

SELECTION OF ALLERGEN PRODUCTS FOR SKIN TESTING

Robert E. Esch, PhD

Skin testing is an essential procedure of an allergy evaluation that is indicated (1) to aid in establishing an allergic basis for the patient's symptoms, (2) assist in establishing specific allergic triggers of the patient's symptoms, and (3) help evaluate the degree of sensitivity to a specific agent.¹² The specificity of diagnostic skin testing is essential for management of allergic diseases especially if immunotherapy or environmental control measures are considered. The knowledge of the aeroallergens that are abundant in a given locale and their clinical relevance is critical. Information regarding cross-reactivity between allergens derived from different source materials can be used to reduce the number of required skin tests.

NUMBER OF SKIN TESTS

In spite of the complexity of making an adequate assessment of the clinically relevant allergen sensitivities by skin testing, attempts have been made to define suitable skin test allergen panels for routine use. These attempts have been complicated by the fact that some regions of the country are more botanically diverse than others.

The Joint Task Force for Practice Parameters, representing the American Academy of Allergy, Asthma, and Immunology and the American College of Allergy, Asthma, and Immunology, suggested the routine use of as many as 70 epicutaneous tests to inhalant allergens, followed by up to 40 intradermal tests if necessary.⁹ Although the incremental cost of additional skin tests is not high, the total number applied at any one time should be limited based on the patient's age, history, allergen

From Greer Laboratories, Inc., Lenoir, North Carolina

IMMUNOLOGY AND ALLERGY CLINICS OF NORTH AMERICA

exposure, and geographic locale in which the patient resides. For screening purposes, the International Consensus Report on the Diagnosis and Management of Rhinitis¹³ recommended the use of only the most common aeroallergens in the patient's environment (e.g., house dust mite, pollens, molds, domestic pets). For a patient requiring immunotherapy, additional testing would be indicated. A study by the Walter Reed Army Medical Center, incorporating over 150 centers across the United States, estimated that 42 tests (including pollens, fungi, and environmentals) would be required for a nationwide panel.¹⁵

PREVALENCE OF AEROALLERGENS BY GEOGRAPHIC AREA

Standardized aeroallergen data are collected in various regions of the United States to establish pollen and fungal spore loads during the year. This type of data, published as part of the Pollen and Spore Committee reports, can be used in conjunction with field surveys by local botanists to determine which plants are important for a given area. Such information can be used with a carefully obtained history to determine the relevance of pollen sensitization as determined by allergy testing. The distribution of allergenic plants by geographic area also can be helpful in identifying potential allergen sources.

Pollens responsible for seasonal allergic symptoms are primarily wind-borne tree pollens in the spring, grass pollens in early to midsummer, and weed pollens in late summer to frost. In the Piedmont of North Carolina, the tree pollen season starts in late January with alder pollen followed by red cedar and elm pollens in February. In March, box elder, hickory, and sweet gum begin to pollinate, and by April, birch, sycamore, and oak are pollinating (Fig. 1). In the South Central region of the United States, pecan pollen is an important aeroallergen in April, and in the West, olive is a major pollinating tree. In most parts of the United States, grass pollens peak in June to July, and in the South, Bermuda and Bahia grass are important aeroallergens. In southern Florida, grasses may pollinate for most of the year. Ragweed pollen can be a major aeroallergen in most areas of the United States between August and October.

Climatic factors influence the pollination patterns, and variations are seen from year to year within a given locale and between geographic areas. For example, an early frost can abruptly end the weed pollen season or an unusually mild winter may initiate an early tree pollen season.

There are limitations of exclusively using such data to construct pollen skin test panels.^{4, 11} There is variability of pollen and spore counts from different sites within a community, and distinguishing individual species by microscopic analysis alone is not possible. Many plant species also are introduced as the human population transforms natural habitats. The patient population is highly mobile, and exposure is not always limited to a single geographic area.

