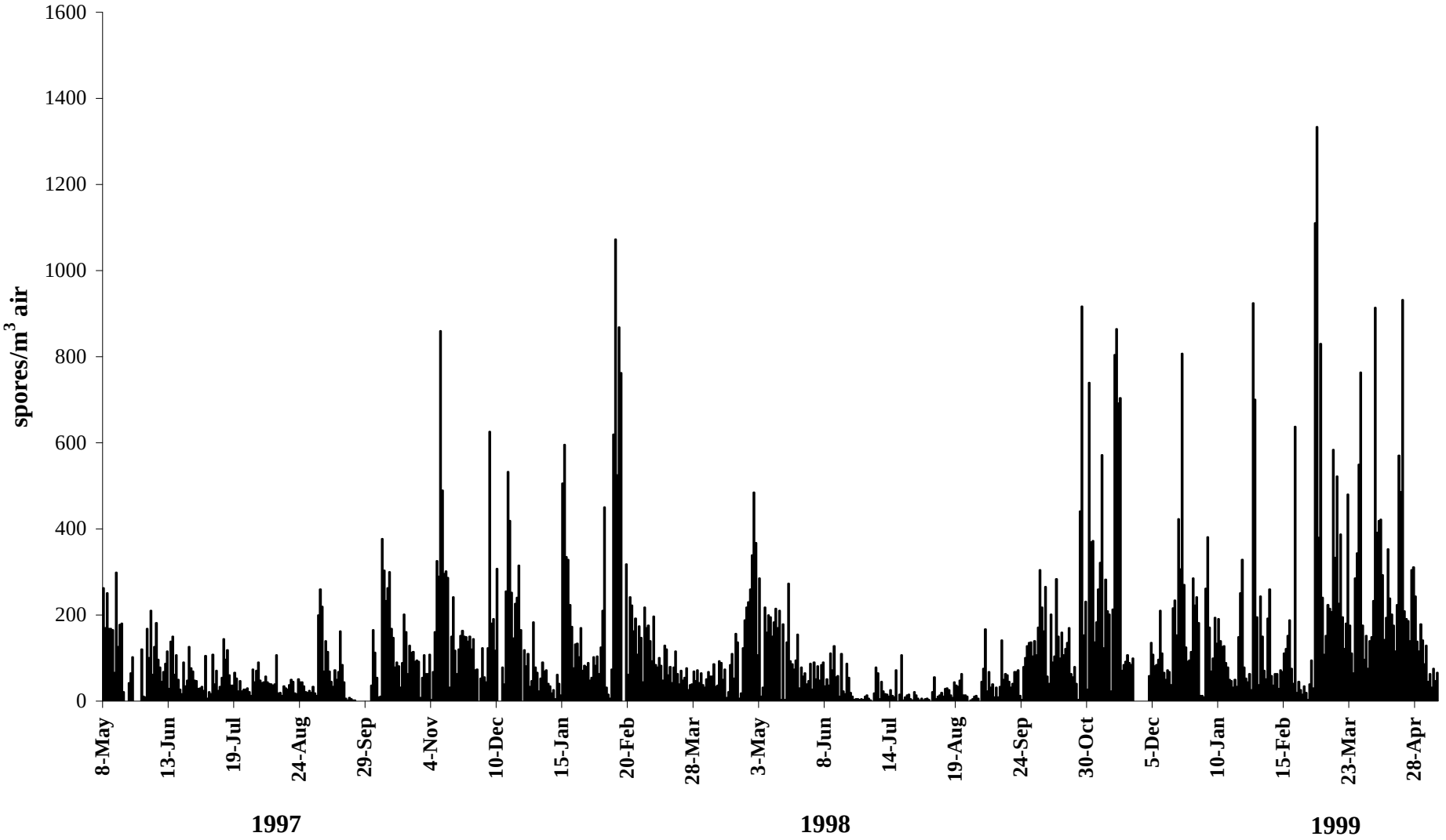
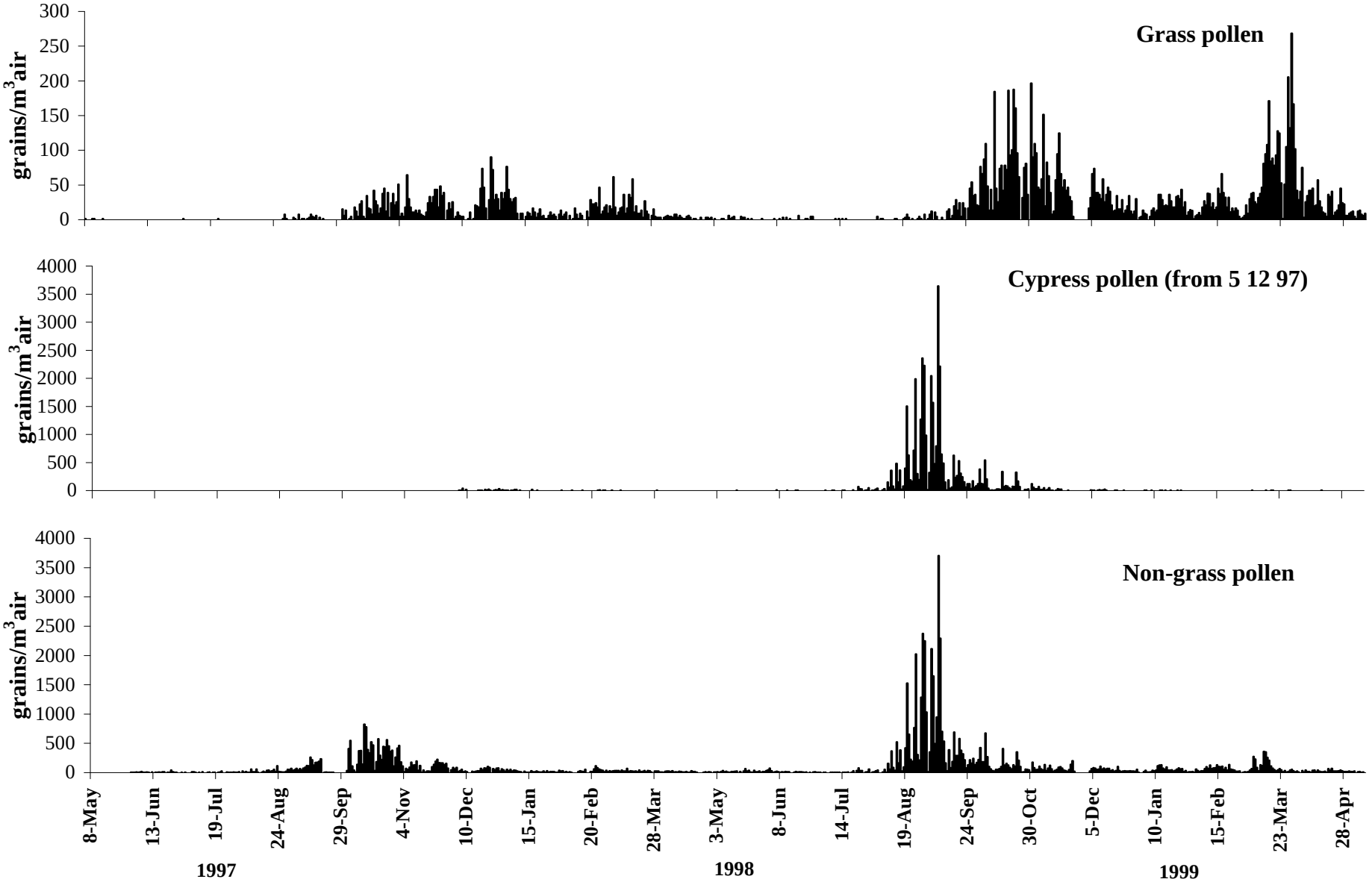
Appendix 2.1 Recipe for Calberla's stain

