

Diagnosis of *Polistes* wasp hypersensitivity

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Patients referred from the Houston, Texas, metropolitan area were evaluated for allergic reactions to insect stings. Forty-eight persons reported at least one systemic reaction caused by a Polistes paper-nest wasp sting. Honey bees, imported fire ants, and other types of Hymenoptera were identified in that order by 19 other subjects with systemic allergic reactions. Life-threatening airway obstruction and/or hypotension were noted by most of our patients. Wasp venom skin testing was positive in 65% of subjects reporting sensitivity to this insect. Skin testing was correlated quantitatively with basophil histamine release, and qualitatively with RAST assays using Polistes wasp venom. Venoms from common species of Polistes were highly cross-reactive as shown by RAST and basophil histamine release. Patients having a positive history and laboratory response (by skin testing, histamine release, or RAST) to Polistes wasp venom also were positive to bee venom about 20% of the time and to another vespid (hornet or yellow jacket) over 50% of the time. (J ALLERGY CLIN IMMUNOL 72:399-406, 1983.)

Venom extracts from honeybees (*Apis*), yellow jackets (*Vespula*) and hornets (*Dolichovespula*) are reasonably effective in establishing sensitivity to these insects by ST, basophil HR, and IgE antibody measurement by RAST.¹⁻⁸ The majority of previous studies have come from geographic regions in which systemic reactions to paper-nest wasp (*Polistes*) stings are rarely encountered; however, in Texas and other parts of the southern United States, *Polistes* insects are most frequently incriminated in human systemic reactions to Hymenoptera stings.⁵⁻⁹ We have documented that reactions to *Polistes* wasps, like those to other stinging insects, are probably caused by IgE antibodies because sensitivity is correlated with HR from basophils challenged with *Polistes* venom in vitro.¹⁰ We have also shown antigenic similarity

among venoms from different species of *Polistes*, and dissimilarity to other Hymenoptera.^{10, 11} This report extends these findings in 67 consecutive persons referred chiefly from the southeastern Texas area for evaluation of Hymenoptera reactions. Additionally, the question of venom cross-reactivity is explored further.

MATERIALS AND METHODS

Subjects

Patients who reported systemic reactions after insect stings were referred to the Allergy Service of the University of Texas Medical Branch by 12 allergists in the greater Houston area. Control subjects were recruited from hospital employees and medical students. In all cases written informed consent was obtained. All protocols were approved by the institutional review board for human research.

Subjects were asked to relate all insect stings that could be recalled. When possible, the locale in which the sting occurred, characteristics of the insect and its nest, and description of the sting were utilized to make a tentative species identification. All symptoms noted after stings were recorded. Patient information and diagnostic data were stored in a computer-based scientific information retrieval system for ease of reporting and statistical analysis.

Venom sources

Insect venoms were supplied by Pharmacia Laboratories, Piscataway, N. J., and Hollister-Stier Laboratories, Spokane, Wash. Small quantities of venom from individual species of *Polistes* insects were supplied by Drs. J. E. Gil-

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Abbreviations used

HR:	Release of histamine from basophils in vitro
RAST:	Radioallergosorbent test
ST:	Skin testing (intracutaneous immediate response)
HEPES:	<i>N</i> -2-hydroxyethylpiperazine- <i>N'</i> -2-ethanesulfonic acid

laspy (Texas A & I University, Kingsville) and A. Benton (Vespa Laboratories, Spring Mills, Pa.).

Venoms were stored at 4° C in lyophilized form until used. Subsequently, venoms were dissolved in a sterile solution containing 0.9% NaCl, 0.03% human serum albumin, and 0.4% phenol, and stored at 4° C. Venom samples used for HR studies were extensively dialyzed against an isotonic HEPES-buffered (pH 7.4) saline containing 2 mM calcium and 1 mM magnesium.

Skin testing

Prick testing was done at 0.01 to 1 μ g/ml. If negative, end point intradermal titration was performed as described by Norman.¹² Venom (0.05 ml) was injected beginning at a concentration of 0.001 μ g/ml. Increasing concentrations were applied until a 2+ reaction was achieved or until a maximal concentration of 1 μ g/ml was reached. An end point of ≤ 1 μ g/ml was considered positive.

