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 quadrimaculatus*, which used to be known as "the Common Malaria Mosquito". It has recently been shown by geneticists at USDA-ARS, Gainesville, FL, that *An. quadrimaculatus* 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. quadrimaculatus* (all sibling species, Q), *An. crucians* (C), and *An. punctipennis* (P). On the left side of the diagram, the *An. quadrimaculatus* 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. quadrimaculatus* 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. Luttrell on attempts to incorporate population dynamics realistically in models for insecticide resistance, to give a true model of insecticidal control failure. While the theory (Mallet & Luttrell, 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 quadrimaculatus* 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 quadrimaculatus* 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. quadrimaculatus* 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 quadrimaculatus* in the Cleveland area.

If these results hold true throughout the range of the *An. quadrimaculatus* 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 quadrimaculatus* apart from species A in the Delta (Fig. 1).

Personnel:

James Mallet, Principal Investigator, Assistant Professor of Entomology.
Robert S. Fritzius, Research Assistant I.
Huang-Jiaxing, Graduate Research Assistant.

Publications:

Mallet, J. & Luttrell, R.G. (1991) A Model of insecticidal control failure: the example of *Heliothis virescens* on cotton. Southwestern Entomologist, Supplement 15: 201-212.

Mallet, J. (1991) Insecticide resistance management: a population genetics approach. 37th Mississippi Insect Control Conference, Proc. I: 10-13.

Mallet, J. (1991) ...And a note from Mississippi (about the presence of *Aedes albopictus* in Starkville, MS). American Mosquito Control Association Newsletter, February 1991: 14.

Mallet, J., Fritzius, R. 1992. Habitat restriction of sibling species of the *Anopheles quadrimaculatus* complex in Mississippi. (in preparation).

Mallet, J., Fritzius, R., and Chittihunsa, T. 1992. Partitioning of swampy woodlands by larval mosquitoes of the genus *Anopheles*. (in preparation).

Mallet, J., Fritzius, R. 1992. Sibling species of *Anopheles quadrimaculatus* complex differ in insecticide susceptibility. (in preparation).

Meetings attended:

Entomological Society of America, Southeastern Branch. Orange Beach, Alabama, 9-13 March 1991. Organized symposium: "Biology and Management of Riceland Mosquitoes", 13 March 1991. Talk: "Biology and genetics of sibling species of *Anopheles quadrimaculatus*".

S-230/RMMP Annual Meeting. Gulf Shores, Alabama, 14-15 March 1991.

Mississippi Mosquito Control Workshop, Jackson, MS. 27 March, 1991.

S-230/RMMP Summer meeting. Stuttgart, Ark., 18-19 July, 1991.

Society for the Study of Evolution. Hilo, Hawaii, 27 July - 3 August, 1991. Talk: "Ecology and genetics of sibling species of *Anopheles quadrimaculatus* complex in Mississippi".

Mississippi Entomological Association Meeting. Starkville, MS. 18-20 November, 1990.

