

The major aeroallergens in Guangxi, China

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Summary

Over a 2-year period we have identified pollen grains from 48 families of grasses, as well as mould spores and mite particles during air sampling in Guangxi Province. The major aeroallergens were *Artemisia*, Moraceae and *Euophorbiaceae*, and the spores of *Aspergillus*, *Penicillium*, *Cephalosporium* and *Helminthosporium*. Mites were probably also one of the major outdoor aeroallergens. Our investigations also included inspection of the vegetation of the geographical area involved, as well as skin testing on 774 subjects using extracts of 37 aeroallergens. We believe that this work has provided fundamental information on seasonal allergy in Southern China and South-east Asia.

Introduction

Although, as elsewhere, aeroallergens occur in China [1–8], a systematic investigation of the numerous aeroallergens involved has not yet been reported. The present paper describes a preliminary investigation on the aeroallergens in Guangxi Province, and includes a study of outdoor airborne mite particles as well as pollen grains and fungal and mould spores.

Materials and methods

Air sampling and counting

The gravity method [9] was used in 2 successive years, from 1 July 1982 to 30 June 1984. There were five sampling sites of varying terrain: Yulin, Nanning, Baise, Liuzhou and Guilin (Fig. 1). At each site a Durham sampler was installed on a 12-m high roof, higher than others in the vicinity. A slide was coated with vaseline and exposed for 24 hr in the sampler, then mounted in glycerojelly plus fuchsin. This is a mixture of gelatine (1 g), glycerine (7 ml) distilled water (6 ml) and fuchsin (a few drops). The latter is a saturated solution of fuchsin in 95% alcohol. The various allergens under a cover-glass of 4.84 cm² were identified and counted microscopically. A few genera of mould

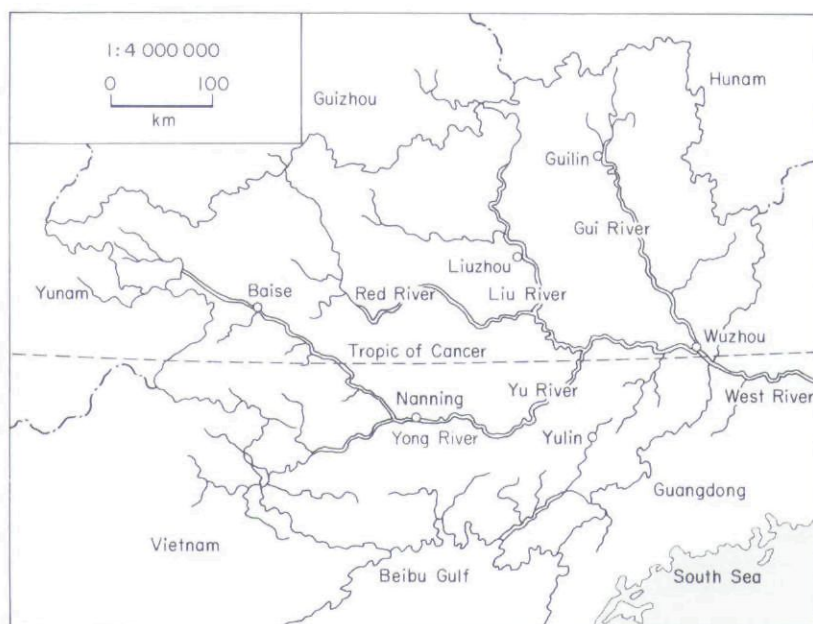


Fig. 1. Map of Guangxi Province.

(e.g. *Penicillium*, *Aspergillus*, *Mucor* and *Rhizopus*) were identified by culture on exposed petri-dishes of Sabourauds medium. The petri-dishes were exposed for 15 min each day.

The environment

The climate, terrain, vegetation and time of flowering were observed, and the temperature, relative humidity, velocity of wind and precipitation were recorded daily during the period studied (Table 1). This was done to determine the relationship between the aeroallergens and climate. The common plants were identified and pollen was collected from the plants in order to identify the airborne pollen and prepare extracts.

