Harmonia axyridis ladybug hypersensitivity in clinical allergy practice.

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ABSTRACT

Background: The imported *Harmonia axyridis* ladybug infests homes in northern West Virginia from fall through spring, causing allergic disease.

Methods: Retrospective single-practice chart reviews: (1) all prick skin 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 employed contingency table analyses and the kappa statistic for concordance.

Results: Ladybug sensitization and allergy occurred at all ages and more often in rural areas. Home infestation with ladybugs is most common in rural areas but does not predict ladybug sensitization (kappa = -0.02). Ladybug sensitization occurred with 21% frequency compared to cat 24%, cockroach 27%, and dust mite 40%. Only ladybug demonstrated 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 mite, 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).

Conclusion: Ladybug is a major allergen in endemic areas, causing rhinoconjunctivitis (8% prevalence), asthma (2%), and urticaria (1%). Ladybug skin-test sensitization is more common in rural areas and is comparable in frequency and age distribution to 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.

INTRODUCTION

Allergen 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 following 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.

In a single-allergist practice in northern West Virginia, patient reports of rhinoconjuctivitis, 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 was often seen in association with cockroach sensitivity. Skin-test data from 2001 through 2004 was 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 four calendar years 2001 through 2004. All patients had been seen by a single allergist (DWG), 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, West Virginia. 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 prick skin tests had been read 15 minutes after placement and recorded using a standard protocol[3] scale of 0 (no wheal or erythema), 1+ (erythema <20mm), 2+ (a wheal <3mm diameter with surrounding erythema), 3+ (a wheal >3mm diameter with surrounding erythema), or 4+ (a wheal >3mm 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, prick skin-test aeroallergen extracts were obtained from ALK-Abello, Round Rock, Texas. Individual prick skin 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 employed for most adult patients and children 6 years of age and older. Individualized skin-test panels were used when patient histories dictated (e.g., with extensive food testing). For children less than 6 years old, individualized skin-test panels of 15 to 24 allergens usually were placed using multiple-test devices according to manufacturer's directions (used initially, Quintest® from Hollister-Stier, Spokane, Washington was later replaced by Multi-Test II® from ALK-Abello). Ladybug extract became a standard allergen on the pediatric panels approximately half way through the 4-year period. All skin tests included diluent negative control and prick 1.0 mg/ml histamine control (ALK-Abello). The patient's upper back was used routinely for testing. During the 4-year period, 4 test allergens changed due to availability of extracts. For these four newest allergens (privet, corn pollen, Curvularia, mouse epithelium) a range of 426 to 477 skin tests were entered in the database, compared to the 1355 to 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 about 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-quart French Press (Bodum® AG, Lelystad, Netherlands). Saline-albumin-phenol diluent (ALK) (ten times the ladybug weight) was added and the container contents were stirred. For 4 to 6 hours, the mix was periodically

stirred at 4 degrees 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 10ml syringes before filtering through 0.45-micron syringe filters (Corning Inc, Corning, NY). After a final filtration through sterile 0.2 micron Pall syringe filters (Gelman Laboratory, Ann Arbor, Michigan), the extract was diluted with an equal volume of 50% glycerin (ALK) 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 above. 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 (DWG)) were recorded, including any ladybug-induced allergic rhinitis, asthma, and/or urticaria.

Statistical Comparisons

Patients were sorted into 8 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%) *D. farinae*, and *D. pteronyssinus* mite 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. US Census Bureau data in numerical and graphical formats from the United States 2000 census was used to categorize each zip code in the database.[5] 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 greater than 140 persons per square-mile. Ninety-six percent of rural database entries included zip code areas with population densities less than 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 employed the statistical software JMP (SAS Institute Inc., Cary, North Carolina). Concordance (agreement) of positive and negative skin tests between two allergens and agreement between history and clinical findings were assessed with the kappa 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 to 17 tests, 248 contained 18 to 48 tests, and 1519 entries contained 49 or more 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, 165 (9%) had two positives, 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%, Kentucky 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 was 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 mite).

