ANNUAL PROJECT SUMMARY REPORT

FOR

RICELAND MOSQUITO MANAGEMENT PROGRAM

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Submitted by

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Mosquito Population Ecology Effort:

A broad investigation of the biology of the *Anopheles quadrimaculatus* complex was undertaken in the state of Mississippi, focusing particularly on Noxubee Wildlife Refuge, an area of sympatry for three sibling species: A, B, and D.

1) Statewide survey of adults.

In order to determine which sibling species were most likely to be pests, collections of adult *An. quadrimaculatus* were made throughout Mississippi and frozen at -80°C. Some of these samples have now been analyzed using starch gel electrophoresis. The enzyme loci IDH-1, IDH-2, GOT-1, GOT-2, GPI, ME, MDH-1, and HAD were stained, because these loci have fixed or nearly fixed genetic differences between sibling species A, B, C and D (see Table 2 of last year's Mississippi State report). The results of species-level identifications are given in Table 1.

In an area of sympatry between species A, B and D, there is again evidence for microhabitat preferences of adults of at least two of the species. For instance, species A and B are found at the different collecting sites in Noxubee Refuge in significantly different proportions (excluding the campground, $G = 15.95$, 4 d.f., $P < 0.01$). Over the whole state, species B and D seem mainly to be found in the Hill Country; elsewhere species A is the main mosquito. This is particularly true of the Delta region, where *An. quadrimaculatus* is a severe nuisance. *An. quadrimaculatus* populations have been collected from a total of 33 sites across Mississippi; most of these remain to be analyzed. From these collections, a fuller picture will emerge than is now available.

2) Larval survey at Noxubee National Wildlife Refuge.

To investigate the microhabitat preferences of *Anopheles* species, larval collections were made in a variety of puddles, ponds, lakes, and rivers in Noxubee Refuge. These larvae were then reared to maturity, and the resulting adults were identified to species using morphological characters. All emerging adult *An. quadrimaculatus* were immediately stored frozen at -80°C in order to preserve enzymes for electrophoretic analysis and sibling species identification. Three sets of collections were made in 1990: May/June, July/August, and September/October. The May/June collection is shown in Table 2. There were low *Anopheles* densities at this time of year, and we had problems rearing those we did collect, so the data are scanty. The other two collections remain to be analyzed, but sample sizes were much greater.

It can be seen from Table 2 that *An. crucians* frequently co-occurs with *An. quadrimaculatus* sibling species, but that *An. punctipennis* is found more often where other *Anopheles* species do not occur. In the May/June collections, we frequently found *An. punctipennis* larvae in muddy roadside puddles, as well as in slow-moving clear, shaded streams. Later in the season, we chiefly found *An. punctipennis* in clear woodland streams; in part perhaps because there was less rain, and thus fewer puddles. *An. quadrimaculatus* and *An. crucians*, on the other hand, were consistently found in more permanent bodies of water. However, it would be hard to separate *An. crucians* and *An. quadrimaculatus* on the basis of the sites examined in Table 2; the two species clearly have similar habitat preferences. Later in the season, *An. crucians* appeared rarer, and *An. quadrimaculatus* was commoner; we found sites where only one or the other were common. In view of the close similarities between the habitats in which *An. quadrimaculatus* and *An. crucians* are found, it seems probable that differences between the sibling species of *An. quadrimaculatus* will be slight. On the basis of Table 2, it would seem that species B is generally found in slowly-moving water, species D in standing water, and species A in both standing and moving water. These possibilities can be confirmed or refuted by means of electrophoretic analysis of the later larval collections. Electrophoresis is now under way.

Economic Impact Assessment Effort:

Work was started in 1989 on the development of a vial test to assess the insecticide resistance potential of natural populations of mosquitoes. This work was continued and amplified in 1990. The method is based on Dr. Bill Plapp's adult vial test as applied to the moth *Heliothis virescens*. Dr. Plapp informs me that the *Heliothis* vial test is itself based on the WHO paper residue tests, as used on insects of medical importance. The mosquito vial test is useful because the apparatus can be made up in many laboratories, and kits do not need to be ordered from Geneva. Known quantities of insecticide are dissolved in acetone and injected into standard disposable scintillation vials. These vials are then rolled on a hot dog roller (with the heating coils disconnected) until the acetone has evaporated. The vials are then capped, and kept cool in the dark until used. (We store vials treated with malathion in the freezer to avoid degradation of the insecticide). We have used two insecticides commonly used as adulticides in mosquito control: malathion and permethrin. We studied the major problem mosquito in the Delta of Mississippi, *An. quadrimaculatus*, a complex of species, some or all of which used to transmit Malaria before its eradication in the U.S.

