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# PP-1053: Pesticide Reduction Through Precision Targeting

## Technical Report Covering Progress through September 1998

Prepared By Dr. Richard J. Brenner [1]
USDA, Agricultural Research Service
Center For Medical, Agricultural & Veterinary Entomology
Gainesville, Florida

### *Introduction*

This project was funded in July 1996 with major objectives that included the development of standardized systems for preventing pollution from pesticides in DoD operations for virtually all pests found in DoD facilities, warehouses, and deployments. The operational engine of the project is the practical application of spatial statistical analysis in a precision targeting strategy to define spatial probabilities of exceeding critical thresholds (risk assessment), and a process of selecting available interventions so as to provide the greatest reduction in risks (comparative risk reduction). Presented as "contour maps" showing isolines of equal risk, the approach offers the advantage of generating 'before' and 'after' maps documenting efficacy of intervention, expressed as spatial areas (qualitative) and the degree that risks were reduced within these areas (quantitative).

This work plan would allow DoD to meet mandates of ***documenting*** pesticide and risk reductions, and also would provide a general framework applicable for characterizing and documenting virtually any other environmental concern of DoD. During the ensuing months, two major pronouncements by DoD further served to define the requirements that would broaden the applications of this technology. The Armed Forces Pest Management Board (AFPMB), under the auspices of the Office of the Deputy Under Secretary for Environmental Security, published their definition of ***integrated pest management*** in 1996. This mandated a shift in operations from application of pesticides on a calendar basis to routine monitoring and treatment only as needed using a variety of tools of a least toxic nature. Secondly, DoD is investigating greater outsourcing of DoD's pest management operations. Therefore, to maximize the return on this SERDP project, we felt the need to interact with private and governmental sectors through all stages of the project. This would ensure the development of standardized, simple, inexpensive pest monitoring strategies in a manner that promoted spatial analysis in a user-friendly software and hardware system that was both ***functional*** and ***economical*** to private sector pest management companies (potential contractors) as well as DoD personnel.

Consequently, research was initiated along two parallel lines: the development and demonstration of standardized monitoring and precision targeting techniques on representative pest insects, and the identification, modification, and development of standardized hardware and software. This technical report describes research activities and the current status of the project. This report complements the previous technical report describing research activities through April 1997, but focuses on three specific areas: detecting pesticide resistance in German cockroaches, managing stored-product pests with less pesticide use, and comparative risks assess-

[1] Contributors: Dr. Terry Arbogast, Capt. Herbert Bolton, Mr. Eric Daniels, Dr. Dana Focks, Dr. Richard Mankin, and Dr. Steven M. Valles.

ments for dengue fever. The latter is a complicated issue, and was selected to represent disease vector concerns of DoD in deployment scenarios.

***Insecticide resistance studies with the German cockroach, Blattella germanica***

The rationale for studying insecticide resistance in this SERDP effort stems directly from the requirement to know the effectiveness of a particular mitigation measure before it can be recommended in a comparative risk reduction environment. The following report deals with insecticide resistance in an important pest, the German cockroach, *Blattella germanica* (L.). This domiciliary pest is found in populations defined by the structure in which they inhabit. Isolation by structure provides the unique opportunity to evaluate a sample of individuals from the population for the presence of insecticide resistance before treatments are chosen or dispensed. Insecticide resistance in German cockroaches has been typically assessed by collecting cockroaches from the field and rearing them in the laboratory to acquire large numbers needed to conduct topical or residual bioassays. These methods are time consuming, labor intensive, and may require special equipment or knowledge. Hence, these techniques are not conducive for use in the field by pest control operators, or for DoD pest management practitioners whose efforts focus on food service facilities on land, and on galleys in ships.

For those concerned with insect pest control, the main objectives of insecticide resistance detection methods are to determine quickly whether insecticide resistance is present and if the level is sufficiently high to result in control failure. From a pest control perspective, the most important information that can be provided about a resistant population is which insecticide or alternative control tactic would be most effective against the population. This also has the potential to detect incipient stages of resistance, allowing DoD pest management practitioners to prevent onset of resistance through periodic monitoring of populations subjected to frequent pesticide exposure.

One of the most difficult problems with development of insecticide resistance detection bioassays is choosing a diagnostic dose. For example, when employing a diagnostic dose bioassay, false negatives and positives may result when a dose is chosen that is too high or low, respectively. The task of assigning a diagnostic dose becomes especially difficult when reference insecticide susceptible strains no longer resemble susceptible insects in the field. Therefore, λ-cyhalothrin at several fixed concentrations were evaluated against 13 German cockroach strains exhibiting a range of resistance levels in an attempt to identify a diagnostic dose or relationship that could be used to provide information about the presence of resistance in field populations.

λ-Cyhalothrin resistance levels of 13 recently-collected field strains of German cockroach, *Blattella germanica*, were determined by topical insecticide bioassay. The resistance levels ($LD_{50}$ field strain/$LD_{50}$ of the susceptible Orlando strain) ranged from 3- to 71-fold. Residual bioassays were conducted simultaneously against the Orlando insecticide susceptible strain, the HRDC strain (carbamate resistant), the Village Green strain (pyrethroid resistant), and the Marietta strain (pyrethroid, carbamate, and organophosphorus resistant) to determine a diagnostic dose for use in resistance detection bioassays. The $LC_{99}$ of the Orlando susceptible strain (0.9 μg/jar) and 5 times the Orlando $LC_{99}$ (4.5 μg/jar) were chosen for use in λ-cyhalothrin resistance detection bioassays. A highly significant relationship was observed between mortality at 5 times the $LC_{99}$ of Orlando (4.5 μg/jar) and resistance ratio ($LD_{50}$ in topical bioassays).

In related research funded by the American Cyanamid company, a novel consumption bioassay was employed to evaluate hydramethylnon tolerance among 14 insecticide-resistant and

1 insecticide-susceptible strain of German cockroach. The feeding bioassay provided the ability to assess physiological changes in hydramethylnon tolerance while simultaneously evaluating German cockroach avoidance behavior toward Siege® gel bait. The cockroach strains, which had been shown previously to be resistant to pyrethroid, carbamate, and organophosphate insecticides, were bioassayed in two groups. The first group was bioassayed by the dose response method and the second group was assayed with a single diagnostic dose. All of the strains evaluated by the dose response consumption bioassay method were more susceptible to hydramethylnon than the Orlando laboratory strain. Although the dose response bioassay data did not indicate physiological resistance among these strains, behavioral resistance (avoidance) was implicated in the Union 511 and Malo strains. In a second group of insecticide-resistant strains, physiological, as well as behavioral resistance to hydramethylnon was implicated when cockroaches were challenged with a diagnostic dose of hydramethylnon in Siege®. At the Orlando $LD_{99}$ (3.5 µg hydramethylnon per cockroach) only 80 and 75% of the NASJAX and Hyd-Sel adult males consumed the bait, respectively. These data indicate that avoidance behavior may be developing in these strains. Furthermore, physiological resistance may be present in the Hyd-Sel strain, as only 89% were killed when fed the $LD_{99}$ Orlando dose.

Basic research has been conducted concomitantly to establish the biochemical mechanisms responsible for resistance in the German cockroach and to possibly identify a common trait among resistant cockroaches (independent of the biochemical basis for resistance). We hope to exploit such a common trait and develop a biochemical test capable of detecting resistance. In pursuit of this goal, topical bioassays with cypermethrin, λ-cyhalothrin, permethrin, propoxur, and chlorpyrifos were conducted on twelve German cockroach strains recently collected from the field. Resistance levels ranged from 3- to 159-fold for cypermethrin, 2- to 88-fold for permethrin, 4- to 55-fold for λ-cyhalothrin, 5- to 33-fold for propoxur, and 3- to 19-fold for chlorpyrifos. The synergists piperonyl butoxide (PBO) and *S,S,S*-tributylphoshorotrithioate (DEF) affected cypermethrin resistance to varying degrees depending on the strain. Piperonyl butoxide pretreatment decreased cypermethrin resistance in only 5 strains, but caused an increase in resistance level in 7 strains. Conversely, DEF pretreatment reduced the resistance level in 10 of the strains and increased the resistance level in only 2 strains. Correlation analysis of resistance ratios for each strain and insecticide indicated a direct relationship between resistance level of one insecticide and another, especially among the pyrethroids. All field strains exhibited significantly higher microsomal oxidase, glutathione *S*-transferase and esterase activities toward surrogate substrates as compared with the insecticide susceptible strain. However, levels of cytochrome P450 content, aldrin epoxidase activity, methoxyresorufin *O*-demethylase activity, and glutathione *S*-transferase activity were not correlated with pyrethroid resistance suggesting that these activities are poor indicators of pyrethroid resistance magnitude. Interestingly, significant correlations were found between general esterase activity and cypermethrin ($P$ = 0.002), permethrin ($P$ = 0.007), cyhalothrin ($P$ = 0.002), and propoxur ($P$ = 0.001) resistance levels. The data support the conclusion of esterase involvement in cypermethrin resistance determined by synergist (DEF) bioassay. However, the significance of this relationship, in the context of resistance detection, requires further examination. This is the focus of our future research.

