

CROSS-ALLERGENICITY AMONG THE GRASSES

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RAST inhibition was employed to determine the allergenic relationship of five common western grasses (brome, quack, western wheat, salt, and grama), three southern grasses (Bermuda, Johnson, and Bahia), and six common northern grasses (timothy, June, rye, red top, meadow fescue, and sweet vernal). The patterns of cross-allergenicity suggested by these studies were compared to those suggested by the botanical classification of these grasses.

There was significant cross-allergenicity among the grasses studied. Brome, western wheat, and quack grasses demonstrated RAST inhibition patterns similar to the northern grasses. Salt and grama grasses exhibited close allergenic relationships to Bermuda grass, which was allergenically distinct from the northern grasses. Johnson grass appeared to share allergenicity with both the northern grasses and Bermuda grass. Bahia shared allergenicity with the northern grasses, but also possessed some unique allergens. Timothy and Bermuda grass appeared to account for all of the RAST-inhibiting activity contained in the other grasses studied with the possible exception of Bahia grass.

Introduction

POLLEN OF BOTANICALLY RELATED PLANTS often contains identical or cross-reacting allergens. Since all of the grasses are members of the Gramineae family, it might be anticipated that similar allergens would occur in the pollens of all members of this group. Indeed, previous investigators have reported common antigens of various grass pollens by *in vitro* techniques of double diffusion in gel^{1,2,3}. Common allergens have been reported by investigators using RAST inhibition^{4,5} and *in vivo* techniques of skin testing⁶ and desensitization of passive transfer sites.^{7,8}

Previous studies of the allergenicity of grasses have concentrated on those species that are predominant in England or the eastern United States. They have, in general, not assessed the allergens of those grasses which

occur predominantly in the western or southern United States. We have investigated the allergenic relationship between five grasses prevalent in the western United States (brome, quack, western wheat, salt, and grama), six grasses commonly found in the northern United States (timothy, June, rye, red top, meadow fescue and sweet vernal) and three grasses prevalent in the southern states (Bermuda, Johnson and Bahia) by RAST inhibition (Table I). We found a high degree of shared allergenicity between brome, western wheat, and quack of the western grasses and the six northern grasses. Grama and salt grasses, on the other hand, demonstrated closer allergenic relationship to Bermuda grass. The related Johnson and Bahia grasses appeared to share allergenicity with both Bermuda grass and the northern grasses.

Materials and Methods

Serum Source. Sera from fifteen patients with strongly positive skin tests to June, timothy, brome, Bermuda, salt, grama, and western wheat grasses were collected, pooled and stored at -70°C until used. These subjects were drawn from a geographically mobile military population residing in the Denver, Colorado area at the time of serum collection. None of the patients

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Table 1. Botanical Classification of Selected Grass Species¹⁴

Family	Subfamily	Tribe	Species (common names)	
Gramineae	Pooideae	Poeae	Bromus inermis (smooth brome)	
			Festuca elatior (meadow fescue)	
			Lolium perenne (rye grass)	
			Poa pratensis (June)	
		Phlarideae	Anthoxanthum odoratum (sweet vernal)	
		Agrostideae	Agrostis alba (red top)	
			Phleum pratense (timothy)	
		Triticeae	Agropyron smithii (western wheat)	
		Chloridoideae	Aeluropodeae	Agropyron repens (quack)
				Distichlis stricta (salt)
	Chlorideae		Cynodon dactylon (Bermuda)	
	Panicoideae		Paniceae	Bouteloua gracilis (grama)
				Paspalum notatum (Bahia)
		Andropogoneae	Sorghum helepense (Johnson)	
	Bambusoideae			
Oryzoideae				
Arundinoideae				

had been on immunotherapy. Non-allergic serum was obtained from a non-allergic subject with negative skin tests and a low total serum IgE (7 IU/ml).