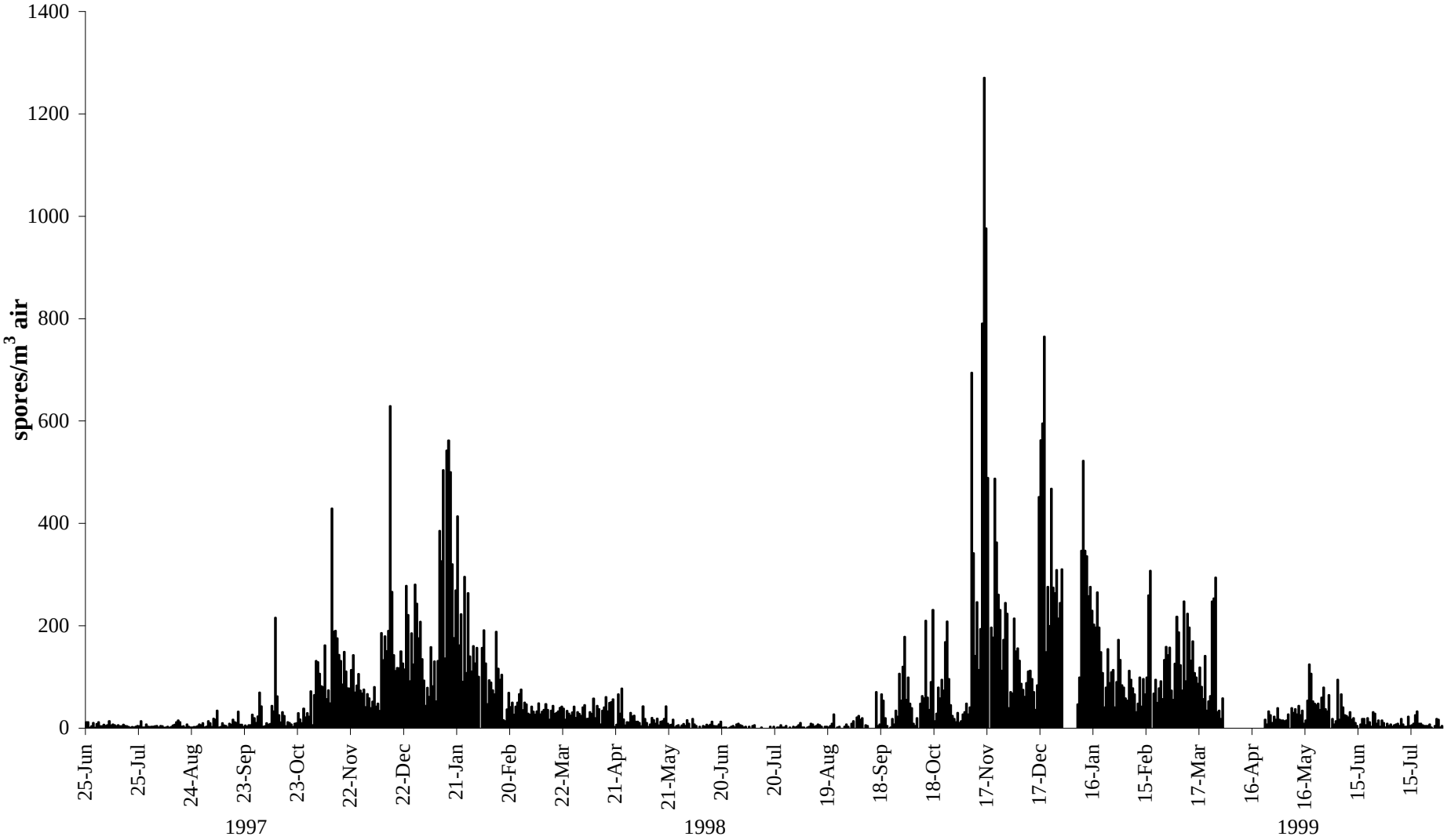
Mix 5 ml glycerine, 10 ml 95% ethanol, 15 ml water and 10 drops basic fuchsin (saturated at 3mg/ml distilled water). Allow to stain for 5 minutes before observing pollens by light microscopy. The dye deposits on the outer wall of pollen grains (Schumacher *et. al*., 1988).



