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Pollen Cross-Reactivity: A Primer for Allergy Specialists

Tricia Sowers, PhD

Introduction

There are over 400,000 land-based plant species that comprise our biodiverse habitat. Only a small subset of those plant species satisfies Thommen's postulates, classifying them as allergens. The number of allergenic species, however, remains so vast that it is prohibitive to both test and treat for all relevant species within a geographical region. Allergy specialists have a valuable tool that can be used to help simplify the management of allergic patients: cross-reactivity.

Cross-reactivity is the ability for an allergen to induce an IgE-mediated response, regardless of previous exposure.[1] Allergens are a complex milieu of proteins, some of which have allergic potential while others do not. A number of proteins are conserved across allergen species. When exposure to these conserved proteins occurs, the immune system recognizes them in a similar manner. For homologous or cross-reactive allergenic proteins, this conserved molecular recognition initiates the allergic cascade. Allergen characterization is critical to the understanding of cross-reactivity. Characterization, in this context, refers to the protein make-up of a particular allergen. The process of allergen characterization began in 1962 with the discovery of antigen E, the first identified allergenic protein.[2] Antigen E, commonly known as Amb a 1, is an allergenic protein in ragweed, and is the primary sensitizing protein for ragweed allergy sufferers. These primary sensitizers are referred to as major allergens and can be defined as such when >50% of the allergic patient population is sensitized to them. An entire branch of research arose from this discovery, which has allowed for major advances to be made in the understanding of cross-reactive relationships among allergen species. Cross-reactivity is not limited to homologous major allergen expression. Rather, minor allergens and panallergens, though less clinically relevant, play a similar role in cross-reactive relationships.[3] This primer will explore the science behind cross-reactivity, as well as provide a general overview of the cross-reactive relationships that have been defined for plant allergens found across North America.

Techniques for Determining Cross-Reactive Relationships

A number of techniques have been used to better understand and define cross-reactive allergens, both *in vitro* and *in vivo*, each providing a varying quality of evidence. Protein sequencing has elucidated conserved proteins among both related and seemingly unrelated allergens, which can explain the cross-reactions observed in clinical practice. In general cross-reactivity appears to occur when there is >70% homology between protein sequences.[4] Proteins with the same cellular function, if allergenic, can induce similar IgE responses, despite coming from dramatically different species. An example of this is the conserved group 1 proteins found in birch (Bet v 1) and apple (Mal d 1). This ability is not only related to function, but also to relative conformation. The 3D structures of cross-reactive allergenic proteins are often very similar. The epitopes can be recognized by the same IgE molecules and provoke the allergic cascade.[5] Cross-referencing allergen databases that include protein sequences, 3D conformations, protein functions, and isoform information has drastically expanded the current knowledge base. It has allowed for a more in-depth characterization of allergens and their cross-reactive relationships.

IgE and IgG inhibition studies enable further evaluation of cross-reactivity. This *in vitro* technique demonstrates how one allergen can inhibit IgE or IgG binding with another allergen. When conducted with maximum rigour, IgE and IgG competitive binding studies evaluate each species independently as the inhibitor. High inhibition suggests cross-reactivity, whereas lower inhibition rates imply less shared IgE or IgG affinity and binding. This methodology has been used extensively to define cross-reactive relationships among grass species and in a more limited capacity with tree and weed species.[6-9]

In vivo methods for evaluating cross-reactivity are largely limited. Comparing skin prick testing results and serum IgE levels across species allows for possible cross-reactivity correlations to be made. However, this approach has limitations, given the vast variability in antigen strength, testing sensitivity and specificity, and the diverse nature of the allergenic components contained within. Positive testing correlations could be attributed to panallergens, rather than allergen-specific proteins, which could lead to the overreporting of cross-reactive relationships.[10] Pan allergens and their contribution to cross-reactivity will be discussed further. Allergen provocation testing can also be used to evaluate cross-reactivity, with a level of rigour that exceeds skin and serum testing correlations. With this approach, atopic patients are exposed to an allergen they have not previously been exposed to, and symptom provocation is recorded.[11] Clinical symptoms can be specifically attributed to the non-sensitizing allergen, demonstrating (or not demonstrating) cross-reactivity.

