

Oilseed rape—a new allergen?

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Summary

Oilseed rape (*Brassica napus*) is a commonly grown crop in Europe and it has been suggested that its pollen may be a potent new allergen. The prevalence of sensitization in a normal exposed population and an objective study of those patients found to be allergic to the rape pollen is described. The results show a low prevalence of allergy to oilseed rape pollen (less than 0.2%) unless the subjects were occupationally exposed. Those affected, with one exception, were already atopic and allergic to other pollens. The role of volatile materials given off by the plant remains to be elucidated.

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Introduction

There is considerable current interest in the effect of oilseed rape (OSR) on health, whether allergic or through other mechanisms. Oilseed rape is a member of the Brassica family and was first grown in England in the 16th century. Since 1972 there has been a dramatic expansion in growth of the crop from 6500 hectares in 1972 to 347 000 hectares in 1988. It has been suggested that this crop was damaging to health through allergic mechanisms [1]. We therefore decided to study three populations of patients who were exposed to the crop and to ascertain by case finding studies the prevalence of oilseed rape pollen allergy in two groups of naturally exposed subjects, and also in a third group of occupationally exposed research workers.

Patients, materials and methods

Patients

The first group was a case finding study of a complete village population who were surrounded by the crop. The second group was a similar study of patients attending a Health Centre during the peak OSR pollen cloud who complained of conjunctivitis and rhinitis. The third group we were able to study was a group of scientists who were occupationally exposed to the OSR plant and pollen.

Group 1: Somerton village The whole population of Somerton village (106 people) were invited to attend the village hall and to complete a simple questionnaire asking if they suffered typical hay fever or respiratory problems during the months of May and June. Seventy out of the total 106 inhabitants were able to attend during the month of October, 1988, filled in the questionnaire, were skin tested and blood was taken for radioallergosorbent test (RAST) to OSR pollen (36 male, mean age 40.6; 34 female, mean age 44.5). All the subjects were contacted in May, 1989, during the rape pollen season and again during the harvest in August to ascertain whether they were symptomatic.

Group 2: Health Centre During May 1989, the peak of the OSR pollen cloud, all patients attending the local Health Centre were questioned as to symptoms of rhinitis and conjunctivitis. Of the 1408 patients who consulted during that time, 20 were found to have symptoms consistent with allergic rhinitis and conjunctivitis (5 males, mean age 22; 15 females, mean age 40). This group also had skin tests performed and blood taken for RAST.

Group 3: Rothamsted Experimental Station This station is actively involved in research on OSR and the workers are handling both plant and pollen on a daily basis. Thirty-seven workers were screened (24 male, mean age 33; 13 females, mean age 35). Twenty-seven complained of symptoms either when handling the plant or during the month of May. These subjects were skin tested and had blood taken for RAST.

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Pollen counts

A volumetric (1952) Hirst [2] spore trap operator on a tower at a height of 10 m was used to collect pollen counts on a daily basis. Pollen is impacted onto a petroleum coated glass slide by an airflow of 10 litres per minute. The slide collects pollen over a 24 hr period and a total for each type of pollen for that period is then calculated. The trap was situated in the middle of the rural community served by Deddington Health Centre. The closest field of OSR was approximately 300 m from the collection point. During a 24 hr period an air flow of 10 litres per minute or a total of 14.4 m³ of air is sampled and the particles impacted on a 48 × 14.4 mm slide. Knowing the width of the microscope field the pollen count can be calculated as a concentration per cubic metre for a 24 hr period. The trap is approximately 80% efficient, and therefore the pollen count [3,4] tends to be underestimated. The pollen slides were read at St Mary's Hospital Medical School, London.