TABLE 1
Statewide survey of adult *An. quadrimaculatus* complex sibling species - 1990 data

Site	Numbers of individuals of species: A	B	C	D	?	Total
A) *Hill Country*						
Noxubee Co., Noxubee Wildlife Refuge						
Hut A	47	53	-	9	2	111
Hut B	54	89	-	8	3	154
Boat Dock	36	30	-	1	6	73
Big Trees Trail	5	6	-	1	-	12
Campground	2	-	-	-	-	2
Tree Holes	-	9	-	1	-	10
Total Noxubee	144	187	-	20	11	362
Union Co., Etta	38	-	-	-	-	38
Madison/Hinds Co., Jackson	27	8	-	-	3	38
Copiah Co., Georgetown	10	21	-	-	5	36
Attala Co., Kosciusko	16	-	-	-	4	20
Panola Co., Sardis Dam	38	-	-	-	-	38
B) *Mississippi Delta*						
Bolivar Co., Boyle	46	-	-	-	3	49
Bolivar Co., Cleveland	27	-	-	-	1	28
Bolivar Co., 3 mi. N. Clvlnd	70	-	-	-	5	75
Sharkey Co., Delta NF, B.L.	89	-	-	-	20	109
Sharkey Co., Delta NF, C.G.	34	-	-	-	2	36
Washington Co., Stoneville	124	-	-	-	6	130
Humphreys Co., Belzoni	21	-	-	-	3	24
Stuttgart, AR.	14	-	-	-	-	14
C) *Coastal Mississippi*						
Pearl River Co., Picayune	14	-	-	-	3	17
Harrison Co., Gulfport	19	-	-	-	-	19
Jackson Co., 3 mi. W. Wade	9	1	-	-	7	17
GRAND TOTAL						1050

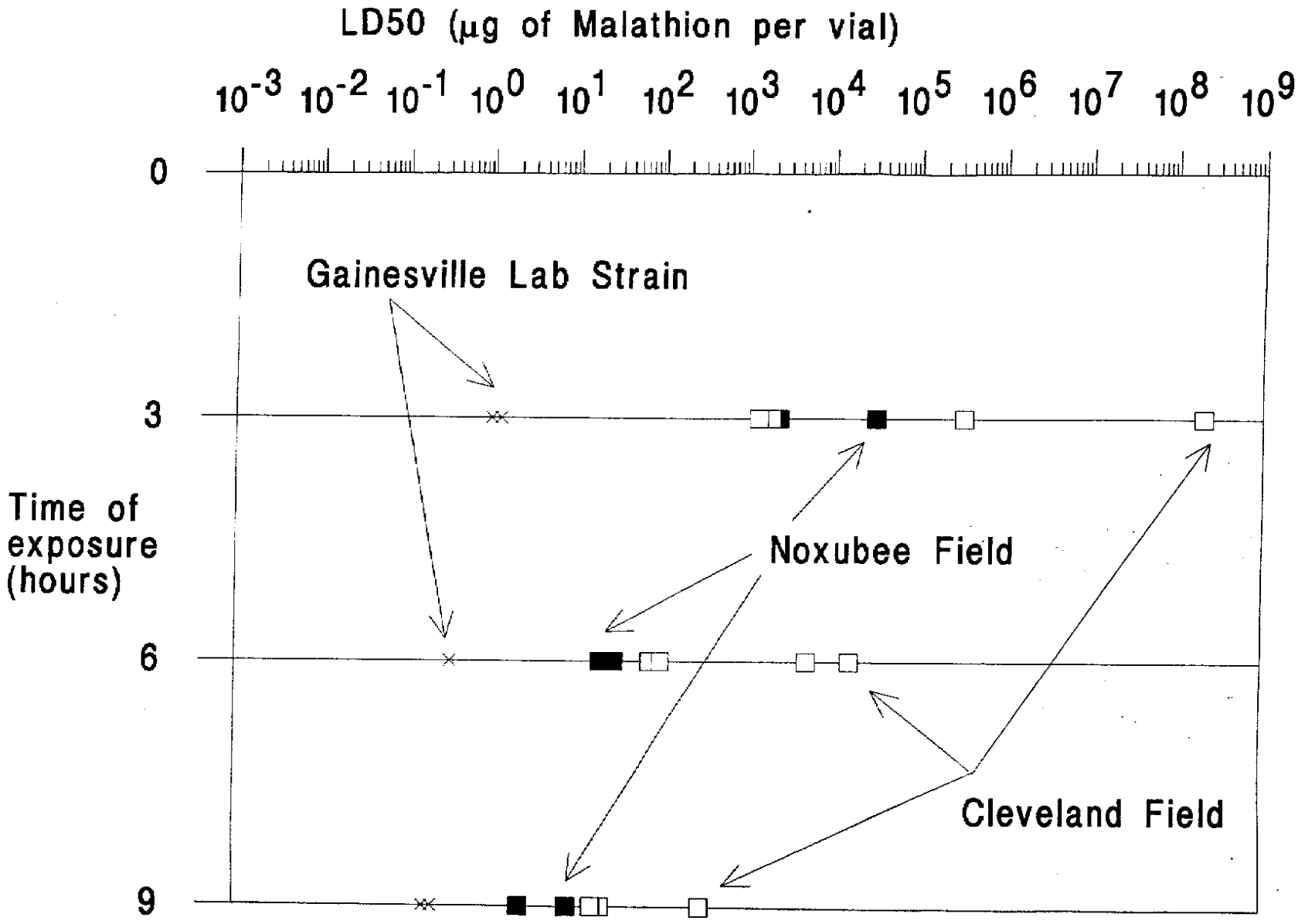
TABLE 2
Larval collections of *Anopheles* at Noxubee Refuge
May/June, 1990