Basophil histamine release

Leukocytes were obtained from fresh venous blood as previously described.^{10, 13} After washing and resuspension in HEPES-buffered (10 mM) normal saline containing 2 mM calcium, 1 mM magnesium, and 0.03% albumin, cells were added to different concentrations of venoms at 37° C. Cells were incubated for 45 min. The percentage of intracellular histamine released into the supernatant was determined by an automated fluorometric technique.¹⁴ The concentration of venom releasing 25% of intracellular histamine was calculated. A value of ≤ 1 μ g/ml was considered positive.

Radioallergosorbent test

IgE antibodies against insect venoms were measured with discs obtained from Pharmacia. In some cases venom from individual species of insects was coupled to microcrystalline cellulose as previously described.¹⁵

RESULTS

Over a 3 yr period, referred patients and volunteers residing in the greater Houston area were evaluated and placed in three groups on the basis of sting history. Forty-eight persons ("wasp allergic") reported at least one systemic allergic reaction after a sting by an insect tentatively identified as a *Polistes* paper-nest wasp. Nineteen other subjects ("other insect allergic") related a systemic reaction(s) after the sting of

another or unknown type of insect. An additional 35 subjects who denied ever experiencing a systemic reaction to stinging insects were recruited as controls. All comparisons were made among these groups.

Table I lists the demographic features of the three groups. Children were intentionally excluded from the control group. Otherwise the groups did not differ significantly with respect to age, race, personal or family allergic history, or other medical disorders.

Sting histories

The major insects inducing systemic reactions in man are reviewed in Table II. All subjects were asked to provide information about every insect sting remembered, and this is described in Table III. *Polistes* paper-nest wasp stings were most frequently implicated, followed by honey bees (*Apis*) and imported fire ants (*Solenopsis*). Stings by Vespinae insects (hornets and yellow jackets) had generally been experienced in other regions of the United States, since these insects are rarely seen in Texas. In the first two groups of patients who had experienced at least one systemic allergic reaction to a stinging insect (*Polistes* or other species), large local reactions (>5 cm) without systemic symptoms were noted on 13 other occasions. Also these groups reported a total of 11 other stings with no local or systemic reaction.

The features of the most severe episode after an insect sting are listed in Table IV. About 75% of the wasp-allergic individuals had experienced respiratory (wheezing, dyspnea) or cardiovascular (light headedness, shock, unconsciousness) symptoms, and 31% had lost consciousness. Generally only a single sting had been noted, and the arm was the most frequent site. Most local reactions were <25 cm, and a third were <5 cm.

Skin testing

The end point obtained for ST with wasp venom is shown in Table V. Thirty out of 48 individuals who had reported a systemic reaction after a wasp sting showed positive responses to wasp venom at a concentration of 1 μ g/ml or lower. Similarly, five out of 18 persons with a history of allergic sensitivity to another insect type and four out of 30 control subjects were positive. ST with a few lots of venom was performed at 10 μ g/ml and gave false positive responses in most normal subjects. No consistent difference was obtained when the results with venoms from the two manufacturers were compared.

The relationship between the interval since the last systemic reaction to a wasp sting and the cutaneous reactivity to wasp venom was evaluated (Table VI). There was a tendency for a higher percentage of posi-

TABLE I. Demographic characteristics of insect-allergic patients and controls

	Wasp-allergic	Other insect-allergic	Controls
No. of subjects	48	19	35
Age (yr) ($\bar{X} \pm 1$ S.D.)	33.4 $\pm$ 17.7	35.1 $\pm$ 15.3	39.5 $\pm$ 14.7
No. <18 yr	12	2	0
Sex			
Male	30	8	15
Female	18	11	20
Race			
White	46	17	32
Black	2		2
Hispanic		2	1
Personal history of allergies			
Asthma	4 (8%)	1 (5%)	2 (6%)
Rhinitis	12 (25%)	6 (31%)	11 (31%)
Urticaria	3 (6%)	2 (11%)	2 (6%)
Eczema	3 (6%)	0	1 (3%)
Drugs	13 (27%)	5 (26%)	6 (17%)

