

Allergy to Pollen from Different Deciduous Trees in Sweden

An Investigation with Skin Tests, Provocation Tests and the Radioallergosorbent Test (RAST) in Springtime Hay Fever Patients

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92 adult patients with springtime allergy were investigated with skin tests, provocation tests and the RAST. Allergen extracts from 12 different deciduous trees were used.

Positive reactions, often of high intensity, were most often found with birch, alder, bog-myrtle, beech and hazel allergens whereas oak, aspen, linden, elm, sawlow, maple and poplar allergens more often gave negative or only weak positive test results. Cross sensitizations were found between botanically related as well as between less related species of the trees. Almost all patients with a clinically relevant tree pollen allergy had positive provocation tests with birch pollen. No patient had positive reactions to all the 12 allergens included in the study. 68% had positive provocation tests with the combination birch, alder and hazel.

It is concluded that for diagnostic screening purposes it is sufficient to use birch pollen allergen. If the majority of relevant tree pollens are to be included in a diagnostic or therapeutic programme in Western Sweden it should contain birch, alder, hazel, beech and bog-myrtle allergens.

Key words: cross-allergy; hay fever; immediate type hypersensitivity; pollen allergy; provocation test; radioallergosorbent test; skin test; tree pollen

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Hay fever in Sweden is mostly caused by allergy to grasses, trees and plants of the *Compositae* family (particularly mugwort). Thirty years ago Juhlin-Dannfelt (13) noticed that of 251 hay fever patients 29% were tree pollen sensitive. Higher relative figures for tree pollen allergies are seen at routine screening at our clinic. Among patients submitted to skin tests (ST) during 1 year, 339 had positive reactions to birch pollen allergen, 484 to timothy pollen and 289 to mugwort pollen allergens. 207 patients had positive ST with both timothy and birch pollen allergen (not published).

Juhlin-Dannfelt (13) performed ST with 15 different tree pollen allergens in 42 patients with springtime hay fever. He found positive reactions to birch and alder pollen in all individuals and to beech or ash pollen in all but one patient. Positive reactions were also often elicited with hazel and oak whereas pollen from maple or pollen from coniferous trees very seldom gave positive reactions.

A mixture of birch, alder and hazel allergen (Allpyral "Northern tree mix") is often used for desensitization therapy in patients with springtime hay fever in Sweden. Although the results of this treatment seem

fairly good in most cases, it is still not known with certainty which tree pollen allergens are of importance for diagnosis and specific therapy.

The purpose of the present study was to evaluate which tree pollen allergens are relevant for hay fever in Western Sweden and to study the pattern of cross-sensitization among tree pollens. Allergens of pollen from 12 different deciduous trees were used for diagnosis with ST, provocation test (PT) and the radioallergosorbent test (RAST) in patients with springtime allergy.

PATIENTS AND METHODS

The study covers 92 untreated hay fever patients (ages 12–71, mean 26 years) living in Gothenburg. They were selected because of a positive ST, PT or RAST result with pollen allergen from one or more of the following trees: birch (*Betula verrucosa*), alder (*Alnus glutinosa*), hazel (*Corylus avellana*), oak (*Quercus robur*), beech (*Fagus sylvatica*), aspen (*Populus tremula*), poplar (*Populus balsamifera*), willow (*Salix caprea*), linden (*Tilia cordata*), bog-myrtle (*Myrica gale*), elm (*Ulmus glabra*) and maple (*Acer platanoides* or *Acer negundo*). Most of the patients reported symptoms of seasonal allergic rhinitis in the month of May and several in April, June and July. Besides reactions with tree pollen allergens, 54 of the patients had positive ST with grass pollen and 29 with mugwort allergen. 53 of the patients had noticed bronchial asthma as well as rhinoconjunctivitis.

