Harmonia axyridis ladybug hypersensitivity in clinical allergy practice

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ABSTRACT

The imported Harmonia axyridis *ladybug infests homes in northern West Virginia from fall through spring, causing allergic disease. Retrospective single-practice chart reviews were performed: (1) all skin prick tests (1400 included ladybug) in a community allergy practice over 4 years and (2) clinical analysis of 400 randomly chosen patients. The usual adult aeroallergen skin test panel included ladybug and 57 other allergens. Statistics used were contingency table analyses and the* κ*-statistic for concordance. Home infestation with ladybugs was most common in rural areas but did not predict ladybug sensitization (*$\kappa = -0.02$*). Ladybug sensitization and allergy occurred at all ages. Ladybug sensitization occurred with 21% frequency compared with cat at 24% frequency, cockroach at 27% frequency, and dust mites at 40% frequency. Only ladybug showed a significant (*$p < 0.0001$*) skin test sensitization decreasing from rural (30%), mixed (21%), to urban (16%) home demographics. Isolated single-positive skin tests constituted 10% of dust mites, 6% of cockroach, 6% of ladybug, and 4% of cat-positive skin tests. Skin test concordance was strongest between the pairs: ladybug–cockroach (*$\kappa = 0.36$*), cockroach–dust mite (*$\kappa = 0.29$*), and dust mite–cat (*$\kappa = 0.25$*). Ladybug is a major allergen in endemic areas, causing rhinoconjunctivitis (8% prevalence), asthma (2% prevalence), and urticaria (1% prevalence). Ladybug skin test sensitization is more common in rural areas and is comparable in frequency and age distribution with cat and cockroach. Cockroach and ladybug have a high degree of skin test concordance. A quality commercial ladybug allergen extract and increased ladybug allergen research are needed.*

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Allergic hypersensitivity to the imported *Harmonia axyridis* ladybug (multicolored Asian ladybeetle) has been reported in case studies.[1,2] Ray and Pence[2] published a recent review of *H. axyridis* allergy and discussed infestation in the United States after multiple agricultural introductions of the beetle for biological pest control during the 20th century.

West Virginia is among the states enduring seasonal marauding swarms of ladybugs for several weeks each fall. Ladybugs preparing for winter hibernation often invade human habitat. Home infestation varies from year to year and by geographical location. Homes painted lighter colors and exterior walls with sun exposure are among factors attracting ladybugs to certain homes. The beetles can squeeze through the smallest cracks and best seals into homes. Ladybugs typically die by the hundreds or thousands during severe home infestations, requiring frequent vacuuming. Ladybugs reaching walls and attics will hibernate until springtime.

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In a single-allergist practice in northern West Virginia, patient reports of rhinoconjunctivitis, asthma, and/or urticaria associated with ladybug exposures were documented from 1998 to 2001. Consequently, the patient environmental-allergy history was expanded to include ladybug allergy. Routine skin testing for ladybug sensitization was initiated at the beginning of 2001. Subsequent experience suggested that ladybug sensitization was common and correlated with clinical ladybug allergy to a degree similar to that seen with cat or other environmental allergens. Ladybug sensitization sometimes presented as an isolated single-positive skin test among the commonly tested 58 allergens. Ladybug sensitization often was seen in association with cockroach sensitivity. Skin test data from 2001 through 2004 were collected and analyzed to confirm these observations and to better describe clinical ladybug sensitization in northern West Virginia. A separate chart review of 400 patients was performed to correlate patient exposures and ladybug skin test sensitization with ladybug clinical allergic rhinitis, asthma, and/or urticaria.

METHODS

Retrospective Skin Test Chart Review

In a retrospective single-practice chart review, skin test results were collected for all allergy patients seen for 4 calendar years (2001 through 2004). All patients

had been seen by a single allergist who read and recorded all skin tests. Patient age in years, home postal zip code, and skin tests were anonymously archived into a database. Patients had been seen in one of three allergy offices located in Morgantown, Clarksburg, or Weston, WV. These communities lie along a 65-mile length of interstate I-79 in northern West Virginia.

Aeroallergen skin tests were recorded as positive or negative in the database. Original skin-prick tests had been read 15 minutes after placement and recorded using a standard protocol[3] scale of 0 (no wheal or erythema), 1+ (erythema <20 mm), 2+ (a wheal <3 mm in diameter with surrounding erythema), 3+ (a wheal >3 mm in diameter with surrounding erythema), or 4+ (a wheal >3 mm in diameter with pseudopods and surrounding erythema). A positive aeroallergen skin test was recorded in the database if the skin test was either (1) a 3+ or 4+ test and the histamine control was 4+; or (2) a 2+, 3+, or 4+ test and the histamine control was either 2+ or 3+ in size. Of all histamine controls for 1902 patients, 3.2% were 2+, 42.5% were 3+, and 54.1% were 4+ in size.

