

8 Weed Pollen Allergens

Shyam S. Mohapatra and Richard F. Lockey

Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida College of Medicine and the James A. Haley Veterans' Hospital, Tampa, Florida, U.S.A.

Florentino Polo

ALK-Abelló, Madrid, Spain

INTRODUCTION

Pollens derived from various ragweed species are one of the most important sources of allergenic proteins in different parts of the world. Although pollination typically begins in midsummer and extends to late autumn, the pollinating seasons vary by geographic location. Immunotherapy with ragweed vaccines is effective therapy for ragweed-allergic patients. There are many other allergic weeds, both related and unrelated; however, ragweed species are by far the most important clinically and, hence, have been studied thoroughly. Using molecular biological techniques, investigators have advanced the knowledge of the allergenic constituents of different ragweed species and provided information on the molecular structure of their allergens and their potential cross-reactivity. Special attention is given in this chapter to the integration of morphological, taxonomical, and aerobiological aspects, as well as, the biochemical and clinically relevant aspects of weed pollen allergens.

TAXONOMY OF WEEDS

Of the families of weeds that have been identified, some have been implicated in pollen allergy. As for others, their importance as allergens is unknown. Table 1 provides a comprehensive list of the most prevalent weeds and their botanical classification (taxonomic family and genus), common names, and relative clinical importance (1). A botanist and/or an aerobiologist should be consulted for detailed information on the prevalent weeds in the surrounding geographical areas. The exposure records, as collected and compiled by the American Academy of Allergy, Asthma, and Immunology, can be obtained by calling the Pollen Hotline, 1-800-POLLEN, or by writing to the American Academy of Allergy, Asthma, and Immunology, 611 East Wells Street, Milwaukee, Wisconsin.

WEED AND POLLEN IDENTIFICATION

Although there are many species of ragweed, their morphologies are strikingly similar (2). Some of the important weeds of the United States are shown in Figure 1. The characteristic features of short ragweed (*Ambrosia artemisiifolia*) include a finely divided leaf, each leaf being subdivided into three or more further divided segments, giving the weed a fernlike appearance. The pyramidal-shaped plants grow in the thousands, mostly on land that has been disturbed. They branch extensively, with a ground spread spanning 3 to 4 ft and a height of 4 to 5 ft, producing greenish-yellow flower spikes. In contrast to short ragweed, giant ragweed (*A. trifida*) features broad leaves, which may be three or five lobed or undivided, with blades of the leaf carried down the leaf stalk as narrow wings on each side. Giant ragweed grows above the ground as a columnar bush frequently attaining a height between 10 and 15 ft. Western ragweed is similar to short ragweed in size and habit. However, these weeds typically have inconsistently divided leaves and grow each spring from the roots rather than from seeds. Western giant ragweed differs from its eastern counterpart by its wingless leaves and its tendency to branch well above the ground, rising from the root on a central stalk. Southern ragweed has slender, lance-shaped leaves with one or more large teeth near its base and is

Table 1 Botanical Classification of Important Allergenic Weeds

Family	Genus	Common name	Clinical importance
Compositae	<i>Ambrosia</i>	Ragweed	+++
	<i>Franseria</i>	False ragweed	—
	<i>Iva</i>	Marsh elder	+
	<i>Xanthicum</i>	Cocklebur	—
	<i>Artemisia</i>	Sage, mugwort	++
	<i>Chrysanthemum</i>	Daisy	+
	<i>Taraxacum</i>	Dandelion	—
	<i>Parthemum</i>	Guayule	+
	<i>Parthenium</i>	American feverfew	++
	<i>Amaranthus</i>	Pigweed, amaranthus	+
Amaranthaceae	<i>Atriplex</i>	Orache, scale	+
	<i>Beta</i>	Beet	+
	<i>Chenopodium</i>	Lamb's quarters	+
		Burning bush	
		Russian thistle	
		Pellitory	
	<i>Kochica</i>	Nettle	+
	<i>Salsola</i>	Patterson's curse	+
	<i>Parietaria</i>	Ram	++
	<i>Urtica</i>	Plantain	+
Urticaceae			
Boraginaceae	<i>Echium</i>	Sorrel, dock	+
Brassicaceae	<i>Brassica</i>	Mustard	+
Plantaginaceae	<i>Plantago</i>	English plantain	+
Polygonaceae	<i>Rumex</i>	Unknown	+

Relative clinical importance of the weeds is as follows: +++, major; ++, moderate; +, minor; and —, no or little importance. Source: From Ref. 2.

much smaller than other ragweed. All of the above ragweed species are to be distinguished from clinically less important species that are essentially similar in their general appearance but distinct by their shinier seedpods.