***Utility of spatial analysis in management of storage pests***

Historically, management of stored-product insects has relied heavily on application of chemical pesticides, but increasing awareness of the risks these chemicals pose to environmental quality and human health, has made it necessary to seek safer methods. Development of insecticide resistance has aggravated the problem and increased the need to develop pest management programs that are less chemical-dependent. These new programs, which would make judicious use of chemical pesticides but rely mainly on alternative methods, will require accurate and comprehensive monitoring of pest populations. Monitoring will not only detect infestation, but also estimate its degree and location. Thus monitoring will guide the timing and targeting of control applications, eliminate the need for routine preventive treatment, reduce the area treated with insecticides, and aid in the application of nonchemical methods.

Research over the last two or three decades has produced a variety of traps that are effective in detecting insect pests in bulk commodities and storage structures. Currently available traps are better for this purpose than conventional grain sampling methods, but development of theory needed to interpret trap catch has lagged behind. Trap catch can be defined as the number of insects captured by a given type of trap in a specified period of time. The value of traps in monitoring pest populations is diminished by our limited ability to relate trap catch to population density, or some action to be taken. This limitation is widely recognized by storage specialists. Wilkin and Fleurat-Lessard showed that detection and estimation of low-level infestation (<5 insects/kg) in grain bulks by spear sampling is unreliable, and noted the serious problem this poses for calibration of trapping methods. They concluded, in fact, that it may be impossible to relate trap catch to population density and suggested that some system of risk factor be devised instead. Their conclusion is supported by the findings of Lippert and Hagstrum in a study of wheat stored on Kansas farms, which showed poor correlation between number of insects trapped and number of insects in grain samples. Yet, Roesli and Jones reported good correlation between numbers of psocids in spear samples of wheat and numbers captured in probe, pitfall and bait bag traps. Also, Haines et al. found that bait bags captured insects in proportion to their population density in stacks of bagged milled rice. Wilkin recognized two categories of risk— the probability that insects would be detected by a customer and the effect of further storage on quality— and presented a scheme for interpreting trap catch in terms of action to be taken. Pinniger pointed out the need, in commercial facilities, for a trapping strategy designed specifically for each trap type, pest species and situation. He outlined a scheme, based on action thresholds set by experience and the needs of industry, that can be adapted to different environments and pests.

There are clearly two types of trap interpretation, for which we propose the terms "representative" and "indicative." Representative interpretation posits that trap catch represents a population density to which it can be converted mathematically. Attempts to determine the required mathematical relationships empirically have had mixed results, as already noted. There has been some progress in deriving the relationships theoretically (from first principles). For example, a self-marking recapture method has been developed based on a Markov chain model, and Jansen proposed a method for interpreting pitfall trap capture by treating insect movements as two-dimensional Brownian motion.

Indicative interpretation posits that trap catch is an indicator of action to be taken. Indicator values may be either numerical ( "When mean trap catch in the stored wheat exceeds two insects, control intervention is indicated.") or numerical-spatial ("Control intervention is indicated for all areas of the store in which the probability of capturing two or more insects per trap exceeds 95 %."). Numerical-spatial values are more useful, because they not only indicate when treatment is needed but also where. This is highly relevant in DoD warehousing and installations.

Pierce successfully located hidden infestations of cigarette beetles and pyralid moths in food warehouses using pheromone-baited traps and triangulation based on the inverse relationship between the number of insects in a trap and the proximity of an infestation. Geostatistical techniques can also be applied to discover the spatial distribution of insect populations. These techniques have not yet been applied extensively in a storage context, but they offer means of describing spatial patterns in grain bulks, warehouses, processing plants, retail stores, and commissaries. Their application will improve pest management capability by locating foci of infestation and points of control failure. The present report treats spatial analysis of insect populations by contour analysis of trap catch. This is a powerful method of indicative interpretation, the utility of which will be illustrated by a number of examples from field studies conducted in private sector facilities for logistical convenience; research findings are applicable to the DoD scenarios described in our SERDP proposal.

Materials and methods.

*Spatial analysis.* Spatial analysis is a three-dimensional analysis in which two dimensions (x, y) represent the positions of points on a horizontal plane, and the third (z) represents data associated with the points. The data can be visualized as elevations above or below the plane. Data consist of: (1) measured numerical values (numbers of insects, temperature, moisture content, insecticide residue, etc.) or derived numerical values (differences, ratios, probabilities, etc.) associated with fixed points on the horizontal plane and (2) the x, y-coordinates of the fixed points. All variables are treated as continuous although insect counts are discrete. The points are fitted to a three-dimensional surface representing the variable (z-axis) as a function of position on the x, y-plane. The surface can be represented in two dimensions by a contour map, which shows the configuration of the surface by means of isolines (contours), drawn at regular intervals of z, on the x, y-plane through the origin. Each contour represents the intersection of a horizontal plane with the surface, which means that all points on a particular contour have the same z-value.

We used Surfer Version 6.02 (Golden Software, Golden, Colorado) for contour analysis because the spatial analyst features of the ArcView GIS program were not yet available when these studies were initiated. This software posts observed z-values to coordinates on a map of the facility (storage bin, warehouse, retail store, etc.), entered as a base map, and then creates a denser grid of z-values by interpolation, using one of several available algorithms. We used radial basis functions with multiquadric algorithm, which is flexible and provides a good overall interpretation of most data sets. After interpolating, the software produces contours of z, based on the interpolated grid values. Goodness of fit is estimated by calculating residuals— differences between observed numbers and numbers predicted by the fitted surface.

Residuals and other derived values, such as differences and probabilities, can also be mapped by means of contour analysis. Spatial changes are best examined by grid subtraction, which is done by subtracting the value of each node in a grid from the corresponding node values of a second, identical grid; the differences are then assigned to corresponding nodes of a third grid and difference contours drawn. Areas of increase are indicated on difference plots by positive contours and areas of decline by negative contours.

In targeting areas for treatment, it is helpful to draw contours of indicator variables, rather than raw trap counts, because indicator variables are affected less by unusually large counts. Indicator variables are obtained by converting trap catch to probability. Trap locations are first sorted in descending order by the number of insects captured, then cumulative and normalized trap catch are calculated for each location. The normalized catch (cumulative trap catch divided by the total number of insects in all traps) gives the probability of an equal or higher catch. An indicator value of 1 is assigned to all locations for which probability equals or exceeds some set level or action threshold (determined by experience and the needs of industry) and a value of 0 is assigned to the remainder.

*Warehouses.* In the spring of 1998, we had an opportunity to study an insect infestation of bagged saw palmetto berries, *Serenoa repens* (Bartram) Small, stored in a steel warehouse (30.5 by 15.2 m.) in central Florida. The berries, which are ground and used as a nutritional supplement for prostate and urinary well-being, are harvested from their natural habitats, largely pine flatwoods in southeastern U. S. They are then heat dried, bagged, and stored until they can be shipped to end processors in Europe and elsewhere.

The infestation in the Florida warehouse involved mainly five species of stored-product insects: *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Lasioderma serricorne* (Fabricius), *Tribolium castaneum* (Herbst), and *Oryzaephilus mercator* (Fauvel). Moths were monitored with pheromone-baited sticky traps (SP-Locator traps with Minimoth lures, AgriSense, Mid Glamorgan, UK), and beetles were monitored with pitfall traps (FLIT-TRAK $M^2$, TRÉCÉ, Salinas, California) baited with *L. serricorne* and *Tribolium* pheromones and a food attractant oil (furnished with the traps). A moth trap and a beetle trap were placed at each of the locations indicated in Fig. 1. Trap location was specified in rectangular coordinates with the origin at one corner of the warehouse. Some moth traps were attached, by means of Velcro, to the walls of the warehouse, with the sticky surface oriented horizontally. Others were attached, to the tops of wooden stakes supported by stands on the floor or to bags on top of the stacks. They were located at heights ranging from 1.2 - 3.8 m. Beetle traps were placed either on the floor or on top of the stacks (0.0 - 3.4 m). Trap catch was recorded daily for 4 days, and insects were removed from the beetle traps. Moth traps (but not the lures) were replaced as necessary, usually every day.

This study illustrates spatial analysis of trap counts for mapping the distributions of several insect species in a warehouse containing packaged raw commodities. Total numbers of *C. cautella* and *O. mercator* trapped over the 4-day period were selected for purposes of illustration.