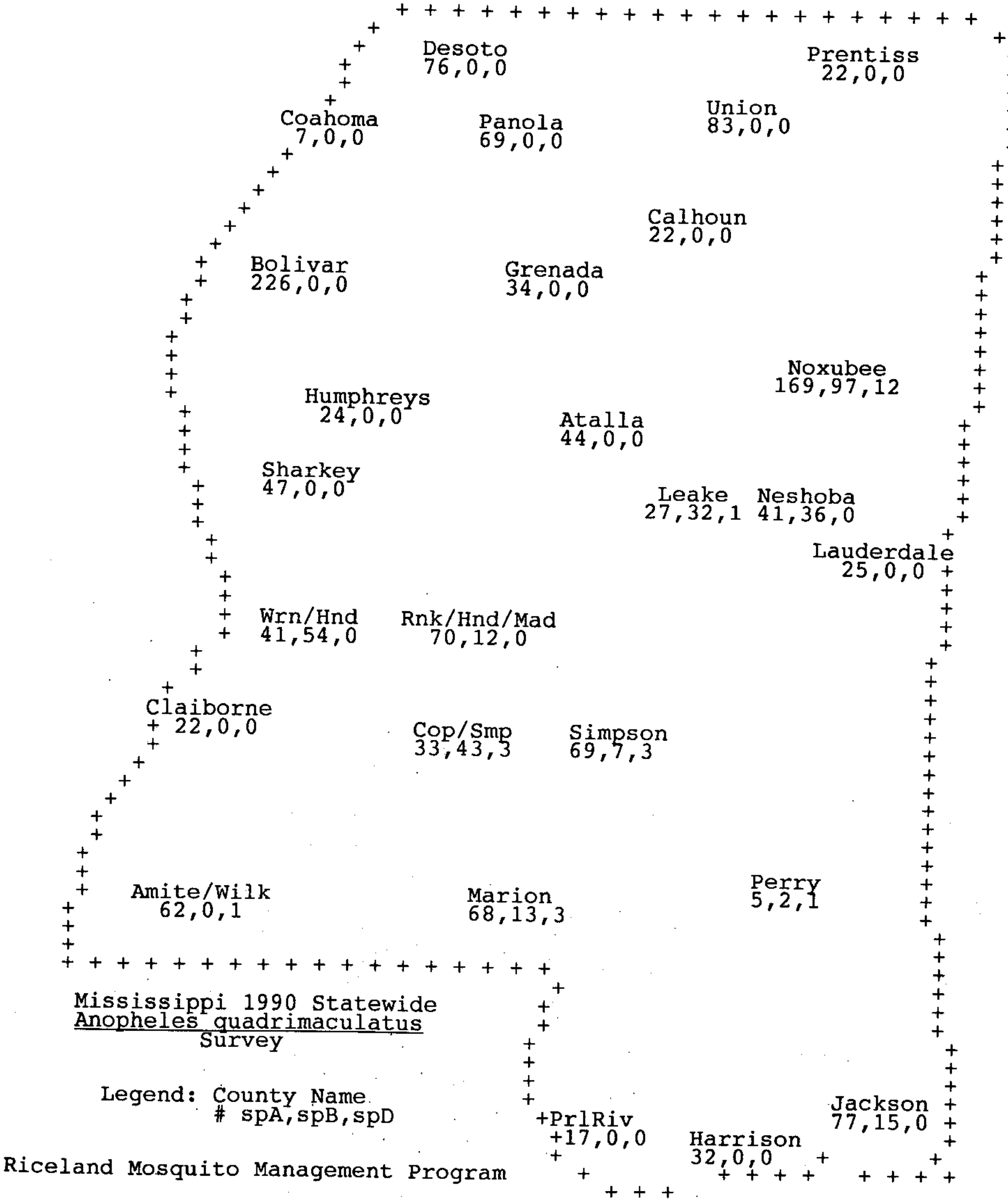


FIGURE 1. Distribution of *Anopheles quadrimaculatus* species A, B, and D across the state of Mississippi.

FIGURE 2. Distribution of sibling species of _Anopheles quadrimaculatus_ in the Noxubee National Wildlife Refuge, Mississippi.