Toward the end of summer, most ragweed produce extremely small flowers borne in immense numbers on small heads and arranged in long spikes at the top of the plant and at the ends of side branches. The spikes stand out in the ragweed plant, and these spike-like structures produce pollen. The pollen grains of the large number of different ragweed species vary considerably in size and form, but they are all light, buoyant, and shed in large quantities over prolonged periods. Table 2 summarizes the anatomical features of ragweed pollen (3). Ragweed pollen causes allergic rhinitis, but its relatively large size (~20 μm) suggests that ragweed pollen may be less likely to penetrate past the glottis to cause an asthma exacerbation (4). However, a study by Agarwal et al. indicates that airborne ragweed allergens are present in particles of less than 10 μm in diameter. Ragweed plant debris exists in different-sized particles both before and after the ragweed season. These allergenic particles may contribute to out-of-pollination-season symptoms in many ragweed-sensitive subjects (5).

Other weeds which also produce pollens and cause hay fever, include mugwort, plantain, pellitory, sunflower, marsh elder, cocklebur, sagebrush, chenopods, amaranths, and Russian thistle. Each of these has morphological features distinct from ragweed (Fig. 1). Most of these weeds are suspected to cause allergic asthma, allergic rhinitis, and conjunctivitis; however, except for a small number of species, few studies have been conducted to confirm their allergenic importance or to characterize their allergens.

Mugwort (*Artemisia vulgaris*), which also belongs to the family Compositae, is an aromatic, perennial weed, with stalks of 2 to 4 ft long, petiolate leaves, and small and inconspicuous flower heads. It preferably grows on disturbed ground often along roadways in urban, suburban, and rural areas, and pollinates in late summer (6). Pollen grains of mugwort are oblate spheroidal, 18 to 22 μm in diameter, and normally tricolporate.

The genus *Plantago* belongs to the family Plantaginaceae and comprises about 250 species. One of the most common species is *Plantago lanceolata* (English plantain or ribwort) and has been associated with hay fever since the beginning of last century (7). English plantain is a biennial or perennial herbaceous weed. It has a basal rosette of ribbed lanceolate leaves and

**Figure 1** Illustrations of allergenic weeds from the Laboratories Guide.**Table 2** The Characteristics of Ragweed Pollen

Tricolporate: three colpi
Subechinate: surface
Small: greatest diameter
Exine: medium size

Source: From Ref. 3.

leafless stems up to 6 ft high. It grows in six to eight foot tall clumps. It occurs in spring and summer.

The genus *Plantago* includes a number of species, including *P. judaica* and *P. lanceolata*. *P. lanceolata* is 3 ft high. The preferred habitat is mountainous zones.

