

Short communication

Allergy to *Diplotaxis eruroides* pollen: occupational sensitization and cross-reactivity with other common pollens

Background: *Diplotaxis eruroides* is a common weed of the Brassicaceae family widespread in southern and central Europe.

Methods: A total of 410 consecutive patients referred for allergy study of rhinoconjunctivitis and/or asthma were skin tested with *D. eruroides* pollen, 14 proving positive. A purified *D. eruroides* pollen extract was prepared to perform quantitative skin tests, provocation tests, immunoblotting, and EIA inhibition in the 14 sensitized patients.

Results: Three patients, directly involved in viniculture, had rhinoconjunctivitis related to *D. eruroides* pollen. No *D. eruroides*-related symptoms were observed in most patients, who were also sensitized to *Artemisia* pollen. RAST was positive in 12/14 patients and nasal provocation tests in 9/12. The molecular masses of the most prevalent IgE-binding proteins ranged from 26 to 27.5 and from 31 to 34 kDa. *D. eruroides* pollen inhibited the IgE-binding of other sensitizing pollens in the three viniculture workers, whereas both *Artemisia* and *D. eruroides* pollen produced similar heterologous inhibition in the pooled serum of the remaining, nonclinically affected, *D. eruroides*-sensitized patients.

Conclusions: *D. eruroides* pollen may be an important prevalent aeroallergen, particularly in rural areas. It may act as an occupational allergen in vineyard workers, in whom it seems to be the primary sensitizing agent, playing a secondary cross-reactive role in other sensitized patients.

**P. García-Ortega¹, B. Bartolomé²,
E. Enrique¹, P. Gaig¹, C. Richart¹**

¹Allergy Unit, Hospital Universitari Joan XXIII, Institut d'Estudis Avançats, Universitat Rovira i Virgili, Tarragona; ²Bial, Bilbao, Spain

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Pilar García-Ortega
Allergy Unit
Hospital Universitari Joan XXIII
Avda Dr Mallafre Guasc, 4
43007 Tarragona
Spain

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Diplotaxis eruroides (white wall rocket) is a 5–50-cm-high weed of the Brassicaceae family, widespread in both rural and urban areas of southwestern and central Europe (1) (Fig. 1). Like other Brassicaceae species, its pollen is prolate or subspheroidal and small in size ($27 \times 18 \mu\text{m}$) (2). The plant is ruderal, growing on roadsides and in sown fields and is frequent in vineyards and stubble (3). It is both insect- and wind-pollinated, and its flowering period is from October to April in southwestern Europe.

Although a common weed, *D. eruroides* has not been related to allergic sensitization or clinical respiratory disease. After one patient, a vineyard worker, reported sudden sneezing, watery rhinorrhea, and conjunctivitis while walking or working near these flowering weeds, a small amount of *D. eruroides* pollen was collected, and a prick test with a 1:20 w/v extract was performed, proving positive. A prospective study to ascertain whether *D. eruroides* pollen could be responsible for allergic respiratory diseases in our area was undertaken.

Material and methods

Patients

Patients were recruited from the outpatient allergy clinic of a general

hospital in Tarragona, a Mediterranean town 98 km south of Barcelona. Patients were referred from urban and rural areas including vineyard areas.

Between November 1998 and March 1999, flowering plants of *D. eruroides* were identified and collected by a botanist. The plants were allowed to pollinate in a warm atmosphere and the pollen was collected. From May 1999 to May 2000, 410 patients admitted for allergy study of asthma, rhinitis, and/or conjunctivitis were prick tested with the *D. eruroides* crude pollen extract and with the routine battery of aeroallergens of our area. The battery included three grass pollens (*Phleum pratense*, *Cynodon dactylon*, and *Phragmites communis*), three tree pollens (*Corylus avellana*, *Cupressus sempervivens*, and *Olea europaea*), seven weed pollens (*Amaranthus retroflexus*, *Artemisia vulgaris*, *Chenopodium album*, *Mercurialis annua*, *Parietaria judaica*, *Plantago lanceolata*, and *Salsola kali*), three house-dust mites (*Dermatophagoides pteronyssinus*, *D. farinae*, and *Lepidoglyphus destructor*), four common molds (*Alternaria alternata*, *Cladosporium herbarum*, *Penicillium* spp., and *Aspergillus* spp.), two animal danders (dog and cat), and latex. Of the 185 patients sensitized to some aeroallergen, 14 (7.5%) were sensitized to *D. eruroides* crude pollen extract. The characteristics and clinical features of these 14 patients who formed the study group are summarized in Table 1.