Skin tests

Standard skin test extracts were obtained from Dome Hollister Stier (Bridgend, U.K.) and used to perform skin prick tests [5] on all the identified patients. Each patient was skin tested to grass-mix '5000 p.n.u./ml (Sweet vernal, Timothy, Orchard, Kentucky Blue), oilseed rape '5000 p.n.u./ml, Nettle (*Urtica*) '5000 p.n.u./ml, Silver Birch (*Betula*) '5000 p.n.u./ml, *Alternaria* '5000 p.n.u./ml, and *Cladosporium*. A positive result was taken as a weal diameter greater than 2 mm measured at 15 min. Normal saline and histamine acid phosphate 1% were used as controls with every subject.

OSR pollen

Material for nasal provocation tests was based on extraction from Coca's solution of OSR pollen. Analar reagents with 1% benzyl alcohol as preservative (BDH Chemicals, Poole) and freshly prepared de-ionized, distilled, autoclaved water were combined in a clean air laboratory. Freeze-dried OSR pollen (Allergon A.B., Engelholm, Sweden) was extracted at 10% w/v at 4°C for 7 days and serially filtered down to 0.2 µm (Schleicher and Schuell, Dassel, West Germany) with the final step being undertaken in a sterile laminar flow cabinet with full aseptic precautions. Dilutions were prepared in the same manner.

Alternaria

The mould *Alternaria alternatum* was also used for nasal challenge as this grows on the OSR plant during the

pollenating season. Standard extracts (Dome Hollister Stier) were used for nasal challenges, dilutions being made in sterile normal saline.

Volatile compounds

These were collected from the air surrounding flowering plants of spring OSR (*Brassica napus*), grown in a glass house. A 'single low' variety of plant was used because it emitted a wide range of volatile substances including 'mustard oils' which are formed by enzymatic degradation of glucosinolates. The volatile compounds were collected by air entrapment enabling a mixture of substances to be collected, the components of which were present in ratios similar to those found in the air around the OSR plant [6].

Other methods commonly used for the isolation of volatiles, e.g. maceration or chopping of plant tissue followed by extraction and distillation were considered undesirable as they can lead to changes in the composition caused by enzymic reactions. The extracted volatiles were dissolved in a glycerol ethyl alcohol carrier in dilutions of 2.5×10^{-3} , 5×10^{-4} and 5×10^{-5} .

Nasal challenge

Before entering this part of the study each subject practised with the nasal inspiratory peak flow meter until there was consistently less than 10% variability in readings.

Each extract was delivered through a standardized delivery system giving 25 µl per dose. Symptoms were measured and scored as sneezing, rhinorrhoea and itching; nasal inspiratory peak flow (NIPF) (Youlten Sniff Meter, Clement Clarke International) was measured 15 min after each challenge. A drop of 20% in NIPF was considered positive, as previously described [7]. Nasal challenges were performed in blocks of placebo (four challenges) or allergens preceded by placebo. These blocks were administered in random order and not known by the tester or the patient.

Lung function tests

Lung function tests were measured on a standard vitalograph and FEV and FVC were measured after each nasal challenge and again 15 min later.

RAST

RAST discs were obtained from Pharmacia (Milton Keynes, U.K.) and the IgE antibody assay was carried out according to published methods [8].