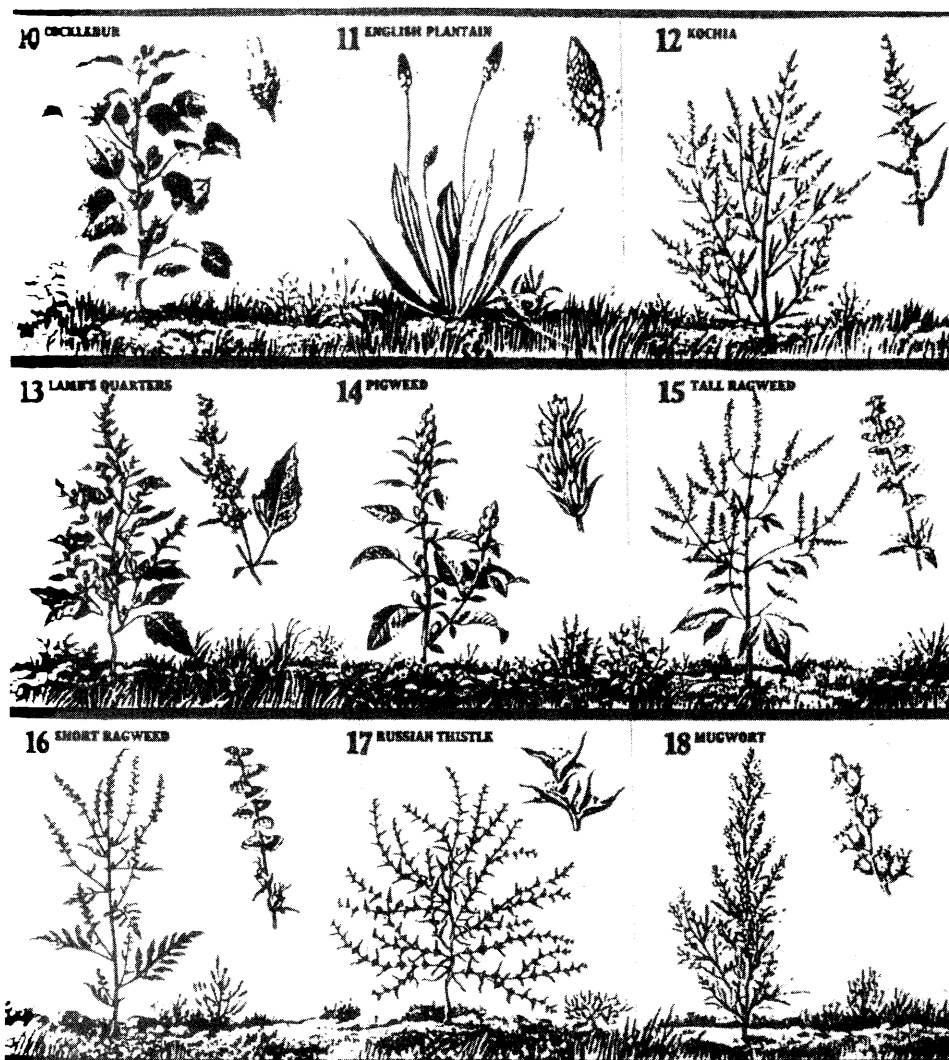


Figure 1 Illustration of some common allergenic weeds in the United States. Source: Poster on Center Laboratories Guide to Allergenic Trees, Weeds, and Grasses of the United States.

Table 2 The Characteristic Features of Ragweed Pollens

Tricolporate: three furrows (boat-shaped portion of grain surface), each enclosing a pore
Subechinate: surface coarsely granular, beset with spines
Small: greatest diameter 18–20 μm
Exine: medium size, subechinate, beset with short spines, approximately 1.7–3.4 μm apart

Source: From Ref. 2.

leafless stems up to 2 ft long bearing dense spikes of hermaphrodite flowers protected by oval bracts. It grows in moist soils, waste places, fields, and pastures. Spheroidal, multipored, with six to eight pores, pollen grains (24–28 μm) are airborne during the pollination period that occurs in spring and summer.

The genus *Parietaria* (pellitory), which belongs to the family Urticaceae, is composed of a number of allergenically related species. The most common and best-studied species are *P. judaica* and *P. officinalis*. They are perennial weeds with a barely branched hairy stem of 1 to 3 ft height. They possess oval or lanceolate leaves and axillary agglomerated flowers. The preferred habitat has been defined for some *Parietaria* species. *P. officinalis* is mainly found in mountainous zones; whereas, *P. judaica* grows on walls in urban coastal areas. Nevertheless,

there are no clear limits to their distribution, and two or more different species may grow simultaneously in most regions. *Parietaria* plants produce large amounts of small (12–18 μm), spheroidal tricolporate pollen grains, which are released through a mechanism of propulsion operated by the elastic filament of the anther, ensuring the success of wind-borne pollination. *Parietaria* has a very long pollen season, which extends over several months. Depending on the climate, the pollination may last nearly the whole year, as is the case in southern Italy, where it starts in February and persists until December and has two cycles of flowering (8).

DISTRIBUTION OF WEEDS

The important and clinically relevant weeds of the world are listed in Table 3 (4,9,10). Ragweed is the most important weed in terms of its allergenic pollen worldwide. There are nearly 60 species included in the ragweed family. In the eastern United States, the common or short ragweed and its relative, the giant ragweed, grow in great profusion in roadside ditches and disturbed areas. These two species are the major ragweed found in the northeastern United States, including parts of Canada, but they are much less abundant in the western states and on the pacific coast. Common ragweed is also widespread in China. Since the 1930s, *A. artemisiifolia* along with *Humulus scandens* (Japanese hops) have become the main source of pollen allergens in China (9). Studies in Croatia also identify common ragweed as an important allergen in Central and Southern Croatia (11–13). Western ragweed prevails in the states west of the Rocky Mountains, on the pacific coast, and in the southern and southwestern states from Louisiana to Arizona. Southern ragweed, with typical lance-shaped leaves, is an important cause of hay fever, but it is mainly confined to the central United States. The slender and bur ragweed are among the false ragweeds and consist of about 25 species in the United States. These are common in the southwest and in Colorado; the latter also ranges northward into Canada, where it is abundant in most arid regions.