The number of database entries for the 8 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 (Figure 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 demographic: urban (1 to 75 years, mean 31.0 years), mixed (1 to 71 years, mean 22.7 years), and rural (1 to 91 years, mean 27.4 years). While most patients were tested with a specific allergen panel, the <2-year-old age group was more often tested with individually chosen allergens. In Figure 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 to cockroach, dust mites, and cat, the higher ladybug positive skin-test frequency in the <2 year-old group is likely due to 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 28 times (6% of 491 positive tests), ladybug 18 times (6% of 299 positive tests), cat 19 times (4% of 440 positive tests), maple 8 times (3% of 316 positive tests), Candida 6 times (7% of 92 positive tests), and other allergens 5 or fewer times. Of these isolated single-positive skin-test entries, 70% contained 48 or more allergen skin tests, while 30% of the entries had 8 to 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: 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 2X2 contingency table analyses and the kappa statistic (Table 1). Ladybug skin tests were most concordant with cockroach (kappa = 0.36) and dust mite (kappa = 0.20). Kappa's for ladybug with Bermuda (0.16), willow (0.16), timothy (0.12), and Johnson grass pollen (0.11) were intermediate, while the average kappa for the other 51 allergens was 0.05. Cockroach skin tests were concordant with ladybug (kappa = 0.36) and dust mite (kappa = 0.29), with smaller kappa values for other allergens. For comparison, strong

agreement is expected for two ragweed species (kappa = 0.67). Within a class, willow was concordant with other trees (average kappa = 0.29) and short ragweed was concordant with other weeds (average kappa = 0.30). Ladybug skin-test concordance with cockroach (kappa = 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 to 57 skin tests per entry. Ladybug most often paired with cockroach (11), dust mite (3), and Alternaria (2) (Table 1). If statistically random, ladybug (or any other allergen) would be present approximately 3 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), ladybug (11), and cat (4 times). In 67 entries, dust mite was dual-positive with cockroach (25), cat (13), and ladybug (3 times). In 31 entries, cat was dual-positive with dust mite (13) and cockroach (4 times). Other allergens were infrequently part of an isolated dual-positive pair: maple (11), Alternaria (10), oak (10) and others (average 1.7). Among all dual-positive entries, 79% contained 48 or more allergen skin tests, while 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 more than 10-times the randomly expected frequency. Parallel to the contingency table analysis results, ladybug and cockroach (kappa = 0.36) demonstrated unusually frequent pairing (7% of all isolated dual-positive skin tests). Two other allergen pairs demonstrated 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 (Figure 2) demonstrated a pattern of significant decreasing skin-test frequency 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 seventeen allergens (alder, ash, birch, maple, olive, Kentucky blue, fescue, orchard, ryegrass, sweet vernal, timothy, yellow dock, lambs quarters, mugwart, 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 was more urban than the 2001-2004 skin-test analysis (58% vs. 42%), mixed (19% vs. 23%), rural (23% vs. 34%). Patient-identified allergy symptom triggers included cat (23%) and dog (16%) with write-ins greater than 1% including ladybug (1%), feathers (2%), rabbit (1%), and horse (1%). Cockroach received no write-ins as a trigger. Patients identified ladybugs as pests in 42% of homes (Table 2), but cockroach in only 2%, and write-ins greater than 1% included ants (4%) and spiders (3%).

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 to the larger 2001-2004 skin-test analysis (e.g. ladybug 20% vs. 21%) even though this mix of individuals was more urban and 59% were not contemporaneous with those in the larger analysis (Table 2). Kappa 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 (28%), and urticaria (16%) with some individuals having multiple diagnoses. Other diagnoses greater than 1% 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 in 1% (Table 2).

In keeping with the findings of Figure 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), while the opposite trend was seen for cat (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%, 64%), asthma (26%, 32%, 28%), and urticaria (15%, 21%, 16%) were similar. But, the frequency of ladybug allergic rhinitis (6%, 8%, 11%), ladybug asthma (1%, 0%, 3%), and ladybug urticaria (<1%, 0%, 1%).were greater in rural areas.

The 60 ladybug skin-test positive patients did not differ from the full group of 400 patients in either year of entry, age, or sex (Table 2). Consistent with the kappa values above and findings in Table 1, ladybug skin-test positive patients were more likely to also be skin test positive to cockroach (67% vs. 32%) and dust mite (60% vs. 38%). Ladybug skin-test positive patients identified ladybugs as home pests no more often than the whole population (42% vs. 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 to all 400 patients, the rates of positive skin tests were comparable for ladybug (19% vs. 20%), cat (24% vs. 27%), cockroach (32% vs. 32%), and dust mite (35% vs. 38%). The positive predictive value (PPV) of having ladybugs as pests was less than chance (42%) for predicting positive skin tests to ladybug. 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 patients (50%) who were slightly younger in age (Table 2). A diagnosis of ladybug asthma was made in 10% of those ladybug skin-test positive, and a diagnosis of ladybug urticaria