Preparation of the extract

Preparation of *D. eruroides* pollen extract was performed as previously described (4). Briefly, defatted pollen was extracted by magnetic stirring in phosphate buffer, clarified by centrifugation, filtered, and dialyzed by ultracentrifugation with a 5-kDa cutoff. The

Table 1. Clinical characteristics of 14 patients sensitized to *Diplotaxis eruroides*

Patient no.	Age (years)	Sex	Symptoms	Season	Aeroallergen sensitization	Food allergy	RAST (class)	NPT (HEP/ml)
1)	30	M	RC	Spring, autumn-winter	<i>Artemisia</i> , <i>Phleum</i> , <i>Olea</i> , <i>Parietaria</i> , <i>Mercurialis</i>	None	5	0.001
2)	39	M	RC	Spring, autumn-winter	<i>Artemisia</i> , <i>Phleum</i> , <i>Olea</i> , <i>Parietaria</i>	None	3	1
3)	41	M	RC	Spring-winter	<i>Phleum</i> , <i>Olea</i>	None	2	0.01
4)	37	M	RC, AN	Autumn	<i>Artemisia</i>	Mustard, cashew, sunflower seed, sesame, pistachio	3	Negative
5)	21	M	RC	Perennial	<i>Artemisia</i> , mites	None	0	1
6)	23	F	RC, U	Perennial	<i>Artemisia</i> , <i>Corylus</i> , <i>Salsola</i> , <i>Platanus</i> , mites	Mustard, cereals, sunflower seed	3	5
7)	20	M	RC/U/AE	Perennial	<i>Artemisia</i> , mites	Sunflower seed	0	1
8)	31	M	RC/U/AE	Perennial	<i>Artemisia</i> , <i>Phleum</i> , mites	Nuts	1	NP
9)	21	F	RC/U/AE	Spring, autumn	<i>Artemisia</i> , <i>Phleum</i> , <i>Olea</i> , <i>Salsola</i> , <i>Plantago</i> mites, cat	Rosaceae fruits	2	0.1
10)	15	M	RC, U, OAS	Perennial	<i>Artemisia</i> , <i>Olea</i> , mites, cat	Rosaceae fruits, sunflower seed, nuts	2	5
11)	17	M	RC, U, OAS	Spring	<i>Artemisia</i> , <i>Olea</i> , <i>Platanus</i>	Mustard, honey, sunflower seed	3	Negative
12)	18	M	RC, A, AE	Perennial	<i>Artemisia</i> , <i>Phleum</i> , <i>Cupress</i> , <i>Parietaria</i> , mites, cat, dog	Egg	1	Negative
13)	19	M	RC, AE	Perennial	<i>Artemisia</i> , <i>Phleum</i> , <i>Olea</i> , <i>Parietaria</i> , mites	Peanuts	1	NP
14)	10	M	RC, A, U	Perennial	<i>Artemisia</i> , <i>Parietaria</i> , mites, cat, dog	Mustard, nuts, sunflower seed	1	5

RC: rhinoconjunctivitis; U: urticaria; AE: angioedema; AN: anaphylaxis; OAS: oral allergy syndrome; NPT: nasal provocation test. RAST and NPT refer to *D. eruroides* pollen extract.

dialyzed extract was sterilized by filtration through an 0.22- μ m pore diameter membrane and freeze-dried. Lyophilized extract of the same batch was used for all *in vitro* and *in vivo* tests performed in the 14 patients identified as sensitized by the crude extract.

Quantitative skin tests

D. eruroides lyophilized pollen extract was diluted in phenol-glycerol saline solution at concentrations of 0.01, 0.1, 1, 10, and 30 mg/ml, and prick tests with standardized lancets were performed. Histamine chlorhydrate at 10 mg/ml, and phenol-glycerol saline solution were used as positive and negative controls, respectively. After 15 min, sellotape was applied over the wheals obtained, and the areas transferred to a blank record sheet. Areas were expressed in square millimeters.

The mean wheal area produced by each allergen concentration was plotted as a function of the allergen concentration in a log-log system, and linear regression was performed. The allergen concentration eliciting a wheal equal to that produced by histamine (10 mg/ml) was defined as one histamine equivalent prick (HEP unit) (5). One HEP unit was found to be equivalent to 1.3 mg/ml of allergen extract. This concentration was used to establish the maximum dose of allergen to be used in nasal provocation tests (5 HEP).