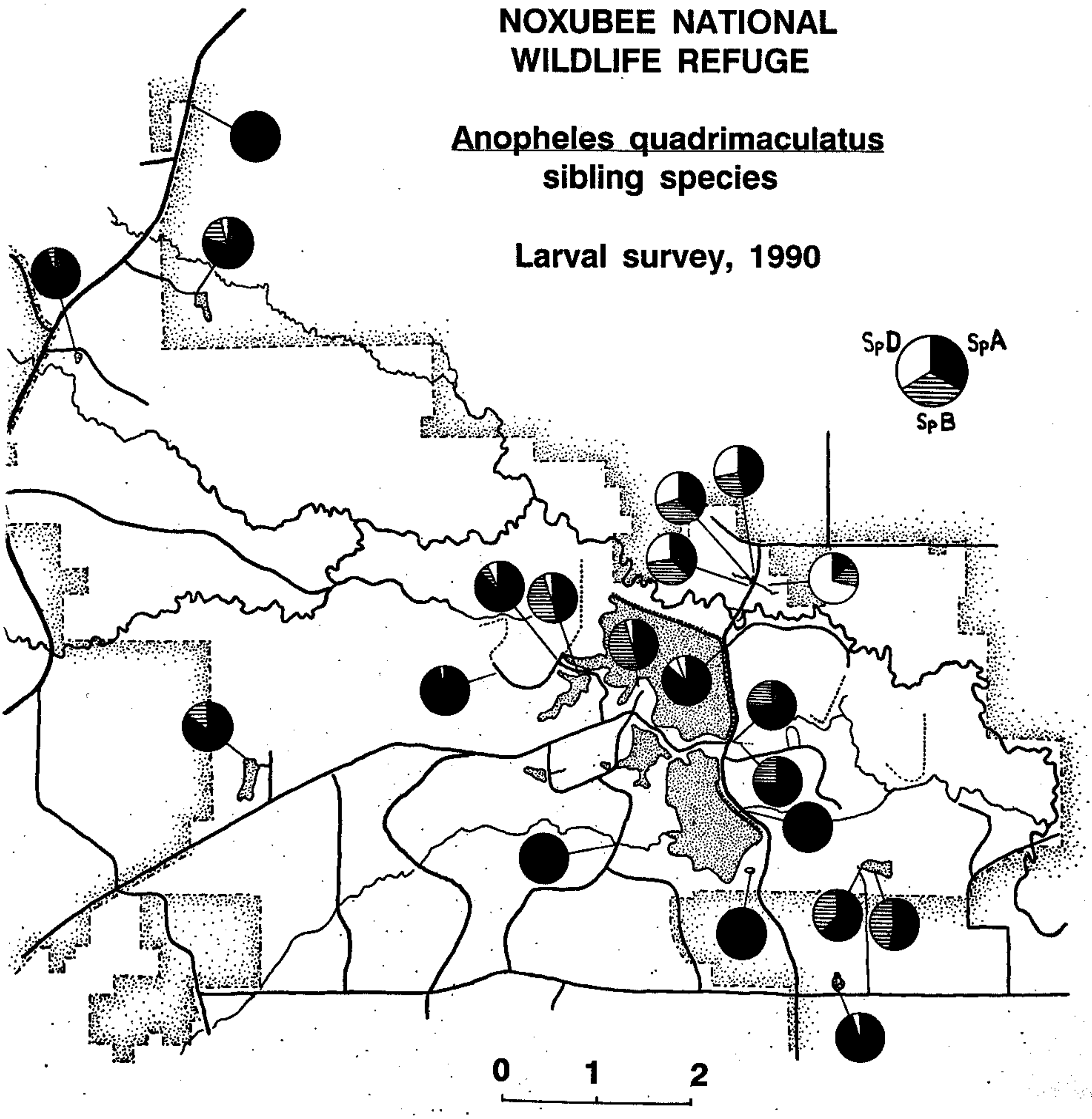


FIGURE 3. Larval survey broken down by environmental components