Allergens

The beech and maple pollen extracts used in the *in vivo* testing were obtained from Vitrum AB, Stockholm, Sweden. The remaining allergens for *in vivo* testing were made by our own laboratory as previously described (10). 10 ml of phosphate-buffered saline were used for the extraction of 1 g non-defatted dry pollen, which was obtained from AB Cernellie, Vegeholm, Sweden. Before the ex-

traction the pollen was disintegrated as a frozen suspension by means of an X-press as earlier described by Belin (3). After an extraction time of 2–4 days during which mechanical agitation was performed for 2 × 2 h insoluble material was separated by centrifugation and discarded. The extracts were finally filtered through Millipore GS filters (0.22), checked for sterility and assayed for the expected allergen activity by intracutaneous ST in earlier documented allergic individuals. The allergens used in the RAST were those supplied with the commercial kit from Pharmacia Diagnostics AB, Uppsala, Sweden. The *Acer* allergen used in the *in vitro* test was *Acer negundo* (box elder) whereas that employed in the *in vivo* tests was *Acer platanoides* (maple), which is the species occurring in Sweden.

In vivo tests

STs (scratch and intracutaneous tests) were performed according to the routines established at our clinic, as earlier described (10). The highest allergen concentration used for intracutaneous tests was 10^{-3} w/v and for scratch tests 10^{-2} w/v. Reactions were recorded after 20 min and graded according to the recommendations of the Standardization Committee of the Northern Society of Allergology (1). Thus, a weal reaction of the same size as that of a histamine reference (histamine 0.1 g/l in intracutaneous tests) was recorded as three plus (+++) and a reaction of the same size as that elicited by a negative reference (isotonic phosphate saline buffer with 0.4% phenol) was recorded as negative (–).

Nasal PTs were performed in the following way: with a 1-ml disposable syringe, 0.1 ml of extraction fluid or allergen extract (from 10^{-3} to 10^{-1} w/v concentration) was instilled in one nostril. In the absence of reactions, instillations were repeated at 10-min intervals with tenfold increasing concentrations of allergen extracts until a positive reaction occurred (sneezing, rhinorrhea, itching, blocking of the nose or

symptoms of conjunctivitis) or the highest concentration (10^{-1} w/v) was reached. The positive results were expressed as the highest dilution giving reaction. In addition, the positive reactions were scored as +=mild, ++=moderate and +++=strong reaction, depending upon the intensity of the symptoms elicited.

The *in vivo* tests were carried out 1–7 months after the pollen season of the deciduous trees. The complete test programme, with 12 allergens, was carried out with ST in 67 and with PT in 62 individuals.

RAST

Specific serum IgE antibodies against birch, alder, hazel, oak, elm, maple and beech were determined by the Phadebas RAST® (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions, with slight modifications (10). The results were expressed as RAST classes 0–4. Class 0 was regarded as negative and classes 1–4 as positive. The complete RAST programme, with seven allergens, was carried out in 87 individuals.

Statistical methods

Fischer's exact test for a two-by-two Table was used for analyses of correlations.

RESULTS

Frequency profiles of skin tests, provocation tests and RAST

Positive PTs were most frequently found with birch (92%) and alder (82%) allergen. Next came bog-myrtle, beech and hazel, followed by oak and aspen, whereas only a few individuals reacted on provocation with linden, elm, willow, maple and poplar. Similar results were obtained employing ST or the RAST (Fig. 1). The distribution of test results with regard to the intensity of nasal sensitivity is shown in Table 1 and with regard to RAST classes in Table 2. As will be seen from the Tables, the positive reactions

Table 1
Distribution of provocation test results with 12 different allergens in 62 patients

Allergen	Provocation test result				
	Negative	Doubtful ^a	Positive at allergen concentration		
			10^{-1}	10^{-2}	10^{-5}
Birch	5	6	23	25	3
Alder	11	6	23	16	6
Hazel	19	9	16	16	2
Bog-myrtle	16	9	19	16	2
Beech	18	7	23	12	2
Oak	41	5	13	3	0
Aspen	42	12	4	3	1
Sallow	55	0	5	2	0
Poplar	55	2	4	1	0
Linden	51	7	3	1	0
Elm	54	2	4	2	0
Maple	55	5	1	1	0

^a Patients reacting with only slight symptoms at the highest allergen concentration (10^{-1}).

to birch, alder, bog-myrtle, beech and hazel were often of high intensity, whereas those to the other allergens mostly indicated low-degree allergy.

