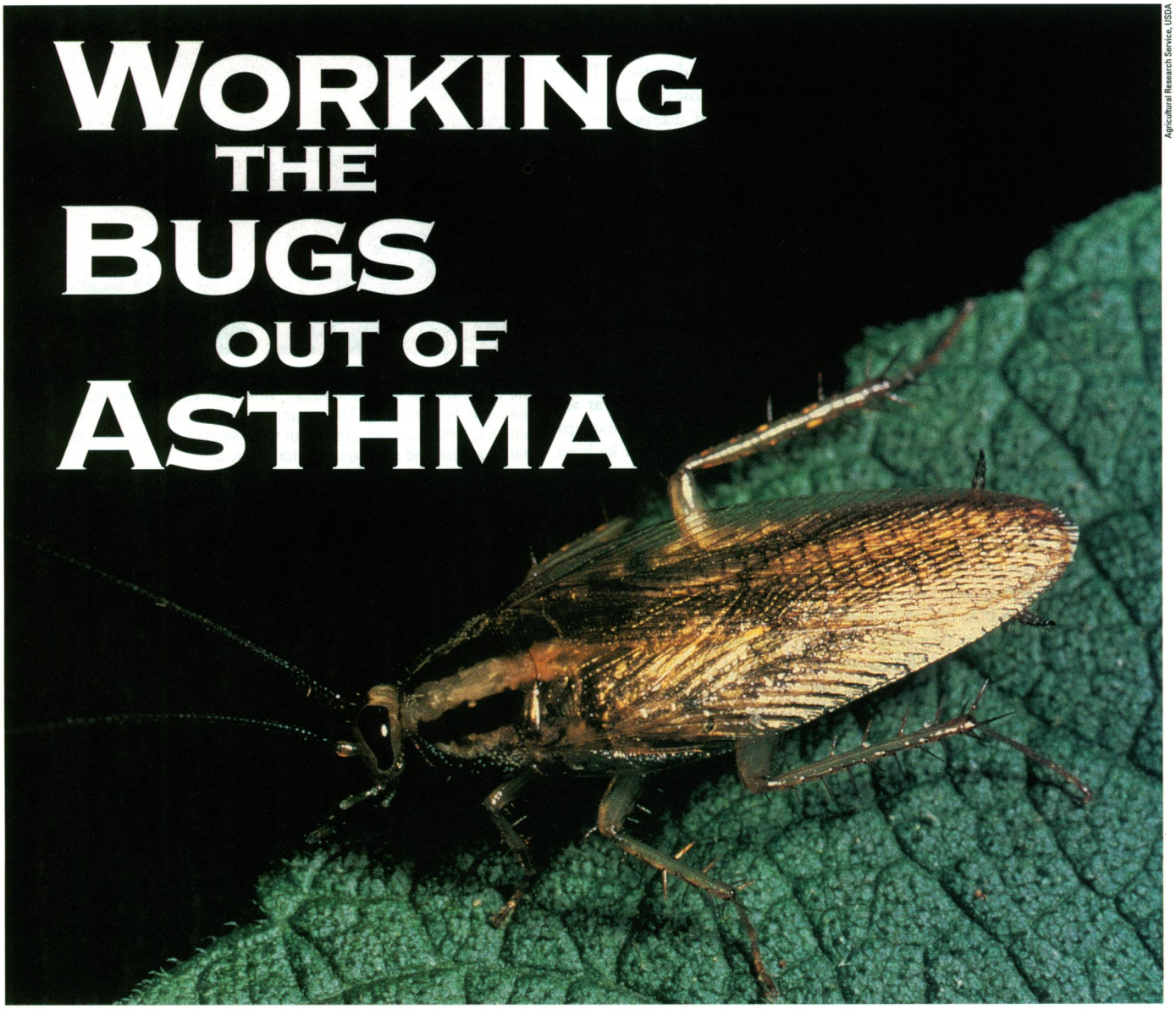
Working the Bugs out of Asthma



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Cockroach antigens are proteins found in the insects' feces, saliva, eggs, and shed cuticles that can trigger allergic reactions (and the corresponding formation of antibodies) when they become airborne and are inhaled by humans. Cockroach antigens produce allergic effects particularly in children, including respiratory symptoms and especially asthma. A recent study concluded that exposure to cockroach antigens may play an important role in asthma-related health problems among inner-city children. Skin tests to detect reactions to cockroach antigens produce positive results at rates second only to house dust mites.