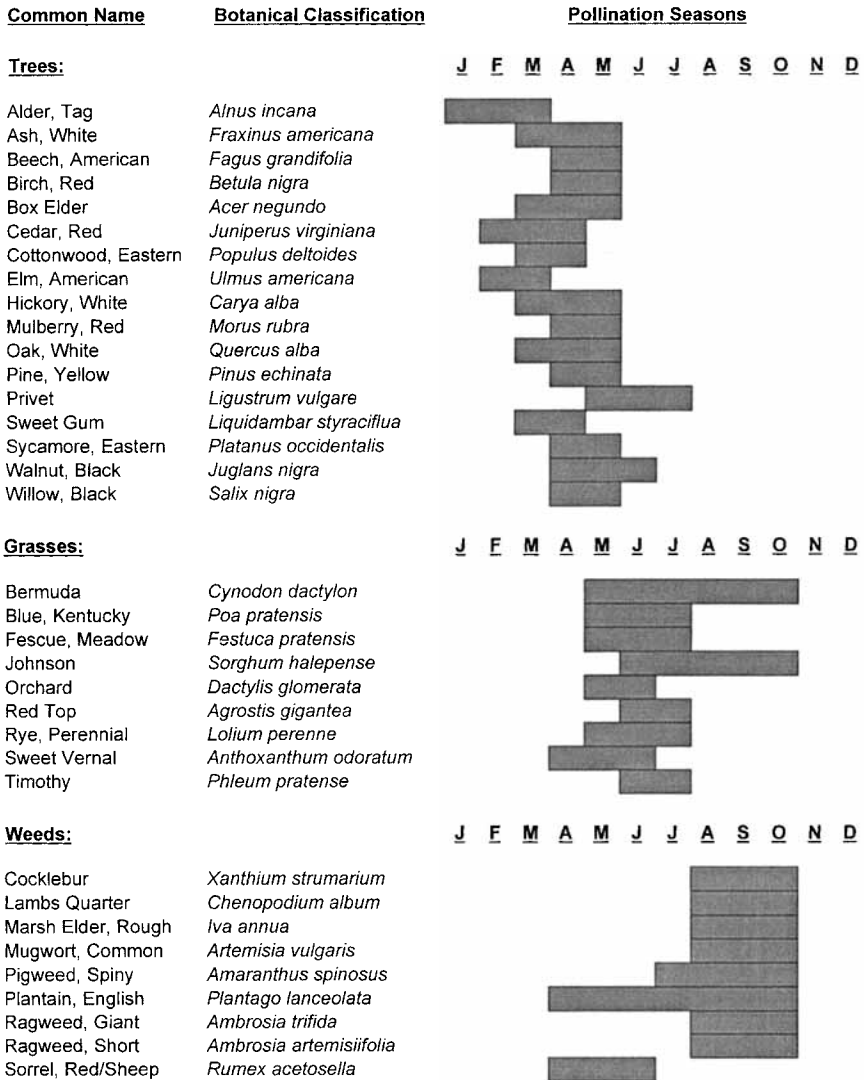


Figure 1. Pollination seasons for species indigenous to Lenoir, North Carolina.

POLLENS

Much evidence exists for cross-allergenicity among pollens from taxonomically related plants.^{10, 16, 20, 27, 29} Among the grasses, a high degree of cross-reactivity exists within the subfamilies Pooideae, Chloridoideae, and Panicoideae. Skin testing with allergenic products derived from multiple species within these groups does not significantly increase the diagnostic efficiency. In addition, there is evidence that immunotherapy with a single representative species within each group is sufficient for therapy. Inclusion of multiple cross-reacting allergens in therapeutic formulations also may increase the risk of adverse reactions caused by overdosing with homologous and cross-reactive allergens and also might limit the dose of other constituents of the immunotherapy mixture. Based on this type of evidence, it would be difficult to justify routine skin testing with multiple allergen products within a cross-reacting group. Other cross-reacting pollen groups are presented in Table 1. In the majority of cases, taxonomic groupings correlate with cross-reactivity. In some cases, cross-reactivity has been shown to exist between groups and across family lines.

FUNGI

Allergenic fungi disperse their dry spores by air currents and are encountered in routine air sampling.⁸ Species of *Alternaria*, *Cladosporium*, *Epicoccum*, *Stemphylium*, *Curvularia*, *Aspergillus*, *Penicillium*, and some of the basidiomycetes are conspicuous for this kind of dispersal. Fungal spores may exceed pollen levels found in outdoor air samples during the damp summer and autumn months. Though fungi are ubiquitous, many species have special nutritional requirements and thus have restricted habitats (Table 2). The common plant pathogens such as the rust fungi and smuts have complex life cycles that are dependent on seasonal conditions and the biology of their host plants.