Site	Description	*Anopheles* species *quadrimaculatus* A	B	D	*crucians*	*punctipennis*
1	lake	1	-	-	5	-
2	puddle	-	-	-	-	15
3	canal	-	-	-	3	3
4	lake	3	-	-	8	-
5	lake	1	-	-	-	-
6	creek	-	-	-	-	2
7	puddle	-	-	-	-	15
8	creek	-	-	-	-	10
9	lake	-	-	-	-	-
10	lake	-	-	-	-	-
11	ditch	-	-	-	-	5
12	ditch	-	-	-	-	5
13	tree hole	-	-	-	-	1
14	ditch	-	-	-	-	2
15	ditch	-	-	-	-	5
16	deep ditch	11	14	2	7	-
17	puddle	-	-	-	-	5
18	lake	1	-	-	6	-
19	ditch	-	-	-	-	4
20	swamp	1	-	-	2	6
21	pond	1	-	2	6	-
22	pond	14	-	-	7	-
23	ditch	-	-	-	-	1
24	lake	2	-	6	10	-
25	puddle	-	-	6	-	-
26	lake nr outfl	-	1	-	3	-
27	lake	-	-	-	-	-
28	pond	-	-	-	-	-

The prepared vials were then used to evaluate insecticide efficacy. For each test, 3-15 vials were used per dose, and 8-9 doses were used per test, plus one zero dose as a control, treated with acetone only. Two mosquitoes were placed in each vial, and vials were kept at 25°C (±2°C) for varying lengths of time. After 24h, over 20% of controls usually died, so shorter times tested (3, 6, and 9 hours) proved more useful. Field-caught *An. quadrimaculatus* were run from two locations: (1) near Cleveland, MS. We have only found species A in the Delta region, including many collections near Cleveland (see above). A mosquito abatement district has been recently formed in Cleveland, and cotton, rice, and other crops are grown in the vicinity. Both mosquito control and agricultural production in the area involve rather heavy pesticide use, so it seems likely that the Cleveland populations of *Anopheles* are exposed to high levels of insecticidal selection by pyrethroids and organophosphates. (2) Noxubee Wildlife Refuge, near Starkville, MS. There are three *An. quadrimaculatus* sibling species at Noxubee, A (41%), B (53%), and D (6%). Noxubee Refuge is situated in the Hill Country of Mississippi, away from centers of agricultural production. In addition, Noxubee Refuge is far from large towns, and no insecticides are used in the Refuge itself. Thus, populations of *Anopheles* inhabiting the refuge are unlikely to encounter much insecticidal pressure. (3) A captive strain of *An. quadrimaculatus* was used as an additional control. The strain was originally collected in Orlando, FL, over 45 years ago, and is species A. This strain was supplied by Johnny Jackson, USDA-ARS, Gainesville, FL.

