

# Oilseed rape pollen is a potentially relevant allergen

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## Summary

Oilseed rape (*Brassica napus*) (OSR) is a partly wind-pollinated crop which has been increasingly cultivated both in Europe and overseas. Allergological data about OSR is scarce and controversial. We evaluated the frequency of sensitization to OSR pollen by skin prick test and RAST over a period of 1 yr. Airborne OSR pollen load and the agricultural role of this crop were analysed. Furthermore, six patients were investigated by immunoblot. In 4468 patients with suspect inhalant allergy investigated between June 1994 and May 1995, routine skin prick testing revealed OSR sensitivity in 7.1% of pollen-allergic patients. In all, monovalent sensitization was detected in nine patients. Routine pollen counts showed daily maxima not exceeding 50 grains/m<sup>3</sup>/24 h, but airborne OSR pollen has continuously increased during the last decade correlating with the increasing acreage. Characterization of OSR allergens by immunoblot revealed major allergens of 6/8 kD, 12/14 kD and in the high molecular weight range at 33, 42, 51, 58/61 and 70 kD. Some OSR proteins may cross-react with birch pollen allergens. In summary, the results suggest that OSR pollen is a moderate but true source of allergy and may sensitize despite low pollen exposure.

**Keywords:** oilseed rape, agricultural allergens, spring pollinosis, pollen allergens, allergen characterization

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## Introduction

Oilseed rape (*Brassica napus*) (OSR) allergy, although already reported in 1954 [1], has been paid little attention so far. This partly wind-pollinated plant has been increasingly cultivated for more than 20 yr as a raw material for grease, motor-oils, fuels, forage and, due to new varieties, also for the production of high-quality oils for human consumption in many European countries and overseas. In spite of this, investigations into OSR allergy have remained limited to a few surveys from Sweden and the UK, resulting in controversial views of its allergological role [2–5]. A review of new agricultural aeroallergens, covering also OSR, seemed to signal an increasing scientific awareness of the potential sensitizing capacity of rape pollen [6]. However, due to frequent failure to prove type I allergy by skin test or RAST, more recent studies have focused on the idea that symptoms experienced during the rape flowering

period may be caused by irritating volatile compounds of this crop rather than by allergy [7–10]. A number of potentially responsible volatile compounds emitted from rape flowers and leaves has been analysed [11].

So far, no data on OSR allergy are available for continental Europe. The aim of the present study was to evaluate the frequency of OSR sensitization by routine skin prick testing and RAST, to measure the load by airborne Brassicaceae pollen and to analyse the agricultural role of cultivated OSR in Austria, which is increasingly affected by the cultivation of this crop. An additional approach to the understanding of OSR allergy was made by determining IgE binding proteins in extracts from OSR pollen by SDS-PAGE and Western blots.

## Material and methods

### *Incidence of OSR sensitization*

A 1 : 100 diluted OSR extract (ALK Soluprick no.317) was

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included into a standard allergy screening tray comprising house dust mite, cat, dog, horse, guinea pig, alternaria, aspergillus, cladosporium, mucor, penicillium, hazel, alder, birch, grass, rye, plantain, nettle, ragweed and mugwort (ALK Soluprick, Copenhagen, Denmark) for 1 yr. The patients' group was made up from subjects suspected of inhalant allergy, including seasonal or perennial conjunctivitis, rhino-conjunctivitis, rhinitis, asthma, persistent cough and recurrent bronchitis. Observation started in June 1994 and ended in May 1995. Skin prick tests were scored as 2++ positive in case of a wheal of at least 3 mm diameter, as 3+ + + positive if equal to or exceeding the positive control (histamine dihydrochloride 10 mg/ml). Wheals <3 mm were judged negative, including questionable positive reactions (1+) showing whealing of 2 to <3 mm.