While these *in vitro* and *in vivo* approaches have greatly advanced our understanding of cross-reactivity, a large number of allergenic species still require characterization. Without an understanding of the protein make-up of an allergen, cross-reactive relationships can only be defined using taxonomy. This approach has been shown to be accurate in the majority of instances. Two assumptions must be made when using taxonomic relationships to define cross-reactivity: **1)** the botanical classification accurately reflects the biological relationship between species; and **2)** cross-reactivity is greatest among plants within the same genus, proceeded by those in the same family (**Figure 1**).[12] This implies that distantly related plants exhibit minimal cross-reactivity. Of course, exceptions do exist, likely driven by panallergens.

Panallergens

Approximately 20% of pollen-allergic patients are polysensitized across tree, weed, and grass species.[3] These patients are not truly sensitized to the diverse array of allergens, rather, they are sensitized to panallergens. Panallergens are ubiquitously expressed proteins that are critical to cellular function. Given their purpose in general organismal processes, their structure and epitope binding capabilities are highly conserved. The presence of panallergens across allergen species complicates testing and treatment of allergic patients. If a patient develops a sensitivity to a panallergen, a multitude of false positive reactions may develop during skin or serum IgE testing.[10] Test interpretation may be further complicated by the disproportionate expression of panallergens across allergenic plant species – concentrations may be low in one species and high in another, resulting in variable test reactivity.[13] Cross-reactivity can also extend beyond pollens to include plant-based foods.

Panallergens are categorized into several protein families, each with varying levels of distribution and cross-reactivity. These families include profilin, polcalcin, pathogenesis-related class 10 (PR-10) related proteins, and non-specific lipid transfer proteins (LTPs).[10] Component-resolved diagnostic testing can help tease apart the role panallergens play in the

