

Investigation of the Involvement of *Echium plantagineum* (Paterson's Curse) in Seasonal Allergy

IgE Antibodies to *Echium* and Other Weed Pollens

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The possible allergenicity of an insect pollinated weed, *Echium plantagineum*, was investigated in a rural area of Australia. Sixty-one subjects with respiratory allergy were studied. Positive skin test reactions to defatted ammonium bicarbonate extract of pollen were found in over 60% of subjects, and positive RAST tests in a similar number. The question of cross-reactivity between weed pollens is discussed. The pollen of *E. plantagineum* was shown to reach the atmosphere in significant amounts about 1 month before the peak grass pollinating period. Evidence that the pollen of *E. plantagineum* becomes airborne and elicits an IgE response suggests that further attention should be directed to weed pollens as potential allergens.

Key words: *Echium* weed pollens; seasonal allergy.

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CLINICAL ASPECTS

The possible allergenicity of an insect pollinated weed, *Echium plantagineum*, was investigated. Forty-six percent of 61 subjects with seasonal respiratory allergy ascribed their symptoms to the weed. Positive skin test and RAST reactions were found in over 60% of subjects. The pollen was shown to reach the atmosphere in significant amounts about 1 month before the peak grass pollinating period. Further attention should be directed to insect pollinated plants as potential allergens in view of these findings that *E. plantagineum* pollen becomes airborne and that it elicits an IgE response.

Seasonal allergy is a major problem in rural New South Wales, Australia. Many patients attribute their symptoms to the weed *Echium plantagineum* which is very noticeable because of its striking purple flowers and profusion of growth. *E. plantagineum* (common names – Paterson's Curse, Salvation Jane, Lady Campbell Weed) is a weed which is widespread in Europe, particularly in the Mediterranean region, and was introduced from Europe to the southern and eastern states of Australia (6, 8, 14, 15) (Fig. 1). The plant is a known dermatological irritant (8).

We decided to investigate the role of this weed in seasonal allergy in rural areas because of the frequently stated belief of patients that *E. plantagineum* was responsible for their allergy and because of the coincidence of its flowering time with the allergy season. We elected to study the subjects in Albury, a city of about 50,000 inhabitants about 500 km from Sydney and an important regional development centre whose population is expected to increase significantly in the coming years.

It seemed doubtful that *E. plantagineum* would prove to be an important allergenic

source because the pollen is entomophilous (insect-pollinated), whereas it is generally accepted (13, 15) that pollens provoking allergic reactions are anemophilous (wind-pollinated). Furthermore, few positive skin test reactions to its pollen have been reported by those performing skin tests with a commercially available extract. As part of the study, we also investigated responses of allergic subjects to pollens from a number of other weeds and grasses.

MATERIAL AND METHODS

Subjects

Patients presenting with allergic symptoms to Albury Base Hospital Casualty Department and to four General Practitioners in the city were asked to participate in the study. Subjects were interviewed by one of us (C.K.). A history of the problem was elicited and subjects were asked to volunteer any factors which they believed contributed to their symptoms (Table 1).

Skin tests

Prick tests were performed on the volar aspect of the left forearm with 28 allergens. The same person (C.K.) performed all skin tests to ensure reproducibility. Prick test solutions of Paterson's Curse and plantain pollens were prepared by dissolving the appropriate quantities of pollen extract in a sterile solution of a 1:1 mixture of saline and glycerin containing 5 mg/ml phenol.

Table 1

Suspected allergen sources contributing to allergic symptoms as named by patients. Results obtained from questionnaire

Suspected allergen source	No. positive
Paterson's Curse	28
Grasses	24
Weeds	7
Garden plants	7
Trees	16
Other	3
Not known	14

Total: 61 questionnaires.

