

# Marker allergens of weed pollen – basic considerations and diagnostic benefits in the clinical routine

## Part 16 of the Series Molecular Allergology

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### Abstract

The term weed is referring to plants used as culinary herbs and medicinal plants as well as ecologically adaptive and invasive segetal plants. In Europe, pollen of ragweed, mugwort, English plantain and pellitory are the main elicitors of weed pollen allergies. Presently, 35 weed pollen allergens have been identified. The most relevant belong to the protein families of pectate lyases, defensin-like proteins, non-specific lipid transfer proteins, and Ole e 1-like proteins. The sensitization frequency depends on geographic regions and might affect more than 50 % of pollen allergic patients in distinct regions. Due to overlapping flowering seasons, similar habitats, polysensitizations and cross-reactive (pan)-allergens, it is difficult to diagnose genuine weed pollen sen-

sitization using pollen extracts. Marker allergens for component-resolved diagnostics are available for the important weed pollen. These are Amb a 1 (ragweed), Art v 1 (mugwort), Pla l 1 (English plantain) and Par j 2 (pellitory). Molecule-based approaches can be used to identify the primary sensitizer and thus enable selection of the appropriate weed pollen extracts for allergen immunotherapy.

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### Background

The term weed does not refer to any particular botanical group of plants. The original term “krut” has its source in the Old High German which simply defines usable plants. In contrast, undesired plants lacking economic or aesthetic values are termed segetal plants. The English term weed encompasses both, and all plants within this termination are meanwhile considered an integrative part of our ecosystem. Weeds are conceptually distinct from herbaceous plants. This group comprises non ligneous plants with succulent, green stems that completely die off or only recover their buried parts after the vegetation period. Generally, the term weed is used for a large variety of different plants and includes culinary herbs, medical plants as well as the economically non-desired but ecologically highly

adaptive segetal plants. Pollen of weeds mediating IgE-related allergies are found in the plant families of Asteraceae, Plantaginaceae, Urticaceae, Amaranthaceae and Euphorbiaceae (**Fig. 1**). Common rag-

### Key words

Weed pollen – marker allergens – mugwort – ragweed – English plantain – pellitory – component resolved diagnostics

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### Abbreviations

AIT	Allergen immunotherapy
CCD	Cross-reactive carbohydrate determinants
IgE	Immunoglobulin E
nsLTP	Non-specific lipid transfer proteins
PR	Pathogenesis-related
SPT	Skin prick test

weed (*Ambrosia* spp) has been imported from the United States and is now persistent predominantly in Southern and Eastern Europe. Due to climatic changes ragweed already demonstrated a prolonged flowering season [1]. The botanically related plant mugwort is growing in the entire Northern hemisphere and represents a relevant allergen source in Europe as well as in Asia [2]. Pellitory is predominantly found in the coastal regions of the Mediterranean Sea and shows a particularly long flowering season. Although English plantain is flowering at the same time as grasses and favors a similar habitat it presents a distinct allergen repertoire [3]. Local exposition and sensitization to pollen of goosefoot, Russian thistle, and annual mercury can be particularly high in distinct geographic areas.

### Allergen nomenclature

To date, 35 molecules originating from twelve different weed pollen are officially acknowledged as allergens (www.allergen.org). **Tab. 1** presents an overview on the clinically most relevant weeds and respective allergen molecules. A comprehensive list can be found in Gadermaier et al. [4]. Since weeds are members of diverse botanical families, they present different allergen panels with major allergens from distinct protein families. Currently, 31 allergenic molecules from weed pollen were purified and/or recombinantly produced. All relevant components are also available for routine allergy diagnostics (**Tab. 1**).