Relationship between test results with different allergens

When the test result achieved with one pollen allergen was correlated to that achieved with

Table 2
Distribution of RAST results with seven different allergens in 87 patients

Allergen	No. of patients in Phadebas RAST class					Phadebas RAST class mean
	0	1	2	3	4	
Birch	12	12	26	31	6	2.1
Alder	18	25	31	9	4	1.5
Hazel	23	25	27	8	4	1.4
Beech	18	28	27	9	5	1.5
Oak	41	29	7	9	1	0.9
Elm	59	20	4	4	0	0.5
Maple	57	16	10	3	1	0.6
Total	228	155	132	73	21	

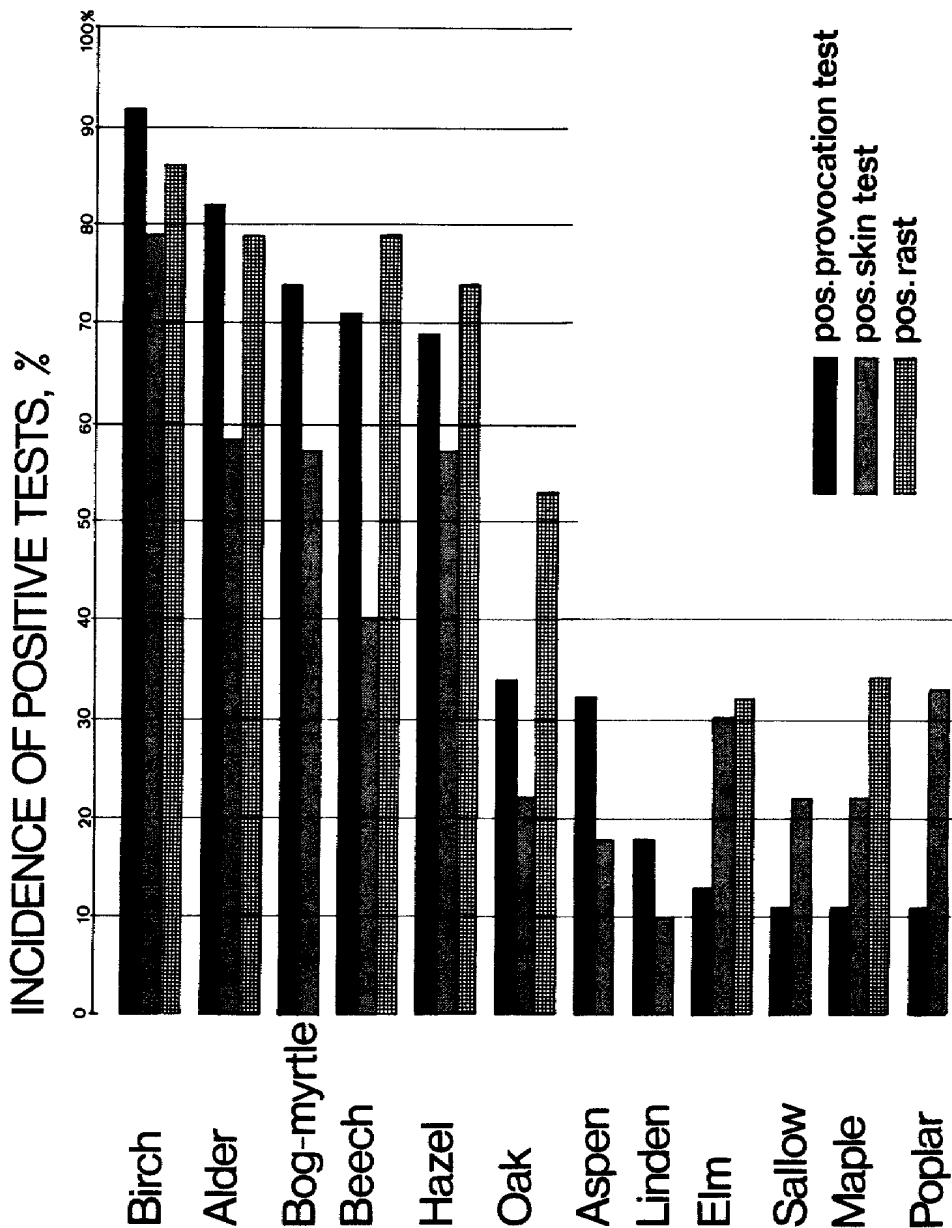


Table 3

Relationship between provocation test results with 12 different tree pollen allergens in 62 patients with positive reaction to one or more of the allergens. The Table is symmetrical. The lower left part consists of 2 x 2 Tables and the upper right part gives the results of statistical analyses of these Tables