Other than the ladybug extract, skin-prick test aeroallergen extracts were obtained from ALK-Abello (Round Rock, TX). Individual skin-prick tests were placed with the AccuSet Device Skin Test System (ALK-Abello) according to the manufacturer's directions. A standard panel of 60 aeroallergen skin tests and controls, including ladybug, was used for most adult patients and children ≥6 years of age. Individualized skin test panels were used when patient histories dictated (*e.g.*, with extensive food testing). For children <6 years old, individualized skin test panels of 15–24 allergens usually were placed using multiple-test devices according to the manufacturer's directions (used initially, Quintest, from Hollister-Stier, Spokane, WA, was later replaced by Multi-Test II, from ALK-Abello). Ladybug extract became a standard allergen on the pediatric panels approximately halfway through the 4-year period. All skin tests included diluent negative control and prick 1.0 mg/mL of histamine control (ALK-Abello). The patient's upper back was used routinely for testing. During the 4-year period, four test allergens changed because of availability of extracts. For these four newest allergens (privet, corn pollen, Curvularia, and mouse epithelium) a range of 426–477 skin tests were entered in the database, compared with the 1355–1836 skin tests entered for the other 54 allergens.

Ladybug Extract

Live multicolor Asian ladybeetles were collected individually by hand from several affected homes in northern West Virginia. Ladybug bites are reported by less than ~10% of patients and occurred rarely during collection. After collection, live ladybugs were frozen until extract preparation. Frozen ladybugs were ground in a coffee-bean grinder before being weighed and placed in a glass 1-qt French Press (Bodum AG, Lelystad, Netherlands). Saline-albumin-phenol diluent (10 times the ladybug weight; ALK-Abello) was added and the container contents were stirred. For 4–6 hours, the mix was periodically stirred at 4°C. The French Press strainer was then passed through the mixture taking care not to compress the solids. The strained aqueous layer was decanted and placed in 10-mL syringes before filtering through 0.45-μm syringe filters (Corning, Inc., Corning, NY). After a final filtration through sterile 0.2-μm Pall syringe filters (Gelman Laboratory, Ann Arbor, MI), the extract was diluted with an equal volume of 50% glycerin (ALK-Abello) to produce the final 1:20 extract of ladybug in 25% glycerin. Over the 4-year period, two extract preparations were used. During simultaneous skin testing of two ladybug-sensitive individuals, the two extractions produced similar wheal and flare responses. Protein per gram of ladybug extract was 55% for the second extraction, as determined by methods previously described.[3]

Descriptive Symptom Chart Review

In a 400-patient retrospective single-office chart review, charts were chosen randomly from an estimated 2000 active charts in the more urban Morgantown, WV office during early 2006. Patients first seen in 2001 or later were included. Fifty-nine percent of the patients were seen first in 2005–2006, resulting in a majority of patients being sampled from outside the pool of patients in the 2001–2004 skin test chart review mentioned previously. At entry all patients completed a history form including experience with allergy symptom triggers and environmental exposures. Anonymously archived data included year of entry; age in years; sex; home postal zip code; patient identification of allergy symptom triggers and home pests; skin tests to ladybug, cat, dog, cockroach, and dust mites; and allergy diagnoses. The entry history form asked for triggers of allergy symptoms and provided choices of "cat," "dog," or "other triggers" with a space for write-ins ("ladybug" was not a specified choice). When identifying pests, patients were given a choice of "ladybug," "cockroach," or "other" with a blank space for write-ins. The patient's final allergy diagnoses (assigned by the single allergist) were recorded, including any ladybug-induced allergic rhinitis, asthma, and/or urticaria.