TABLE II. Taxonomic classification of Hymenoptera causing human allergic reactions

Family	Subfamily	Genus	Common name
Vespidae (wasps)	Vespinae	<i>Vespula</i>	Concealed nesting yellow jacket
		<i>Dolichovespula</i>	Aerial nesting hornet
		<i>Vespa</i>	Old World hornet
Apidae	Polistinae	<i>Polistes</i>	Paper-nest wasp
		<i>Apis</i>	Honeybee
		<i>Bombus</i>	Bumblebee
Formicidae		<i>Solenopsis</i>	Fire ant
		<i>Pogonomyrmex</i>	Harvester ant

TABLE III. Summary of total number of stings related by insect allergic patients and controls

Insect type	Wasp-allergic (N = 48)			Other insect-allergic (N = 19)			Controls (N = 35)	
	Systemic	Local only*	Neg.	Systemic	Local only*	Neg.	Local only*	Neg.
<i>Polistes</i>	67	8	6				7	8
<i>Apis</i>	2	0	1	15	1	2	2	8
<i>Solenopsis</i>	8			5				1
Vespinae	3	1	0	3	1			2
Other	2			1				1
Unknown	1	1	1	3	1	1	3	2
Total	83	10	8	27	3	3	12	22

* Local reaction of >5 cm.

tive skin tests in wasp-allergic subjects who had been stung within the past 6 mo ($\chi^2 = 3.71$, $df = 1$, $p < 0.057$ by two-tailed analysis). An insufficient number of controls could recall wasp stings for us to reach any conclusion.

Basophil histamine release

Thirty-four individuals randomly selected from those giving a history of a systemic reaction to a wasp

sting were tested for sensitivity to wasp venom by HR from basophils in vitro. A comparison was made between the intradermal end point by using wasp venom and the concentration of the same lot of wasp venom necessary to release 25% of intracellular histamine (Fig. 1). As with ST, we observed that basophils from normal subjects frequently release histamine at concentrations above 1 $\mu\text{g/ml}$, and thus such a response was considered negative.

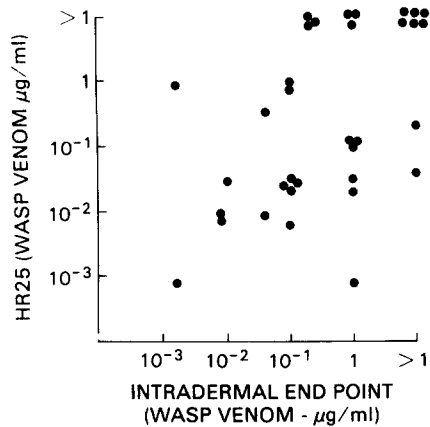


FIG. 1. Comparison of HR and ST end point testing with *Polistes* wasp venom antigens in patients reporting a systemic reaction to a paper-nest wasp sting. The concentration of wasp venom causing release of 25% of total basophil histamine (HR25) was estimated from a dose-response analysis for each patient.

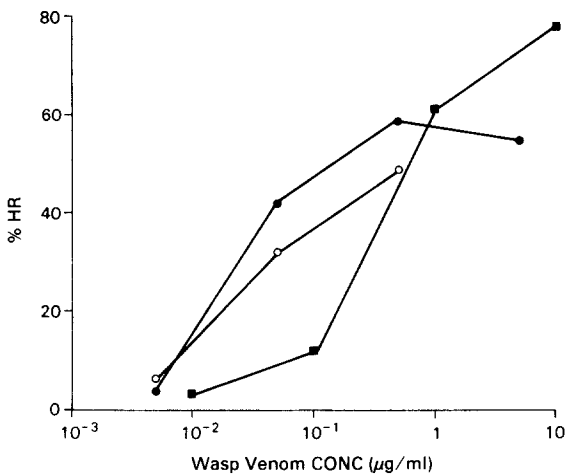


FIG. 2. Leukocyte HR of two patients with a history of an anaphylactic reaction to a wasp sting but with negative cutaneous responses to wasp venom. One patient was studied 2 mo (open circles) and 4 mo (closed circles) after a systemic reaction to a sting. On both occasions he was skin test-negative to wasp venom. The second patient (squares) was seen 3 mo after a systemic reaction but was not available for subsequent evaluation.