### Structure, biological function and clinical relevance

#### Pectate lyases

Amb a 1 and Art v 6, the allergenic pectate lyases from ragweed and mugwort pollen are in contrast to homologous representatives from cedar and cypress non-glycosylated. Pectate lyases possess a characteristic three dimensional fold resembling a tunnel-like structure [5]. The natural as well as the recombinant molecule is frequently processed into two proteolytic subunits [5]. Pectate lyases play an important role in the maturation and rotting process of plant tissue. In pollen, these enzymes are expressed during the late developmental phase and enable growth and emergence of the pollen tube after loosening the pollen cell wall. Amb a 1 is the most important allergen in pollen of ragweed demonstrating a sensitization rate of > 95 %. The homologous molecule Art v 6 plays however only a minor role in mugwort pollen allergy.

#### Defensin-like proteins

Allergenic molecules consisting of a fusion between a defensin-like and proline rich domain have so far only been identified in the Asteraceae family. The

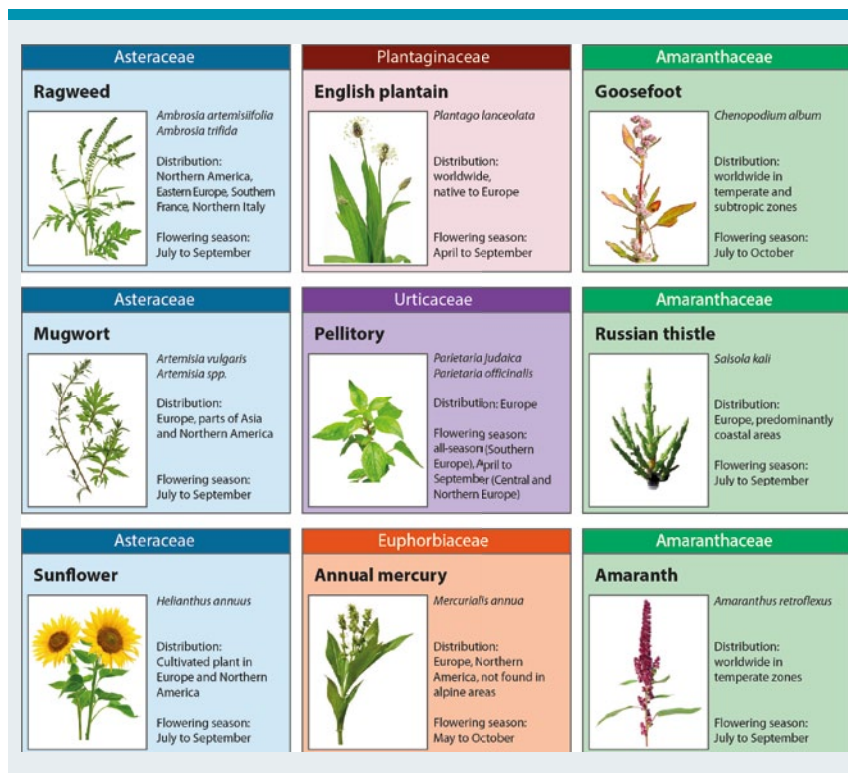


Fig. 1: Allergy eliciting weeds in Europe

compact defensin domain is stabilized by four disulfide bridges and presents a typical  $\alpha/\beta$  motif [6]. The C-terminal part is comparably flexible and carries different O-glycans specific for plants [7]. Defensin-like proteins are frequently found in peripheral cell layers. The localization suggest that those molecules act as first line of defense and they were clustered into the pathogenesis-related (PR)-12 protein family. However, an antibacterial or antifungal mode of action regarding allergenic defensin-like proteins has not been proven yet.

Sensitization to Art v 1 from mugwort pollen ranges from 70 to 95 % rendering it the most important as well as best studied allergen in this family [7, 8, 9, 10]. The vast majority of conformational IgE-binding epitopes is localized on the defensin domain, while the C-terminal region displaying the glycan moiety has only minor clinical relevance [6, 11]. Art v 1 possesses a dominant T cell epitope which is a quite unique feature for allergens [12]. Homologous allergens are present in pollen of ragweed (*Amb a 4*) and sunflower [13, 14].