To compare specificity and sensitivity of *in vivo* and *in vitro* tests for OSR allergy, sera of 30 consecutive patients showing a 3+ + + and 2++ skin test to OSR were examined for specific IgE using RAST (Pharmacia, Uppsala, Sweden, CAP Rw 203; Biodiagnostics, Freiburg, FRG, W31). RAST was also done in 20 randomly chosen patients showing a questionable positive prick test (1+) to OSR and not reporting symptoms to be caused by rape. Furthermore, RAST for OSR was performed in a group of patients ( $n = 30$ ) with a history of spring pollinosis but negative skin prick test results to all tested aeroallergens and negative RASTs to birch, grass and house dust mite. RAST values  $\geq 0.7$  kU/l ( $\geq$  class 2) were scored positive.

Since flowering periods of rape and birch overlap, missed sensitization to rape might influence the clinical outcome of immunotherapy against tree pollen (hazel-alder-birch). Eleven patients, having finished 2 yr of specific immunotherapy against hazel-alder-birch in autumn 1993 and reporting clinical improvement of less than 40% in spring 1994, were investigated for OSR sensitivity by both skin prick test and RAST in summer 1994.

#### *Acreage of cultivated OSR and pollen load*

Data on the acreage of cultivated OSR in Austria were provided by the Austrian Ministry of Agriculture and the Austrian Directorate of Statistics. Pollen load by OSR is estimated from routine Brassicaceae pollen counts performed by the aerobiological section of the ENT department, University of Vienna. Continuous data were available for the City of Vienna from 1976 on, and two rural sites with respectively low (Rosalia) and high (Mistelbach) rape field density.

#### *Allergen characterization*

Aqueous extracts from rape pollen were prepared by shaking 1 g pollen (Allergon AB, Engelholm, Sweden) in 10 ml

phosphate buffered saline for 1 h at room temperature. Raw extracts were centrifuged for 30 min at 13,500 rpm, the supernatants dialyzed for 3 days against distilled water, lyophilized, and stored at  $-20^{\circ}\text{C}$  until use; 130  $\mu\text{g}$  of resuspended protein were separated by SDS-PAGE [10] using 12% mini-gels ( $10.5 \times 8.0 \times 0.1$  cm), and blotted onto nitrocellulose [11]. For immunoblotting, transferred proteins were incubated with 1:10 diluted sera of OSR positive patients ( $n = 6$ ), pollen allergic but OSR negative patients, and non-atopic healthy controls; 1:10 diluted  $^{125}\text{I}$ -labelled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden) was employed for IgE detection.

## Results

### *Incidence of sensitization*

In all, 4468 patients with suspect inhalant allergy were tested; 2608 (58.4%) reacted positively to any aeroallergen, 1974 subjects were sensitized to pollen allergens in particular (75.7% of positive tests). One hundred and forty patients had a positive skin prick test to OSR, establishing an incidence of OSR sensitivity of 3.1% in patients with suspect inhalant allergy, 5.4% in patients with a positive response to any aeroallergen, and 7.1% in pollen-allergic patients, respectively (Table 1). In most cases sensitization to OSR was associated with multiple allergy to other pollen antigens (Table 2). Grass pollen was the most frequent concomitant allergen (88%), followed by weeds (78%) and birch pollen (67%). Three cases of OSR monosensitization were found by routine screening, two more patients showed sensitization to house dust mite/animal dander and OSR as the only pollen allergen.

Out of 30 patients with a 3+ + + prick test, 67% (20/30) were positive in RAST, while only 40% (12/30) of those with a 2++ prick test were positive (Table 3). RAST scores in seropositive patients were not strikingly different between the

**Table 1.** One-year average incidence of pollen allergens in pollen sensitive patients ( $n = 1974$ )

| Allergen          | Incidence (%) |
|-------------------|---------------|
| Grass-rye         | 78.8          |
| Hazel-alder-birch | 52.7          |
| Mugwort           | 26.8          |
| Plantain          | 13.1          |
| Ragweed           | 9.7           |
| Oilseed rape      | 7.1           |
| Nettle            | 6.4           |
| Plane-tree        | 1.8           |

