SPATIAL ENVIRONMENTAL ASSESSMENT AND MITIGATION OF GERMAN COCKROACHES (BLATTARIA: BLATTELLIDAE) AND ALLERGENS USING POLYCLONAL DETECTION ASSAYS AND PRECISION TARGETING SOFTWARE

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Abstract - Preliminary studies in experimental structures at Agricultural Research Service in Gainesville resulted in the development of a rabbit-anti-cockroach polyclonal ELISA inhibition system for assessing spatial distribution of cockroach antigens for subsequent mitigation interventions. The system was tested in homes of adolescent asthmatics selected because of a history of cockroach infestation. The goal was to determine the impact of pest mitigation and professional cleaning on antigen load. Data were collected via pentablet using a customized hardware and software system. German cockroach distributions were determined using sticky traps. Spatial analysis was used to determine distributions for each floor of two homes. About 50 environmental samples were taken from each floor of each home, and assays were referenced to standards. Cockroaches were removed and baits were applied. Antigen levels in the home without current infestation were below 150 cockroach-hr equivalents (c-h equiv); no recent asthmatic episodes were reported, and no intervention was conducted. In the infested home, antigen assays revealed values as high as 2200 c-h equiv; approximately 85% of the estimated antigen load was contained in less than 30% of the floor space. Post cleaning assays revealed areas where antigen load had decreased, and other areas where antigen load had increased. Levels in the carpet were reduced while those on non-floor hard surfaces were unchanged or higher, suggesting that improper rinsing of cleaning rags redistributed antigens. Variability in results among assay technicians and laboratories was negligible. We conclude that the antigen detection system allowed rigorous characterization of antigen loads, and that a standardized cleaning system could be developed and verified for efficacy.
Key words - Precision targeting, cockroach asthma, IPM, spatial analysis

INTRODUCTION

Recent studies on a national scope revealed that allergy to German cockroaches, *Blattella germanica* (L.) is the most important home-related allergy among inner city asthmatic children (Rosenstreich *et al.*, 1997). The potency of cockroach allergens is such that even low levels, traditionally measured with monoclonal assays to only 1 or 2 of the numerous allergens, can be life threatening (Platts-Mills *et al.*, 1997). Therefore, methods of assessing the presence of cockroaches and their attendant antigens in the environment are critical for the medical profession in evaluating asthma, and for the medical entomologist in developing and demonstrating reduced-risk management strategies that truly reduce the composite of risks associated with pests, their attendant allergens (or pathogens), and with the biocides used in interventions.

We contend that these goals are best achieved through a spatial characterization of the pests and allergens, and that a highly sensitive antigen environmental detection system is necessary. Monoclonal assays cannot provide that resolution. Preliminary studies in experimental structures at ARS in Gainesville resulted in the development of a rabbit-anti-cockroach polyclonal ELISA inhibition system for assessing spatial distribution of cockroach antigens (allergens) for subsequent mitigation interventions.

Herein, we describe how this spatially-based system was tested in actual homes of adolescent asthmatics in Baltimore, MD, that were selected because of a history of cockroach infestation. The goal was to assess pests and antigens spatially, reduce cockroach populations with least-toxic technologies, and

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

to determine whether we could detect changes in antigen load and distribution following a professional cleaning. We also tested a field-deployable hardware and software system for data and risk assessment under development by ARS for the Department of Defense (Pesticide Reduction Through Precision Targeting, PP-1053, funded under the Strategic Environmental Research and Development Program).

MATERIALS AND METHODS

Floor plans of the homes were prepared using an inexpensive home architect computer program (FloorPlan 3D, Ver 3, IMSI, San Rafael, CA 94901 USA). Data were collected using a hardware and customized software system via a Fujitsu Pentablet® that incorporates ArcView® Geographic Information System (ver. 3.1, Environmental Systems Research Institute, Redlands, CA, 92373 USA) and customized Visual Basic® programs. German cockroach distributions were determined using sticky traps baited and placed overnight. Spatial analysis (Brenner *et al.*, 1998a) was used to determine distributions for each floor of two homes. About 50 environmental samples were taken on each floor of each 2-story home.