Statistical Comparisons

Patients were sorted into eight age categories: <2 years, 2–10 years, 11–20 years, 21–30 years, 31–40

years, 41–50 years, 51–60 years, and >60 years. Dust mite was considered positive if either (21%) or both (79%) *Dermatophagoides farinae* and *D. pteronyssinus* were positive. Geographic analysis of the skin test database began with transformation of each zip code into one of three population categories: urban, mixed urban/rural, or rural. U.S. Census Bureau data in numerical and graphical formats from the United States 2000 census was used to categorize each zip code in the database (U.S. Census Bureau: Fact sheets for the 2000 census, factfinder.census.gov/servlet/SAFFFacts; last accessed June 2005). U.S. Census Bureau "urban" areas were geographically overlaid onto zip code areas. A zip code was classified as urban if two-thirds or more of the zip code area was urban in the 2000 census. A zip code was classified as rural if it contained no urban areas in the 2000 database. Zip codes were classified as "mixed" urban/rural if they fell between the criteria for urban and rural. Ninety-six percent of urban database entries included zip code areas with population densities >140 persons per square mile. Ninety-six percent of rural database entries included zip code areas with population densities <40 persons per square mile. Mixed urban/rural database entries included zip code areas with population densities between 40 and 140 persons per square mile 75% of the time.

Descriptive data and contingency table analyses between allergens used the statistical software JMP (SAS Institute, Inc., Cary, NC). Concordance (agreement) of positive and negative skin tests between two allergens and agreement between history and clinical findings were assessed with the κ-statistic.[4]

RESULTS

Frequency of Positive Skin Tests

The skin test database contained 1902 entries (all individuals skin tested in 2001 through 2004). Individual patient ages ranged from infancy to 92 years, with a mean and median age of 27 and 23 years, respectively. Of the entries, 39 included fewer than 10 allergen skin tests, 96 contained 10–17 tests, 248 contained 18–48 tests, and 1519 entries contained ≥49 skin test entries. Excluding food allergens, the database contained 1839 entries, of which 440 (24%) had no positive allergen skin tests, 229 (12%) had a single-positive skin test, 165 (9%) had two positive skin tests, and 1005 (55%) had three or more positive skin tests.

Positive skin test frequencies for tree pollens ranged from 4 (pine) to 23% (hickory) with intermediate frequencies for cedar, 6%; mulberry, 8%; poplar, 9%; privet, beech, and alder, 10%; cottonwood, 11%; sycamore and elm, 12%; ash and walnut, 13%; willow and birch, 14%; olive, 15%; oak, 17%; and maple, 21%. At least one tree was positive 56% of the time. Positive skin test frequencies for grass pollens ranged from 15 (Johnson) to 28% (orchard) with intermediate frequencies for corn pollen, 16%; Bermuda, 21%; Bahia, 23%; fescue and sweet vernal, 26%; timothy, 27%; and KY blue and ryegrass, 28%. At least one grass was positive 47% of the time. Positive skin test frequencies for weed pollens ranged from 4 (nettle) to 22% (short ragweed) with intermediate frequencies for firebush, 8%; pigweed and English plantain, 10%; mugwort, 11%; lambs quarters and yellow dock, 12%; cocklebur, 14%; and tall ragweed, 17%. At least one weed was positive 40% of the time. Positive skin test frequencies for molds ranged from 3 (*Rhizopus*) to 12% (*Alternaria*) with intermediate frequencies for *Helminthosporium*, *Fusarium*, and *Aureobasidium*, 4%; *Cladosporium* and *Epicoccum*, 5%; *Curvularia* and *Aspergillus fumigatus*, 6%; and *Candida albicans*, 7%. At least one mold was positive 30% of the time.

Positive skin test frequencies for animal danders ranged from 6 (mouse and dog) to 24% (cat) with intermediate frequencies for rabbit, 7%; and horse, 9%. Mixed feathers were positive in 3% of database entries. Positive skin test frequencies for insects included 21% ladybug, 27% cockroach, and 40% dust mites (34% *D. farinae*, and 37% *D. pteronyssinus*).

The number of database entries for the eight age categories were <2 years, 78; 2–10 years, 517; 11–20 years, 282; 21–30 years, 249; 31–40 years, 224; 41–50 years, 232; 51–60 years, 183; and >60 years, 137. The age-specific frequencies of positive skin tests were similar for ladybug and major aeroallergens (Fig. 1). Ladybug-sensitized individuals had an age distribution almost identical to the full population, ranging from infancy to 91 years, with a mean and median age of 28 and 23 years, respectively. The ages were similar by demographics: urban (1–75 years; mean, 31.0 years), mixed (1–71 years; mean, 22.7 years), and rural (1–91 years; mean, 27.4 years). Although most patients were tested with a specific allergen panel, the <2-year-old age group was tested more often with individually chosen allergens. In Fig. 1, only cockroach, dust mites, cat, and ladybug were skin tested frequently in this age group (26 ladybug and 72 times for the others). Compared with cockroach, dust mites, and cat, the higher ladybug positive skin test frequency in the <2-year-old group is likely caused by a testing selection bias.