Seasonal pollen dispersal is important since it suggests the critical period of avoidance for ragweed-sensitive individuals in various locations. Frenz and associates (14) compared

Table 3 Allergenicity Relevant Weeds of the World

Country	Weeds
Argentina	Short ragweed
Australia	Capeweed, wattle, plantain, dock, goosefoot family, Paterson's curse, wild mustard
Brazil	Ragweed
Canada	Ragweed, goosefoot family, pigweed, Russian thistle, sage, dock, plantain
Chile	Lambs quarters, English plantain, sweet clover, cocklebur, dandelion, dock, sorrell
China	Japanese hop, common ragweed
Colombia	Pigweed family
Cuba	Short ragweed, goosefoot, pigweed family
Ecuador	Goosefoot and pigweed families
Egypt	Goosefoot and pigweed families
France	Ragweed, sagebrush, plantain, Pellitory, goosefoot, and pigweed families
Russia	Sagebrush, Ragweed
Germany	Dock, nettle, plantain, goosefoot family, sagebrush
Great Britain	Sunflower family, plantain, sorrels and docks, nettle, goosefoot, mugwort
Hawaii	Goosefoot and pigweed families
Hungary	Plantain, dock, goosefoot, hemp, ragweed, sagebrush, pigweed, cocklebur
India	Cocklebur, hemp, sagebrush, dock, goosefoot and pigweed family, castor bean, mugwort
Italy	Pellitory, mugwort, sunflower, warnwood, burweed
Japan	Sagebrush, ragweed, English plantain
The Netherlands	Ragweed, sagebrush, sedges, cattails
New Zealand	Plantain, dock, goosefoot family
Portugal	Goosefoot and pigweed families, cocklebur, plantain, sage, pellitory
Romania	Goosefoot, sages, ragweed
Sweden	Dock, sagebrush, goosefoot family, pellitory
United States	Ragweeds (short, giant, western, southern, slender, and false ragweeds), burweed, marsh elder, cocklebur, sages, pigweed, Russian thistle, firebush
Yugoslavia	Hemp, pellitory, dock, goosefoot and pigweed families, ragweed, plantain, castor bean

Source: From Ref. 1.

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Ragweed pollen dispersal in the United States using volumetric techniques and recorded the date of first and final pollen capture and the date of maximum airborne pollen concentration at locations ranging from 30° to 45° north latitude. Sixteen cities located 38° N have similar peak dates, achieving maximal pollen concentration in late August or early September. Four cities, located south of 38° N, experienced later peak dates, reaching maximum pollen levels in mid-October.

Mugwort is widely spread, especially in the temperate and humid zones of the northern hemisphere and along the Mediterranean basin. Mugwort is considered the most important cause of pollinosis at the end of summer and the beginning of autumn in Europe. Pollination occurs at the end of July and through August in Central Europe and takes place mostly in September and beginning of October (15) in the Mediterranean areas.

English plantain is distributed in the temperate zones of both hemispheres. It was introduced from Europe into North America and is now found throughout Canada and the United States. Furthermore, English plantain is widespread in Asia, Australia, and Japan. The pollen season begins in April or early May, peaking in May–July, depending on the latitude and climatic conditions, and continues throughout the remainder of summer and early fall. The clinical importance of pollinosis caused by English plantain has been underestimated for a long time because of the low frequency of monosensitized patients and the overlap of the pollen season with that of grasses. Nevertheless, a high incidence of allergy to English plantain pollen has been reported in the last two decades in different geographic areas, particularly in the Mediterranean basin, Australia, and Japan (10,16,17).

Parietaria is the most important allergenic plant in most regions of the Mediterranean surrounding countries (8). The abundance of this plant, and hence its allergenic relevance, decreases in Europe as the latitude increases, although sensitization to *Parietaria* has been reported to occur as far north as southern England (18). Only one report identifying *Parietaria* as a possible cause of respiratory allergy in the United States has been published so far, in which 8 out of 100 sequential patients with seasonal respiratory allergy referred to the allergist practice were prick tested positive to *Parietaria* (19). One important characteristic of *Parietaria* is the very long pollination period, resulting in multiseasonal or almost perennial symptoms shown by allergic patients, ranging from mild rhinoconjunctivitis to severe asthma. The prevalence of asthma is very high (40–60%) in regions in which this plant is prevalent, such as central and southern Italy (20).

MOLECULAR CHARACTERISTICS OF RAGWEED AND OTHER WEED POLLEN ALLERGENS

Ragweed

Although a number of allergens from diverse weeds have been studied, the most extensively investigated is short ragweed. The allergens described from various weeds are listed in Table 4 (16–19,21,22). Crossed-radioimmuno-electrophoresis of aqueous short ragweed pollen extract detected 22 distinct proteins which bound to specific human IgE antibodies (23,24). However, not all of these allergens are fully characterized.