#### Non-specific lipid transfer proteins (nsLTP)

NsLTP are members of the prolamin superfamily and constitute small, basic proteins with a compact,  $\alpha$ -helical structure. Despite considerable variability in the primary sequence, they present a highly con-

© Ragweed: Elenathewise; English Plantain: Schliemer; Goosefoot: tosoth; Mugwort: Joachim Opelka; Pellitory: MIMOH; Russian thistle: pete pahham; Sunflower: ksenia32; Annual mercury: Alois; Amaranth: Alois / i9 x/fotolia.com

Table 1

Relevant allergens in weed pollen							
	Pectate lyases	Defensin-prolin fusion (PR-12)	Ole e 1-like proteins	nsLTP (PR-14)	Profilins	Polcalcins	Pectin-methylesterase
Ragweed <i>Ambrosia artemisiifolia</i>	<b>Amb a 1<sup>a,b</sup></b>	Amb a 4		Amb a 6	Amb a 8	Amb a 9 Amb a 10	
Mugwort <i>Artemisia vulgaris</i>	Art v 6	<b>Art v 1<sup>a,b</sup></b>		Art v 3 <sup>a,b</sup>	Art v 4	Art v 5	
Sun flower <i>Helianthus annuus</i>		Hel a 1			Hel a 2		
English plantain <i>Plantago lanceolata</i>			<b>Pla l 1<sup>a,b</sup></b>				
Pellitory <i>Parietaria judaica</i>				<b>Par j 1</b> <b>Par j 2<sup>a,b</sup></b>	Par j 3	Par j 4	
Goosefoot <i>Chenopodium album</i>			<b>Che a 1<sup>b</sup></b>		Che a 2	Che a 3	
Russian thistle <i>Salsola kali</i>					Sal k 4	Sal k 5	<b>Sal k 1<sup>a,b</sup></b>
Amaranth <i>Amaranthus retroflexus</i>					Ama r 2		
Annual mercury <i>Mercurialis annua</i>					Mer a 1 <sup>b</sup>		

*nsLTP, non-specific lipid transfer proteins; PR, pathogenesis-related*

**bold:** Major allergens  
<sup>a</sup>ImmunoCAP allergens, Thermo Scientific  
<sup>b</sup>ImmunoCAP ISAC, Thermo Scientific

served, cysteine-stabilized three dimensional fold. This compact structure renders the molecule its particularly high resistance to thermal and proteolytic treatment. High concentrations of nsLTPs can be found in peripheral cell layers. The hydrophobic cavity enables the accommodation of different fatty acids and thus binding and transport of phospholipids. However the role in plant defense against fungi and bacteria (PR-14 proteins) might be biologically more relevant and nsLTP expression was shown to be induced upon stress and injury [15].

Allergenic members of this protein family are predominantly found in plant food (e.g. Pru p 3 from peach), while expression in pollen is restricted to weeds, olive and plane. Par j 1 and Par j 2 (48–50% sequence identity) demonstrate a sensitization frequency of 95% and 83%, respectively and thus represent the major allergens of pellitory pollen [16, 17]. In contrast to other allergenic nsLTP, they both show a higher molecular mass and no IgE cross-reactivity with other representatives of this protein family [18]. Art v 3, the nsLTP from mugwort pollen can trigger respiratory symptoms in sensitized patients [19].

### Ole e 1-like proteins

Proteins assigned to the Ole e 1-like family share a short, conserved consensus stretch while the remaining part of the primary sequence varies considerably among members. Representatives of this family contain one N-glycosylation site and they are usually partially glycosylated [3]. The biological function of Ole e 1-like proteins is to date unknown. Ole e 1-like proteins represent major allergens in pollen of English plantain (Pla l 1) and goosefoot (Che a 1).