Fungal diagnostic reagents may not be available for some major fungal allergens, which may in part explain why some patients who have symptoms consistent with fungal allergy have negative fungal skin tests. Fungal ascospores and basidiospores are frequently the dominant airborne spores detected in air samplers during certain seasons, but they are not widely available nor used in skin test panels.¹⁷ Commercially available fungal skin test preparations, manufactured from laboratory-grown cultures, may not contain allergens produced under natural conditions. Fungal allergen products are not standardized. Thus, the specificity and potency of fungal diagnostic reagents obtained from different manufacturers can differ even when they have identical labeling. Nonetheless, skin testing with commercially available fungal allergen preparations can be useful in confirming the role of fungi in respiratory allergy. The ten most common allergenic fungi based on prevalence and skin sensitization among allergic subjects in the Southeastern United States

Table 1. CROSS-REACTING POLLEN ALLERGEN GROUPS*

	Cross-Reacting Groups	Representative Genera
Grass Pollens	<i>Pooideae</i>	<i>Poa</i> (bluegrass), <i>Bromus</i> (brome), <i>Dactylis</i> (orchard), <i>Festuca</i> (fescue), <i>Lolium</i> (perennial rye), <i>Agrostis</i> (redtop), <i>Anthoxanthum</i> (vernal), <i>Avena</i> (cultivated oat), <i>Holcus</i> (velvet), <i>Phalaris</i> (canary), <i>Phleum</i> (timothy), <i>Agropyron</i> (quack), <i>Elymus</i> (wild rye), <i>Secale</i> (cultivated rye), <i>Triticum</i> (cultivated wheat)
	<i>Chloridoideae</i>	<i>Cynodon</i> (Bermuda), <i>Bouteloua</i> (grama), <i>Distichlis</i> (salt)
	<i>Panicoideae</i>	<i>Paspalum</i> (Bahia), <i>Sorghum</i> (Johnson), <i>Panicum</i> (Para), <i>Zea</i> (corn)
Tree Pollens	<i>Aceraceae</i>	<i>Acer</i> (maples and box elder)
	<i>Betulaceae</i>	<i>Alnus</i> (alder), <i>Betula</i> (birches), <i>Corylus</i> (hazelnut)
	<i>Cupressaceae</i>	<i>Cupressus</i> (cypress), <i>Juniperus</i> (junipers and cedars), <i>Taxodium</i> (bald-cypress)
	<i>Fabaceae</i>	<i>Acacia</i> (mimosa), <i>Robinia</i> (locust), <i>Prosopis</i> (mesquite)
	<i>Fagaceae</i>	<i>Quercus</i> (oaks), <i>Fagus</i> (beech)
	<i>Juglandaceae</i>	<i>Carya</i> (hickory and pecan), <i>Juglans</i> (walnut)
	<i>Moraceae</i>	<i>Morus</i> (mulberry), <i>Broussonetia</i> (paper mulberry)
	<i>Oleaceae</i>	<i>Olea</i> (olive), <i>Fraxinus</i> (ash), <i>Ligustrum</i> (privet)
	<i>Pinaceae</i>	<i>Pinus</i> (pines)
	<i>Platanaceae</i>	<i>Platanus</i> (sycamore)
Weed Pollens	<i>Salicaceae</i>	<i>Populus</i> (cottonwood and poplars), <i>Salix</i> (willows)
	<i>Ulmaceae</i>	<i>Ulmus</i> (elms)
	<i>Chenopodiaceae</i>	<i>Atriplex</i> (scales and saltbush), <i>Chenopodium</i> (lamb's quarter), <i>Salsola</i> (Russian thistle), <i>Kochia</i> (firebush), <i>Allenrolfea</i> (iodine bush)
	<i>Asteraceae:</i> <i>Artemisia</i>	<i>Artemisia</i> (mugworts, wormwood, sages)
	<i>Asteraceae:</i> <i>Ambrosia</i>	<i>Ambrosia</i> (ragweeds) <i>Xanthium</i> (cocklebur), <i>Iva</i> (poverty weed, marsh elders)
	<i>Amaranthaceae</i>	<i>Amaranthus</i> (careless weed, pigweeds), <i>Acnida</i> (Western water hemp)
	<i>Plantaginaceae</i>	<i>Plantago</i> (plantain)
	<i>Polygonaceae</i>	<i>Rumex</i> (dock and sorrel)

Note: *Eupatorium* (fennel), *Hymenoclea* (burrobush), *Urtica* (nettle), *Cocos* (Queen palm), *Tamarix* (salt cedar), *Myrica* (bayberry), and *Casuarina* (Australian pine) were excluded because of their high specificity, limited diversity, or lack of information regarding their cross-allergenicity.