Amb a 1 is the predominant allergen from short ragweed pollen. Amb a 1 (formerly known as antigen E) first purified and identified in 1964 (23–25), consists of an alpha and a beta chain. Amb a 1 constitutes about 6% of the protein content of short ragweed pollen, and about 90% of ragweed-sensitive subjects have Amb a 1-specific antibodies. Using site-specific monoclonal antibodies, a small number of major antigenic determinants were found on the native Amb a 1 molecule (26). The cloning of Amb a 1 polypeptide subunits reveal that short ragweed pollen contains four isoforms of Amb a 1, designated as 1.1, 1.2, 1.3, and 1.4 (25). The IgE binding of these isoforms indicates that they differ in their capacity to bind human IgE with a ranking of 1.1 > 1.3 > 1.4 > 1.2. T-cell responses to these individual isoforms of Amb a 1, measured by the stimulation index (SI), which is an index of T-cell proliferation compared with control cells of T-cell lines, show a different ranking: 1.1 (SI 25), 1.2 (SI 4.2), 1.3 (SI 9.1), and 1.4 (SI 8.3). Together, these studies confirm that Amb a 1 is the dominant allergen of short ragweed pollen (25).

Amb a 2 is the second most important short ragweed allergen and is closely related to Amb a 1 (65% amino acid identity). Amb a 1 is present in both the pollen and flower heads of

Table 4 Weed Pollen Allergens

Botanical name (common name)	Allergens	Molecular weight (kDa)
<i>Ambrosia artemisiifolia</i> (short ragweed)	Amb a 1 (antigen E)	38
	Amb a 2 (antigen K)	38
	Amb a 3 (Ra 3)	11
	Amb a 5 (Ra 5)	5
	Amb a 6 (Ra 6)	10
	Amb a 7 (Ra 7)	12
	Amb a	11
	Amb a 8	14
	Amb a 9	9
<i>Ambrosia trifida</i> (giant ragweed)	Amb t 5 (Ra 5G)	4.4
<i>Artemisia vulgaris</i> (mugwort)	Art v 1	27-29
	Art v 2 (Ag 7)	35
	Art v 3 (LTP)	12
	Art v 4 (profilin)	14
	Art v (unclassified)	60
	Art v 5	9
	Art v 6	42
	Par j 1	10-15
	Par j 2	11.3
<i>Parietaria judaica</i> (pellitory of the wall)	Par j 3 (profilin)	14
	Par o 1	11-15
	Pla l 1	17-20
<i>Parietaria officinalis</i>	Che a 1	17
<i>Plantago lanceolata</i> (English plantain)	Sal k 1	43
<i>Chenopodium album</i> (Lamb's quarter)	Hel a 1	34
<i>Salsola kali</i> (Russian thistle)	Hel a 2 (profilin)	15.7
<i>Helianthus annuus</i> (sunflower)	Mer a 1 (profilin)	14-15
<i>Mercurialis annua</i>		

short ragweed, while Amb a 2 is detectable only in flower heads. *Escherichia coli* recombinant and native Amb a 2 differ in their ability to bind human IgE antibodies (27), indicating that the recombinant protein is not as allergenic as the native protein. More than 50% of the T-cell lines could be stimulated with Amb a 2, exhibiting an average SI of 14 (25).

Amb a 3 is a basic glycoprotein, having a single polypeptide chain composed of 101 amino acid residues (28). Clinical testing shows that Amb a 3 is highly allergenic in about 30% to 50% of short ragweed-sensitive patients (29) and therefore, is a minor allergen. The antibody and T lymphocyte recognition regions on short ragweed allergen Amb a 3 (Ra3) are characterized (30).

Amb a 5 is one of the most studied among the minor ragweed allergens. About 10% to 20% of short ragweed-allergic subjects are sensitized to this allergen (31,32). The Amb a 5 allergens are cloned and sequenced from different species of ragweed and are characterized with respect to their B- and T-cell epitopes (33). The three-dimensional structures of Amb a 1 and Amb a 5 were also derived by two-dimensional spectroscopy (34,35). The HLA association study of the human allergic immune response demonstrates that all Amb a 5 allergen immune responses are restricted by the same DR molecule (36).

Amb a 6 and four other minor allergens have also been defined in the pollens of short ragweed. By Radioallergosorbent test (RAST) analysis, between 17% and 51% of ragweed-allergic patients exhibit IgE antibodies that bind to these minor allergens (21,37,38). Amb a 7 is highly basic, blue protein and is postulated to be a plastocyanin. Approximately 15% to 20% of ragweed-allergic individuals have antibodies that react to Amb a 7 (39). Three additional allergens have been identified by molecular weight (Table 4). One of these allergens with molecular weight 11 kDa may actually be an isoform of Amb a 3. Two additional allergens have been tentatively identified as Amb a 8 and Amb a 9 in Table 4.

Other Weed Pollen Allergens

Allergens from other weeds are important in different geographic regions of the world. These include mugwort (40-47), English plantain (48-51), *Parietaria* (52-61), sunflower (62,63), lamb's quarter (64), Russian thistle (65), and parthenium (66).