*Retail stores.* Our second example is from a study of *P. interpunctella* infestations in retail department stores. Much of the area in these stores is devoted to merchandise not susceptible to moth infestation, but the stores also carry highly susceptible items such as pet food, and these items are sometimes damaged and contaminated by insects. Flying moths can occur anywhere in a store, and because retail department stores often include restaurant facilities, the moths sometimes cause problems with health inspectors. Retailers and pest control operators that service retail stores need monitoring methods that are quick, easy to use, inexpensive, and inconspicuous. This scenario is analogous to DoD commissaries.

We monitored moth populations in three stores using SP-Locator traps. These traps are small enough (7 by 10 by 1.5 cm) to conceal under shelves, to which they were attached with Velcro for easy removal and replacement when making counts. For spatial analysis of trap counts, trap locations were specified in rectangular coordinates with the origin at one corner of the store. The traps were distributed as well as possible throughout each store, but locations suitable for trap placement were not uniformly available. To minimize the number of traps with no captures, we placed more in areas likely to support insect infestation, such as in the pet food and grocery departments. Moths were counted 1 hour after the traps were set and again after 4, 24, 48, 72 and 96 hours. Moths were detected in all three stores, but the level of infestation varied considerably from one to another. In the most heavily infested store, we did two trapping tests, separated by about five weeks. The first test involved the entire store, and the second was limited to pet supplies and adjacent sections. Our example is based on the cumulative number of moths captured over a 96-hour period during the second test. Twenty-five traps were deployed in these sections as shown in Fig. 2, with the 14 traps in pet supplies located as in the initial test.

Results and discussion.

*Warehouses.* Trapping and contour analysis effectively mapped the distribution of each pest species in the warehouse, and located foci of infestation, mostly in the stacks of bagged saw palmetto berries (Fig. 1). *Oryzaephilus mercator* was limited to stacks S-2, S-3, and S-4, with the major center of infestation in S-4 (Fig. 1A). *Cadra cautella* was widely distributed with major centers in stacks S-3 and S-5 and in an area, adjacent to the store room and rest room, used for storage of empty burlap bags and equipment (Fig. 1B). Subsequent visual inspection revealed that insecdts were arising from accumulated organic debris behind plywood panes that had been affixed to the wall surfaces to protect them from fork lift operations. These insects then infested or re-infested any product brought into the warehouse, necessitating frequent intervention. Consequently, based on this precision targeting study, plywood was removed to facilitate cleaning of the debris to eliminate these residual insect populations; plywood was then reinstalled to protect wall surfaces.

The situation at the time of our study presented a difficult pest management problem. Consumers want a pure product free of chemical contamination, so no pesticides could be applied to the berries. Treatment with modified atmospheres was considered, but rejected as economically unfeasible. Finally, the berries were run through the drier a second time (at about 52 to 57°C) to eliminate the insect infestation before shipment. The costs accrued (moisture loss, fuel, and labor) were significant.

We learned of the infestation after most of the berries in the warehouse had been shipped. The infestation had become severe, and the remainder of the berries were due to be shipped. We conducted this study to determine if trapping and spatial analysis would be effective in locating foci of infestation, thus indicating potential for the method in long term monitoring to reduce pesticide use. Because our results were positive, we are now planning a study to determine the effectiveness of the method in detecting and pinpointing low levels of infestation in natural product warehouses, so least-toxic spot interventions can be applied when and where they are needed.

*Retail stores.* Initial trapping in the retail store indicated a heavy infestation of *P. interpunctella* involving most of the pet supply department. The infestation was apparently restricted to this department, because only three moths were captured elsewhere, and two of these were captured nearby. Follow-up trapping, which was restricted to pet supplies and adjacent areas, showed four well-defined foci of infestation (Fig. 2). These encompassed shelves with birdseed and dog food as well as items not susceptible to infestation, such as cat litter and flea treatments. However, in all foci, the enclosed space between the bottom shelves and the floor held accumulations of infested pet food and birdseed. A few moths were captured in the garden shop and in the pharmacy area immediately adjacent to pet supplies, and these captures are reflected in the contours of raw trap counts (Fig. 2A). However, most of the traps in the pharmacy captured no moths, and the contours near the left-front corner of the store are an artifact of interpolation. This artifact illustrates the importance of assuring that the edges of an area to be mapped are adequately represented by traps. The artifact could have been avoided by placing a few traps along the wall near the origin. This was not done, because this corner of the store was occupied by the prescription center and was not accessible.

The foci of infestation are shown more clearly by contours of indicator variables (Fig. 2B) calculated from the raw trap counts (Fig. 2A). Indicator variables are defined to suit the needs of a particular situation, as determined by some action threshold. Here they were defined so that the contours representing a value of 1 enclose an area in which > 95 % of the moths were captured. Stated another way, these contours enclose an area in which 95% of trap captures are expected to occur. This is the area that requires treatment. Treatment in this case would consist of removing infested items, cleaning the floor under the bottom shelves, and perhaps applying an insecticide in some areas. Long-term pest management would require improved sanitation and better rotation of stock. Sanitation would be made easier by eliminating the kickplates that cover the space beneath the bottom shelves.

The results of this study suggest that a sufficient number of well-distributed pheromone traps followed by spatial analysis of trap counts is enough to detect infestations of *P. interpunctella* and locate foci of infestation, which are two of the main objectives of monitoring in retail stores. A third objective is to assess the effectiveness of treatment by follow-up monitoring. Spatial analysis of trap counts serves all three purposes, and no other form of trap interpretation is required. This simplified proactive management process greatly reduces the need for routine applications of pesticides.

Conclusions.

The two cases presented illustrate the utility of spatial analysis of trap counts in monitoring storage pests. Contour maps provide excellent visuals that show storage and retail store managers the extent and location of pest problems. They provide decision support in determining the type, timing, and targeting of least-toxic control interventions. They document the results of control intervention and indicate the extent and location of control failure. Finally, they reduce pesticide risk by eliminating the need for routine chemical treatment by making it possible to precisely target pesticide applications, and by suggesting and guiding the application of nonchemical methods.

### *Dengue transmission thresholds*

The need for thresholds for the risk assessment.

Today, most dengue control efforts in military settings and in private sector settings are based on suppression of *Aedes aegypti* (L.) and not eradication; increasingly, these efforts rely on reducing the number of larval breeding habitats and not on insecticides. How should such *source reduction* efforts be monitored? In terms of risk assessment, what levels of elimination are necessary to preclude transmission? Several authors have recently argued that the *Stegomyia* indices as epidemiologic indicators of dengue transmission should be viewed with caution as they have a number of serious shortcomings. By way of background, the Container Index (percentage of water-holding containers infested with active immatures) is probably the poorest since it reflects only the proportion of containers positive in an area and it does not take into account the number of containers per area, per house, or per person. The House Index (percentage of houses with one or more positive containers) is perhaps better, but this index fails to give the number of positive containers per positive house. Of the indices, the Breteau Index (number of positive containers per 100 houses) is arguably the best, combining information on containers and houses. However, all three indices fail to take into account that containers vary in the production of adult

*Ae. aegypti*. For example, two very different containers, an indoor flower vase, commonly found with larvae but seldom producing an adult because of frequent water changes, and, say, a large outdoor drum under a fig tree which supports a standing crop of 10 or 20 or 50 pupae, are for the purposes of calculating indices, equally positive. Field observations bear this out: Southwood et al. reported for a temple area in Bangkok a ca. 23-fold difference in the most and the least productive types of container. A six-fold difference was seen in Honduras. Connor and Monroe, in their original paper on indices, recognized these shortcomings and, in 1923, pointed out that herd immunity was an additional and important epidemiologic factor not considered by the *Stegomyia* indices. There are two additional shortcoming- the indices fail to adequately provide information on either a *per area* or a *per person* basis nor do they take into account the role of temperature, both factors known to relate to levels of transmission. Finally, in an island-wide study in Trinidad involving 20 urban sites, the three traditional indices did not correlate with each other, and more importantly, they were completely uncorrelated with the observed densities of the vector.

As a result of the foregoing work, the case has recently been made by us for the SERDP effort that a pupal and demographic survey is more appropriate for assessing risk and directing control operations. This work focused on using pupae for several reasons: 1) Unlike any of the other life stages, it is possible to actually count the absolute number of *Ae. aegypti* pupae in most domestic environments. 2) Container-inhabiting *Stegomyia* pupae are easily and inexpensively separated from other genera and identified to species as emerged adults or pupae. 3) Because pupal mortality is slight and well-characterized, the number of pupae per person is highly correlated with the number of adults per person. 4) Estimates of transmission thresholds for dengue based on simulation models were being developed for tropical locations which are based on, among other factors, herd immunity, the number of *Ae. aegypti* pupae per person, and ambient air temperatures.

The topic of transmission thresholds is the subject of the present work- what levels of pupae per person will permit transmission under various conditions of temperature, herd immunity, and the magnitude of the viral introduction(s). A second issue concerns the importance of the stochastic nature of nascent epidemics.