Environmental samples. Each consisted of a Q-tip® cotton swab dipped in standard phosphate buffered saline (PBS) and repeatedly swabbed over a 10 x 10 cm area for approximately 10 seconds. Assays were referenced to internal standards (dilutions of German cockroach antigens). Swab samples were extracted in 3 ml of a 50% glycerol and phosphate buffered saline solution for 24 hours at 2 - 8ºC. The swabs were removed and the solution was filtered through a 0.45 micrometer syringe filter to remove any solids from the swab that may continue to be extracted by the remaining solution. The extractions were then stored at 2-8 ºC until assayed. A blind professional cleaning was conducted of carpets and hard surfaces.

Enzyme-linked Immunosorbent assay (ELISA) for cockroach antigens. The ELISA-inhibition system consisted of a rabbit-anti-cockroach IgG antibodies prepared at FDA by injecting rabbits with an extract prepared from German cockroach colony debris. Rabbits were bled at 6 weeks, and serum was maintained at -20 ºC in 0.7 ml aliquots. Briefly, assay procedures were as follows. Wells of microtiter plates were sensitized with reference cockroach antigen (colony extract). These were rinsed, and 50 µl of the extract from environmental sample (swab) was added into wells (2 reps in each of 3 plates). 50 µl of the primary antibody (polyclonal rabbit-anti-cockroach IgG serum 1:250 dilution in PBS) was added and was incubate overnight. Following a rinse, the secondary antibody (peroxidase conjugated goat anti-rabbit IgG) was added and incubated for 2 hr. The peroxidase substrate was added, the reaction stopped at 2 min, and the optical density was read using a Spectral Max 250 (Molecular Devices, Sunnyvale, CA) set at 450 nanometers. Optical density was converted to % inhibition using internal standards (dilutions) for a calibration curve. The % inhibition was converted to c-hr equiv based on extracts from reference timed samples consisting of 50 mixed stages of German cockroaches confined in petri dishes for various amounts of time.

Data analysis. Spatial distributions were estimated for cockroaches using trap counts, and for antigen load using c-hr equiv. Procedures used were generally those of Brenner *et al.* (1998a) except that inverse distance weighted was used for interpolation. Kriging algorithms are not yet supported in ArcView ver. 3.1. Pre- and post-intervention swab assays were compared using a Wilcoxon signed rank test on paired swabs (Sigma Stat®, ver. 2.0, Jandel Scientific Software, San Rafael, CA, 94912 USA) For the control home, these observations reflect 2 independent assessments of antigen load.

RESULTS AND DISCUSSION

German cockroaches were captured only in one home, and populations were minimal in the kitchen (evidence of previous baiting). However, 2nd floor infestations were detected in a nightstand and desk of bedrooms, and in closet door tracks (Fig. 1). Using hot air and vacuum (Brenner *et al.*, 1998b)