Extracts. Extracts of the following grass pollens were obtained from either Hollister-Stier Laboratories, Livermore, CA or Greer Laboratories, Lenoir, NC: timothy (*Phleum pratense*), Bermuda (*Cynodon dactylon*), June (*Poa pratensis*), rye (*Lolium perenne*), red top (*Agrostis alba*), meadow fescue (*Festuca elatior*), sweet vernal (*Anthoxanthum odoratum*), brome (*bromus inermis*), grama (*Bouteloua gracilis*), western wheat (*Agropyron smithii*), quack (*Agropyron repens*), salt (*Distichlis stricta*), Bahia (*Paspalum notatum*), and Johnson (*Sorghum halepense*). *Alternaria* (*Alternaria tenuis*), extract obtained from Hollister-Stier Laboratories, was used as an unrelated antigen control. The extracts were lyophilized in 10 cc aliquots and stored at 20° F until use. They were reconstituted to a 1:4 weight per volume just prior to use in the RAST inhibitions. The same grass extracts were used for binding to the RAST discs and for the inhibition studies. None of the extracts used in these procedures were dialyzed.

RAST Allergen Discs. Cellulose discs 6 mm in diameter were activated with cyanogen bromide.⁹ Grass extracts (timothy, Bermuda, brome, quack, salt, grama, western wheat, Johnson, and Bahia grasses) were reacted with the activated discs according to the method of Ceska, Eriksson, and Varga.⁹

RAST Inhibition. Serial 2-fold dilutions of the pooled allergic serum were prepared with saline. For the different allergen discs, the dilutions employed ranged from full strength to between 1:4 and 1:32 depending upon the reactivity of the pooled allergic serum with that particular allergen disc. Fifty microliters of each serum dilution were mixed with 50 microliters of each extract (1:4 w/v) in duplicate. The mixtures were incubated for 6 hours at room temperature. The allergen discs were then added and allowed to react for 3 hours. Throughout all of the incubations the vials were agitated by a shaker at a slow speed. Standard RAST procedure was followed after this initial inhibition procedure.⁹ ¹²⁵I-antihuman IgE was obtained from Pharmacia Diagnos-

tics, Piscataway, NJ. Uninhibited RAST binding of each dilution of the pooled allergic serum was determined by adding 50 microliters of saline instead of extract to the serial dilutions of serum. This control representing 100% binding for each dilution was done in triplicate. Nonspecific binding by dilutions of the non-allergic serum was determined in triplicate in a similar manner. Nonspecific binding was subtracted from the positive controls and from all of the extract-inhibited values. The counts for the serum with extract added were then divided by the counts of the serum without added extract to give a percentage of activity not inhibited by the extract. This procedure was repeated for each of the nine extract discs: timothy, Bermuda, brome, western wheat, quack, salt, grama, Johnson, and Bahia. *Alternaria* extract was used in each series of inhibitions to determine the amount of inhibition by an unrelated antigen extract. Because close allergenic relationships were demonstrated among some grasses in preliminary studies, not all extracts were used in the inhibitions of the salt grass, Bahia grass, and quack grass RASTs.

Statistical Analysis. Statistical analysis was done by analysis of variance and ranges of similarity at the 5% level were determined using the Duncan multiple range test.¹⁰

Results

RAST Inhibition. The results of the RAST inhibitions are shown in Figure 1 A through I. Each quantitative bar graph depicts the inhibition by the grass extracts and *Alternaria* extract of the RAST titer of one individual grass. For each extract the bars represent, from left to right, the inhibition of full strength and serial 2-fold dilutions of the serum. The inhibition of the timothy RAST by the fourteen grass pollens and *Alternaria* is shown in Figure 1A. The pollen extracts of the other northern grasses exhibited a similar degree of inhibition to that produced by timothy with the exception of sweet vernal which inhibited somewhat less. There was a moderate degree of inhibition by western wheat, quack, salt, and brome of the western grass group and Bahia