*Use of epidemiologic models to estimate transmission thresholds.* The estimates of transmission thresholds are based on a pair of simulation models (CIMSiM/DENSiM) developed to provide site- and weather-specific insight into the dynamics and control of dengue viruses and their vectors. These models reflect a long history of mathematical modeling of epidemiologic phenomenon. As early as 1906, Hamer postulated that the course of an epidemic depended on the rate of contact between susceptible and infectious individuals; this notion, *the mass action principal*, has become a central concept in mathematical epidemiology- the rate of spread of an infection within a population is proportional to the product of the density of susceptible and infectious people. Ross used this principal in his pioneering work on the dynamics of malaria transmission. The insight of Hamer and Ross were further developed by Kermack and McKendrick in 1927 into an understanding of the concept of thresholds. Anderson and May consider this *threshold theory*, coupled with the mass action principal, to be the cornerstone upon which modern epidemiological theory is built. The notion of thresholds indicates that the introduction of a few infectious individuals into a community of susceptibles will not give rise to an epidemic outbreak unless the density of susceptibles (or vectors) is above a certain critical level. More recent advances in the rapid growth of mathematical epidemiology involve recognition that variation and the elements of chance are important determinants of the spread and persistence of infection and have led to the development of stochastic models.

Citing the abstract nature of much of the theoretical work and the lack of ties to field data, Anderson and May make the observation that little use of this theoretical understanding has been made in empirical studies and the development of public health policy regarding infectious human diseases. They find that "in view of the successes achieved by combining empirical and theoretical work in the physical sciences, it is surprising that many people still question the potential usefulness of mathematical models in epidemiology." The dengue transmission models (CIMSiM/DENSiM) incorporate the theoretical principals outlined above, but in a computer simulation environment that permits site-specific information to be used. These models were designed to provide support to DoD preventive medicine specialists, to public health policy decision makers, and to help answer research and control questions.

*Vectorial capacity models of dengue- uses and limitations.* The validity and utility of the concept of transmission thresholds for dengue was recently used in efforts to gain some understanding of the potential influences of warming temperatures on the intensity and distribution of dengue transmission throughout the world. The parameter values used in this analysis were derived from those used in the construction of CIMSiM and DENSiM. In these studies, an expression of vectorial capacity (used originally in the study of malaria to estimate the mean number of potentially infective contacts made by a mosquito population per infectious person per unit of time) was modified to reflect the role of temperature on development and survival of the vector and virus. The traditional vectorial capacity expression was rearranged to develop an equation for the *critical density threshold*, an estimate of the number of adult female vectors required to just maintain the virus in a susceptible human population. In this expression, temperature influences adult survival, the lengths of the gonotrophic cycle and the extrinsic incubation period of the virus in the vector, and vector size, a factor that indirectly influences the biting rate. Before making estimates of the consequences of global warming, we validated this method by successfully comparing model projections and the observed spatial, temporal, and altitudinal distribution of dengue using current climate in 5 cities that are endemic or have had epidemics in the past.

A significant limitation to using a vectorial capacity approach to the study of transmission thresholds for dengue is that there is no provision for estimating actual threshold densities of vector in an environment where everyone is not susceptible. For this type of analysis we turn to the simulation models CIMSiM and DENSiM.

*Overview of CIMSiM and DENSiM.* The threshold analysis uses two, interrelated simulation models, CIMSiM (Container-Inhabiting Mosquito Simulation Model) and DENSiM (Dengue Simulation Model). The entomological model (CIMSiM) is a dynamic life-table simulation model which produces mean-value estimates of various parameters for all cohorts of a single species of *Aedes* mosquito within a representative one-hectare area. Like DENSiM, the entomology model is basically a accounting program. For each cohort, depending on the life stage, CIMSiM maintains information on abundance, age, development with respect to temperature and size, weight, fecundity, and gonotrophic status. With few exceptions, the various processes are simulated mechanistically. The accounting is made dynamic by calculating on a daily basis the number of each cohort which will pass to the next age or stage as a function of a number of variables and relationships. For example, development times of eggs, larvae, pupae, and gonotrophic cycle are based on temperature using an enzyme kinetics approach. The basis of larval weight gain, food depletion, and fasting are differential equations modified to compensate for the influence of temperature. Fecundity is modeled as a function of pupal size which in turn is a function of the recent history of larval abundance, food, temperature, and fasting in the larval habitat. All survivals are tied to temperature, and for adults and eggs, saturation deficit as well; larval survival is also a function of fasting and fat body reserves. The heterogeneity of the larval habitat is depicted by modeling the cohorts of eggs, larvae, and pupae within up to 9 different types of containers, each of which serves to represent an important type of mosquito-producing container in the field. The adult production from these representative containers is combined, the output of each type being scaled to reflect its relative abundance in the environment. Because microclimate is a key determinant of survival and development for all stages, CIMSiM also contains an extensive database of daily weather information. Adult microclimate is assumed to be the same as the daily local weather. For immatures, however, CIMSiM calculates daily water temperatures and water gains/losses for each of the representative containers based on local weather and container characteristics and location. Validation of CIMSiM involved comparing simulation results with several independent series of laboratory and field data on the dynamics of *Ae. aegypti* which were not used in model development.

Whereas CIMSiM is basically a habitat- and weather-driven accounting program of the population dynamics of *Ae. aegypti*, DENSiM is essentially the corresponding account of the dynamics of a human population driven by country- and age-specific birth and death rates. For example, in a simulation run of a small village or barrio in a developing country, over the course of 15-20 years, the human population will grow from 10,000 to ca. 17,000 people. An accounting of individual serologies is maintained, reflecting infection and birth to seropositive mothers. In DENSiM, the entomological factors passed from CIMSiM, daily estimates of *Ae. aegypti* adult female survival, gonotrophic development, weight, and emergence on a per hectare basis, are used to create the biting mosquito population. The survival and emergence values dictate the dynamic size of the vector population within DENSiM while the gonotrophic development and weight estimates influence the rate at which these females bite. Temperature and titer of virus in the human influences the extrinsic incubation period (EIP) in the mosquito; titer is also seen as influencing the probability of transfer of virus from human to mosquito. The infection model accounts for the development of virus within individuals and its passage between the vector and human populations. Parameters estimated by DENSiM included demographic, entomologic, serologic, and infection information on a human age-class and/or time basis. As in the case of CIMSiM, DENSiM is a stochastic model.

Methods.

*Definitions of transmission thresholds used and limitations of their use.* In this analysis, CIMSiM and DENSiM are used to estimate transmission thresholds as a function of herd immunity, ambient temperatures, and the magnitude of the viral introduction(s). We begin with a definition of an epidemic that is arbitrary but useful from a public health point of view- any single year where seroprevalence rises by at least 10% over the previous year is considered to be an epidemic year. Ten percent was selected because any disease involving that proportion of the population would be considered an epidemic and this level of transmission would result in peak prevalence of cases to be just slightly more than 1% of the population- a minimum value that has been suggested as sufficient for the detection of transmission. Just how many mosquitoes per person are required to support this level of transmission is a function of many factors, but the ones considered key determinants are the number, size, and timing of viral introductions during the year, seroprevalence of dengue antibody, and temperature. We will develop estimates for scenarios where 1, 2, 4, or 8 viremic person(s) are introduced into an area on day 90 of the year (ca. the end of March) and for the case where a single viremic person is introduced once-a-month throughout the year; for convenience, we will name these *Single Introduction*$_{1,\ 2,\ 4,\ \&\ 8}$ and

*Monthly Introductions*, respectively. In these assessments we make several important assumptions that are likely to be true in most tropical locations: 1) Vector competence is adequate, 2) blood feeding by *Ae. aegypti* occurs primarily (>90%) on humans, and 3) essentially all hosts are at risk of being bitten. The conditions in the southeastern United States are an obvious exception to these assumptions.

*Gonotrophic development rate, adult size and daily survival.* CIMSiM was used to estimate the entomological parameters that were passed to DENSiM using constant temperatures between 20 and 34°C in 2° steps. To mimic the average daily temperature fluctuation between the daytime high and the nighttime low typically seen in our weather files for tropical locations, the maximum and minimum temperatures used in CIMSiM and DENSiM were plus and minus 5°C of the desired constant temperature. The parameters estimated using CIMSiM were values for gonotrophic development rate, adult size and survival for each of the temperatures used. We assumed that atmospheric moisture was adequate so as to not limit adult daily survival (0.89). The representative container types used in CIMSiM to make these estimates were those described in Focks et al. for Honduras.