A significant correlation was noted between these two tests for wasp sensitivity (Fig. 1; $R = 0.57$, $p < 0.001$). In six individuals, we obtained positive ST but no significant HR. It has previously been observed that the basophils of allergic subjects may be unresponsive to allergens and to anti-IgE.^{6, 16} Of concern was the observation that two individuals were unresponsive to wasp venom ST but were significantly sensitive as measured by basophil HR. The percentages of HR by wasp venom for these two patients are shown in Fig. 2. The responses were well

TABLE IV. Characteristics of most severe reaction to a stinging insect

	Percentage of subjects	
	Wasp-allergic (N = 48)	Other insect-allergic (N = 19)
Symptoms		
Urticaria	52	78
Diffuse angioedema	38	37
Edema about the upper airway	60	47
Dyspnea	73	58
Wheezing	35	37
Pruritus	67	74
Erythema	42	63
Shock	48	26
Light-headed	75	68
Unconscious	31	5
Nausea	48	32
No. of stings		
1	73	74
2-5	17	21
>5	10	5
Site stung		
Head	26	16
Torso	9	11
Arm	60	42
Leg	17	42
Size of local reaction (cm)		
<5	36	28
5-25	44	68
25-50	8	0
>50	11	6

within the range thought necessary to demonstrate sensitivity. One of these patients (circles) had a history of two nearly fatal reactions to a wasp sting within the preceding 4 mo. Repeat ST and HR gave similar results on two additional occasions. Furthermore, this patient subsequently sustained a severe anaphylactic reaction to a wasp sting while receiving maintenance venom therapy.

Radioallergosorbent test

Detection of IgE antibodies to wasp venom antigens by Pharmacia RAST discs is compared to ST results in Fig. 3. The percentage of anti-IgE bound in the 19 control subjects was 2% or less, corresponding to RAST class 0. Twenty-five out of 40 persons studied who had reported a systemic reaction after a wasp sting showed a response of class 1 or higher. All 10 of the history-positive, but ST-negative subjects had a RAST score of class 1 or lower. Three out of 18

TABLE V. Intradermal end point responses to *Polistes* wasp venom

Concentration ($\mu\text{g/ml}$)	No. of subjects (%)		
	Wasp-allergic (N = 48)	Other insect-allergic (N = 18)	Controls (N = 35)
1	11(23)	4(22)	2(6)
0.1	10(21)	1(6)	1(3)
0.01	7(15)		1(3)
0.001	2(4)		
Negative*	18(37)	13(72)	31(88)

*No 2+ reaction to 1 $\mu\text{g/ml}$ or lower concentration.

TABLE VI. Relationship of time interval since last wasp sting and skin reactivity to wasp venom

Interval (yr)	Wasp-allergic		Control	
	ST+	ST-	ST+	ST-
<0.5	17	5	1	3
0.5-1	4	4	1	
1-3	4	4		2
>3	5	5	1	5

ST+ = 2+ reaction to 1 $\mu\text{g/ml}$ or lower concentration; ST- = negative reaction to 1 $\mu\text{g/ml}$.

persons having a reaction to another species had a positive RAST to *Polistes*, and one was borderline. Overall there was a significant correlation ($\chi^2 = 8.7$, $p < 0.001$) between ST and RAST in systemic reactors.

The precision of our determinations with the Pharmacia discs was confirmed in a blind-fashion comparison of 40 samples, with wasp venom coupled to microcrystalline cellulose ($r = 0.76$, $z = 4.76$, $p < 0.0001$ by Spearman rank correlation test).

Table VII summarizes our results in 32 subjects who reported definitive systemic reactions to wasp stings and who were studied by all three diagnostic procedures. ST was most sensitive, and 78% had a positive response to wasp venom at 1 $\mu\text{g/ml}$ or less. Sixty-seven percent of subjects had a RAST score of 1 or higher. Finally, HR was positive in 59% of subjects (25% release by venom at 1 $\mu\text{g/ml}$ or less). All three diagnostic tests gave identical results (either positive or negative) 53% of the time. It should again be emphasized that ST was negative in two individuals who were identified as positive by both HR and RAST.