Both pollen extracts were tested at concentrations of 1 mg/ml and 10 mg/ml. Other allergen extracts were obtained from commercial sources. Grass mix 10,000 PNU/ml, meadow fescue (*Festuca elatior*) 5,000 PNU/ml, *Acacia* sp. 10,000 PNU/ml, Bermuda grass (*Cynodon dactylon*) 5% w/v, perennial rye grass (*Lolium perenne*) 5,000 PNU/ml, timothy grass (*Phleum pratense*) 5,000 PNU/ml, *Aspergillus fumigatus* 4,000 PNU/ml, house dust 5,000 PNU/ml, *Dermatophagoides pteronyssinus* 1,500 PNU/ml and *D. farinae* 1,500 PNU/ml were obtained from Dome Laboratories, Mulgrave, Australia. Privet (*Ligustrum* spp.) 10,000 U/ml, capeweed (*Cryptostemma calendula*) 10,000 U/ml and Paterson's Curse (*E. plantagineum*) 10,000 U/ml were supplied by CSL, Melbourne, Australia. Sweet vernal (*Anthoxanthum odoratum*) 1:20 w/v, short ragweed (*Ambrosia elatior*) 1:20 w/v and dandelion (*Taraxacum officinale*) 1:20 w/v were supplied by Hollister Stier Laboratories, Spokane, Washington. Clover (*Trifolium* spp.) 2.5% and sorrel (*Rumex acetosa*) 2.5% were supplied by Bencard, Brentford, U.K. Extracts were not standardised in weight or in terms of biological activity so no attempt was made to compare reactions to various extracts by size on a quantitative basis.

Histamine acid phosphate 1 mg/ml in saline was used as a positive control and a solution of 4 mg/ml phenol and 50% glycerin in saline (Dome Laboratories, Sydney) served as a negative control. Wheal sizes calculated from the average of greatest and least diameters, were recorded 15 min after testing. A positive reaction was accepted as a wheal $\geq$ 3 mm diameter. For perennial rye grass, plantain and *Echium* extracts potency was estimated by comparing the wheal diameters to that of histamine acid phosphate 1 mg/ml.

Extraction of *E. plantagineum* and *Plantago lanceolata* (plantain) pollens

Crude extracts and fractions from whole dry pollen (*E. plantagineum*) from CSL, Melbourne, and *Plantago lanceolata* from Greer Laboratories, Lenoir, North Carolina, were prepared by the method of Marsh et al. (13), except that the defatting step was omitted (Fig. 2).

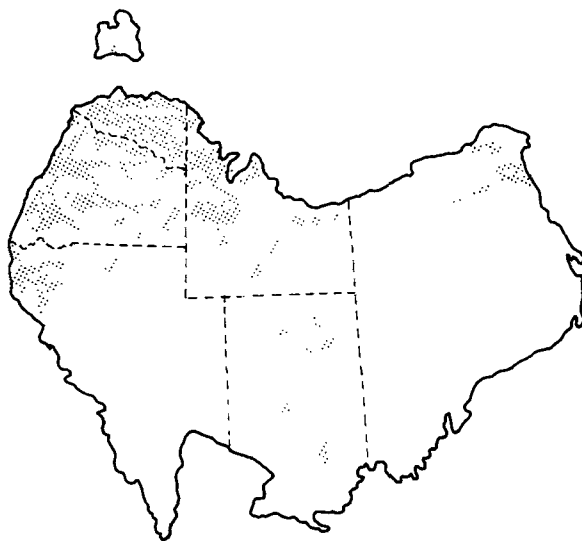


Fig. 1. Distribution of *E. plantagineum* in Australia (adapted from Piggin (16)).

Pollen count

Pollen collection was performed using a 7-day volumetric spore trap (Burkard Industries, Kent, U.K.), situated on the roof of a seven storey building in the centre of Albury. This was operated continuously from August to January. Counts were done as outlined by Hirst (4, 10).

Allergen discs

E. plantagineum whole plant and pollen and *P. lanceolata* pollen extracts were reacted with cyanogen bromide-activated Whatman grade 44 6 mm paper discs as described by Ceska et al. (5). *A. elatior*, *Artemisia vulgaris* (mugwort), *Chenopodium album* (goosefoot or fat hen), *Chrysanthemum leucanthemum* (marguerite or ox eye daisy), *Salsola kali* (Russian thistle or soft roly-poly), *Solidago virgaurea* (golden rod), *Taraxicum vulgare*, *L. perenne*, *P. pratense* and *C. dactylon* RAST allergen discs were obtained from Pharmacia, Uppsala, Sweden.

Radioallergosorbent test (RAST)

RAST examinations (17) were carried out as described by Baldo & Uhlenbruck (3) using 50

µl quantities of sera. Cord serum, used as an IgE-“free” control, was obtained from St. John of God Hospital, Perth.