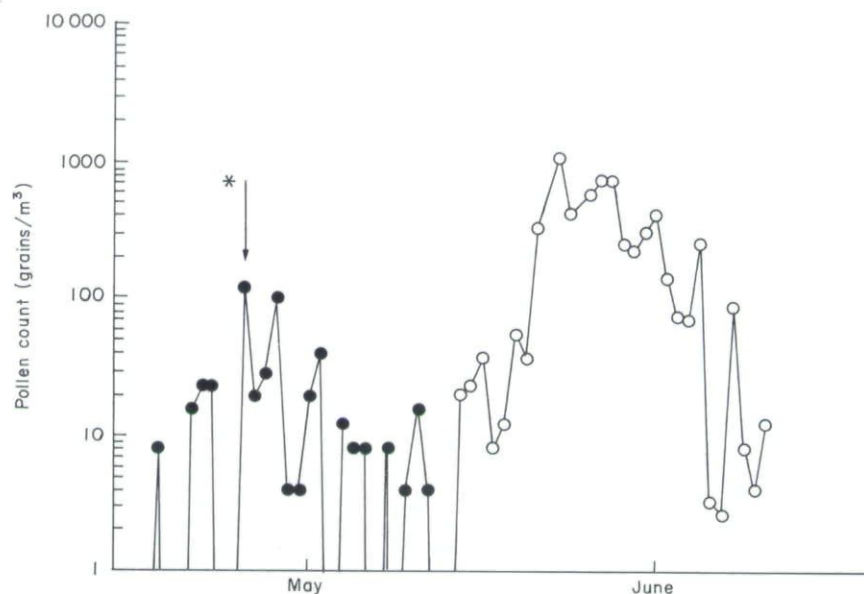


Fig. 1. Pollen count for OSR (●) and grass (○) during the 1989 flowering season.

Table 1. Pollen counts including other allergens for the peak day of OSR pollen, 14 May 1989

Allergen	Count/m ³ of air
Pine	480
<i>Alternaria</i>	480
Mugwort	96
Silver birch	96
OSR	90
Grass	450
<i>Cladosporium</i>	360
Nettle	300

Results

Pollen counts

As can be seen from Fig. 1 the peak of the OSR pollen season is in the middle of May and there is no overlap between that and the peak of the grass pollen season which is in the middle of June. OSR is not the predominant pollen in the month of May, with others such as silver birch (*Betula*), plane, pine (*Pinus*), mugwort (*Artemisia vulgaris*) and spores from the mould *Alternaria* being as much, or more, prevalent. To demonstrate this phenomenon Table 1 shows the pollen counts for other allergens on the peak day of OSR pollen (May 14th) and as can be seen, some of these far outnumber the counts of OSR pollen.

Skin tests and RAST

Group 1 Of the 70 subjects skin tested from the village of Somerton, four complained of hay fever like symptoms.

Table 2. Results of nasal challenge studies with possible OSR associated allergen in subjects + healthy volunteers

	Group A Positive total	Group B Positive total	Group C Positive total
OSR pollen	0/4	2/5	5/5
<i>Alternaria</i>	0/4	1/5	0/5
Placebo	0/4	0/5	1/5

Group A, controls, non-symptomatic, negative skin tests, negative RAST to OSR pollen.

Group B, symptomatic, negative skin tests, negative RAST to OSR pollen.

Group C, symptomatic, positive skin tests, positive RAST to OSR pollen.

One of these gave a positive skin test to OSR pollen and also showed a positive RAST to OSR pollen, but we were unable to perform a nasal provocation on this patient. The other three subjects were skin test and RAST negative to OSR pollen. Although only one out of the 70 subjects gave a positive skin test to OSR pollen six subjects did show positive reactions to the other allergens, i.e. approximately 9% of the population.

Group 2 Of the 1408 subjects attending the Health Centre during the month of May, 20 complained of symptoms of rhinitis and conjunctivitis with eight being skin test positive to a variety of seasonal pollens. Three of the eight were also skin test positive to OSR pollen. In addition, two of those three were RAST positive to OSR.

Group 3 Of the 29 (out of 37, 78%) symptomatic subjects at Rothamsted Experimental Station, 16 were skin test positive to a variety of inhalants and nine were also positive to OSR pollen. One unique patient showed a single positive skin test to OSR pollen alone. This particular worker had spent four years in close contact with the plant.

Nasal challenge versus allergic status (Table 2)

Because there were so few subjects sensitive to OSR pollen it was necessary to select subjects from across the groups to determine three categories of patient for direct nasal challenge.

Group A: no symptoms: RAST -ve: skin test -ve

Group B: symptoms: RAST -ve: skin test -ve

Group C: symptoms: RAST +ve: skin test +ve

Fourteen subjects were challenged with the alcohol solution containing volatile material and 12 responded with a positive challenge. This clearly indicates a sensitivity to not only the volatiles but also the carrier and consequently these results shed no light on whether volatile materials cause a problem, i.e. the control reaction was too great.