Cross-reactivity

Five subgenera and 16 different species of *Polistes* wasps have been identified within the United States.^{17, 18} We were concerned whether the antigens

TABLE VII. Summary of results with three diagnostic tests using wasp venom in 32 wasp-allergic patients*

	Histamine release +		Histamine release -	
	RAST+	RAST-	RAST+	RAST-
Skin test+	13	4	5	3
Skin test -	2	0	1	4

*Wasp venom was obtained from A. Benton. The same lot of venom was used for ST and HR studies in a given patient.

in venom from these different species would be related. Rabbit antibodies were raised against venom of individual *Polistes* species. As reported previously, lines of identity usually were observed, although some species appeared to have unique antigens.¹¹

We have extended our previous investigations of cross-reactivity by measurement of IgE antibodies. RAST values for 22 patients who were wasp-sensitive by history were determined with venom from five species of *Polistes* wasps, and the responses (percent of total counts bound) were highly correlated (Table VIII). Correlation tended to be best among species from the same subgenus. By Spearman nonparametric analysis, *Polistes annularis* and *Polistes exclamans* of the *Polistarchus* subgenus were the most highly correlated ($r = 0.96$). In a previous study¹¹ we observed that some sera reacted to a lesser degree to venom from certain *Polistes* species. In the present study using different species of wasps from a different entomologist, we did not see unique reduction in responses to individual species.

Cross-reactivity also has been analyzed by HR from basophils of sensitive subjects. We previously reported that the pattern of HR for seven individuals was similar for three common species of paper wasps collected in Texas.¹⁰ Subsequently we compared the concentration of venom required for 25% HR from basophils exposed to venom from the two subgenera

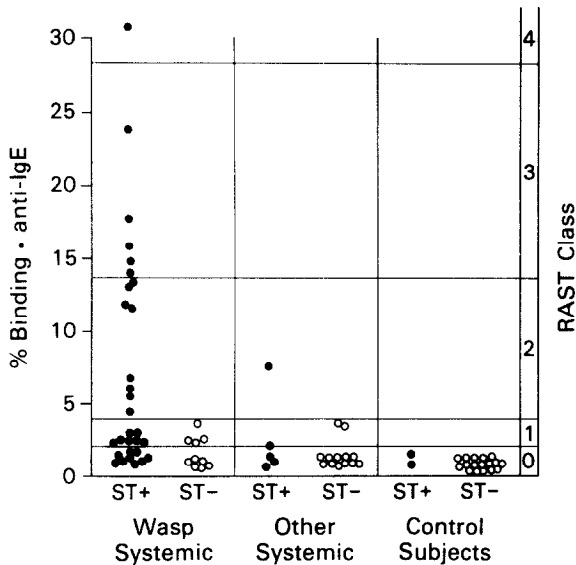


FIG. 3. IgE antibodies against *Polistes* venom measured by RAST. Closed circles, Persons having positive skin tests (ST+) to *Polistes* venom; open circles, persons with negative (ST-) skin responses.

of *Polistes* wasps responsible for most allergic reactions. *P. exclamans* from *Polistarchus* and *Polistes fuscatus* from *Fuscolistes* were highly correlated in 20 individuals (Fig. 4).

The antigenic properties of venom from *Polistes* wasps and other Hymenoptera were compared. We previously reported that rabbit antibodies to venoms from insects of the Vespinae subfamily (see Table II) showed limited reactivity with *Polistes* venom.¹¹ When patients sensitive to *Polistes* wasps were studied for possible reactivity to other insects, two patterns were generally seen: positive responses to *Polistes* as well as *Vespula* and *Dolichovespula* (Fig. 5, A), and responses only to *Polistes* (Fig. 5, B). Table IX summarizes our findings. Persons giving a history of a systemic reaction to *Polistes* and showing an immunologic response to venom from this species (by ST, HR, or RAST) were examined for sensitivity to other Hymenoptera. Concomitant sensitivity to both *Polistes* and *Apis* venoms occurred in less than 20% of patients, whereas more than half responded to venoms of both Polistinae (*Polistes*) and Vespinae (*Vespula* and *Dolichovespula*) subfamilies.

