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IN MEMORIAM

HILTON B. MUNNS
1929-1999
A unique first name.
A unique last name.
Munzy was popularly known as “MUNZY,” a
unique nickname!

It seems that the common denominator is unique,
in eulogizing this remarkable human being, a kind,
compassionate gentleman, and a genuine friend.

Munzy was born on 26 February 1929, in
Fennimore, Wisconsin. On his final day, 9 September
1999, Munzy was once again in Fennimore. He was 70
years old. His hometown paper, the Fennimore Times
characterized Munzy for living “…an exemplary life
with that quiet dignity that identified him as a man
among men.”

Following a postwar stint in the army and college,
Munzy began his career as a “cheese-maker” in
Fennimore. He came to California in 1963, first working
in the dairy section of the food processing chemicals
department for Wyandotte Chemicals (now a subsidiary
of the giant BASF Chemical Company). He then
worked for Ralston—Purina until he formed Fennimore
Chemicals in 1971. As the hometown paper characterized
Munzy: “He loved Fennimore with a
passion and took advantage of every opportunity to
bring honor to his native home.” And, until the very
end, many years after leaving Fennimore, his Fennimore
friends always received calendars which proudly
included the name of their town in his company name.

A good friend of Munzy’s, Dr. Maria Zagomba
(from far away Yugoslavia), expressed her, including
other colleagues’ sentiments as follows: “…it is
unbearable to think that there won’t be any more
of his vivid, charming personality to meet and enjoy
Munzy’s discreet, nonintrusion generosity wherever he
appears.…” We too, share the same sentiments.

When his daughters eulogized their father, they
quoted Horace Mann, who said: “Be ashamed to die
until you have won some great victory for humanity.”
His family, as well as all of his friends share the same
feeling; Munzy passed on unashamed.

Most of the sentiments expressed above, including
many more by all those who knew Munzy, were
mirrored in my hurriedly prepared eulogy I delivered
at the XIIth. Euro-SOVE Conference in Wageningen,
Holland, on Saturday 11 September 1999. It was

Munzy’s genuine friendship and his great affection for
the SOVE that earned him lifelong friendships with
many of us here and abroad, plus the honor of being one
of the most beloved members of the Society. Munzy
was presented with the Distinguished Service Award

I first met Munzy sometime in 1980 or soon
thereafter, our friendship was instant. We traveled all
through the USA and Europe many times together. But
it never really mattered how long one had known
Munzy—whether it was decades, years, months, or
even a day or two, one often experienced the feeling
that the friendship with him would be a long-lasting
one.

Warm, gentle, kind, compassionate, genuine, and
most of all, giving—Munzy always gave, not because
he felt he had to—he gave for the sake of giving, never
really expecting anything in return. He made it a habit
of usually being there to extend a helping hand to those
that needed it. He acquired and fostered the friendship
of many of us ever since he first became a member of
the SOVE in the early 1980’s.

Although many sponsors from the private industry
graciously contributed, Munzy always “walked that
extra mile” to assure that the hosted socials (“attitude
adjustment hours”) were enjoyable, and these became
an integral part of the SOVE meetings. He left this
legacy behind, therefore, we must strive to keep it
alive. Munzy was also a golf enthusiast. I am sure that
his golfing buddies will surely “feel his presence” at
the “19th hole” whenever they happen to be there.

Munzy is survived by his wife, Jessica; his three
daughters, Meridith, Andrea, and Melanie and their
mother LuAnn; Jessica’s three children David, Glenn,
and LoriAnne; 12 grandchildren; and 4 sisters.

Munzy is not amongst us anymore, but we will
always remember his warm and friendly smile, his
genuine friendship, and now we must bid him fare-
well.

Minoo B. Madon.
Greater Los Angeles County
Vector Control District
12545 Florence Avenue
Santa Fe Springs, California 90760
Effects of Partial Blood Engorgement and Pretest Carbohydrate Availability on the Repellency of Deet to Aedes albopictus

Rui-De Xue and Donald R. Barnard

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Received 21 September 1998; Accepted 16 April 1999

ABSTRACT: The pretest availability of 10% sucrose solution and/or partial blood engorgement in Aedes albopictus Skuse significantly influenced mosquito attack rates and the time of repellent protection in laboratory bioassays. In 46 cm L x 38 cm W x 37 cm H cages used in USDA repellent tests, non-blood-fed and partially blood-fed mosquitoes attempted to bite at similar rates. In small cages (5 cm dia. x 4 cm H), holding individual females, mean mosquito attack rates were reduced when females were partially blood fed, compared with those not blood fed. The protection period from bites by Ae. albopictus using 25% ethanolic deet (N,N diethyl-3-methylbenzamide) increased significantly in small and USDA standard cages when females had pretest access to sucrose solution, compared with females starved for 12 h. Partial blood engorgement in mosquitoes affected repellent protection time in USDA standard test cages but not in small cages.

Keyword Index: Mosquito, sucrose, carbohydrate, blood, feeding, repellents.

INTRODUCTION

Two pretest conditions that can affect mosquito host-seeking behavior and repellency in repellent bioassays are carbohydrate availability and partial blood engorgement (Scott et al. 1993). In Aedes aegypti L., repellent efficacy is correlated with the pretest availability of sugar solution (Khan et al. 1975); repellents are most effective against this species when the mosquitoes have access to sugar water before testing, and are least effective when females have been starved.

The effects of pretest sugar availability and partial blood engorgement on repellent activity against Aedes albopictus Skuse are unknown. In the study presented here, we determined if repellent protection time against this species was affected by either factor when deet (N,N diethyl-3-methylbenzamide) was applied to human skin and tested against caged populations of mosquitoes. Our findings will be used to standardize repellent bioassays against Ae. albopictus and may be useful for revision of standard E951-94 (American Society for Testing and Materials 1994). This standard presumes the use of nulliparous mosquitoes but does not specify carbohydrate availability during the pretest period.

MATERIALS AND METHODS

Immature and adult Ae. albopictus (=F2) were maintained in the laboratory at 27°C and 80% relative humidity in a 14:10 (L:D) photoperiod. Larvae were reared in groups of 200 using 30 cm long x 19 cm wide x 5 cm high trays containing 1000 ml of well water. Adults emerged in screened cages and were provided continuous access to 10% sucrose/water solution. Blood meals were obtained from restrained 5-7 week old chicks.

Mosquito responses to deet were evaluated in two different test arenas: (1) a screened cage (the “USDA standard” cage), 46 cm long x 38 cm wide x 37 cm high (volume: 64,676 cm3), containing 200 female mosquitoes, and (2) small cages (5 cm dia. x 4 cm H; volume: 78.4 cm3), each containing a single female and with fine mesh (1.7 mm) cloth over the open end. The latter test arena approximated the space and mosquito density conditions defined by the above cited ASTM standard; specifically, individual small cages were used to eliminate the positional bias resulting from preference by landing and probing mosquitoes for the outmost ports of the five feeding port, box-shaped apparatus specified in the standard.
Mosquito Attack Rates and Deet Repellency in USDA Standard Cages

Four treatments (10% sugar water, no sugar water, partial blood meal, no blood meal) were used in this experiment, one treatment per cage, with each treatment replicated repeated three times. Each replicate required 100 female mosquitoes, which were partially blood fed using the following technique: the feathers on an adult chicken were clipped from one side of the abdomen and from adjacent areas on the wing. A small cage (5 cm dia. x 4 cm H), with the one open end covered by 1.7-mm mesh cloth, was used to hold a single female mosquito. The screened end of the small cage was placed against the chicken’s skin while the chicken was restrained. Once the mosquito began to feed, engorgement was allowed until the mosquito gut appeared one-quarter to one-third full of blood. Feeding was interrupted by lifting the small cage containing the mosquito from the chicken’s skin. The first group of 50 females partially blood fed in this manner was transferred to a test cage and provided cotton saturated with 10% sucrose solution for 12 hours before testing. A second group of 50 partially blood-fed females was transferred to a test cage and provided cotton saturated with water only for 12 hours before testing. The remaining 100 non-blood-fed mosquitoes were divided equally into the third and fourth cages; those in the third cage were provided sucrose solution for 12 hours before testing and those in the fourth cage were provided water only for 12 hours before testing.

**Mosquito Attack Rates.** To determine mosquito attack rates, the arm of a human volunteer was presented to the mosquitoes in each cage for 1 min. Mosquitoes that probed the skin exposed in a 9.8 x 4.8 cm, 1.7 mm mesh-covered window in a vinyl glove, that otherwise protected the forearm and hand from mosquito bite, were categorized as attempting to feed. The mean attack rate was calculated for each treatment as the average percentage of mosquitoes that probed the skin in three replicates.

**Repellent Test.** One ml of 25% deet in ethanol solution was applied to the forearm of a human volunteer between the wrist and the elbow (the hand was protected from mosquito bite by covering it with a latex glove). Mosquitoes inside each cage were allowed access to the treated forearm for 3 min. (Xue et al. 1995) with observations repeated every 30 min. The test for a cage was stopped when a cumulative total of three mosquitoes from the cage attempted to feed. Repellent protection time (hours) was calculated for each cage as that elapsed between deet application and the end of the test. The mean protection time from mosquito bite was calculated for each treatment as the average for three replicates.

Mosquito Attack Rates and Deet Repellency in Small Cages

Four treatments (10% sugar water, no sugar water, partial blood meal, no blood meal) were used in this experiment with each treatment replicated three times. Small cages, as described above, were used to cage individual female mosquitoes.

One hundred mosquitoes were used in each replicate; 50 of these were not blood fed and 50 were partially blood fed immediately before testing. Cages were separated into four groups of 25 cages per group with each group placed into one of four plastic trays (56 cm L x 43 cm W x 8 cm H) according to pretest sugar availability and blood-feeding status of the female. All trays were lined with paper towels. In two of the trays (with 25 blood-fed females; 25 non-blood-fed females), the paper towels were soaked to runoff with a 10% sucrose solution. Towels in the remaining two trays (25 blood-fed females; 25 non-blood-fed females) were soaked with water only.

**Mosquito Attack Rates.** To test mosquito attack rates, the screened end of each cage was individually pressed against the skin on the forearm of a human volunteer. If the mosquito landed and probed the skin surface, the observation was recorded as an attempted bite. If the mosquito did not probe the skin within the 1 min. test period, a non-bite was recorded.

**Repellent Test.** One ml of 25% ethanolic deet was applied to the forearm skin of a human volunteer between the wrist and the elbow (approximately 650 cm²). The screened end of each cage was held against the treated skin for 3 min. and the mosquito observed for probing. Observations were repeated at 30-min. intervals. The repellency test for each tray ended when a cumulative total of three female mosquitoes from both groups of the 25 cages in the tray had probed the skin. Protection time was calculated as above.

**Design and Data Analysis.** USDA standard cage and small cage experiments each were made as a 2 x 2 factorial using a split plot design (Steel and Torrie 1980). Factor one was sugar availability (10% sucrose solution; water only), factor two was blood engorgement status (non blood fed, partially blood fed). Mean percentage attack rate and repellent protection time were analyzed separately and by cage type using analysis of variance procedures (Gustafson, 1989).

**RESULTS**

Mosquito Attack Rates and Deet Repellency in USDA Standard Cages

**Mosquito Attack Rates.** Carbohydrate availability significantly influenced attack rates by *Ae. albopictus*
(F_{1,8} = 65.19, P < 0.0001) (TABLE 1) with mean rates higher in the water only group compared with the sucrose group. Interaction between sugar availability and blood engorgement status was not significant.

**Repellent Test.** The protection time of deet against *Ae. albopictus* was affected by sugar availability (F_{1,8} = 55.13, P < 0.001) and blood engorgement status (F_{1,8} = 6.13, P < 0.05) (TABLE 1). Mosquitoes with pretest access to sucrose were repelled by deet longer than females provided water only, regardless of blood engorgement status, whereas, repellent protection time was longer against partially blooded females than against non-blood-fed females, regardless of sugar availability. There was no significant interaction between sugar availability and blood-engorgement status.

**Mosquito Attack Rates and Deet Repellency in Small Test Cages**

**Mosquito Attack Rates.** In small cages (TABLE 2), sugar availability (F_{1,8} = 57.31, P < 0.0001) and blood engorgement status (F_{1,8} = 111.33, P < 0.0001) influenced mosquito attack rates. The rates were highest in sugar-starved females, regardless of blood-engorgement status, and lowest in partially blood-fed females, regardless of sugar availability. There was no significant interaction between sugar availability and blood-engorgement status.

**Repellent Test.** The protection time of deet against *Ae. albopictus* was affected by sugar availability (F_{1,8} = 73.51, P < 0.0001) (TABLE 2) and was longest in mosquitoes with access to sugar, compared with those without access to sugar, regardless of blood-engorgement status. Interaction between sugar availability and blood-engorgement status was not significant.

**DISCUSSION**

If the attack rate in a population of mosquitoes used in a repellent bioassay is low, the protection time will be overestimated (Kalmus and Hocking 1960). Thus, it is important to know the hunger status of the mosquitoes before a repellent test is made. From other studies (Xue et al. 1995, Xue and Barnard 1996) we know that blood feeding in *Ae. albopictus* is affected by mosquito body size and age, parity, and by the time of day when observations are made. In the present study, we have shown that attack rates in *Ae. albopictus* are affected by the pretest availability of carbohydrates. In addition, we showed that partial blood feeding in mosquitoes affects attack rates in small cage tests but not in tests with USDA standard cages. One reason for this difference may be the forced proximity of mosquitoes to the skin surface in small cages, which facilitates orientation to a feeding site.

### TABLE 1. Mean mosquito attack rates by, and repellent protection times against, partially blood-fed and non-blood-fed female *Aedes albopictus* provided water or sucrose solution for 12 hours before testing in USDA cages.

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<td>Mosquito attack rate</td>
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<tr>
<td>(％±SE)</td>
<td>25.0 ± 3.1</td>
<td>11.0 ± 2.3</td>
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<td>Repellent protection time (hour ± SE)</td>
<td>7.0 ± 0.5</td>
<td>8.5 ± 0.5</td>
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### TABLE 2. Mean mosquito attack rates by, and repellent protection times against, partially blood fed and non-blood fed female *Aedes albopictus* provided water or sucrose solution for 12 hours before testing in small cages.

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<td>Sucrose</td>
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<tr>
<td>Mosquito attack rate</td>
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<tr>
<td>(％±SE)</td>
<td>60.0 ± 10.1</td>
<td>23.0 ± 7.0</td>
</tr>
<tr>
<td>Repellent protection time (hour ± SE)</td>
<td>8.3 ± 0.3</td>
<td>10.0 ± 0.2</td>
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Pretest sucrose availability affected the protection time of deet against *Ae. albopictus*. Although a comparison of repellent protection time according to cage type was not an objective of this study, we did note that blood-engorgement status affected protection time in USDA cages, but not small cages; and that protection time was longest in the small cages. These disparate responses among cage types could be explained on the basis of neuronal inhibition in mosquitoes (Davis 1985) in small cages resulting from the small space and poor air exchange and the inability of the mosquito to move away from the deet treated skin surface.

We conclude that sucrose availability to mosquitoes prior to a repellent test influences the period of repellent effectiveness. We also conclude that mosquitoes that have access to sugar solution and to blood before a repellent test is made are repelled longer, by the same dose of deet, than mosquitoes receiving neither sugar nor blood.

The results of this study are important because they underscore the need to control pretest conditions for mosquitoes if the results of laboratory repellent bioassays are to be considered reliable. They also suggest the need to amend the ASTM standard with regard to carbohydrate availability to mosquitoes before a repellent test is made because this factor affects mosquito responses to a repellent.

REFERENCES CITED


A World Checklist of Genera, Subgenera, and Species of Ticks (Acari: Ixodida) Published from 1973-1997

James E. Keirans¹ and Richard G. Robbins²

¹U.S. National Tick Collection, Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, Georgia 30460-8056, USA.
²Armed Forces Pest Management Board, Walter Reed Army Medical Center, Washington, D.C. 20307-5001, USA.

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ABSTRACT: Researchers on ticks and tickborne diseases have been extremely fortunate in having at their fingertips the tick bibliographies produced by Harry Hoogstraal and his coworkers at the U.S. Naval facility at Cairo, Egypt, and by Mildred Doss and her colleagues at the U.S. Department of Agriculture laboratory at Beltsville, Maryland, USA. The Doss checklist of tick families, genera, species, and subsppecies is now 25 years out of date, and the following checklist of one new genus, nine new subgenera, and 110 new species of Ixodida brings together the nomenclature on ticks produced during the last quarter century.

Keyword Index: Tick taxa (Ixodida) since 1973.

INTRODUCTION

During the 1970s, a team of bibliographers, headed by the late Mildred Doss (September 2, 1903-December 21, 1993), Animal Parasitology Institute (now Biosystematics and National Parasite Collection Unit (BNPCU), Livestock and Poultry Sciences Institute), Agricultural Research Service, U.S. Department of Agriculture, and by George Anastos, Department of Zoology, University of Maryland, compiled a series of subject indices on the genera and species of ticks, their hosts, and distributions. These works were issued as Special Publications of the USDA’s Index-Catalogue of Medical and Veterinary Zoology. After collating references to tick genera and species (Part I, 3 volumes, January 1974) and tick hosts (Part II, 3 volumes, July 1974), Doss and colleagues produced a checklist of tick families, genera, species, and subspecies (Part III, May 1977), based largely on the contents of the preceding parts. Their fourth and final installment on tick distribution appeared in 1978.

Users of the “Doss bibliographies” generally agree that they are invaluable. Despite the enormity of their task, the USDA-University of Maryland team succeeded in listing by category much of the world literature on ticks and tickborne diseases. As well, they cross-referenced their entries to two other acarological bibliographies: the Index-Catalogue itself (Authors, Parts 1-18, and Supplements 1-17) and the Bibliography of Ticks and Tickborne Diseases by the late Harry Hoogstraal (1917-1986). In their checklist, the Doss team cite most references to new tick taxa published up to 1972 (and some subsequently), but through usage we have determined that the year 1973 marks the point at which the Doss list ceases to be an all-inclusive work. With the passage of a quarter century, we believe that the time has come to augment our predecessors’ compilation.

Since 1973, descriptions of 1 new tick genus, 9 new subgenera, and 110 new species have appeared in 96 scientific papers, worldwide. Of the new species, 34 (31%) are argasids, described in 5 genera, while 76 (69%) are ixodids, described in 10 genera. Ornithodoros antiquus is known only from Dominican amber; the remaining 109 new species represent all zoogeographic regions, as follows: Afrotropical 31, Australian 10, Nearctic 4, Neotropical 21, Oriental 15, and Paleartic 28. In our checklist, tick taxa and hosts are listed alphabetically, without regard to phylogenetic propinquity. However, depositories appear in the sequence given in the original descriptions, using the following acronyms for collections cited more than once:
ANIC, CSIRO: Australian National Insect Collection, Commonwealth Scientific and Industrial Research Organisation, Canberra.
BMNH: The Natural History Museum, London; formerly British Museum (Natural History).
CASP: Institute of Parasitology, Czech (formerly Czechoslovak) Academy of Sciences, Prague.
FMNH: Field Museum of Natural History, Chicago, USA.
FVZ: Department of Parasitology, Veterinary Faculty at Zaragoza, Spain.
IFAN: L'Institut Fondamental d'Afrique Noire.
ICT: Centro de Zoologia do Instituto de Investigação Científica Tropical, Lisbon, Portugal.
IZAC: Instituto de Zoología, Academia de Ciencias de Cuba, Havana.
MBB: Museum Bogoriense, Bogor, Java, Indonesia.
MNHM: Museum of Natural History, Maputo, Mozambique.
NAMRU-3: United States Naval Medical Research Unit Number Three, Cairo, Egypt.
NTMD: Northern Territory Museum, Darwin, Australia.
ORSSTOM: Office de la Recherche Scientifique et Technique Outre-Mer.
OVI: Veterinary Research Institute, Onderstepoort, South Africa.
USNTC: United States National Tick Collection, Statesboro, Georgia.
VRLH: Veterinary Research Laboratory, Harare, Zimbabwe.
WAMP: Western Australia Museum, Perth.
ZISP: Zoological Institute, St. Petersburg, Russia.

We take pleasure in dedicating this update to the memory of Mildred Doss, whose conscientiousness and charm greatly facilitated the always arduous task of bibliography. Our thanks also to J. Ralph Lichtenfels, Research Leader and Supervisory Zoologist, BNPCU, who provided important background information on Ms. Doss and her career, and to Renjie Hu for obtaining and translating various Chinese publications.
Antricola habanensis de la Cruz, 1976. Poeyana (151): 1-8, figs. 1a, d.
HOST: Collected in a bat cave.
DISTRIBUTION: Cuba, La Habana Province, Catalina de Güines, Cueva del Mudo.
DEPOSITORY: IZAC.

HOST: Mormoops megalophylla (Peters).
DISTRIBUTION: Venezuela, Curaçao, Hato, Cueva di Rato.
DEPOSITORY: USNTC.

Antricola martelorum de la Cruz, 1978. Poeyana (184): 1-17, fig. 1f.
HOST: Collected in a bat cave.
DISTRIBUTION: Cuba, La Habana, Santa Cruz del Norte, Finca Galera, Cueva de los Murciélagos.
DEPOSITORY: IZAC.

Antricola naomiae de la Cruz, 1978. Poeyana (184): 1-17, fig. 1d.
HOST: Collected in a bat cave.
DISTRIBUTION: Cuba, Matanzas, Camarioca, Cueva de Santa Catalina.
DEPOSITORY: IZAC.

Antricola occidentalis de la Cruz, 1978. Poeyana (184): 1-17, fig. 1a.
HOST: Collected in a bat cave.
DISTRIBUTION: Cuba, Pinar del Rio, San Andrés de Caiguanaabo, Galalón, Cuevà de los Majáes.
DEPOSITORY: IZAC.

HOST: Collected on bat guano.
DISTRIBUTION: Cuba, Santiago de Cuba, Siboney, Cuevà de los Majáes.
DEPOSITORIES: USNTC; IES; FVZ; BMNH.

HOSTS: Collected from nests of Columba guinea phaeonota Gray, Geronticus calvus (Boddart), and Pytonoprobe fuligula rufigula (Fischer and Reichenow).
DEPOSITORIES: USNTC; OVI; South African Institute for Medical Research, Johannesburg, South Africa; BMNH; ZISP.

HOST: Hirundo daurica japonica Temminck and Schlegel.
DISTRIBUTION: People’s Republic of China, Jiangxi Province, Tonggu County.
DEPOSITORIES: IZAS; Department of Parasitology, Jiangxi Medical College.

HOST: Collected from roosts of Columba livia Gmelin.
DISTRIBUTION: People’s Republic of China, Shijing mountain region of Beijing.
DEPOSITORY: Not stated but probably IZAS.

HOST: Collected from roosts of Speotyto cunicularia nanodes Berlepsch and Stolzmann.
DISTRIBUTION: Peru, Lima, La Molina (12° 05’S, 76° 57’W).
DEPOSITORIES: USNTC; Museo de Entomología, Universidad Nacional Agraria, Lima, Peru.

HOSTS: Chalinolobus dwoyeri Ryan, C. gouldii Gray, Eptesicus pumilus Gray, Nyctophilus geoffroyi Leach, Pipistrellus tasmaniensis (Gould), Rhinolophus megaphyllus Gray.
DISTRIBUTION: Australia, New South Wales, Victoria, Tasmania.
DEPOSITORIES: ANIC, CSIRO; Queen Victoria Museum, Launceston, Tasmania; USNTC.

HOST: Falco cenchroides Vigors and Horsfield.
DISTRIBUTION: Australia, Western Australia, Cue Shire, Flemington Breakaway (26° 28’S,
117° 35'E).

DEPOSITORIES: ANIC, CSIRO; USNTC; BMNH.


HOST: *Gyps fulvus* L.

DISTRIBUTION: Spain, Zaragoza Province, 15 km NW of Tabuenca (41°45'N, 01°32'W).

DEPOSITORIES: Unidad de Parasitologfa, Facultad de Veterinaria, Zaragoza, Spain; USNTC.


HOST: *Falco cenchroides* Vigors and Horsfield.

DISTRIBUTION: Australia, Western Australia, Nullarbor Plain, Horseshoe Cave (31°39'S, 127°26'E).

DEPOSITORIES: ANIC, CSIRO; BMNH; USNTC.


HOST: *Macroderma gigas* Dobson.

DISTRIBUTION: Australia, Queensland, Limestone Ridge, Johannsen's Cave (23°10'S, 150°27'E) near Rockhampton.

DEPOSITORIES: Queensland Museum, Brisbane; Department of Parasitology, University of Queensland, Brisbane; BMNH; USNTC.


HOST: Collected under and around nests of *Larus californicus* Lawrence.

DISTRIBUTION: U.S.A., California, Mono County, islands in Mono Lake.

DEPOSITORY: USNTC.


HOSTS: Unknown; found on the wall of a house and from unspecified locales. Wild birds and domestic chickens are probable hosts.

DISTRIBUTION: Peru, Junin Province, Quebrada de Gagaracca (10°57'S, 76°01'W). Also from Arequipa Province.

DEPOSITORY: BMNH.


HOST: *Columba livia* Gmelin.

DISTRIBUTION: Poland, Krakow (50°03'N, 19°58'E).

DEPOSITORIES: USNTC; Polish Academy of Sciences, Institute of Systematic and Experimental Zoology, Krakow; Department of Animal Morphology, Institute of Biology, Adam Mickiewicz University, Poznan; senior author's collection; ZISP; BMNH; FMNH.


DISTRIBUTION: U.S.A., Texas, Zavala County, 3.5 mi. (5.6 km) E of La Pryor (28°55'N, 99°47'W) on Highway 57. Also from Bastrop, Bexar, Kimble, Menard, and Travis Counties.

DEPOSITORIES: USNTC; Entomology Department, Texas A & M University, College Station; FMNH.


HOST: *Pipistrellus abramus* (Temminck) [now *P. javanicus* (Gray)].

DISTRIBUTION: People's Republic of China, Sichuan Province.

DEPOSITORY: Department of Parasitology, Chongqing Medical College, Chongqing, Sichuan.


HOSTS: *Cynopterus brachyotis* (Müller), *Dobsonia viridis* (Heude), *Rousettus amplexicaudatus* (E. Geoffroy).

DISTRIBUTION: Indonesia, Halmahera Island, Jailolo District, Kampung Pasir Putih; Lombok Island, Bilekedit; Java.

DEPOSITORY: USNTC.


HOSTS: *Dobsonia moluccensis* (Quoy and Gaimard), *D. viridis* (Heude), *Dobsonia* sp.

DISTRIBUTION: Papua New Guinea, Madang.
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Ornithodoros

HOST: Indonesia, Irian Jaya, Owi Island, Biak Island, Sarwon and Sorido; Indonesia, Seram Island, Manusela National Park; Indonesia, Halmahera Island, Jailolo District, Kampung Pasir Putih.

DEPOSITORY: USNTC.


Acarologia 36: 25-40, figs. 1-5.

HOSTS: Dobsonia moluccensis (Quoy and Gaimard), D. viridis (Heude).

DISTRIBUTION: Indonesia, Halmahera Island, Jailolo District, Kampung Pasir Putih; Papua New Guinea, Central District, Kairuku, Kukuba Cave.

DEPOSITORY: USNTC.


Experientia 51: 384-387, figs. 1-5.