Isolated Single-positive Skin Tests

Of the 229 isolated single-positive skin tests in the aeroallergen database, dust mite was single positive 72 times (10% of 728 positive dust mite tests in the database), cockroach was single positive 28 times (6% of 491 positive tests), ladybug was single positive 18 times (6% of 299 positive tests), cat was single positive 19 times (4% of 440 positive tests), maple was single

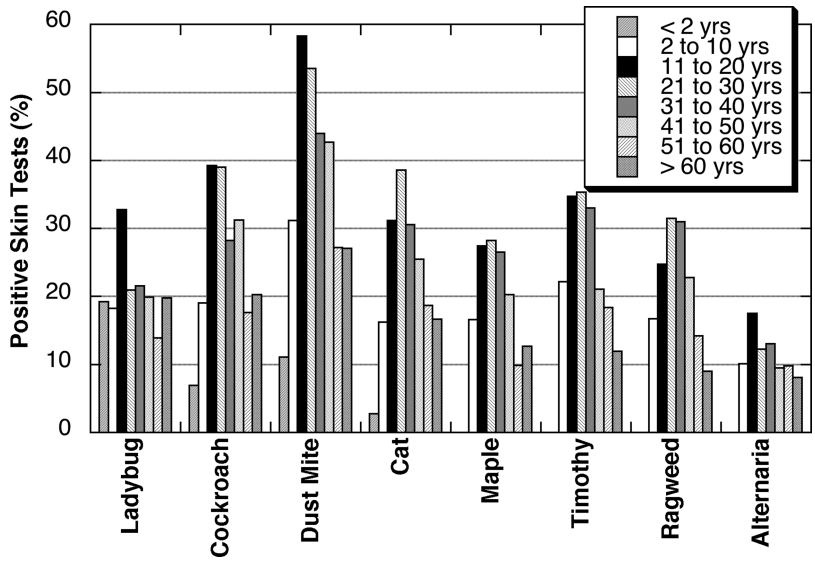

Figure 1. *Percent allergen sensitization by age group. Database entries: <2 years (1–72), 2–10 years (278–504), 11–20 years (232–276), 21–30 years (191–241), 31–40 years (181–216), 41–50 years (191–225), 51–60 years (144–171), and >60 years (106–133). Ages >6 years and 2–6 years: usually skin tested with the adult and pediatric standard panel respectively. Age <2 years: individual allergens chosen, limiting comparisons among allergens for this youngest age group.*

Table 1 **Allergen pair κ* and percent of isolated dual-positive skin tests#**

	κ			Dual-Positive Skin Tests		
	Cockroach	**Dust Mite**	**Cat**	**Cockroach**	**Dust Mite**	**Cat**
Ladybug	0.36	0.20	0.09	7%	2%	1%
Cockroach		0.29	0.14		15%	2%
Dust mite			0.25			8%

**κ-statistic for the allergen-pair concordance (agreement) in the full skin test database (if identical, κ = 1.0).*
#Statistically random expected value is <1%.

positive 8 times (3% of 316 positive tests), *Candida* was single positive 6 times (7% of 92 positive tests), and other allergens were single positive ≤5 times. Of these isolated single-positive skin test entries, 70% contained ≥48 allergen skin tests, and 30% of the entries had 8–47 skin tests. The ladybug single-positive individuals ranged from 1 to 85 years of age, with a mean of 27.7 years. Their homes were more rural (58%) than all ladybug-positive patients (45%) or all patients (35%). If statistically random, the expected frequency of ladybug (or any other allergen) among the 229 single-positive skin tests is approximately 5 times (2% of single positives). Only four single-positive allergens exhibited greater than twice the random frequency: dust mites, 31%; cockroach, 12%; and ladybug and cat, each 8% of the isolated single positives.

Concordance of Ladybug and Other Allergen-Positive Skin Tests

Concordance (agreement) between pairs of allergens in the database was addressed first using 2 × 2 contingency table analyses and the κ-statistic (Table 1). Ladybug skin tests were most concordant with cockroach (κ = 0.36) and dust mites (κ = 0.20). The κ's for ladybug with Bermuda (0.16), willow (0.16), timothy (0.12), and Johnson grass pollen (0.11) were intermediate, and the average κ for the other 51 allergens was 0.05. Cockroach skin tests were concordant with ladybug (κ = 0.36) and dust mite (κ = 0.29) skin tests, with smaller κ-values for other allergens. For comparison, strong agreement was seen as expected for two ragweed species (κ = 0.67). Within a class, willow was concordant with other trees (average κ = 0.29) and short ragweed was concordant with other weeds (average κ = 0.30). Ladybug skin test concordance with cockroach (κ = 0.36) is uniquely strong among ladybug-allergen pairs.