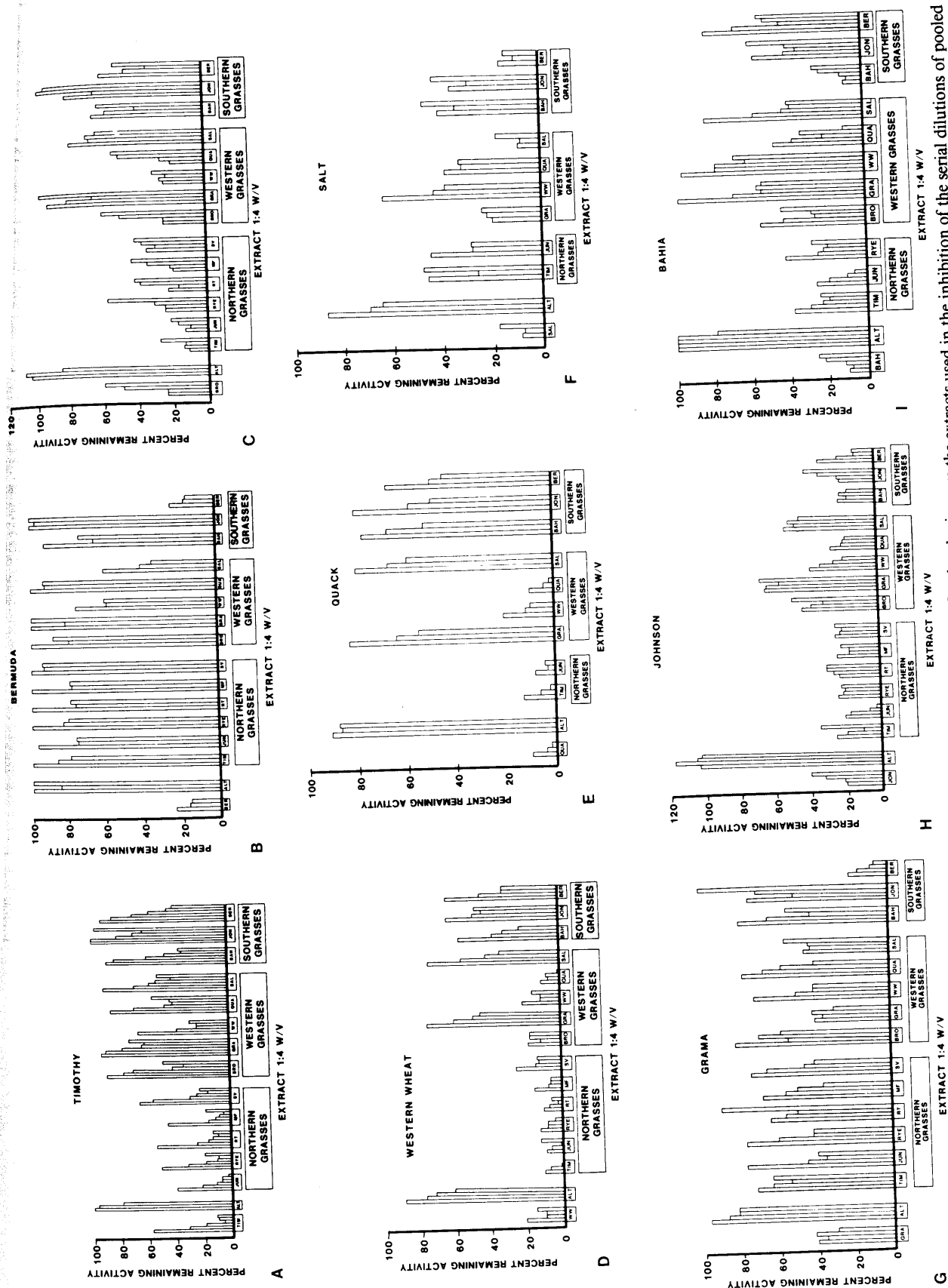


Figure 1. RAST Inhibition. On the ordinate is the percentage of remaining specific activity. On the abscissa are the extracts used in the inhibition of the serial dilutions of pooled allergic serum. The extract tagged discs are the following: (A) timothy, (B) Bermuda, (C) quack, (D) western wheat, (E) quack, (F) salt, (G) grama, (H) Johnson, and (I) Bahia. Abbreviations: TIM, timothy; ALT, *Alternaria*; JUN, June; RYE, rye; RT, red top; MF, meadow fescue; SV, sweet vernal; BRO, bromine; GRA, grama; WW, western wheat; QUA, quack; SAL, salt; BAH, Bahia, JON, Johnson; BER, Bermuda.