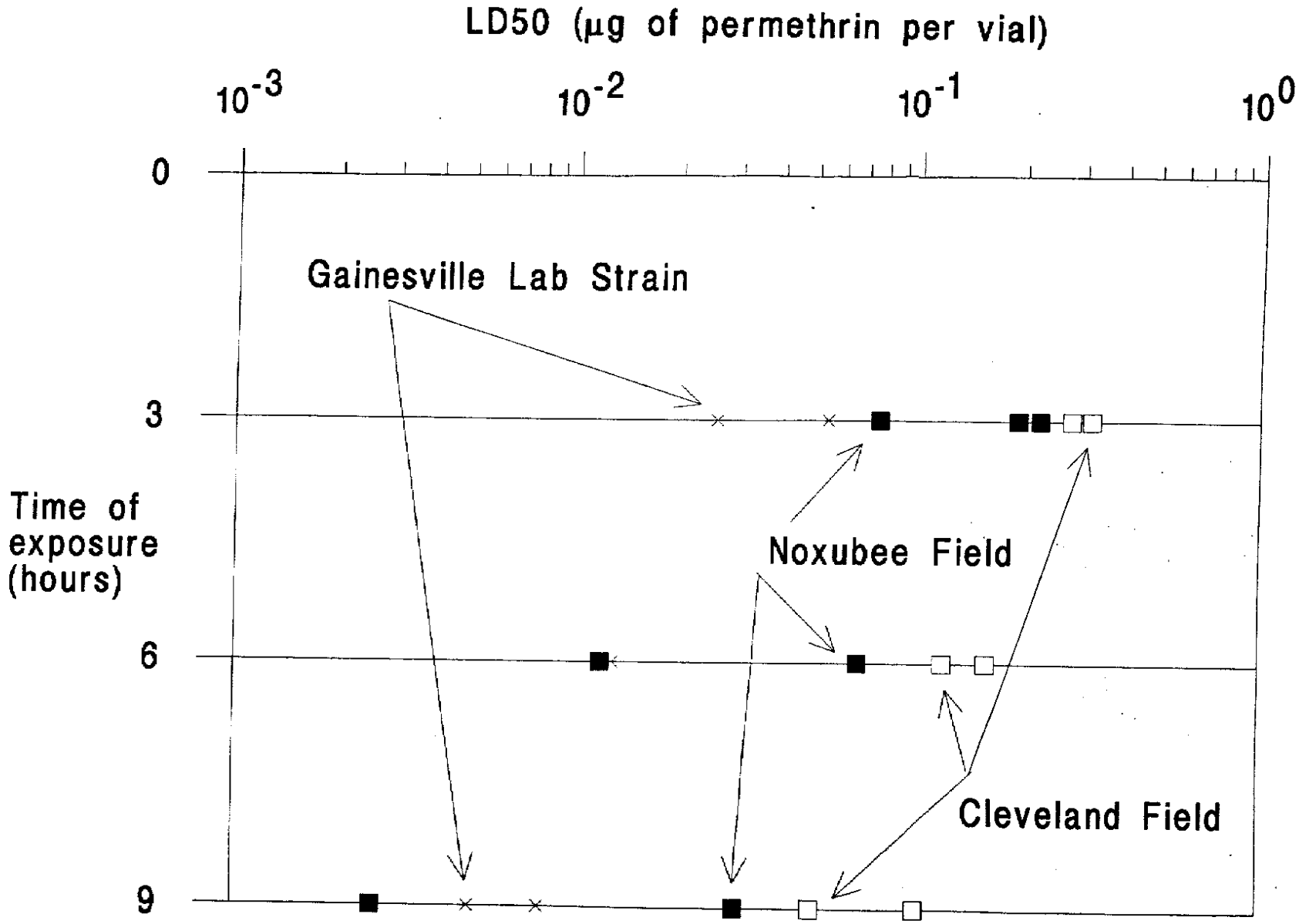
LD_{50} values from all the tests performed in 1990 are shown in Figs. 1 and 2. For malathion (Fig. 1), it can be seen that the Gainesville lab strain is far more susceptible to Malathion than are either of the field strains, by factors of 100-1000. There also appears to be some tendency for the Cleveland field collections to be more tolerant of insecticides than those from Noxubee, though this difference does not show up clearly at 3h of exposure. It should be noted that LD_{50} values greater than about 100 µg/vial are highly suspect, because the highest dose we used was 1000 µg/vial; a dose at which droplets of the insecticide coating the glass can easily be seen with the naked eye. Another feature of the data for malathion (not shown in the figure) is that the slope of the dose-response curves for the field strains were extremely shallow, suggesting a great deal of individual variation in susceptibility. This shallowness of slope, together with the extreme tolerance, explains why the LD_{50} measurements are erratic. In contrast, the lab strain had a steep slope, indicative of very little individual variation.