A second indication of concordance between two allergen skin tests may be their isolated dual-positive occurrence. There were 165 database entries with only two positive allergen skin tests. Of these, 24 included ladybug among the 51–57 skin tests per entry. Ladybug

most often paired with cockroach (11 times), dust mite (3 times), and *Alternaria* (2 times; Table 1). If statistically random, ladybug (or any other allergen) would be present approximately three times among the 165 pairs, while the expected frequency for a specific pair of allergens is <1%. In 59 entries, cockroach was dual-positive with dust mite (25 times), ladybug (11 times), and cat (4 times). In 67 entries, dust mite was dual-positive with cockroach (25 times), cat (13 times), and ladybug (3 times). In 31 entries, cat was dual-positive with dust mite (13 times) and cockroach (4 times). Other allergens were infrequently part of an isolated dual-positive pair: maple (11 times), *Alternaria* (10 times), oak (10 times), and others (average, 1.7 times). Among all dual-positive entries, 79% contained 48 or more allergen skin tests, and 21% contained between 8 and 47 skin tests. Dust mites, cockroach, ladybug, and cat were together present in 122 of 165 (74%) isolated dual-positive skin tests, which was >10 times the randomly expected frequency. Parallel to the contingency table analysis results, ladybug and cockroach ($\kappa = 0.36$) showed unusually frequent pairing (7% of all isolated dual-positive skin tests). Two other allergen pairs showed frequent pairing: dust mite–cockroach (15% of dual-positives; $\kappa = 0.29$) and dust mite–cat (8% of dual-positives; $\kappa = 0.25$; Table 1).

Geographic Variation of Ladybug Sensitization

Of all aeroallergens in the database, only ladybug (Fig. 2) showed a pattern of significant decreasing skin test frequency that was highest (30%) in rural, intermediate (21%) in mixed, and lowest (16%) in urban zip codes ($p < 0.0001$). The opposite pattern of significant ($p < 0.05$) increasing skin test frequencies from lowest in rural to highest in urban zip codes was seen for 17 allergens (alder, ash, birch, maple, olive, KY blue, fescue, orchard, ryegrass, sweet vernal, timothy, yellow dock, lambs quarters, mugwort, short ragweed, tall ragweed, and cat) and was highly significant ($p < 0.0001$) only for cat and ash pollen (pollen data not shown).

Symptom Review of Ladybug Allergy

As anticipated, the 400-chart descriptive symptom review that was limited to the Morgantown office in 2006 was more urban (58% versus 42%) than the skin test chart review from 2001 to 2004. Patient-identified allergy symptom triggers included cats (23%) and dogs (16%) with write-ins greater than 1% including ladybugs (1%), feathers (2%), rabbits (1%), and horses (1%). Cockroaches received no write-ins as a trigger. Patients identified ladybugs as pests in 42% of homes (Table 2) but identified cockroaches in only 2%, and write-ins >1% included ants (4%) and spiders (3%).

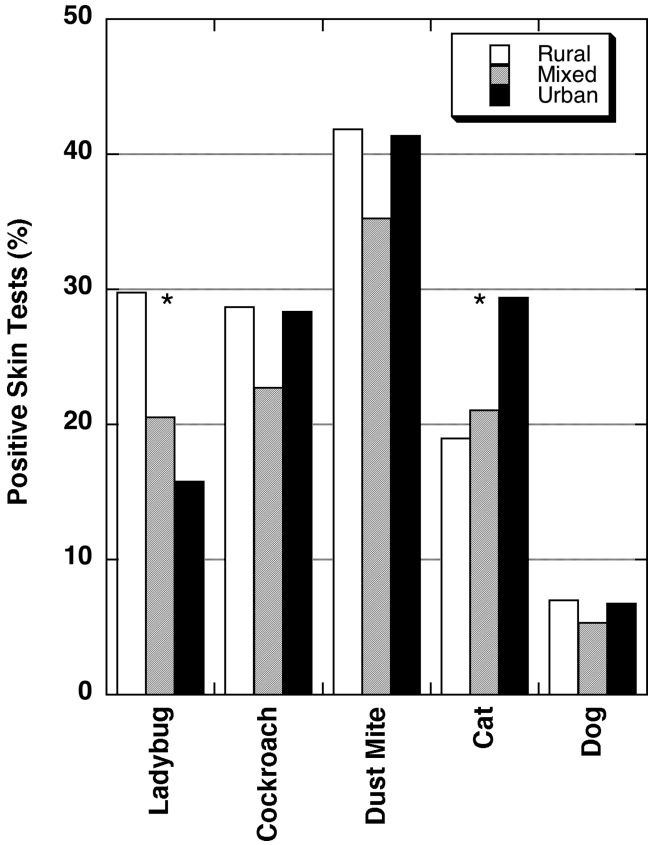

Figure 2. *Percent allergen sensitization by urbanization of patient's domicile zip code. Database entries: 413–417 rural, 429–546 mixed, 558–774 urban; *$p < 0.0001$*