HOST: Found in two pieces of amber.

DISTRIBUTION: Dominican Republic, Cordillera Septentriional mountain range, La Toca mine.

DEPOSITORY: In the private collection of Jim Work, Ashland, Oregon, U.S.A.


HOST: Collected in and near nests of Collocalia esculenta linchi Horsfield and Moore.

DISTRIBUTION: Indonesia, East Java, Baluran, Wonoredojo (07°56'S, 114°22'E), and West Java, Palabuhanratu.

DEPOSITORIES: MBB; USNTC; BMNH.

Ornithodoros cyclurae De la Cruz, 1984. 

Poeyana (227): 1-6, figs. 1-2d.

HOST: Collected in nasal cavity of Cyclura nubila (Gray).

DISTRIBUTION: Cuba, Granma Province, Cabo Cruz.

DEPOSITORY: IZAC.


HOST: Spheniscus humboldti Meyen.

DISTRIBUTION: Peru, Ica, Punta San Juan (15°21'S, 75°11'W), Punta Blanca (05°49'S, 81°05'W) and Punta San Fernando (15°08'S, 70°21'W).

DEPOSITORY: USNTC; Universidad Nacional Agraria, La Molina, Lima, Peru; Florida State Collection of Arthropods, Bureau of Entomology, Gainesville, Florida, USA.


Annals of the Entomological Society of America 70: 221-228, figs. 1-14.

HOSTS: Collected in nests of Hirundo abyssinica unitatis Sclater and Mackworth-Praed, H. daurica emini Reichenow, and Myrmecocichla cinamomeiventris subrufipennis Reichenow.

DISTRIBUTION: Kenya, Eastern Province, in cave, Ngomeni Rock (04°08'S, 38°35'E), and in cave, peak of Ukazzi Hill (00°50'S, 38°35'E).


HOST: Collected in marine bird nesting site, probably of Sula nebuchii Milne-Edwards.

DISTRIBUTION: Ecuador, Galapagos Islands, Isabella (Albemarle) Island, Punta Vicente Roca (00°03'S, 91°33'W). Also on the islands of Culpepper (Darwin), Plaza Sur, Santa Cruz, Espanola, Daphne Major, Fernandina, and Seymour Norte.

DEPOSITORIES: USNTC; BMNH.

NOTE: Also on and around nesting areas of Diomedea irrorata Salvin, Nanopterum (now more commonly placed in the genus Phalacrocorax) harrisi Rothschild, Spheniscus mendiculus Sundevall, Sterna lunata Peale, and Sula dactylatra Lesson. Collections from Amblyrhynchus cristatus Bell and Zalophus wollebaeki Sivertsen (now Z. californianus (Lesson)) are probably accidental.


Annals of the Entomological Society of America 70: 221-228. [Subgenus of Ornithodoros. Type - O. (P.) vansomereni]

Ixodidae

Africanaella Santos Dias, 1974. 


Amblyommaarianae Keirans and Garris, 1986. 

Journal
of Medical Entomology 23: 622-625, figs. 1-8.
HOST: Alsophis portoricensis Reinhardt and Lütken.
DISTRIBUTION: Puerto Rico, Barrio Puyano Adentro, Municipio of Vega Baja.
DEPOSITORY: USNTC.

*Amblyomma glauerti* Keirans, King, and Sharrad, 1994. *Journal of Medical Entomology* 31: 132-147, figs. 7-12, tab. 2.
HOSTS: Varanus glauerti Mertens, V. glebopalma Mitchell.
DISTRIBUTION: Australia, Western Australia, Buccaneer Archipelago, Lachlan Island (16° 37'S, 123° 31'E).
DEPOSITORIES: WAMP; NTMD; USNTC; BMNH.
NOTE: Coordinates for numerous other localities are detailed in Table 2 of the original article.

HOST: Undetermined species of snake.
DISTRIBUTION: People's Republic of China, Guangdong Province, and on Hainan Island.
DEPOSITORY: IZAS.
NOTE: Hainan Island is also a Province of the P.R.C.

HOSTS: Dolichotis salinicola Burmeister. Also found on cattle.
DISTRIBUTION: Argentina, Salta Province, Department of Rivadavia, Rivadavia (24°11'S, 62° 53' W). Also in Department of Anta.
DEPOSITORIES: USNTC; MBR.

HOST: Egermia stokesii (Gray).
DISTRIBUTION: Australia, South Australia, Flinders Ranges near Hawker, Warruwarldunha Range (31° 54'S, 138° 25'E).
DEPOSITORIES: South Australia Museum, Adelaide, Australia; USNTC.
NOTE: Coordinates for several other collection localities are detailed in Table 1 of the original article.

HOST: Cricetulus migratorius (Pallas).
DISTRIBUTION: People's Republic of China, Xinjiang Uygur Autonomous Region, Hashi District.
DEPOSITORY: IZAS.

HOST: Alticola argentatus (Severtzov).
DISTRIBUTION: Tajikistan, Peter the First Ridge.
DEPOSITORY: ZISP.

HOSTS: Varanus glauerti Mertens, V. glebopalma Mitchell.
DISTRIBUTION: Australia, Western Australia, Prince Regent River Reserve (15° 32'S, 125° 19'E).
DEPOSITORIES: WAMP; NTMD; USNTC; BMNH.
NOTE: Coordinates for numerous other localities are detailed in Table 1 of the original article.

HOST: Varanus exanthematicus (Bosc).
DISTRIBUTION: Democratic Republic of Congo, Monga.
DEPOSITORY: Musée Royal de l'Afrique Centrale, Tervuren, Belgium.

HOST: Python molurus bivittatus Kuhl.
DISTRIBUTION: Vietnam, Shonla(21°20'N, 103° 50'E).
DEPOSITORY: ZISP.

of Dermacentor; type - D. (A.) pavlovskyi Olenev].

HOST: *Aepeceros melampus* (Lichtenstein).
DISTRIBUTION: Mozambique, Gaza Province, Govuro District, Parque Nacional do Zinave.
DEPOSITORY: IICT; BMNH; Museum d'Histoire Naturelle de Paris; USNTC.
NOTE: In all probability a junior synonym of *Boophilus decoloratus* (Koch).

HOST: *Petrodromus tetradoxylus beirae* Roberts [now *P. tetradoxylus* Peters].
DISTRIBUTION: Mozambique, Sofala Province, Lagoa Ura.
DEPOSITORIES: IICT; BMNH; OVI.
NOTE: In all probability a junior synonym of *Boophilus decoloratus* (Koch).

[Subgenus of Aponomma; type - A. (B.) glebopalma Keirans, King and Sharrad].


HOST: *Ochotona rutila* (Severtzov).
DISTRIBUTION: Tajikistan, southern slopes of Peter the First Ridge near the middle course of the Obikhingou River.
DEPOSITORY: ZISP.

HOST: Collected on vegetation.
DISTRIBUTION: Kazakhstan, valley of the Chilik River. Also from Kyrgyzstan and Turkmenistan.
DEPOSITORY: ZISP.


HOST: Not stated, probably collected on vegetation.
DISTRIBUTION: People's Republic of China, Yunnan Province, wilderness of Lushui County.
DEPOSITORY: IZAS.

Mo ta va phan laoi. (In Vietnamese).
HOST: *Chloropsis cochinchinensis* (Gmelin).
DISTRIBUTION: Vietnam, Bacthai.
DEPOSITORY: Unknown.
NOTE: We have been unable to acquire the publication by Phan. Kolonin (1992) *In: V. E. Sokolov, ed. Zoological Researches in Vietnam*, Nauka Publishers, Moscow, considers *Haemaphysalis bacthaeni* to be a junior synonym of *H. ornithophila* Hoogstraal and Kohls.

DISTRIBUTION: Pakistan, Swat, Tirich-Mir Valley (36° 15'N, 71° 51'E). Collections also from Wakhan area of Badakhshan Province, Afghanistan.
DEPOSITORIES: CASP; USNTC.

HOSTS: *Citellus* [now *Spermophilus*] *dauricus* Brandt. Also on *Ochotona pallasi* (Gray), "Pamir high-mountain vole," and "Siberian soulik."
DISTRIBUTION: Mongolia, southern Khangai, Khure-Marl. Also in the Tarbagatai and Taishir regions.
DEPOSITORY: Not stated.

HOST: Not stated.
DISTRIBUTION: Russia, Primor'ye, Nadezhdinsky region, on the northern slope of the lower Anan'evka River valley (right tributary of the Razdno'l' naya River).
DEPOSITORY: ZISP.

HOST: Cattle.
DISTRIBUTION: Vietnam, Laocai.
DEPOSITORY: ZISP.

HOSTS: *Rattus dominator* Thomas [now *Paruromys dominator* (Thomas)], *Echinothrix leucura* Gray.
DISTRIBUTION: Indonesia, Central Sulawesi.
DEPOSITORIES: MBB; USNTC; BMNH.

HOST: *Hystrix cristata* L.
DISTRIBUTION: Ethiopia, near Awash National Park (08° 50'N, 39° 50'E).
DEPOSITORIES: Zoological Museum of Moscow University, Moscow, Russia; author's collection.

HOST: Collected by flagging vegetation.
DISTRIBUTION: Japan, Kagoshima Prefecture, Mage Shima (Mage Island), Kumage-gun (30° 45'N, 130° 51'E).
DEPOSITORIES: Medical Zoology Department, Niigata University School of Medicine; USNTC.
NOTE: It is presumed that Sika deer, *Cervus nippon* Temminck, are hosts for this tick species.

HOST: *Cervus* sp.
DISTRIBUTION: People’s Republic of China, Yunnan Province, Meng La.
DEPOSITORY: Institute of Microbiology and Epidemiology, Academy of Military Medical Science, Beijing.

HOST: *Moschus berezovskii* Florov.
DISTRIBUTION: People’s Republic of China, Qinghai Province, and Qusum, Xizang Autonomous Region.
DEPOSITORY: IZAS.

HOST: *Erinaceus frontalis* Smith [now *Atelerix frontalis* (Smith)].
DISTRIBUTION: Zimbabwe, Matabeleland, Bulawayo District. Also from Matopos National Park, Victoria Falls Road, and Kumalo.
DEPOSITORIES: USNTC; VRLH; OVI; ORSTOM, Bondy, France.

HOST: *Viverra civetta* (Schreber).
DISTRIBUTION: Cameroon, Nanga-Eboko (04° 41'N, 12° 22'E).
DEPOSITORIES: USNTC; J.-L. Camicas Collection (ORSTOM); P.-C. Morel Collection.

HOST: *Phasianus versicolor* Vieillot.
DISTRIBUTION: Japan, Niigata Prefecture, Sado Island, Kanai (38° 03'N, 138° 22'E).
DEPOSITORY: USNTC.
NOTE: Russia, China and Korea are listed along with other hosts for tick specimens that are tentatively regarded as *H. phasiana*.


HOSTS: For adults, Ailax paludinosus (G. Cuvier), Herpetes sanguineus (Rüppell) [now Galerella sanguinea (Rüppell)], Genetta maculata (Gray), Herpetes ichneumon (L.), Ichneumia albicuda (G. Cuvier), Mungos mungo (Gmelin); for immatures, Aethomys chrysophilus (de Winton), Praomys natalensis (Smith) [now Mastomys natalensis (Smith)], Tachyoryctes splendens Rüppell.


DEPOSITORIES: USNTC; H. Hoogstraal Collection at NAMRU-3, Cairo, Egypt; J.-L. Camicas Collection at ORSTOM.

NOTE: One nymph collected from *Galerella sanguinea* (Rüppell).


HOSTS: Hystrix brachyura L., Sus scrofa L.

DISTRIBUTION: Vietnam, Dalac, Dac-Genh (12° 30’N, 107° 50’E). Also from Gailai Contum, Buon-Loi (14° 10’N, 108° 30’E); Quangninh, Island Bamun (21° 05’N, 107° 40’E).

DEPOSITORIES: ZISP; BMNH; Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; USNTC.


HOST: Collected in an alpine pasture and off a wild goat.

DISTRIBUTION: People’s Republic of China, southern part of Xinjiang Uygur Autonomous Region.

DEPOSITORY: IZAS.


HOSTS: Goats and sheep.

DISTRIBUTION: Yemen Arab Republic, Ta’izz Province, southern tihama (lowland) foothills, Al Hamilee (Al Murco) (13° 20’N, 43° 35’E), Misgab as Seloo (13° 20’N, 44° 20’E), and Saudi Arabia, Mecca.

DEPOSITORIES: USNTC; BMNH; Department
of Biology, Faculty of Science, King Abd el Aziz University, Jidda, Saudi Arabia. 
NOTE: It is postulated that the original host for this tick species was the Nubian ibex, *Capra nubiana* F. Cuvier.

HOST: *Oryctolagus cuniculus* (L.).
DISTRIBUTION: Portugal, Leiria District, Porto de Mós.
DEPOSITORIES: IICT; BMNH.

DISTRIBUTION: Uganda, Buganda, Namulange (00° 32'N, 32° 37'E). Also Democratic Republic of Congo and Congo.
DEPOSITORIES: USNTC; BMNH; OVI.

**Ixodes calcarhebes** Arthur and Zulu, 1980. *Systematic Parasitology* 1: 241-244, text figs. 1-2; pl. 1, figs. 1-5, pl. 2.
HOST: *Praomys natalensis* Smith [now *Mastomys natalensis* (Smith)].
DISTRIBUTION: Zambia, found in the tick collection of the Pest Research Unit Laboratories of the National Council for Scientific Research, Chilanga, Lusaka.
DEPOSITORY: Same as for distribution.

HOST: *Lepus saxatilis* F. Cuvier.
DISTRIBUTION: Republic of South Africa, East Cape Province, Clark's Siding (31° 25'S, 27° 08'E).
DEPOSITORIES: OVI; USNTC.

**Ixodes columnae** Takada and Fujita, 1992. *Journal of the Acarological Society of Japan* 1: 37-44, fig. 1a-c, fig. 2a-c, fig. 3a-d.
HOSTS: Collected by flagging vegetation and from *Apodemus argenteus* (Temminck), *A. speciosus* (Temminck), *Clethrionomys* [now *Phaupomys*] *andersoni* (Thomas), *Sciurus* [now *Petaurista*] *leucogenys* (Temminck), *Sciurus lis* Temminck, and *Phasianus colchicus* L.
DEPOSITORY: Fukui Medical School, Fukui; Laboratory of Ohara General Hospital, Omachi, Fukushima, Japan.
NOTE: Specific collecting localities in the various prefectures are given in Table 1 of the original description.

HOST: *Mimus polyglottos* (L.).
DISTRIBUTION: Jamaica, St. Thomas Parish, Blue Mountains, Penlyne, Whitfield Hall.
DEPOSITORY: USNTC.

HOSTS: *Aonyx capensis* (Schinz). Also on *Galerella pulverulenta* (Wagner), *G. sanguinea* (Rüppell), *Genetta genetta* (L.), and *G. tigrina* (Schreber).
DISTRIBUTION: Republic of South Africa, Tsitsikama National Park (33°58'S, 23°45'E).
DEPOSITORIES: OVI; USNTC; BMNH.

HOST: Collected by flagging vegetation.
DISTRIBUTION: USA, Massachusetts, Nantucket Island (41°20'N, 70°02'W).
DEPOSITORY: USNTC.
NOTE: Numerous hosts and localities are listed in Tables 1 and 2 of the original description. A junior synonym of *Ixodes scapularis* Say.

**Ixodes donarthuri** Santos Dias, 1980. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre"* (151): 1-11, fig. 1a-e.
HOSTS: *Redunca arundinum* (Boddart), *Sylvicapra grimmia* (L.).
HOSTS: Collected by flagging vegetation. Also on goats, cattle and *Taurotragus oryx* (Pallas).
DISTRIBUTION: Republic of South Africa, Natal (now Kwa-Zulu Natal), Giant’s Castle Nature Reserve (29° 16’ S, 29° 30’ E), and Tank Area 118 (29° 35’ S, 29° 50’ E).
DEPOSITORY: OVI; USNTC; BMNH.

HOSTS: Clethrionomys gapperi (Vigors), Microtus longicaudus (Merriam), M. pennsylvanicus (Ord), Neotoma cinerea (Ord), Sorex cinereus Kerr, Tamias minimus Bachman, Zapus hudsonius (Zimmermann).
DISTRIBUTION: USA, South Dakota, Lawrence County, Spearfish Canyon (44° 25’ N, 103° 52’ W), also Pennington Co., Black Hills National Forest; Wyoming, Weston Co.
DEPOSITORY: USNTC.

HOST: Oryzomys galapagoensis bauri (Allen).
DISTRIBUTION: Ecuador, Galapagos Islands, Santa Fe (Barrington) Island (00° 49’ N, 90° 04’ W).
DEPOSITORY: USNTC.

HOST: Chionomys gud (Satunin).
DISTRIBUTION: Russia, Caucasus Mountains, South Daghestan.
DEPOSITORY: ZISP.

HOSTS: Genetta tigrina rubiginosa [now Genetta maculata (Gray)]. Also found on Cephalophus sylvicultor (Afzelius).
DEPOSITORY: USNTC; OVI; BMNH.

HOST: Oreotragus oreotragus (Zimmermann).
DISTRIBUTION: Zimbabwe, Matabeleland South Province, Rhodes Matopos (now Matopos) National Park, Maleme Dam (20° 33’ S, 28° 30’ E).
DEPOSITORY: OVI; USNTC; BMNH.
NOTE: Ticks congregate on twigs containing pre-orbital gland secretions of this host.

HOST: Moschus berezovskii Flerov.
DISTRIBUTION: Xizang, Zham and Nylam.
DEPOSITORY: IZAS.

HOST: Myosplax fontanierii (Milne-Edwards).
DISTRIBUTION: People’s Republic of China, Gansu Province, Pingliang County (35° 30’ N, 106° 40’ E). Also in Ningxia Huizu Autonomous Region and Shanxi Province.
DEPOSITORY: IZAS.

HOST: Reduncia fulvorthula (Afzelius).
DEPOSITORY: OVI; USNTC; BMNH.

HOST: Sylvicapra grimmia (L.)
DISTRIBUTION: Mozambique, Inhambane Province, Mambone.
DEPOSITORY: MNHM.

HOST: Moschus berezovski Flerov.
DISTRIBUTION: People's Republic of China, Sichuan Province, Rangtang County.
DEPOSITORY: IZAS.

HOSTS: Anourosorex squamipes Milne-Edwards, Mus pahari Thomas.
DISTRIBUTION: Thailand, Doi Inthanon.
DEPOSITORIES: National Science Museum, Natural History Institute, Shinjuku, Tokyo; National Institute of Animal Health, Tsukuba, Ibaraki, Japan.

HOSTS: Abrocoma bennetti Waterhouse, Acroamys fuscus (Waterhouse), Octodon degus (Molina), Phyllotis sp., probably P. darwini (Waterhouse).
DISTRIBUTION: Chile, Malleco Province, Parque Nahuelbuta (37° 50'S, 72° 57'W), also in Santiago and Maule Provinces.
DEPOSITORIES: USNTC; Texas Tech University, Lubbock, Texas, USA.

HOSTS: Cattle. Also on goats.
DEPOSITORIES: IZAS.

HOSTS: Crocidura flavescens (I. Geoffroy) and Crocidura sp.
DISTRIBUTION: Democratic Republic of Congo, Gemena Zone, Tandala (02° 58'N, 19° 21'E).
DEPOSITORIES: USNTC; BMNH; OVI.


HOST: Erinaceus dauuricus [now Mesechinus dauuricus (Sundevall)].
DISTRIBUTION: Mongolia, northeastern steppes. Also found in the southeastern Transbaikal.
DEPOSITORY: Not stated.

**HOSTS:** *Tragelaphus spekii* Scelater, occasionally from *Panthera leo* (L.), *P. pardus* (L.) and cattle.

**DISTRIBUTION:** Uganda, Tanzania, and Zambia in semi-aquatic habitats.

**DEPOSITORIES:** BMNH; OVI; USNTC.


**HOST:** *Rattus rattus* (L.).

**DISTRIBUTION:** India, Gujarat, Anand.

**DEPOSITORY:** Zoological Survey of India, Calcutta.

**NOTE:** A junior synonym of *Rhipicephalus ramachandrai* Dhanda.


**HOST:** cattle.

**DISTRIBUTION:** Ethiopia, Harrar, Hubeta. Also from Bagemder, Bale, Gemu Gofa, Gojam, and Sidamo.

**DEPOSITORY:** Not stated.

**Rhipicephalus camicasi** Morel, Mouchet, and Rodhain, 1976.

*Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* 29: 337-340, fig. 1.

**HOSTS:** Sheep. Also on goats and *Lepus capensis* L.

**DISTRIBUTION:** Afars and Issas (now Djibouti), Randa. Also Ethiopia, Harrar, and Shoa.

**DEPOSITORY:** Not stated.


**HOSTS:** Cattle, sheep, occasionally goats; wild artiodactyls such as *Antidorcas marsupialis* (Zimmermann), *Oryx gazella* (L.), and *Tragelaphus strepsiceros* (Pallas); leporids such as *Lepus saxatilis* F. Cuvier.

**DISTRIBUTION:** Namibia, Botswana, South Africa, and Angola.

**DEPOSITORIES:** BMNH; OVI; USNTC.


**HOST:** Cattle.

**DISTRIBUTION:** Uganda, Masaka District, Kawoko-Masaka (00° 30’ S, 31° 35’ E); Tanzania, Igula Village, Ihimbu Gunguli (07° 50’ S, 35° 47’ E); Zambia, Lutale, Mumbwa (15° 16’ S, 26° 50’ E).

**DEPOSITORIES:** USNTC; BMNH; OVI.


**HOSTS:** Sheep. Also on various antelope species.

**DISTRIBUTION:** Republic of South Africa, Eastern Cape Province, Dordrecht (31° 22’ S, 27° 02’ E).

**DEPOSITORIES:** OVI; USNTC.

**NOTE:** Ticks are found on the feet of these animals, usually between the toes and on the heels. Numerous other collecting localities in South Africa are cited on pg. 64 of the original description.


**HOSTS:** Sheep. Also on goats to a lesser extent.

**DISTRIBUTION:** Namibia, Bethanien District, farm “Soutdoringvlei” (c. 26°05’ S, 17°10’ E). Also in the Northern, Western, and Eastern Cape Provinces, Republic of South Africa.

**DEPOSITORIES:** OVI; USNTC; BMNH.

**NOTE:** Ticks are found on the feet of these animals, usually between the toes. Numerous other collecting localities are cited on pp. 72 and 74 of the original description.


**DISTRIBUTION:** Thailand, Doi Inthanon.

**DEPOSITORY:** National Science Museum, Natural History Institute, Shinjuku, Tokyo, Japan.

Countries Where These Tick Species are Found

AFGHANISTAN: *Haemaphysalis danieli*.

ANGOLA: *Rhipicephalus exophthalmos*.

ARGENTINA: *Amblyomma pseudoparvum, Ixodes pararicinus*.


BOTSWANA: *Rhipicephalus exophthalmos, R. zambeziensis*.

CAMEROON: *Haemaphysalis paraleachi*.

CHILE: *Ixodes sigelos*.

CONGO: *Ixodes brevisterea*.


DEMOCRATIC REPUBLIC OF CONGO: *Aponomma inopinatum, Haemaphysalis subterra, Ixodes brevisterea, I. macfarlanei, I. zairensis*.

DJIBOUTI: *Rhipicephalus camicasi*.

DOMINICAN REPUBLIC: *Ornithodoros antiquus*.

ECUADOR: *Ornithodoros yunikeri, Ixodes galapagoensis*.

ETHIOPIA: *Haemaphysalis lobachovi, H. subterra, Rhipicephalus bergeoni, R. camicasi*.

INDIA: *Hyalomma hystricis, Rhipicephalus arakeri*.

INDONESIA: *Carios hadiae, C. multisetosus, C. papuensis, Ornithodoros collocaliae, Haemaphysalis karsarsi*.

JAPAN: *Haemaphysalis mageshimaensis, H. phasiana, Ixodes columnae*.

KAZAKHSTAN: *Dermacentor ushakovae*.

KENYA: *Ornithodoros vansomereni, Haemaphysalis subterra*.

KYRGYZSTAN: *Dermacentor ushakovae*.

MADAGASCAR: *Haemaphysalis simplicicima*.

MONGOLIA: *Haemaphysalis demidovae, Pholeoixodes prokop'yevi*.

MOZAMBIQUE: *Boophilus floriae, B. scheepersi, Ixodes donarthuri, I. nicolasi*.

NAMIBIA: *Rhipicephalus exophthalmos, R. neumannii, R. zambeziensis*.

PAKISTAN: *Haemaphysalis danieli, H. sindensis*.

PAPUA NEW GUINEA: *Carios multisetosus*.


PERU: *Argas dalei, A. moreli, Ornithodoros spheniscus*.

POLAND: *Argas polonicus*.

PORTUGAL: *Ixodes bivari*.

PUERTO RICO: *Amblyomma arianae*.


RUSSIA: *Haemaphysalis filippovae, Ixodes ghilarovi*.

SAUDI ARABIA: *Hyalomma arabica*.

SENEGAL: *Alectorobius camicasi*.

SPAIN: *Argas gilcolladoi*.

TAJIKISTAN: *Anomalohimalaya lotozkyi, Dermacentor montanus*.

TANZANIA: *Haemaphysalis subterra, Rhipicephalus aquatilis, R. interventus*.

TIBET (XIZANG): *Ixodes moscharius*.

THAILAND: *Ixodes siamensis, Rhipicephalus tetracornus*.

TURKMENISTAN: *Dermacentor ushakovae*.

UGANDA: *Ixodes brevisterea, I. macfarlanei, Rhipicephalus aquatilis, R. interventus*.

URUGUAY: *Ixodes pararicinus*.

USA: *Argas monolakensis, A. ricei, Ixodes dammini, I. eastoni*.

VENEZUELA: *Antricola hummelincki*.

VIETNAM: *Aponomma orlovi, Haemaphysalis bacthaensis, H. grochovskajae, H. quadraculeata, H. suntzovi*.

YEMEN ARAB REPUBLIC: *Hyalomma arabica*.

ZAMBIA: *Haemaphysalis subterra, Ixodes calcicarbehas, Rhipicephalus aquatilis, R. interventus, R. zambeziensis*.

ZIMBABWE: *Haemaphysalis norvali, H. subterra, Ixodes matopi, Rhipicephalus zambeziensis*.

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Larval Habitats of Anopheleline Mosquitoes in the Upper Orinoco, Venezuela

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ABSTRACT: Survey of larval habitats of anopheline mosquitoes was conducted in Ocamo in the State of Amazonas, southern Venezuela. The sampled habitats belonged to three different hydrological types: lagoons (26 habitats), forest pools including flooded forest (16 habitats), and forest streams (4 habitats). Out of 46 habitats surveyed, 31 contained anopheline larvae. Six species were found: Anopheles darlingi, Anopheles triannulatus, Anopheles oswaldoi, Anopheles peryassui, Anopheles punctimacula, and Anopheles mediopunctatus. Anopheles triannulatus was the most abundant species. Significantly higher numbers of anopheline larvae, in general, and of An. triannulatus specifically were found in lagoons with submersed macrophytes and sparse emergent graminoids than in forest pools with detritus.