RESULTS

Information from questionnaire

Sixty-one subjects were studied, comprising 33 females and 28 males. Their ages ranged from 3 to 64 with a mean of 27 years. Subjects had been in Albury from 0.5 to 52 years with a mean of 13. Paterson's Curse was volunteered by 28 patients (46%) as a major contributing factor to their symptoms. November was the peak month for symptoms in 55 of 58 patients (95%). This corresponds to the highest grass pollen counts. However, 75% (46/61) experienced their first symptoms of allergic rhinitis or asthma in September-October during the period corresponding to the flowering time of *E. plantagineum*, and antedating the grass pollinating season.

Pollen counts

Fig. 3 shows the pollen counts for *Echium* and Gramineae species for the Spring/Summer of 1979. Significant numbers of *Echium* pollen

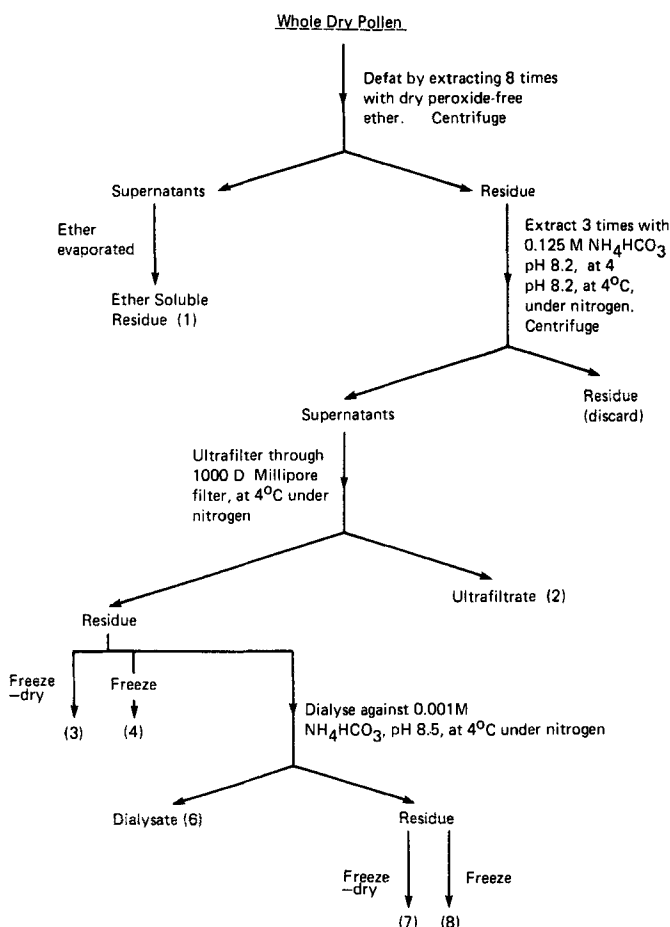


Fig. 2. Fractionation procedure for preparing crude pollen extracts.

grains enter the atmosphere for a period in September-October before the rise of the Gramineae pollen in late October. The peak for grasses was considerably greater than for *Echium*.

Skin prick test studies

Results of prick tests are set out in Table 2. The subjects proved to be highly atopic in that all reacted to more than one allergen with 57% (35/61) having 16–20 positive reactions out of 28 allergens tested. Grass pollens evoked the greatest number of reactions (91.8%), and in this population only about 40% reacted to house dust mite extracts. Reactions to weed pollens ranged from 19.7% for capeweed to

78.6% for clover pollen extract. Reactions to *Echium* varied according to the extract and dosage (Table 3). A number of patients who responded to the prepared extract even at the lower concentration (1 mg/ml) did not respond to the commercial extract. Potencies of the skin prick test solutions of *Echium* and plantain were compared with rye grass by relating diameter of reactions to the diameter of histamine 1 mg/ml (Table 4). The majority of subjects who showed reactions to *E. plantagineum* also reacted to other weeds, in particular plantain. However, several subjects showed positive reactions to one weed and not to others (Table 5). A number of subjects also showed reactions to short ragweed which is not found in the Albury district.