### Sensitization frequencies

The clinical relevance of weed pollen allergy in Europe is highly dependent on geographic regions and pollen exposure. Mugwort and ragweed are both important elicitors of weed pollen allergy in Eastern Austria. In Western Austria however they demonstrate only a minor relevance as pollen of English plantain is the major trigger of weed pollen allergies in this region. A recent study investigated IgE sensitization to 112 different allergens among 378 randomly selected 13–20 year old school children from

Salzburg (Western Austria) using the ImmunoCAP® ISAC [20]. Notably, 23.5% of subjects were sensitized to weed pollen allergens while the overall sensitization rate was 57%. Highest prevalence in weed sensitized individuals was found against Pla l 1 (11.6%), which confirms the importance of English plantain as relevant allergen source. Further weed pollen allergen sensitizations were against Art v 1 (8.2%), Mer a 1 (7.1%), Che a 1 (6.3%), Amb a 1 (1.3%) and Sal k 1 (0.5%). No IgE reactivity was found against Par j 2. Another study conducted in South-West Germany evaluating 1,039 randomly selected adults from the population register showed a sensitization rate of 4.4% against Art v 1 and 0.7% against Amb a 1 [21]. Similarly distributed sensitization rates against Art v 1 and Amb a 1 were observed in patients from Northern (84% and 20%) and Southern Europe (74% and 16%) [9]. A different sensitization prevalence is however found in Northern America; 46% of patients are reactive to Art v 1 while 68% are positive to Amb a 1 [9]. The frequency of a genuine mugwort pollen sensitization (68% reactive to Art v 1, 8% reactive to Amb a 1) was also confirmed in a further study investigating weed pollen allergic patients from Germany [22]. Sensitizations to Par j 2 appear almost exclusively in Southern Europe where sensitization rates in some coastal areas can reach 60–90% [4, 9].

### Cross-reactive versus marker allergens

Marker allergens have been identified for all relevant weed pollen; they concurrently constitute the major allergens of respective pollen sources (**Tab. 1**). Although Pla l 1 demonstrates moderate sequence identity with Che a 1 and Ole e 1, IgE cross-reactivity against the partially glycosylated allergens on protein level seems to be rather low [23]. Latest microarray data from ImmunoCAP ISAC showed no correlation in reactivity to Pla l 1 and other Ole e 1-like allergens [20].

Analogous to tree and grass pollen also weed pollen contain the cross-reactive pan-allergens profilin and polcalcins. Those allergens give rise to positive test results in extract based diagnostics, while they are clinically of minor relevance [17, 24]. An intermediate position is attributed to nsLTPs. Par j 2 from pellitory is not cross-reactive with other nsLTPs and thus represents a valid and specific marker allergen [17, 18]. On the other hand, IgE cross-reactivity of Art v 3 and plant food nsLTPs is frequently observed. In Central Europe, clinically manifested mugwort pollen allergies are almost exclusively associated with sensitization to Art v 1.

In pollen of mugwort and ragweed, homologs of the major allergens Art v 1 and Amb a 1 can be found showing moderate cross-reactivity. The defensin-like domain of Amb a 4 from ragweed presents 69% sequence identity with Art v 1 and partial

IgE cross-reactivity was demonstrated [13]. Inhibition experiments predominantly point at primary sensitization with Art v 1, while genuine sensitization to Amb a 4 seems to be uncommon [10, 25]. On the other hand, cross-reactivity was also observed between Amb a 1 and Art v 6 (65% sequence identity). Sixty-three percent of Amb a 1-positive patients suffering from late summer pollinosis demonstrated in vitro reactivity to Art v 6. T cell and inhibition experiments investigating a limited number of patients point at a frequent primary sensitization with Amb a 1. However, in rare cases primary sensitization with Art v 6 and cross-reactivity to Amb a 1 seems possible [26]. In summary, the mentioned cross-reactivities are a plausible explanation for the frequently observed double sensitizations to mugwort and ragweed pollen in routine extract diagnostics. Existing studies demonstrated the strong primary sensitizing capacities of Art v 1 and Amb a 1, while genuine sensitization with the cross-reactive homologs Amb a 4 and Art v 6 seems to be rather uncommon. Thus, Art v 1 and Amb a 1 can in the vast majority be considered genuine marker allergens for mugwort and ragweed pollen allergy. To which extend genuine mugwort and ragweed pollen allergy is diagnosed inappropriately needs to be investigated in further studies. Whether double sensitizations to mugwort and ragweed pollen are due to co- or cross-reactivity highly depends on pollen exposure and the study population [10, 22, 27].