DISCUSSION

In 1960, Micks⁹ polled physicians within Texas and determined that the *Polistes* wasp most frequently was incriminated as the cause of serious reactions to stinging insects. We report an evaluation of individuals from the Houston metropolitan area, 67 of whom had experienced systemic reactions to Hymenoptera

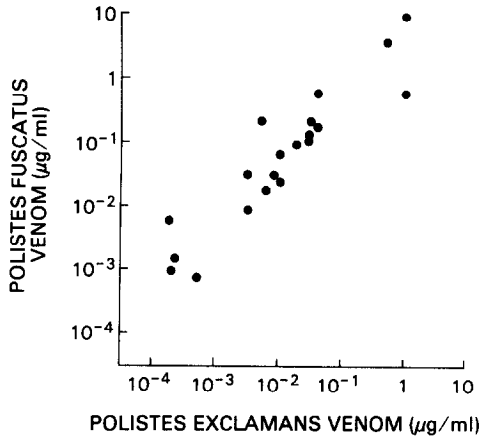


FIG. 4. Comparison of the basophil HR of wasp-allergic subjects challenged with venom from *P. fuscatus* and *P. exclamans*. These are representative species from the two subgenera of *Polistes* responsible for most allergic reactions. Data represent the estimated concentration of venom releasing 25% of cellular histamine.

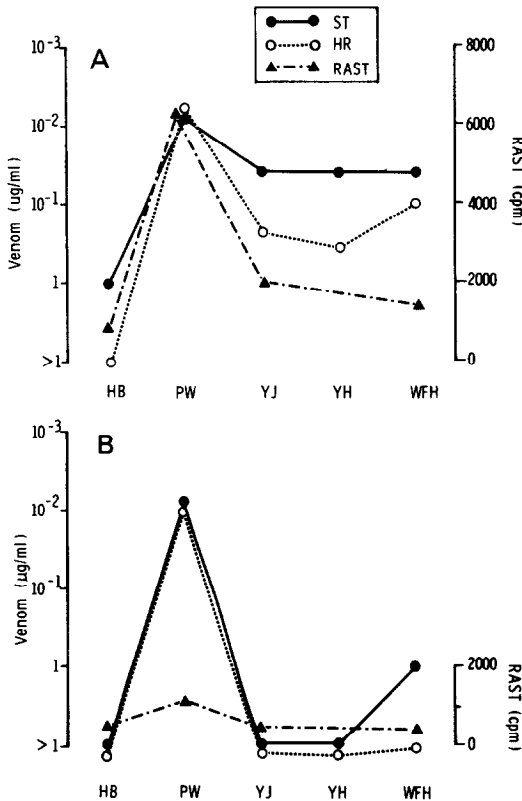


FIG. 5. Results of ST (minimum concentration of venom giving $\geq 2+$ reaction), HR (venom concentration causing release of 25% of intracellular histamine), and RAST (total counts bound; >900 is a class 1 response) in two patients sensitive to *Polistes* wasps (PW). Responses to *Apis* (HB), *Vespula* (YJ) and *Dolichovespula* (YH and WFH) venoms are also shown. A, Patient with sensitivity to multiple venoms. B, Patient with allergy principally to *Polistes* venom.

TABLE VIII. Pearson correlation coefficients of RAST values for venom from five species of *Polistes* wasps in 22 wasp-allergic subjects*

Subgenus	Species	<i>P. exclamans</i>	<i>P. fuscatus</i>	<i>P. metricus</i>	<i>P. perplexus</i>
<i>Polistarchus</i>	<i>annularis</i>	0.88	0.83	0.90	0.87
	<i>exclamans</i>		0.78	0.81	0.89
<i>Fuscolistes</i>	<i>fuscatus</i>			0.96	0.94
	<i>metricus</i>				0.93
	<i>perplexus</i>				

*RAST values for venom samples bound to microcrystalline cellulose were compared. Venom samples were provided by A. Benton.