For permethrin, the Gainesville lab strain was again among the most susceptible of strains, but was in this case scarcely distinguishable from Noxubee field collections, which exhibited highly variable LD_{50}s. In contrast, populations



Malathion / An. quadrimaculatus: Mississippi, 1990

FIGURE 1



Permethrin / An. quadrimaculatus: Mississippi 1990

FIGURE 2

collected from near Cleveland displayed a 2 - 10-fold increase in tolerance over the other strains.

It is still unclear whether resistance to permethrin or malathion has evolved in field populations of *An. quadrimaculatus*. The analyses were carried out using the probit analysis routine in the statistical package SAS, which cannot properly incorporate control mortality. Further analyses will be performed using POLO, a more powerful program for probit analysis. It is also possible that laboratory rearing increases susceptibility to insecticides, which might in part cause the greater susceptibility of the Gainesville strain. Future studies will be performed by making field collections of eggs from gravid females or larvae, rearing adults from the larvae, and exposing the emerging adults to the insecticides. The adults collected from Noxubee contain at least three sibling species: thus the great variability and shallow slopes at Noxubee could be due to strong differences between species. Work is planned to investigate species-specific differences in tolerance. In spite of these caveats, it does seem that there is strong evidence for a greater susceptibility of the laboratory strain, especially to malathion. The Gainesville lab strain was collected before organic insecticides were used widely in agriculture or public health pest control. Unless the laboratory strain has a tendency to evolve susceptibility to insecticides, this strain could represent the primitive susceptible condition in *An. quadrimaculatus* species A.

These vial test results should be validated under field conditions. If field tests indicate that *An. quadrimaculatus* from near Cleveland are resistant to malathion, as is suggested by the results above, malathion will not be useful for control of this species in the area. The susceptibility of these mosquitoes to permethrin should also be monitored continuously, and if the LD_{50} continues to rise the efficacy of pyrethroids should be assessed. These results will have important applications to mosquito control practice in the Cleveland area.

Personnel:

James Mallet, Principal Investigator, Assistant Professor of Entomology.
Robert S. Fritzius, Research Assistant I.
Titiya Chittihunsa, Graduate Research Assistant.

Publications:

Mallet, J. 1990. Evolution of insecticide resistance. Reply. Trends Ecol. Evol. 5:164-165.

Mallet, J. 1991. Insecticide resistance management: a population genetics approach. Proc. Miss. Ent. Assoc. 10 (in press).

Mallet, J., Fritzius, R. 1991. Habitat restriction of sibling species of the *Anopheles quadrimaculatus* complex in Mississippi. (in preparation).

Mallet, J., Fritzius, R., and Chittihunsa, T. 1991. Partitioning of swampy woodlands by larval mosquitoes of the genus *Anopheles*. (in preparation).

Meetings attended:

S-230/RMMP Annual Meeting, Baton Rouge, LA. 15-18 January, 1990.

University of California at Davis. 21-23 February, 1990. Seminar: "Managing insecticide resistance: can population genetics help?"

Mississippi Mosquito Control Workshop, Jackson, MS. 22 March, 1990.

American Mosquito Control Association Meeting, Lexington, KY. 2-6 April, 1990.

Arkansas Mosquito Control Conference, Stuttgart, AR. 19-20 April, 1990. Talk: "Riceland Mosquito Management Project research at Mississippi State University".

Industry-sponsored Insecticide Resistance Management Conference, New Orleans, LA. 26-27 April, 1990.

International Congress of Systematic and Evolutionary Biology, College Park, MD. 1-7 July, 1990.

Collaborative research and informal meeting with Mark Ponder, Director of Mosquito Abatement District, Cleveland, MS. 4-7 September, 1990.

Mississippi Entomological Association Meeting, Starkville, MS. Talk: "Insecticide resistance management: a population genetics approach". 12-14 November, 1990.