*The relationship between standing crops of female adults and pupae.* Because temperature influences the duration of pupal development ($PD_{temp}$), temperature also influences the relationship between the standing crops of pupae and adults. Under cool conditions a smaller proportion of the standing crop of pupae emerge each day than at higher temperatures; specifically, under steady-state conditions, the proportion emerging daily is $1/PD_{temp}$. As a consequence, under cooler conditions, a larger standing crop of pupae will be associated with a given adult population than at warmer conditions. Under equilibrium conditions of constant recruitment and survival, the number of female adult *Ae. aegypti* emerging each day will be the product of the number of pupae present, $1/PD_{temp}$, i.e., that portion of the standing crop of pupae expected to emerge on a given day, the proportion of pupae that are females (0.50), and the rate of successful emergence (0.83). The resulting steady-state standing crop of female adults of all ages would be this daily number of emerging females times the expected lifespan of adult ($1/-\log_e$ [Sa]) where Sa is the daily adult survival probability, 0.89).

*Threshold determinations using DENSiM.* DENSiM was parameterized with the previously-published default parameters values, with three exceptions based on unpublished studies: 1. Virus was assumed to titer in the human host at $10^5$ mosquito infection doses ($MID_{50}$). 2. The number of interrupted feeding attempts per replete blood meal was set to 3.0. 3. The probability that an interrupted feeding attempt was resumed on a different host was 0.35. Each simulation iteration began with an initial human population of 10,000 with an age distribution and age-specific birth and death rates typical of Honduras in the 1980. Because the human population grew at an approximate rate of 3.7% during the year, the *density* of people remained constant as DENSiM was set to dynamically model a corresponding larger area- population growth was accompanied by "urban sprawl" during the year. The initial seroprevalence of antibody was set to either 0, 33, or 67% in all age classes depending on the analysis being run. For each temperature, DENSiM was provided the appropriate temperature-specific values for daily gonotrophic development rate and weight of emerging females (Table 1) and on each day, the number of newly-emerged females that were associated with a standing crop of 100 *Ae. aegypti* pupae per hectare (Table 4). By varying the number of people per hectare within DENSiM, we could evaluate the consequences various ratios of pupae per person; e.g., telling DENSiM that the human population was 200 per hectare would correspond to 100 pupae per 200 people or 0.50 pupae per person.

DENSiM was then iteratively run using these various ratios of *Ae. aegypti* and humans under conditions of *monthly introductions* of a single viremic individual and an initial seroprevalence of all age classes of dengue antibody to be 0, 33, or 67%, our *Monthly Introductions* threshold. In a similar fashion, the second type of threshold, *Single Introduction*$_{1, 2, 4, or 8}$, was estimated, the number of pupae per person necessary to lead to an epidemic at least 50% of the time due to introduction(s) on calendar day 90 of 1, 2, 4, or 8 individual(s) at each the 3 levels of initial seroprevalence. Iterative runs (ca. 14,000) were necessary because of the stochastic nature of incipient epidemics; typically, at the threshold ratio of pupae per person, only a portion (the goal being 50%) of the runs resulted in an epidemic rise of >10% over the initial seroprevalence of dengue antibody.

Results.

*Gonotrophic development rate, adult size, and daily survivals.* Given that the daily adult survival of females is independent of temperatures ranging between 22 and 32°C, a shorter gonotrophic cycle (Table 1) results in females at higher temperatures being expected to feed more times during their life times than at lower temperatures. For example, the results in Table 1 indicate that females at 32°C will attempt to take more than twice as many replete feeds as females at 24 ° (0.411179 / 0.199184 = 2.064); a difference of only 4°C is significant- females at 30° will take an average of ca. 30% more feeds than females at 26°.

The size of adult *Ae. aegypti* females has been suggested to influence the proportion of females requiring two replete feeds on the first gonotrophic cycle; smaller females are therefore expected to be slightly more likely to transmit virus because of higher biting rates during the first cycle. Under larval rearing conditions where food is not limiting, CIMSiM generates estimates of smaller females at elevated temperatures (results not shown). However, CIMSiM's projections of the weight of emerging females under field conditions (Table 1) does not indicate such a decline; the weights are essentially uniform at temperatures ranging between 22 and 30 °C. This observation is a result of lower larval competition at higher temperatures due to reduced survivals in the egg stage (Table 2).

Table 2 presents the daily survival of adults ($S_{adult}$), eggs ($S_{egg}$), larvae ($S_{larval}$), and pupae ($S_{pupal}$), the possibility of egg hatch, and the possibility of adult populations under conditions of constant temperatures as indicated by CIMSiM. Parameter values in CIMSiM and the field and laboratory observations upon which they were based were presented earlier. Temperatures at or below 20 °C do not permit adult populations to develop because of the failure of eggs to hatch. Temperatures >34 or 35°C eliminate the possibility of adult populations because the aggregate survival of all stages combine to drive the population growth rate below replacement. Note at the temperatures considered herein, only egg survival varies significantly with temperatures ranging between 22 and 34°C. Adult survival begins to decline at temperatures higher than 36° and larval and pupal survivals begin to decline as a function of elevated temperatures only at temperatures in excess of 39 or 40°.

*The relationship between standing crops of female adults and pupae as a function of temperature.* The length of the pupal development ($PD_{temp}$) period influences the relationship between the standing crops of pupae and adults. Table 3 presents observed and predicted pupal development periods as a function of temperature based on data from Rueda et al. Note that the development period is the nominal ca. 2-days length only at 28°C, at 25° it is ca. 50% longer and at 32°, the period is only half of that at 28°. The significance of this in terms of transmission can be see in Table 4 where the number of newly-emerged females and associated standing crop of adult females arising from a standing crop of 100 *Ae. aegypti* pupae under steady-state equilibrium conditions as a function of temperature is presented. Here we see in addition to the reduction in gonotrophic development rate with temperature, another significant cause of increased transmission with elevated temperatures- a given standing crop of pupae observed in the field will be associated with significantly higher female populations at elevated temperatures. The number of adult female *Ae. aegypti* associated with each pupa at 22° is less than one; at 28°, it has almost doubled to 1.75 females per pupa. Even a small temperature difference of only 4°C is important; for example, there are ca. 45% more females at 30° than at 26° for a given standing crop of pupa. Put another way, 100 pupae at 22°C are associated with a standing crop of ca. 90 females whereas at 30° the associated number is ca. 240.

*The Monthly Introduction threshold.* Under the conditions of our study, we have seen that temperature would be expected to influence probability of transmission in two ways, through 1) its role in determining the length of the gonotrophic cycle and hence the daily biting rate and through the impact of the pupal development period and 2) its relationship to the ratio of number of pupae observed in a survey to the number of adult female *Ae. aegypti* mosquitoes in the same survey area. Another factor not documented in this study but incorporated in DENSiM is the influence of temperature on the extrinsic incubation period (EIP) of the virus in the female. At higher temperatures, infected females become infectious through viral dissemination at a significantly faster rate; hence the probability of an infected female living long enough to become infectious goes up significantly with temperature. Specifically, the probability of surviving the incubation period is $Sa^{EIP}$. Using values for EIP from earlier work by Focks et al. for 22 and 32°C of 16.67 and 8.33 days, respectively, the associated survival probabilities are 0.89 or 0.143 at 22° and $0.89^{8.33}$ or 0.379 at 32°; females incubating virus at the higher temperature are 2.64 times more likely to survive long enough to potentially infect human hosts.

Table 5 presents estimates of the *Monthly Introduction* thresholds incorporating the above-listed factors as a function of initial seroprevalence of dengue antibody and temperature. We have limited our range of estimates to temperatures between 22 and 32°C because field and laboratory observations serving as the basis of the parameter estimates in CIMSiM and DENSiM often did not include temperatures outside of this range. Initially, we will confine our comments to the thresholds at 26 and 28°C- temperatures in the range of average annual temperatures experienced in many dengue-endemic areas (Table 6). Our first observation is simply how very low these estimates are- among dengue-naïve populations, the *Monthly Introduction* thresholds are estimated to be 1.05 and 0.42 *Ae. aegypti* pupae per person, respectively; given a low level of herd immunity of 33%, the estimates rise to only 1.55 and 0.66, respectively. A comparison with observed numbers of pupae per person in various locations emphasizes this point (Table 6).

In light of the many factors increasing the probability of transmission at higher temperatures, it is not surprising that the threshold estimates for temperatures >28°C fall quickly. Note that there is approximately a 4-fold decrease in the required number of pupae per person between 28 and 30°C but less than a 2-fold attenuation between 30 and 32°; this is the result of a flattening of the EIP-temperature relationship at higher temperatures in the 28 to 32° range and not to a reduction in daily adult survival at the more elevated temperatures. At temperatures below 26°, the thresholds rise sharply and provide a partial explanation of the seasonal nature of transmission even in tropical locations where seasonal variation is not greater than a few degrees.