Allergen extracts and skin testing

Pollen extracts were prepared in our laboratory from 32 species (17 families) that were found to be common both in the air and in the fields. Before the moulds were extracted they were identified and cultured in Czapek Dox Broth medium. The mites were obtained from a flour mill. After the mites were identified they were separated, cultured and extracted for skin testing. The potency of the extracts were standardized by the micro-Kjeldahl total nitrogen method.

The extracts were used in 774 subjects who suffered from allergic rhinitis and/or asthma. The out-patients were tested with the pollen, moulds and mites in random sampling. In the grain processing factories and textile mills only the extract of *Dermatophagoides farinae* was used for skin testing because the majority of patients from factories suffered from perennial allergic rhinitis and asthma.

Concentrations of extracts of 1:10 000 or 1:100 000 (w/v) were used in children and sensitive patients; another concentration (1:1000) of extracts was used for most

Table 1. Climate data

Months	Average temperature (°C)	Relative humidity (%)	Precipitation (Monthly sum: mm)
<i>1983</i>			
Jan.	11.5	78.2	77.8
Feb.	12.7	84.2	125.4
Mar.	15.8	81.1	101.6
Apr.	23.1	80.4	59.9
May	26.6	79.9	64.4
Jun.	28.5	79.3	186.1
Jul.	29.8	76.6	113.6
Aug.	28.8	78.6	131.3
Sep.	27.7	80.2	174.6
Oct.	24.2	79.9	112.7
Nov.	18.1	76.0	46.2
Dec.	13.1	74.5	28.2
Average	21.7°C	79.1	1221.8 (total)
<i>1984</i>			
Jan.	9.3	75.6	13.2
Feb.	10.8	84.8	35.7
Mar.	18.4	81.3	22.7
Apr.	22.0	82.8	37.1
May	24.9	89.2	205.3
June	27.9	83.2	247.7
<i>1982</i>			
July	28.6	82.1	170.2
Aug.	28.1	84.5	180.2
Sep.	26.6	81.8	236.4
Oct.	24.9	81.0	52.6
Nov.	19.8	80.7	58.1
Dec.	11.7	76.2	24.9
Average	21.1	81.2	1284.1 (total)

other patients. Intradermal injection was performed in the arms and 10–12 tests were made in rows. A weal reaction was compared with a positive histamine control (0.1 mg/ml) and a negative control (extraction solution without allergen). A weal of the same size as that induced by the histamine control is indicated as +++ and a reaction similar to that evoked by the negative control is indicated by ÷. The grading + and ++ are used for reactions between the negative and the positive control. For reactions larger than +++, a plus is added for each doubling of the average diameter. This standardized graduation of skin tests is proposed by the standardization committee of Northern Europe (Scandinavian) Society of Allergy [7].

Results

Environment and vegetation

Guangxi Province is situated in the tropical and subtropical district and in 21°–26°