*Representative genera are members of the same botanical family or subfamily. Manufacturers currently offer allergen products derived from one or more species of each listed genus.

are *Alternaria*, *Helminthosporium*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Epicoccum*, *Fusarium*, *Stemphylium*, *Botrytis*, and *Curvularia*.

INDOOR ALLERGENS

A variety of indoor allergens derived from pet animals, fungi, and insects may be important causes of allergic symptoms.²¹ The significance

Table 2. PREVALENCE AND HABITAT OF COMMON ALLERGENIC FUNGI

Fungi	Prevalence/Habitat
<i>Alternaria alternata</i> (<i>Alternaria tenuis</i>)	Cosmopolitan; isolated from a variety of plants and from soil
<i>Aspergillus amstelodami</i> (<i>Aspergillus glaucus</i>)	Cosmopolitan; isolated primarily from soil, plants, house dust
<i>Aspergillus flavus</i>	Cosmopolitan; isolated mainly from plants and soil; found on peanuts
<i>Aspergillus fumigatus</i>	Ubiquitous; peaks during high humidity and warm weather; often found in humidifiers, dehumidifiers, basements, attics, plants, food
<i>Aspergillus niger</i>	Ubiquitous; common black bread mould; isolated from soil and decomposing plant material
<i>Aureobasidium pullulans</i> (<i>Pullularia pullulans</i>)	Cosmopolitan; found in bark of sequoia trees and on plant leaves
<i>Bipolaris sorokiniana</i> (<i>Helminthosporium sativum</i>)	Cosmopolitan; isolated from soil and plants; found more in the tropical and subtropical areas
<i>Botrytis cinerea</i>	Cosmopolitan; often found in regions of high humidity; can be found indoors on decaying fruits and vegetable matter
<i>Candida albicans</i>	Common; normal flora on mucous membranes of warm-blooded animals
<i>Chaetomium globosum</i>	Common; isolated from soil and decomposing plant materials, especially straw, mulch, and from herbivore dung
<i>Cladosporium herbarum</i>	Ubiquitous; found frequently in air, soil, plant debris
<i>Cladosporium sphaerospermum</i> (<i>Hormodendrum hordei</i>)	Ubiquitous; found frequently in air, soil, plant debris
<i>Drechslera spicifera</i> (<i>Curvularia spicifera</i>)	Cosmopolitan; isolated from soil and plants; found more in the tropical and subtropical areas
<i>Epicoccum nigrum</i>	Cosmopolitan; isolated from soil and plants
<i>Fusarium moniliforme</i>	Cosmopolitan; isolated from soil and plants
<i>Mucor</i> spp	Cosmopolitan; isolated from soil and decaying organic material
<i>Penicillium chrysogenum</i>	Ubiquitous; found on food products, spores are often airborne
<i>Penicillium digitatum</i>	Cosmopolitan; found on citrus fruits
<i>Penicillium notatum</i>	Ubiquitous; found on citrus fruits and food products
<i>Phoma betae</i>	Common; parasitizes sugar beets
<i>Puccinia graminis</i>	Common; rusts; produces rust colored spores on the leaves and stems of the primary host (oats, wheat)
<i>Rhizopus stolonifer</i> (<i>Rhizopus nigricans</i>)	Ubiquitous; causes spoilage of refrigerated foods; found on cereal grains
<i>Saccharomyces cerevisiae</i>	Common; common bread yeast (baker's and brewer's yeast)
<i>Stemphylium solani</i>	Common; plant pathogen; causes leaf spot on tomatoes
<i>Trichophyton mentagrophytes</i> (<i>Trichophyton interdigitale</i>)	Common; causes athlete's foot and inflammatory skin lesions
<i>Ustilago</i> spp	Common; smuts sp. = <i>cynodontis</i> (Bermuda grass), <i>maydis</i> (corn), <i>avenae</i> (oat), <i>tritici</i> (wheat)