Nasal provocation tests

Nasal provocation tests were performed at 9 a.m. in the 13 patients with rhinitis. Active anterior rhinomanometry was performed according to the criteria of the Committee Report on Standardization of Rhinomanometry (6). A Rhinospir 64 (Sibel, Barcelona, Spain) programmed to perform nasal provocation tests was used. Airflow and resistances were recorded in an X-Y mirror image (6). After spraying 0.2 ml of diluent, increasing concentrations of allergen of 0.01, 0.1, 1, and 5 HEP/ml were sprayed into the same nostril every 15 min until symptoms (over five sneezes) appeared or resistances doubled those induced by the diluent. Patients were monitored with nasal resistance measurements for 6 h to detect possible late responses.

Specific IgE

Solid-phase antigen was obtained by coupling the extract solution (10 mg/ml) to the 6-mm diameter CNBr-activated paper disks by the method of Ceska & Lundqvist (7), and EIA was performed as described by Wide et al. (8) with HY-TEC EIA (Hycor Biomedical, Inc., Irvine, CA, USA).

SDS-PAGE immunoblotting

SDS-PAGE was carried out by the method of Laemmli (9). Polyacrylamide concentrations of 12.5% and 3% were used for separating and stacking gels, respectively. Samples were dissociated with 0.1% SDS and 5% v/v β -mercaptoethanol by treatment at 100°C for 5 min. Separated protein bands were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Milford, MA, USA), essentially by the method described by Towbin et al. (10). Immunochemical staining was performed by first blocking the Immobilon membrane with Tween-20 0.05%, and then using a chemiluminescent detection reagent (Lumigen PS-3) (11).

Cross-reactivity studies

As only three patients (nos. 1, 2, and 3) claimed an association between *D. eruroides* flowering and respiratory symptoms, we deduced that two different groups of patients could exist: one, probably primarily sensitized to *D. eruroides* pollen in an occupational setting, and the other, probably sensitized to *D. eruroides* pollen by means of cross-reactivity with other major pollens or vegetables. To test this hypothesis, individual EIA inhibition studies were performed, whenever possible (respective specific IgE levels $\geq$ class 2), in the three mentioned patients, using *D. eruroides* pollen and each of the other major pollens to which each patient was sensitized (*Artemisia*, *Parietaria*, *Olea*, and/or *Phleum*) as a solid (inhibited) phase. In addition, a pooled patient serum from patients 5–14 was used to perform EIA inhibition studies between *D. eruroides* and a major pollen to which all these patients were sensitized (*Artemisia*).



Figure 1. *Diplotaxis eruroides* plant.

Results

All patients were polysensitized to other pollens, mites, and/or animal danders (Table 1). Patients 1, 2, and 3, who were directly involved in viticulture, identified *D. eruroides* as a common weed growing in and near vineyards and claimed that its flowers were directly responsible for their rhinitis or conjunctivitis in the months from autumn to spring, and patient 13 referred to a possible relationship with occasional sudden urticaria. The remaining 10 patients did not recognize the weed and were not aware of any health problem related to it, and most of them had concomitant food allergy (Table 1).

D. eruroides-specific IgE was detected in 12 patients (Table 1). SDS-PAGE immunoblotting of the 14 sera showed different IgE-binding proteins (Fig. 2). Cross-reactivity studies performed with sera of patients 1, 2, and 3, when *Phleum*-, *Olea*-, *Parietaria*-, or *Artemisia*-specific IgE levels were high enough to be used as solid (inhibited) phase, showed that *D. eruroides* extract was able to induce partial inhibition ranging from 0 to 80%, whereas the inverse situation (*D. eruroides* as solid phase) did not induce inhibition. However, cross-reactivity assays performed with the pooled serum from patients 5 to 14 showed that both *Artemisia* and *D. eruroides* pollen extracts produced

heterologous inhibition of more than 70% in both cases, which was greater (90%) when *Artemisia* pollen extract was used as free (inhibition) phase.

Discussion

Our study proves that *D. eruroides* pollen elicits allergic sensitization and clinical symptoms (rhinoconjunctivitis or urticaria) in heavily exposed patients. The fact that three of the four patients who unequivocally related exposure to the flowering weed to allergic symptoms were involved in viticulture, while other sensitized patients suffered no symptoms in the pollination season of *D. eruroides*, suggests that this pollen might rather be considered an occupational allergen.