INTRODUCTION

An ecological approach that relates (in predictive models) vector distribution, environmental characteristics, and human activities to malaria transmission has recently been advocated for malaria control programs (Roberts and Rodriguez 1994, Rubio-Palis and Zimmerman 1997). Defining environmental conditions that characterize larval habitats of malaria vectors is an integral part of this approach. While we know a lot about distribution and behavior of adult mosquitoes, larval habitat studies have often been neglected.

An increase in numbers of malaria cases has occurred in many countries where malaria has been previously under control (PAHO 1998). One of the regions that has experienced recent increase in the incidence of malaria is the Upper Orinoco, specifically the Yanomami settlements in the Biospheric Reserve Alto Orinoco-Casiquiare in the State of Amazonas, southern Venezuela. Adult seasonal abundance, behavior, and entomological inoculation rate of the principal malaria vector of this region, Anopheles darlingi, have been recently documented from the Ocamo area by Rubio-Palis (1995) and Rubio-Palis et al. (1997), but the larval habitats have not been described. In this paper we report results of a systematic survey of anopheline larval habitats conducted in Ocamo in July, 1997. The results of this survey are supported by additional data collected over several trips to the region by one of the authors (YRP). This study provides the only available data on larval ecology of malaria vectors from this region and should be viewed as a potential starting point for more detailed investigations of larval habitats in the Upper Orinoco.

METHODS

Site Description

The survey of larval habitats was carried out in July, 1997 along the Ocamo and Orinoco Rivers (Fig. 1) in the Alto Orinoco-Casiquiare Biospheric Reserve, southern Venezuela. No high resolution maps/remote sensing images are available for the region; the most detailed map is on a scale of 1:500,000. We created our own map by plotting positions of the rivers, villages, and sampling
Figure 1. Map of the study area. Sampling locations are indicated by numbers.
locations as we recorded them with the GPS (Trimble Navigation). The area includes several villages, Santa Maria de los Guácharos - Ocamo, located close to the confluence of Ocamo and Orinoco rivers; Clavotheri, Carlitos, Kashora, and Tumbala along Ocamo river; and Shashana, San Benito, Lechoza, and Yochope along the Orinoco river.

Rio Ocamo, one of the major tributaries of the Upper Orinoco river is draining the central and southwest slopes of Sierra Parima in east-central Amazonas. Both the Upper Orinoco and Ocamo rivers can be characterized as clear water rivers with yellow-brown color, relatively low suspended solids, low nutrients, and circumneutral pH. These conditions reflect low mineral content of parent rocks (igneous metamorphic basement of granite overlain with layers of sand). Geomorphologically the study area is part of the Upper Orinoco lowlands (mean elevation about 135 m above see level) typified by a mosaic of periodically flooded forests including many palm species (Steyermark et al. 1995). Climate is macrothermic ombrophilous (hot and rainy) with mean annual T > 24°C and mean annual precipitation > 2200 mm (Ministerio del Ambiente station in Ocamo, 1997). The area is sparsely inhabited by Amerindians of Yanomami ethnic group practicing a shifting agriculture. Forests in the vicinity of villages are interspersed with small fields, “conucos.” Numerous lagoons are part of the fringing river floodplain. The floodplain is seasonally inundated by flow from the main river channels. When the floodwater recedes, the lagoons are isolated from the river until the following inundation. During the isolation period, water level is primarily controlled by rainfall, evapotranspiration, and groundwater seepage (Hamilton and Lewis 1990). In addition to lagoons, flooded forest (usually a transient habitat formed after a rain), small forest pools, and forest streams represent potential larval habitats. The lagoons support diverse aquatic vegetation, including emergent macrophytes (graminoids, Ludwigia spp., Montrichardia arborescens); submersed plant communities represented by genera Elodea, Mayaca, Utricularia, and Cabomba; and occasional floating macrophytes (Lemma, Salvinia). Forest pools and flooded forests provide habitats dominated by detritus, i.e., an accumulation of predominantly organic debris on the water surface.

Larval Sampling

Surveys of larval habitats were conducted at the beginning of the wet season (July, 1997). Sampling was concentrated in about 1 km buffer zones along the rivers (see Fig. 1). All potential habitats encountered when walking around the villages and through the forest with a Yanomami guide, well familiar with the area, were sampled. Thirty dips for mosquito larvae were taken from each habitat with a standard mosquito dipper. Larvae were reared to adults and some were preserved in 80% ethanol. Identified voucher specimens are deposited in the collection of the Entomology Laboratory, Research Division, School of Malariology in Maracay, Venezuela, and at the Museum Support Center, Smithsonian Institution.

Environmental Factors

The environmental data were recorded for each site (habitat) either as nominal values (hydrology type, surrounding environment, degree of shade), estimated percentages (tree cover, shrub cover, detritus cover), or measured water characteristics (pH, conductivity, ion concentrations). Standard methods of water analyses were used for determining the concentrations of cations (APHA 1985).

Field Data Analysis

The individual habitats, defined by dominant plant species/forms and hydrology/water chemistry, were categorized into higher units referred to as habitat types (Rejmankova et al. 1992). Cluster analysis (average linkage, chord Euclidean distance, Podani 1996) was conducted. The environmental variables measured as percentages were subjected to the angular transformation. The two-tailed t-test was used to compare the means of environmental variables for sites with or without larvae. Null hypothesis of independence of larval occurrence on types of habitats was tested with chi-square criterion (Sokal and Rolf 1995).

RESULTS

In July, 1997, we sampled 46 potential larval habitats in the Ocamo area (see the map, Fig. 1 for sampling locations). These habitats belonged to three different hydrological types: lagoons (26 habitats), forest pools including flooded forest (16 habitats), and forest streams (4 habitats). The environmental differences among these three types of habitats are summarized in TABLE 1. Lagoon habitats were significantly deeper than the other two types and they had water mineral content (specific conductivity and potassium, calcium and magnesium cations) comparable to streams but lower than forest pools. Lagoons were also significantly less shaded than forest pools and streams. They usually contained diverse communities of submersed macrophytes, which were absent in forest pools and streams.

Anopheline larvae were present in 31 out of 46 sampled habitats. Six species were identified: Anopheles...


Table 1. Means and standard deviations of environmental variables measured in habitats belonging to the three hydrological types. Means sharing the same letter are not significantly different (Scheffe’s test).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lagoons (n=26)</th>
<th>Forest Pools (n=16)</th>
<th>Forest Streams (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Depth [cm]</td>
<td>57.0 (23.6) a</td>
<td>16.0 (1.0) b</td>
<td>30.0 (18.5) b</td>
</tr>
<tr>
<td>pH</td>
<td>5.7 (0.5) a</td>
<td>5.7 (0.3) a</td>
<td>5.2 (0.4) b</td>
</tr>
<tr>
<td>Special Conditions [µS cm⁻¹]</td>
<td>14.6 (3.5) a</td>
<td>20.7 (12.3) b</td>
<td>9.0 (1.3) a</td>
</tr>
<tr>
<td>K⁺ [ppm]</td>
<td>0.8 (0.7) a</td>
<td>1.7 (1.5) b</td>
<td>0.5 (0.2) a</td>
</tr>
<tr>
<td>Ca++ [ppm]</td>
<td>1.5 (0.9) a</td>
<td>2.6 (2.1) b</td>
<td>1.2 (1.1) a</td>
</tr>
<tr>
<td>Mg++] [ppm]</td>
<td>0.2 (0.1) a</td>
<td>0.4 (0.3) b</td>
<td>0.1 (0.08) a</td>
</tr>
</tbody>
</table>

Tree Cover [%] 20.6 (28.6) a
Shrub Cover [%] 2.1 (3.9) a
Submersed [%] 33.3 (39.0)
Detritus [%] 13.9 (23.4) a

(Nyssorhynchus) darlingi Root, An. (Nyssorhynchus) triannulatus (Neiva and Pinto), An. (Nyssorhynchus) oswaldoi (Peryass), An. (Anopheles) peryassui Dryar and Knab, An. (Anopheles) punctimacula Dryar and Knab, and An. (Anopheles) mediopunctatus (Theobald). In addition, one of the authors collected An. (Nyssorhynchus) marajoara Galvao and Damasceno and An. (Nyssorhynchus) argyritarsis Robineau-Desvoidy in the lagoons (locations 1–3, Fig. 1) on a previous date (Yasmin Rubio-Palis, unpublished data). Not all the larvae were identified to species level since many collections consisted only of first or second larval instars. The abundances of other identified species, except for An. triannulatus, were too low to allow for testing of associations between individual mosquito species and habitat types. Consequently, only associations for An. triannulatus and for total anophelines are presented. The frequency analysis of larval occurrence in the three types of hydrologically defined habitats (TABLE 2) showed that larvae of An. triannulatus were significantly more frequent in lagoons than in forest pools or streams. The same analysis using data for all anopheline larvae showed similar trend but the association was only marginally significant (chi-square 4.99; DF = 2; p=0.08).

The cluster analysis of habitats based on environmental variables revealed two main clusters (A and B) differing mainly in the tree cover and water depth (Fig. 2). Cluster A can be further divided into three distinct smaller clusters dominated by submersed macrophytes (cluster #1), grasses (cluster #2), and sedges (cluster #3). Cluster B is characterized by a high tree cover, low water depth, and frequent presence of detritus. Cluster A closely overlaps with the hydrological type “lagoon.” Correspondingly, the frequency analysis of larval occurrence in the two main clusters showed that significantly more An. triannulatus, as well as more of total anopheline larvae were found in cluster A (TABLE 2). Frequency analysis testing the deviation from randomness of larval distribution among all four clusters (clusters 1, 2, 3, and cluster B) showed the heterogeneity of distribution of An. triannulatus among individual clusters to be only marginally significant (chi-square 7.18; DF = 3; p=0.06).

TABLE 3 shows the variables that significantly varied between habitats with An. triannulatus present versus absent. Tree cover and consequently a degree of shade, cover of broad-leaved macrophytes, total cover of submersed macrophytes, as well as cover of individual submersed species Utricularia spp. and Mayaca spp., cover of detritus, and cover of filamentous algae were significantly different between habitats with and without An. triannulatus larvae. Differences in water depth and pH were not quite significant (p=0.08), and specific conductivity as well as individual cations did not differ between positive and negative habitats.

**DISCUSSION**

There are several reasons why lagoons would provide favorable habitats for immature stages of mosquitoes. Vegetation is usually well developed in lagoons, since they are more permanent than ephemeral forest pools and have a higher light availability. Higher...
structural complexity provides more refuge from predation (Hall 1972). In addition, a well-developed periphyton layer including bacterial assemblages is most probably supplying food for larvae (Merritt et al. 1992), which may be especially important in this generally nutrient poor system. Tadei et al. (1998), in their paper on ecological observations on anophelines in the Brazilian Amazon, suggest that the higher species diversity of mosquitoes at human impacted locations is caused by increased input of nutrients from agricultural activities.

The main regional vector of malaria in the Upper Orinoco is An. darlingi. In our July survey we found this species only at one location (a lagoon with submersed macrophytes). Anopheles darlingi larvae were found in our study area in December, 1997 in similar habitats (locations 4, 5, and 6; Fig. 1) (Rubio-Palis, unpublished data). Habitats with submersed macrophytes were also reported for An. darlingi larvae from Belize by Manguin et al. (1996). Low abundance of An. darlingi in early July could be a consequence of a generally lower incidence of this species in the dry season (Rubio-Palis et al. 1997, Magris et al., unpublished data). Regardless of the paucity of larvae found, this species is clearly the dominant human biting anopheline in the area (Rubio-Palis 1996, Rubio-Palis et al., unpublished data).

### TABLE 2. Frequency analysis (ch-square test) of larval occurrence in habitats classified by cluster analysis and according to hydrology.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Anopheles triannulatus</th>
<th>Total anopheline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-square</td>
<td>p&lt;</td>
</tr>
<tr>
<td>CLUSTER A vs. B (see Fig. 2)</td>
<td>6.04</td>
<td>0.01</td>
</tr>
<tr>
<td>CLUSTER 1, 2, 3, 4 (see fig. 2)</td>
<td>7.18</td>
<td>0.06</td>
</tr>
<tr>
<td>HYDROLOGY (lagoon, forest pool, forest stream)</td>
<td>20.10</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### TABLE 3. Comparison of group means (+/-SD) of environmental variables from habitats with Anopheles triannulatus present and absent (two-tailed t-test).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Anopheles triannulatus present</th>
<th>Anopheles triannulatus absent</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td>Tree cover [%]</td>
<td></td>
<td>10.5</td>
<td>(21.4)</td>
</tr>
<tr>
<td>Submersed total [%]</td>
<td></td>
<td>40.4</td>
<td>(40.7)</td>
</tr>
<tr>
<td>Utricularia spp. [%]</td>
<td></td>
<td>7.0</td>
<td>(14.0)</td>
</tr>
<tr>
<td>Mayaca sp. [%]</td>
<td></td>
<td>12.2</td>
<td>(25.3)</td>
</tr>
<tr>
<td>Filamentous algae [%]</td>
<td></td>
<td>0.7</td>
<td>(1.6)</td>
</tr>
<tr>
<td>Detritus [%]</td>
<td></td>
<td>8.0</td>
<td>(9.4)</td>
</tr>
<tr>
<td>Shade</td>
<td></td>
<td>1.7</td>
<td>(0.7)</td>
</tr>
<tr>
<td>Broadleaved [%]</td>
<td></td>
<td>5.6</td>
<td>(16.6)</td>
</tr>
<tr>
<td>Water depth [cm]</td>
<td></td>
<td>53.9</td>
<td>(22.4)</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>5.9</td>
<td>(0.5)</td>
</tr>
</tbody>
</table>
Figure 2. Average linkage classification of 46 larval habitats based on 21 environmental variables and chord Euclidean distance.
The most frequent larval species in the study area was *An. triannulatus*. This species is regarded as a secondary vector of malaria by many authors from similar regions in Brazil (Tadei et al. 1983, Arruda et al. 1986, Deane et al. 1988, Oliveira-Ferreira et al. 1990, Pövon 1993) and Peru (Hayes et al. 1987). Nevertheless, it has not been collected during intensive human landing and CDC trap catches inside human dwellings in the study area (Rubio-Palis et al., unpublished data), which documents its exophilic behavior. As shown in TABLE 3 and the frequency analyses (TABLE 2), this species was mostly associated with submersed macrophytes and sparse growth of graminoids in lagoons. This is in agreement with Conn (1991) who reports collecting larvae of *An. triannulatus* from ponds with floating and emergent macrophytes. Tadei et al. (1983) report *An. triannulatus* to be among the most frequently occurring species in their study area (northern Amazon Basin).

*Anopheles mediopunctatus* was found only twice during the survey, each time in a shaded forest pool in detritus. Deane et al. (1988) reported this species from similar habitats, i.e., shaded forest pools and streams with clear water. *Anopheles punctimacula* was collected from lagoons with submersed macrophytes and detritus, which is in agreement with Gorham et al. (1967) and Rejmánková et al. (1998). Each, *An. oswaldoi* and *An. peryassui* were only found once in this survey, associated with submersed macrophytes and detritus, respectively. Rubio-Palis (1991) collected these species in ground pools with floating vegetation in shaded areas.

Habitat description, as provided here and in other papers (Rejmánková et al. 1993, 1998), is based primarily on hydrology and aquatic vegetation present. One of the advantages of using the aquatic vegetation for characterization of larval habitats is its integrating and therefore more permanent indication value. For example, stands of submersed *Mayaca* require certain water depth, nutrients, salinity, light, and time to get established. By finding certain anopheline species repeatedly in habitats defined by this submersed macrophytes, we can infer specific chemical and physical characteristics of the habitat and its relative permanence. This is important especially in situations when time or other constrains do not allow us to do repeated surveys of the same habitats.

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We thank Mr. James Pecor, Walter Reed Biosystematics Unit, for verifying the identifications of early stage larvae. Professor Justiniano Velasquez, Central University, Venezuela, kindly assisted with the identification of submersed macrophytes. Jae Kim, University of California, Davis, conducted the cation analysis. Remi was a great guide through the Ocama region. Yasmin Rubio-Palis and Leopoldo Villegas were funded by Proyecto Control de Enfermedades Endémicas/Malariología.

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Mosquito Control and Bacterial Flora in Water Enriched with Organic Matter and Treated with *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* Formulations

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**ABSTRACT:** Three tests were conducted during July 17 to October 30, 1998 to study the impact of two mosquitocidal microbial agents on mosquito larvae and their contribution to bacterial flora in aquatic microcosms. Formulations of *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Bacillus sphaericus* strain 2362 (Bsph) were applied at various rates to outdoor tubs enriched with rabbit pellets and filled with irrigation water from a reservoir. Mosquito larvae were effectively controlled by all treatments; the magnitude of initial and persistent control depended on materials and dosages applied. Bacterial flora were assessed in the irrigation water as well as water in the enriched tubs before and after treatment with the microbial agents. The irrigation water contained 800-1000 total bacterial cells/ml. The populations of total bacteria and spore formers peaked on day 3 after enriching and filling the tubs, then declined progressively to the low levels at the end of the tests. After treatment, the numbers of Bti and Bsph spores in treated tubs prevailed at a dosage-dependent manner, their populations peaked at three hours after treatment, and declined progressively thereafter. The contribution of Bti and Bsph spores to the total bacterial flora was negligible but significant to the counts of spore-forming bacteria. The gram-negative bacteria made up more than 80% of the total bacterial flora during the test periods; and, of these, gram-negative rods constituted the greatest proportion, gradually increasing from the time of flooding to the end of the tests. Gram-negative cocci also occurred in relatively great proportion, but showed a reverse trend as compared with the gram-negative rods, declining gradually from pretreatment to the end of the tests. Gram-positive rods (spore formers), including Bti and Bsph, occurred in low numbers in all the tests but increased slightly in treated tubs due to the addition of Bti and Bsph spores. Gram-positive cocci occurred occasionally in some water samples.

**Keyword Index:** *Bacillus thuringiensis* subsp. *israelensis*, *Bacillus sphaericus*, bacterial flora, spore-forming bacteria, *Culex* mosquitoes.

**INTRODUCTION**

*Bacillus thuringiensis* subsp. *israelensis* de Barjac (Bti) and *Bacillus sphaericus* Neide (Bsph) are two entomopathogenic gram-positive, aerobic, endospore-forming bacteria that produce parasporal proteinaceous crystals toxic to mosquito larvae. Bti has been evaluated for mosquito and black fly control in many countries (de Barjac and Sutherland 1990, Mulla 1990). It was registered in 1980 by the US-EPA for use against mosquitoes and black flies and has been found to be effective against these two groups of insects in many types of habitats (de Barjac and Sutherland 1990, Karch et al. 1991, Mulla et al. 1999). The most studied mosquitocidal strains of Bsph are strains 1593, 2297, and 2362 (Singer 1990). These strains have high activity against larvae of *Culex* mosquitoes. Despite its more limited host range, Bsph provides more persistent larvicidal activity than Bti under polluted water conditions (Yap 1990; Mulla 1991; Mulla et al. 1997, 1999). *Bacillus sphaericus* strain 2362 has been more extensively studied than the other strains and was registered by the US-EPA in 1991 for mosquito control. It has been successfully tested and used in mosquito control programs in Spain (Aranda and Eritja 1992), Thailand (Mulla et al. 1997, 1999),
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United States (Mulla 1991), West Africa (Karch et al. 1991, Skovmand and Bauduin 1997), and other countries.

Mosquito larvae breed in a variety of aquatic habitats and their food consists of microorganisms, detritus, algae, protozoans, bits of leaves, and small living and dead invertebrates (Clements 1992, Merritt et al. 1996). Bacteria are dominant microorganisms in the aquatic natural habitats of mosquito larvae. Bacterial densities have been estimated by direct microscopic count method (DMC), and have been reported to range from 18.8-65.4 x 10^7 cells/ml in surface microlayer of water in marsh habitats where anopheline larvae were found. In subsurface water, counts have ranged from 3.8-14.0 x 10^6 cells/ml (Walker and Merritt 1993). In another study, two different methods of bacterial assessment yielded different cell counts. Smith et al. (1998) collected surface water samples from a fresh water marsh habitat supporting anopheles larvae and made bacterial counts using the DMC and culturing on trypticase soy agar medium. The culture method yielded bacterial density in the range of 1.0-1.5 x 10^7 cells/ml, while the DMC method gave bacterial counts from 9.7 x 10^5-1.3 x 10^6 cells/ml. Bacteria constitute a major source of food of mosquito larvae. Using acridine orange and epifluorescence microscopy, Nilsson (1987) found that the number of bacteria per gut in some *Aedes*, *Culex*, *Caliseta*, and *Anopheles* species larvae ranged from 6.68 x 10^6-2.18 x 10^7. In field-collected *Aedes triseriatus* Say and *Anopheles quadrimaculatus* Say 4th-instar larvae, the number of bacteria averaged 2.2 x 10^6 and 2.0 x 10^5 cells/gut, respectively (Walker et al. 1988). In another study, four food types consisting of bacteria, detritus, euglenoid protozoans, and algae were found in the gut of 4th-instar larvae of *Cocquillettidia perturbans* Walker. Bacteria were found in highest numbers averaging 7.07 x 10^7 cells/gut. Next in numbers found were detrital particles, euglenoid protozoans, and algae (Merritt et al. 1990).

Natural populations of bacteria play an important role in the breakdown of organic matter and eutrophication of aquatic habitats and constitute a major source of food for mosquito larvae. We initiated the present studies to determine: 1) the relationship between natural microbial flora and mosquito breeding; and 2) the impact of various mosquitocidal formulations of Bti (VectoBac) and Bsph (VectoLex) on bacterial abundance in microcosms sustaining heavy breeding of natural populations of mosquitoes. The VectoBac and VectoLex formulations were applied to outdoor tubs enriched with organic matter, and the population trends of mosquito larvae, total bacterial flora, spore-forming bacteria, as well as Bti and Bsph spores were studied over time.

MATERIALS AND METHODS

Test Facilities

Three tests were conducted during the summer and fall seasons, from the middle of July to the end of October 1998. In total, 24 fiberglass tubs measuring 1.0 x 1.0 x 0.4 m deep, placed in an open sunlit area at the Aquatic and Vector Control Research Facility (Mudgeville, University of California, Riverside) were used. The tubs were enriched with rabbit pellets (Brookhurst®, Brookhurst Mill, Riverside, CA, crude protein 17%) at the rate of 100 grams per tub (0.04%) to facilitate continuous and sustained oviposition by wild gravid mosquitoes. The tubs were then filled to a depth of 30 cm (236 liters) of water from an irrigation reservoir. A constant water level was maintained in each tub by float valves. Within a day or two after flooding in warmer months, wild populations of *Culex stigmatosoma* Dyar, *Culex quinquefasciatus* Say, and *Culex tarsalis* Coquillett oviposited nightly in these tubs. During the course of the tests, the tubs also supported the production of algae and some aquatic macroinvertebrates, such as ephyrid flies, chironomid midges, mayfly naiads, dragonfly naiads, and others. The tubs were treated with Bti formulation on day 7 postflooding in tests 1 and 2 or both Bti and Bsph formulations on day 10 postflooding in test 3, when 3rd-4th-instar mosquito larvae were present in large numbers. The check and treatment tubs were assigned randomly before flooding using six (tests 1 and 2) or four (test 3) replicates. After termination of a given test, the tubs were cleaned and kept dry for three to seven days before starting a new test.

Bacterial Insecticides and Application

Three preparations of Bti and Bsph (provided by Abbott Laboratories, North Chicago, IL) were used in the tests. The water dispersible granules (WDG) of Bti (VectoBac WDG, ABG-6490, Lot # 30-067-BR, 4,000 ITU/mg, received on 7/10/1997) were applied at the rates of 0.27, 0.53, and 1.1 lb/ac, equaling 25, 50, and 100 mg per tub, respectively. The Bsph WDG formulation (VectoLex WDG, ABG 6491, Lot # 32-094-BR, 600 ITU/mg, received 8/5/1998) was applied at the rates of 0.05 and 0.1 lb/ac, equaling 5 and 10 mg per tub, respectively. The commercial product VectoLex CG (corn grit) (Lot # 29-853-N8, 50 ITU/mg, received 8/4/1998) was applied at the rates of 1.4 and 2.7 lb/ac, equaling 125 and 250 mg per tub, respectively.

The WDG formulations were brownish fine sized granules with a loose appearance that readily dispersed in water by gentle stirring or shaking. Before application, the WDG formulations were suspended in distilled water at 1% for VectoBac WDG or 0.1% for VectoLex
WDG. The required aqueous aliquots based on the treatment rates were slowly applied to the corners and edges of the tubs using a 5 ml pipette. The required amounts of the VectoLex CG granules were broadcast by hand to the corners and edges of the tubs getting good coverage.

Abiotic Water Parameters
Maximum-minimum water temperatures were measured during each test period by leaving a minimum-maximum thermometer submerged in the water located in the center of the tub arrangement. In every tub, dissolved oxygen (DO, ppm), electrical conductivity (EC, μS), and salinity (ppt) were measured by YSI Model 85 Handheld Water Quality Meter on every sampling day. The pH values of the tub water were determined by pH test strips.

Mosquito Control
In VectoBac WDG tests (1 and 2), immature mosquitoes (larvae and pupae) were sampled just before treatment (day 0 or day 7 postflooding) and on days 3, 7, and 14 posttreatment. In the test of VectoBac and VectoLex (test 3), immature mosquitoes were sampled just before treatment (day 0 or day 10 postflooding) and on days 3, 7, 14, 21, and 28 posttreatment. Five dip samples were taken from each tub; one dip from each corner and one from elsewhere where mosquito larvae were noted in large numbers. The mosquito immatures were counted and divided into three categories as early instars (1st and 2nd), late instars (3rd and 4th), and pupae.

Bacterial Assessment
Water Samples: To study bacterial density in tests 1 and 2, both top and bottom water from assigned check and treated tubs was collected on day 4 pretreatment (day 3 postflooding), just before treatment (day 0 posttreatment or day 7 postflooding), and on days 3, 7, and 14 posttreatment. Additionally, in test 3 we took samples of water flowing from the float valves to determine bacterial density in the water coming from the reservoir. Water from assigned check and treated tubs was collected on day 10 pretreatment (3 h after start of filling), day 7 pretreatment (day 3 postflooding), just before treatment (day 0 or day 10 postflooding), and on days 3, 7, 14, 21, and 28 posttreatment for study of bacterial density. For determination of Bti and Bsph spores soon after treatment with VectoBac and VectoLex formulations, additional water samples from check and treated tubs were also collected at three hours after treatment. Using sterile pipettes fitted with a pro-pipette, 5 ml of water was collected at 0.5-1.0 cm below the surface (not surface film) and about 0.5-1.0 cm above the bottom of the tub. From each tub, five samples were collected at the four corners and middle for the top and bottom of each tub. The samples from the top or the bottom of all replicates were composited in 250 ml sterile flasks, each composite sample amounting to 150 ml (tests 1 and 2) or 100 ml (test 3) of water from six or four replicates of each treatment as well as check. The water samples were kept in a refrigerator at 5°C and processed a few hours after collection.