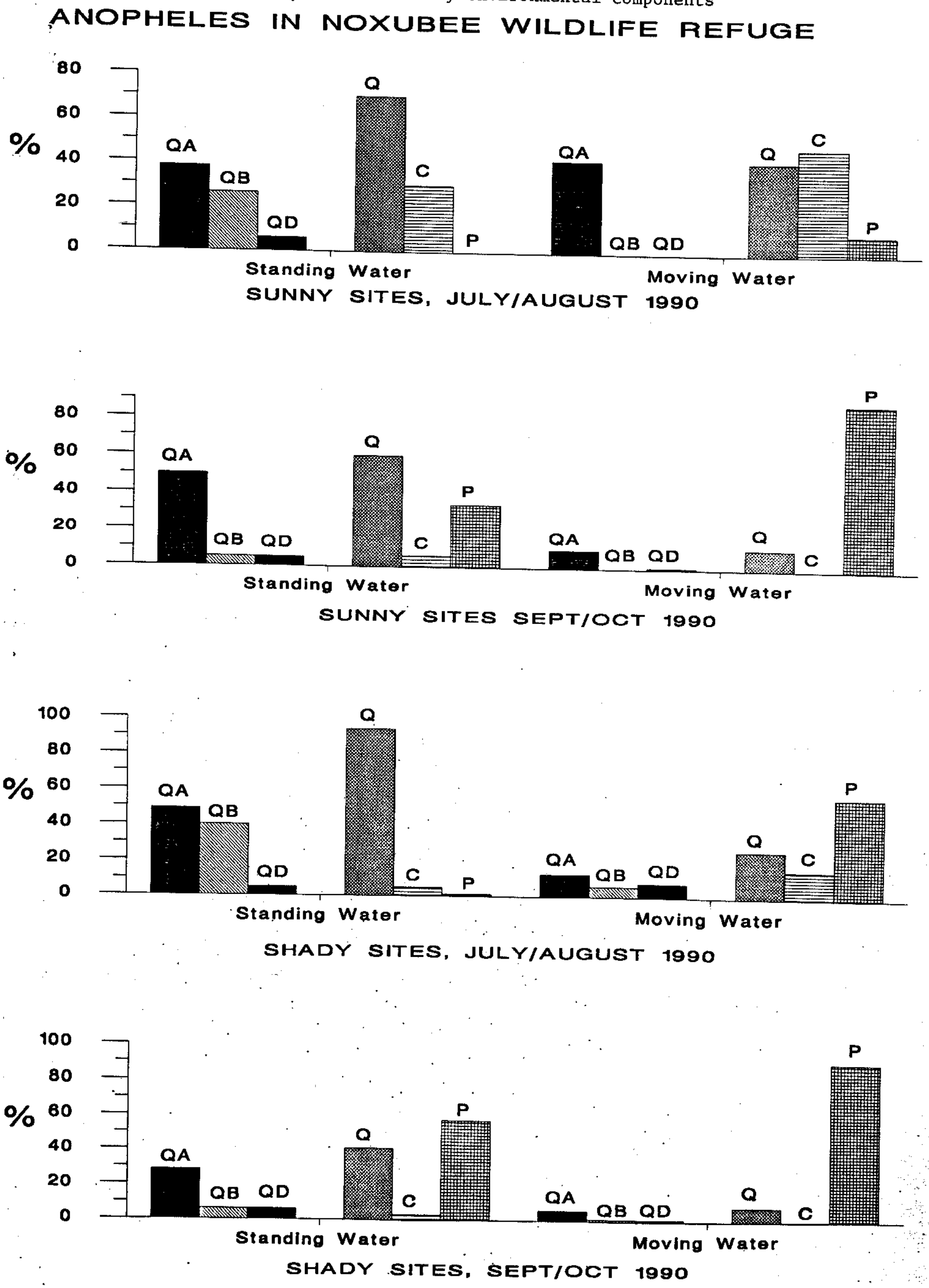


FIGURE 4. Dosage mortality curves from vial tests after 3, 6, and 9 hours.