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

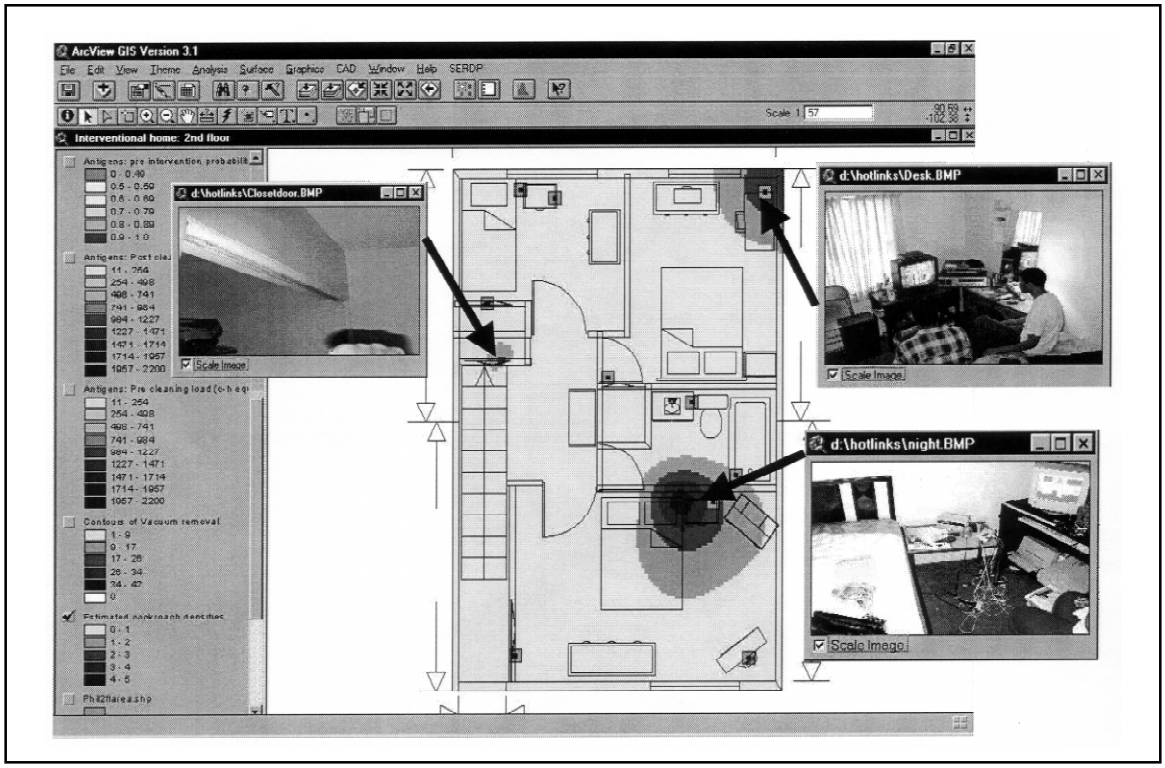


Figure 1. Output of ArcView showing second floor floorplan and precision targeting contours revealing areas of German cockroach infestation (max contour = 5). Inset photos are digital images showing where cockroaches were removed (nightstand, wooden desk, and upper track of folding closet door).

42 cockroaches were removed from the hollow legs of the nightstand, and 5 from the desk. In all, 29 and 67were removed from 1st and 2nd floors, respectively. Residual gel baits were applied to foci (Siege®).

Antigen values were as high as 2200 c-hr equiv in the home with current cockroach populations, but were less than 130 in the home without a current infestation. Residents in high antigen home reported emergency room visit due to asthma within previous few weeks; residents in other home reported no asthma-related problems during the previous several months. The remainder of this paper will discuss only the home that had cockroach infestations.

First floor antigen loads exhibited a spatial edge effect consistent with behavior of foraging and harboraging cockroaches (Fig. 2). High values in living room may have been associated with residents' common activities of eating while engaging in phone conversations or television watching. The highest 2nd floor value (Fig. 3) was on the carpeted floor (near infested closet); residents often accumulated dirty dishes at top of stairs before removing them to kitchen. Conversion of data to probability contours delineates areas that cumulatively account for ca. 85% of antigen load (all swabs >90 and 54 c-hr equiv for 1st and 2nd floors, respectively). During our visits, we noted that the asthmatic 3 yr old played by sliding down the stairs head first; this clearly illustrates the concept of risk associated with concentration and exposure. Televisions were used frequently in bedrooms of adolescent asthmatics, where food also was consumed routinely.