Plant food allergies related to weed pollen sensitization are predominantly observed in patients allergic to mugwort and ragweed pollen [28]. So far members of the profilin and nsLTP family as well as high molecular weight (glycan)-components were identified as causative cross-reactive allergens.

### Allergy diagnostics

According to the current GA<sup>2</sup>LEN recommendations for harmonization of skin prick tests in Europe, mugwort, ragweed, and pellitory pollen are included in routine diagnostics, while plantain, goosefoot and Russian thistle are not considered [29]. Since the clinical relevance of distinct weeds can considerably vary among regions, local modifications are considered useful and necessary. Weed pollen sensitization is commonly observed in polysensitized patients, while monosensitization is rather infrequent. Thus, molecule-based allergy diagnostics offers a valuable tool and should be consequently used for discrimination. To date, all major allergens of weed pollen are commercially available for component resolved diagnostics (**Tab. 1**). Che a 1 is presently the only exception being available only on the ImmunoCAP ISAC® and not as single component for ImmunoCAP testing. Components are either available as recombinant molecules (rPla l 1, rChe a



1, rPar j 2) or CCD (N-glycan)-free, natural molecules (nArt v 1, nAmb a 1). In the case of Sal k 1, false-positive results due to the partial N-glycan moiety might arise. Specific diagnostics of profilins and polcalcins is currently available for the profilin of annual mercury (Mer a 1). Due to the high IgE cross-reactivity with grass and birch pollen profilin, interpretation of these results might be limited.

**Added value of molecular allergy diagnostics**

Molecule-based allergy diagnostics is particularly advantageous in diagnosis of weed pollen sensitizations as patients are frequently polysensitized and the clinical history is not providing unequivocal results due to overlapping flowering seasons. In clinical practice, Art v 1 and Amb a 1 are particularly useful as specific marker allergens for mugwort and ragweed pollen allergy, since identification of the culprit plant is difficult to assess using extract based diagnostics (Fig. 2). Although a misleading diagnosis due to cross-reactivity cannot entirely be ruled out, the primary sensitizer is correctly identified for the vast majority of patients. Thus unnecessary (double) immunotherapies can efficiently be prevented. Although Art v 3 does not constitute a marker for mugwort pollen allergy, it is considered a useful diagnostic option for patients suffering from mugwort pollen associated food allergies (e.g. celery allergy) [15, 30].

So far only limited data are available suggesting Pla l 1 as useful allergen for the diagnosis of plantain allergy in Central Europe [3]. However, lack of IgE cross-reactivity with Che a 1 and Ole e 1 indicates that non-glycosylated rPla l 1 is a highly specific marker for genuine plantain allergy [20, 23].