A final point of interest concerns the rise in thresholds with increasing initial seroprevalence of antibody to dengue virus. Independent of temperature, threshold values rise an average of 1.51-fold when the initial seroprevalence increases from zero to 33%, 2.05-fold when going from 33 to 67%, and 3.15-fold when comparing an initial seroprevalence of zero to 67%. In light of the frequently high numbers of *Ae. aegypti* pupae per person observed (Table 6), it is not surprising that epidemic transmission is reported at even high levels of herd immunity. This would be especially true among infants and adolescents because these ages rapidly lose levels of immunity due to the steady recruitment of sero-negative infants into their classes.

*Multiple Introduction thresholds.* The patterns seen the among the transmission thresholds for the case of monthly introductions are also true for the single introductions of varying sizes (Tables 7-10). For each size of introduction, 1, 2, 4, or 8 individuals, there is a very uniform relationship between the threshold required for an initial seroprevalence of 0, 33, and 67%. Thresholds are an average of 150% higher for 33% than 0%, 206% higher for 67% than 33%, and 310% higher for 67% than in the case of an initial seroprevalence of 0%. The surprising result to us was the only slight decline in threshold values with increasing numbers of viremic individuals introduced each year (Fig. 3). It would appear that the probability of significant transmission is only slightly influenced by the size of the introduction. In practical terms this means that any of our thresholds, *Multiple* or *Monthly* thresholds, can be used for risk assessment; this is especially true for the cases where the initial seroprevalence is at or below 33%. In the case of an initial seroprevalence of zero, the thresholds only decline some 21% when comparing an introduction of 1 or 12 people; for initial seroprevalences of 33 and 67%, the reductions are 19 and 25%, respectively.

Discussion and Conclusions.

In the present work models are used to estimate transmission thresholds for dengue. This is important in assessing comparative risks as to the impact of "doing nothing." In many potential DoD deployments, careful comparative risk assessments likely will alleviate the need for *any* pesticide use when risks of transmission are unequivocally low. In other situations, the risks will be high and the transmission levels many-fold above thresholds. Even in these circumstances, this spatially-based risk assessment approach will be useful in guiding DoD preventive medicine practitioners in selecting least-toxic environmental interventions.

The estimates of transmission thresholds for dengue presented here, at least for ambient air temperatures ranging between 26 and 30°C, here are consistent with the only field estimate available, <0.25 *Ae. aegypti* pupae per person in Honduras, and with a theoretical estimate made by Newton and Reiter for Puerto Rico. We hope our estimates will be of value in directing control operations and influencing policies pertaining to dengue. In our view and in the view of others, much of what is currently accepted or recommended regarding source reduction for control is questionable and in need of modification. So, while the acceptance of a model by others cannot vouch for its validity, a partial list of the current use of CIMSiM and DENSiM in various projects is given here to help others evaluate their adequacy for this task: The entomological assumptions of dengue control are being evaluated in an NIH-funded project in Peru involving the U. S. Navy, University of California-Davis, and USDA. A second NIH-funded study of the biting behavior of *Ae. aegypti* employs the models for analysis. The impact of anticipated climate change on dengue is being evaluated in an EPA/ORD-funded project in Indonesia, the southern United States, Puerto Rico, and northern Mexico. The models are also being used to investigate the etiology of DHF/DSS, the impact of El Niño Southern Oscillations (ENSO), and provide guidance in vaccine trials and control in Indonesia in a cooperative project involving the Gadjah Mada University, the U. S. Navy, and USDA. They have or are being used for operational or risk assessment purposes in Colombia, Venezuela, Brazil, Costa Rica, Honduras, Mexico, Trinidad, Argentina, Australia, and New Zealand. And finally, the models are a component of an dengue early warning system being developed by the Interagency Research Program on Infectious Diseases (IntRePID).

In light of estimates of the actual density of *Ae. aegypti* per person seen in various dengue-prone areas (Table 6), the results of this analysis are only somewhat encouraging from the perspective of controlling dengue through sustained suppression of the vector via source reduction. At best, they provide a basis for evaluating the probability of success of source reduction efforts. The ratio of *Ae. aegypti* pupae to human density has been observed in limited field studies to range between 0.34 and >60 in 25 sites in dengue-endemic or dengue-susceptible areas in the Caribbean, Central America, and Southeast Asia. These areas reflect diverse risks for potential DoD troop deployments. If, for purposes of illustration, we assume an initial seroprevalence of 33%, in Puerto Rico, Honduras, and Bangkok, Thailand, the degree of suppression required to essentially eliminate the possibility of summertime transmission was estimated to range between 10 and 83%; however in Mexico and Trinidad, reductions of >90% would be required. DoD deployments in these areas would require different preventive medicine strategies --- source reduction in the former, and personal repellent use in the latter. A clearer picture of the actual magnitude of the reductions required to eliminate the threat of transmission is provided by the ratio of the observed standing crop of *Ae. aegypti* pupae per person and the threshold. For example, in a site in Mayaguez, Puerto Rico, the ratio of observed and threshold was 1.7- roughly meaning that about 7 out of every 17 breeding containers would have to be eliminated. For Reynosa, Mexico, with a ratio of ca. 10, 9 containers out of every 10 would have to be eliminated. For sites in Trinidad with ratios averaging ca. 25, the elimination of 24 of every 25 would be required. With the exceptions of Cuba and Singapore, no published reports of sustained source reduction efforts have achieved anything near these levels of reductions in breeding containers.

Finally, these results should be useful in anticipating the consequences of proposed climate change and in estimating the consequences of the somewhat predictable temperature anomalies associated with El Niño Southern Oscillation (ENSO) events.

Tables.

Table 1. Estimates from CIMSiM of the daily gonotrophic development rate and wet weight of emerging *Ae. aegypti* females as a function of constant temperatures.

| Temperature (°C) | Development rate ($da^{-1}$) | Weight (mg) |
|---|---|---|
| 22 | .165 | .233 |
| 24 | .199 | .232 |
| 26 | .240 | .233 |
| 28 | .288 | .236 |
| 30 | .344 | .244 |
| 32 | .411 | .275 |

Table 2. Daily percentage survival of adults ($S_{adult}$), eggs ($S_{egg}$), larvae ($S_{larval}$), and pupae ($S_{pupal}$), the possibility of egg hatch, and the possibility of an adult population under conditions of constant temperatures as indicated by CIMSiM. Parameter values in CIMSiM and the field and laboratory observations upon which they were based have been presented earlier. Temperatures at or below 20 °C do not permit adult populations to develop because of the failure of eggs to hatch. Temperatures >34 or 35 °C eliminate the possibility of adult populations because the aggregate survivals of all stages are insufficient.

| Temperature (°C) | Population | $S_{adult}$ | $S_{egg}$ | $S_{larval}$ | $S_{pupal}$ | Egg hatch |
|---|---|---|---|---|---|---|
| 20 | - | 89 | 96.5 | 99 | 99 | - |
| 22 | + | 89 | 92 | 99 | 99 | + |
| 24 | + | 89 | 88 | 99 | 99 | + |
| 26 | + | 89 | 84 | 99 | 99 | + |
| 28 | + | 89 | 78-80 | 99 | 99 | + |
| 30 | + | 89 | 68-72 | 99 | 99 | + |
| 32 | + | 89 | 58-62 | 99 | 99 | + |
| 34 | + | 89 | 52-55 | 99 | 99 | + |
| 36 | - | 81 | 46-49 | 99 | 99 | + |
| 38 | - | 64 | 39-42 | 99 | 99 | + |
| 40 | - | 47 | 32-35 | 88-99 | 88-99 | + |
| 42 | - | 30 | 25-28 | 53-70 | 53-70 | + |
| 44 | - | 13 | 13 | 18 | 18 | + |

Table 3. Observed [a] and predicted [b] lengths of the pupal development ($PD_{temp}$) period.

| Temperature (°C) | $\log_e$(Temperature) | $PD_{temp}$ (days) Predicted [b] | Observed [a] |
|---|---|---|---|
| 15 | 2.7080 | 7.27 | 8.49 |
| 20 | 2.9957 | 4.86 | 3.11 |
| 22 | 3.0910 | 4.06 | - |
| 24 | 3.1780 | 3.33 | - |
| 25 | 3.2188 | 2.99 | 3.03 |
| 26 | 3.2581 | 2.66 | - |
| 27 | 3.2958 | 2.34 | 1.79 |
| 28 | 3.3322 | 2.04 | - |
| 30 | 3.4012 | 1.46 | 1.82 |
| 32 | 3.4657 | 0.92 | - |
| 34 | 3.5264 | 0.41 | 1.09 |

[a] Data from Rueda et al.

[b] Regression model used was $PD_{temp} = 29.97723 - 8.38467*\log_e(\text{temperature})$; $R^2 = 0.850$, standard errors and $P$ values for the intercept and temperature coefficients were 5.6340 and $P = 0.006$, and 1.7593 and $P = 0.009$, respectively.