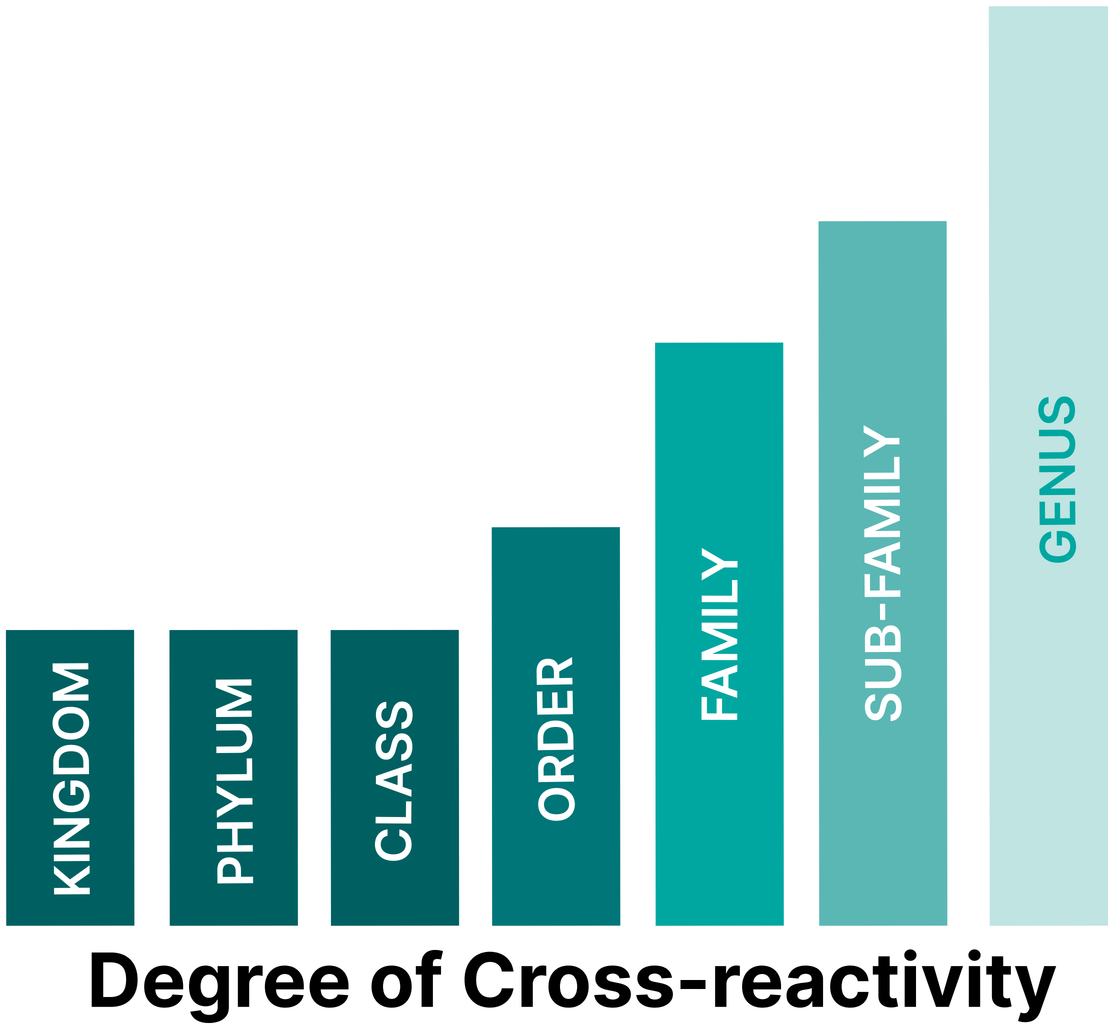

Figure 1. Cross-reactivity as it related to taxonomic relationships. Species that are more closely related are more cross-reactive. Distantly related species typically have little to no cross-reactivity, with the exception of panallergen expression. Little cross-reactivity is observed among plant species in the same Order; *courtesy of Tricia Sowers, PhD*.

sensitization status of allergic patients. Molecular diagnostic panels contain panallergen proteins that are expressed across multiple allergen species. An example is profilin-related proteins, which include Bet v 2 (birch), Pho d 2 (palm), Phl p 12 (Timothy grass) and Amb a 8 (ragweed). When a patient is panallergic, component-resolved diagnostic testing results will be consistently positive for the panallergen-related protein, regardless of the plant species. While component-based testing is becoming commonplace for food allergy diagnosis, it is less frequently employed for pollen-allergic patients.

As an alternative to component-resolved diagnostic testing, queen palm extract can be used as a diagnostic tool to screen for panallergy, specifically profilin sensitization. Queen palm extract contains a high concentration of profilin. If a patient without prior exposure produces a positive reaction to queen palm and most other pollens used for testing, it can be surmised that the patient is profilin sensitized. Theoretically, patient treatment can be significantly simplified, using an extract with high profilin expression (e.g., Timothy grass).[13] Integrating knowledge of panallergens and their relative expression into clinical practice is important when diagnosing and treating allergic patients. Inclusion of a large number of species into patient treatment can create a dilution effect and compromise patient outcomes. Ruling out panallergen involvement can allow for the use of individual species, rather than comprehensive pollen mixes. Further refining treatment formulations by considering cross-reactive relationships can enhance the effectiveness of allergy treatments.