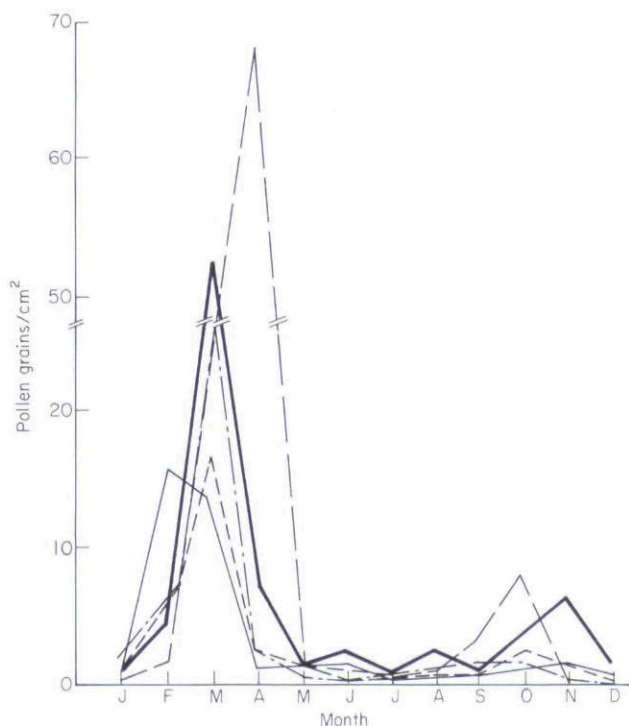


Fig. 2. Change in the pollen count in Guangxi in 1983. (—) Yulin, (---) Nanning, (—) Blaise, (- - -) Liuzhou and (- · -) Guilin.

north latitude. The Tropic of Cancer is across the middle of Guangxi. It has a subtropical monsoon climate. The average annual temperature is 17°–23°C. The average maximum temperature is about 29°C in July, and the minimum temperature is about 15°C in January. The average maximum relative humidity is about 84·8% in February, and the minimum relative humidity is about 75% in December. The average annual precipitation is 1520 mm. The winter lasts for 10–30 days and the summer lasts for 160–180 days. A warm humid climate is conducive to the growing of plants and moulds and flourishing of mites.

The height of this province is 100–200 m or 300–400 m above sea level. More than two-thirds of the province are mountainous district. Other districts include river basin. There are more trees than herbs in the mountainous district. Generally, the flowering period of the trees is more concentrated in spring, but the herbs have a long flowering period. Some herbs (e.g. *Ageratum conyzoides*) flower all year.

Count of aeroallergens

In the 2 years of study, 3637 slides were collected from five sites. There were 76 137 pollen grains, 1745 spores of fern (497 monoletes and 1248 triletes), 78 124 spores of mold and 925 mites on the slides. The airborne pollen grains were found all year but there were two pollen peaks February–April and September–November (Fig. 2). In the former peak tree pollen (Pinaceae, Moraceae and Casuarinaceae) was predominant and in the latter herb pollen (Gramineae and *Artemisia*) was predominant.

Table 2. The result of skin testing in pollen

Families	Species	Case	Positive count				Positive rate (%)
			+	++	+++ or ++++	++++	
Gramineae	<i>Cynodon dactylon</i> P.	158	19	27	5		32.3
	<i>Echinochloa crusgalli</i> R.	147	23	26	4		36.0
	<i>Zea mays</i> L.	110	11	18	1		27.3
	<i>Arundo donax</i> L.	96	13	7	0		20.8
	<i>Artemisia annua</i> L.	221	27	58	8		42.0
Compositae	<i>Artemisia princeps</i> P.	224	24	53	6		37.0
	<i>Ageratum conyzoides</i> L.	204	22	55	5		40.2
	<i>Xanthium sibiricum</i> P.	219	31	25	3		26.9
	<i>Helianthus annuus</i> L.	107	11	7	0		16.8
	<i>Chenopodium album</i> L.	160	24	26	4		33.7
Chenopodiaceae	<i>Chenopodium ambrosioides</i> L.	212	28	51	6		40.1
	<i>Beta vulgaris</i> L.	110	8	13	1		20.0
	<i>Spinacia oleracea</i> L.	118	7	11	0		15.3
Cyperaceae	<i>Cyperus exaltatus</i> R.	109	12	9	0		21.1
	<i>Rhynchospora corymbosa</i> B.	109	5	12	0		15.6
Amaranthaceae	<i>Amaranthus spinosus</i> L.	218	25	41	3		31.6
Plantaginaceae	<i>Plantago major</i> L.	115	12	25	2		25.1
Humulu	<i>Humulus scandens</i> L.	159	11	23	0		21.3
Polygonaceae	<i>Polygonum orientale</i> L.	128	22	15	0		28.9
Moraceae	<i>Morus alba</i> L.	165	20	31	2		32.1
	<i>Broussonetia papyrifera</i> V.	161	14	33	3		31.0
	<i>Ricinus communis</i> L.	222	11	32	2		20.2
Euphorbiaceae	<i>Aleurites moluccana</i> W.	99	8	27	3		38.3
	<i>Sapium sebiferum</i> R.	108	9	16	1		24.1
	<i>Cleistanthus oliverianus</i> L.	96	13	11	0		25.0
Pinaceae	<i>Pinus massoniana</i> L.	222	27	9	0		16.2
Fagaceae	<i>Castanea mollissima</i> Bl.	159	22	9	0		19.4
Myrtaceae	<i>Callistemon rigidus</i> R.	64	7	4	0		17.1
Mimosaceae	<i>Acacia confusa</i> M.	195	32	19	0		23.8
Meliaceae	<i>Melia azedarach</i> L.	159	21	32	1		33.9
Sapindaceae	<i>Sapindus mukorossi</i> C.	96	11	6	0		17.7
Caesalpinaceae	<i>Cassia surattensis</i> B.	226	31	13	0		19.4