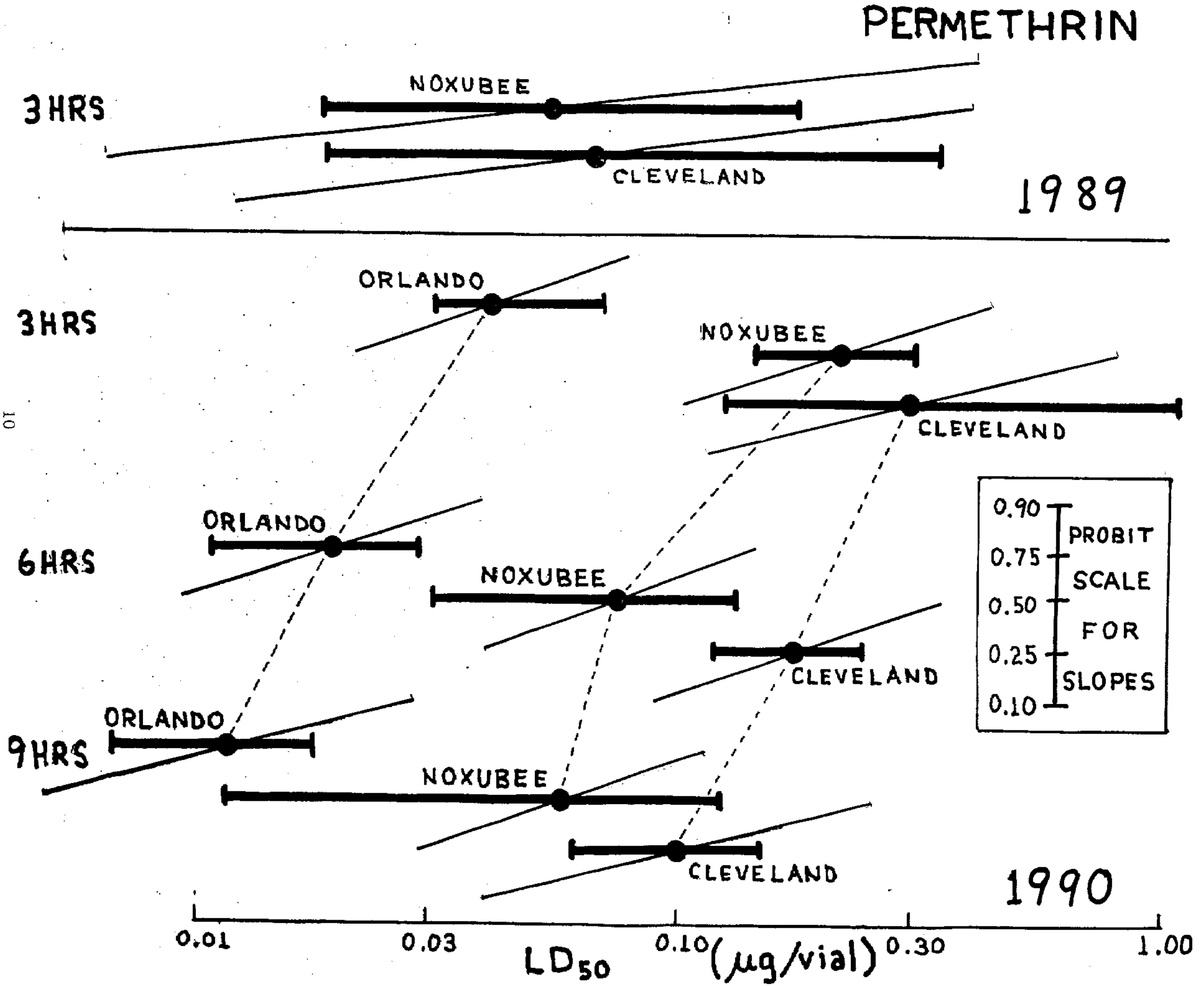