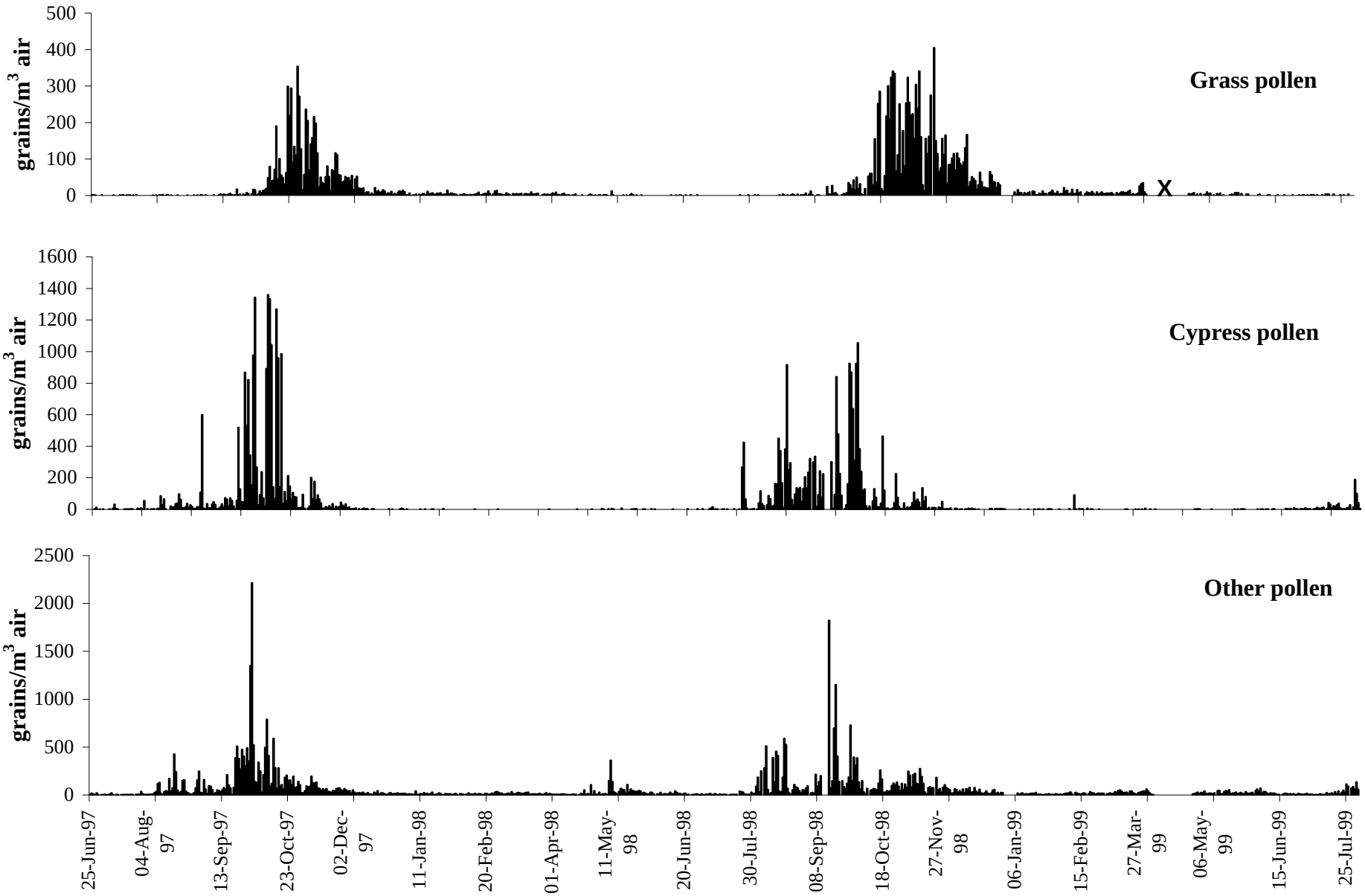
Appendix 2.2 Daily spore counts of *Alternaria* at Moree hospital, 1997 -1999