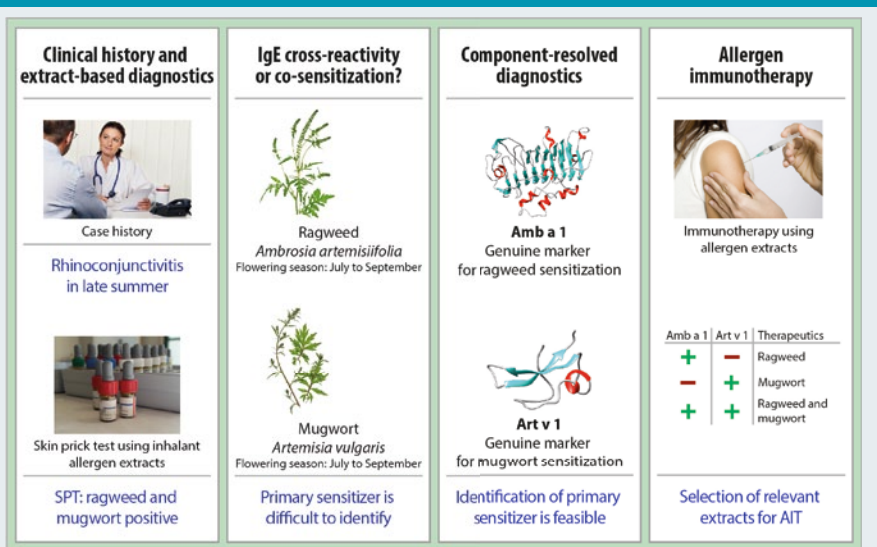
Par j 2 and Sal k 1 are playing only a minor role in Central Europe since respective weeds are absent or show a low prevalence. Even though goose-foot is ubiquitously present, its role as allergy elicitor in a Central European population is barely investigated.

**Therapy and recommendations**

Identification of the primary sensitizer is usually supported using marker allergens specific for weed pollen (Fig. 2). In difficult or doubtful cases of multi-sensitizations, allergen extracts of plants triggering most profound symptoms are typically selected for therapy. Presently, a decrease in the availability of weed pollen extracts for subcutaneous immunotherapy is noticed. Due to regulations and enhanced standardizations for allergen products in hand with economic considerations, several providers (particularly in Germany) already have or are planning to withdraw their weed pollen extracts. In one company selling in Austria, weed pollen extracts only constitute 0.9% of the total annual turnover. On the other hand, the same provider specified 25.1% for Greece and 73.7% for Hungary regarding weed pollen extracts for therapy. On a speculative basis these products might be available further on due to less restrictive provisions in these countries. On average, the European market for weed pollen allergens is indicated with 2.6% for this provider. Currently, 5 single extracts and 9 combination products (mugwort, pellitory, and plantain pollen) for subcutaneous immunization are available and registered in the German market (Paul-Ehrlich-Institut, www.pei.de). In the United States, various different standardized subcutaneous weed pollen solutions and recently also a tablet for sublingual immunotherapy of ragweed pollen allergy are on the market (www.fda.gov).

**Perspectives**

The use of purified allergen molecules in allergen immunotherapy as patient tailored application has been investigated in clinical studies. The efficiency of natural, recombinant or hypoallergenic molecules has already been demonstrated in phase III studies of birch and grass pollen allergic patients [31]. Regarding weed pollen allergies, hypoallergenic derivatives of relevant allergens from ragweed, mugwort and pellitory pollen have been engineered. These molecules demonstrate lower IgE



IgE, immunoglobulin E; AIT, allergen immunotherapy; SPT, skin prick test

© Ragweed: Elenathewise; Mugwort : Joachim Opelka; Case history: Gina Sanders; Immunotherapy: Adam Gregor / (4 x) fotolia.com | Prick test & molecular structures: Gadermaier G.

**Fig. 2:** Added value of molecular based diagnostics for allergen immunotherapy selection using ragweed and mugwort as example, depictions of molecules prepared with UCSF Chimera.

binding capacity and thus potentially less side effects for treatment [4]. Clinical investigations of these hypoallergenic weed pollen molecules are not yet available. However, the efficacy and safety of Amb a 1-T cell epitope based peptide immunotherapy has been demonstrated in 275 ragweed allergic patients [32].

### Conclusion for the clinical routine

Overlapping flowering periods, polysensitizations and geographic differences regarding the clinical relevance of weeds can impede the vaccine choice for subcutaneous immunotherapy. Specific marker allergens are available for all relevant weed pollen supporting the laboratory diagnosis of clinically relevant sensitizations by identification of the primary sensitizing plant. Based on results of the molecule-based diagnostics as well as the clinical symptoms, appropriate therapeutic extracts can be selected for allergen immunotherapy.

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#### Conflict of interest

The authors state to have no conflict of interest.

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