and prevalence of aeroallergens in indoor environments is frequently difficult to establish because this requires more sophisticated methods for allergen detection and measurement.⁵ Assays that use house dust samples collected from a patient's home are available, but the usefulness of such results generally has not been established. In most cases, the presence of furry pets, insect infestation, water damage, and visible mold contamination is used to identify potentially relevant allergens. Among the most frequently encountered and important allergens are the *Dermatophagoides* mites, cockroaches, and cat and dog allergens. Standardized skin test allergen products prepared from *Dermatophagoides farinae* and *D. pteromyssinus* are commercially available. Because of their high degree of cross-reactivity, the use of a 50/50 mixture of the two species may be a reasonable alternative to testing with each individually. Two standardized products derived from cat are also available: cat hair and cat pelt. Both products are standardized based on their major allergen, Fel d 1, content. Cat pelt may contain certain proteins (e.g., cat albumin) at higher concentrations than in cat hair preparations. The clinical significance of this difference has not yet been established.

STINGING INSECTS

Skin testing with insect venoms in subjects who experience systemic reactions to insect stings is the most rapid, sensitive, and economic method to demonstrate sensitization to stinging insects.¹⁴ Standardized venom preparations derived from honey bee, yellow jacket, yellow hornet, white-faced hornet, and paper wasp have been commercially available since 1975. Frequently, skin testing is performed with all of the commercially available preparations because uncertainties exist with identifying the causative species. The need for skin testing with individual *Vespula* or *Dolichovespula* species may be questioned because a high degree of allergenic cross-reactivity has been established between hornet and yellow jacket venoms.^{7, 22} In contrast, cross-sensitization between wasp (*Polistes*), vespine (*Vespula* and *Dolichovespula*), and honey bee (*Apis*) venoms is not likely to occur.^{23, 24} The recommended 1.0- μ g/mL epicutaneous test concentration for Hymenoptera venoms rarely elicit positive skin test reactions in sensitized subjects. For this reason, intradermal skin tests are required in nearly all cases.

FOODS

Almost all children who have immunoglobulin E (IgE)-mediated allergic reactions to foods by double-blind, placebo-controlled food challenge have positive skin test reactions to that food.²⁵ The most important foods associated with anaphylaxis and certain cutaneous disorders are cow's milk, egg, and the legumes, soybean and peanuts.²⁶ No standardized food allergen products are currently available for skin testing.

Skin testing, when indicated, should be performed exclusively by epicutaneous (prick or puncture) methods, because use of intradermal tests increases the frequency of nonspecific positive reactions using food extracts. In addition, allergens derived from some food sources (i.e., fruits and vegetables) are highly unstable and difficult to prepare.²⁸ In these cases, pricking the fresh fruit with a needle and then pricking the skin of the patient (the prick-and-prick method) has been proposed as the preferred method.⁶

Although clinical reactivity to food can be self-evident and specific, cross-reactivity to various unrelated plant or animal allergens may occur in some patients, especially among those with oral allergy syndrome.³ The association between the birch pollen allergen Bet v 1 and homologous proteins found in foods (e.g., apples, cherries, raw potatoes, carrots, celery, parsley, hazelnuts) can be clinically relevant. Similarly, homologous proteins in latex, bananas, avocado, and chestnut have been identified as basic class I chitinases and are used to explain the apparent allergenic cross-reactivity between them. These and other biochemical and molecular studies with plant-derived food allergens may allow the delineation of sensitivities among some patients based on a limited number of well-defined, cross-reactive allergens.²

USE OF STANDARDIZED ALLERGEN PRODUCTS

The allergen preparations that are manufactured and marketed for diagnostic use are available as standardized or nonstandardized products in a variety of formulations that are labeled with a variety of potency units (Table 3). Standardized allergen products should be used for skin testing when available.¹ Since the 1980s, the standardization of