Media for Bacterial Isolation: To estimate the bacterial densities in water samples, nutrient agar medium and selective media containing antibiotics were used (Jones 1970, Yousten et al. 1985). Nutrient agar medium (NA medium: 2.3% Difco nutrient agar) was used for identifying diversity of total bacteria, including spore-forming bacteria. For recovery of Bti spores, we used selective medium (NYPC medium) containing 2.3% nutrient agar, 0.05% yeast extract, 0.01% polymixin B sulfate, 0.0001% chloramphenicol. Selective nutrient-yeast medium with streptomycin (NYST medium: 2.3% nutrient agar, 0.05% yeast extract, 0.01% streptomycin) was used for recovery of Bsph spores. The media were prepared, autoclaved, and poured into Petri dishes three days before plating out water samples onto the media. This time was necessary for drying the medium surface and detecting contamination of Petri dishes if any. Dishes showing any contaminations were discarded.

Bacterial culture and count: Prior to plating the water samples onto microbiological media, a 1.0-ml aliquot from each water sample was serially diluted in 9 ml autoclaved water. For detection of bacterial cells, dilutions ranged from 10-105 times. Three Petri dishes containing 20 ml NA medium were inoculated with 0.1 ml aliquot of each serial dilution, and incubated at 28-30°C for 48-72 hours. Autoclaved water for dilution was also plated on NA as a control. After the incubation period, colony-forming units (CFUs) were counted visually and characterized by color, form, elevation, edge, and diameter. Cultivable cell densities were averaged from the CFUs and adjusted for dilutions and inoculum volume and were expressed as CFUs/ml. Cell morphology and gram-stained cells were examined under light microscopy at 400x and under oil immersion at 1,000x. Bacteria were categorized as rods prevailing singly or in chains and cocci consisting of monococci, diplococci, and tetracocci.

For determination of the densities of spore-forming bacteria including Bti and Bsph, water samples were pasteurized at 65°C for 30 min to kill vegetative and non-spore-forming bacteria before diluting and plating. Pasteurized water samples were spread on NA medium for recovery of total spore-forming bacteria as well as on
selective media NYPC and NYST for recovery of Bri and Bsph spores. Colonies were counted visually and characterized 48-72 hours after incubation at 28-30°C. Cell morphology and gram-stained cells were observed microscopically. In a few cases where the identification of cells and spores was uncertain, isolated bacterial colonies were removed for culturing on agar slants at 30°C for 24 hours, and then were kept at 5°C to reconfirm identification by microscopic examination.

Data Analysis

Average mosquito larval densities (larvae/dip) and average bacterial densities (CFU/ml) in the check and treated tubs were analyzed for significance by one factor ANOVA (Scheffé F test) for repeated measurements (Abacus Concepts, Inc. 1987). For mosquito control, the reduction in the population of immature mosquitoes was calculated using Mulla’s formula (Mulla et al. 1971): \( \% R = 100 - \frac{[C1/T1] x (T2/C2)}{100} \), where \( C1 \) = mean number of larvae in control pretreatment, \( T1 \) = mean number of larvae in treated pretreatment, \( T2 \) = mean number of larvae in treated posttreatment, and \( C2 \) = mean number of larvae in control posttreatment.

RESULTS

VectoBac WDG Tests (Test 1 and 2)

Abiotic Water Parameters

During the course of these tests, maximum water temperatures ranged from 32.2-37.8°C and minimum ranged from 22.1-23.3°C. No significant differences were detected in abiotic water parameters (pH, DO, EC, and salinity) among various treatment regimens on every sampling day (data omitted). But it was noted that oxygen tension due to microbial degradation of the organic matter reached an almost anoxic level on day 3 postflooding. Oxygen tension increased after this initial period when the organic matter decomposition had passed the peak period.

Mosquito Control

The population trends of mosquito larvae in test 1 and 2 were essentially the same, and the data of the two experiments are combined and the averages are presented (Fig. 1A). Immediately prior to treatment, larval densities in all the assigned check and treatments were statistically the same. On days 3 and 7 posttreatment, all three rates (0.27, 0.53, and 1.1 lb/ac) of VectoBac WDG yielded good control. The number of larvae was reduced by 94.3, 94.9, and 72.7% (day 3) and 93.6, 91.3, and 68.7% (day 7), respectively. It was clear that the lower rate (0.27 lb/ac) was less effective than the two higher rates that demonstrated equal efficacy. The larval densities in the checks declined naturally on day 14 posttreatment, and no control due to the treatment was indicated on this sampling day.

Bacterial Assessment

Total bacteria: Since the bacterial flora and trends were essentially the same in tests 1 and 2, we have combined the data of the two experiments and report their averages. The average numbers of total bacteria (CFUs/ml) in top water cultivable on NA medium peaked on day 4 pretreatment (day 3 postflooding), and declined sharply by 91-93% on day 0 pretreatment (day 7 postflooding). The total bacterial counts declined gradually afterward to the end of the test periods (day 14 posttreatment) when the reductions were 97-98% as compared with the peak populations on day 4 pretreatment (Fig. 1B). The changing pattern of bacterial numbers in bottom water was similar to that in top water (Fig. 1B).

As to the impact of microbial treatment on bacterial flora, the total bacterial counts in the check tubes were slightly but significantly lower than in treated tubes with the highest rate of VectoBac WDG (1.1 lb/ac) on day 3 posttreatment in top water. The same situation held true on day 7 posttreatment. No treatment-related differences occurred on day 14 posttreatment. In bottom water, treatment-related differences in total bacterial counts were only noted on day 7 posttreatment, where the tubes treated with the middle rate of VectoBac WDG (0.53 lb/ac) contained more spore formers than check and other treatments (Fig. 1B).

Spore-forming bacteria: In water samples collected on day 4 pretreatment (day 3 postflooding), we recovered some spore-forming bacteria, which produced big elongated ovoid spores. These bacteria occurred singly or in long chains. The average numbers of all spore-forming bacteria in top water increased gradually from day 4 pretreatment, peaked on day 3 posttreatment, and declined thereafter (Fig. 2A). The changing pattern in populations of total spore formers in bottom water was similar to that in top water (Fig. 2A).

With regard to the impact of VectoBac WDG treatment on total spore-former counting after treatment, the spore formers in treated tubs with the highest rate of 1.1 lb/ac were significantly higher than check and other treatments in top water on days 3 and 14 posttreatment. No treatment-related differences were indicated on day 7 posttreatment (Fig. 2A). In bottom water, the same impact was only seen on day 3 posttreatment. No treatment-related differences were indicated on the following sampling days (Fig. 2A).

Bti spores: After treatment with VectoBac WDG,
Figure 1. Average numbers of mosquito larvae (A) and total bacteria (B) in water of tubs enriched with organic matter and treated with VectoBac G formulation. * Unshared letters indicate significant differences by 1-factor ANOVA (Scheffe F test) for repeated measurements at the 0.05 level.
Figure 2. Average numbers of spore-forming bacteria including Bti (A) and Bti spores alone (B) present in water enriched with organic matter and treated with VectoBac WDG formulation (tubs flooded 7 days before treatment, day 0 indicates just before treatment). *Unshared letters indicate significant differences by 1-factor ANOVA (Scheffe F test) for repeated measurements at the 0.05 level.
we isolated Bti spores on NA as well as on selective NYPC medium in all treated tubs. Overall, Bti spore counts in treatment samples gradually decreased from day 3 posttreatment to the end of the tests in both top and bottom water (Fig. 2B). In top water of treated tubs, the average counts of Bti spores showed a dosage-dependent manner on every sampling day posttreatment. The high rate of 1.1 lb/ac contributed significantly more Bti spores than the middle rate of 0.53 lb/ac and the low rate of 0.27 lb/ac. No differences were indicated between the middle and low rates. Similar trends were noted in bottom water. On day 3 posttreatment, significant differences in Bti spore counting were detected among three treatment rates. On day 7 posttreatment, significant difference only showed between the lowest and the highest rates. The situation on the last sampling day was the same as that in top water on this sampling day, i.e., the significantly higher count of Bti spore was encountered in the highest rate of 1.1 lb/ac (Fig. 2B).

Gram-stained bacteria: As to the bacterial types in top and bottom water, it was noted that gram-negative (Gr-ve) bacteria constituted 90-99% of the populations on different sampling days during the course of the two tests. In general, while Gr-ve rods increased from 34-52% on day 4 pretreatment to 81-93% on day 14 posttreatment. The Gr-ve cocci proportion decreased steadily from 48-66% to 5-16% during the same period of time. Gram-positive (Gr+ve) rods determined in our tests were spore-forming bacteria, to which Bti belongs, occurred in very low density and never exceeded 4% of the total bacterial flora. Gr+ve cocci occurred only occasionally in some water samples, constituting 4-9% of total bacterial flora (Fig. 3).

**VectoBac and VectoLex Test (Test 3)**

**Abiotic Water Parameter**

Water temperatures during the 39 days of evaluation were considerably cooler than the previous two tests in the summer, ranging from 12.2-17.8°C and 25.6-27.8°C for the minimum and the maximum, respectively. As in the previous tests, no significant differences were detected in abiotic water parameters (pH, DO, EC, and salinity) among various treatment regimens on every sampling day (data omitted). But as noted in test 1 and 2, the oxygen tension reached to an almost anoxic level on day 3 postflooding, then increased after this initial period upon the completion of decomposition of enriched organic materials.

**Mosquito Control**

In this test, the numbers of total larvae on day 0 (before treatment) in assigned check and treatments were statistically the same. Significant mosquito larval control was achieved on days 3 (75.7-97.4% reductions) and 7 (50.0-65.0% reductions) posttreatment in all treatments. On day 14 posttreatment, only VectoLex WDG gave partial but significant control (50.0-59.0% reductions) at both rates. Other treatments (VectoBac WDG and VectoLex CG) did not show any more control. On days 21 and 28 posttreatment, no more control was indicated in all treatments (Fig. 4).

**Bacterial Flora**

**Total bacteria:** In the third test, in addition to assessing the bacterial population in the water enriched with rabbit pellets, we also took samples of water flowing from the float valves, which contained very few bacteria averaging 0.8-1.0 x 10³ cells/ml.

In top water of assigned check and treated tubs, the average numbers of total bacteria increased from very low counts on day 10 pretreatment (at 3 h after start of filling) to the peak populations on day 7 pretreatment. On day 0 (before treatment), bacterial densities decreased sharply by 81-83% as compared with day 7 pretreatment. From day 3 posttreatment to the end of the test, the number of total bacteria continued to decrease slightly. On day 28 posttreatment, when the test was concluded, the reductions in total bacterial populations were 96-97% as compared with peak populations on day 7 pretreatment (Fig. 5). The overall changing trends of total bacteria in bottom water were essentially the same as those in top water (Fig. 5).

After treatment with VectoBac and VectoLex formulations, differences in bacterial population declines were noted between check and treatments. In the top water on days 3, 7, 14, and 21 posttreatment, the total bacterial counts in check tubs were slightly but significantly lower than in treated tubs. On the last sampling day (day 28 posttreatment), the tubs treated with VectoLex WDG at 0.1 lb/ac contained more total bacteria than the check and other treatments (Fig. 5). As to the impact of microbial treatments on total bacterial counts in bottom water, some slight but significant differences were indicated on some occasions. On day 3 posttreatment, the tubs treated with VectoBac WDG showed higher bacterial counts than the check and VectoLex WDG at the low rate (0.05 lb/ac). On day 7 posttreatment, however, all treatments showed higher total bacterial counts than the check. On day 14 posttreatment, the tubs treated with high rates of VectoLex products (both WDG and CG) showed higher total bacterial counts than the check and other treatments. The treatment of VectoLex WDG at the high rate still showed higher counts of total bacteria than check and other treatments on days 21 posttreatment. The situation
Figure 3. Proportion of gram-negative (Gr-ve) and gram-positive (Gr+ve) bacteria present in water from untreated (A) and treated tubs with VectoBacWDG formulation at 0.27 lb/ac (B), 0.53 lb/ac (C), and 1.1 lb/ac (D) (tubs flooded 7 days before treatment, day 0 indicates just before treatment).
on day 28 posttreatment was the same as that on day 21 posttreatment (Fig. 5).

*Spore-forming bacteria:* Presence of spore-forming bacteria was noted at three hours after start of filling the tubs in all water samples. The numbers of total spore formers in top water increased progressively from very low levels at three hours after start of filling to the peak populations on day 3 posttreatment. From day 3 posttreatment to the end of the test, densities of total spore formers decreased gradually and reached the low levels on day 28 posttreatment (Fig. 6). The trends of total spore formers in bottom water were essentially the same as in top water (Fig. 6).

The treatment using VectoBac and VectoLex formulations at various dosages made some contributions to the counts of total spore formers. In top water, on days 3 and 7 posttreatment, all treatments yielded significantly higher counts of total spore formers than the check. The treatments of VectoBac WDG and VectoLex CG at the high rate of 2.7 lb/ac added more to the counts of total spore formers than other treatments on day 7 posttreatment. On day 14 posttreatment, unexpectedly, the treatment with VectoLex CG at the low rate of 1.4 lb/ac slightly but significantly increased the populations of total spore formers as compared with the high rate of this material. On day 21 posttreatment, the samples from the treatment of VectoBac WDG contained more spore formers than those from the treatments of VectoLex WDG at 0.1 lb/ac and VectoLex at 2.7 lb/ac. On the last sampling day, only the treatment of VectoBac WDG resulted in higher counts of total spore formers than check and the treatments of VectoLex products (both WDG and CG) at the high rates (Fig. 6). In bottom water, essentially the same situations as in top water existed. On day 3 posttreatment, all treatments increased the counts of total spore formers as compared with the check, and the impacts of VectoBac WDG and VectoLex at 2.7 lb/ac were stronger than other treatments. On day 7 posttreatment, only the treatments of VectoBac WDG and VectoLex WDG at the high rate of 0.1 lb/ac increased the counts of total spore formers. The counts on day 14 posttreatment was unexpected where the treatment of
Figure 5. Average numbers of total bacteria present in water of tubs enriched with organic matter and treated with VectoBac and VectoLex formulations (tubs flooded 10 days before treatment, day 0 indicates just before treatment). * Unshared letters indicate significant differences by 1-factor ANOVA (Scheffe F test) at the 0.05 level.
Figure 6. Average numbers of spore-forming bacteria including Bti present in water enriched with organic matter and treated with VectoBac G formulation (tubs flooded 10 days before treatment, day 0 indicates just before treatment). *Unshared letters indicate significant differences by 1-factor ANOVA (Scheffe F test) for repeated measurements at the 0.05 level.
VectoLex CG at the high rate of 2.7 lb/ac showed lowest total spore former counts. On days 21 and 28 posttreatment, the treatment of VectoBac WDG still increased the counts of total spore formers as compared with check and other treatments (Fig. 6).

_Bti and Bsph spores:_ In this test, as expected, no Bti and Bsph spores were found in any samples collected before treatment, and the samples from check after treatment. However, after treatment with VectoBac WDG and VectoLex formulations, the spores were recovered on selective media. In top water samples, the spore counts of Bti and Bsph were the highest at three hours after treatment, then declined progressively to very low levels at the end of the test (Fig. 7). The similar population trends of Bti and Bsph spores were indicated in bottom water (Fig. 7).

With respect to the spore counts of Bti and Bsph in the check and various treatments, in top water, the tubs treated with VectoLex WDG at the low rate of 0.05 lb/ac contained fewer spores than those treated with VectoBac WDG at three hours posttreatment. On the following sampling days, highest spore counts were encountered in the treatment of VectoBac WDG (Fig. 7). In bottom water, at three hours posttreatment, VectoBac WDG treatment produced higher spore counts than other treatments except VectoLex WDG at the high rate of 0.1 lb/ac. From day 3 through 28 posttreatment, more spore counts were encountered in the treatment of VectoBac WDG than in other treatments (Fig. 7).

_Gram stained bacteria:_ The data obtained with regard to the changes in bacterial types in top and bottom water in the third test showed that Gr-ve bacteria as in previous tests, accounted for 80-99% of the total bacteria on different sampling days during the course of the test. Gr-ve rods increased from 40-60% at three hours after start of filling the tubs and day 7 pretreatment to 97-98% on day 28 posttreatment (Fig. 8). Gr-ve cocci increased from 20-24% at three hours after start of filling the tubs to 55-60% on day 7 pretreatment, then decreased to 4-16% on day 21 posttreatment. On day 28 posttreatment, no Gr-ve cocci were recovered in all water samples. As in the first and second tests, Gr+ve rods found in the third test were spore-forming bacteria, their numbers made up no more than 4% of the total bacteria. Gr+ve cocci were found in low numbers most during the earlier phase of the test period (Fig. 8).

**DISCUSSION**

Initially, the Bti WDG formulation yielded a high level of mosquito control at the two higher rates (0.53 and 1.1 lb/ac). The extent of control was still high at 7 days posttreatment. At 14 days posttreatment the larval density in the check declined drastically due to natural phenomena, and larval abundance in the treated was not markedly different from that in the check. Because of the natural decline, it cannot be concluded with certainty whether the low prevalence of larvae in treatments was due to Bti or natural decline (lack of oviposition) or both.

In another test, low dosages of the VectoLex WDG formulation produced a persistent control of larvae up to 14 days posttreatment. But in this test, as before, the larval abundance from day 21 posttreatment decreased substantially in the check approximating that of larvae in the treatments. This declining trend of larvae as a result of reduced oviposition is partly responsible for the low larval abundance in both the treated and untreated regimens.

Embarking on the present studies, we hypothesized that mosquito ovipositional activity and, in turn, larval abundance are a function of the decomposition of organic matter and abundance of bacterial flora. Since we enriched the tubs with rabbit pellets prior to filling, we hypothesized that drastic changes in bacterial flora will ensue following flooding. Extensive bacteriological assessment lent support to this hypothesis. In the first place, the irrigation water from the reservoir used to fill the tubs had low bacterial counts, averaging 800-1000 cells/ml. However, soon after the start of filling (3 h), a large number of bacteria were detected in the top and bottom water in the enriched tubs (0.3-0.4 x 10^5 cells/ml). This increase in bacterial numbers is very likely the result of bacteria present before filling, and due to bacteria present on or in the rabbit pellets added to the tubs. The bacterial flora propagated rapidly and reached peak populations on day 4 pretreatment (day 3 postflooding). Bacterial abundance was positively correlated with ovipositional activity of mosquitoes (data omitted). After reaching a peak, the bacterial flora followed a natural decline, reaching the low levels at the end of the tests. This natural decline pattern was similar in both the check and treatment regimens.

In tests 1 and 2 in the summer season, treatments with VectoBac WDG formulation contributed little if any to the total bacterial flora. This was because of the application of relatively small quantities of the products necessary for the control of mosquito larvae. The spores of Bti were not detected in the check or other tubs before treatment, but they were found in all treated tubs after treatment. On day 3 posttreatment, a higher number of Bti spores was found in both top and bottom water. It is possible that the peak was reached soon after treatment.

Concerning the dynamic changes in the abundance of bacterial types, it was noted that gram-negative (Gr-ve) rods and Gr-ve cocci constituted most of the total...
Figure 7. Average numbers of Bti and Bsph spores present in water enriched with organic matter and treated with VectoBac G formulation (tubs flooded 10 days before treatment, day 0 indicates just before treatment). *Unshared letters indicate significant differences by 1-factor ANOVA (Scheffe F test) for repeated measurements at the 0.05 level.
Figure 8. Proportion of gram-negative (Gr-ve) and gram-positive (Gr+ve) bacteria present in water from untreated (A) and treated tubs with VectoBac WDG at 0.53 lb/ac (B), VectoLex WDG 0.1 lb/ac (C), and VectoLex CG 1.4 lb/ac (D) (tubs flooded 10 days before treatment, day 0 indicates just before treatment).
bacterial flora during the experimental period. Gr-ve rods increased gradually in their abundance over time while the Gr-ve cocci showed the reverse trend declining in abundance at the same time. Gr-positive (Gr+ve) rods (spore forming to which Bti and Bsph belong) occurred in very low densities. In general, the numbers of total spore-forming bacteria in treated tubs increased slightly after treatment with Bti formulation. Gr+ve cocci were noted occasionally, and no obvious trend was noted in their populations during the course of the tests.

In the experiment conducted in the cooler season when both Bti and Bsph formulations were applied, the bacterial populations also peaked on day 7 pretreatment (day 3 postflooding). Just before treatment (10 days postflooding), the total bacterial flora had declined sharply and further slight declines continued on subsequent sampling days as in the previous experiments. Spore-forming bacteria reached a peak in all treatments and the check on day 3 posttreatment, then declined steadily as did the total bacteria on subsequent sampling days. However, the numbers of total spore formers in Bti and Bsph treatment regimens were higher than those of the check on most sampling days. Soon after treatment (3 h), high numbers of Bti and Bsph spores were found in the top and bottom water of treated tubs. None of their spores were detected in the check. The decline of Bti and Bsph spores in both top and bottom water was very pronounced on day 3 after treatment. Bti and Bsph spore counts declined further on subsequent sampling days. It was also noted that the populations of Gr-ve and Gr+ve bacteria followed the similar trends to those found in the previous experiments (tests 1 and 2), but the Gr+ve cocci were found most during the earlier phase of the test.

In all three tests, total bacterial flora was higher in the treated regimens than in the checks in most of the samples, especially in top water in test 3. This difference was very likely due to the elimination of mosquito larvae in the treated tubs. Abundant larval populations in checks were responsible for ingesting bacterial cells, resulting in reduced numbers of bacteria.

From these studies, it is clear that bacterial flora play an important role in the oviposition activity of gravid mosquitoes as indicated by larval production and as a source of food for larvae. Bacterial decomposition of organic matter produces transspecific behavior modifying substances that strongly influence mosquito oviposition. Bacterial flora also serves as a source of food for mosquito larvae. Oviposition cues provided by the decomposition of organic matter also are signaling abundant supply of food for larvae. These studies also showed that the addition of spores by treatments with Bti and Bsph formulations add very little to the already large number of total existing natural populations of bacteria, especially at the peak population level. However, if one considers the spore-forming bacteria alone, then the treatments made with larvicidal dosages can increase the number of spores in the top and bottom water of the treated habitats. This increase, however, could be influenced by biotic and edaphic factors in mosquito breeding habitats.

Acknowledgments

We wish to thank John D. Chaney (Department of Entomology, University of California, Riverside) for assistance in field and laboratory during the course of this study.

REFERENCES CITED


Field Efficacy of Fipronil 3G, Lambda-cyhalothrin 10% CS, and Sumithion 50EC Against the Dengue Vector

*Aedes albopictus* in Discarded Tires

S. Sulaiman, Z. A. Pawanchee, A. Wahab, J. Jamal, and A. R. Sohadi

**ABSTRACT:** The efficacy of three insecticides, fipronil 3G, lambda-cyhalothrin 10%CS, and sumithion 50EC were evaluated against the dengue vector *Aedes albopictus* in discarded tires in Kuala Lumpur, Malaysia. The dosage given for each insecticide was 0.01 g of active ingredient/m². Fipronil 3G was the most effective larvicide with a residual activity of up to two weeks, causing 88% mortality in *Aedes albopictus*. Lambda-cyhalothrin 10% CS was effective for one week causing 92% larval mortality and two weeks with 63% larval mortality. Sumithion 50EC had a residual efficacy of one week with 79% larval mortality.

**Keyword Index:** Fipronil 3G, lambda-cyhalothrin 10% CS, sumithion 50EC, *Aedes albopictus*, discarded tires

**INTRODUCTION**

In Southeast Asia, *Aedes albopictus* (Skuse) has been incriminated as a secondary vector of dengue fever and *Aedes aegypti* (Linnaeus) as the principal vector of dengue viruses (Russell et al. 1969, Chan et al. 1971, Jumali et al. 1979, Harinasuta 1984). Human-made habitats, such as discarded tires, are becoming important sources for the prolific breeding and dispersal of *Ae. albopictus* into new geographical areas (Reiter and Sprenger 1987, Laird et al. 1994).

Vythilingam (1988) used fenithrothin in fogging trials in the field against *Ae. aegypti* adults and reported 100% mortality. With the same insecticide it caused 89 to 100% mortality in *Ae. aegypti* larvae. Lam and Tham (1988) conducted a field trial with ULV application of malathion 96% technical grade and sumithion L-40S against *Ae. aegypti* and *Ae. albopictus* at residential areas in Ipoh municipality, Perak, Malaysia. Malathion 96% TG showed little larvicidal activity, whereas, sumithion L-40S appears to have good larvicidal and adulticidal effects both indoors and outdoors.

The objective of this study was to compare the efficacy of fipronil 3G, lambda-cyhalothrin 10% CS, and sumithion 50EC against the dengue vector *Ae. albopictus* in discarded tires in a dengue endemic area in Kuala Lumpur.

**MATERIALS AND METHODS**

The experiment was conducted on flat ground under shade. About 280 discarded tires were placed horizontally on the ground in each of four plots selected for the study. Each plot measured 5x10m (50m²) and the distance between plots was 5m. Three plots were assigned to receive one control agent each, namely, fipronil 3G (Regent®) granular formulation (manufactured by Rhone-Poulenc Sector Agro Rue Pierre Biazet, BP 9163 Lyon, France), lambda-cyhalothrin 10% CS (IconCS®) (manufactured by Zeneca Agrochemicals, United Kingdom), and Sumithion 50EC (manufactured by Sumitomo Chemical Company, Ltd., Japan). Both lambda-cyhalothrin 10% CS and sumithion 50EC were diluted with water and applied as a residual spray inside
each tire at a concentration of 0.01 gm a.i/m² and a 
volume of 5 liters per plot. The fipronil G granules were 
placed inside each tire by hand at a rate of 0.01 g a.i/m². 
The dosage was based on the area of the plot.

Prior to spraying, the tires in each plot were left 
undisturbed in the open for about three weeks for Ae. 
albopictus breeding to take place. Between 2 and 3 liters 
of rainwater collected in each tire. No other mosquito 
species was identified in this study. All the tires in each 
plot were sampled for immatures before spraying, 24 
hours after spraying, and weekly thereafter. Sampling 
was conducted by taking only one dip with a ladle from 
each tire for every plot, and the numbers of dead and live 
immatures were recorded from each dip. Live immatures 
were replaced into their respective tires. The percentage 
mortality was based on dead immature stages sampled 
by the ladle from each tire sampled. The above 
experiment was conducted three times at different time 
periods. Statistical analysis was performed using the 
ANOVA and LSD test (Choi, 1978).

RESULTS AND DISCUSSION

Fipronil 3G produced the highest mortality of Ae. 
albopictus of 95% (290 died out of 304 immature 
stages), lambda-cyhalothrin 10%CS of 90% (247 died 
out of 275 immature stages), sumithion 50EC killed 
92% (244 died out of 264 immature stages), and controls 
were without any mortality (381 alive, no mortality) 24 
hours after treatment, respectively (TABLE 1). There 
were no significant differences (P>0.05) among the 
activities of the three insecticides, but they were 
comparatively different from the controls (P<0.0005) 24 
hours after treatment. A week after spraying fipronil 3G 
the highest mortality of 92% resulted (48 died out of 52 
immature stages), with lambda-cyhalothrin 10%CS 
mortality was 92% (45 died out of 49 immature stages), 
sumithion 50EC produced 79% mortality (65 died out of 
82 immature stages), and controls had 2% mortality (5 
died out of 331 immature stages). There were again no 
significant differences (P>0.05) between the three 
insecticides, but all were significantly different from the 
controls (P<0.0005). Two weeks after spraying, fipronil 
3G yielded the highest mortality of 88% (28 died out 
of 32 immature stages), lambda-cyhalothrin 10%CS of 
63% (15 died out of 24 immature stages), sumithion 
50EC of 42% (19 died out of 45 immature stages), while 
the controls had no mortality (354 alive). There was no 
significant difference between the three insecticides 
fipronil 3G, lambda-cyhalothrin 10%CS, and sumithion 
50EC (P>0.05). However, all the insecticides were 
significantly different from the controls (P<0.05).