There were three patterns of airborne pollen movement. (i) Pollen that is abundant in the air for a long period of time, e.g. Gramineae, *Artemisia* and Moraceae pollen, most of which belong to classes of sensitizing pollen. (ii) Pollen present in the air for a long period of time, but in small quantities, e.g. Salicaceae and Juglandaceae, which are sensitizing agents. (iii) Pollen present in the air for short but concentrated periods, e.g. Pinaceae and Cupressaceae. In different sampling districts the quantity of pollen varies but the types are similar. In Guilin the pollen grains are far more than in Yulin.

Mould spores were found in the air all year. Every year the spores begin to increase in February and keep on a high level until December. The total quantity of spores did not have any definite annual peaks. Thirty-four genus spores were definitely identified from the slides.

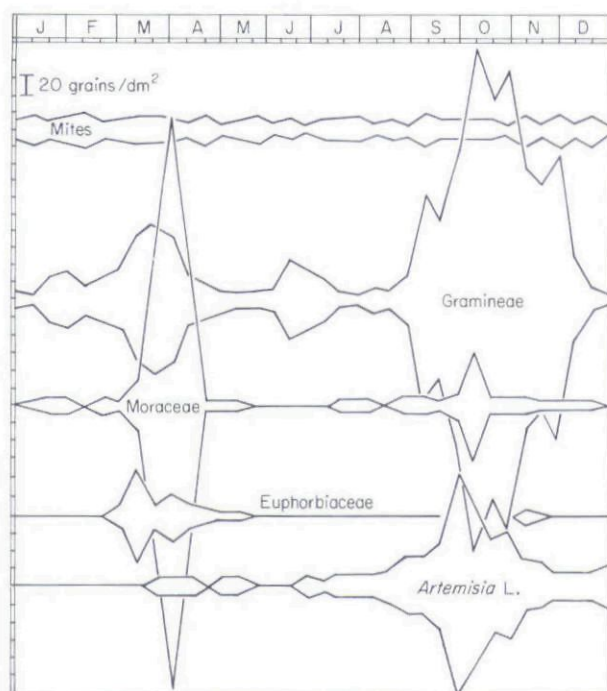


Fig. 3. Calendar of major sensitizing pollen and mites in Guangxi. Data are based on the average of 10-day counts from five Durham samplers over 2 years, from July 1982 to June 1984.

Mites were found on the slides every month. In addition to many fragments of mites, there were 849 larvae, nymphs or imagoes, and 76 eggs. Over 30 species of mites were found but only seven genera were definitely identified: *Tyroglyphus*, *Chortoglyphus*, *Choyletus*, *Melichares*, *Tydeus*, *Acarophenax* and *Aleuroglyphus*.