Among the 400 charts, 305 had been skin tested to all five antigens: ladybug, cat, dog, cockroach, and dust mite. Positive skin tests for ladybug, cockroach, cat and dust mite were very comparable with the larger 2001–2004 skin test analysis (*e.g.*, ladybug, 20% versus 21% frequency) even though this mix of individuals was more urban and 59% were not contemporaneous with those in the larger analysis (Table 2). The κ-agreement for pairs of allergen skin tests mirrored the results of Table 1: ladybug–cockroach ($\kappa = 0.35$), ladybug–dust mite ($\kappa = 0.20$), ladybug–cat ($\kappa = 0.08$), cockroach–dust mite ($\kappa = 0.26$), cockroach–cat ($\kappa = 0.15$), and dust mite–cat ($\kappa = 0.38$).

Among the 400 charts reviewed, a final diagnosis of allergic rhinitis was made in 62%, asthma in 28%, and urticaria in 16%, with some individuals having multiple diagnoses. Other diagnoses with >1% prevalence included food allergy (10%), immune deficiency (4%), contact dermatitis (4%), hymenoptera hypersensitivity (4%), atopic dermatitis or rash (4%), drug reactions (2%), cholinergic rhinitis (1%), delayed pressure urticaria (1%), and environmental intolerance (1%). Ladybug allergic rhinitis was diagnosed in 8% of the 400 patients. A diagnosis of ladybug asthma was made in 2%, and a diagnosis of ladybug urticaria was made in 1% (Table 2).

Table 2 **Retrospective single-office chart review results by group**

Group	Total	Skin Tested	Ladybug Skin Test Positive	Ladybug Allergy
Patient History by Population Group				
Patients	400	305	60	31
Age range (yr)	1–90	1–90	1–76	1–76
Mean age (yr)	33.6	31.6	32.1	27.1
Female	57%	54%	53%	52%
Urban home	58%	56%	53%	45%
Mixed home	19%	21%	17%	19%
Rural home	23%	23%	30%	36%
Cat triggers symptoms	23%	28%	30%	39%
Dog triggers symptoms	16%	20%	20%	23%
Ladybug triggers symptoms	1%	2%	8%	16%
Ladybug pests reported	42%	44%	42%	48%
Cockroach pests reported	2%	2%	3%	6%
Skin-Test Results and Diagnoses				
Ladybug-positive skin test		20%	100%	100%
Cockroach-positive skin test		32%	67%	58%
Dust mite–positive skin test		38%	60%	52%
Cat-positive skin test		27%	35%	35%
Allergic rhinitis	62%	76%	93%	97%
Asthma	28%	33%	33%	39%
Urticaria	16%	11%	13%	10%
Ladybug allergic rhinitis	8%	10%	50%	97%
Ladybug asthma	2%	2%	10%	19%
Ladybug urticaria	1%	1%	5%	10%

In keeping with the findings of Fig. 2, among the three demographic groups the rates of positive ladybug skin tests were greatest in rural areas (19% urban, 16% mixed, and 25% rural), whereas the opposite trend was seen for cat skin tests (32% urban, 22% mixed, and 21% rural). Ladybugs were identified as pests more often in rural areas (33% urban, 47% mixed, and 60% rural). Among the 400 patients in urban, mixed, and rural areas, the frequency of diagnosis for all allergic rhinitis (63, 56, and 64%), asthma (26, 32, and 28%), and urticaria (15, 21, and 16%) were similar. But, the frequency of ladybug allergic rhinitis (6, 8, and 11%), ladybug asthma (1, 0, and 3%), and ladybug urticaria (<1, 0, and 1%) were greater in rural areas.