Mugwort pollen allergens (40). Five allergens included in the official list because no sequence information is termed Art v 1 in the allergen glycoprotein of 60 kDa in allergic patients. The allergen with 108 amino acid residues is allergic to mugwort pollen. N-terminal cysteine-rich in hydroxyproline moiety is highly heterogeneous. are observed in mass spectrometry. the electrophoretic pattern on PAGE is as large as 100 kDa. allergenicity of the allergen of affected patients.

Art v 2 is also an identical polypeptide chain with different isoforms, and 33% of sera from mugwort allergic patients.

Two plantain allergens (species), lipid-transfer protein belongs to the LTP family. than one-third of its amino acid the family Rosaceae. mugwort allergic patients have IgE antibodies. been tentatively listed.

English plantain specific IgE to the allergen mixture of isoforms. Pla l 1 variants are found in *europa* pollen allergen present in *Parietaria* allergen weight in the range of *Parietaria* allergen and Par o 1, isolated immunochemical analysis both from the native and Par j 2, sharing 43% have been produced as LTP family. The protein (61). A calcium-binding panallergen (68).

WEED POLLEN

Plants with a common homologous sequence are also positive for reactivity among different strains. analyses of pollen interspecies cross reactions 2 allergens of short

Mugwort pollens contain approximately 40 extractable proteins of which 10 appear to be allergens (40). Five allergens from mugwort are characterized although none of them are yet included in the official list of allergens of the International Union of Immunological Societies because no sequence information is available. The first allergen isolated from mugwort is termed Art v 1 in the article dealing with its purification (41). It is a monomeric, acidic glycoprotein of 60 kDa in SDS-PAGE that is recognized by the IgE from 73% of mugwort allergic patients. The allergen, Art v 1, in the official list of allergens, is a different glycoprotein, with 108 amino acid residues and high sugar content (30–40%), which 95% of the individuals allergic to mugwort have specific IgE. The official Art v 1 is a modular glycoprotein with an N-terminal cysteine-rich domain homologous to plant defensins and a C-terminal domain rich in hydroxyproline residues, some of which are O-glycosylated (42). The carbohydrate moiety is highly heterogeneous (two major series of peaks centered around 13.4 and 15.6 kDa are observed in mass spectra of the natural allergen), and the carbohydrate greatly influences the electrophoretic mobility of the allergen, since the apparent molecular weight in SDS-PAGE is as large as 27 to 29 kDa. The carbohydrate moiety is likely important in the allergenicity of the Art v (42). A single immunodominant T-cell epitope is recognized by 81% of affected patients (43).

Art v 2 is also a glycosylated protein (10% carbohydrate content) that consists of two identical polypeptide chains covalently linked by disulfide bridges. It exists in at least six different isoforms, and it cannot be considered a major allergen, since it binds IgE from only 33% of sera from mugwort allergic patients (44).

Two plant panallergens (a panallergen is an allergen present in multiple unrelated species), lipid-transfer protein (LTP) and profilin, are present in mugwort pollen. Art v 3 belongs to the LTP family. The N-terminal amino acid sequence of this allergen, covering more than one-third of its complete sequence, has a 40% to 50% sequence identity with LTPs from the family Rosaceae fruits (40). A positive skin prick test to Art v 3 is present in 40% of mugwort allergic patients (46). Art v 4 is mugwort profilin, and 36% of mugwort-sensitive patients have IgE antibodies against this allergen (47). Two additional mugwort allergens have been tentatively listed as Art v 5 and Art v 6 because of their distinct molecular weights (Table 4).

English plantain pollen contains 5 to 10 allergenic proteins (48–50). The prevalence of specific IgE to the major allergen, Pla l 1, in plantain allergic patients is about 90%. Pla l 1 is a mixture of isoforms that may occur in glycosylated and unglycosylated forms (45,46). Three Pla l 1 variants are sequenced and display about 40% sequence identity with the major *Olea europaea* pollen allergen, Ole e 1 (51). Although authors differ about the number of allergens present in *Parietaria* pollen, all agree that a highly heterogeneous glycoprotein with a molecular weight in the range 10 to 15 kDa is the main allergen, inducing an IgE response in at least 95% of *Parietaria* allergic patients (52,53). The major allergens from *P. judaica* and *P. officinalis*, Par j 1 and Par o 1, isolated from their respective pollens, exhibit very similar physicochemical and immunochemical properties (54–56). Different Par j 1 isoforms and variants have been isolated both from the natural source and through recombinant expression (57–59). Another allergen, Par j 2, sharing 45% sequence identity and an immunodominant IgE epitope with Par j 1, has been produced as a recombinant protein (60,67). Both Par j 1 and Par j 2 are related to the plant LTP family. The panallergen profilin has been identified in *P. judaica* pollen and named Par j 3 (61). A calcium-binding protein (see chap. 6) in *Parietaria* has also been identified as a panallergen (68).

