ANNUAL PROJECT SUMMARY REPORT

FOR

RICELAND MOSQUITO MANAGEMENT PROGRAM

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Submitted by

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Mosquito Population Ecology Effort:

This laboratory is continuing its program to understand the distribution and pest status of sibling species of *Anopheles quadrrimaculatus*, which used to be known as "the Common Malaria Mosquito". It has recently been shown by geneticists at USDA-ARS, Gainesville, FL, that *An. quadrrimaculatus* actually consists of at least four species: A, B, C, and D. These species cannot be identified morphologically, although electrophoresis of isozyme loci, breeding tests, and chromosomal studies have shown the species to be quite distinct. Analysis of mosquito samples collected from Mississippi during 1990 was completed using starch gel electrophoresis during the first six months of 1991.

1) Statewide survey of adults.

A survey across the state of Mississippi has revealed the presence of only three of the four sibling species: A, B, and D (Fig. 1). *Anopheles* mosquitoes are a major pest of man and animals in the Mississippi Delta; in this part of Mississippi, represented in our samples by Coahoma, Bolivar, Grenada, Humphreys, and Sharkey Cos., species A is the only species found. Species B, and more rarely, species D show sporadic appearances in the central Hill Country and in the Southern part of Mississippi. It is clear that species A is the major pest in all areas.

2) Larval survey at Noxubee National Wildlife Refuge.

In trying to understand the pest status of the sibling *Anopheles* species, we have sampled water bodies in Noxubee National Wildlife Refuge in great detail, where all three of Mississippi's sibling species occur in relatively high frequencies. Here (Fig. 2) the species distributions are similar to those in the state as a whole. In all areas, species A is much the commonest. In a small group of sites in forest near the Noxubee River, species D makes up over 25% of the numbers, but is otherwise rare. Species B is also rare in most sites.

In Fig. 3, the data from Noxubee Refuge has been split into collections made in July and August and those made, often at the same sites, in September and October. The environments were classified into standing and moving water, and sunny and shady sites. On the right hand side of each 6-bar chart can be seen the relative frequencies of the three species *An. quadrrimaculatus* (all sibling species, Q), *An. crucians* (C), and *An. punctipennis* (P). On the left side of the diagram, the *An. quadrrimaculatus* bar is split into its subsidiary sibling species (QA, QB, QC). Several conclusions can be drawn from this diagram. (1) *An. punctipennis* is relatively commoner in moving water than in standing water, in shady sites than in sunny sites, and later in the season than earlier. (2) *An. crucians* is commoner in sunny sites in July and August than at any other time or place. (3) The *An. quadrrimaculatus* sibling species are commoner in still water in July and August than at other times and places. (4) There doesn't seem to be any
clear distinguishing environmental feature favouring one sibling species of 
*Anopheles quadrimaculatus* over another.

**Survey, Prediction and Modeling Effort:**

Work has continued using population genetic models to understand and 
predict the evolution of insecticide resistance, with a view to aiding decisions of 
resistance management. There has been a controversy between those who claim 
that mixtures of insecticides can control insects prone to resistance for longer than 
rotations, and those who claim the reverse. Recently, much attention has been 
given to the claim that mixtures do not usually work under field conditions. The 
work in this laboratory indicates that while rotations of insecticides may sometimes 
outperform mixtures, a rotation is theoretically limited to giving control for more 
than twice as long as a mixture. In contrast, under some conditions mixtures may 
give thousands of times the control period of a rotation.

Work was also performed in collaboration with Dr. R.G. Luttrel on 
approaches to incorporate population dynamics realistically in models for insecticide 
resistance, to give a true model of insecticidal control failure. While the theory 
(Mallet & Luttrel, 1991) was built around *Heliothis* on cotton, the models are 
general. General conclusions are: (1) Population dynamic realism makes little 
difference to control failure provided that populations tend to erupt after 
resistance evolves; the simplest gene frequency model is the most important 
determinant of population outbreak under these conditions. (2) Many strategies 
for resistance management which depend on altering the dominance (e.g. "high 
dose", "low dose") will not work because shallow dosage response curves in the 
field are liable to cause intermediate dominance in almost all cases.

**Economic Impact Assessment Effort:**

Work continued testing laboratory strains for the efficacy of two 
insecticides, permethrin and malathion, on *Anopheles quadrimaculatus* in 
Mississippi. The insides of vials were coated with various doses of insecticide, and 
stored in freezers (malathion) or in cool, dark rooms (permethrin). The 
mosquitoes were placed in the vials for varying lengths of time (3, 6, and 9 hrs). 
The results show that the Orlando laboratory strain is considerably more 
susceptible both to malathion and to permethrin than adult mosquitoes brought in 
from Noxubee and Cleveland (Figs. 4,5); in addition, the malathion dosage-
mortality curves of wild mosquitoes have a much lower slope than the those of the 
Orlando lab strain (Fig. 5), suggesting that individuals collected from the field are 
extremely heterogeneous for resistance. In contrast, the slopes for permethrin 
dosage mortality curves are similar between laboratory (Orlando species A) strains 
and wild-caught individuals (Noxubee and Cleveland) (Fig. 4). These results do 
suggest that wild populations of *An. quadrimaculatus* are tolerant to malathion,
and, to a lesser extent, permethrin; alternative control measures should be adopted if possible.