Table 4. The pupal development ($PD_{temp}$) period, expected daily number of newly-emerged females and associated standing crop of females of all ages for a standing crop of 100 *Ae. aegypti* pupae as a function of temperature. Methods of calculation are presented in the text and assume steady-state conditions and constant temperatures.

| Temperature | $PD_{temp}$ (days) | Number of adult females | | Ratios of standing crops | |
|---|---|---|---|---|---|
| | | New | Standing crop | Pupae/female | Females/pupa |
| 22 | 4.06 | 10.22 | 87.7 | 1.14 | 0.88 |
| 24 | 3.33 | 12.46 | 106.9 | 0.94 | 1.07 |
| 26 | 2.66 | 15.61 | 133.9 | 0.75 | 1.34 |
| 28 | 2.04 | 20.37 | 174.8 | 0.57 | 1.75 |
| 30 | 1.46 | 28.44 | 244.0 | 0.41 | 2.44 |
| 32 | 0.92 | 45.20 | 387.9 | 0.26 | 3.88 |

Table 5. Estimated number of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year resulting from 12 monthly viral introductions of a single viremic individual, the *Monthly Introduction* threshold. In a series of simulations in DENSiM, these values resulted in a 10% or greater rise in prevalence ca. 50% of the time.

| Temperature (°C) | Transmission threshold by initial seroprevalence of antibody | | |
|---|---|---|---|
| | 0% | 33% | 67% |
| 22 | 7.13 | 10.70 | 23.32 |
| 24 | 2.20 | 3.47 | 7.11 |
| 26 | 1.05 | 1.55 | 3.41 |
| 28 | 0.42 | 0.61 | 1.27 |
| 30 | 0.10 | 0.15 | 0.30 |
| 32 | 0.06 | 0.09 | 0.16 |

Table 6. Comparison of observed numbers of *Ae. aegypti* pupae per person in various dengue-endemic or dengue-receptive locations with estimated transmission thresholds based on average summertime temperatures and an initial seroprevalence of 33%.

| Location | Temp (°C) [a] | Pupae per person [b] | Threshold [c] | Ratio [d] | % Control [e] |
|---|---|---|---|---|---|
| Reynosa, Mexico [f] | 29.4 | 2.75 | 0.26 | 10.4 | 90 |
| Mayaguez, Puerto Rico [f] | 26.6 | 1.73 | 1.05 | 1.7 | 40 |
| Trinidad (20 sites) | 27.0 | 22.7 [g] | 0.86 | 26.4 | 96 |
| El Progreso, Honduras | 29.1 | 0.34 | 0.31 | 1.1 | 10 |
| San Juan, Puerto Rico [f] | 27.8 | 2.75 | 0.58 | 4.7 | 79 |
| Bangkok, Thailand | 29.2 | 1.69 | 0.29 | 5.8 | 83 |

[a] *Temp* refers to average temperature during the months of June through August or December through February in locations above and below the equator, respectively.

[b] *Pupae per person* refers to the average number of *Ae. aegypti* pupae per person observed in survey.

[c] *Threshold* refers to the estimated transmission threshold for 12 monthly introductions, assuming an initial seroprevalence of 33%.

[d] *Ratio* is the ratio of observed pupae per person and the estimated temperature- and seroprevalence-specific threshold.

[e] *% Control* is the degree of reduction in pupae per person necessary to reduce observed field level to that of the threshold.

[f] Unpublished studies conducted by DAF in collaboration with others. Surveys in Puerto Rico and Mexico were limited and preliminary.

[g] Observed range: 1.4 – 63.4 pupae per person; the island-wide average is used for calculation.

Table 7. Estimated number of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year under conditions of a single viral introduction of one viremic individual on day 90 of the year- the *Single Introduction$_1$* threshold. In a series of simulations in DENSiM, these values resulted in a 10% or greater rise in prevalence ca. 50% of the time.

| Temperature (°C) | Transmission threshold by initial seroprevalence of antibody | | |
|---|---|---|---|
| | 0% | 33% | 67% |
| 22 | 9.57 | 14.10 | 30.55 |
| 24 | 2.92 | 4.47 | 9.22 |
| 26 | 1.42 | 2.03 | 4.26 |
| 28 | 0.53 | 0.75 | 1.69 |
| 30 | 0.13 | 0.19 | 0.38 |
| 32 | 0.07 | 0.10 | 0.26 |

Table 8. Estimated number of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year under conditions of a single viral introduction of 2 viremic individuals on day 90 of the year- the *Single Introduction$_2$* threshold. In a series of simulations in DENSiM, these values resulted in a 10% or greater rise in prevalence ca. 50% of the time.

| Temperature (°C) | Transmission threshold by initial seroprevalence of antibody | | |
|---|---|---|---|
| | 0% | 33% | 67% |
| 22 | 9.16 | 12.83 | 29.15 |
| 24 | 2.68 | 4.21 | 8.68 |
| 26 | 1.23 | 1.98 | 4.01 |
| 28 | 0.48 | 0.72 | 1.38 |
| 30 | 0.12 | 0.18 | 0.35 |
| 32 | 0.07 | 0.10 | 0.18 |

Table 9. Estimated number of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year under conditions of a single viral introduction of 4 viremic individuals on day 90 of the year- the *Single Introduction$_4$* threshold. In a series of simulations in DENSiM, these values resulted in a 10% or greater rise in prevalence ca. 50% of the time.

| Temperature (°C) | Transmission threshold by initial seroprevalence of antibody | | |
|---|---|---|---|
| | 0% | 33% | 67% |
| 22 | 8.02 | 11.66 | 24.66 |
| 24 | 2.52 | 3.69 | 7.76 |
| 26 | 1.09 | 1.80 | 3.79 |
| 28 | 0.47 | 0.63 | 1.33 |
| 30 | 0.11 | 0.18 | 0.33 |
| 32 | 0.06 | 0.09 | 0.18 |

Table 10. Estimated number of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year under conditions of a single viral introduction of 8 viremic individuals on day 90 of the year- the *Single Introduction$_8$* threshold. In a series of simulations in DENSiM, these values resulted in a 10% or greater rise in prevalence ca. 50% of the time.

| Temperature (°C) | Transmission threshold by initial seroprevalence of antibody | | |
|---|---|---|---|
| | 0% | 33% | 67% |
| 22 | 7.13 | 10.69 | 22.11 |
| 24 | 2.20 | 3.27 | 7.02 |
| 26 | 1.08 | 1.57 | 3.24 |
| 28 | 0.41 | 0.62 | 1.27 |
| 30 | 0.09 | 0.15 | 0.31 |
| 32 | 0.06 | 0.09 | 0.16 |

Figures.