Table 3. SKIN TEST CONCENTRATIONS OF STANDARDIZED AND NONSTANDARDIZED ALLERGENS*

Allergen Product	Epicutaneous Test Concentration	Intradermal Test Concentration†
Standardized short ragweed	1:20 w/v	1:1,000 w/v
Standardized cat hair	10,000 BAU/mL	200 BAU/mL
Standardized grass pollens	10,000–100,000 BAU/mL‡	200 BAU/mL
Standardized Hymenopteran venoms	1.0 µg/mL protein	0.1–10 µg/mL protein
Standardized mites	10,000 AU/mL	200 AU/mL
Nonstandardized allergens	1:40–1:20 w/v	1:1000 w/v

*Consult product package inserts and labeling for specific manufacturer's recommendations for skin testing.

†Intradermal tests should be preceded by epicutaneous tests. Concentrations presented are based on recommendations for patients who do not react to epicutaneous tests.

‡The 10,000 BAU/mL concentration will detect the more sensitive patients. Inconclusive results at the 10,000 BAU/mL concentration may be followed by epicutaneous tests at 100,000 BAU/mL.

allergen products in the United States has been based on their total allergenic potency, which is determined by comparing production lots to an established reference preparation using validated immunoassays. A reference preparation, assigned potency units (BAUs) based on its skin test reactivity in highly sensitive allergic subjects, serves as the standard. This approach has been applied to allergens derived from cat, *Dermatophagoides* mites, and grass pollens. The potencies of these standardized skin test reagents are more predictable and clinically useful. Nonstandardized allergen products continue to be labeled in terms of weight/volume (w/v) or protein nitrogen units (PNUs) and may possess varying biologic potencies.

Another difference between standardized and nonstandardized allergen products is that the shelf-life of standardized products is supported by stability studies using methods approved by the Food and Drug Administration (FDA). The expiration dating of nonstandardized preparations are arbitrarily given as 36 months for glycerinated prick test reagents and 18 months for aqueous intradermal test reagents without formal studies to support the dating. The extended shelf-life of glycerinated formulations used for prick testing is well established, but the stability of diluted aqueous products used for intradermal tests can be highly variable.^{18, 19} Dilutions for intradermal testing should be prepared on a routine basis to ensure adequate potency.

SUMMARY

The selection of allergens for diagnostic skin testing requires knowledge of the environmental allergens to which the patient is exposed. The number of allergens may vary depending on the clinical history and assessment of each patient and whether immunotherapy is contemplated. A total number of as few as six and as many as 70 allergens could be justified on the basis of these variables. The optimal or proper number varies depending on the location of the practice, the clinical history and age of the patient, whether allergen immunotherapy is considered, and the physician's training and clinical experience. Care should be taken not to overdiagnose by using diagnostic reagents known to contain highly cross-reacting allergens. Based on current information, the maximum number of pollen skin tests required to detect most of the major causes of pollinosis in the United States is approximately 25.

Standardized allergen products should be used whenever possible. Without them, an accurate assessment of patient sensitivity cannot be made. Clinicians should institute practices that ensure the stability of diagnostic reagents, as their allergenic activity may be affected adversely by temperature and dilution. Standardized allergen products derived from fungi and foods urgently are needed for definitive studies on their prevalence and clinical relevance.