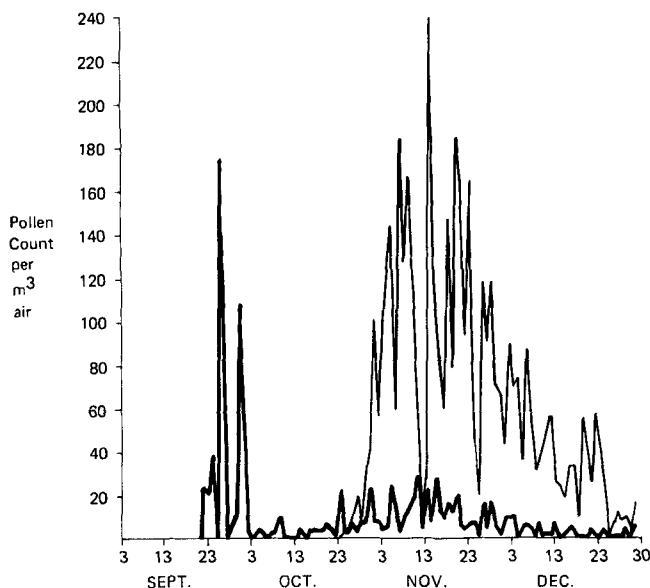


Fig. 3. Pollen counts (per m^3 air) for *Echium plantagineum* and Graminae species from August–December. *Echium* counts (heavy line) peaked from the last week in September to the first week in October. Graminae species pollen counts (light line) increased by mid-October and high counts continued until late December.

RAST studies

Preliminary experiments designed to detect potent RAST-positive pollen fractions and to assess the effects of ether extraction, dialysis, freezing and lyophilisation on allergenic reactivity were carried out using *E. plantagineum* and *P. lanceolata* pollens. Eight fractions (numbered (1)–(8) in Fig. 2) were examined in RAST experiments using activated discs that had been incubated with 50–500 μg of extract. Preparations (7) and (8) proved equally active, being slightly more potent than fractions (1), (2) and (6). The freeze-dried preparations (fraction 7) of *E. plantagineum* and *P. lanceolata* pollen extracts were therefore used in all subsequent RAST experiments described in this paper. Optimum coupling ratios for the freeze-dried fractions from both pollens were examined in direct RAST experiments with *E. plantagineum*- and *P. lanceolata*-positive sera and shown to be 200 μg per disc for *E. plantagineum* and 50 μg per disc for *P. lanceolata*. Direct RAST experiments with weed and grass pollen extracts for pooled cord and normal sera showed no significant uptake.

Fifty-three of the 61 allergic subjects had RAST studies performed. Thirty-one of the 53 had positive uptakes with *Echium*. Fig. 4 demonstrates the results in 24 of these patients. Sera reacted with between two and eight different species of weed pollens and with extracts from three grasses, *L. perenne*, *P. pratense* and *C. dactylon*. Eight other weed species were examined. *P. lanceolata* allergen discs reacted with all 24 of the *Echium*-positive sera, while reactions with other weeds were seen less frequently (Fig. 4). Twenty-two sera (41.5%) did not react with *Echium* discs. Positive reactions to other weed pollens were rarely seen in this group, although all 22 sera showed clear reactivity with grass pollen discs. The correlation between positive RAST uptakes for *Echium* and positive skin prick tests was 64.5%. Of those with negative RAST uptakes, 50% had negative skin prick tests.

DISCUSSION

Paterson's Curse pollen has not been considered to be an important source of allergens in Australia in the past, despite the obvious and abun-

Table 2

Skin prick test results in allergic subjects showing percentage positive for each extract

Extract ^a from		% Positive ^b
Grass pollens:	grass mix	91.8 ^d
	meadow fescue	91.8
	Bermuda grass	72.1
	perennial rye grass	90.2
	timothy grass	85.2
	sweet vernal grass	92.8 ^c
Weed pollens:	capeweed	19.7
	clover	78.6 ^c
	sorrel	69.6 ^c
	short ragweed	39.3
	dandelion	58.9 ^c
	plantain (commercial)	44.3
	plantain 1 mg/ml	58.2
	plantain 10 mg/ml	62.5 ^c
	Paterson's Curse (commercial)	24.6 ^c
	Paterson's Curse 1 mg/ml	64.8 ^c
	Paterson's Curse 10 mg/ml	88.5 ^c
Fungi:	<i>Aspergillus</i> sp.	9.8
	<i>Cladosporium</i> sp.	8.9 ^c
	<i>Alternaria</i> sp.	11.5
Mites:	<i>Dermatophagoides pteronyssinus</i>	42.6
	<i>D. farinae</i>	44.3
Miscellaneous:	histamine	86.8
	negative control	8.2
	privet	13.1
	acacia	37.7
	house dust	34.4

^a For details of extracts used, see Material and Methods.