Group A subjects showed no reactions to placebo, *Alternaria* or OSR pollen. The intermediate group B who had symptoms but were RAST and skin test negative showed two out of five subjects reacting to OSR and one out of the five to *Alternaria* pollen.

The final group C who had symptoms and were RAST and skin test positive showed most reactivity. Five out of five reacted to OSR pollen, none to *Alternaria* and one also reacted to placebo control.

Lung function tests

These remained unchanged after nasal challenge in all subjects with one exception. This was a patient who was atopic and also sensitive to OSR pollen. Mild asthmatic symptoms developed which responded quickly to one puff of Ventolin.

Discussion

The main points at issue with the problem of OSR is the incidence of sensitization. Our data has not shown that the pollen is a major problem as a sole inducer of allergic reactions in subjects who are naturally exposed to the pollen. Of the two groups of subjects who were naturally exposed (i.e. the inhabitants of Somerton village and those attending the local Health Centre, 1478 subjects), 24 thought that they had symptoms due to OSR pollen, but

only four showed positive skin tests to OSR pollen. Three of these four were RAST positive and it was only these three who had a positive nasal provocation test, i.e. 6% of the symptomatic subjects. Thus, we only found three clinically proven allergic subjects in the whole group, giving an incidence of the order of 0.2% of the population studied.

In contrast the incidence of OSR sensitivity in those subjects occupationally exposed is very much higher. Of the 29 subjects at Rothamsted Experimental Station who had upper respiratory tract and conjunctival symptoms, 16 were skin test positive to a range of inhalant allergens, nine of whom were also skin test positive to OSR pollen. Interestingly, only five of those gave a positive nasal challenge to the OSR pollen, and those five subjects were also RAST positive.

The data that we have from nasal challenge with volatile material from the plant is less clear cut. Both the non-allergic controls and the symptomatic subjects showed nasal symptoms following challenge but this may be an artefact of the material preparation which contained a high concentration of ethyl alcohol, necessary to dissolve the volatile material. The inhalation of volatile material which may be attached to the pollen grain itself is clearly important in the elicitation of nasal symptoms in both atopic and non-atopic subjects. This needs further investigation as the volatile may prime the nasal mucosa of atopics for the allergic response to other airborne allergens, in the similar way that cigarette smoking has been shown to increase IgE levels [9].

The plant (OSR), a member of the *Brassica* family, has been introduced to a number of countries and cultivated widely. The genus includes broccoli, kale, cabbage, mustard, turnip as well as rape, and there are 56 additional genera under cultivation [10]. Members of the *Brassica* family can cause a variety of allergic reactions depending on the route of contact. Anaphylaxis following ingestion has been reported with cabbage [11] and mustard [12-14], and contact sensitivity to members of the *Brassica* family can occur, whether exposure is domestic [15,16], or industrial [17,18]. There is an interesting cross-reactivity between isothiocyanates (mustard oils) and food where those sensitized and also having asthma become sensitive to *Brassica* species, in particular mustard [19]. In these subjects, food allergy was difficult to diagnose in the context of occupational asthma and the association may be underestimated [20].

Colldahl [21] reported the first patient with asthma due to OSR pollen in 1954. This patient responded well to hyposensitization. Rape plants are widely cultivated in Scandinavia and a survey of 366 atopic asthmatics showed 85 (23%) to be skin test positive to OSR pollen and 54 of the 85 also gave positive response on bronchial

provocation (15% of the total group). There was no data in this study of the specific serum IgE [22].

The Scottish survey [1] was of sera sent to a reference laboratory for routine RAST analysis to common allergens but with no specific request for OSR and with no clinical details given. The overall incidence of RAST positivity of the sera to OSR was 17% and there was no data to show whether this particular group was in fact clinically sensitive to OSR pollen. In our group only half of those who were RAST positive gave a positive nasal provocation test, suggesting the overall incidence of OSR sensitivity in the Scottish survey may well be less than half of the 17% suggested and closer to the Scandinavian survey. In contrast to this, of the nearly 1500 naturally exposed population we have studied, the incidence of proven clinical sensitivity was well below 1%.