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of the southern grass group. Bermuda, grama, and Johnson grasses exhibited even less inhibition but greater than the *Alternaria* or saline control. The statistical analysis of these data is shown in Figure 2A. The extract inhibitions are arranged with the extract exhibiting the greatest degree of inhibition on the left and progressively less inhibition proceeding to the right. Each line connects those extracts that produce inhibitions that were not significantly different at the 5% level. The distance of the line from the listed extract was chosen for clarity of presentation and does not reflect the actual degree of inhibition.

The Bermuda RAST (Fig. 1B, 2B) was inhibited strongly only by Bermuda grass extract. Salt grass exhibited a moderate degree of inhibition. Otherwise only western wheat and Bahia extracts demonstrated inhibition which was greater than the *Alternaria* control.

The brome RAST (Fig. 1C, 2C) was inhibited to a similar degree by the grass extracts of the northern group and by brome, western wheat, and quack of the western group. Salt and grama of the western group and Bermuda, Bahia, and Johnson of the southern group exhibited less inhibition than the others studied but greater than the saline or *Alternaria* control.

A similar pattern was exhibited in the inhibition of the western wheat RAST (Fig. 1D, 2D). All of the grasses of the northern grass group and brome, quack, and western wheat of the western group produced a similar degree of inhibition. Bahia, Bermuda, and Johnson of the southern group and salt and grama of the western group exhibited a lesser degree of inhibition than that produced by the other grass extracts but greater than the controls.