**Table 2.** Concomitant pollen allergy in OSR-sensitive patients (OSR = oilseed rape, GR = grass, WE = weeds, BI = birch, HDM = house dust mite)

| Allergy            | % of patients |
|--------------------|---------------|
| OSR + GR           | 10            |
| OSR + GR + WE      | 16            |
| OSR + GR + WE + BI | 55            |
| OSR + GR + BI      | 7             |
| OSR + WE + BI      | 4             |
| OSR + BI           | 1             |
| OSR + WE           | 3             |
| OSR + GR total     | 88            |
| OSR + WE total     | 78            |
| OSR + BI total     | 67            |
| OSR + HDM/dander   | 2             |
| OSR isolated       | 2             |

two groups (3+ + +: medium 3.28 kU/l,  $Q(0.25/0.75) = 1.52/8.45$ ); 2+ +: medium 2.15 kU/l,  $Q(0.25/0.75) = 1.08/13.45$ ). A positive RAST for OSR was found in 15% (3/20) of patients with a questionable positive skin reaction to OSR (medium RAST score 1.27 kU/l). Investigation of patients with completely negative prick test results but a specific history of spring pollinosis revealed seropositivity for OSR specific IgE in 20% (6/30; medium RAST score 1.8 kU/l). All these patients were monosensitized to OSR.

Three out of eleven (27%) patients reporting insufficient

**Table 3.** Correlation of skin prick test results and RAST in patients with positive (3+ + +, 2+ +), questionable positive (1+) and negative skin reactions to oilseed rape

| Skin prick test             | Positive RAST       |
|-----------------------------|---------------------|
| 3+ + + pos.                 | (n = 30) 20 (66.7%) |
| 2+ + pos.                   | (n = 30) 12 (40.0%) |
| 1+ pos.                     | (n = 20) 3 (15.0%)  |
| Negative, spring pollinosis | (n = 30) 6 (20.0%)  |

clinical efficacy of specific immunotherapy against birch were found positive to OSR in RAST. Skin testing was positive (2+ +) in two of them but negative in the third patient.

#### *Acreage of cultivated OSR and pollen counts*

Cultivation of OSR is concentrated to the north and east of Austria. The national overall acreage increased from a baseline around 3500 ha to 70 000 in 1994; 90 000 ha is estimated for 1995 (Fig. 1). Local density of rape fields calculated from the proportion in the total geographical and agricultural area on the level of political districts varies considerably (Table 4). Interestingly, high values were evaluated for several towns, which presumably is a consequence of intensified planting of this crop in urban outskirts characterized by declining husbandry.

Load by Brassicaceae pollen was found low in routine

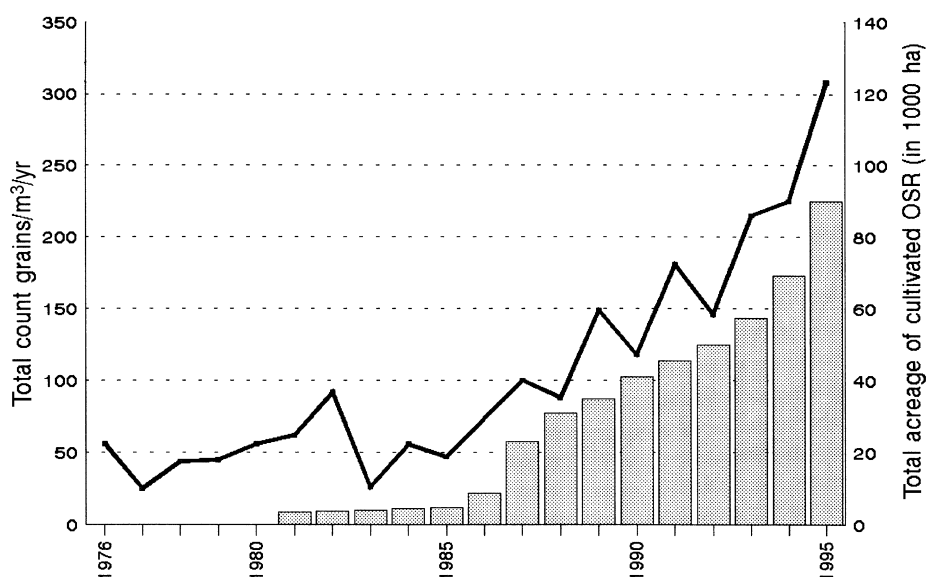
**Fig. 1.** Pollen load by airborne Brassicaceae pollen in Vienna City, and total acreage of cultivated OSR in Austria.