Three weeks after spraying, fipronil 3G showed the 
highest mortality among all the insecticides evaluated 
with 51% mortality (43 died out of 85 immature stages), 
lambda-cyhalothrin 10%CS killed 48% (19 died out of 
40 immature stages), sumithion 50EC of 13% (13 died 
out of 98 immature stages), and controls had no mortality 
(370 alive). There was no significant difference between 
fipronil 3G and lambda-cyhalothrin 10%CS (P>0.05), 
but fipronil 3G was significantly different from sumithion 
50EC (P<0.05). Similarly, lambda-cyhalothrin 10%CS 
was significantly different from sumithion 50EC 
(P<0.05) three weeks after spraying. However, all the 
insecticides were significantly different from the controls 
(P<0.05).

Four weeks after spraying, fipronil 3G yielded the 
highest mortality of 41% (11 died out of 27 immature 
stages), lambda-cyhalothrin 10%CS of 3% (3 died out of 
115 immature stages), sumithion 50EC of 5% (7 died

<table>
<thead>
<tr>
<th>Weeks After Treatment</th>
<th>Fipronil 3G</th>
<th>Lambda-cyhalothrin 10%CS</th>
<th>Sumithion 50EC</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>95</td>
<td>90</td>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td>1 week</td>
<td>92</td>
<td>92</td>
<td>79</td>
<td>2</td>
</tr>
<tr>
<td>2 weeks</td>
<td>88</td>
<td>63</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>3 weeks</td>
<td>51</td>
<td>48</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>4 weeks</td>
<td>41</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>5 weeks</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
out of 130 immature stages), and controls showed no mortality (230 alive). Fipronil 3G was significantly different from both lambda-cyhalothrin 10% CS (P<0.001) and sumithion 50EC (P<0.001). However, lambda-cyhalothrin 10% CS was not significantly different from sumithion 50EC (P>0.05). All three insecticides were significantly different from controls (P<0.05). TABLE 2 indicated that there were more reductions in the population of live immature stages in the treated tires compared to the control 24 hours after treatment. Fipronil 3G showed the least population of live immatures two weeks after treatment compared to the treated tires with sumithion 50EC, lambda-cyhalothrin 10% CS, and the controls, respectively.

In this study, fipronil 3G appeared to be the most effective larvicide, followed by lambda-cyhalothrin 10% CS and sumithion 50EC. Our previous study also indicated that fipronil 3G was more effective than a formulation of Bacillus thuringiensis var. israelensis H-14 in controlling Aedes albopictus populations in tires (Sulaiman et al. 1997). Our previous study indicated fipronil 3G (Regent) and fipronil 50SC (Regent) was effective against Aedes albopictus larvae in discarded tires (Sulaiman et al. 1997). The fipronil 3G formulation was a more effective larvicide compared to the fipronil 50SC, having up to two weeks of residual activity with more than 80% mortality while the latter formulation was effective up to one week only. The present study indicated that the fipronil 3G produced similar results with residual activity of up to two weeks with 88% mortality of the immature stages.

Thus, the present study indicated that fipronil 3G was effective for two weeks, causing 88% mortality in Aedes albopictus in the discarded tires. Lambda-cyhalothrin 10% CS was effective for a week with 92% mortality and two weeks with 63% mortality. Sumithion 50EC was effective for one week with 79% mortality.

Acknowledgments

We wish to thank the Universiti Kebangsaan Malaysia for providing research facilities and the Ministry of Science Technology and Environment Malaysia for awarding the research grant IRPA 06-02-02-0006 to support this study. We also appreciate the support of the technical staff of the Vector Control Unit, Municipality of Kuala Lumpur. We also appreciate Rhone-Poulenc Malaysia Sdn. Bhd. for providing the sample of fipronil 3G, Agricultural Chemicals (M) Sdn. Bhd. for providing sumithion 50EC, and CCM Bioscience Sdn. Bhd. for providing the lambda-cyhalothrin 10% CS. We also thank Mrs. Zaleha Nipah for technical support and Fatimah Atan for typing the manuscript.

REFERENCES CITED


TABLE 2. Distribution of live immature stages of Aedes albopictus in tires over time after treatments with fipronil 3G, sumithion 50EC, and lambda-cyhalothrin 10% CS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Number of Live Immature Stages Before and After Treatment [% in Parenthesis]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before spraying</td>
</tr>
<tr>
<td>Fipronil 3G</td>
<td>1490(100)</td>
</tr>
<tr>
<td>Sumithion 50EC</td>
<td>1448(100)</td>
</tr>
<tr>
<td>Lambda-cyhalothrin 10% CS</td>
<td>111(100)</td>
</tr>
<tr>
<td>Control</td>
<td>685(100)</td>
</tr>
</tbody>
</table>
Reproductive Biology of *Lutzomyia shannoni* (Dyar) (Diptera: Psychodidae) Under Experimental Conditions

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ABSTRACT. Baseline biological growth data of *Lutzomyia shannoni* (Dyar) were compared under two experimental conditions within insulated styrofoam chests and in standard laboratory incubators. The developmental time from egg to adult was 67 and 52 days, respectively. Based on cohorts of 100 females in each experiment, horizontal life tables were constructed. The following predictive parameters were obtained under each of the two conditions: net rate of reproduction (23.5 and 18.0 females per cohort female), generation time (11.4 and 9.4 weeks), intrinsic rate of population increase (0.27 and 0.30), and finite rate of population increment (1.31 and 1.36). The reproductive value for each class age of the cohort females was calculated. The observed parameters were obtained under each experimental condition: net rate of reproduction (1.9 and 2.5 females per cohort female), generation time (11.7 and 9.6 weeks), intrinsic rate of population increase (0.05 and 0.09), and finite rate of population increment (1.06 and 1.10). Vertical life tables were elaborated and mortality was described for every generation in each cohort. In addition, for two successive generations, additive variance and heritability for fecundity were estimated.

Keyword Index: *Lutzomyia shannoni*, phlebotomine sand flies, life cycle, fecundity, intrinsic rate of increase, oviposition, heritability.

INTRODUCTION

*Lutzomyia shannoni* is a zoophilic species with a broad geographical distribution in the Western Hemisphere, extending from the southeastern United States to northern Argentina (Young and Duncan 1994). Local distribution limits are dependent on factors of rainfall, temperature, physical barriers to migration, forest habitat, and abundance of appropriate vertebrate hosts (Young and Arias 1991). In the United States, it is a vector of vesicular stomatitis virus (VSV), a disease affecting cattle, horses, and swine (Comer et al. 1990, 1991). In Colombia, *Lu. shannoni* is a species of medical and veterinary importance and occurs from sea level up to approximately 1,300 m (Young 1979). The role of *Lu. shannoni* in VSV epidemiology in Colombia is unknown, but the Instituto Colombiano Agropecuario (ICA) has recorded cattle infected with the New Jersey serotype of VSV in nearly 100 communities (ICA 1999). In addition, under laboratory conditions, *Lu. shannoni* can support the development of at least three species of *Leishmania*: *Le. mexicana*, *Le. panamensis* and *Le. chagasi* (Ferro et al. 1998).

The reproductive cycles of vector arthropods play a fundamental role in the epidemiology of the disease they transmit. The parameters affecting transmission of disease include fecundity rate, mortality rate, density, distribution by ages, migration rate, and genetic variation of the vector arthropods (Black and Moore 1996, Tabachnick and Black 1996). However, none of this information is available for *Lu. shannoni*. To better understand baseline parameters of the life cycle, growth under two experimental conditions was compared. Horizontal life tables were constructed based on the developmental time of each instar in cohorts of 100 females, observed and predictive population parameters were calculated. Stage specific mortality data were collected, and, for two successive generations, the additive variance and heritability of fecundity were estimated.
MATERIAL AND METHODS

Maintenance of Cohorts in the Laboratory

The specimens of Lu. shannoni came from a colony maintained since 1992 in the Entomology Laboratory, Instituto Nacional de Salud (INS), Bogotá, Colombia. A paired comparison was made under two experimental conditions, in a styrofoam chest and in incubator.

The females were fed on hamster blood two days after pupal emergence. Females were blood fed as follows: first, the adults were transferred from the rearing chambers to cloth cages (20 cm x 20 cm), then a hamster was anesthetized (sodium pentothal, 10 mg/kg animal weight) and introduced into the cloth cages for one hour. After blood feeding, a 60% glucose solution was provided daily on cotton swabs placed on the muslin covering of each rearing chamber. The rearing and feeding of adults and larvae of Lu. shannoni followed the methods of Modi and Tesh (1983), Endris et al. (1982), and modifications of Ferro et al. (1998).

Each day, data were collected on the number of females remaining alive, date of emergence of the pupae of the each female, date of blood feeding of each female, date of the oviposition of each female, number of oviposited eggs for each female, number of retained eggs per female, number of non-embryonated eggs per female, number of embryonated eggs per female, and number of days that each female survived in the adult stage. The life cycle parameters were estimated by tracking the development of each cohort female progeny and averaging these data. Fecundity was estimated by summing eggs oviposited and those retained by the female. Retained eggs were counted by dissection after the females died.

In Experiment 1, the cohort of 100 females was taken from the 22nd generation of the colony. After blood feeding, individual females were separated into rearing chambers (5.5 cm high and 6.6 cm in diameter). The rearing chambers were placed in a styrofoam chest. Inside, relative humidity and temperature were continuously monitored with a hygrometer and maximum–minimum thermometer. In Experiment 2, the cohort consisted of 100 couples (one female and one male) taken from the 27th generation of the colony. Immediately after blood feeding the females, each couple was separated into a rearing chamber (5.5 cm high and 6.6 cm in diameter). The rearing chambers were placed in plastic boxes with lids, and temperature and humidity were monitored within each box. The plastic boxes were maintained inside a temperature-controlled incubator. Changes in relative humidity and temperature in each experiment are summed in TABLE 1, and the daily oscillations of these parameters are shown in the Figures. 1, 2.

Calculation of Predictive Population Parameters Based on Horizontal Life Tables

In groups of organisms with intervals of discrete ages, the net reproductive rate ($R_o$) was calculated with the following equation:

$$ R_o = \sum_{x=0}^{\infty} l_x m_x $$

where $l_x$ is the survival proportion if the cohort females, and $m_x$ is the average of the cohort females. The generation time ($T_c$) was calculated with the following equation:

$$ T_c = \frac{\sum x l_x m_x}{R_o} $$

where $x$ is the age class of the cohort female, $l_x$ is the survival proportion if the cohort females, $m_x$ is the average of the cohort females, and $R_o$ is the net reproductive rate. The intrinsic rate of population increase ($r_c$) was calculated from the following equation:

$$ r_c = \frac{l_0 R_o}{T_c} $$

The maximum possible value of the parameter ($r$) for species in given biotic and physical environments was denoted by ($r_m$). This value was calculated from the ($r_c$) as the $r$ initial ($r_i$) and then, with an iterative method, each $r_i$ was proved simultaneously until the left side of the Euler and Lotka equation was equaled to 1. At this point, the maximum value of the intrinsic natural rate of increase $r_m$ was obtained (for its calculation, see Rabinovich 1980).

Euler-Lotka equation:

$$ \sum_{x=0}^{\infty} l_x m_x e^{-r_m x} = 1 $$

where ($e$) is the base of the natural logarithm, and $l_x m_x$ is obtained from the horizontal life table of the cohort females. The finite rate of increase was calculated with the equation:

$$ \lambda = e^{r_m} $$

where ($e$) is the base of natural logarithms and $r_m$ is the
TABLE 1. Variation, standard deviation, average relative humidity, and temperature in two experimental conditions for rearing *Lutzomyia shannoni*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental Conditions</th>
<th>Styrofoam Chest</th>
<th>Incubator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range (°C)</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>80-96%</td>
<td>3.4%</td>
<td>88.1%</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>21-27°C</td>
<td>1.2°C</td>
<td>25.1°C</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>27-30°C</td>
<td>0.84°C</td>
<td>28°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Styrofoam Chest</th>
<th>Incubator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>SD</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>90-100%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>22-27°C</td>
<td>0.9°C</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>26-28°C</td>
<td>0.5°C</td>
</tr>
</tbody>
</table>

Figure 1. Longevity of the cohort females and developmental time of each instar in one generation of *Lutzomyia shannoni* maintained in a styrofoam chest environment in relation to relative humidity and the temperature. CF, cohort females; L1, Larvae I; L2, larvae II; L3, larvae III; L4, larvae IV; P, pupae; A, adults. RH, relative humidity; MT, maximum temperature; mT, minimum temperature.

maximum rate of increase. According to Fisher (cited by Pianka 1988), the reproductive value ($V_x$) of the cohort females at the age ($x$) was formerly expressed as the relative reproductive value at the moment of the birth $V_0$ (in practice equals 1). $V_x$ was calculated by the following equation:

$$V_x = \frac{e^{r_m x}}{V_0} \sum_{x=0}^{\infty} e^{r_m x} l_x m_x$$

where ($e$) is the base of natural logarithm, ($r_m$) is the maximum natural rate of increase, ($x$) is the age of the cohort females, and $l_x m_x$ is the product of the survivorship of the each cohort female by its fecundity at an age ($x$).

The construction of horizontal life tables and the calculation of the predictive population parameters were based on methods of Pianka (1988), Rabinovich (1980), and Southwood (1978). This was assisted with a small computer program (E. Cárdenas and M.

Calculation of Observed Population Parameters

The calculation of observed population parameters was carried out with the following equations modified of Southwood (1978). The observed net reproductive rate \( R_o' \) was estimated as follows:

\[
R_o' = \frac{N_t'}{N_o'}
\]

where \( (N_t') \) is the number of females daughters obtained and \( (N_o') \) the number of cohort females. The observed intrinsic rate of population increase \( r_c' \) was calculated as follows:

\[
r_c' = \frac{l_t R_o'}{t}
\]

where \( (R_o') \) is the observed net reproductive rate, and \( (t) \) is the observed generation time. The observed finite rate of increase \( (\lambda) \) was estimated as follows:

\[
\lambda' = e^{r_c'}
\]

where \( (e) \) is the base of natural logarithms, and \( (r_c') \) is the observed intrinsic rate of population increase. The statistical comparison of the population parameters was tested by a minimum significant difference of \( p \leq 0.01 \) (Steel and Torrie 1985).

Vertical Life Tables and Calculation of Mortality

A vertical or temporal life table for arthropod populations is based on discrete developmental stages. The mathematical protocols of Southwood (1978) were followed to express the equations for the calculation of mortality. Apparent mortality was expressed as percentage of dead individuals in a particular stage, relative to the survivors of the same stage. For each stage, the apparent mortality is obtained as follows:

\[
\text{% Apparent Mortality} = \frac{d_j \times 100}{l_j}
\]

where \( d_j \) is the number of dead individuals in the \( j^{th} \) stage and \( l_j \) is the number of survivors in the \( j^{th} \) stage. The real mortality is presented as an additive percentage and serves to compare the role of different mortality factors in the same generation (Southwood 1978). For each stage it is obtained as follows:

\[
\text{% Real Mortality} = \frac{d_j \times 100}{l_j}
\]

where \( d_j \) is the number of dead individuals in the \( j^{th} \) stage, and \( l_j \) is the number of eggs at the beginning of the generation.
The irrereplaceable or indispensable mortality is that part of the generation mortality that will not occur if the mortality factor in question were removed from the life system. Allowance is made for the action of subsequent mortality factors (Southwood 1978). The irrereplaceable mortality is calculated as follows:

\[
\% \text{ Irreplaceable Mortality} = \frac{A_k \times 100}{l_i}
\]

where \(A_k\) is the number of adults over the observed if mortality had not occurred at the \(k\)th stage, and \(l_i\) is the number of eggs at the beginning of the generation.

**Calculation of Genetic Parameters**

The genetic parameters additive variance (\(\sigma^2_a\)) and heritability (\(h^2\)) for the fecundity of *Lu. shannonii* of the laboratory colony were estimated, following the method father-descending Linear Simple Regression (Falconer 1970, Strickberger 1978). For this purpose, the number of oviposited eggs, retained eggs, and total eggs was recorded for 30 parent females; these data were averaged for three daughters of each line.

**RESULTS**

**Life Cycle**

The oscillations of the relative humidity and temperature were not drastic in the two experiments (Fig. 1, 2); however, these oscillations were reduced in the incubator where the average life cycle time was shorter by two weeks (Fig. 2). The longevity of cohort females never extended beyond two weeks in either experiment. The curves labeled "Egg" in Figures 1 and 2 showed the distribution percentage of oviposited eggs in each cohort. In both, the maximum peak of oviposition occurred at the ninth day of the female adult life. Oviposition pattern and emergence times of the 1st and 2nd-instar larvae were similar in the two micro-environments. The 3rd, 4th larval instars, and pupae in the styrofoam chests required an average of one additional day each over those in the incubator; moreover, these three stages demonstrated greater variance in time within stage (Figs. 1, 2).

In TABLE 2, the life cycle averages for *Lu. shannonii* obtained in an incubator were compared with those obtained by Ferro et al. (1998) in styrofoam chests. No significant differences were obtained of the average duration in the following stages: eggs, 1st-instar larvae, and adults; however, significant differences were obtained for 2nd, 3rd, and 4th larval instars, and pupae between the two experiments.

**Horizontal Life Tables and Predictive Population Parameters**

Under the assumption that the cohort parental females were reared in styrofoam chests or an incubator, their pre-reproductive ages will approximate those of their respective progeny (see Pianka 1988). The pre-reproductive ages of cohort females were estimated based on the average pre-reproductive ages of their respective progeny—10.7 weeks for styrofoam chests and 8.6 weeks for the incubator. With these data, together with the average survivor data of cohort females (2 weeks for both), the class ages column (\(c\)) of the horizontal life tables was constructed. The pre-reproductive data provided an estimate of reproductive value (\(V_x\)) (TABLES 3, 4). The \(m_x\) column was formed according to Rabinovich (1972). The predictive population parameters were estimated in absence of mortality according to Southwood (1978).

From TABLE 3, the following values were obtained for predictive population parameters: net reproductive rate \((R_0) = 23.50\) daughter females per cohort female, generation time \((T_0) = 11.44\) weeks, the natural rate of increase \((r_m) = 0.27\) daughter females for each cohort female per week, and the finite rate of increase for each cohort was \(\lambda = 1.31\) individuals per female per week.

From TABLE 4, the following values were obtained for predictive population parameters: net reproductive rate \((R_0) = 18\) daughter females per cohort female, generation time \((T_0) = 9.42\) weeks, the natural rate of increase \((r_m) = 0.30\) daughter females for each cohort female per week, and the finite rate of increase for each cohort was \(\lambda = 1.36\) individuals per female per week.

The last columns of TABLES 3 and 4 showed estimates of the reproductive values per age class of the cohort females. For cohort maintained in a styrofoam chest, the increase in reproductive value was exponential (despite a fecundity of zero between 0.5 to 9.5 weeks) until the females reached an age of 11.5 weeks. For the incubator cohort, the increase in reproductive value was also exponential (note zero fecundity between 0.5 to 7.5 weeks) until the females reached an age of 9.5 weeks (Figs. 3, 4). These maximum peaks of \(V_x\) coincided with the week in which oviposition was initiated, confirming the theoretical expectation. \(V_x\) then abruptly decreased in the following week when the majority of the cohort females died. This population parameter provided a clear estimate of the increase potential of *Lu. shannonii* laboratory colony.

**Observed Population Parameters**

In the styrofoam chest, 192 adult daughter females were obtained from the 100 cohort; therefore, the net
TABLE 2. Statistical comparison of the developmental time of each instar of *Lutzomyia shannoni* obtained in two laboratory microenvironments.

<table>
<thead>
<tr>
<th>Development Stage</th>
<th>Styrofoam Chest (Ave. days)</th>
<th>Incubator (Ave. days)</th>
<th>Difference</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>8.5</td>
<td>8.3</td>
<td>0.20</td>
<td>Ns</td>
</tr>
<tr>
<td>Larva I</td>
<td>9.6</td>
<td>9.1</td>
<td>0.47</td>
<td>Ns</td>
</tr>
<tr>
<td>Larva II</td>
<td>9.2</td>
<td>7.9</td>
<td>1.30</td>
<td>**</td>
</tr>
<tr>
<td>Larva III</td>
<td>11.8</td>
<td>8.1</td>
<td>3.65</td>
<td>**</td>
</tr>
<tr>
<td>Larva IV</td>
<td>19.9</td>
<td>14.3</td>
<td>5.60</td>
<td>**</td>
</tr>
<tr>
<td>Pupa</td>
<td>15.2</td>
<td>6.7</td>
<td>8.46</td>
<td>**</td>
</tr>
<tr>
<td>Adult</td>
<td>8.6</td>
<td>8.1</td>
<td>0.45</td>
<td>Ns</td>
</tr>
</tbody>
</table>

** = *p* ≤ 0.01

TABLE 3. Life table of 100 cohort females of *Lutzomyia shannoni*, maintained in a styrofoam chest environment.

<table>
<thead>
<tr>
<th>x</th>
<th>l_x</th>
<th>m'_x</th>
<th>l xm</th>
<th>Xl xm</th>
<th>V_x</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.15</td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.51</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.00</td>
</tr>
<tr>
<td>3.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.63</td>
</tr>
<tr>
<td>4.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.46</td>
</tr>
<tr>
<td>5.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.56</td>
</tr>
<tr>
<td>6.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6.02</td>
</tr>
<tr>
<td>7.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.93</td>
</tr>
<tr>
<td>8.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10.45</td>
</tr>
<tr>
<td>9.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13.77</td>
</tr>
<tr>
<td>10.5</td>
<td>1</td>
<td>1.35</td>
<td>13.35</td>
<td>14.17</td>
<td>18.15</td>
</tr>
<tr>
<td>11.5</td>
<td>0.93</td>
<td>23.77</td>
<td>22.10</td>
<td>254.22</td>
<td>23.81</td>
</tr>
<tr>
<td>12.5</td>
<td>0.01</td>
<td>5.5</td>
<td>0.05</td>
<td>0.68</td>
<td>5.5</td>
</tr>
<tr>
<td>13.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>23.50</td>
<td>269.07</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

x, age class of the cohort females in weeks,
l_x, the survival proportion of the cohort females at the age (x),
m'_x, average of the females fecundity (corrected for sex ratio) by each cohort female of age (x),
V_x, the reproductive value of the cohort females of age (x).

reproductive rate (R'_o) was 1.92 females per female. For the 100 cohort females maintained in incubator, 253 adult daughter females were obtained, for a net reproductive rate (R'_o) of 2.53 females per female (TABLE 5).

In the styrofoam chest, the intrinsic rate (r'_c) was 0.05 females per female per week, while in the incubator r'_c reached 0.09 females per female per week (TABLE 5). Because, the net reproductive rate was >1, the population of the laboratory colony was increasing, but at a rather low rate.

The generation time T_c' = 11.70 weeks in the styrofoam chest versus T_c' = 9.6 weeks in the incubator. The finite rate of population increase (λ') in the
TABLE 4. Age-specific life table of 100 cohort females of *Lutzomyia shannoni*, maintained in an incubator environment.

<table>
<thead>
<tr>
<th>$x$</th>
<th>$l_x$</th>
<th>$m_x$</th>
<th>$l_xm_x$</th>
<th>$Xl_xm_x$</th>
<th>$V_x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.17</td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.59</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.16</td>
</tr>
<tr>
<td>3.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.93</td>
</tr>
<tr>
<td>4.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.98</td>
</tr>
<tr>
<td>5.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5.41</td>
</tr>
<tr>
<td>6.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.35</td>
</tr>
<tr>
<td>7.5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.99</td>
</tr>
<tr>
<td>8.5</td>
<td>1</td>
<td>1.34</td>
<td>1.34</td>
<td>11.39</td>
<td>13.58</td>
</tr>
<tr>
<td>9.5</td>
<td>0.95</td>
<td>17.51</td>
<td>16.66</td>
<td>158.03</td>
<td>17.51</td>
</tr>
<tr>
<td>10.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TOTAL  18.00  169.42

$x$, age class of the cohort females in weeks,

$l_x$, the survival proportion of the cohort females at the age ($x$),

$m_x$, average of the females fecundity (corrected for sex ratio) by each cohort female of age ($x$),

$V_x$, the reproductive value of the cohort females of age ($x$).

---

Figure 3. Survival ($l_x$), fecundity ($m_x$), and reproductive value ($V_x$) as a function of age (weeks) of the cohort females of *Lutzomyia shannoni* maintained in a styrofoam chest environment.

The styrofoam chest was 1.06 versus 1.10 in the incubator. These values were very similar to those demonstrated in the horizontal life tables (TABLE 5).

**Statistical Comparison of Population Parameters**

Significant statistical difference was obtained for the net rate of replacement ($R_0$), between the two rearing experiments. However, no significant differences were obtained for ($r_m$) between the two experiments (TABLE 5). These findings corroborated the intrinsic nature of the parameter, ($r_m$) as a genetically determined value for each species, since it was not
affected by changes in environment. Furthermore, the 
\( r_m \) values remained unaffected when data for 
non-ovipositing females, including those with retained eggs, 
were removed from the analysis (8 females in the 
styrofoam chest—\( r_m = 0.28 \), and 5 females in the 
incubator—\( r_m = 0.31 \)).

The predicted value for the intrinsic rate of 
population increase (\( r_m \)) was greater than the observed 
value (\( r_e \)); however, the value of the finite rate of 
population increase (\( \lambda \)) not was affected (TABLE 5).

Overall, the Lu. shannoni colony is currently increasing 
at an estimated rate of one individual per female per 
week.

Analysis of the Stage-Specific Mortality

Vertical life tables were constructed based on the 
\( l_x \) (live individuals) and \( d_x \) (mortality) at each stage. 
Stage-specific mortality was based on data in the vertical 
life tables according to Southwood (1978) (TABLES 6 
and 7).

Egg mortality - The overall egg mortality in the 
styrofoam chest environment was 91.2\% versus 67.1\% 
in incubator and occurred at three distinct levels 
(TABLES 6 and 7). The first was due to retained eggs 
by the cohort females, and whose real mortality was 
51.6\% in the styrofoam chest versus 28.4\% in the 
incubator. The second level consisted of oviposited, 
but unembryonated eggs (real mortality—33.8\% in the 
styrofoam chest versus 25.9\% in the incubator). The 
third level of real mortality occurred as embryonic 
death—5.8\% in the styrofoam chest versus 12.7\% in 
the incubator.

Larval mortality - The second type of significant 
mortality occurred during the 1st-instar larval (TABLES 
6 and 7). The conditions in the styrofoam chest 
environment were favorable for 1st-instar larval survival 
with a mortality of only 0.7\%. However, in the incubator, 
the mortality was almost 39\%. The mortality at this 
stage was probably a consequence of an inability to 
feed on the food provided, since most of the dead 1st-
instar larvae had no food in the gut. For 2nd to 4th-
larval instars, the mortality was very low in both 
experiments and was probably due to bacterial infection. 
We noted that affected larvae fed at a lower rate, 
demonstrated a loss of mobility, and became 
progressively darkened. These observations parallel those 
described by Killick-Kendrick et al. (1977) for Lu. 
longipalpis.