On the other hand, the patients sensitized to *D. eruroides* pollen with no symptoms in the flowering season showed sensitization to other pollens (mainly *Artemisia*, *Phleum*, and *Olea*) as well as to *Artemisia* and/or *Phleum* pollen-related foods (spices, nuts, Rosaceae fruits, and cereals). Moreover, most of these patients had clinically relevant food allergy, suffering not only oral allergy syndrome, but urticaria, angioedema, and even anaphylaxis when eating foods such as sunflower seed, mustard, nuts, fruits, peanuts, or cashews. These facts suggest that, in these patients,

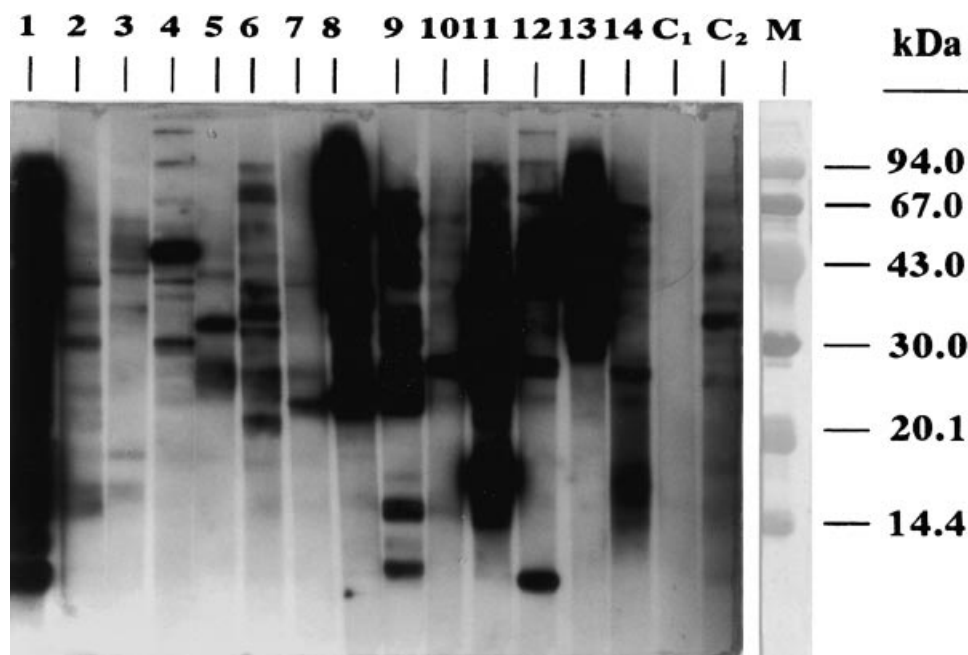


Figure 2. SDS-PAGE immunoblotting of sera of 14 *D. erucoides*-allergic patients, showing IgE-binding proteins ranging from 10 to 90 kDa. Two prevalent ranges of 26–27.5- and 31–34-kDa bands are observed. Lanes 1–14) patients 1–14; lane C₁) control lane (incubation buffer without serum); lane C₂) control serum (pool from nonatopic subjects' sera); lane M) molecular mass marker.

sensitization to *D. erucoides* pollen is clinically irrelevant and merely a consequence of cross-reactivity with other pollens and vegetables carrying similar structural or functional allergenic proteins in the context of pollen-food syndromes (12). As all these patients were sensitized to *Artemisia* (mugwort) pollen, cross-reactivity to *Helianthus*-derived allergens and sunflower seed food allergy are not surprising (13). On the other hand, as *D. erucoides* belongs to the Brassicaceae family, immunologic similarities between proteins from this and other botanically related plants, such as mustard (14) or rapeseed (15), may be expected. Furthermore, an association between sensitization to *Artemisia* pollen and mustard allergy has been found (16).

The clinical differences between patients with and without *D. erucoides* pollen-related symptoms were in accord with the *in vitro* cross-reactivity results. In the three patients with occupational exposure, the presence of respiratory allergic symptoms during the *D. erucoides* flowering season suggests a primary sensitization. This feature concurs with the EIA inhibition results, which demonstrated partial inhibition of other pollens by *D. erucoides* pollen extract, whereas the opposite did not occur. In patients without symptoms during the *D. erucoides* pollen season and also

sensitized to *Artemisia*, *D. erucoides* and *Artemisia* pollen both significantly inhibited the other in cross-inhibition studies. Moreover, a greater degree of inhibition was obtained when *Artemisia* pollen extract was the free inhibitor phase, thus pointing to the probable primary sensitizing role of *Artemisia* proteins in these polysensitized, food-allergic patients.

In conclusion, *D. erucoides* pollen may be an important prevalent allergen in suburban and rural areas, particularly in those dedicated to vine growing, and should be included in panels of allergenic pollens. In addition, the high prevalence of allergy to other Brassicaceae species and the cross-reactivity with *Artemisia* (Compositaceae) found in patients sensitized to *D. erucoides* pollen may help to explain some cluster-association clinical phenomena in food- and food-pollen-allergic patients living in the Mediterranean basin.

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