Grass Cross-Reactivity

There is a high degree of cross-reactivity among many grass species – particularly among those grasses that are considered temperate or Northern pasture grasses (e.g., Timothy, perennial ryegrass, orchard, Kentucky bluegrass). These grass species are the most highly abundant species across Canada and are the most clinically relevant. IgE and IgG inhibition studies have demonstrated a 98–100% homology among temperate grass species, meaning that a single species can serve as a representative for both allergen screening and allergy immunotherapy.[6,7] This high degree of homology is likely attributed to the conservation of both group 1 and group 5 proteins, across members of this Pooideae sub-family (**Table 1**). While there is a high degree of cross-reactivity, the major allergen concentration does vary, making certain species more favourable for use in clinical diagnosis and treatment. Although concentrations vary among extract manufacturers, in general, Timothy and orchard grass report the most robust group 5 concentrations.[14]

Southern grasses are less predominant in Canada; however, Bermuda and Johnson grass can be found in multiple provinces. Group 1 proteins, which are homologous to those found in temperate grass species, are the major allergens associated with subtropical grass sensitization. Several studies have demonstrated a high prevalence of co-sensitization when evaluating temperate grass and Southern grass species, particularly with perennial ryegrass and Timothy grass.[11,15,16] Furthermore, treatment with temperate grasses has been shown to reduce clinical symptoms associated with Bermuda grass pollen exposure in co-sensitized patients, suggesting that cross-reactivity does occur among temperate and subtropical grass species.[15] The degree to which cross-reactivity is observed is limited, largely due to the exclusion of group 5 proteins from the Southern grass species.

Tree Cross-Reactivity

While cross-reactivity among grass species is quite extensive, tree species present a more complex landscape. In general, tree allergens are not as well characterized, resulting in greater dependence on taxonomical associations and less in-depth knowledge of major and minor allergen homologies. **Table 1** summarizes tree cross-reactivity using the current body of literature. Species within the same family are generally assumed to be cross-reactive. Intrafamilial cross-reactivity has been well-established, using multiple *in vivo* and *in vitro* methods, for both the *Cupressaceae* (e.g., cedar, cypress, juniper) and *Oleaceae* (e.g., olive, ash, privet) families.[12] Given these findings, a single representative species can be used for both testing and treatment. Some evidence for interfamilial cross-reactivity has been elucidated; however, further allergen-specific characterization is generally required to alter clinical approaches to testing and treatment. The exception to this is among members of the *Betulaceae* and *Fabaceae* families.

The cross-reactive relationship among birch-homologous species is the most well-documented. Birch and alder have long been classified as cross-reactive within the *Betulaceae* family. Recent studies have extended this cross-reactivity to include members of the *Fabaceae* family (e.g., beech, oak).[8] IgE inhibition studies have demonstrated a high degree of homology across these species. In addition, provocation studies have also shown that oak pollen-related symptoms can be alleviated using birch-specific immunotherapy.[17] With both birch-related and oak species being prevalent across Canada, this finding has significant clinical impact.

Of note, there is conflicting evidence in the literature concerning box elder and maple cross-reactivity. Earlier research suggests that these species might have unique, unrelated allergens; however, more recent literature indicates that cross-reactivity is sufficient to allow for the selection of individual species for treatment.[12,18] Until further characterization studies produce conclusive results, it remains at the discretion of the allergy specialist to determine rules for inclusion or exclusion of *Acer* family members.