Researchers at the United States Department of Agriculture's Agricultural Research Service (ARS), the Arkansas Children's Hospital Research Institute in Little Rock, Arkansas, and the FDA have teamed up to identify cockroach antigens associated with triggering asthma. Their goal is to reduce asthma symptoms by finding and removing cockroach antigens from dwellings. A key milestone of their work is the development of a home test kit that uses polyclonal antibodies to detect cockroach antigens, which can then be eliminated with common household cleaners.

Controlling cockroach-induced asthma is difficult. Immunotherapy with injections of cockroach extracts brings little relief. And killing cockroaches by fumigating homes with pesticides fails to significantly reduce allergic symptoms. During their search for better ways to prevent cockroach-induced asthma, the ARS researchers and their colleagues discovered that cockroach antigens persist in buildings for at least five years, even in the absence of cockroaches.

Tracking Cockroaches

Richard Brenner, a medical entomologist and research leader in the imported fire ants and household insects research unit of the ARS in Gainesville, Florida, set out to demonstrate the tenacity of cockroach antigens. In August 1990, Brenner and his colleagues infested a 1,040-square-foot building with 600 German cockroaches (*Blattella germanica*) captured at a Miami housing project. The facility was furnished with wall cabinets, countertops, a sink, refrigerator, electric stove, waste basket, and table. The activity of the cockroaches and their antigen distribution was monitored for five months while moving food and water sources.

Because insect pests redistribute themselves to optimize their survival in any environment, "all infestations are spatial

in nature," says Brenner. However, research procedures and statistics traditionally used by entomologists fail to address these spatial relationships fully. So Brenner adapted spatial analysis, a statistical method developed by mining engineers to precisely target the subsurface distribution of minerals, to study the cockroaches.

Unlike traditional statistical methods that assume random sampling and independent observations, spatial analysis recognizes "spatial continuity," which is the phenomenon that samples taken close to each other will appear similar. This parallels ecological systems—trees tend to grow together in groves, grass occurs in continuous patches, and German cockroaches aggregate.

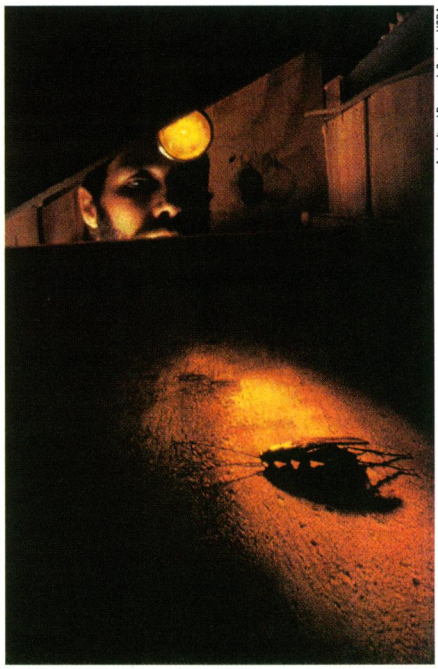
Spatial analysis is modeled by computer programs that generate a contour map showing areas of varying density, similar to topographic maps. Within a data set, sample locations are defined by X and Y spatial coordinates. Spatial analysis pairs each observation with each of the other observations, calculates the distance between the members of each pair, then determines how similar the values are in each pair. The information is then used to estimate values at unsampled locations within the study site.

In Brenner's German cockroach experiment, about 100 live traps, baited with bread soaked in beer, were placed overnight in a grid that covered the scope of ecological diversity in the test building. The next morning, trapped cockroaches were counted. Using these counts and spatial analysis, the researchers estimated the number of cockroaches at one-foot intervals, which added up to 1,040 locations within the facility.

Through spatial analysis Brenner learned that the cockroaches, which are very adaptable creatures, redistributed themselves rapidly after their food and water were relocated. This meant that allergens associated with them were probably broadly distributed as well. The next step was to devise a detection system that could determine the allergen distribution and how long the allergens persisted.