TABLE IX. Reactivity of *Polistes*-sensitive patients to venom from other species*

<i>Polistes</i> -sensitive by		Percent also positive to			
		<i>Apis</i>	<i>Vespula</i>	<i>D. arenaria</i>	<i>D. maculata</i>
ST	(26)	19	58	50	73
HR	(9)	11	67	33	56
RAST	(21)	19	52	N.T.	N.T.

N.T. = not tested; number of subjects in parentheses.
*Patients who were identified as sensitive to *Polistes* wasp venom by ST, HR, or RAST were also studied for reactivity to other Hymenoptera [*Apis*, *Vespula*, and *Dolichovespula* (see Table II)] by the same procedure.

stings and 35 nonallergic controls. We confirmed the observation of Micks in both our allergic and control groups (Table III), although the *Solenopsis* imported fire ant is of increasing medical importance in this region. Insects from the Vespinae subfamily (see Table II) are rarely seen in this area but are of major significance in other parts of North America and Europe. Hoffman et al.⁵ showed that only 4% of serum samples from insect-allergic donors collected from the entire United States showed a significant RAST to *Polistes* venom, whereas 26% of samples collected in Alabama and Florida were *Polistes*-positive.

Intradermal challenge with *Polistes* venom antigens was the most sensitive assay for demonstrating immunologic responses in our population: 65% of individuals relating a systemic reaction to a wasp sting showed a positive response at ≤ 1 μ g/ml. Twelve percent of controls also responded at ≤ 1 μ g/ml and most reacted at 10 μ g/ml (a dose generally considered toxic). These findings are in agreement with previous reports,^{3, 5-7, 19} including a cooperative study.⁸ Individuals who have noted large local, but not systemic reactions to Hymenoptera also show a high incidence of skin reactivity; this point was not investigated in our study.

We also found that HR from basophils of *Polistes*-sensitive subjects was significantly correlated with cutaneous reactivity. Positive RAST values ($\geq$ class 1) with wasp venom were observed in 63% of persons giving a history of reacting to a sting by this species,

and RAST and ST were correlated qualitatively. Our findings are in agreement with previous comparisons of these diagnostic tests performed principally with honey bee and vespid venoms.¹⁻⁸

Clearly ST is the initial diagnostic test of choice. However, occasional patients with unequivocal sensitivity to Hymenoptera venoms will be negative by this assay but positive by HR or RAST testing. These persons are obviously candidates for immunotherapy.

It is essential that patients be tested with reagents containing the venom antigens inducing anaphylactic sensitivity. We have demonstrated significant cross-reactivity among several common species of *Polistes* wasps by immunoprecipitation with rabbit antibodies, basophil HR, and RAST. Hoffman and McDonald²⁰ also observed considerable similarity in responses to different *Polistes* venoms, using direct RAST and RAST inhibition. Both of our studies found greater correlation for venoms within subgenera of *Polistes*. An occasional subject appeared to be uniquely sensitive to antigens from one species. For this reason it is necessary that commercial extracts of *Polistes* wasp venom contain several representative species. Both sources in the United States currently meet this criterion.

Some individuals who give a history of an allergic reaction to wasp venom demonstrate immunoreactivity only to venom from this insect. Less than 20% also respond to honeybee venom, although there seems to be little similarity between *Polistes* and *Apis* venoms.

In agreement with previous studies,^{7, 8, 20} we ob-

served that a significant majority of *Polistes*-sensitive individuals also react to one or more venoms from the Vespinae subfamily (hornets and yellow jackets). Vespinae venoms share many antigenically related proteins.²¹⁻²³ *Polistes* venom has been only partly characterized.²⁴ It appears that some persons are sensitive to antigens unique to *Polistes*, but most respond to antigens common to both Polistinae and Vespinae venoms. Hoffman and McDonald²⁰ have shown that the pattern of response can be accurately identified only by RAST inhibition, a procedure not appropriate for routine clinical evaluation. The choice of venoms for immunotherapy is a difficult one. The allergist may elect to administer all venoms giving a positive response, but this somewhat increases the expense and difficulty of management. Alternatively, venom of species uncommon to the region (e.g., Vespinae insects in Texas and other regions of the southern United States) may be omitted with caution.

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