Pupal mortality - Very little mortality occurred at 
this stage, with the percentage developing to adults 
exceeding 97\% in both experiments (TABLES 6 and 
7). The real mortality of Lu. shannoni pupae was 0.04\% 
and 0.42\% in the two respective experiments, in contrast 
with a pupal mortality in Lu. longipalpis of 88 to 96\% 
(Killick-Kendrick et al. 1977).

The highest irreplaceable mortality (18.5\%) 
ocurred in oviposited eggs of cohort females 
maintained in the styrofoam chest (TABLE 6). This is 
mortality due to unfertilized (unembryonated) eggs, 
resulting in a loss of 420 eggs. If they had been fertile,
TABLE 5. Statistical comparison of Lutzomyia shannoni population parameters obtained in two laboratory environments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Laboratory Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Styrofoam Chest</td>
</tr>
<tr>
<td>Predicted</td>
<td></td>
</tr>
<tr>
<td>Net reproductive rate ($R_0$)</td>
<td>23.50</td>
</tr>
<tr>
<td>Generation time ($T_c$)</td>
<td>11.44</td>
</tr>
<tr>
<td>Intrinsic rate of population increase ($r_m$)</td>
<td>0.27</td>
</tr>
<tr>
<td>Finite rate of population increase ($\lambda$)</td>
<td>1.31</td>
</tr>
<tr>
<td>Observed</td>
<td></td>
</tr>
<tr>
<td>Net reproductive rate ($R_0'$)</td>
<td>1.92</td>
</tr>
<tr>
<td>Generation time ($T_c'$)</td>
<td>11.70</td>
</tr>
<tr>
<td>Intrinsic rate of population increase ($r_m'$)</td>
<td>0.05</td>
</tr>
<tr>
<td>Finite rate of population increase ($\lambda'$)</td>
<td>1.06</td>
</tr>
</tbody>
</table>

*, $p \leq 0.01$

Ns = Non significant

TABLE 6. Temporal life table of one generation of a cohort of Lutzomyia shannoni reared in a styrofoam chest environment to analyze the mortality. A: eggs retained; B: eggs without embryonic development; C: death of embryonated eggs; D: 1st-instar mortality; E: 2nd-instar mortality; F: 3rd-instar mortality; G: 4th-instar mortality; H: pupal mortality.

<table>
<thead>
<tr>
<th>Development Stages</th>
<th>$l_X$</th>
<th>$d_X$</th>
<th>Percent Apparent Mortality</th>
<th>Percent Real Mortality</th>
<th>Percent Irreplaceable Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total eggs</td>
<td>4,703</td>
<td>A</td>
<td>2,429</td>
<td>51.64</td>
<td>51.64</td>
</tr>
<tr>
<td>Oviposited eggs</td>
<td>2,274</td>
<td>B</td>
<td>1,591</td>
<td>70.00</td>
<td>33.83</td>
</tr>
<tr>
<td>Embryonated eggs</td>
<td>683</td>
<td>C</td>
<td>271</td>
<td>39.70</td>
<td>5.80</td>
</tr>
<tr>
<td>Larvae I</td>
<td>412</td>
<td>D</td>
<td>33</td>
<td>8.00</td>
<td>0.70</td>
</tr>
<tr>
<td>Larvae II</td>
<td>379</td>
<td>E</td>
<td>4</td>
<td>1.00</td>
<td>0.08</td>
</tr>
<tr>
<td>Larvae III</td>
<td>375</td>
<td>F</td>
<td>6</td>
<td>1.60</td>
<td>0.13</td>
</tr>
<tr>
<td>Larvae IV</td>
<td>369</td>
<td>G</td>
<td>3</td>
<td>0.80</td>
<td>0.06</td>
</tr>
<tr>
<td>Pupae</td>
<td>366</td>
<td>H</td>
<td>2</td>
<td>0.50</td>
<td>0.04</td>
</tr>
<tr>
<td>Adults</td>
<td>364</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$l_X$, the number of individuals that enter a specific stage.

$d_X$, the number of individuals that die within a specific stage.

A total of 1,103 embryonated eggs will have been retrieved from the styrofoam chest. The second highest irreplaceable mortality (15.6%) occurred in the 1st-instar larvae maintained in incubator (TABLE 7). Without this mortality, an additional 185 larvae will have been available for a total of 731 2nd-instar larvae.

Analysis of the Fecundity Heritability

In the previous analysis, the potential population increment was estimated, based on the production of female daughters per cohort female. How much of the fecundity potential is hereditary, and how much due to environment? To answer this question, the number of
TABLE 7. Temporal life table of one generation of a cohort of incubator-reared *Lutzomyia shannoni* to analyze mortality. A: eggs retained; B: eggs without embryonic development; C: death of embryonated eggs; D: 1st-instar mortality; E: 2nd-instar mortality; F: 3rd-instar mortality; G: 4th-instar mortality; H: pupal mortality.

<table>
<thead>
<tr>
<th>Development stages</th>
<th>$l_x$</th>
<th>$d_x$</th>
<th>Percent Apparent Mortality</th>
<th>Percent Real Mortality</th>
<th>Percent Irreplaceable Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Totals eggs</td>
<td>3,595</td>
<td>A</td>
<td>1,022</td>
<td>28.43</td>
<td>28.43</td>
</tr>
<tr>
<td>Oviposited eggs</td>
<td>2,573</td>
<td>B</td>
<td>933</td>
<td>36.26</td>
<td>25.95</td>
</tr>
<tr>
<td>Embryonated eggs</td>
<td>1,640</td>
<td>C</td>
<td>459</td>
<td>28.00</td>
<td>12.77</td>
</tr>
<tr>
<td>Larvae I</td>
<td>1,181</td>
<td>D</td>
<td>635</td>
<td>53.80</td>
<td>38.72</td>
</tr>
<tr>
<td>Larvae II</td>
<td>546</td>
<td>E</td>
<td>29</td>
<td>5.31</td>
<td>0.80</td>
</tr>
<tr>
<td>Larvae III</td>
<td>517</td>
<td>F</td>
<td>6</td>
<td>1.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Larvae IV</td>
<td>511</td>
<td>G</td>
<td>13</td>
<td>2.54</td>
<td>0.36</td>
</tr>
<tr>
<td>Pupae</td>
<td>498</td>
<td>H</td>
<td>15</td>
<td>3.00</td>
<td>0.42</td>
</tr>
<tr>
<td>Adults</td>
<td>483</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$l_x$, the number of individuals that enter a specific stage.

$d_x$, the number of individuals that die within a specific stage.

oviposited eggs, the number of retained eggs, and the number total eggs were recorded for each of 30 females, and compared with the average of three of their daughters.

The linear regression for the oviposition is shown in Figure 5. The heritability for the oviposition of *Lu. shannoni* was 32%. In *Drosophila melanogaster*, the heritability for oviposition was 18% (Strickberger 1978). Strickberger considered that heritability is low for characters essential for survival, such as the rapidity of conception or size of litter because if this value is incremented, the survival is decreased. The additive variance for the oviposition was $\sigma_A^2 = 50.3$. An F test was applied to the regression coefficient for oviposition; the null hypothesis $H_0$ was accepted at the levels 95% and 99%. Therefore, the observed variability in oviposited eggs was not due to heritable factors, but rather a function of environmental variables.

The regression of the retained eggs in mothers and daughters is shown in Figure 6. The negative slope indicates that the character has not heritable component. However, for total fecundity, the regression was highly positive and significant (Fig. 7). The heritability value for total fecundity was $h^2 = 77.8\%$. When the regression coefficient of total fecundity was tested, the null hypothesis was rejected at the levels 95% and 99%. The confidence interval for heritability of total fecundity was between 26.5% to 129.1%.

DISCUSSION

Laboratory colonies of phlebotomine sand flies are necessary for several aspects of vector research, such as transmission of the disease agents, population genetics comparisons, systematic, insecticide susceptibility, and so forth. However, due to great difficulty of maintaining most sand fly colonies for longer than a few generations, life-table studies on sand flies are rare. For New World sand flies, only the study of Killick-Kendrick et al. (1977) on *Lutzomyia longipalpis* (Lutz & Neiva) has focused on development times and reproductive rate. Data on life cycle and biology reproductive for phlebotomines are nearly impossible to obtain in field conditions, since immatures have been found only rarely.

This is the first work that has focused on the details of reproductive biology of *Lu. shannoni*. Comer et al. (1994) described annual cycles of abundance of *Lu. shannoni* adults in field conditions on Ossabaw Island (USA) and concluded that up to three generations occurred each year. The first generation emerges in spring as diapause is terminated, a second generation occurs in mid-summer, and third generation may occur near the summer’s end. The difference in developmental time for the second and a third generations (10 week versus 8 week) probably reflects differences in ambient temperatures between the spring and early summer months. In the present study, the difference in developmental time in the two experimental conditions
The maximum intrinsic rate of population increase ($r_m = 0.27$ and $0.30$ in the respective experiments) is an estimate of the fitness of the colony after 22 and 27 generations, respectively. Neither the additional time in colony nor the experimental conditions had a significant effect on $r_m$. The high heritability of the total fecundity is reflected in the maximum intrinsic rate of population increase ($r_m$), and the data support the assertions of Southwood (1978) that this parameter is genetically determined.

The irrepealceable mortality in the styrofoam chest environment was higher in oviposited, but unembryonated eggs, whereas, in the incubator it was highest in the 1st-instar larvae. The egg mortality in the styrofoam chest environment was due probably to the lack of mating. This may have been a consequence of isolating the females in the rearing chambers immediately after blood feeding on the hamster, although frequent mating activity is observed prior to blood feeding. In the incubator environment, the male and female pair were kept together in the rearing chamber after blood feeding. This indicates that an optimum strategy for the rearing phlebotomines is to keep males with females before and after blood feeding to ensure maximum egg fertilization.

To what extent can the observations obtained under these laboratory conditions be extrapolated to *Lutzomyia shannoni* in the field environment? Probably the stress of colonization has reduced the genetic variability that occurs naturally in field conditions, as has been
demonstrated for several colonies of *Lu. longipalpis* (Mukhopadhyay et al. 1997, Morrison et al. 1995) and several mosquito species (Munstermann 1994). The colony environment cannot duplicate conditions of larval nutrition, temperature, humidity, and host availability; and, consequently, will strongly affect adult behavior, egg mortality, larval growth period, and adult mortality. Furthermore, female longevity in the field is undoubtedly greater than that observed in the laboratory, with an associated low egg mortality. This conclusion is based on the necessary chronology of disease transmission by these insect vectors. The field females must ingest blood at least twice—first to obtain the parasite, then (after a defined parasite incubation and sand fly oviposition period) to transmit the disease agent to healthy hosts. Nonetheless, the laboratory data have provided essential baseline information concerning the reproductive potential for *Lu. shannoni*. For laboratory colonies, it suggests more efficient rearing practices and at which points in the life cycle are more critical to colony maintenance. For studies of phlebotomine population biology in the field, these observations provide guidelines for approximating the boundaries of expected biological behaviors. Finally these data provide clues to how and where this species might survive in the field and, eventually, documenting its complete life cycle in the natural habitat.

**Acknowledgments**

We thank the anonymous reviewers whose suggestions greatly improved the presentation. Marco Fidel Suarez is acknowledged for his continuous maintenance of the laboratory colony. We also want to thank Margarita Cárdenas for her collaboration in the elaboration of a small computer program. This research was supported by the United States National Institutes of Health (AI-34521 to L.E.M) and the Colombian Instituto Nacional de Salud (Project No. 3100302 to C.F. of the Entomology Laboratory).

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Identification and Geographic Distribution of  
Lutzomyia Sand Flies in Mexico, the West Indies,  
Central and South America (Diptera: Psychodidae).  
The Fleas (Siphonaptera) of South Carolina with an Assessment of Their Vectorial Importance

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ABSTRACT: We document 25 species of fleas from South Carolina including new state records for two species, the ctenophthalmids Epitedia caevernicola and Rhadinopsylla arama. Host and other collection data, by county, are provided, including many new records amassed from 1992-1998 and some older records gleaned from the Clemson University Arthropod Collection. One flea species, the rhopalopsyllid Polygenis gwyni, is especially common and widespread in South Carolina, particularly on the cotton rat (Sigmodon hispidus) in coastal plain habitats. The largest number of flea species (8) from a single host species was recorded from the cotton mouse, Peromyscus gossypinus. Several flea species of potential medical or veterinary importance were recorded, some of which are potential vectors of pathogens, such as the agents of murine typhus, murine typhus-like disease, sylvatic epidemic typhus, cat scratch disease, and rodent bartonellosis. A host-flea list for South Carolina is included.

Keyword Index: Fleas, Siphonaptera, South Carolina, faunal analysis, vectorial importance.

INTRODUCTION

The flea fauna of South Carolina has not previously been documented in any detail. Benton (1980) depicted collection localities for 18 species of fleas from South Carolina in his flea atlas of the eastern United States. Additionally, a few miscellaneous flea records for South Carolina have been documented, particularly by Fox (1940), Trembley and Bishop (1940), Johnson and Traub (1954), Pratt and Good (1954), Whitaker et al. (1994), and Durden et al. (1997a). Because South Carolina encompasses several physiographic realms ranging from coastal plain to montane (Appalachian) zones, a relatively rich fauna of eastern North American fleas could feasibly inhabit this state. Numbers of flea species documented from adjacent states include 19 for Georgia, 17 for North Carolina (Benton 1980), and 33 for Tennessee (Durden and Kollars 1997).

Except for murine typhus, which was a widespread zoonosis in South Carolina until the early 1950s (Pratt 1958), the status of flea-borne diseases in the state is poorly documented. Knowledge of the flea species occurring in South Carolina will permit an analysis of the flea-borne pathogens that may be present in the state.

MATERIALS AND METHODS

In order to survey South Carolina’s flea fauna, we first made detailed literature searches for previously documented flea records from the state. Next, we initiated a statewide mammal live-trapping program from 1994-1998 to survey fleas associated with various species of mammals. We also asked field mammalogists working in the state to collect fleas for this study. Mist-netted birds were also examined for fleas (Durden et al. 1997b). Lastly, we accessed the Clemson University Arthropod Collection and recorded collection data for any flea specimens deposited there, some of which are historically noteworthy.

In the lists that follow, flea species are presented alphabetically followed in parentheses by the Entomological Society of America-approved common name (Bosik 1997) if one has been assigned, and then by the family designation. Collection data are then presented by county (in upper case); South Carolina counties are shown in Figure 1. Literature citations for South Carolina flea records mostly provide the author and date of publication but also include the number and sex of flea specimens, the host, collection date, and name of
The same data are presented for the new collections including the slide-mounted material held at Clemson University. In a few cases, especially for older material, the host species, name of the collector, or full collection date were unavailable. Collection data from a single host individual are separated by backslashes, whereas, data from different host individuals are separated by a hyphen.

In the collection lists, initials of collectors are given according to the following key: AD = Amy Dye (South Carolina Dept. of Natural Resources, Clemson), ARB = Amy R. Banks (Georgia Southern University, Statesboro); CFR = C. F. Rainwater; CWB = Craig W. Banks (Georgia Southern University, Statesboro); DD = D. Dunavan; DCW = Dwight C. Williams (Cypress Gardens, Moncks Corner SC); DV = David Vaughan (South Carolina Dept. of Health & Environmental Control, Columbia); EVH = E. V. Horton; GRW = G. R. Wilkie III; JCH = J. C. Hartzell, Jr.; KLC = Kerry L. Clark (University of North Florida, Jacksonville); LAD = Lance A. Durden (Georgia Southern University, Statesboro); LR = Laurie Reid; MB = Mary Bunch (South Carolina Dept. of Natural Resources, Clemson); SL = Susan Loeb (U. S. Dept. of Forest Resources, Clemson, SC); RBC = R. B. Casey; REW = R. E. Ware; SCH = Schroeder (other initials unknown); SM = Stanley Miller (Campbell Museum of Natural Resources, Clemson University, Clemson, SC); TC = T. Currin; WED = W. E. Dove; WW = William Wills (Columbia, SC); WVB = W. Wilson Baker.

Throughout this work, we follow the flea classification of Lewis (1993) and the mammal classification in Wilson and Reeder (1993). Flea specimens from this survey are deposited in the Clemson University Arthropod Collection, Clemson, SC, or in the Institute of Arthropodology and Parasitology at Georgia Southern University, Statesboro, GA. All of the flea species recorded here from South Carolina can be identified using the key prepared by Benton (1983).

Figure 1. Map of South Carolina showing county locations.
FLEA RECORDS FOR SOUTH CAROLINA

Cediopsylla simplex (Baker, 1895) (rabbit flea) - Family Pulicidae.

This rabbit flea is widespread in eastern North America (Lewis 1972). It is sometimes also recovered from predators of rabbits such as foxes, coyotes, and dogs.

COLLECTION RECORDS:
AIKEN CO.: 1♂ ex domestic dog\7 Oct. 1979.
ANDERSON CO.: 2♀ ex “fox”\RBC\Dec. 1939 (Fox 1940); Benton (1980).
BEAUFORT CO.: 2♂ ex domestic dog\29 May 1945 - 1♀\31 May 1945 (Carpenter et al. 1945).
BERKELEY CO.: 2♀ in house\DCW\12 Aug. 1998.
CHARLESTON CO.: Trembley and Bishopp (1940).
PICKENS CO.: 2♂,9♀ ex Virginia opossum, Didelphis virginiana\MB\28 Oct. 1998 - 1♀ ex domestic dog\1924.
RICHLAND CO.: 3♂,6♀ ex domestic dog\WW\13 May 1995.
SUMTER CO.: Trembley and Bishopp (1940).

Ctenophthalmus pseudagyrtes Baker, 1904 - Family Ctenophthalmidae.

This flea was distributed in eastern North America where it parasitizes many species of small mammals, particularly shrews, moles, voles, chipmunks, and native mice (Lewis 1974a). Durden and Kollars (1997) reported that it was abundant on these hosts in Tennessee.

COLLECTION RECORDS:
BARNWELL CO.: 10♂ ex cotton mouse, Peromyscus gossypinus\WW & SL\22 Jan. 1998.
CHARLESTON CO.: Benton (1980).
OCONEE CO.: 1♂ ex “mole”\REW\1950 - 1♂,1♀ ex pine vole, Microtus pinetorum\WW\7 Aug. 1996.
PICKENS CO.: Benton (1980).

Doratopsylla clarinacae C. Fox, 1914 - Family Ctenophthalmidae.

This flea is a widespread ectoparasite of Blarina spp. short-tailed shrews in eastern North America (Durden and Kollars 1997). However, there are few records of it from the southeastern United States (Benton 1980).

COLLECTION RECORDS:
AIKEN CO.: 12 specimens from 9 of 50 individual southeastern short-tailed shrews, Blarina carolinensis, examined (Whitaker et al. 1994).

Echidnophaga gallinacea (Westwood, 1875) (sticktight flea) - Family Pulicidae.

Ctenocephalides felis (Bouché, 1835) (cat flea) - Family Pulicidae.

Unlike the previous species, this flea, another cosmopolitan ectoparasite (Lewis 1972) of veterinary importance, is common in South Carolina. We recorded it from the Virginia opossum, a fox, domestic dogs, and from inside a house in South Carolina.

COLLECTION RECORDS:
AIKEN CO.: 1♀ ex domestic dog\7 Oct. 1979.
ANDERSON CO.: 2♀ ex “fox”\RBC\Dec. 1939 (Fox 1940); Benton (1980).
BEAUFORT CO.: 2♂ ex domestic dog\29 May 1945 - 1♀\31 May 1945 (Carpenter et al. 1945).
BERKELEY CO.: 2♀ in house\DCW\12 Aug. 1998.
CHARLESTON CO.: Trembley and Bishopp (1940).
PICKENS CO.: 2♂,9♀ ex Virginia opossum, Didelphis virginiana\MB\28 Oct. 1998 - 1♀ ex domestic dog\1924.
RICHLAND CO.: 3♂,6♀ ex domestic dog\WW\13 May 1995.
SUMTER CO.: Trembley and Bishopp (1940).

Ctenocephalides canis (Curtis, 1826) (dog flea) - Family Pulicidae.

The dog flea, a cosmopolitan ectoparasite (Lewis 1972) of veterinary significance, does not appear to be common in South Carolina especially on domestic dogs. However, it may parasitize foxes and coyotes more frequently.

COLLECTION RECORDS:
CHARLESTON CO.: Trembley and Bishopp (1940).
KERSHAW CO.: Trembley and Bishop (1940).

Conorhinopsylla stanfordi Stewart, 1930 - Family Ctenophthalmidae.

This is a flea of squirrels, especially flying squirrels (Glaucomys spp.) in eastern North America (Lewis 1974a). There are few records of it from the southern United States (Benton 1980).

COLLECTION RECORD:
PICKENS CO.: Benton (1980).

Doratopsylla clarinacae C. Fox, 1914 - Family Ctenophthalmidae.

This flea is a widespread ectoparasite of Blarina spp. short-tailed shrews in eastern North America (Durden and Kollars 1997). However, there are few records of it from the southeastern United States (Benton 1980).

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COLLECTION RECORDS:
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BEAUFORT CO.: 2♂ ex domestic dog\29 May 1945 - 1♀\31 May 1945 (Carpenter et al. 1945).
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CHARLESTON CO.: Trembley and Bishopp (1940).
PICKENS CO.: 2♂,9♀ ex Virginia opossum, Didelphis virginiana\MB\28 Oct. 1998 - 1♀ ex domestic dog\1924.
RICHLAND CO.: 3♂,6♀ ex domestic dog\WW\13 May 1995.
SUMTER CO.: Trembley and Bishopp (1940).

Ctenocephalides canis (Curtis, 1826) (dog flea) - Family Pulicidae.

The dog flea, a cosmopolitan ectoparasite (Lewis 1972) of veterinary significance, does not appear to be common in South Carolina especially on domestic dogs. However, it may parasitize foxes and coyotes more frequently.

COLLECTION RECORDS:
CHARLESTON CO.: Trembley and Bishopp (1940).
KERSHAW CO.: Trembley and Bishop (1940).
This small flea is globally widespread in temperate and subtropical climates (Lewis 1972). It parasitizes a wide variety of bird and mammal species. Evidently, it is widespread in South Carolina.

COLLECTION RECORDS:
AIKEN, ANDERSON, BEAUFORT, CALHOUN, CHARLESTON, COLLETON, DARLINGTON, FLORENCE, GEORGETOWN, HAMPTON, Horry, Jasper, Kershaw, Lexington, Marlboro, Orangeburg, Richland, and Sumter Counties: Trembley and Bishop (1940), Carpenter et al. (1945), Pratt and Good (1954), Benton (1980).

DILLON CO.: 1♀ ex feral kitten, Felis sylvestris WW \ 11 Jul. 1996.

FLORENCE CO.: 1♀ ex “jaybird” \ CFR\ 1894 (Fox 1940).

ORANGEBURG CO.: 99 ex horses, Equus caballus \ UCH\ Nov. 1894 (Fox 1940, Hopkins and Rothschild 1953).

UNSPECIFIED County/Counties: Trembley and Bishop (1940); ex blue jay, Cyanocitta cristata (Boyd et al. 1956) - ex horses, mules, and asses (Becklund 1964).

Epitedia cavernicola Traub, 1957 - Family Ctenophthalmidae.

This is a host-specific flea of the eastern woodrat, Neotoma floridana. Because of its nidicolous habits and apparent winter phenology (Benton 1980) there are relatively few records of it. The single specimen we report here was taken at fairly high altitude.

COLLECTION RECORD:
PICKENS CO.: 1♀ ex N. floridana MB \ 8 Nov. 1998 \ 1,067 m elevation (NEW STATE RECORD).

Epitedia wenmanni testor (Rothschild, 1915) - Family Ctenophthalmidae.

This is a widespread North American flea that mainly parasitizes Peromyscus spp. mice (Lewis 1974a).

COLLECTION RECORDS:
ANDERSON CO.: Benton (1980).
BARNWELL CO.: Both collections ex P. gossypinus by WW & SL on 25 Jan. 1998 - 1♂, 4♀ - 4♂, 2♀.

Eupholopsyllus glacialis affinis (Baker, 1904) - Family Pulicidae.

This flea is mainly an ectoparasite of native rabbits and hares in the Rocky Mountain region (Lewis 1972). Its occurrence in South Carolina must be considered unusual but Durden and Kollars (1997) recorded this flea from eastern cottontails in western Tennessee. Further collecting should clarify the status of this flea in South Carolina.

COLLECTION RECORDS:
UNSPECIFIED County/Counties: ex S. floridanus (Andrews et al. 1980).

Leptopsylla segnis (Schönherr, 1811) (European mouse flea) - Family Leptopsyllidae.

This cosmopolitan flea mainly parasitizes the house mouse, Mus musculus, but it is sometimes recovered from other small rodents (Lewis 1974b). It appears to be widespread in South Carolina.

COLLECTION RECORDS:
AIKEN, ALLendale, ANDERSON, BAMBERG, BARNWELL, BEAUFORT, BERKELEY, CHARLESTON, COLLETON, DARLINGTON, DORCHESTER, FAIRFIELD, GEORGETOWN, HAMPTON, Horry, MARLBORO, ORANGEBURG, and UNION Counties: Pratt and Good (1954), Benton (1980).

CHARLESTON CO.: 2♂ ex Mus sp. \ 12 Jan. 1938 (Fox 1940) - 2♂, 6♀ ex “rat” \ 2 Feb. 1944 (Carpenter et al. 1945).

BEAUFORT CO.: 1♂ ex “rat” \ 1945 - 2♀ ex “rat” \ 1945 - 1♂, 3♀ ex “rat” \ 1945.

PICKENS CO.: 1♀ ex house mouse, Mus musculus \ ADD \ 18 May 1933.

Nosopsyllus fasciatus (Bosc, 1800) (northern rat flea) - Family Ceratophyllidae.

This flea is a cosmopolitan ectoparasite of Old World domestic rats but it is typically more prevalent in temperate or cooler regions (Traub et al. 1983). It appears to be widely distributed in South Carolina and probably occurs wherever commensal rats are found within the state.

COLLECTION RECORDS:

CHARLESTON CO.: 3♂, 1♀ \ 21 Nov. 1945.

UNSPECIFIED Counties: Traub et al. (1983).

Odontopsyllus multispinosus (Baker, 1898) - Family Leptopsyllidae.

This rabbit flea is widespread in the eastern United States (Lewis 1974b, Benton 1980). It appears to be
fairly widely distributed in South Carolina.

COLLECTION RECORDS:


Oconee CO.: 1♀ ex *S. transitionalis* AD & MD 23 Feb. 1999.

Orangeburg CO.: Ex *S. floridanus* WED & SCH 8 Feb. 1931 (Fox 1940, Benton 1980).

Unspecified County/Counties: ex *S. floridanus* (Andrews et al. 1980).

**Orchopeas howardi** (Baker, 1895) - Family Ceratophyllidae.

This flea parasitizes tree squirrels throughout much of North America and is especially common in the central and eastern United States (Lewis 1975) on the eastern gray squirrel, *Sciurus carolinensis*, and fox squirrel, *Sciurus niger*, as discussed by Traub et al. (1983). It is probably widespread on these hosts and on the southern flying squirrel in South Carolina.