**Table 4.** Proportion of OSR fields in the total agricultural and geographical area on the level of political districts (values for 1990; *n* = number of political districts, *SD* = standard deviation, 'urban' = towns with >30 000 inhabitants)

| Federal state        | <i>n</i> | Acreage of OSR   |           |         |                        |           |         |
|----------------------|----------|------------------|-----------|---------|------------------------|-----------|---------|
|                      |          | % of arable land |           |         | % of geographical area |           |         |
|                      |          | Mean             | <i>SD</i> | Min-max | Mean                   | <i>SD</i> | Min-max |
| Vienna (urban)       | 1        | 2.5              |           | 0.6     |                        |           |         |
| Burgenland           | 7        | 2.6 ± 1.2        |           | 0.8–4.6 | 1.3 ± 0.6              |           | 0.4–2.2 |
| Lower Austria, rural | 21       | 2.1 ± 1.7        |           | 0.0–5.2 | 1.2 ± 1.1              |           | 0.0–3.3 |
| Lower Austria, urban | 2        | 1.4 ± 0.5        |           | 0.9–1.8 | 0.8 ± 0.3              |           | 0.5–1.1 |
| Upper Austria, rural | 15       | 2.1 ± 2.2        |           | 0.0–8.9 | 1.3 ± 1.5              |           | 0.0–6.1 |
| Upper Austria, urban | 3        | 8.0 ± 1.7        |           | 5.8–9.9 | 3.3 ± 1.2              |           | 1.7–4.2 |

pollen counts; however, a continuous rise during the last decade proceeding with the increase in OSR cultivation is evident (Fig. 1). In Vienna, total counts increased from a baseline of about 50 before 1987 to 308 in 1995. Similar values were recorded for Rosalia, a site with moderate cultivation of OSR (287 grains/m<sup>3</sup>/year, daily maximum 28, 1990: acreage 1.3% of arable land). Total counts and daily maxima were highest in Mistelbach, a rural site with more rape fields (435 grains/m<sup>3</sup>/year 1995, daily maximum 49, 1990: acreage 4.5% of arable land).

#### OSR allergens

Gel electrophoresis of pollen extracts stained with Coomassie-blue reveals a number of faint protein bands equally distributed from the low to the high molecular weight range between 14 and 90 kD. Prominent bands were found in the very low molecular weight range below 10 kD (Fig. 2).

IgE binding in sera from OSR sensitized patients occurs predominantly in the high or in the low molecular weight range. In the high molecular weight range, major binding

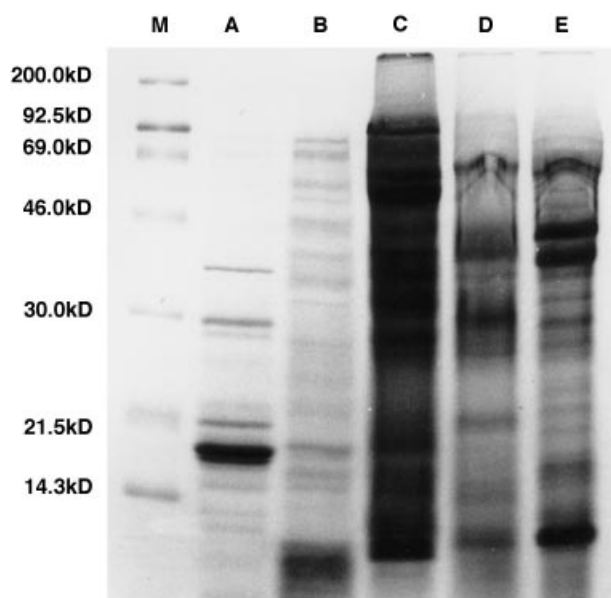


**Fig. 2.** SDS-PAGE analysis of protein extracts from OSR and other allergenic pollen (Coomassie-blue staining): M = marker proteins, A = birch, B = oilseed rape, C = grass, D = mugwort, E = ragweed.