Allergen	Birch	Alder	Hazel	Bog-myrtle	Beech	Oak	Aspen	Sallow	Poplar	Linden	Elm	Maple
	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +
Birch	- 5 — + — 57	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Alder	- 3 8 + 2 49	11 — — 51	***	**	***	*	ns	ns	ns	ns	ns	ns
Hazel	- 4 15 + 1 42	10 9 1 42	19 — — 43	ns	***	***	ns	ns	ns	ns	ns	ns
Bog-myrtle	- 3 13 + 2 44	7 9 4 42	12 4 7 39	16 — — 46	**	**	ns	ns	ns	ns	ns	ns
Beech	- 4 14 + 1 43	9 9 2 42	14 4 5 39	12 6 4 40	18 — — 44	**	ns	ns	ns	ns	ns	ns
Oak	- 5 36 + 0 21	11 30 0 21	19 22 0 21	16 25 0 21	17 24 1 20	41 — — 21	*	**	ns	**	*	**
Aspen	- 4 38 + 1 19	10 32 1 19	16 26 5 17	12 30 4 16	13 29 5 15	32 10 9 11	42 — — 20	ns	ns	ns	ns	**
Sallow	- 4 51 + 1 6	10 45 1 6	18 37 1 6	15 40 1 6	17 38 1 6	40 15 1 6	40 15 2 5	55 — — 7	**	**	**	**
Poplar	- 3 52 + 2 5	9 46 2 5	18 37 1 6	15 40 1 6	17 38 1 6	39 16 2 5	40 15 2 5	53 2 2 5	55 — — 7	**	**	**
Linden	- 2 49 + 3 8	8 43 3 8	17 34 2 9	14 37 2 9	16 35 2 9	38 13 3 8	37 14 5 6	50 1 5 6	50 1 5 6	51 — — 11	**	**
Elm	- 3 51 + 2 6	10 44 1 7	18 36 1 7	15 39 1 7	16 38 2 6	39 15 2 6	41 13 1 7	51 3 4 4	52 2 3 5	49 5 2 6	54 — — 8	**
Maple	- 4 51 + 1 6	10 45 1 6	18 37 1 6	16 39 0 7	18 37 0 7	40 15 1 6	41 14 1 6	52 3 3 4	53 2 2 5	50 5 1 6	52 3 2 5	55 — — 7

- = Provocation test negative, + = provocation test positive.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

ns = not significant.

another pollen allergen for each patient the results shown in the two-by-two Tables of Table 3 (PT results), Table 4 (ST results) and Table 5 (RAST results) were obtained. As will be seen from the Tables, significant correlations were found between pollen from

botanically related trees (e.g. birch-alder, oak-beech) as well as between pollen from less related trees (e.g. birch-beech, oak-elm, maple-linden). It should be noticed that for methodological reasons it is not possible to achieve statistical significance in two-by-two Tables where the expected number in any column or row is very small.

As a rule, there was a quantitative relationship between the test results with different allergens: the stronger the reaction

Fig. 1. Frequency profiles for different tree pollen allergens with provocation test ($n=62$), skin test ($n=67$) and the RAST ($n=87$). The length of the bars indicate the percentage of patients with positive test results.