^b Sixty-one patients examined except where indicated. A positive response was calculated as the average of two diameters. A wheal size of ≥ 3 mm was recorded as positive.

^c Fifty-six patients tested.

^d Grass mix contained cocksfoot, timothy, Kentucky blue, sweet vernal, perennial rye, meadow fescue and bent grass.

dant presence of the plant during the spring and early summer. This has been due to two factors. Firstly, it is an insect-pollinated plant (personal communication, J. M. Cullen, Division of Entomology, Commonwealth Scientific and Industrial Research Organisation) and therefore was thought not to fulfil one of the important criteria for aeroallergens, namely that the pollen

Table 3

Difference in skin prick test reactivity using two different extracts of *Echium plantagineum*

Extract from	No. positive reactions	$\frac{D+d}{2} \geq 3$ mm
	<i>n</i>	%
PC ₁	15/61	24.6
PC ₂	35/54	64.8
PC ₃	54/61	88.5

PC₁ = Commercially available extract of *E. plantagineum*.

PC₂ = *E. plantagineum* at a concentration of 1 mg dry weight/ml.

PC₃ = *E. plantagineum* at a concentration of 10 mg dry weight/ml.

D = The greatest diameter of the wheal.

d = The smallest diameter.

Table 4

Comparison of potencies for *L. perenne*, *P. lanceolata* and *E. plantagineum*

HEP* value	No. of patients		
	<i>L. perenne</i>	<i>P. lanceolata</i>	<i>E. plantagineum</i>
≤ 1.0	14	38	36
$> 1.0 \geq 2.0$	24	10	9
$> 2.0 \geq 3.0$	14	2	5
> 3.0	8	2	2
Total	60	52	52

* Histamine equivalent: 1 HEP potency elicits a reaction of similar size to histamine acid phosphate 1 mg/m (2).

must be sufficiently buoyant to become airborne (10). The second factor has been the poor response to skin prick tests using the commercially available extract.

Pollen counts performed during this study have shown that Paterson's Curse pollen does reach the atmosphere in large amounts. Furthermore, we have demonstrated a high degree of reactivity in this rural population to an extract prepared as described. The commercially available extract failed to elicit a skin reaction in patients who had positive skin tests to the 1 mg/ml extract. These factors indicate that the pollen is present in the atmosphere, is allergenic