After five months of monitoring the cockroaches, Brenner's crew removed all the insects, closed the building without cleaning it, and set out to build a detection system. In December 1996, they returned with a prototype of the cockroach antigen test kit to determine whether these potential cockroach allergens persisted years later in the absence of cockroaches, and, if so, where they were distributed. Because German cockroaches only live where there are people, Brenner was certain no new ones had taken up residence in the closed building.

A 16-square-inch surface was swabbed at 110 locations, and the swabs were shipped to the laboratory of standards and testing at the FDA's Center for Biologics Evaluation and Research in Bethesda, Maryland, where they were analyzed with an enzyme-linked immunoadsorbent assay (ELISA) using polyclonal antibodies to detect cockroach antigens. When the results were returned to Brenner and plugged into the spatial analysis computer program, the distribution of cockroach antigens matched to a remarkable degree the cumulative distribution of cockroaches found five years earlier. The researchers concluded that the allergen load, expressed by a newly created index called "cockroach hour equivalents," measured as high as 4,100 units five years after all cockroaches were removed. "The allergen load was still enormous. You can see why even when cockroaches are removed, asthma does not get better," says Brenner.



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Creatures in the attic. Researchers can use filtered yellow light to observe the behavior of a common smokybrown cockroach without disturbing it.

Next, the ARS researchers cleaned the facility with a common household cleaner. They took test swabs of the surfaces after cleaning and sent the samples to the FDA for ELISA testing. Spatial analysis showed that cleaning removed 90% of the cockroach antigens and highlighted where the rest remained. The researchers cleaned again, swabbed again, and tested with ELISA. This second cleaning reduced the allergen load to zero cockroach hour equivalents, and confirmed that common cleaners adequately eliminate cockroach antigens.

Perfecting a Home Test Kit

Testing for potential cockroach allergens in homes requires a detection probe that is both highly specific and environmentally sensitive. A monoclonal antibody to a single cockroach antigen fails to detect many others, because 15–20 proteins from several types of cockroaches, including German, American, and Asian varieties, are suspected of causing human allergic reactions, says microbiologist Chris Anderson, chief of the FDA's laboratory of standards and testing. To be environmentally sensitive to cockroach allergens in homes, a probe must be polyclonal, or capable of detecting all major cockroach antigens.

Perfecting the laboratory-based ELISA devised by Anderson's team into a simple,

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A home fit for a roach. ARS researchers constructed a test house with about 100 sensors and probes to track the movements and location of cockroaches.

home-based test kit is the next challenge facing the cockroach researchers. The polyclonal ELISA includes two major cockroach antigens characterized and cloned by immunologist Ricki Helm, an associate professor of pediatrics at the Arkansas Children's Hospital Research Institute. Using physicochemical methods such as isoelectric focusing and gel electrophoresis, Helm identified a 36-kilodalton (kd) and a 90-kd antigen, both of which recognize IgE antibodies in serum from people with known cockroach allergies. The 90-kd allergen bound IgE in 77% (17 of 22) of patient sera tested. By definition, a major allergen produces an IgE response in more than 50% of allergic patients.

Other known cockroach antigens range in size from 6 to 100 kd. "Some people react to only one, others to 15 allergens," says Anderson. At this point, the researchers do not know the final combination of cockroach antigens that will be included in a home test kit, but the final mixture has to cover a practical, broad base, says Anderson.

In addition, the team needs to verify that cockroach antigens recovered from swabbed floors that test positive in the ELISA are allergens that trigger asthma. Work is underway to correlate the relationship between these antigens and asthma allergens. "This needs to be done before a test kit can be successful," says Anderson.