WEED POLLEN ALLERGEN CROSS-REACTIVITY

Plants with a close taxonomic relationship will most likely have pollen proteins with homologous sequences. Clinical studies reveal that skin test-positive, ragweed-allergic patients are also positive to pollen proteins derived from several distinct plant families (69). Cross-reactivity among geographically distant pollen allergens also occurs (70). The cross-reactivity among weed pollen allergens may be categorized to between species (interspecies) and in different strains within a species (intraspecies). Table 5 summarizes the western blotting analyses of pollen proteins from different ragweeds that demonstrate both intra- and interspecies cross-reactivity (25). The results of these studies show that the Amb a 1 and Amb a 2 allergens of short ragweed not only share significant homologies with each other but also

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Table 5 Cross-Reactivity Among Weed Pollen Allergens

	Anti-Amb a 1	Anti-Amb a 2	Anti-Amb a 2
Ragweed species	pAbs	PAbs	mAb
False ragweed (<i>Franseria acanthicarpa</i>)	Yes	Yes	No
Slender ragweed (<i>F. ensifolia</i>)	Yes	Yes	No
Wooly ragweed (<i>F. tomentosa</i>)	Yes	Yes	No
Short ragweed (<i>A. artemisiifolia</i>)	Yes	Yes	Yes
Southern ragweed (<i>A. bidentata</i>)	Yes	Yes	No
Western ragweed (<i>A. psilostachya</i>)	Yes	Yes	Yes
Western giant ragweed (<i>A. aptera</i>)	Yes	Yes	No
Giant ragweed (<i>A. trifida</i>)	Yes	Yes	No

Abbreviations: pAb, polyclonal antibodies; mAb, monoclonal antibody.

Table 6 Interspecies Cross-Reactivity of Weed Pollen

Species (common name)	Ragweed allergens	Remarks
<i>Phleum pratense</i> (Phl p 4, timothy grass)	Amb a 1	Basis for cross-reactivity between grass and weed allergens
<i>Chamaecyparis obtusa</i> (Cha a I, Japanese cypress)	Amb a 1 Amb a 2	46–49% sequence identity
<i>Cryptomeria japonica</i> (Cry j 1, Japanese cedar)	Amb a 1 Amb a 2	46–49% sequence identity
<i>Zea mays</i> (corn)	Amb a 1	Sequence homology
<i>Parthenium hysterophorus</i> (American feverfew)	Amb a	82–94% cross-inhibition

with the equivalent allergens from other ragweed species (25). Thus, these two allergens have not diverged significantly throughout the evolution of different ragweed species. Similarly, Amb a 5 and Amb t 5 (*A. trifida*) share 49% identity in their amino acid sequence (33).

In addition to the cross-reactivity among related ragweeds, the cross-reactivity of ragweed allergens and the allergens of other plants have been reported. Some of these studies are listed in Table 6. Analysis by RAST and immunoblotting inhibition reveals cross-reactivity between sunflower pollen and other pollens of the Compositae family (mugwort, marguerite, goldenrod, and short ragweed). Mugwort pollen exhibits the greatest degree of allergenic homology with sunflower pollen; whereas, at the other end of the spectrum, short ragweed shows less cross-reactive epitopes (71). One study shows that there is no cross-reactivity between mugwort and ragweed pollens (72); whereas, another indicates that the pollens contain a number of cross-reactive allergens, including the major mugwort allergen Art v 1 and profilin (73). Another study by Asero et al. demonstrates that mugwort reactivity on skin prick test was strongly associated with ragweed hypersensitivity (74).

Skin tests and tests for IgE antibodies of ragweed-sensitive subjects are usually positive to a number of different pollens, frequently from taxonomically diverse species, which are assumed to be allergenically not cross-reactive (75–78). Cross-reactivity occurs between ragweed and a number of vegetables, including fennel, parsley, and carrot (79). Several other weed pollen food syndromes include mugwort-celery-spice (80,81) and ragweed-melon-banana syndromes, the former being the best characterized (81). Parthenium, a weed introduced from the United States into India, is the major aeroallergen in southern India. Parthenium allergens are cross-reactive with short ragweed pollen (82). Similarly, recombinant proteins from *H. scandens* in Japan react with sera from patients allergic to *A. artemisiifolia* (9). The panallergens, profilin and calcium-binding proteins, present in weed pollen may be responsible for the extensive cross-reactivity among pollen-sensitized patients (83). One study demonstrates that villin-related proteins (cytoskeletal proteins) occur in pollens of weeds, namely, mugwort, trees, and grasses; thus, villin-related proteins may represent a novel family of cross-reactive allergens (84). In effect, the presence of pollen-reactive IgE antibodies may not necessarily reflect the sensitizing pollen species. This information is clinically important in view of the increased and rapid migration of people throughout the world.