We can conclude from our study that classical type 1 hypersensitivity to the OSR pollen allergen is not a common phenomenon and in the population at large it only occurs in atopics who are already allergic to other airborne pollens. In the occupationally exposed this is also true with one exception; a non-atopic subject who had worked in close contact with the plant for four years. Sole sensitization to this pollen in the naturally exposed is unlikely. Time will tell whether increasing exposure leads to increasing sensitivity. The role of volatile chemicals given off by the plant requires further investigation.

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References

- Parratt D, Thomson T, Saunders C, McSharry C, Cobb S. Oilseed rape as a potent allergen. *Lancet* 1990; i:121–2.
- Hirst JM. An automatic volumetric spore trap. *Ann Appl Biol* 1952; 39:257–65.
- Davies RR. Air sampling for pollens and bacteria. In: Broth C, ed. *Methods in microbiology*, Vol. 4. London: Academic Press, 1971: 367–404.
- Davies RR, Deung MJ, Newton LM. A comparison between the summer and autumn air-spores of London and Liverpool. *Acta Allergologica* 1963; 18:131–47.
- Pepys J. Skin testing. *Br J Hosp Med* 1975; 14: 412–17.
- Blight MM. Techniques for isolation and characterisation of volatile semiochemicals of phytophagous insects. In: McCaffrey AR, Wilson ID, eds. *Chromatography and isolation of insect hormones and pheromones*. New York: Plenum Press, 1990: 281–88.
- Krayenbuhl M, Hudspeth BN, Scadding GK, Brostoff J. Nasal response to allergen and hyperosmolar challenge. *Clin Allergy* 1988; 18:157–64.
- Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 1967; ii:1105.
- Burrows B, Halonen M, Barbe ERA, Lebowitz MD. The relationship of serum Immunoglobulin E to cigarette smoking. *Am Rev Resp Dis* 1981; 124:523–5.
- Lewis WH, Vinay P. North American pollinosis due to insect pollinated plants. *Annals of Allergy* 1979; 42:309–18.
- Blaiss MS, McCants ML, Lehrer SB. Anaphylaxis to cabbage: detection of allergens. *Ann Allergy* 1987; 58:248–50.
- Widstrom L, Johansson SGO. IgE mediated anaphylaxis to mustard. *Acta Derm Venerol (Stockh)* 1986; 68:70–1.
- Meding B. Immediate hypersensitivity to mustard and rape. *Contact Dermatitis* 1980; 6:294–5.
- Panconesi E, Sertoli A, Fabbri P, Giorgini S, Spallanzani P. *Contact Dermatitis* 1980; 6:294–5.
- Calnan CD. Contact urticaria from cabbage (Brassica). *Contact Dermatitis* 1981; 7:279.
- Leanizbarrutia I, Munoz D, Fernandez-de-Corres L. Cutaneous allergy to mustard. *Contact Dermatitis* 1987; 17:262–3.
- Kavli G, Moseng D. Contact urticaria from mustard in fish stick production. *Contact Dermatitis* 1987; 17:153–5.
- Dannaker CJ, White IR. Cutaneous allergy to mustard in a salad maker. *Contact Dermatitis* 1987; 16:212–14.
- Gervais P, Rosenberg N, Chabaux C. Le role initiateur de certains contaminants respiratoires et cutanes dans les allergies alimentaires. *Allerg-Immunol (Paris)* 1987; 19:7–11.
- Rosenberg N, Gervais P. Une frequence sous-estimee; L'association allergie alimentaire asthme professionnel. *Presse-Med* 1986; 15:1712–14.
- Colladhi H. Rape pollen allergy. *Acta Allergol* 1954; 7:367.
- Bucur I, Arner B. Rape pollen allergy. *Scand J Resp Dis* 1978; 59:222–7.

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