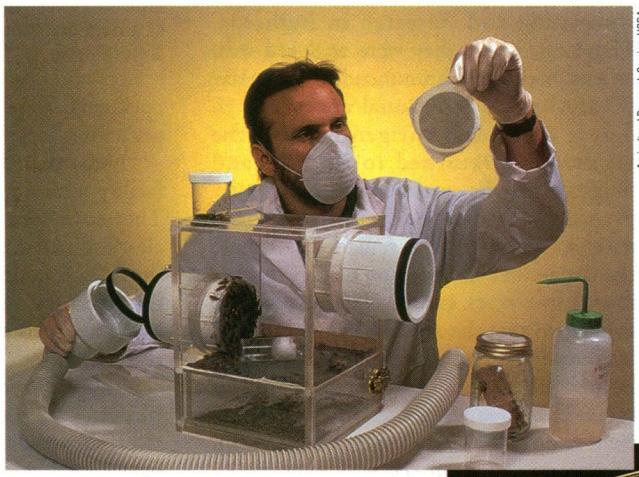
Preventing Cockroach-induced Asthma

A study published in the 8 May 1997 issue of the *New England Journal of Medicine* highlighted the need to detect cockroach allergens in homes. Dust samples from the bedrooms of 476 inner-city asthmatic children were analyzed for dust mite, cat, and cockroach allergens. Multivariate analysis revealed that cockroaches were the most common cause of the children's asthma. In the children's bedrooms, 50.2% had cockroach allergen levels that exceeded the disease-induction threshold, compared with 9.7% for dust mite allergen levels and 12.6% for cat allergen levels. The rate of hospitalization for asthma was 3.4 times higher among children whose skin tested positive to cockroach allergens and whose bedrooms had high cockroach-allergen levels. The same group also had 78% more visits to health care providers, experienced significantly more wheezing, and missed more school because of asthma.

Brenner proposes that a cockroach allergen home test kit can help relieve asthmatic symptoms by precisely targeting the allergens and directing cleaning efforts. "We can have a positive impact on human health by eliminating the pests and their attendant allergens," he says. By analogy, he compares cockroach allergen contamination to having a nuclear waste spill in a home. Both are extremely dangerous even in small quantities—a picogram of cockroach allergen can kill a sensitive asthmatic—and both require clean-up procedures that find and remove all of the contamination. For example, he says, in the case of allergens, removing 95% clearly is good. But the remaining 5% could conceivably be entirely concentrated in one small location—for example, the corner of a child's playroom. Consequently, the remaining 5% is potentially a concentration that could be life-threatening to a severely cockroach-sensitive asthmatic. Brenner says, "That is the essence of our spatial analysis. It tells you exactly where the problem is, not just the magnitude."

Brenner foresees the test kit including many probes to check many sites in a home before and after cleaning with household cleansers. The cockroach allergen test kit could also be adapted by the pest control industry to assess, target, clean, resample, and verify that all cockroach allergens are eliminated. The technology is also helping to develop and test new cockroach repellents that are environmentally friendly. The exact design of a commercial home test kit will be determined once partners from private industry are selected and patents are obtained. This novel approach of combining polyclonal antibodies for cockroach antigens with spatial analysis "allows us to take a holistic view of indoor environmental quality by examining surface contaminants," says Brenner, "and goes beyond simply testing indoor air quality."

Carol Potera



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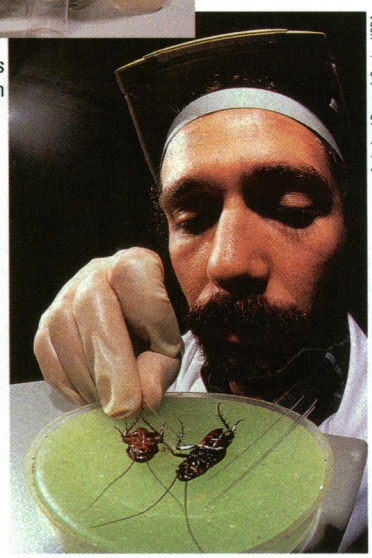
Observing the evidence. A filter full of allergens is obtained from an enclosed colony of German cockroaches.



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Isolating allergens. Cockroaches produce 8–13 antigens that can cause allergic reactions in people.

Suggested Reading

Brenner RJ. Preparing for the 21st century: research methods in developing management strategies for arthropods and allergens in the structural environment. In: Proceedings of the 1st International Conference on Insect Pests in the Urban Environment (Wildey KB, Robinson WH, eds.). Exeter:BPCC Wheatons Ltd.,1993;57–69.

Rosenstreich DL, Eggleston P, Kattan M, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 336:1356–1363 (1997).

Helm R, Cockrell G, Stanley JS, et al. Isolation and characterization of a clone encoding a major allergen (Bla g Bd90K) involved in IgE-mediated cockroach hypersensitivity. J Allergy Clin Immunol 98:172–180 (1996).