in 5%. Consistent with the findings of Figure 2, the demographic distributions for the 31 ladybug allergic 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 five patients wrote-in ladybug as a trigger, and all five were among the skin-test positive patients diagnosed as ladybug allergic. For prediction of a positive skin test, the PPV of patients' identification of ladybug as a trigger was 100%. Cat was an identified allergy trigger by 22.5% of patients and dog by 15.5%. However, for prediction of a positive skin test, the PPV of patients' identification of cat or dog 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, while 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 ladybug can be as great as that seen with cockroach or cat. This review confirms that approximately 20% of skin-tested patients in endemic areas are ladybug hypersensitive and at least 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%) (Figure 1). These skin-test frequencies are very similar to recently reported NHANES III data for 9 aeroallergens and over 10,000 skin tests in the general U.S. population ages 6 to 59 years.[6] Except for a greater dust mite sensitivity in the allergy clinic population, the rates of positive skin tests for the 9 aeroallergens are very similar in the two data sets (NHANES III vs. WV allergy): dust mite (28% vs. 40%), cockroach (26% vs. 27%), cat (17% vs. 24%), rye grass (27% vs. 28%), short ragweed (26% vs. 22%), Bermuda grass (18% vs. 21%), oak (13% vs. 17%), Alternaria (13% vs. 12%), and Russian thistle (15% in NHANES III) versus Lambs quarters (12% in this database). The NHANES III age distribution results were reported for 6 aeroallergens, and the age of peak sensitization was

similar in the two data sets (NHANES III vs. WV allergy): Alternaria (10-20 vs. 10-20 years), Bermuda grass (20-30 vs. 10-40 years), cat (20-30 vs. 20-30 years), oak (20-30 va. 20-30 years), ragweed (20-30 vs. 20-30 years), and rye grass (20-40 vs. 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 mite, 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.[7] 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 (kappa = .36 and .35) and further supported by the high percentage of ladybug-cockroach pairings in Table 2 and in 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 greater than 20%, (2) were the most frequent isolated single-positive skin tests, 4% to 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 (Figure 1, Tables 1 and 2). Cross-reactivity among other insects has been extensively researched and might similarly account for the ladybug-cockroach association, but confirmation awaits further research.

Ladybug allergy and skin-test sensitization is present in all age groups (Figure 1, Table 2). Unlike any other aeroallergen, ladybug sensitization was greatest in rural home areas (Figure 2). Until further 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 due to 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, cat allergy was more common in urban areas in both reviews. In contrast to a strong PPV for identification of ladybug as an allergy trigger, identification of cat or dog 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 animals' high visibility. Cockroach was never identified as an allergy trigger and rarely was 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. But, identification of ladybug pests in a home was no better than chance in predicting a

positive skin test to ladybug or clinical allergy to ladybug. Apparently, allergic sensitization to ladybugs is a result of more exposures than just those at home. Unlike cockroach that was 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 mite, cockroach, and other allergens, skin testing to ladybug then provided significant post-test confidence in diagnosis and recommendations for allergen avoidance and treatment. As the notoriety of ladybug as an allergen trigger grows in public awareness, the identification of ladybug as a trigger may well rise to that level of misidentification currently seen for cat and dog.

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. While outcomes for ladybug immunotherapy are anecdotal, the clinical improvement seen with ladybug immunotherapy was as great or greater than that with cat (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 ladybug as a significant allergen in allergy practices within endemic areas. Allergen sensitization and clinical importance is comparable to 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.

REFERENCES

1. Yarbrough JA, Armstrong JL, Blumberg MZ, Phillips AE, McGahee E, Dolen WK. Allergic rhinoconjunctivitis caused by *Harmonia axyridis* (Asian lady beetle, Japanese lady beetle, or lady bug). J Allergy Clin Immunol 1999; 104:704-705.

2. Ray JN, Pence HL. Ladybug hypersensitivity: Report of a case and review of the literature. Allergy and Asthma Proc 2004; 25:133-136.

3. Reid MJ, Nish WA, Whisman BA, Goetz DW, Hylander RD, Parker WA, Freeman TM. HLA-DR4-associated nonresponsiveness to mountain-cedar allergen. J Allergy Clin Immunol 1992; 89:593-598.

4. Agresti A. 1990. Categorical data analysis. John Wiley and Sons, Inc., New York.

5. U.S. Census Bureau: Fact Sheets for the 2000 Census. (Accessed June 2005 at http://factfinder.census.gov/servlet/SAFFFacts)

6. Arbes SJ, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: Results from the Third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005; 116:377-383.

7. Kelso JM. Interpretation of isolated positive skin-test results for mesquite tree pollen. J Allergy Clin Immunol 2004; 114:452-454.

Figure Legends

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.

Figure 2. Percent allergen sensitization by urbanization of patient's domicile zip code.

Database entries: rural (413-517), mixed (429-546), urban (558-774); *P < 0.0001