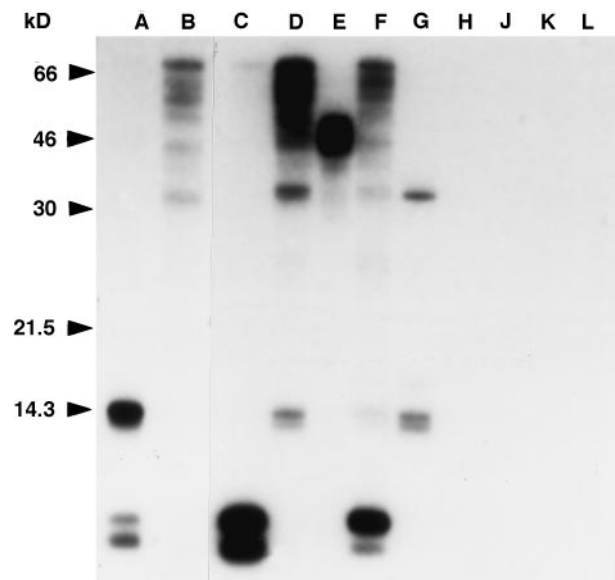


**Fig. 3.** Autoradiography of IgE immunoblots probed with human sera from OSR sensitized patients (lanes a–f), serum from a birch pollen monosensitized patient (lane g), serum from a mugwort monosensitized patient (lane h), a non-atopic control (lane j), a pool of non-atopic controls (*n* = 5, lane k), buffer control (lane l).

sites were found at 70, 58/61, 51, 42 and 33 kD. In 50% of the patients a double band at 6/8 kD occurred. Additionally, a double band at 12/14 kD was present in half of the investigated sera (Fig. 3, lanes a–f). Incubation with serum from a birch pollen monosensitized subject (lane g) revealed IgE binding to OSR proteins at 12/14 kD and 36 kD, with faint staining also at 17 kD. No IgE binding was seen with serum from a mugwort monosensitized patient (lane h) and with non-atopic controls (lanes j–k).

## Discussion

The present viewpoints concerning OSR allergy are controversial regarding the allergological importance of OSR, as there is still a lack of sufficient clinical and experimental data on this plant. Recent investigations have focused on the conception that respiratory and hay fever-like complaints coinciding with the flowering of rape are not allergic but primarily caused by irritant volatiles set free by the plant [8–10,14]. Notwithstanding this irritating effect of OSR volatiles on rhino-conjunctival and bronchial mucosae and their potential involvement as a priming cofactor in allergic sensitization to other aeroallergens [14], our results provide further evidence that OSR pollen does act as an allergen and hence should be considered as a potentially relevant factor in pollen allergy and OSR attributed adverse effects in particular.

In this study, we found positive skin prick test results to OSR in 3.1% of patients tested for suspect inhalant allergy. With the exception of the data given by Ninan *et al.* [4], this value is low compared to earlier papers reporting frequencies of 15% or more in naturally exposed persons [2,3,5]. Rape field density is still low in Austria, covering approximately 4% of arable land at the present. Therefore these conflicting results may reflect differences in allergen exposure but may also be due to the different methodology of different investigators.

Furthermore, it has to be born in mind that so far no biologically standardized OSR extracts are commercially available. An in-house prick test reference solution from a self-prepared extract which was used parallelly in many patients frequently gave stronger reactions than the commercial product, implying a variable number of false negative or false positive results depending on the particular test solution. In the same way, the frequent incongruence between skin prick and RAST results in our study may be due to the present use of non-standardized extracts in allergological work. Future efforts to standardize rape extracts may become intricate since various allergen isoforms may exist among the dozens of cultivated OSR breeds.