Extracts of timothy and June of the northern grass

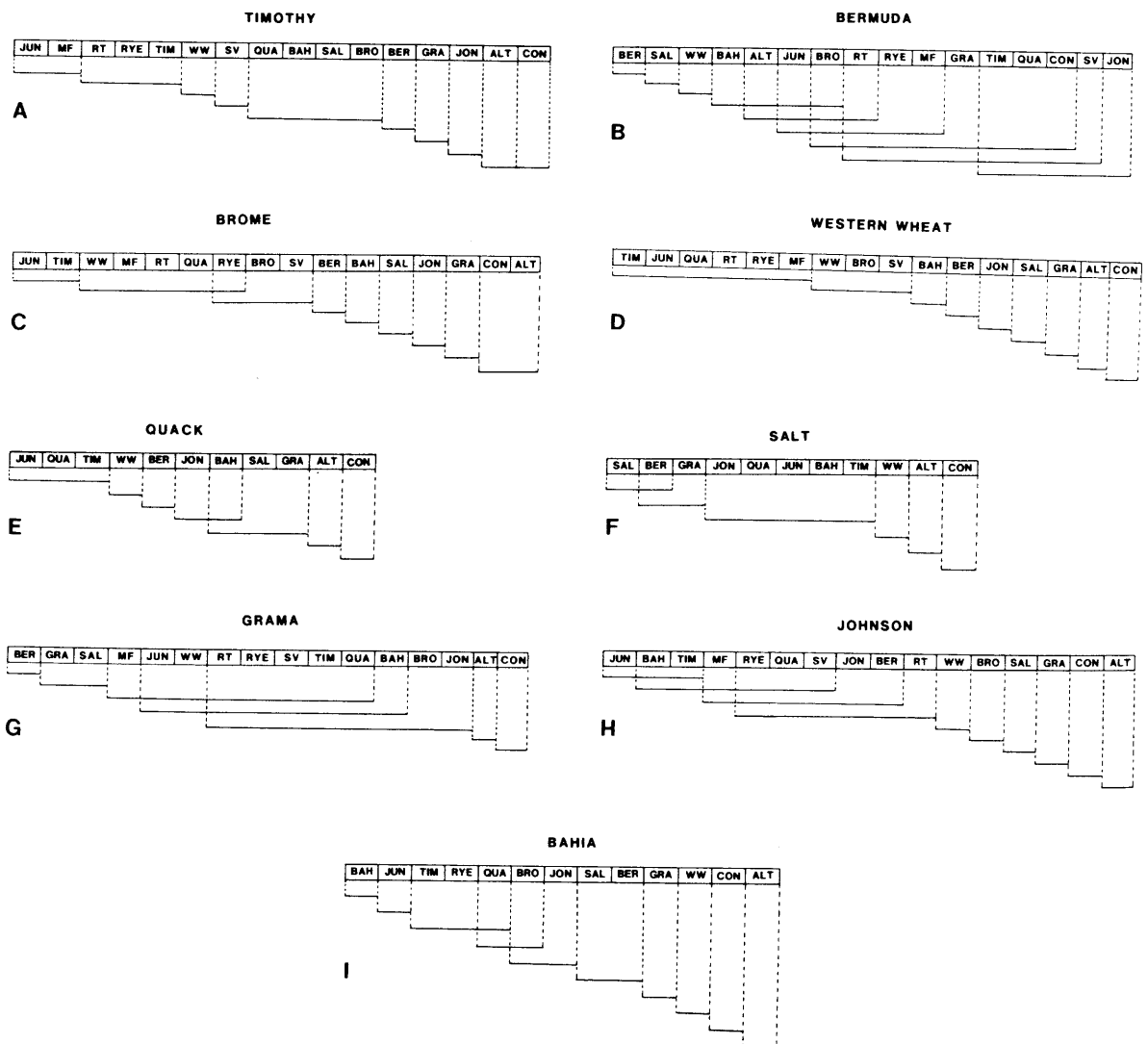


Figure 2. Statistical analysis of RAST Inhibitions. Continuous line indicates statistical similarity at the 5% level of those grasses listed in the boxes above the line. (A) timothy, (B) Bermuda, (C) brome, (D) western wheat, (E) quack, (F) salt, (G) grama, (H) Johnson, (I) Bahia. Abbreviations: CON, saline control; see Fig. 1 for others.

group exhibited an equal degree of inhibition of the quack RAST to that produced by the homologous extract (Fig. 1E, 2E). Only slightly less inhibition was produced by western wheat extract. Bermuda, Johnson, and Bahia of the southern group and salt and grama of the western group exhibited a degree of inhibition which was only slightly greater than the controls.

The greatest degree of inhibition of the salt grass RAST was by Bermuda and salt grass extracts (Fig. 1F, 2F). Grama inhibited only to a slightly less extent. The remainder of the grasses produce a moderate degree of inhibition, greater than the controls.

The grama RAST was inhibited to the greatest degree by Bermuda, grama, and salt grass extracts (Fig. 1G, 2G). The northern grasses, western wheat and quack, exhibited a moderate degree of inhibition. Bahia, brome, and Johnson grasses exhibited slightly less inhibition but greater than the controls. All six northern grasses, three southern grasses and quack of the western grasses exhibited a similar degree of inhibition of the Johnson grass RAST (Fig. 1H, 2H). Inhibition by western wheat, brome, salt and grama extracts produced decreasing degrees of inhibition, all much greater than the controls.

Bahia extract exhibited the greatest degree of inhibition of the Bahia RAST (Fig. 1I, 2I). The northern grasses (June, timothy, and rye) and quack and brome of the western group exhibited slightly less inhibition than Bahia. Johnson and Bermuda of the southern grass group and salt grass of the western group produced a moderate degree of inhibition. Grama and western wheat grasses exhibited the least inhibition, but all of the grasses inhibited the Bahia RAST more than the controls.