**Orchopeas leucopus** (Baker, 1904) - Family Ceratophyllidae.

This flea is distributed across North America but it is especially common in the eastern half of the United States (Lewis 1975) as a parasite of *Peromyscus* spp. mice (Durden and Kollars 1997). Surprisingly, it does not appear to be common in South Carolina.

**Orchopeas sexdentatus pensylvanicus** (Jordan, 1928) - Family Ceratophyllidae.

This eastern woodrat flea is widespread in eastern North America (Benton 1980, Lewis 1975), but it was only recently recorded from South Carolina (Durden et al. 1997a). Nevertheless, it appears to be quite widespread on *N. floridanus* within the state. Some of the South Carolina specimens have been examined by Robert E. Lewis (Ames, IA) who has noted some morphological variation in them (R. E. Lewis, pers. com.) but advocates retaining them as *O. s. pensylvanicus* until a systemic revision of the genus *Orchopeas* has been completed.

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parasite of *Peromyscus* spp. mice (Johnson and Traub 1954). It occurs throughout most of the eastern United States with several records in southern states (Benton 1980).

**COLLECTION RECORDS:**


**GEORGETOWN CO.:** ex *Peromyscus* sp. (Johnson and Traub 1954); Benton (1980).

**SUMTER CO.:** all collected from individual *P. gossypinus* by KLC as follows - 1♂, 1♀ 16 Jan. 1995 - 2♀ 12 Apr. 1995.

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**Polygenis gwyni** (C. Fox, 1914) - Family Rhopalomyidae.

This flea is mainly a parasite of the cotton rat, *S. hispidus*, although it is also frequently recovered from the ecological associates of this rodent and on Virginia opossums (Lewis and Lewis 1994a) throughout much of the southern United States. It was the most abundant flea during our survey being collected in all months except November and December and from several rodent species, but particularly from *S. hispidus*.

**COLLECTION RECORDS:**


**BARNWELL CO.:** 3♀ ex *S. hispidus* SL 15 Jan. 1999.


**CHESTER CO.:** all collected from individual *S. hispidus* by KLC on 15 Oct. 1994 - 1♂ - 3♂, 2♀.


**DILLON CO.:** all collected from individual *S. hispidus* by WW & DV as follows - 1♂ 16 Jul. 1996 - 2♂, 2♀ 18 Jul. 1996.


**JASPER CO.:** 1♂, 1♀ ex *N. floridana* ARB, CBW & LAD 28 Nov. 1996.

**McCORMICK CO.:** all collected from individual *S. hispidus* by WW & DV on 31 Jul. 1996 as follows - 1♂, 3♀ - 4♂, 1♀ - 4♂, 1♀ - 10♂, 10♀.
NEWBERRY CO.: 1♀ ex S. hispidus\WW\23 Jan. 1999.
ORANGEBURG CO.: all collected from individual S. hispidus by KLC as follows - 1♂2♀12 Sep. 1994 - 3♂,3♀13 Sep. 1994.

Pulex irritans L., 1758 (human flea) - Family Pulicidae.
Prior to 1958, P. irritans and P. simulates were not recognized as separate species (Smit 1958); instead, all specimens were assigned to P. irritans. Therefore, it cannot be definitively stated that the records cited below are attributable to P. irritans. Nevertheless, given its almost cosmopolitan distribution (Lewis 1972), it is likely that P. irritans occurs in South Carolina.
COLLECTION RECORDS:
CHARLESTON CO.: Trembley and Bishopp (1940).
FLORENCE CO.: Trembley and Bishop (1940).

Pulex simulates Baker, 1895 - Family Pulicidae.
This New World flea parasitizes a wide spectrum of mammalian hosts, especially carnivores, deer, and large rodents (Hopla 1980). It appears to be widely distributed, especially on carnivores, in the southern United States (Durden and Kollars 1997).

Rhadinopsylla orama Smit, 1957 - Family Ctenocephalidae.
This eastern North American vole flea (Lewis 1974a) was collected at the Savannah River Site in South Carolina close to the Georgia border. This represents the most southern record of this flea documented to date and is several hundred kilometers south of the next closest record in northeastern Tennessee (Benton 1980, Durden and Kollars 1997).

Stenoponia americana (Baker, 1899) - Family Ctenocephalidae.
This large flea is relatively common on small rodents, especially Peromyscus spp. and Microtus spp., in eastern North America (Lewis 1974a). It appears to be common on P. gossypinus in South Carolina especially during the cooler months.

COLLECTION RECORDS:
CHESTER CO.: 1♂ ex O. palustris\KLC\10 Nov. 1994 - remainder collected from individual S. hispidus on 11 Nov. 1994 by KLC as follows - 1♂ - 1♀.
EDGEFIELD CO.: Benton (1980).
RICHLAND CO.: 1♂ in pitfall trap\LR\Feb. 1999.
SUMTER CO.: 1♂ ex P. gossypinus\KLC\16 Jan. 1995.

Sternopsylla distincta texana (Rothschild, 1903) - Family Ischnopsyllidae.
This is the only species of bat flea recorded from South Carolina (Benton 1980). Its range appears to include much of the southern United States (Lewis and Lewis 1994b) in tandem with that of its principal host, the Brazilian free-tailed bat, Tadarida brasiliensis.

COLLECTION RECORD:

Xenopsylla cheopis (Rothschild, 1903) (Oriental rat flea) - Family Pulicidae.
The Oriental rat flea, virtually cosmopolitan in distribution, is especially common in warmer climates (Lewis 1972). It is principally an ectoparasite of Old World domestic rats, with many records from these rodents in sea ports such as Charleston, South Carolina.

COLLECTION RECORDS:
AIKEN, ALLENDALE, BAMBERG, BARNWELL, BEAUFORT, BERKELEY, CALHOUN, CHARLESTON, DORCHESTER, FLORENCE, GEORGETOWN, HAMPTON, Horry, JASPER, Kershaw, Marion, NEWBERRY, ORANGEBURG, and RICHLAND Counties: Carpenter et al. (1945), Pratt and Good (1954), Benton (1980).


HOST-FLEA LIST FOR SOUTH CAROLINA

Blue jay, Cyanocitta cristata: Echidophaga gallinacea.
Virginia opossum, Didelphis virginiana: Ctenocephalides felis.
“Bat”: Sternopsylla distincta texana.
Southeastern short-tailed shrew, Blarina carolinensis: Doratopsylla blarinae.
“Mole”: Ctenopthalmus pseudagyrtetes.
Domestic cat, Felis sylvester: Echidophaga gallinacea.
Domestic dog, Canis lupus: Cediopsylla simplex, Ctenocephalides felis, Pulex simulans.
Bobcat, Lynx rufus: Odontopsyllus multispinosus.
“Fox”: Ctenocephalides felis.
Gray fox, Urocyon cinereoargenteus: Cediopsylla simplex, Pulex simulans.
Red fox, Vulpes vulpes: Cediopsylla simplex.
Coyote, Canis latrans: Pulex simulans.
Horse, Equus caballus, mule and ass: Echidophaga gallinacea.
Norway rat, Rattus norvegicus: Polygenis gwyni.
“Rat”: Leptopsylla segnis, Nosopsyllus fasciatus, Xenopsylla cheopis.
House mouse, Mus musculus: Leptopsylla segnis.
Cotton rat, Sigmodon hispidus: Orchopeas sexdentatus pennsylvanicus, Polygenis gwyni, Stenoponia americana.
Eastern woodrat, Neotoma floridana: Epitedia cavernicola, Orchopeas sexdentatus pennsylvanicus, Polygenis gwyni.
Rice rat, Oryzomys palustris: Polygenis gwyni, Stenoponia americana.
Cotton mouse, Peromyscus gossypinus: Ctenopthalmus pseudagyrtetes, Epitedia weynmanni testor, Orchopeas leucopus, Peromyscopsylla hesperomys, Peromyscopsylla scotti, Polygenis gwyni, Rhadinopsylla orama, Stenoponia americana.
“Peromyscus sp.”: Peromyscopsylla scotti.
Golden mouse, Ochrotomys nuttalli: Peromyscopsylla scotti.
Eastern harvest mouse, Reithrodontomys humulis: Peromyscopsylla scotti.
Pine vole, Microtus pinetorum: Ctenopthalmus pseudagyrtetes.
“Squirrel”: Conorhinopsylla stanfordii, Orchopeas howardi.
Southern flying squirrel, Glaucomys volans: Orchopeas howardi.

Eastern cottontail rabbit, Sylvilagus floridanus: Cediopsylla simplex, Euhoplopsyllus glacialis affinis, Odontopsyllus multispinosus.
Marsh rabbit, Sylvilagus palustris: Cediopsylla simplex, Odontopsyllus multispinosus.
New England cottontail rabbit, Sylvilagus transitionalis: Cediopsylla simplex, Odontopsyllus multispinosus.
Unspecified hosts: Ctenocephalides canis, Pulex irritans.

DISCUSSION

The Flea Fauna of South Carolina

We have documented a relatively rich fauna of 25 species of fleas from South Carolina. This compares favorably with the 19 recorded flea species for adjoining Georgia and 17 species for North Carolina (Benton 1980), but less impressively with the 33 flea species known from adjacent Tennessee (Durden and Kollars, 1997). We hypothesize that approximately 5 additional flea species are denizens of South Carolina’s higher elevations in the western part of the state. Further collecting in Appalachian South Carolina will provide a test of this assumption.

Whereas some of the fleas we recorded are widespread in South Carolina, others appear to be characteristic of coastal plain or montane zones. South Carolina’s widespread flea taxa appear to include C. simplex, C. felis, E. gallinacea, X. cheopis, S. americana, L. segnis, N. fasciatus, O. sexdentatus pennsylvanicus, and perhaps also C. pseudagyrtetes, O. multispinosus, P. scotti, and O. howardi. Montane flea species that we recorded in South Carolina are C. stanfordii and E. cavernicola. One flea species, P. gwyni, was distinctly more common in coastal plain habitats, although it was also recorded from other physiographic zones.

Polygenis gwyni was clearly the most abundant flea in our survey and it was mainly associated with the cotton rat. This species, and the cat flea, C. felis, appear to be the 2 most common fleas in South Carolina. Multiple flea species infestations were recorded for several mammalian hosts, most notably for the cotton mouse which hosted 8 flea species, but also for the domestic dog, cotton rat, eastern woodrat, and eastern cottontail, each of which was host to 3 flea species. Also, Old World domestic rats (Rattus spp.) appear to be parasitized by 4 flea species in South Carolina but host data are not sufficiently definitive to be certain.

Some of the flea species we report from South Carolina have medical and/or veterinary importance.
Several of the species, such as *C. canis*, *C. felis*, *E. gallinacea*, and *P. irritans* will feed on pets, livestock animals, or humans, and may reach pest status.

**South Carolina Fleas as Potential Vectors**

Some of the flea species we recorded are proven or suspected vectors of zoonotic pathogens. One of these pathogens, *Rickettsia typhi*, the etiologic agent of murine typhus, is usually transmitted by *X. cheopis*, but *C. felis*, *L. segnis*, *N. fasciatus*, and *P. gwyni*, all of which were recorded in this survey, can also transmit this pathogen (Azad 1990). Murine typhus was formerly a widespread and relatively common infectious disease in most southern states including South Carolina, but human cases declined significantly after the 1940s largely as a result of rat control and rat-flea control programs (Mohr et al. 1953, Pratt 1958, Traub et al. 1978). Although *Polygenis gwyni* has sometimes been perceived as an important historical vector of *R. typhi* in the southern United States, its role as a vector of this pathogen in nature may have been overestimated according to Lewis and Lewis (1994a). Murine typhus probably still persists in enzootic mammal and flea foci in parts of South Carolina and it could still represent a zoonotic threat within the state.

Another flea-borne rickettsial agent, *Rickettsia felis*, which causes murine typhus-like disease, is also almost certainly present in South Carolina. It has been detected in cat fleas recovered from opossums, bobcats, and domestic dogs in neighboring Georgia and is also known from cat fleas collected in other southern states (Azad et al. 1997).

Some species of *Bartonella* may be transmitted by fleas in South Carolina. One of these is *Bartonella henselae*, the causative agent of cat scratch disease, which can be transmitted by cat fleas in the laboratory (Chomel et al. 1996). Several other species of *Bartonella* are known to infect rodents in the southeastern United States (Kosoy et al. 1997) and other species of fleas may be vectors of these agents. For example, *O. leucopus* is known to be a laboratory vector of *Bartonella peromysci* to mice (Katavolos and Telford 1998).

The strain of *Rickettsia prowazekii* that causes sylvatic epidemic typhus in humans and asymptomatic transient infections in its enzootic southern flying squirrel reservoir host (Bozeman et al. 1975) is almost certainly present in South Carolina. McDade (1987) recorded human infections by this rickettsial agent from several states including adjacent Georgia, North Carolina, and Tennessee. In the laboratory, the squirrel flea, *O. howardi*, which we found to be widespread in South Carolina, is a competent vector of this pathogen (McDade 1987).

Several members of the apicomplexan protozoan genus *Hepatozoon* have been detected in fleas and some are known to be transmitted by fleas (Smith 1996). Of relevance to flea species and hosts recorded in South Carolina, is the squirrel parasite, *Hepatozoon sciuri*, which has been found in *O. howardi*. *Hepatozoon erhardovae* can be transmitted by 5 species of fleas to European bank voles (Krampitz and Wongchari 1980) so it is likely that additional species of *Hepatozoon* are transmitted to other mammals by various species of fleas.

Some trypanosomes are similarly known to be transmitted to rodents or lagomorphs by fleas. Molyneux (1970) discussed 11 species of trypanosomes known to be transmitted by fleas to hosts belonging to these two Orders. Of relevance to the flea species and hosts we recorded for South Carolina, he listed *N. fasciatus* and *X. cheopis* as vectors of *Trypanosoma lewisi* to domestic rats, *N. fasciatus* as a vector of both *T. microti* and *T. evotomys* to voles, and *C. simplex* as a vector of an unnamed trypanosome to the eastern cottontail.

Other pathogens detected in fleas in the southern United States, such as *Borrelia burgdorferi*, the etiologic agent of Lyme disease, are not thought to be transmitted by fleas but may instead reflect a recent bloodmeal by these fleas from, for example, a spirochetalic host (Teltow et al. 1991). Similarly, although plague is not present in South Carolina, potential flea vectors, especially *X. cheopis*, are evidently common in the state. Overall, we advocate that fleas have a persistent but underestimated role as vectors of pathogens to humans, pets, livestock, and wild mammals in South Carolina.

**Acknowledgments**

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Experimental Studies of Interactions Between Wild Turkeys and Black-Legged Ticks

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ABSTRACT: Wild turkeys are increasing in abundance and distribution in eastern North America, but their potential role as hosts for ticks, or as predators on ticks, is unknown. We performed two experiments, one to determine whether juvenile black-legged ticks (Ixodes scapularis) feed successfully on turkeys, and the other to determine if turkeys depredate adult black-legged ticks in forest habitats. Of 550 larval ticks placed directly on 5 captive wild turkeys, none engorged and only 7 (1.3%) were recovered; the remainder apparently were consumed during preening. Of 165 nymphal ticks placed on the turkeys, 5 engorged and 8 unengorged ticks were collected; 152 (93.3%) were apparently consumed. Of 250 adult ticks introduced into forest enclosures exposed to turkey foraging, 89.5% were recaptured, which was not significantly different from the 92.2% recaptured in control enclosures from which turkeys were excluded. We conclude that wild turkeys are unlikely to host juvenile black-legged ticks in nature, and that turkey foraging is unlikely to reduce local density of adult ticks.

Keyword Index: Host, Lyme disease, parasite-host interactions, vector.

INTRODUCTION

Historically, the wild turkey (Meleagris gallopavo) was an abundant member of vertebrate communities within forests and grasslands of eastern and central North America (Healy 1992). However, this species was decimated throughout its historical range during the 18th through early 20th centuries as a result of hunting and habitat destruction (Kennenmer et al. 1992). Regrowth of forests following deforestation, combined with reintroductions, transplantations, and subsequent population growth and dispersal of the birds, has resulted in rapid increases in both density and range of this species. Between about 1930 and 1990, the turkey population in the eastern United States and southeastern Canada increased from extreme scarcity to more than 2.5 x 10^6 individuals, which may equal or exceed the abundance they maintained in precolonial times (Kennenmer et al. 1992).

Regrowth of eastern forests and the consequent expansion of populations of other vertebrates, most notably white-tailed deer (Odocoileus virginianus) and white-footed mice (Peromyscus leucopus), have resulted in the emergence of Lyme disease, a zoonotic disease transmitted by tick vectors (Ixodes spp.) (Barbour and Fish 1993, Ostfeld 1997). Forest floor vertebrates play key roles in the Lyme-disease epizootic both as hosts for ticks and as sources from which feeding ticks obtain the bacterium (Borrelia burgdorferi) that causes the disease (Lane et al. 1991, Piesman and Gray 1994). Because wild turkeys are abundant, large, and active on the forest floor where ticks seek hosts, this species may interact strongly with Ixodes ticks. If turkeys serve as hosts for ticks or as reservoirs of Lyme-disease bacteria, they may enhance the density and infection prevalence of the ticks, thus increasing risk of human exposure to Lyme disease. Alternatively, if turkeys consume the ticks they encounter while foraging, they may reduce tick density and hence disease risk. We are not aware of any studies on trophic interactions between turkeys and Ixodes ticks. To evaluate the possibility that turkeys may influence the abundance or infection prevalence of ticks, we undertook a study to determine experimentally whether and how these species interact.

In summer and autumn 1998, we conducted two experiments on trophic interactions between turkeys...
and black-legged ticks (*Ixodes scapularis*—formerly *I. dammini*), the primary vector of Lyme disease in eastern and central North America. In the first experiment, we sought to determine whether turkeys are suitable hosts for juvenile ticks. If so, our intention was to determine whether turkeys are a competent reservoir for *Borrelia burgdorferi*. In the second experiment, we asked whether turkeys depredate unfed adult ticks and consequently reduce their abundance within local areas.

**MATERIALS AND METHODS**

**Experimental Animals**

Five yearling female eastern wild turkeys were obtained in June 1998 from Quattro's Game Birds in Pleasant Valley, NY. The breeding stock consisted of 10th generation turkeys originating from wild-caught birds native to Wisconsin, and was bred periodically with wild stock captured in Massachusetts. These birds were hatched in incubators and raised in large groups. We housed the five birds together in a 2m wide by 5m long by 2m high coop made of welded wire on a wood frame and containing a wooden perch and rain shelter. The coop was placed in a native old-field at the Institute of Ecosystem Studies in Millbrook, NY, ~500 m from the experimental forest plots (see below). Turkeys were supplied with commercial turkey feed, scratch grain, and water ad lib.

Host-seeking ticks (*Ixodes scapularis*) were obtained for both experiments by dragging a 1 m² piece of white corduroy cloth in a forested site ~3 km from the experimental site. Ticks were placed in glass vials with moistened plaster of paris, and were used within five days of collection. The same collection and maintenance procedure in prior experiments resulted in high feeding success of larval ticks on two species of *Peromyscus* mice (Hazler and Ostfeld 1995).

**Experiment I - Turkeys as Hosts for Ticks**

The primary purpose of this experiment was to determine whether juvenile (larval and nymphal) black-legged ticks can feed successfully on turkeys. In the initial phase, we used nymphal ticks known from our prior studies at this site to have a 30-40% infection prevalence with Lyme-disease bacteria (R. S. Ostfeld, unpublished data). If nymphal ticks fed successfully, our secondary purpose was to determine using xenodiagnosis the probability that uninfected larval ticks feeding two weeks later would acquire the bacteria.

In August 1998, we placed 27 nymphal *I. scapularis* ticks on each of the five turkeys. While the birds were restrained, ticks were placed on the nape of the turkeys' necks with a fine brush. The nape of the neck was used to reduce the possibility that turkeys would immediately remove ticks by preening. The turkeys were then held individually for five days (125 hours) in wire-mesh cages (40 cm wide by 56 cm long by 80 cm high) that were suspended over tubs of water. Cages were covered by plastic tarps for protection and shading. The turkeys had ad lib access to food and water through a 10 cm by 15 cm opening in the front of each cage. The tubs of water beneath cages were examined for fed or unfed ticks once each day for five days. We used a five-day criterion because juvenile ticks may require up to a day to attach and typically remain attached to hosts for about three days (Lane et al. 1991, Ostfeld 1997). After a two-week resting period, we placed 110 larval and 6 nymphal *I. scapularis* on each turkey (again, on the nape of the neck), returned the turkeys to their individual cages, and repeated the daily collection procedure for seven days (168 hours).

**Experiment II - Turkeys as Predators on Ticks**

The purpose of this experiment was to evaluate the effectiveness of turkeys as predators on adult *I. scapularis* in a natural forest habitat. We used adult ticks because this largest life stage (ca 2 mm long in *I. scapularis*) is the most likely to be visible and attractive to turkeys as food. To this end, we established six 10 m by 10 m enclosures in a mature oak forest site about 500 m from the turkey coop. The oak forest sites are described thoroughly in Ostfeld et al. (1995, 1996a). Enclosures were arranged as three blocks with 30-100 m between blocks and 15 m between the two enclosures within each block. Enclosures were constructed of plastic snow-fencing 2.5 m high that was supported at the corners by stapling the fence to tree trunks. For each pair of enclosures within a block, we used a coin toss to determine which would be the experimental (with turkeys) and control (no turkeys) unit.

We performed a pilot experiment in October 1998, in which we introduced 50 adult ticks (1:1 sex ratio) into the center of one of the experimental enclosures and allowed them to acclimate for 15 hours. We then introduced two turkeys into the enclosure and left them undisturbed for eight hours. The turkeys were then placed individually in cages suspended over water for 48 hours (see methods for Experiment 1) to determine whether any of the ticks had parasitized the turkeys. We then estimated the number of ticks that remained in the forest plots using a standard drag-sampling technique (e.g., Falco and Fish 1992). We exhaustively sampled the forest floor of both experimental and control enclosures by dragging a 1 m² corduroy cloth in concentric circles, checking the cloth for ticks every 10 paces. Because recovery rates were low, we followed
the pilot experiment with an additional experiment using carbon dioxide-baited tick traps (Falco and Fish 1992) in addition to drag-sampling.

We performed the full experiment in early to mid November, 1998, which is the time of peak activity of adult ticks at our sites (Ostfeld et al. 1996a,b). We placed 100 adult ticks (1:1 sex ratio) in the center of each of the six enclosures and allowed them to acclimate for 15 hours. We then released two turkeys into each of the three experimental enclosures and left them undisturbed for 8 hours per day for the following two days (16 hours total). Immediately following the 16 hours of foraging, the birds were placed in individual cages suspended over water for 48 hours to collect any ticks having parasitized the turkeys. The morning after turkey removal, three CO₂-baited tick traps were placed 2.5 m from the center of each enclosure, with at least 4.5 m between adjacent traps. Each trap was charged with ~1 kg of dry ice and was checked after seven hours. Immediately after the traps were checked, we dragged-sampled the entire plots to collect any remaining ticks. We also drag-sampled the outer perimeter of the enclosures to determine whether any ticks had escaped.

The following day the tick traps were recharged and the trapping and dragging procedures were repeated. We repeated this entire process in all six enclosures one week later. To be conservative in our analysis, we did not consider the repetitions of the tick introductions to be experimental replicates. Instead, we used a paired t-test with the presence or absence of turkeys as the independent variable, the experimental and control enclosure within a block comprising the pairs (N = 3 pairs), and the total number of ticks recovered in the two introductions as the dependent variable.

RESULTS

Experiment I - Turkeys as Hosts for Ticks

Very few larval or nymphal *Ixodes scapularis* ticks were recovered from the turkeys. Of the initial 27 nymphs placed on each of the five turkeys (135 ticks altogether), only six (4.4%) were recovered. On average, 1.20 (0.74 (SE; range 0-4) ticks per bird were recovered after 125 hours. Of the six nymphs that were recovered, four were engorged and two were unfed; the four engorged nymphs all came from one of the birds.

Of the 110 larvae and six nymphs placed on each turkey in phase two (580 ticks altogether), only 15 ticks (2.6%) were recovered after 168 hours. None of the 550 larvae engorged; all seven of the larvae recovered were unfed (mean = 1.40 ± 1.17 larvae per bird). Of the eight nymphs that were recovered, only one was engorged (TABLE 1). A thorough visual examination of one of the turkeys immediately following the seven days over water pans revealed no ticks. Because of the low success rate in feeding nymphs to repletion on the birds, and because no engorged larvae were recovered, we could not conduct a xenodiagnosis to test for the reservoir competence of wild turkeys.

**Experiment II - Turkeys as Predators on Ticks**

Turkeys were ineffective predators on adult *I. scapularis* ticks. On average, 179 adult ticks were recovered from the three forest enclosures in which turkeys foraged, compared to 184 ticks in the control enclosures (paired t-test, t = -0.23, P = 0.84; Fig. 1). The proportion of introduced ticks recovered after 16 hours of access by turkeys was 89.5% in experimental enclosures (with turkeys) and 92.2% in the turkey-free controls. No ticks were recovered from the turkeys during the 48 hours they were held over water immediately following the experiments.

**DISCUSSION**

Results of Experiment I suggest that wild turkeys are a poor host for juvenile *Ixodes scapularis* ticks, and are likely to be parasitized only rarely in nature. In the first phase, only four (3.0%) of 135 nymphs placed on turkeys fed to repletion, and in the second phase, only

### TABLE 1. Recovery of juvenile *Ixodes scapularis* ticks placed on five captive wild turkeys held individually over collecting pans of water.

<table>
<thead>
<tr>
<th>Tick Life Stage</th>
<th>Number of Ticks Introduced</th>
<th>Number (%) of Engorged Ticks Recovered</th>
<th>Number (%) of Unengorged Ticks Recovered</th>
<th>Total Number (%) of Ticks Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval</td>
<td>550</td>
<td>0 (0)</td>
<td>7 (1.3)</td>
<td>7 (1.3)</td>
</tr>
<tr>
<td>Nymphal</td>
<td>30</td>
<td>1 (3.3)</td>
<td>7 (23.3)</td>
<td>8 (26.7)</td>
</tr>
</tbody>
</table>
one (3.3%) of 30 nymphs and none of 550 larvae fed to repletion. The low recovery rates for ticks in either an engorged or unengorged state strongly suggest that the ticks we placed on turkeys were preened off and swallowed by the hosts. The ticks had no other route of escape. Casual observations revealed that the turkeys commonly autopreened and allopreened while housed in the coop; however, observations of the birds while they were held in individual cages were prevented by protective tarps surrounding the cages. Grooming behavior is known or suspected to be responsible for reductions in infestation rates by ixodid ticks on other hosts (Ostfeld et al. 1993, Sonenshine 1993).

*Ixodes scapularis* ticks are known to parasitize dozens of species of vertebrate hosts, including several species of ground-dwelling songbirds (reviewed by Lane et al. 1991, Fish 1993, Tälleklint 1996). However, we are not aware of any studies reporting *I. scapularis* parasitizing *M. galloppavo*. Davidson and Wentworth (1992) reviewed studies of ectoparasites on turkeys, and reported the occurrence of three species of other ixodid ticks and one species of argasid on wild turkeys in the southeastern United States. The poor performance of these ticks on turkeys, and the likelihood that they were consumed during autopreening, suggests that continued range expansion and population growth of wild turkeys will not increase the number of feeding opportunities for questing *I. scapularis*.