In the majority of cases, sensitization to OSR was found together with multiple sensitization to other inhalant allergens supporting the view that OSR hypersensitivity

typically is an additional phenomenon in atopics [5]. However, a number of oligosensitized patients showing combinations such as birch + OSR, grass + OSR, or weeds + OSR alone could be observed, and our data corroborate previous reports [2,5] that isolated OSR sensitization does exist. Our findings agree with most earlier publications that this crop does not seem to be an extremely potent allergen; however, considering the comparatively low amounts of airborne Brassicaceae pollen, the existence of oligo- and monosensitization, and the finding of high prevalences of rape sensitization in cases of occupational exposure [5], the allergenicity of this plant should not be underestimated.

In our survey the incidence of OSR sensitivity was 5.4% among allergic individuals in general and 7.1% among cases of pollinosis in particular. Accordingly, OSR sensitization was of a similar magnitude like allergy to ragweed and nettle, which are considered relevant allergens in Austria and are covered by specific immunotherapy. We therefore consider OSR should be included in routine allergy screening series in countries with rape cultivation. The data from our birch pollen immunotherapy patients seem to indicate that OSR sensitivity should be also considered as influencing the clinical outcome of specific immunotherapy against birch, although the number of investigated patients was small and the results may be also explained by the higher percentage of atopics in this group.

Unlike in other countries, OSR has not yet attracted public attention as a potential cause of adverse effects in Austria. This might explain why only four patients within the study period definitely blamed OSR for their complaints. Three of them actually proved sensitized. Irritant rape volatiles have been repeatedly blamed as the cause for seasonal illness in rural dwellers, although differences in self-reported symptoms between persons living in areas with and without OSR cultivation may be small [8] and atopy is common in symptomatics [9,10]. The frequent failure to demonstrate allergic sensitization in individuals with a history of symptoms during the rape flowering period thus may also reflect a high degree of psychological sensitization to this plant in the population.

In contrast to previous reports [3], we could not diagnose OSR sensitization more often in patients from rural sites, but all patients with a history of OSR-induced symptoms did have rural addresses. This is important in view of the observation that adjacent to rape fields pollen density may outnumber average values by far [8]. In the same study, a distinct fall in airborne rape pollen has been measured 300 metres downwind of fields. Accordingly, daily maxima around 30 grains/m<sup>3</sup>/24 h as measured in the centre of Vienna several kilometers away from rape fields seem comparatively high. Whether this load is sufficient to provoke allergic symptoms remains unclear at the moment. As cross-reactivity may exist between OSR

and birch, airborne Brassicaceae pollen could be a factor aggravating and protracting allergic symptoms in birch pollen-sensitized patients. Recently some OSR proteins were shown to be cross-reactive with grass pollen allergens [15]. Rape pollen therefore might also have a booster effect in the early grass pollen season in grass pollen-sensitized subjects.

For the first time, binding of human serum IgE to OSR pollen proteins has been demonstrated. Future studies will have to investigate which proteins represent species-specific entities and which have cross-reacting properties. Binding of monoclonal anti-*Lol p* IV (57 kD) and anti-*Poa p* IX (35, 30, 28 kD) IgG is known from a 55 and 65 kD rape protein [15] which could explain why we found grass pollen the most frequent allergen associated with OSR sensitivity. The 12/14 kD protein was also recognized by a birch pollen monosensitized patient, suggesting that this protein, considering its molecular size and its double-band structure, is profilin-like. From the present data, the low molecular weight proteins localized at 6 and 8 kD are the most likely candidates to represent OSR unique allergens. Apart from ragweed *Amb V* allergens [16], proteins of that low molecular weight have hardly been documented as major pollen allergens so far.

In summary, we found skin sensitivity to oilseed rape pollen in 7.1% (140/1974) of pollinotic patients. Less than 20% showed mono- and oligosensitization. Increasing acreages were shown to correlate with increasing but still low pollen load. Immunoblots with patients' sera proved the existence of IgE-binding allergens in OSR pollen, some of which may be cross-reactive with birch pollen proteins. OSR pollen should be kept in view as a modest but true source of spring pollinosis in areas affected by extensive or increasing cultivation of this plant.

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