Appendix 2.3 Pollen calendars collected from the Moree hospital site, 1997 -1999.



Appendix 2.4 Daily spore concentrations of *Alternaria* at Wagga Wagga hospital



Appendix 2.5 Daily Pollen counts for Wagga Wagga hospital site, June 1997 - July 1999.

Appendix 4.1 Relative risk of attendance for asthma (7-60 year olds) at Wagga Wagga Base Hospital Emergency Department associated with an increase in *Alternaria* spore concentrations of 100 spores/m^3air/day, July 1st 1997 to December 31st 1998.

	Relative risk	95% CI
All Subjects	1.22	1.06 to 1.39
Subjects atopic to *Alternaria*	1.37	1.09 to 1.70
Subjects non-atopic to *Alternaria*	1.09	0.82 to 1.45

Based on Poisson model estimates from days when grass pollen concentrations were less than 50 grains/m^3/air and adjusts for sinusoidal terms, epidemic days, temperature, humidity, cloud cover, day of the week and a five autoregressive terms (Downs, 2000).

Appendix 4.2 Odds ratios for wheeze on some days or more each week during the preceding month and airway hyper-responsiveness in 399 school children (7-12 years) associated with an increase in *Alternaria* spore concentrations of 100 spores/m^3air/day for one month (Wagga Wagga and Moree combined)

	atopic to *Alternaria*		non-atopic to *Alternaria* (atopic to other allergens)		p value for difference
	Odds ratio	95% CI	Odds ratio	95% CI	
AHR	1.26	(1.14 to 1.39)	1.07	(0.97 to 1.19)	0.04
Wheeze	1.17	(1.03 to 1.32)	1.02	(0.90 to 1.16)	0.14

Calculated using generalised estimating equations (Downs, 2000)

Appendix 5.1 Initial Halogen procedure as developed by David Taylor

Sprinkle spore stock onto 0.45µm PVDF and coat with Reveal adhesive

Wet membrane : soak in 80% methanol 1-3 minutes, wash in dH_2O x 3 then

soak in PBS for 10 minutes

Cut membrane with a hole puncher to fit 96 well microtitre plates. Elute in Coca's solution for 4 hours