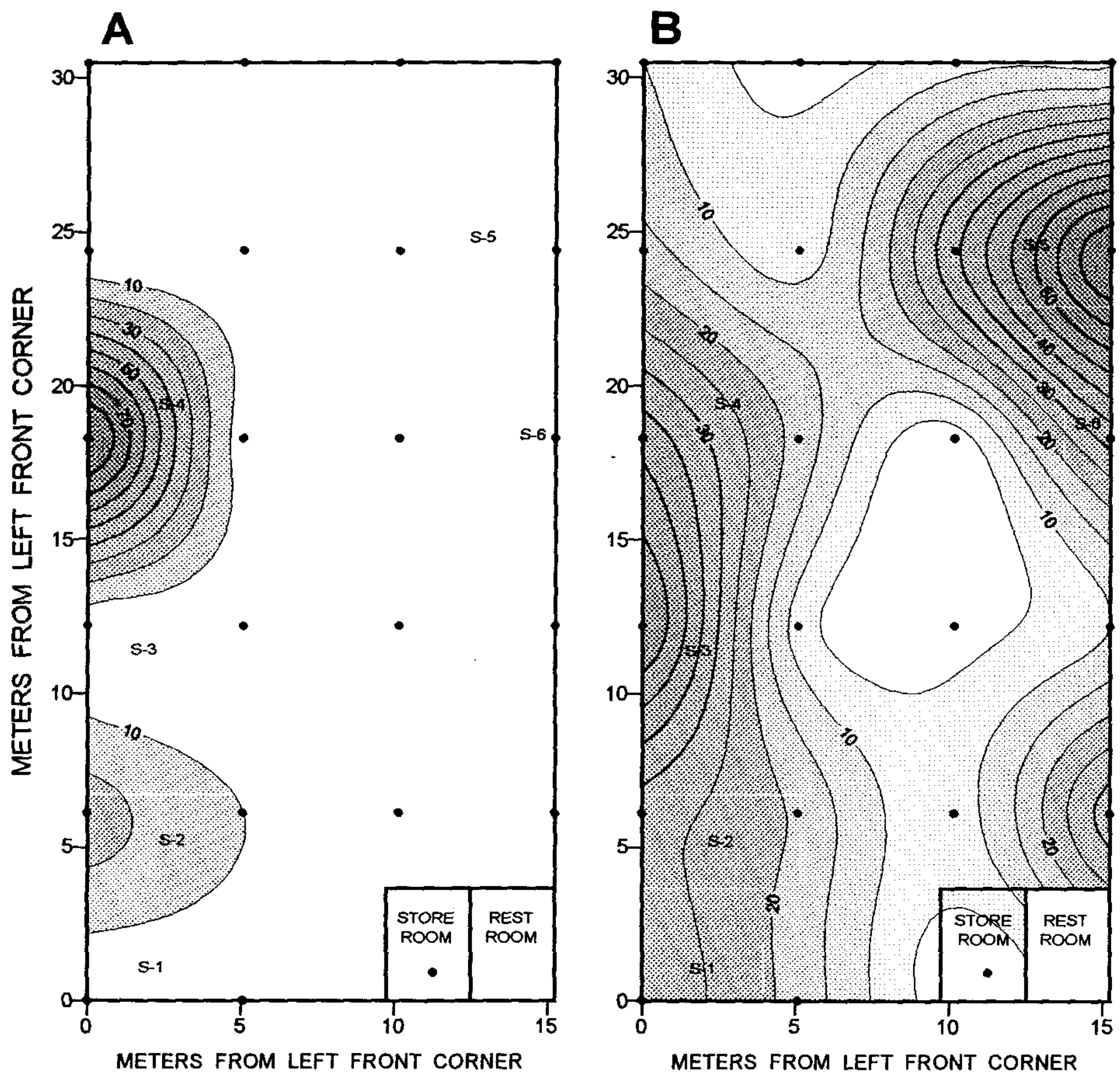


Figure 1. Spatial distribution of insects infesting bagged saw palmetto berries stored in a warehouse in central Florida. (A) *Oryzaephilus mercator* sampled with baited pitfall traps. (B) *Cadra cautella* sampled with pheromone-baited sticky traps. Solid dots indicate trap positions. Fine lines enclosing designations S-1, S-2, ... indicate stacks of bags.

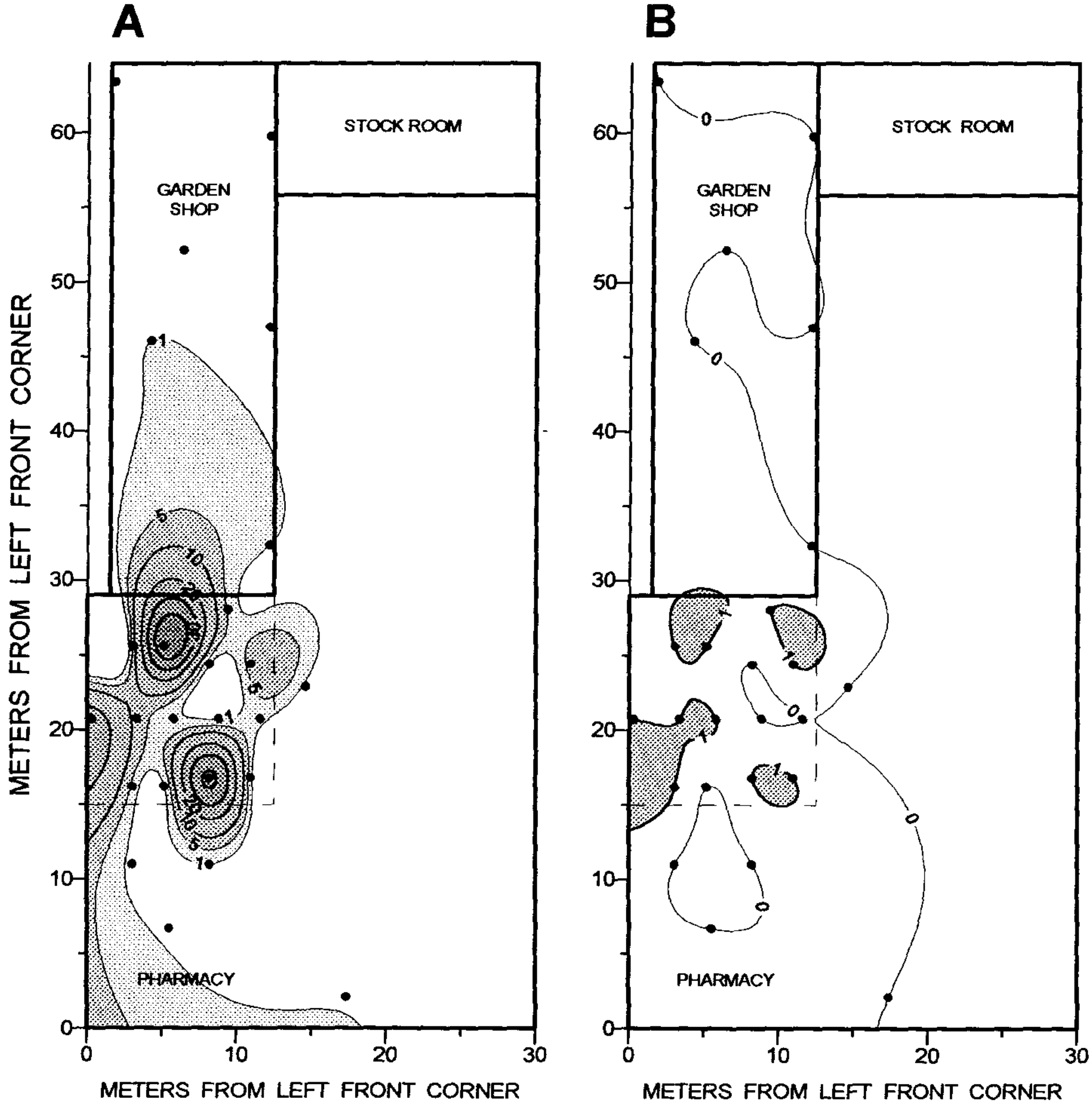


Figure 2. Spatial distribution of *Plodia interpunctella* infesting the pet supply department and adjacent areas of a large retail department store. The contours represent the cumulative totals of moths captured in pheromone-baited sticky traps over a 96-hr period. Trap locations are indicated by solid dots. Dashed lines indicate the extent of the pet supply department. (A) Contours of raw trap counts. (B) Indicator contours obtained by assigning a value of 1 to all trap locations with normalized catch $\leq 0.95$ and a value of 0 to all others. At least 95 % of captures are expected to occur within the shaded areas enclosed by the contours with a value of 1. In this particular case, the probability of capturing two or more moths in any trap within the shaded areas is about 95 %.

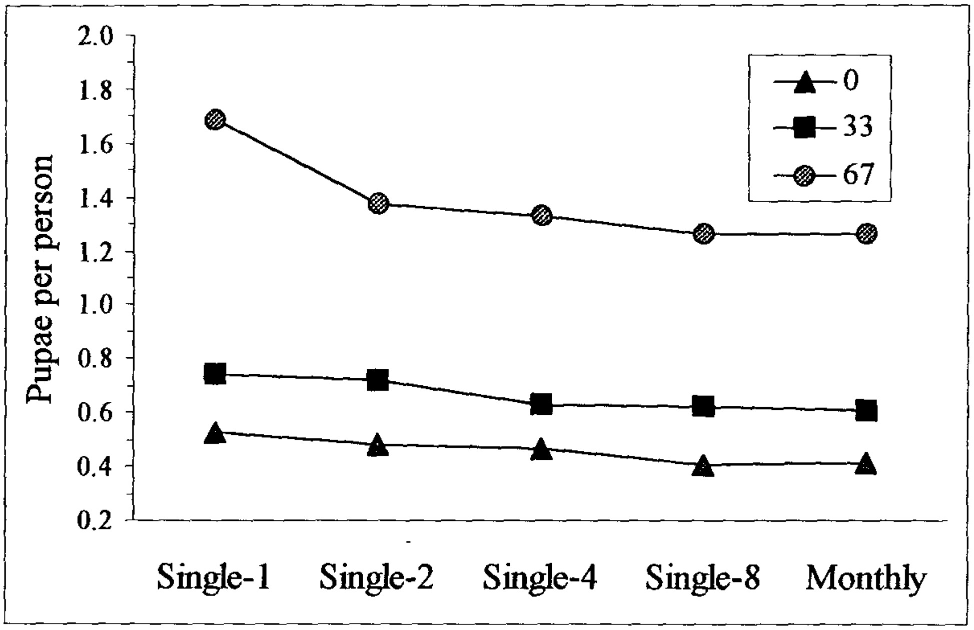


Figure 3. Transmission thresholds at 28°C for each type of introduction by initial seroprevalence of antibody (see text for definitions of introduction types).