Grasses	Trees	Weeds
Pooideae Sub-Family	**Aceraceae Family**	**Asteraceae Family**
Agrostideae Tribe	Box Elder	*Iva-Xanthium Genera*
Redtop	Maple	Marshelder
Timothy	**Betulaceae Family**	Cocklebur
Poceae Tribe	Alder	*Artemisia Genus*
Brome	Birch	Mugwort
Orchard	Hazelnut	Sagebrush
Meadow Rescue	**Fagaceae Family**	Prairie Sage
Perennial Ryegrass	Beech	*Eupatorium Genus*
Tritceae Tribe	Oak	Dog Fennel
Quack Grass	**Cupressaceae Family**	Baccharis
Wheat Pollen	Cedar	*Ambrosia Genus*
Phalarideae Tribe	Cypress	Ragweed
Sweet Vernal	Juniper	Rabbit Bush
Canary-Reed	**Juglandaceae Family**	Burrobush
Aveneae Tribe	Hickory	*Solidago Genus*
Oat Grain	Pecan	Goldenrod
Panicodieae Sub-Family	Walnut	*Polygonaceae Genus*
Paniceae-Andropogoneae Genera	**Moraceae Family**	Sheep Sorrel
Bahia	Mulberry	Yellow Dock
Johnson	**Oleaceae Family**	*Plantaginacea Genus*
Corn	Ash	English Plantain
Chloridoideae Sub-Family	Olive	**Amaranthaceae Family**
Cynodonteae Tribe	Privet	*Amaranthus Genus*
Bermuda	**Plantanaceae Family**	Careless Weed
	Sycamore	Pigweed
	Saliaceae Family	W. Waterhemp
	Aspen	*Atriplex-Chenopodium-Kochia-Salsola Genera*
	Cottonwood	Wingscale
	Poplar	Lenscale
	Willow	Allscale
	Fabaceae Family	Saltbrush
	Acacia	Lamb's Quarters
	Locust	Kochia
	Mesquite	Russian Thistle
	Ulmaceae Family	
	Elm	
	Cannabaceae Family	
	Hackberry	
	Fabaceae Family	
	Bottlebrush	
	Eucalyptus	
	Melaleuca	
	Arecaceae Family	
	Queen Palm	
	Pinaceae Family	
	Pine	
	Atingiaceae Family	
	Sweetgum	

Table 1. Cross-reactive relationships among grass, tree, and weed species. Related species are grouped accordingly; *courtesy of Tricia Sowers, PhD.*

Weed Cross-Reactivity

Cross-reactivity among weed species is better defined than the cross-reactive relationships among tree species. This is largely due to enhanced characterization of relevant allergenic proteins. Generally speaking, intrafamilial cross-reactivity exists among members of the common weed families such as *Asteraceae, Amaranthaceae*, and *Polygonaceae*. The degree of cross-reactivity is enhanced at the genus level (**Table 1**).[12]

A significant body of research has been dedicated to the characterization of ragweed and ragweed-related species. Proteins within the *Ambrosia* genus are highly conserved, with 12 identified allergenic proteins that contribute to patient sensitization.[19] Clinically, a single representative ragweed species can be used for both testing and treatment. Mugwort shares many homologous proteins with ragweed and numerous studies report high levels of cross-reactivity. However, it is important to note that Art v 1, the mugwort major allergen, is not homologous with Amb a 1, the ragweed major allergen.[20] While this does not negate cross-reactivity, there are likely patients who would benefit from mugwort-specific immunotherapy, rather than relying upon ragweed and cross-reactivity for desensitization. Significant cross-reactivity has been demonstrated between ragweed and cocklebur using inhibition assays.[21]

Cross-reactivity among members of the *Amaranthaceae* family is also well-documented, particularly with respect to lamb's quarters, kochia, and Russian thistle.[22,23] Russian thistle is the most highly characterized of the allergens, however, there are a number of conserved proteins across the three aforementioned species. Profilin and an Ole e-1 like protein appear to be conserved across the *Amaranthaceae* family members.[23] This homology extends to carelessweed and pigweed as well, and likely accounts for the high degree of cross-reactivity that is observed clinically.

Conclusion

Understanding cross-reactive relationships among grass, tree and weed species can provide substantial opportunities for simplifying the clinical approach to allergy testing and treatment. Cross-reactivity is extensive within allergen families and is even further enhanced at the genus level. The evolution of proteomic research has greatly enhanced our understanding of allergic sensitization and advanced our knowledge concerning cross-reactivity. This research has led to the characterization of both major and minor allergens, as well as elucidating the role of panallergens. In addition, panallergen sensitization should be carefully considered when diagnosing and treating allergic patients. While the clinical relevance of panallergens appears to be less significant, ignorance of panallergen contributions to skin and serum testing results can potentially complicate patient treatment. When component-resolved diagnostic testing is added to the diagnostic process, immunotherapy prescriptions are changed in >50% of patients, often leading to significant treatment simplification.[24] It is important to remember that allergen sensitization is patient-specific. Cross-reactivity will only translate when the relevant, sensitizing allergenic proteins are conserved across species.

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