The cleaning process, conducted by a local Baltimore cleaning company, was performed blind. The workers received no special instruction, nor knowledge on cockroach allergy, nor the objectives of the pilot test. Hot water extraction (60°C) removed water soluble materials directly into a truck-mounted tank. Hard surfaces were wiped with wet cloths, periodically dipped and wrung from a bucket of rinse water. We noted that over ca. 80% of their time was spent in the kitchen and on carpeted flooring in the

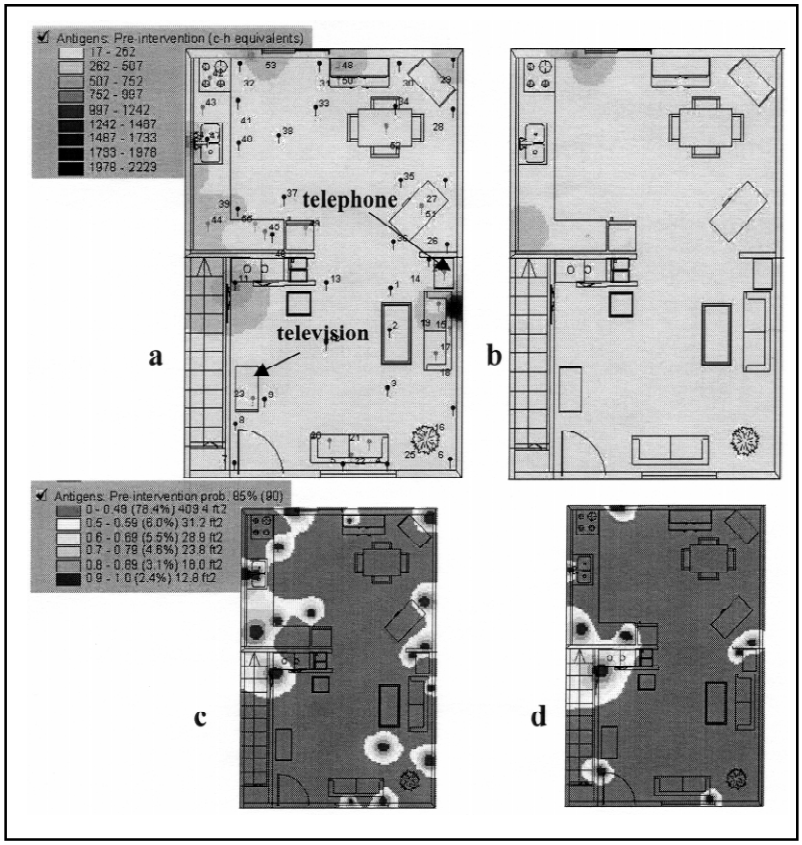


Figure 2. Contour maps showing pre-cleaning (panels a and c) and post-cleaning (panels b and d) distribution of antigens on the 1st floor of the home in Baltimore where German cockroach infestation was confirmed. Swab locations (numbered) are shown in panel a. The living room was carpeted, whereas the kitchen floor was vinyl. Panels a and b are absolute values in cockroach-hour equivalents (c-h equiv; maximum of 2200). Panels c and d are probability maps showing the probability that any given area exceeds 90 c-h equiv. Cumulatively, all areas with swab values >90 c-h equiv comprised 85% of the antigen load on the 1st floor. The post-cleaning probability map shows those areas that still exceed 90 c-h equiv. See text for description of the cleaning process.