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In vitro and in vivo studies suggest that sensitization to the cross-reactive mugwort LTP, Art v 3, may extend the recognition pattern of these patients to more distantly related species (45-46). Although Par j 1 and Par j 2 are also related to the LTP family, an association between sensitization to *Parietaria* and Rosaceae fruits has not been demonstrated. It is worth mentioning that sensitization to *Parietaria* normally means sensitization to several species of this genus, since a strong cross-reactivity occurs among the major allergens from different species within the genus (85). The major allergens implicated in cross reactivity among the species are Par j 1 and Par j 2 (86).

A 30-kDa allergen in English plantain cross-reacts with the grass Group 5 allergens, yet this cross-reactivity shows little or no clinical relevance (49). In the same way, there is a rather limited allergenic cross-reactivity between Pla l 1 and Ole e 1, despite structural similarity (51).

WEED IMMUNOTHERAPY

The effectiveness of ragweed immunotherapy was established in the 1960s, and simultaneously the allergenic composition of the extract was determined (87-89). There have been attempts, however, to investigate the efficacy and safety of variations in the approach to immunotherapy. In an effort to increase the safety of allergen immunotherapy, some clinical studies have been performed using chemically modified (90) peptide fragments of ragweed vaccine (91), or encapsulated allergens (92). Studies involve the use of immunostimulatory adjuvants, such as monophosphoryl lipid A (MPL), a detoxified form of lipid A from the lipopolysaccharide of *Salmonella minnesota*; a ragweed-Toll-like receptor ligand; and other forms of chemical modification that entail the use of immunostimulatory DNA sequences (93-95). Allergens modified with immunostimulatory DNA mask IgE epitopes and stimulate a Th-1 immune response (95). The goal of these studies is to produce safer and more efficacious immunotherapy; however, none of these modified products are utilized currently in clinical practice. The original immunotherapy protocols for ragweed-allergic subjects remain unchanged, except that ragweed allergens used today are standardized in the United States with respect to the content of Amb a 1, the major ragweed allergen (88,92). Similarly, methods to determine the concentration of the major allergens have been applied to *Parietaria*, mugwort, and English plantain pollens, and some companies market allergenic products of these species that are standardized with their own "in-house" process of standardization (85,96-98).

Sublingual immunotherapy experience with weeds is limited. Evidence exists regarding safety and efficacy of sublingual immunotherapy for ragweed rhinoconjunctivitis (99).

Short and giant ragweeds are largely cross-reactive, and usually one species is used for skin testing and immunotherapy (100). However, in a small group of patients, Asero et al. demonstrated that short and giant ragweeds are not totally allergenically equivalent. They suggest that diagnostic and therapeutic procedures be conducted using both species (101).

Rush subcutaneous ragweed immunotherapy, used in conjunction with the humanized monoclonal anti-IgE, omalizumab, demonstrates enhanced safety of immunotherapy, presumably by downregulating the expression of the IgE receptors on mast cells and basophils as well as reducing ragweed-specific IgE (102,103). Studies have also been conducted to produce a safer and more efficacious immunotherapy against *Parietaria* allergens. Reduced IgE binding and reduced allergenic activity have been observed using the hybrid proteins Par j 1 and Par j 2 (104). Clinical studies are underway for evaluating the effectiveness of these proteins in *Parietaria* allergic patients.

SALIENT POINTS

- A large number of weed species contribute to the seasonal increases in weed pollen allergens in the air. The most important allergenic pollens are derived from ragweed and its relatives, mugwort, and pellitory.
- The identification of local and regional weed plants and weed pollens is important for clinical practice.
- The cross-reactivity of weed allergens should be considered in the management of weed-allergic subjects.

- The most important allergens of short ragweed are the major allergens, Amb a 1 and Amb a 2. These two major allergens and other minor short ragweed allergens have been characterized in terms of their molecular structure and cross-reactivity.
- Many weed pollen allergens are cross-reactive. Amb a 1, the major allergen of ragweed, cross-reacts with other allergens of ragweed pollen and also cross-reacts with allergens from taxonomically diverse genera and species. On the basis of cross-reactivity, weed allergens can be categorized into three classes: (i) ragweed and related plants, including parthenium; (ii) mugwort and sunflower; and (iii) *Parietaria*.
- The immunotherapy of weed-allergic subjects in the United States is conducted with ragweed allergen vaccines standardized with respect to Amb a 1 content.

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