The 60 ladybug skin test–positive patients did not differ from all skin-tested patients in either year of entry, age, or sex (Table 2). Consistent with the κ-values mentioned previously and findings in Table 1, ladybug skin test–positive patients were more likely to be skin test positive also to cockroach (67% versus 32%) and dust mites (60% versus 38%). Ladybug skin test–positive patients identified ladybugs as home pests no more often than the whole population (42% versus 42%). The lack of association between ladybug skin tests and ladybug pest identification was confirmed further by a $\kappa = -0.02$ ($p = 0.73$). If the 166 patients who reported a ladybug pest problem were compared with all 400 patients, the rates of positive skin tests were comparable for ladybug (19% versus 20%), cat (24% versus 27%), cockroach (32% versus 32%), and dust mites (35% versus 38%). The positive predictive value (PPV) of having ladybugs as pests was less than chance (42%) for predicting positive skin tests to ladybugs. Similarly, the PPV of having ladybugs as pests was no better than chance for predicting ladybug allergic rhinitis (47%), ladybug asthma (50%), and ladybug urticaria (33%).

The totality of clinical evidence resulted in the diagnosis of ladybug allergic rhinitis in 30 (50%) of ladybug skin test-positive patients (Table 2). A diagnosis of ladybug asthma was made in 10% of those patients who were ladybug skin test positive, and a diagnosis of ladybug urticaria was made in 5%. Consistent with the findings of Fig. 2, the demographic distributions for the 31 ladybug-allergic patients and the 60 ladybug skin test–positive patients were more rural than that of the full review group of 400 (36, 30, and 23%, respectively).

Among the full group of 400 patients only 5 patients wrote in ladybug as a trigger, and all 5 were among the skin test–positive patients diagnosed as ladybug allergic. For prediction of a positive skin test, the PPV of patients' identification of ladybugs as a trigger was 100%. Cats were an identified allergy trigger by 22.5% of patients and dogs were an identified allergy trigger by 15.5%. However, for prediction of a positive skin test, the PPV of patients' identification of cats or dogs as a trigger was about the same as chance (54 and 53%).

DISCUSSION

Two retrospective reviews provide a descriptive overview of ladybug hypersensitivity in this West Virginia allergy practice. The smaller chart review sampled patient history and diagnosis of ladybug allergy, and the larger 4-year inclusive skin test study contrasted the results of ladybug skin testing to 57 commonly skin-tested allergens. Ladybug-allergic patients in northern West Virginia most often present with fall and spring allergic rhinoconjunctivitis and less often with asthma or urticaria. Clinical experience in this allergy practice previously suggested that the population burden of allergic disease attributable to ladybugs can be as great as that seen with cockroaches or cats. This review confirms that ~20% of skin-tested patients in endemic areas are ladybug hypersensitive and at least one-half (10%) are ladybug allergic.

Based on the 4-year skin test data in northern West Virginia, ladybug sensitization is prevalent (21%) among allergy patients and similar to cat (24%) and cockroach (27%) sensitization (Fig. 1). These skin test frequencies are very similar to recently reported National Health and Nutrition Examination Survey III (NHANES III) data for 9 aeroallergens and over 10,000 skin tests in the general U.S. population aged 6–59 years.[5] Except for a greater dust mite sensitivity in the allergy clinic population, the rates of positive skin tests for the nine aeroallergens are very similar in the two data sets (NHANES III versus this database review): dust mite (28% versus 40%), cockroach (26% versus 27%), cat (17% versus 24%), rye grass (27% versus. 28%), short ragweed (26% versus 22%), Bermuda grass (18% versus 21%), oak (13% versus 17%), *Alternaria* (13% versus 12%), and Russian thistle (15% in NHANES III) versus Lambs quarters (12% in this database). The NHANES III age distribution results were reported for six aeroallergens, and the age of peak sensitization was similar in the two data sets (NHANES III versus this database review): *Alternaria* (10–20 years versus 10–20 years), Bermuda grass (20–30 years versus 10–40 years), cat (20–30 years versus 20–30 years), oak (20–30 years versus 20–30 years), ragweed (20–30 years versus 20–30 years), and rye grass (20–40 years versus 20–30 years).

Isolated single-positive skin tests occurred in 12% of tested patients, making this a common event. Four allergens—dust mite, cockroach, ladybug, and cat—accounted for 60% of such events among the usually tested 58 allergens. The single-positive skin test represented 10% of all positive skin tests for dust mites, 6% of cockroach and ladybug–positive skin tests, and 4% of all positive cat skin tests. The finding of 50% ladybug allergy among ladybug skin test–positive patients in the smaller chart review rules out the possibility that single-positive skin tests are false positive tests, as has been reported for mesquite tree pollen.[6] Frequent isolated single-positive skin tests for ladybug (dust mites, cockroach, and cat) raise potential research questions of unique and particularly strongly allergenic epitopes, genetic host susceptibilities, and vagaries of human ecology.