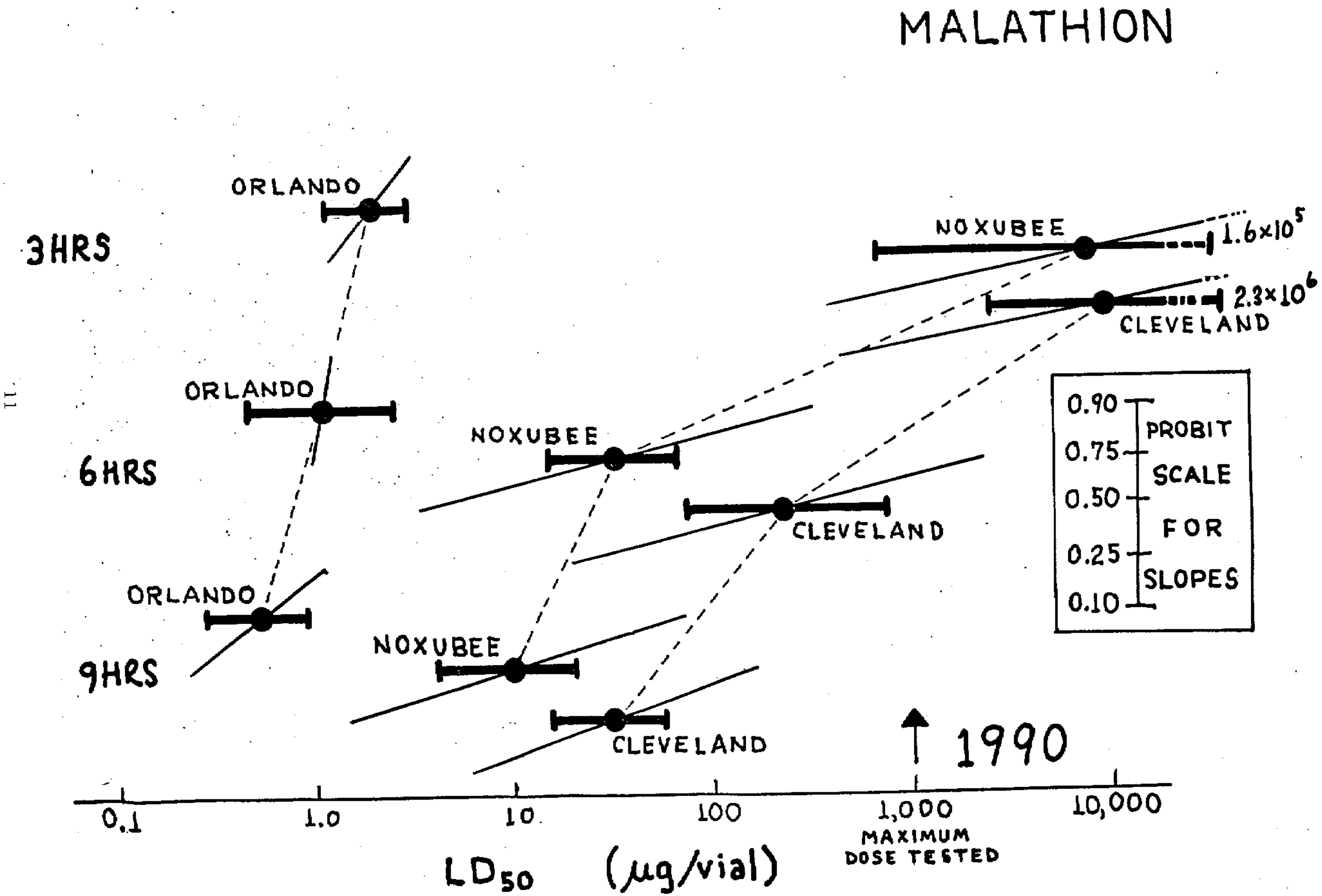