References

1. AAAAI Committee on Allergen Standardization: The use of standardized allergen extracts. *J Allergy Clin Immunol* 99:583-586, 1997
2. Bernhisel-Broadbent J: Allergenic cross-reactivity of foods and characterization of food allergens and extracts. *Ann Allergy Asthma Immunol* 75:295-303, 1995
3. Breiteneder H, Ebner C: Molecular and biochemical classification of plant-derived food allergens. *J Allergy Clin Immunol* 106:27-36, 2000
4. Chapman JA: How relevant are pollen and mold spore counts to clinical practice? *Ann Allergy Asthma Immunol* 84:467-468, 2000
5. Chapman MD, Vailes LD, Ichikawa K: Immunoassays for indoor allergens. *Clin Rev Allergy Immunol* 18:285-300, 2000
6. Dreborg S, Foucard T: Allergy to apple, carrot, and potato in children with birch-pollen allergy. *Allergy* 38:167-172, 1983
7. Hamilton RG, Wisenauer JA, Golden DBK, et al: Selection of hymenoptera venoms for immunotherapy based on patient's IgE antibody cross-reactivity. *J Allergy Clin Immunol* 92:651-659, 1993
8. Horner WE, Helbling A, Salvaggio JE, et al: Fungal allergens. *Clin Microbiol Rev* 8:161-179, 1995
9. International Rhinitis Management Working Group: International consensus report on the diagnosis and management of rhinitis. *Allergy* 19 (suppl): 1-34, 1994
10. Ipsen H, Lowenstein H: Basic features of crossreactivity in tree and grass pollen allergy. *Clin Rev Allergy Immunol* 15:389-396, 1997
11. Jelks ML: Interpretation of pollen counts. *Ann Allergy Asthma Immunol* 67:1-2, 1991
12. Joint Task Force for Practice Parameters: Practice parameters for allergy diagnostic testing. *Ann Allergy Asthma Immunol* 75:543-625, 1995
13. Joint Task Force for Practice Parameters: Preliminary proposal for practice parameters. *Ann Allergy Asthma Immunol* 75:482-484, 1995
14. Joint Task Force on Practice Parameters for Allergy and Immunology: Stinging insect hypersensitivity: A practice parameter. *J Allergy Clin Immunol* 103:963-980, 1999
15. Kosisky SE, Squire EN, Bienlein AA, et al: Nationwide standardized skin test panel: A panel for all seasons [abstract]. *J Allergy Clin Immunol* 104:376, 2000
16. Leavengood DC, Renard RL, Martin BG, et al: Cross allergenicity among grasses determined by tissue threshold changes. *J Allergy Clin Immunol* 76:789-794, 1985
17. Lehrer SB, Hughes JM, Altman LC, et al: Prevalence of basidiomycete allergy in the USA and Europe and its relationship to allergic respiratory symptoms. *Allergy* 49:460-465, 1994
18. Nelson HS: Effect of preservatives and conditions of storage on the potency of allergy extracts. *J Allergy Clin Immunol* 67:64-69, 1981
19. Niemeijer NR, Kauffman HF, van Hove W, et al: Effect of dilution, temperature, and preservatives on the long-term stability of standardized inhalant allergen extracts. *Ann Allergy Asthma Immunol* 76:535-540, 1996
20. Pham NH, Baldo BA: Allergenic relationship between taxonomically diverse pollens. *Clin Exp Allergy* 25:599-606, 1995
21. Platts-Mills TA, Vaughn JW, Carter MC, et al: The role of intervention in established allergy: Avoidance of indoor allergens in the treatment of chronic allergic disease. *J Allergy Clin Immunol* 106:787-804, 2000
22. Reisman R, Muller U, Wypych J, et al: Comparison of the allergenicity and antigenicity of yellow jacket and hornet venoms. *J Allergy Clin Immunol* 69:268-274, 1982
23. Reisman R, Muller U, Wypych J, et al: Studies of coexisting honey bee and vespid-venom sensitivity. *J Allergy Clin Immunol* 73:246-252, 1984
24. Reisman R, Wypych J, Muller U, et al: Comparison of the allergenicity and antigenicity of *Polistes* venom and other vespid venoms. *J Allergy Clin Immunol* 70:281-287, 1982
25. Sampson HA, Albergo R: Comparison of results of skin tests, RAST and double-blind, placebo-controlled food challenge in children with atopic dermatitis. *J Allergy Clin Immunol* 74:26, 1984
26. Sampson HA, Metcalfe DD: Food allergies. *JAMA* 268:2840, 1992

27. Van Ree R, van Leeuwen WA, Aalberse RC: How far can we simplify in vitro diagnostics for grass pollen allergy? A study with 17 whole pollen extracts and purified natural and recombinant major allergens. *J Allergy Clin Immunol* 102:184-190, 1998
28. Veiths S, Hoffman A, Holzhauser T, et al: Factors influencing the quality of food extracts for in vitro and in vivo diagnosis. *Allergy* 53:65-71, 1998
29. Weber RW, Nelson HS: Pollen allergens and their interrelationships. *Clin Rev Allergy* 3:2191-318, 1985

Address reprint requests to

Robert E. Esch, PhD
Research and Development
Greer Laboratories
PO Box 800, 639 Nuway Circle
Lenoir, NC 28645

e-mail: esch@greerlabs.com