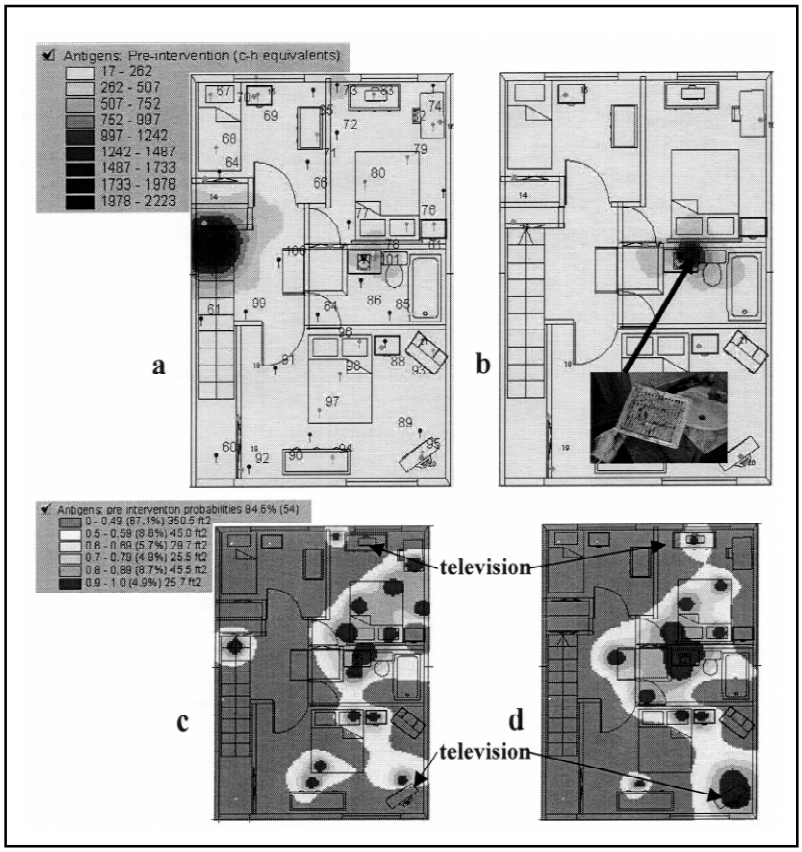
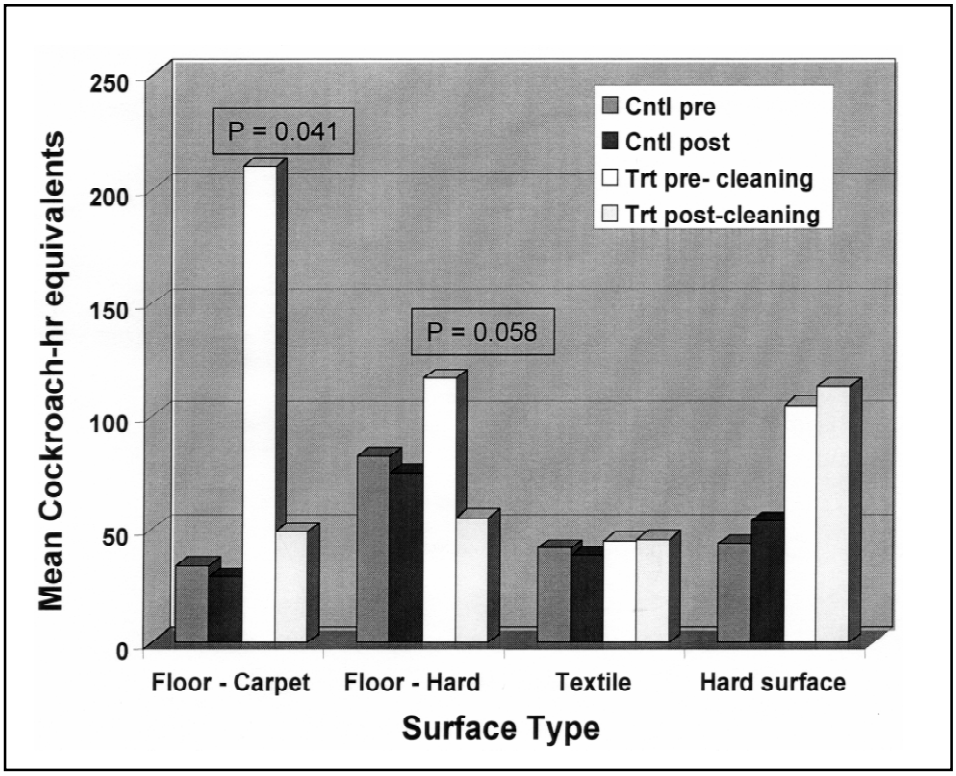