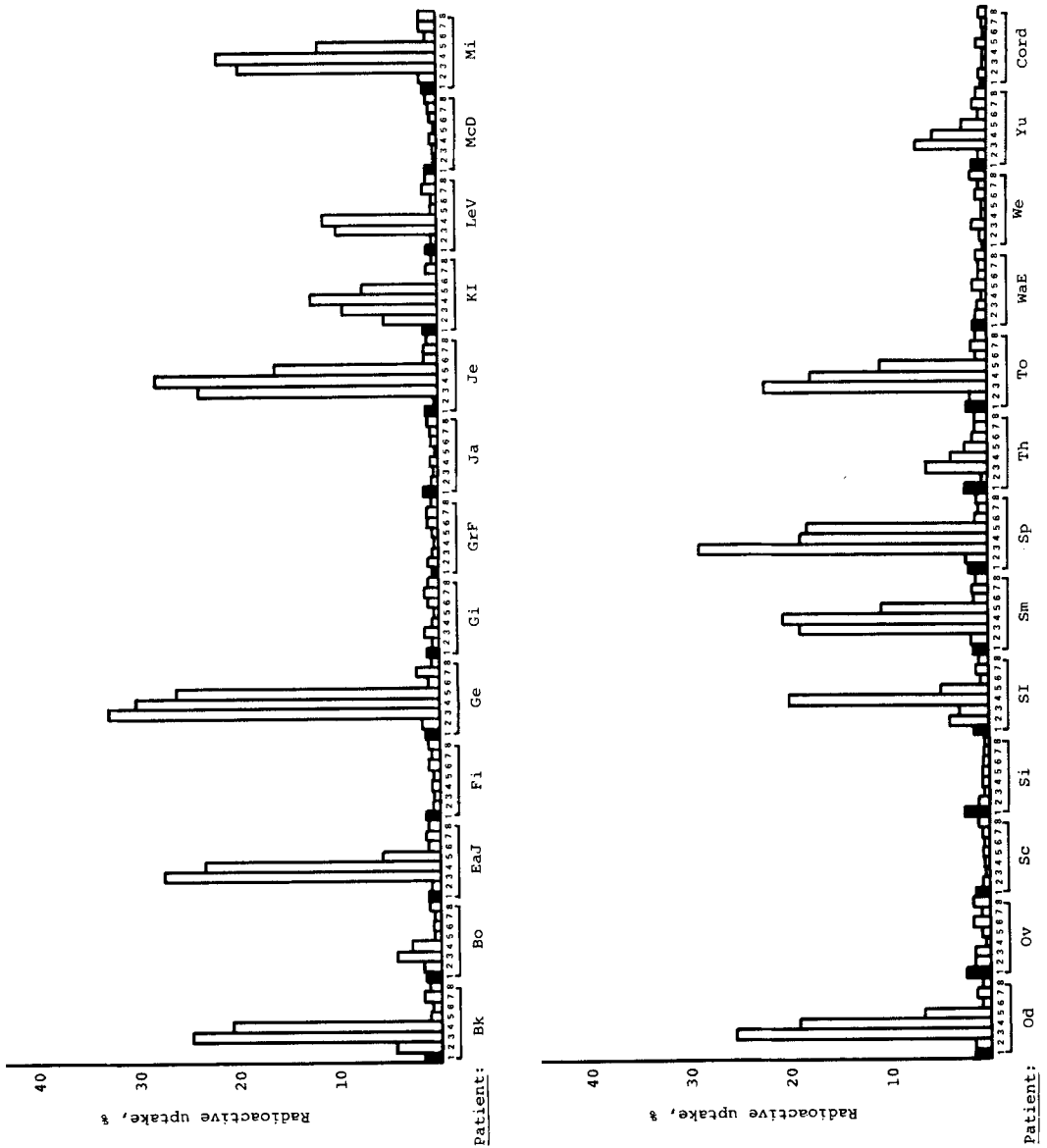


Fig. 4. RAST results in 49 individual patients for weed and grass pollens.

Pollens:

- | | |
|-------------------------------------|-----------------------------|
| 1 <i>Echium plantagineum</i> | 7 <i>Salsola kali</i> |
| 2 <i>Ambrosia elatior</i> | 8 <i>Solidago virgaurea</i> |
| 3 <i>Artemisia vulgaris</i> | 9 <i>Taraxacum vulgare</i> |
| 4 <i>Chenopodium album</i> | 10 <i>Lolium perenne</i> |
| 5 <i>Chrysanthemum leucanthemum</i> | 11 <i>Phleum pratense</i> |
| 6 <i>Plantago lanceolata</i> | 12 <i>Cynodon dactylon</i> |

and therefore may be potentially an important allergen clinically.

Correlation between skin prick tests and

RAST uptakes range from 40% to over 90% depending on the allergen and its purity (1).

The positive correlation of 64.5% between prick

testing and RAST uptakes for *Echium* in this study is in keeping with published results for other weeds (15).

Patients who showed positive RAST reactions to *E. plantagineum*, also had IgE antibodies to several other weed pollens. Patients RAST negative to *E. plantagineum* were also negative to all other weeds. The same did not hold for skin prick test reactions. There were subjects who reacted to *Echium* and not to other weeds, e.g., *P. lanceolata* or *A. elatior*. These were chosen for comparison because the former is a ubiquitous weed and the latter an unknown weed in this region. Allergic cross-reactivity may exist between some of the weed pollens or separate sensitization to individual weed species may occur. Further investigations are needed to clarify which of these possibilities exists.

Apart from ragweed species (4, 11, 12), there has been unjustified neglect of weed pollens both in research directed at isolating pure allergens, in determining their potential clinical significance and in investigating cross-reactive pollen allergens. Identification of common weed allergens could prove to be of great significance in immunotherapy.

The observation that *Echium* pollen is an allergen accessible for inhalation suggests that more attention should be directed to determining whether it may be of clinical relevance. The observation that symptoms preceded the onset of the grass pollinating season make this a distinct possibility.

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