Wash in PBS/0.05% Tween x 3

Block in milk on vibrator for 45 mins

Wash in PBS/0.05% Tween x 3

Soak samples in neat anti-*Alternaria* human sera overnight in fridge

Wash in PBS/0.05% Tween x 3

Soak in 1:500 biotinylated goat anti- human IgE in 2% skim milk/PBS/Tween for 1.5 hours on shaker

Wash in PBS/0.05% Tween x 3

Soak in 1:1000 Extravidin alkaline phosphatase in 2% skim milk/PBS/Tween for 1.5 hours on shaker

Wash in PBS/0.05% Tween x 3

Incubate in BCIP/NBT checking for stain

Stop by rinsing in dH_20

Count percentage haloes

Appendix 5.2 Recipes for agars and buffers

V8 juice agar

200 ml V8 juice

800 ml dH_2O

20 g agar

mix together, adjust to pH 6.0 and autoclave

Potato Dextrose Agar

16 g PDA powder

1 L dH_2O

mix together and autoclave

Malt Marmite Agar

20g malt extract

12g vegemite

15g agar

1L dH_2O

dissolve ingredients in hot water

then autoclave

NDY/6

0.34g $NaNO_3$

0.17g KH_2PO_4

0.08g KCl

0.08g $MgSO_4.7H_2O$

1 drop FeEDTA

5.0 sucrose

20 g agar

make up to 1L with dH_2O

mix together and autoclave

Water Agar

15g agar

1 L dH_2O

mix together and autoclave

Coca's solution

0.28g $NaHCO_3$

0.82g NaCl

0.46g Phenol

1L dH_2O

Borate buffer (0.2M, pH 8.2)

12.366g of boric acid

1000ml deionised water

adjust pH to 8.2

PBS (pH 7.4)

8g NaCl

0.2g KH_2PO_4

1.15g Na_2HPO_4

0.2g KCl

1000ml deionised water

adjust pH to 7.4

PBS/0.05%Tween

to 1 litre of PBS (pH 7.4)

add 0.5ml of Tween 20

Appendix 5.3 Allergen detection protocol for spores of *Alternaria alternata*

1. grow *Alternaria* culture, aged between 8 to 20 days old, grown on V8 agar.
2. touch 0.45µm PVDF to culture then press with second piece of filter (any kind) to PVDF which will separate out the chained spores to single spores
3. Apply Reveal adhesive, binding it firmly with roller, removing bubbles
4. cut out filter pieces for 3 replicates per variable (approximately 7mm^2, fitting a 24 well microtitre wells)
5. wet in 80% methanol for 1 min
6. wash in dH_2O 3 times
7. soak in PBS 5 mins on rocker
8. Transfer to microtitre tray with Borate buffer- elute for 4 hours on shaker
9. wash in PBS/0.05% Tween x 3
10. block in 5% skim milk on shaker for 45 mins
11. wash in PBS/0.05% Tween x 3
12. incubate samples in 350µl (24 well plate) anti-A. alt human sera diluted 1:3 in 2% skim milk/PBS/0.05% Tween (Pool B), leave on shaker or rocker overnight at room temperature

Day 2

1. wash in PBS/0.05% Tween x 3
2. incubate samples with biotinylated goat anti- human IgE (1:500, diluted with 2% skim milk/PBS/0.05% Tween) for 90 minutes on shaker
3. wash in PBS/0.05% Tween x 3
4. incubate in 1:1000 Extravidin alkaline phosphatase in 2% skim milk/PBS/0.05% Tween for 90 mins on shaker
5. wash in PBS/0.05% Tween x 3

6.incubate in BCIP/NBT checking for stain ≈ 30 minutes

7.stop by rinsing in dH_20 x 3, leaving samples in dH_20

8. Clear samples in 9:1 ethylene glycol/glycerol (App 1/5.1)

8. count percentage haloes: count 100 spores, calculate the average of 3 replicates

note: if necessary this assay can be modified so that it may be completed in one day. The overnight incubation with primary antibody may be reduced to 3 hours.

Appendix 5.4 Succinate dehydrogenase (NBT) stain for spore viability

2.5ml nitroblue tetrazolium salts (4mg/ml)

2.5ml 0.2M Tris/HCl buffer (pH 7.4)

1.0ml 5mM MgCl

3.0ml dH_2O

1.0ml 2.5M disodium succinate

mix.