Skin testing

Two hundred and thirty-three out-patients, equal to 4900 person time, were tested with pollen extracts. The positive reactions are shown in Table 2. Sixty-three subjects were tested with mould extracts and the positive reactions were as follows: *Penicilium* 29.6%, *Alternaria* 26.4%, *Aspergillus* 24.9% and *Mucor* 20.1%. Seventy-one subjects were tested with the extract of *Dermatophagoides farinae* and the positive reaction was 64.7%. In the grain processing factories and textile mills, 478 subjects were tested with the extract of *Dermatophagoides farinae*. The rate of positive reaction was 48.9% but that of the control group (65 persons working in the same factory) was 15.4%. In general, the rate of positive reaction to pollen is higher than that of moulds and lower than that of mites.

Discussion

In regard to pollen peaks and quantity, Nanning is approximate to Yulin, and Baise is

approximate to Liuzhou. Perhaps the reason is that their latitudes are similar. Generally, the temperature, relative humidity and precipitation are dependent on the latitudes.

In Yulin the first pollen peak is in February but in Guilin it is in April. In low latitudes the pollen peak is more early than in high latitudes. There was more airborne pollen in Guilin than in Yulin (Fig. 2), but with airborne mites it was the reverse. Perhaps the reason is that Guilin is situated in mountainous district, which is covered mostly with trees, but Yulin is situated in a river basin and is affected by sea wind for most of the year.

The rate of positive reaction to *Artemisia* in Guangxi (39.7%) was lower than in Beijing (78.0%). This finding was in accordance with the fact that the amount of *Artemisia* pollen was less in Guangxi. Thus, sensitization of a certain pollen is influenced by its quantity in air. However, the quantity of pollen grains is influenced by the vegetation so the sensitization of aeroallergen is also influenced by geographic environments.

If properly performed, the intradermal injection for skin testing is a simple, safe method, with relatively little risk of a serious constitutional reaction. It is sensitive, economical and quick to provide the information for clinical judgment [10]. Similar to nasal provocation and RAST, skin testing aims to detecting specific allergens [11]. In the grain processing factories and textile mills mite extract was used for skin tests. This concentration (1:1000, w/v) of extract is rather strong. The difference between the control group and the patients is significant ($\chi^2 = 26.02$, $P < 0.01$), which indicates that the result of skin testing is convincing and that mites are also a major sensitizing allergen in the grain processing factories and textile mills.

House dust can be responsible for perennial allergic rhinitis and asthma as mites found in houses are a major source of dust allergens. Several investigations have been directed towards the distribution of the mites inside houses [7,8]. However, studies on sensitizing mites floating in the atmosphere outside have so far been neglected. We found many sensitizing mites in the atmosphere; most of them as fragments and larvae. The excreta, horny layer and fragments of mites are lighter than the larvae but they are more potent sensitizers. In view of these facts, we feel the mites are also important outdoor allergens. Sensitization of pollen is stronger than that of moulds and weaker than that of mites in the skin testing.

After comparing the results of skin testing with the pollen in the air and the distribution of plants on field, it is ascertained that the major sensitizing pollens are Gramineae, *Artemisia*, Moraceae and Eupobiaceae (Fig. 3). Of the airborne pollen, 54.6% was that of Pinaceae. Over 80% of Pinaceae pollen is concentrated in the first pollen peak (Fig. 2). Pinaceae pollen was most abundant but its rate of positive reaction was rather low. It is obvious that Pinaceae pollen is not a significant aeroallergen. The major sensitizing moulds are *Aspergillus*, *Penicillium*, *Cephalosporium*, *Helminthosporium*, *Alternaria*, *Mucor* and *Hormodendrum*.

There were 48 families of airborne pollen in our collection but only 19 families in Beijing. There were 2217 grains of pollen on one (4.84 cm²) of our slides but only 725 grains of pollen in northern China. In Guangxi the types and quantity of airborne pollen are far more numerous than those in northern China [6] and in California [8]. This indicates that in tropics and subtropics the sensitizing pollen is more complex than other regions. The symptoms of pollinosis and asthma in northern China and the United State disappear when the pollen season is over, but in Guangxi the symptoms

persist for a longer period. Perhaps the reason is that the airborne pollens are numerous in type and quantity and the major sensitizing pollens are present in the air for a long time in Guangxi.

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