The clinical correlation of ladybug with cockroach sensitization was confirmed in both retrospective reviews. The strong agreement of ladybug and cockroach skin test results was both statistically apparent (κ = 0.36 and 0.35) and further supported by the high percentage of ladybug–cockroach pairings in Table 2 and by isolated dual-positive skin tests in Table 1. The four allergens—dust mites, cockroach, ladybug, and cat—were prominent allergens in the skin test database. Each (1) had a total frequency of positive skin tests of >20%, (2) were the most frequent isolated single-positive skin tests 4–10% of the time, (3) were a constituent in 74% of the isolated dual-positive skin tests, and (4) exhibited highly coincident pairings for ladybug–cockroach, dust mite–cockroach, and dust mite–cat (Fig. 1; Tables 1 and 2). Cross-reactivity among other insects has been researched extensively and might similarly account for the ladybug–cockroach association, but confirmation awaits additional research.

Ladybug allergy and skin test sensitization are present in all age groups (Fig. 1; Table 2). Unlike any other aeroallergen, ladybug sensitization was greatest in rural home areas (Fig. 2). Until additional research is available, perhaps the best hypothesis for this unique geographic prevalence for ladybug sensitization in rural areas is that ladybug exposures are greater in more rural areas, perhaps because of the insect preference for rural environments. This is consistent with patient reports of extreme infestations of cabins and homes in deep-wooded areas and the insect's known behavior in its native territories.[2]

In contrast to ladybug allergy, cat allergy was more common in urban areas in both reviews. In contrast to a strong PPV for identification of ladybugs as an allergy trigger, identification of cats or dogs as an allergy trigger was no better than chance at identifying those who were skin test positive to either animal. This misidentification of animals as allergy triggers may result from forceful publicizing of animal allergy and ani-

mals' high visibility. Cockroaches were never identified as an allergy trigger and rarely were identified as a pest.

The prevalence of ladybug home infestation was 42% overall and greatest in rural homes. Ladybug-positive skin tests and ladybug allergies also were more common in rural areas. However, identification of ladybug pests in a home was no better than chance in predicting a positive skin test to ladybug or clinical allergy to ladybugs. Apparently, allergic sensitization to ladybugs is a result of more exposures than just those at home. Unlike cockroaches that were acknowledged as a pest only 2% of the time, ladybugs are highly visible when not in hibernation. Despite their visibility, from 2001 to 2006 ladybugs were seldom recognized as triggers of allergy symptoms. Only after discussion of potential allergen triggers with the physician did most patients entertain the possibility. Once considered, patients with ladybug allergy often linked their symptoms with ladybug exposures at home, school, daycare, visits with relatives, or in other settings. Just as with cat, dust mites, cockroach, and other allergens, skin testing to ladybug then provided significant posttest confidence in diagnosis and recommendations for allergen avoidance and treatment. As the notoriety of ladybugs as an allergen trigger grows in public awareness, the identification of ladybugs as a trigger may well rise to that level of misidentification currently seen for cats and dogs.

In this allergy practice between 2001 and 2004, allergen vaccine therapy was prepared for 481 patients, of whom 88 (18%) received allergen vaccine therapy including ladybug plus any other clinically relevant allergens. All 88 patients strongly correlated their allergy symptoms with ladybug exposures. Although outcomes for ladybug immunotherapy are anecdotal, the clinical improvement seen with ladybug immunotherapy was as great as or greater than that with cats (which is usually highly successful). No adverse reactions occurred. No individuals reaching and maintaining maintenance immunotherapy reported failure. All were able to tolerate greater ladybug exposures with fewer allergen symptoms. At the extreme, several individuals unable to live in their home during ladybug infestation were able to return to live in their homes.

These retrospective reviews extend the clinical experience with ladybug allergy from the previously reported individual cases and spotlight ladybugs as a significant allergen in allergy practices within endemic areas. Allergen sensitization and clinical importance is comparable with cat and cockroach in West Virginia. Ladybug allergy presents most often as allergic rhinoconjunctivitis (8% prevalence), less often as asthma (2%), and least often as urticaria (1%). Ladybug sensitization and allergy occurs at any age and are greater in patients living in rural versus urban environments. Ladybug home infestation was more common in rural areas but did not predict ladybug sensitization or allergy. Isolated single-positive skin tests are fairly common not only for ladybug, but also for dust mites, cockroach, and cat. Cockroach and ladybug have a high degree of skin test concordance. Availability of a quality commercial ladybug allergen extract is paramount for future patient care. Ladybug allergen investigations and controlled prospective studies of ladybug allergy are needed.

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