Table 4

Relationship between skin test results with 12 different tree pollen allergens in 67 patients with positive skin test reaction to one or more of the allergens. For explanations, see Table 3

Allergen	Birch		Alder		Hazel		Bog-myrtle		Beech		Oak		Aspen		Sallow		Poplar		Linden		Elm		Maple		
	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	
Birch	-	14	—	°		ns		ns		ns		ns		ns		ns		ns		ns		ns		ns	
	+	—	53																						
Alder	-	10	18	28	—	°°		ns		ns		ns		ns		ns		***		ns		°		*	
	+	4	35	—	39																				
Hazel	-	8	21	18	11	29	—	°°		ns		ns		***		ns		°°		ns		***		ns	
	+	6	32	10	28	—	38																		
Bog-myrtle	-	8	21	19	10	19	10	29	—		ns		ns		ns		ns		***		ns		°°		ns
	+	6	32	9	29	10	28	—	38																
Beech	-	10	30	20	20	19	21	17	23	40	—		ns		ns		ns				°		ns		°°
	+	4	23	8	19	10	17	12	15	—	27														
Oak	-	9	43	24	28	24	28	24	28	29	23	52	—		ns		°		°°		ns		ns		ns
	+	5	10	4	11	5	10	5	10	11	4	—	15												
Aspen	-	13	42	24	31	29	26	25	30	34	21	45	10	55	—		ns		ns		ns		°		**
	+	1	11	4	8	0	12	4	8	6	6	7	5	—	12										
Sallow	-	12	40	23	29	24	28	24	28	34	18	44	8	45	7	52	—		ns		ns		ns		ns
	+	2	13	5	10	5	10	5	10	6	9	8	7	10	5	—	15								
Poplar	-	12	33	25	20	25	20	27	18	24	21	40	5	40	5	37	8	45	—		ns		°°		ns
	+	2	20	3	19	4	18	2	20	16	6	12	10	15	7	15	7	—	22						
Linden	-	13	47	27	33	27	33	27	33	39	21	48	12	51	9	49	11	43	17	60	—		°		***
	+	1	6	1	6	2	5	2	5	1	6	4	3	4	3	3	4	2	5	—	7				
Elm	-	12	35	24	23	27	20	26	21	26	21	40	7	42	5	38	9	41	6	45	2	47	—		ns
	+	2	18	4	16	2	18	3	17	14	6	12	8	13	7	14	6	4	16	15	5	—	20		
Maple	-	11	42	26	27	25	28	24	29	37	16	44	9	48	5	44	9	38	15	52	1	40	13	53	—
	+	3	11	2	12	4	10	5	9	3	11	8	6	7	7	8	6	7	7	8	6	7	7	—	14

— = Skin test negative, + = Skin test positive.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

ns = not significant.

to one allergen, the stronger it was to other allergens. As an example of this, the correlation between RAST with alder and with birch pollen allergen is shown in Table 6. All the patients with RAST class 4 with any of the other allergens also had RAST class 4 with birch pollen.

Of 62 patients submitted to PT, five had negative reactions to birch pollen but a positive PT to one or more of the other allergens tested. These positive reactions, however, were of very low intensity and of

questionable clinical significance. ST and RAST results with the corresponding allergens were often negative (Table 7).

No patient had positive reactions to all 12 allergens. 42 out of 62 patients (68%) had positive PT with the combination birch, alder and hazel (the "Northern tree mix"). Of these 42 patients, 39 also had positive PT with bog-myrtle, 39 with beech, 21 with oak and 17 with aspen pollen allergen. 37 individuals reacted to bog-myrtle and beech in addition to birch, alder and hazel.

Table 5

Relationship between RAST results with seven different tree pollen allergens in 87 patients with positive RAST results (class 1-4) with one or more of the allergens. For explanations, see Table 3

Allergen	Birch - +	Alder - +	Hazel - +	Beech - +	Oak - +	Elm - +	Maple - +
Birch - +	12 — — 75	***	***	**	ns	ns	ns
Alder - +	10 8 2 67	18 — — 69	***	***	**	ns	ns
Hazel - +	9 14 3 61	14 9 4 60	23 — — 64	***	**	ns	ns
Beech - +	7 11 5 64	11 7 7 62	15 3 8 61	18 — — 69	***	ns	ns
Oak - +	9 32 3 43	15 26 3 43	18 23 5 41	14 27 4 42	41 — — 46	°	°
Elm - +	9 50 3 25	13 46 5 23	17 42 6 22	14 45 4 24	33 26 8 20	59 — — 28	***
Maple - +	8 49 4 26	13 44 5 25	18 39 5 25	15 42 3 27	33 24 8 22	50 7 9 21	57 — — 30

— = Phadebas RAST class 0; + = Phadebas RAST class 1-4.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

ns = not significant.