Figure 3. Contour maps showing pre-cleaning (panels a and c) and post-cleaning (panels b and d) distribution of antigens on the 2nd floor of the home in Baltimore where German cockroach infestation was confirmed. The bathroom flooring was vinyl, and the remainder of the upstairs was carpeted. Panels a and b are absolute values in cockroach-hour equivalents (c-h equiv; maximum of 2200). Panels c and d are probability maps showing the probability that any given area exceeds 54 c-h equiv. Cumulatively, all areas with swab values >54 c-h equiv comprised 85% of the antigen load on the 2nd floor. The post-cleaning probability map shows those areas that still exceed 90 c-h equiv. The hot spot missed by the cleaning was a bathroom vent (inset photo). See text for description of cleaning process.

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.



living room and stairway. Residents reported that limited time was spent upstairs, and that little-to-no effort was directed toward bedrooms and bathroom. One of the residents cleaned the top of the wooden desk (see Fig. 1); pre- and post-cleaning antigen levels were quite dramatic at this location (see Fig. 3). We also noted that televisions were not dusted, and appear to have acquired increased amounts of antigen, perhaps due to static charge that normally develops when a television is used.

Statistical analysis of pre- and post cleaning efficacy are presented in Figure 4 by surface type. Data for the control house were not statistically significant. These data indicate that the polyclonal cockroach antigen detection system was highly reproducible, based on analysis of the 2 independent environmental assessments conducted 2 days apart in this home that received no cleaning intervention. Even though the cleaning process was not standardized in any manner, antigen loads were reduced significantly in carpeted areas (see stairway on 2nd floor in Fig. 3), and moderately on hard floor surfaces elsewhere in home. In some areas, such as the tops of the televisions mentioned earlier, we detected higher post-cleaning antigen levels. In one such instance, the antigen level beneath the microwave on the kitchen countertop was higher because cockroach debris fell onto the countertop as the unit was returned to its normal location after cleaning. Obviously, the manner in which cleaning is conducted may have an impact on redistribution of remaining antigens.

From this study, we conclude that the impact of pest and antigen intervention (cleaning) was clearly documented using spatial analysis and precision targeting procedures. The antigen detection system is feasible to define areas of risk, and to target those areas that need intervention. However, by experimental design, the cleaning process for these water-soluble antigens was inadequately defined and implemented. We would recommend that precision targeting maps could be used in conjunction with a standardized cleaning process that incorporate quality control measures taken during the cleaning process. Subsequently, precision targeting of pests with reduced-risk pest management strategies, and targeting antigens (allergens) may allow sustained indoor environmental management.

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

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REFERENCES CITED

Brenner, R. J., D. A. Focks, R. T. Arbogast, D. K. Weaver, and D. Shuman. 1998a. Practical use of spatial analysis in precision targeting for integrated pest management. American Entomologist 44 (2): 79-101.

Brenner, R. J., S. A. Hedges and D. E. Milne. 1998b. Biological vacuum device to enhance environmental quality. U.S. Patent 5,806,238.

Platts-Mills, T. A. E., D. Vervloet, W. R. Thomas, R. C. Aalberse, and M. D. Chapman. 1997. Indoor allergens and asthma: Report of the 3rd International Workshop. J. Allergy and Clinical Immunology (supplement): 100 (6) part 1: s1-s24.

Rosenstreich, D. L., P. Eggleston, M. Kattan, D. Baker, R. G. Slavin, P. Gergen, H. Mitchell, K. McNiff-Mortimer, H. Lynn, D. Ownby, and F. Malveauxet. 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N. Eng. l. J. Med. 336:1356-1363 .