FIGURE 5. Dosage mortality curves from vial tests after 3, 6, and 9 hours.



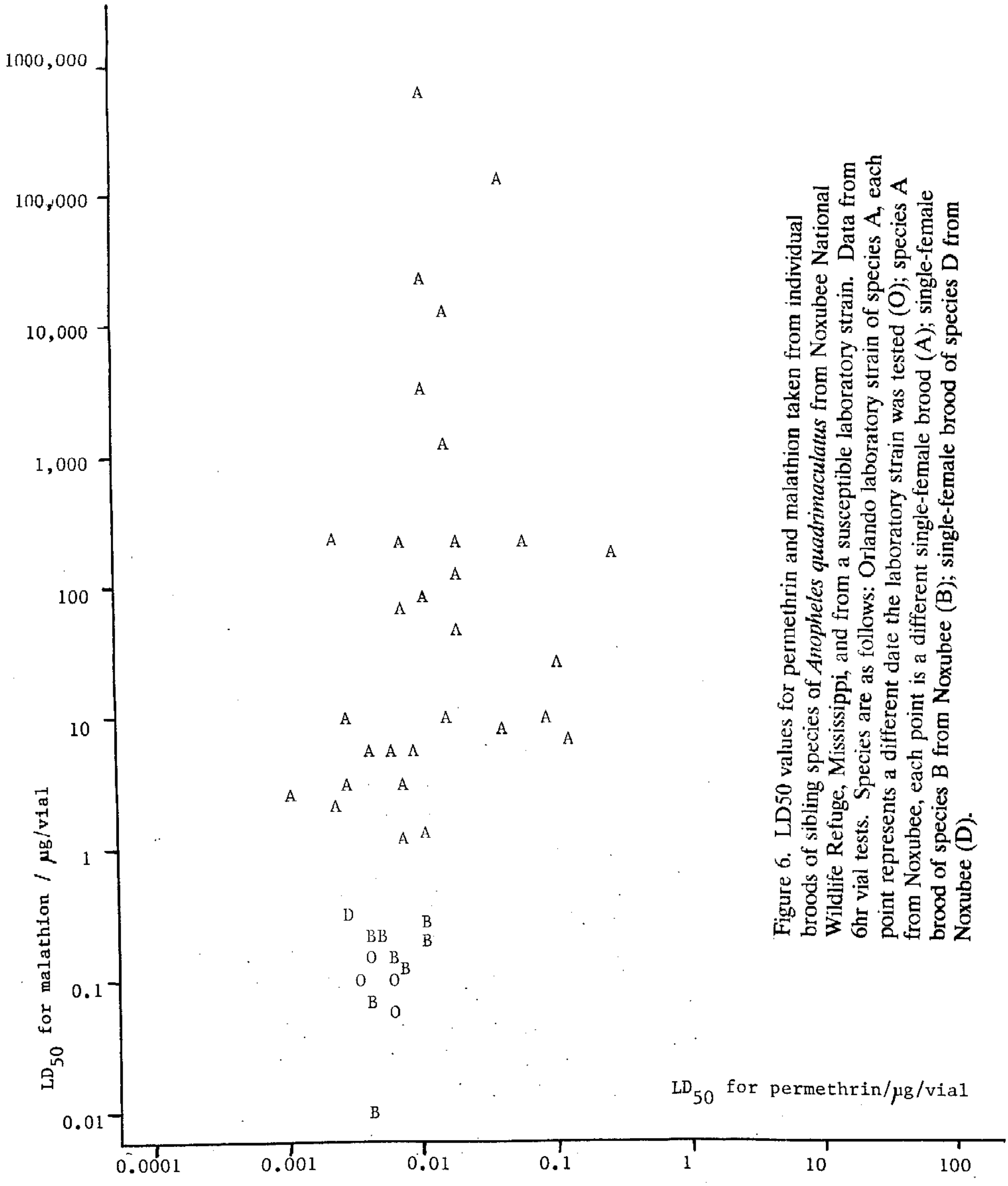


Figure 6. LD50 values for permethrin and malathion taken from individual broods of sibling species of *Anopheles quadrimaculatus* from Noxubee National Wildlife Refuge, Mississippi, and from a susceptible laboratory strain. Data from 6hr vial tests. Species are as follows: Orlando laboratory strain of 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).