Discussion

In this study we demonstrated that there is a large degree of shared allergenicity among the grasses studied. The results indicate that the western and southern grasses studied can be arranged in three groups. The first group includes those cross-reacting with the northern pasture grasses which includes: brome, western wheat, and quack. The second group is identified by a close allergenic relationship to Bermuda and consists of Bermuda, salt, and grama grasses. The third group, consisting of Johnson and Bahia grasses, exhibited shared allergenicity with both Bermuda and the northern pasture grasses.

Comparison of the patterns of cross-allergenicity to the botanical classification of the grasses studied is summarized in Table I.¹⁴ Brome, quack, and western wheat were shown to share allergenicity with other members of their respective tribes and the Pooideae subfamily. A close allergenic relationship between grama and Bermuda, both members of the Chloridoideae tribe, was demonstrated. In addition, salt grass, a member of the Aeluropodeae tribe of the Chloridoideae subfamily showed allergenic similarity to these mem-

bers of the Chloridoideae tribe (Bermuda and grama) and very little cross-allergenicity with species of the Pooideae subfamily. This demonstrates that these three grasses, members of two tribes of the subfamily Chloridoideae have a great degree of shared allergens differing from the grasses of the other subfamilies. Johnson grass showed similar allergenic content to not only Bahia grass, the closest related grass studied and a member of the Panicoideae subfamily, but also to Bermuda grass, and the members of the subfamily Pooideae. Bahia grass was most closely related allergenically to species of the Pooideae subfamily, but also strongly inhibited the RAST of the closely related Johnson grass.

In previous studies, broad cross-reactivity was demonstrated among allergens of grass pollens.^{8,11,12} Timothy grass was shown to share allergens of the common northern grasses¹ and, in addition, to have additional allergens not present in the other grasses.^{4,7,13} Our study confirms the extensive cross-allergenicity but failed to confirm the uniqueness of timothy. Bermuda grass has been shown to be allergenically quite distinct from the common northern grasses (timothy, June, rye, red top, meadow fescue, and sweet vernal) in previous investigations and this was again confirmed by our study. In addition, we have shown that brome, western wheat, quack, Johnson, and Bahia grasses show close allergenic similarity to this group of northern grasses as indicated by the ability of extracts of the northern grasses to inhibit the RAST for these grasses. On the other hand, brome, western wheat, quack, and Bahia extracts induced only a moderate degree of inhibition and Johnson extract inhibited the timothy RAST very little. This indicates that although they share allergens, timothy has additional allergens not present in these other grasses. Failure to demonstrate unique allergenicity in brome, western wheat, quack, Johnson, and to a lesser degree Bahia may indicate that the patients who contributed to the serum pool had been primarily sensitized by the northern pasture grasses. The Johnson grass RAST, in particular, was inhibited by most of the grass extracts studied, although to a lesser degree by grama and salt grass; however, Johnson grass extract did not strongly inhibit any of the other grass RASTS. This suggests that Johnson grass contains fewer allergens than the other grasses studied, or alternatively, that our allergic population had not had significant sensitization by Johnson grass and reactivity with the Johnson grass disc was only to allergens shared with the other grasses. Bahia grass appeared to cross-react to varying degrees with the other grasses studied, but in every case possessed some unique allergenicity.

In summary, our studies with RAST inhibition have not demonstrated any inhibiting capacity by the extracts of the five western grasses or Johnson grass which was not present in extracts of timothy or of Bermuda grass. The reactivity of our pooled sera to Bahia grass was inhibited strongly by timothy and Bermuda but not as completely as by Bahia extract itself. The clinical

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implications of our findings are that it is perhaps not necessary to include the extracts of these five western grasses and Johnson grass in skin testing or immunotherapy. The small degree of allergenic uniqueness exhibited by Bahia grass may also not be important clinically for most individuals. Further investigation is needed to determine if the implications of this study are confirmed by clinical studies.

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Sir William Osler
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