Table 6

Relationship between the RAST results with birch pollen and the RAST results with alder pollen in 87 patients

Birch pollen (Phadebas RAST class)	Alder pollen (Phadebas RAST class)				
	0	1	2	3	4
0	10	2	—	—	—
1	4	8	—	—	—
2	3	10	11	2	—
3	1	4	20	6	—
4	—	1	—	1	4

DISCUSSION

The study included pollen allergens of most of the deciduous trees common in Southern and Western Sweden. Their pollen season starts in March with hazel, followed by alder, willow, aspen, elm and poplar in April, beech, birch and maple in May and oak in

May and June (13). Bog-myrtle, which is a small bush and not a tree, pollinates in April or May. Linden, pollinating in July, is an exception to the rule that the trees have a springtime pollen season. Coniferous trees were not included in the study since they are known from many years' experience to be of little importance in allergy. Among 1376 patients submitted to ST with pollen allergens at our clinic during 1 year, only 39 had clearcut positive reactions to coniferous pollen allergens (not published).

The most common tree pollens in Gothenburg are those from coniferous trees and birch, followed by alder and — with considerably lower concentrations — oak, elm, willow, aspen and beech (17). With some of the pollen (e.g. birch, alder and oak) a relationship is found between pollen counts and the results of the present study; the frequency profile of sensitization reflects that

Table 7

Some pertinent test data for five patients with negative provocation tests with birch pollen but positive provocation tests with one or more of the other pollens from deciduous trees

Patient	Age	Provocation test		Skin test positive	Phadebas RAST class ^b
		Doubtful ^a	Positive		
A. B.	22	maple beech	linden bog-myrtle poplar	sallow maple beech	birch 1 maple 1 beech 1 others 0
B. L.	23	hazel linden poplar elm		birch	all allergens 1
B. G.	22	alder		bog-myrtle maple	all allergens 0
H. O.	30	alder aspen elm	bog-myrtle	alder bog-myrtle beech	elm 1 maple 1 beech 1 others 0
S-G. E.	15	linden	sallow	bog-myrtle poplar maple beech	all allergens 0

^a Only weak reactions elicited on challenge with the highest allergen concentration (10^{-1}).

^b RAST analysis was performed only with birch, alder, hazel, oak, elm, maple and beech allergens.

of the pollen count. With bog-myrtle on the other hand no such relationship exists; sensitization with bog-myrtle allergen is common in spite of very low count values for the corresponding pollen. The explanation for the high frequency of positive test results with bog-myrtle could be the existence of cross-reactions with other allergens, as further discussed below.

Birch and alder pollen were the ones most frequently giving rise to positive reactions, a finding which is in accordance with those of Juhlin-Dannfelt (13) and of Zetterström et al. (18), who studied pollen allergy in patients from Stockholm on the Eastern side of Sweden. For other allergens, some differences were found between the studies: beech and hazel allergy was common in our patients whereas Zetterström et al. found more oak allergy among their springtime hay

fever patients. The discrepancies might be explained by differences in allergen extract potency or by geographical variation. The importance of allergen quality is shown by the different results achieved with the different methods of diagnosis, as earlier reported (8, 10). The importance of geographical variation is shown e.g. by the findings of Bernstein et al. (4), who carried out RAST analyses with eight different tree pollens. They found the highest incidence of positive reactions with allergens from box elder (*Acer negundo*) while birch seldom gave positive reactions in their material. They concluded that the rank order among the tree allergens coincided with the ecology of the tree flora in the Cincinnati area, where their patients were living.

Another factor of importance is the character of the pollen. Trees such as linden,

maple, poplar and willow, which are not wind pollinated, cannot be expected to be of great importance in allergy. In the present study pollen from these trees very seldom gave positive PT reactions.