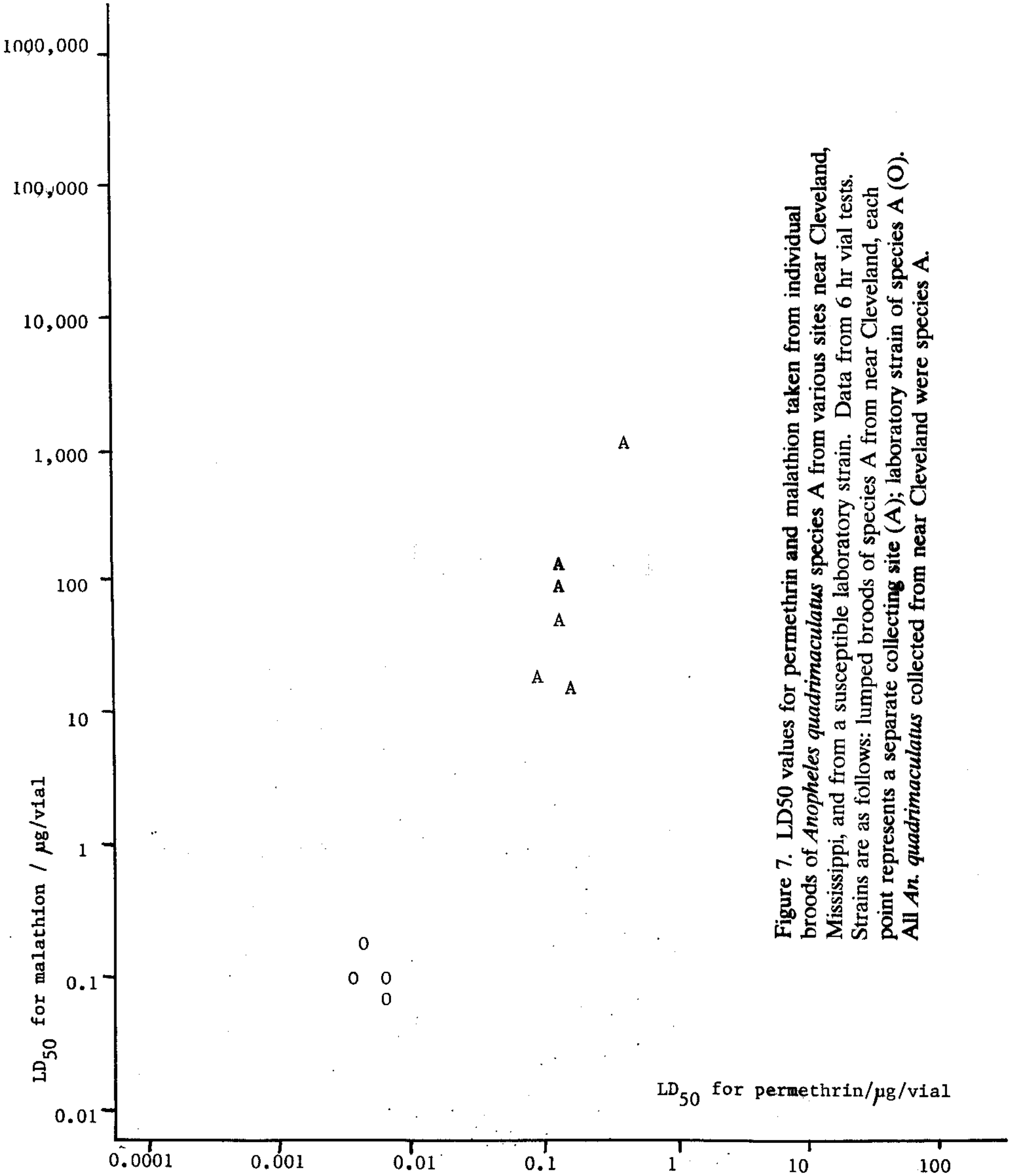


Figure 7. LD50 values for permethrin and malathion taken from individual broods of *Anopheles quadrimaculatus* species A from various sites near Cleveland, Mississippi, and from a susceptible laboratory strain. Data from 6 hr vial tests. Strains are as follows: lumped broods of species A from near Cleveland, each point represents a separate collecting site (A); laboratory strain of species A (O). All *An. quadrimaculatus* collected from near Cleveland were species A.