The data of Figs. 4, 5 were based on adults collected in the wild, and were compared to laboratory-reared Orlando Anopheles quadrinaculatus species A. In summer 1991 we performed tests using adults reared from eggs laid by individual females collected in the wild in order to (1) test whether the previous evidence for resistance was a spurious result of laboratory rearing, and (2) test whether Anopheles quadrinaculatus species A, B, and D differed in susceptibility to insecticides.

Results of these brood-by-brood analyses are shown from Noxubee (Fig. 6) and Cleveland (Fig. 7). The results clearly show that resistance to malathion is not an artifact of laboratory rearing; laboratory reared broods of An. quadrinaculatus species A from Noxubee were about 10x to 100,000x resistant compared with a laboratory strain of species A (Fig. 6). Broods of species B and one brood of species D differed little from the susceptible laboratory strain of species A, suggesting that these other species have no resistance to malathion in the field. All species were similar in their susceptibility to permethrin, but once again, wild species A from Noxubee were marginally more tolerant (approx. 2x-10x) than the laboratory strain of species A or the other wild species. In the vicinity of Cleveland, Ms., only species A has been found. Here tests of species A broods showed an approximately 100x-10,000x resistance to malathion, and 50-100x resistance to permethrin (Fig. 7). There also seems to be approximately 10x resistance to permethrin in Cleveland species A compared to Noxubee species A (Figs. 6, 7). Although this resistance currently causes little reduction in control using synergized resmethrin ULV (J. Olson & M. Ponder, pers. comm.), the results do not bode well for the continued future control of Anopheles quadrinaculatus in the Cleveland area.

If these results hold true throughout the range of the An. quadrinaculatus species complex, it seems likely that species B and D will be restricted to areas of low pesticide input (especially of organophosphates and pyrethroids). This suggests that Anopheles could be used as bioindicators of water quality. Our results from the statewide survey certainly indicate that species A is the commonest species throughout the state, and that species B and D are restricted, in general, to wooded areas where agriculture is less intensive. The Mississippi Delta is the major cotton-growing area of the state, and many applications of parathion, malathion, guthion, and synthetic pyrethroids are used there for boll weevil and Heliothis control. We have not detected any species of Anopheles quadrinaculatus apart from species A in the Delta (Fig. 1).

Personnel:
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Publications:


Meetings attended:


FIGURE 1. Distribution of *Anopheles quadrimaculatus* species A, B, and D across the state of Mississippi.
FIGURE 2. Distribution of sibling species of Anopheles quadrimaculatus in the Noxubee National Wildlife Refuge, Mississippi.

NOXUBEE NATIONAL WILDLIFE REFUGE

Anopheles quadrimaculatus sibling species

Larval survey, 1990

0 1 2
FIGURE 3. Larval survey broken down by environmental components

ANOPHELES IN NOXUBEE WILDLIFE REFUGE

SUNNY SITES, JULY/AUGUST 1990

SUNNY SITES SEPT/OCT 1990

SHADY SITES, JULY/AUGUST 1990

SHADY SITES, SEPT/OCT 1990
FIGURE 4. Dosage mortality curves from vial tests after 3, 6, and 9 hours.
FIGURE 5. Dosage mortality curves from vial tests after 3, 6, and 9 hours.

MALATHION

LD_{50} (µg/vial)
Figure 6. LD50 values for permethrin and malathion taken from individual broods of sibling species of *Anopheles quadrinaculatus* from Noxubee National Wildlife Refuge, Mississippi, and from a susceptible laboratory strain. Data from 6hr vial tests. Species are as follows: Orlando laboratory strain of species A, each point represents a different date the laboratory strain was tested (O); species A from Noxubee, each point is a different single-female brood (A); single-female brood of species B from Noxubee (B); single-female brood of species D from Noxubee (D).
Figure 7. LD50 values for permethrin and malathion taken from individual broods of *Anopheles quadrimaculatus* species A from various sites near Cleveland, Mississippi, and from a susceptible laboratory strain. Data from 6 hr vial tests. Strains are as follows: lumped broods of species A from near Cleveland, each point represents a separate collecting site (A); laboratory strain of species A (O). All *An. quadrimaculatus* collected from near Cleveland were species A.