In our experience, as well as according to the study of Juhlin-Dannfelt (13), patients seldom or never have springtime hay fever without birch pollen allergy. In the present study five patients out of 62 were found to have positive PT with one or more other tree pollen allergens in spite of a negative PT with birch pollen. Their PT with other tree allergens were of low intensity and of questionable clinical relevance, however, and they all had a grass pollen allergy with hay fever symptoms mainly in the summer. It is thus obvious that for purposes of screening patients with springtime hay fever it is sufficient to use only one pollen allergen, namely birch pollen.

In addition to birch pollen, most of the patients showed positive reactions to one or more of the other tree pollen allergens employed, especially alder, hazel, beech and bog-myrtle. Atopic individuals seem to have a tendency to develop allergies to groups of allergens such as pollen, animal epithelium, house dust, mould and food allergens (5, 11). The reason for this is unknown, although genetic predisposition is assumed to be important for both IgE production and antigenic preference. Some authors have suggested that the month of birth is of importance for development of pollen allergy (6). Cross-reactivity between different species of major classes of pollen allergens would, to a large extent, explain the multi-sensitization, at least as far as grasses and ragweeds are concerned (4, 15).

Although tree pollens are more heterogeneous than grass pollens (4, 15), there is undoubtedly some degree of cross-reactivity between tree pollens and even between birch pollen and some nuts and fruits (9, 12). Rackemann & Wagner (14), studying the ability of one pollen to block the Prausnitz-Küstner reactivity of

potentially related pollens, found that birch pollen desensitized to oak, maple and willow in most instances. Segal et al. (16), using the haemagglutination inhibition technique and passive cutaneous anaphylaxis inhibition technique, found minor cross-reactivity among the orders of trees but a considerable cross-reactivity among families within each order (poplar, alder and oak). Belin (3), employing immunodiffusion and the passive haemagglutination inhibition technique, found antigenic relationship between birch pollen and hazel nut as well as between birch pollen and pollen of alder, aspen and hazel. Zetterström et al. (18) showed with the RAST inhibition technique that birch pollen contains allergenic determinants of which at least one is present in alder, ash, beech, elm, hazel, oak and willow pollen. The sera from some of the patients of the present study have been used for RAST inhibition experiments (2). It was found that all the allergens studied (birch, bog-myrtle, oak, alder, elm, hazel, maple and beech) could to some extent inhibit the RAST reaction for one another. A considerable individual variance between different patients' sera was evident, however. The alder and the birch extracts showed the strongest inhibitory capacity.

Trees from seven families were represented in the present study: *Salicaceae* (poplar, aspen and willow), *Betulaceae* (birch, alder and hazel), *Fagaceae* (beech and oak), *Myricaceae* (bog-myrtle), *Aceraceae* (maple), *Tiliaceae* (linden) and *Ulmaceae* (elm). Cross-sensitization was found between species within families as well as between different families. The pattern of cross-sensitization found is consistent with the existence of a considerable cross-allergy among tree pollens, although similar reactivity to several pollens in large numbers of individuals could, of course, be due to similar environmental exposure rather than antigenic relationship between pollens.

The object of submitting hay fever patients to diagnostic tests is to find out which

allergens should be included in a hyposensitization programme. In Sweden the combination of birch, alder and hazel ("Northern tree mix") is, for practical reasons, often used for Allpyral immunotherapy in springtime hay fever. The results of this study indicate that if the majority of relevant pollens are to be included, allergens of beech and bog-myrtle should be added to the mixture. On the other hand, injection of mixtures containing allergens to which the patient is not sensitized is theoretically undesirable, owing to the risk of inducing sensitization. If an expensive and time-consuming diagnostic and therapeutic programme, employing many different pollen allergens, is to be avoided, a more limited approach, using only the most important allergen(s), might be better. Since pollen counts show that birch pollen predominates during the springtime pollen season (7, 13, 17), and since the present as well as other studies (13, 18) indicate that birch pollen gives the most pronounced allergies among the tree pollens, it is not certain that the therapeutic efficacy of hyposensitization would be improved by adding other allergens to the treatment extract.

Some of the patients of the present study have been submitted to an immunotherapy study in order to find out whether the therapeutic efficacy of hyposensitization is improved when "Northern tree mix" is supplemented, with other tree pollen allergens. The results, which indicate that this is not the case, will be published in another paper.

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