.02 (−9.32, 10.25)	−0.18 (−9.52, 10.11)					
		Category 3	6.29 (−6.29, 20.55)	2.49 (−11.61, 18.85)	−11.05 (−22.36, 1.91)					
		Category 4	−2.38 (−15.1, 12.24)	−12.17 (−24.94, 2.77)	−8.79 (−20.14, 4.19)					
Lag 1	Linear	Interquartile range rise		−1.6 (−4.48, 1.36)		5.07 (1.04, 9.25)		0.04 (−1.46, 1.57)	0.76 (−0.35, 1.88)	
	Non-linear[a]	Category 2	−5.03 (−13.42, 4.17)		2.1 (−7.46, 12.66)		10.98 (−0.73, 24.06)			7.63 (−25.96, 56.46)
		Category 3	−1.4 (−13.06, 11.84)		−4.43 (−16.59, 9.51)		4.26 (−9.89, 20.64)			0.87 (−8.97, 11.77)
		Category 4	9.88 (−3.84, 25.55)		0.83 (−11.51, 14.88)		15.85 (−2.48, 37.62)			6.25 (−7.09, 21.51)
Lag 2	Linear	Interquartile range rise	2.14 (−2.88, 7.42)	−0.93 (−3.79, 2.01)		4.07 (0.01, 8.29)	0.24 (−1.02, 1.51)	−0.91 (−2.47, 0.68)		0.35 (−2.42, 3.2)
	Non-linear[a]	Category 2			1.87 (−7.76, 12.51)				−11 (−31.21, 15.14)	
		Category 3			11.55 (−1.91, 26.87)				5.92 (−4.24, 17.15)	
		Category 4			0.9 (−11.28, 14.75)				10.42 (−4.33, 27.45)	
Lag 3	Linear	Interquartile range rise					0.03 (−1.23, 1.3)	0.12 (−1.59, 1.86)		
	Non-linear[a]	Category 2	−3.34 (−11.73, 5.85)	1.93 (−7.63, 12.47)	−3.53 (−12.41, 6.24)	−7.64 (−16.33, 1.96)			0.62 (−21.36, 28.75)	7.47 (−4.14, 20.49)
		Category 3	−1.81 (−13.37, 11.3)	−12.93 (−25.2, 1.36)	−9.32 (−20.49, 3.41)	1.11 (−13.45, 18.12)			−12.9 (−21.5, −3.35)	18.53 (3.74, 35.42)
		Category 4	−9.12 (−20.8, 4.29)	−11.4 (−23.95, 3.22)	−11.59 (−22.35, 0.66)	1.93 (−14.43, 21.41)			0.67 (−12.54, 15.88)	3.78 (−9.4, 18.88)

[a]Non-linear relationships were quantified using ordinal variables created from the distributions of the pollen or spore counts and categorized as follows: Category 1 (reference), 0–50th centile; Category 2, 50–75th centile; Category 3, 75–90th centile; Category 4, ≥90th centile

gayensis and *Sarga* spp.) are both C4 grasses, the allergenic effect of grass pollen at the low levels we measured in Darwin is consistent with the notion that C4 grasses could be more allergenic than C3 grasses.

We are confident of the validity of our finding of the allergenic effects of grass pollen because of the detailed taxonomic identification of the overall pollen count, the measurement of pollen at two sites to assess their regional variation, and the ability to include data for several important confounders in the analyses. These included the daily air temperature and humidity, particulate air pollution, and respiratory viral illness, all of which can be seasonally associated with rhinitis (Cashel et al., 2004; Salib et al., 2008). Although the pattern of pollen distribution recorded by our two monitors was generally similar, with fixed monitors, sampling is inevitably biased towards widely dispersed, wind pollinated species such as Poaceae. This could have limited our ability to detect associations with bird or insect pollinated taxa, such as Arecaceae, Myrtaceae, and *Acacia* that have more limited pollen dispersal.

Previous studies have validated the use of time series studies of medication sales for assessing the community-wide impact of exposure to pollens (Fuhrman et al., 2007). There were some limitations with the use of this outcome measure in our study because not all pharmacies participated. Nonetheless, those that participated had high sales turnover, were geographically distributed throughout the urban residential areas and all had similar temporal patterns in their daily product sales. Collectively, these factors suggest that data from the participating pharmacies were representative of the wider pattern of sales of these products in Darwin. Not all available products for the treatment for allergic rhinitis were monitored for logistical reasons; we focused on those products most frequently sold for this condition. This constrained our sample size and may have limited the power of the study to detect an association with less abundant plant taxa.

Conclusions

The rising prevalence of allergic diseases in tropical regions demands greater understanding of the potential role of locally abundant allergens and how they interact with environmental and demographic changes, such as increasing urbanization (Nicolaou et al., 2005). A potent and largely overlooked factor is the rapid transformation of natural vegetation via a grass-fire cycle. The ongoing broadscale use of fires in tropical regions (van der Werf et al., 2006; Turner et al., 2007) produces severe recurrent episodes of particulate air pollution, which can further potentiate the clinical response to allergen exposure (Hauser et al., 2003; Saxon and Diaz-Sanchez, 2005). It might also be possible that tropical grasses with the C4 photosynthetic pathway are more allergenic than temperate grasses, explaining the strong association we observed with very low concentrations of grass pollen. The global spread of tropical grasses due to ongoing loss of tropical forests, introduced species, and changing fire regimes could be contributing to the increased burden of disease due to allergic rhinitis in these regions. Testing this hypothesis will require further study of the pollen flux, plant allergens, and the epidemiology of allergic diseases in tropical settings.

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