Captive wild turkeys in our forest enclosures did not reduce abundance of adult *I. scapularis*, nor did the ticks parasitize the turkeys during their foraging bouts. Prior studies with the same individual turkeys in the same forest enclosures revealed that the turkeys spent much of their time in the enclosures feeding, and that they consumed substantial numbers of tree seeds experimentally introduced into the enclosures (F. Keesing, E. Brownold, and R. Ostfeld, unpublished data). In both prior and current studies, turkeys began pecking and scratching at the forest floor within several minutes of being introduced into enclosures (D. Lewis and R. Ostfeld, pers. obs.). Therefore, the lack of removal of adult ticks was not caused by a lack of foraging effort. We tentatively conclude that natural populations of turkeys are unlikely to reduce population size of questing adult *I. scapularis* through predation. Because both turkeys and ticks occur naturally in aggregations (Ostfeld et al. 1996a, Lewis and Ostfeld, pers. obs.), it remains possible that turkey flocks may encounter and depredate clumps of ticks.

Natural enemies of ticks appear to be common (Carroll 1995, Hu et al. 1993, Samish and Rehacek 1999, Zhioua et al. 1995), although their impacts on tick populations are poorly understood. Duffy et al. (1992) found that helmeted guineafowl (*Numida meleagris*) reduced abundance of adult *I. scapularis* ticks on grass lawns. However, this species of tick is substantially more abundant in forests than on lawns (Adler et al. 1992, Maupin et al. 1991, Ostfeld et al. 1995). Although >40 species of birds are reported to feed on ticks, evidence suggesting regulation of tick populations by birds is scarce (Samish and Rehacek 1999). Further studies of the regulatory capacity of birds and other predators and pathogens on ticks are warranted.

![Graph](image)

**Figure 1.** Number (+1 SE) of adult *Ixodes scapularis* ticks recovered using CO₂-baited traps and cloth drag-sampling inside 10m by 10m forest enclosures. Three replicates (experimental enclosures) had turkeys present for 16 hours over two days, and three (controls) had no turkeys.
Acknowledgements

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Susceptibility of the Malaria Vector Anopheles culicifacies (Diptera: Culicidae) to DDT, Dieldrin, Malathion, and Lambda-Cyhalothrin

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ABSTRACT: The susceptibility of the malaria vector Anopheles culicifacies to DDT, dieldrin, malathion, and lambda-cyhalothrin was determined in Karnal, Yamunanagar, and Ambala districts of Haryana State, India. The vector population showed a high degree of resistance to DDT and dieldrin. The test mortality to DDT and dieldrin ranged from 25% to 28% and 18% to 20%, respectively, in Nadasahib of Ambala districts. The mortality of An. culicifacies to malathion ranged between 65% and 68%. All the tests with lambda-cyhalothrin resulted in 100% mortality of An. culicifacies. DDT and dieldrin resistance did not confer cross-resistance to lambda-cyhalothrin in An. culicifacies.

Keyword Index: DDT, Malathion, Anopheles culicifacies.

INTRODUCTION

Insecticides continue to be the mainstay of vector-borne disease control programs in India. The extensive and intensive use of residual insecticides reduced the vector population, and malaria control was achieved to a great extent in India by the mid-sixties. Afterwards, however, malaria incidence started rising to a peak level of 6.4 million by 1976. Vector resistance to DDT, BHC, and malathion was considered to be one of the contributory factors for the resurgence of malaria in India. The steady development of resistance to an increasing number of insecticides by an increasing number of vector species over wider geographical areas has impeded malaria control programs in India. Anopheles culicifacies was highly susceptible to malathion in Haryana State (Sharma 1990, Sharma et al. 1996), where malathion was introduced in 1981 for regular indoor residual spraying for DDT and BHC resistant populations of An. culicifacies. Over the years, the An. culicifacies populations have reacted differently under different ecological and environmental conditions ranging from the development of resistant populations to the selection of refractory behavior, affecting malaria transmission to varying degrees (Bang 1985). In India, the first report of malathion resistance came from Gujarat state (Rajagopal 1977). The development of resistance in An. culicifacies to malathion was found to be widespread in Gujarat and Maharastra (Vittal and Deshpande 1983, Das et al. 1986).

Synthetic pyrethroids have been found to be effective for the control of mosquitoes (Chester et al. 1992). During the 1996 malaria epidemic in the Gurgaon district of Haryana State, HCH and malathion were replaced by synthetic pyrethroids, which controlled An. culicifacies (Sharma et al. 1997). The response of multi-resistant An. culicifacies to lambda-cyhalothrin is not known in Haryana State. The present field study on the susceptibility of An. culicifacies populations using the diagnostic dosage of 4% DDT, 0.4% dieldrin, 0.1% lambda-cyhalothrin, and 5% malathion was undertaken in different districts of Haryana State.

MATERIALS AND METHODS

The study was carried out in Ambala, Karnal, and Yamunanagar districts of Haryana State. The diagnostic concentration for the detection of resistance in Anopheles culicifacies was 5% malathion. The susceptibility tests were carried out according to the WHO standard test (WHO 1975), in which the mosquitoes were exposed to impregnated papers with an oil solution containing the diagnostic concentration of the test insecticide. The impregnated papers were supplied by WHO. The exposure period was one hour in all the tests. Fully fed An. culicifacies collected from the field were used for
tests. Mortality counts were made at the end of a 24-hour period and mosquitoes unable to walk were considered as dead. Mortality was adjusted by applying Abbott's formula when mortality in controls was recorded at up to 20%. The insecticide pressure (% coverage) was sprayable area in all the three rounds for malathion.

RESULTS AND DISCUSSION

The epidemiological situation in collections from study districts by the District Malaria Officer is shown in TABLE 1. The annual blood examination rate (ABER) was more than 10% in all the districts. The annual parasite (API) and slide positive (SPR) rates were highest in the Karnal district in 1995. In all the districts sampled, *An. culicifacies* showed mortality of between 65% and 88% from exposure to malathion (TABLE 2). The species showed a higher degree of resistance to dieldrin with mortality ranging from 18 to 28%, and from 25 to 28% with DDT. With lambda-cyhalothrin all tests showed 100% mortality (Fig. 1).

The DDT and dieldrin resistance in *An. culicifacies* did not confer cross-resistance to lambda-cyhalothrin.

The *An. culicifacies* showed resistance to malathion in Haryana State. The malathion spray was started with three cycles per year since 1981 in Haryana State. During the 1981-1982 and 1996 epidemics of malaria in Sonepat and Gurgaon districts of Haryana State, HCH was replaced with malathion and synthetic pyrethroids (deltamethrin and cyfluthrin), respectively, which successfully controlled *An. culicifacies*. Later, three rounds of malathion spraying resulted in *An. culicifacies* becoming resistant. The impact of malathion pressure in study districts under the malaria control program ranged between 26-79% (Fig. 2). In 1993-1994, a focal spray of malathion was also carried out in the state. The presence of possible resistance to malathion was expected because a residual malathion spray was also used frequently in agriculture throughout the state. Resistance of *An. culicifacies* to malathion was observed after 13 cycles of

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**TABLE 1. Epidemiological situation of study districts.**

<table>
<thead>
<tr>
<th>Districts</th>
<th>Year</th>
<th>BSE&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Number Positive</th>
<th>PF&lt;sup&gt;2&lt;/sup&gt;</th>
<th>ABER</th>
<th>API</th>
<th>SPR&lt;sup&gt;3&lt;/sup&gt;</th>
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<td>165130</td>
<td>492</td>
<td>17</td>
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</tr>
<tr>
<td></td>
<td>1995</td>
<td>168135</td>
<td>1441</td>
<td>66</td>
<td>13.26</td>
<td>1.14</td>
<td>0.86</td>
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<tr>
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<td>4461</td>
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<td>17.66</td>
<td>3.98</td>
<td>2.26</td>
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<tr>
<td></td>
<td>1995</td>
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<td>10067</td>
<td>2050</td>
<td>19.21</td>
<td>8.81</td>
<td>4.59</td>
</tr>
<tr>
<td>Yamuna Nagar</td>
<td>1994</td>
<td>103032</td>
<td>198</td>
<td>7</td>
<td>11.62</td>
<td>0.22</td>
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</tr>
<tr>
<td></td>
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<td>639</td>
<td>2</td>
<td>11.62</td>
<td>0.71</td>
<td>0.61</td>
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</table>

<sup>1</sup>BSE = Blood Slide Examination  
<sup>2</sup>PF = *Plasmodium falciparum*  
<sup>3</sup>SPR = Slide Positive Rate

**TABLE 2. Susceptibility status of *Anopheles culicifacies* to malathion in Haryana.**

<table>
<thead>
<tr>
<th>Date of Test</th>
<th>District/Village</th>
<th>No. Exposed (No. of Replicates)</th>
<th>% Mortality</th>
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<td>35(3)</td>
<td>88</td>
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<tr>
<td>July 13, 1994</td>
<td>Karnal/Gharaulna</td>
<td>34(3)</td>
<td>85</td>
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<tr>
<td>July 26, 1994</td>
<td>Yamuna Nagar/Todarpur</td>
<td>26(2)</td>
<td>65</td>
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<tr>
<td>July 27, 1994</td>
<td>Ambala/Finjore</td>
<td>30(3)</td>
<td>75</td>
</tr>
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</table>
Figure 1. Susceptibility status of *Anopheles culicifacies* to DDT, dieldrin, malathion, lamdacyhalotrin and the mosquitoes exposed.

Figure 2. Insecticide pressure against *Anopheles culicifacies* in the study area.
spray in the Thane district of Maharashtra (Vittal and Despande 1983). The development of resistance in An. culicifacies to malathion in Haryana State is due to prolonged selection pressure maintained by the Control Program and the predominant indoor resting and biting habits of the vector species.

Acknowledgements

The authors are thankful to Shri P. K. Jena and Shri Y. S. Parihar for their dedicated assistance in the field. The authors are also thankful to Shri Jarnail Singh and Mr. S. K. Katyal for secretarial assistance.

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Bacteria and Mosquito Abundance in Microcosms Enriched with Organic Matter and Treated with a Bacillus thuringiensis subsp. israelensis Formulation

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ABSTRACT: Bacteria and mosquito abundance were studied in outdoor tubs unenriched and enriched with 0.04% rabbit pellets during the winter, 1999. The irrigation water used to fill the tubs contained a total bacterial count of 1.15-1.35 x 10^3 cells/ml. Adding rabbit pellets for enrichment yielded a total bacterial count of 5.50-7.63 x 10^3 cells/g. Bacterial densities in unenriched water were significantly lower than in enriched tubs on every sampling day. When bacterial densities in both enriched and unenriched regimens reached peak populations on day 3 post-flooding, their numbers in enriched water were 25-fold higher than the unenriched water. Under cool weather conditions, mosquito oviposition activity was low and larval development was very slow. Egg raft counts and larval densities in enriched water were nevertheless higher than those in unenriched water. After reaching peak populations on day 3 post-flooding, the natural decline in bacterial densities in the top portion of enriched water without mosquito larvae was lower compared with that in water with larvae. In water with larval present, the decline of bacterial levels in top water was greater than in bottom water on day 7 post-flooding. VectoBac G, a granular formulation of Bacillus thuringiensis subsp. israelensis caused a reduction in larval numbers of 80, 93, 73% at the rate of 5.5 lb/ac and a reduction of 94, 93, 86% at the rate of 10.6 lb/ac on days 1, 3, 7 posttreatment, respectively. After treatment, the reductions of bacterial densities in untreated tubs were greater than treated tubs. These results indicate that mosquito larvae play an important role in the decline of bacterial populations by their feeding activity.

Keyword Index: Organic matter, enriched water, oviposition, Bacillus thuringiensis subsp. israelensis, mosquitoes, bacteria.

INTRODUCTION

Organic matter has been used in field experiments as ovipositional attractants and stimulants to obtain sustained production of mosquito larvae (Beehler and Mulla 1995, Rodcharoen et al. 1997). Supplemental enrichment of mosquito breeding sources with chicken lay mash, alfalfa pellets, and rabbit pellets produces large numbers of Culex mosquitoes (Culex quinquefasciatus Say, Culex tarsalis Coquillet, Culex stigmatosoma Dyar) for field evaluation of microbial agents and mosquito fish as larval predators (Walton and Mulla 1991, Su and Mulla 1999, Nguyen et al. 1999).

Bacterial activity can modify the ovipositional behavior of gravid mosquitoes. The bacterium Aerobacter aerogenes isolated from hay infusion produced chemicals attractive to ovipositing Cx. quinquefasciatus and Aedes aegypti L. (Hazard et al. 1967). Ikeshoji et al. (1975) isolated the bacterium Pseudomonas aeruginosa from fatty acid substrates, which produces ovipositional attractants for both Cx. pipiens molestus Forskal and Ae. aegypti. In addition, bacteria also served as an important component of food sources of mosquito larvae (Nilsson 1987, Walker et al. 1988, Merrit et al. 1990, 1996).

We recently studied the bacterial flora and the contribution of the microbial mosquitoicidal formulations of Bacillus thuringiensis subsp. israelensis de Barjac (B.t.i.) and Bacillus sphericus Neide strain 2362 to bacterial abundance in microcosms sustaining heavy natural populations of mosquito larvae in the summer and fall seasons (Nguyen et al. 1999). In the present study, we investigated the bacterial abundance in water in microcosms unenriched and enriched with organic matter to address the relationship between organic pollution and bacterial abundance. To determine the effect of mosquito activity on bacterial densities, bacterial abundance was assessed in enriched water in the presence
or absence of mosquito larvae, and tubs treated with the microbial agent B.t.i., where mosquito larval densities were reduced, were also examined. The experiments were carried out in the winter season of 1999, when water temperatures and mosquito larval breeding were lower than in the summer and fall seasons.

MATERIALS AND METHODS

Test Facilities and Material

Two tests were carried out in the winter season of 1999 in outdoor fiber-glass tubs measuring 1.0 x 1.0 x 0.4 m deep at the Aquatic and Vector Control Research Facility at University of California, Riverside. In test 1, because of lack of adequate mosquito production due to cool weather, only bacterial abundance in water unenriched and enriched with organic matter was assessed. In total, 24 tubs were used in this experiment. Twelve of them were selected at random to be enriched with rabbit pellets (Brookhurst®, Brookhurst Mill, Riverside, CA, crude protein 17%) at the rate of 0.04%, equaling 100 grams per tub. The other 12 tubs were left unenriched. The tubs were filled with water from an irrigation reservoir and water depth was kept constant at 30 cm (236 liters) using float valves. In test 2, 16 tubs were enriched with rabbit pellets at the same rate as in test 1. The purpose of this experiment was to determine bacterial abundance in enriched water with or without mosquito larvae and in microbial treatments using B.t.i. granular formulation (VectoBac G). Eight randomized tubs were treated with two rates (four tubs for each rate) of VectoBac G, while the other eight tubs were left untreated. Among the untreated tubs, four tubs were assigned as check 1 with mosquito larvae present, while the other four tubs were used as check 2 where mosquito egg rafts were removed every other day during the test period to prevent larval breeding. Wild populations of Culex stigmatosoma, Cx. quinquefasciatus, and Cx. tarsalis oviposited nightly in these filled tubs.

The B.t.i. granular formulation (VectoBac G, corn cob granules, Lot# 25-801-N8, 200 ITU/mg, Abbott Laboratories, North Chicago, IL., received 7/10/1997) was applied to the eight tubs in test 2, 13 days after enriching and filling the tubs, when 3rd-4th-instar mosquito larvae were present in large numbers. The formulation was applied at two rates of 5.3 and 10.6 lb/ac, equaling 0.5 and 1.0 g per tub, respectively. The required amounts of VectoBac G for each rate were gently broadcast by hand on the water surface at the corners and middle of each tub.

Water Quality Determination

During the course of the tests, water quality parameters were measured for all tubs on every sampling day. The mean values for dissolved oxygen (DO) and corrected electrical conductivity at 25°C (EC) were determined with a YSI Model 85 Handheld Water Quality Meter. Water temperature was monitored with a minimum-maximum thermometer submerged in a tub located in the center of the tub arrangement, and temperatures were recorded each time the tubs were sampled for mosquito larvae and/or bacterial density.

Bacterial Assessment

In order to assess the background bacterial populations in the water used for tub filling, three water samples, each of which consisted of 120-150 ml, were directly collected from the float valves of three randomized tubs during the period of filling the tubs in both test 1 and 2.

To determine the background bacterial density in the dry rabbit pellets used for tub enrichment in test 1 and 2, about 2 kg of pellets were placed in a plastic bucket of 10 quarts capacity before enriching the tubs. Three 10 g samples were collected from the periphery and center of the top layer of the pellets in plastic bucket and put in separate sterile plastic Petri dishes. Rabbit pellets were then ground to powder in the laboratory with a porcelain mortar and pestle and processed for isolation and determination of bacterial numbers present in the pellets.

To determine bacterial abundance in the tubs after filling in test 1, water from both the top and bottom of enriched and unenriched tubs was collected at three hours after filling the tubs and on days 3, 8, 15, and 22 post-flooding. No treatments were made with B.t.i. in this test. In test 2, water samples were collected at three hours after enriching and filling the tubs and on days 3, 7, and 13 post-flooding. After treatment with VectoBac G, water samples were collected at three hours and on days 1, 3, and 7 posttreatment. Sterile pipets (5ml) fitted with a pro-pipette were used to collect five samples, one at each corner and one at the middle of the undisturbed water at 0.3-0.5 cm below the surface and about 0.5-1.0 cm above the bottom of each tub. The top and bottom water samples of all replicates were composited separately in 250 ml sterile flasks, each composite sample amounting to 100 ml of water from the four replicates.

To estimate the bacterial densities in water samples and in rabbit pellets, the surface spread plated method on nutrient agar was used (Jones 1970). For quantitative determination of bacterial cells, 1 ml from each water sample was serially diluted in 9 ml of autoclaved water and dilutions ranged from 10⁶ to 10¹⁰ times to facilitate colony counting. One gram of powdered rabbit pellets
was weighed and suspended in 99 ml sterile water. After shaking for 5 min, 1 ml of this aliquot was serially diluted in test tubes containing 9 ml sterile water. Three Petri dishes containing 20 ml NA medium (2.3% Difco nutrient agar) were inoculated with 0.1 ml aliquot of each serial dilution, and incubated at 30°C for 48 hours. Autoclaved water for dilution was also plated on NA as a control. After an incubation period of 48 hours, the colony-forming units (CFUs) were counted visually and adjusted for dilutions and inoculum volume, and were expressed as mean number of CFUs/ml of water sample or as mean number of CFUs/gram of rabbit pellets.

To determine densities of spore-forming bacteria including *B. t.i.*, water samples were heated to 65°C for 30 minutes to kill vegetative and non-spore-forming bacteria before diluting and plating. These water samples were diluted from 10^0 to 10^6 times and spread on NA medium. After incubation at 30°C for 48 hours, colonies were counted visually and expressed as CFUs/ml.

**Mosquito Production and Control**

Mosquito oviposition was assessed by counting the total number of egg rafts (presented as mean number of egg raft per tub) on every sampling day in test 1 and 2 (except check 2) in each tub and placing them in plastic sentinel cups, which were partially submerged in the water of the tubs using floatation collars. The bottom of these cups was removed previously to allow the hatched larvae to swim freely out into the water. This technique allowed us to count the newly laid egg rafts at each sampling interval. In test 2, in order to investigate the influence of mosquito larvae on microbial abundance, the egg rafts were removed and discarded every other day from four enriched tubs, which were assigned as check 2 to determine bacterial abundance without mosquito larvae. These tubs were devoid of mosquito larvae throughout the experiment.

Mosquito larvae were assessed by taking five-dip samples per tub, one dip from each corner and one from where larvae were noted in large numbers on every sampling day. In order to minimize the dipping disturbance to the water surface, during a dipping cycle for a treatment assignment, one dip was taken from each tub, so there was a 3-5 minute period between each dipping in a given tub. Larval populations were categorized into early (1st and 2nd) and late (3rd and 4th) instars, then counted and recorded as mean number of larvae per dip. The pupae, if any, were excluded from counting because no feeding activity was occurring at pupal stage. The percentage reduction (%R) of mosquito larvae due to *B. t.i.* treatments was calculated using the formula given by Mulla et al. (1971).

**Statistical Analyses**

Water quality parameters (DO and CEC), average bacterial densities (CFU/ml), and average egg raft counts (egg rafts/tub) as well as average larval densities (larvae/dip) in the check and treated tubs were analyzed for significance by one factor ANOVA (Scheffé *F* test) for repeated measurements.

**RESULTS**

**TEST 1**

**Water Quality**

Test 1 was carried out from January 11 to February 2, 1999. The water temperatures during the test period were low, minimum, and maximum ranged 6.8-10.2°C and 15.7-20.2°C, respectively (Fig. 1A). Marked differences in dissolved oxygen (DO) values were noted between unenriched and enriched water. In unenriched water, the DO was 6.7 ppm at three hours after filling the tubs and progressively increased to 14.1-14.2 ppm on days 15-22 post-flooding (Fig. 1B). The DO in enriched water, however, was similar to that in the unenriched water at three hours after enriching and filling the tubs (6.5 ppm), but the readings sharply decreased to 0.04, 0.11, and 0.49 ppm DO on days 3, 8, and 15 post-flooding, respectively, then increased to 9.8 ppm on day 22 post-flooding. On days 3, 8, 15, and 22 post-flooding, DO readings in enriched water were significantly lower than in unenriched water (Fig. 1B). The CEC in unenriched water increased from 528 mS at three hours after start of filling the tubs to 545.7 mS on day 8 post-flooding, then rapidly decreased to 420.6 mS on day 22 post-flooding. In enriched water, the CEC increased from 557.8 mS at three hours after enriching and filling the tubs to 590 mS on day 8 post-flooding, then declined to 552.6 mS on day 22 post-flooding. Significant difference in CEC was indicated between unenriched and enriched water on days 15 and 22 post-flooding (Fig. 1C).

**Bacterial Abundance**

When the background bacterial population in reservoir water used to fill the tubs was monitored, the bacterial densities in water flowing from the float valves averaged 1.15 x 10^3 total bacterial cells/ml. Rabbit pellets used for enrichment contained a large number of bacteria, averaging 5.5 x 10^8 total bacterial cells/g. After filling the tubs, the bacterial density in water of the unenriched regimen was significantly lower than that in the enriched regimen on every sampling day during the test period (Fig. 2A). In top water of unenriched tubs at three hours after filling the tubs, total bacterial numbers were 1.80 x 10^5 cells/ml (equal to the incoming water),
Figure 1. Water quality parameters in tubs enriched and unenriched with organic matter. Asterisks indicated significant differences between unenriched and enriched tubs by 1-factor ANOVA (Scheffé F test) for repeated measurements at the 0.05 level.
which peaked at 6.22 x 10^4 cells/ml on day 3 post-flooding, then progressively declined to 1.10 x 10^5 cells/ml at the end of the test on day 22 post-flooding. However, in top water of tubs enriched with rabbit pellets, the total bacterial numbers were 2.20 x 10^6 cells/ml at three hours after enriching and filling the tubs, which sharply increased and peaked at 1.53 x 10^9 cells/ml on days 3 and 8 post-flooding, maintaining a high count of 1.10 x 10^6 cells/ml on day 15 and finally declining to 1.45 x 10^5 cells/ml on day 22 post-flooding (Fig. 2A).

Total bacterial densities in the bottom water of unenriched as well as enriched tubs were slightly higher than those in top water on every sampling day, however these data were not included in figures. In bottom water of unenriched tubs, bacterial numbers increased from 2.30 x 10^4 cells/ml at three hours after filling the tubs to the peak population of 6.55 x 10^9 cells/ml on day 3 post-flooding, then gradually declined to 1.10 x 10^6 cells/ml at the end of the test. Bacterial numbers in bottom water of enriched tubs sharply increased from 2.40 x 10^6 cells/ml at three hours after enriching and filling the tubs to the peak population of 1.66 x 10^9 cells/ml on day 3 post-flooding. High numbers continued to prevail with 1.64 x 10^6 cells/ml on day 8 post-flooding, then declined to 1.21 x 10^6 cells/ml on day 15 and 1.48 x 10^6 cells/ml on day 22 post-flooding. The growth and decline trends of total bacterial abundance over time were essentially the same in the top and bottom waters.

As with the total bacteria, densities of spore-formers in unenriched water samples were significantly lower than those in enriched water on every sampling day. In unenriched water, spore-formers densities increased slightly from 0.76 x 10^2 cells/ml at three hours after filling the tubs to 0.80 x 10^2 cells/ml on day 3 post-flooding, then progressively declined to 0.30 x 10^2 cells/ml on day 22 post-flooding (Fig. 2B). In top water of enriched tubs, the average count of spore-formers was 1.20 x 10^3 cells/ml at three hours after enriching and filling the tubs, increasing to 1.75 x 10^3 cells/ml on day 3 post-flooding, then gradually declining to 0.75 x 10^3 cells/ml on day 22 post-flooding (Fig. 2B).

Spore-forming bacterial densities in bottom water of unenriched and enriched tubs were essentially the same as or slightly higher than in densities in top water, depending on sampling days. The spore-formers reached peak populations on day 3 post-flooding (0.80 x 10^2 and 2.05 x 10^2 cells/ml in unenriched and enriched water, respectively), gradually declining to the low levels at the end of the test on day 22 post-flooding. The overall growth and decline patterns of spore-forming bacteria over time were similar in top and bottom waters.

**Mosquito Production**

Because of cool temperatures during test 1, mosquito oviposition levels and larval numbers were low, these data are not presented in figure form. Despite this, on day 8 post-flooding, the mean number of egg rafts in unenriched water (4.9 ± 1.4 egg rafts/tub) was significantly lower than in the enriched (17.8 ± 2.6 egg rafts/tub). Similarly, the number of larvae in unenriched water was significantly lower than in the enriched tubs amounting to 6.1 ± 2.7 and 19.6 ± 5.8 larvae/dip on day 22 post-flooding, respectively. No species identification of the mosquito larvae was conducted because of low larval densities in this test.

**TEST 2**

**Water Quality**

Test 2 was conducted from February 23 to March 15, 1999. Water temperatures fluctuated from a minimum of 8.5-10.8°C to a maximum of 16.8-25°C. No difference in water quality among the various treatments was detected. DO readings were 3.6 ppm at three hours after enriching and filling the tubs, decreasing to almost anoxic conditions (0.04 and 0.06 ppm) on days 3 and 7 post-flooding, then gradually increasing to 15.9 ppm at the end of the test on day 7 posttreatment (day 20 post-flooding). CEC values increased progressively from 549.1 mS at three hours after enriching and filling the tubs to 614.8 mS on day 1 posttreatment (day 14 post-flooding), then decreased slightly to 564.9 mS on day 7 posttreatment. These data are not presented in figure form.

**Bacterial Abundance**

In test 2, irrigation water contained an average of 1.35 x 10^3 total bacterial cells/ml and rabbit pellets contained 7.63 x 10^3 total bacterial cells/g. After filling the tubs, a marked decline in total bacterial populations was noted in all treatment regimens (tubs without mosquito larvae, tubs with larvae and tubs treated with *B.t.i.* formulation). In top water, the bacterial densities increased from 1.5-2.30 x 10^4 cells/ml at three hours after enriching and filling the tubs to the peak populations of 1.94-2.26 x 10^6 cells/ml on day 3 post-flooding (Fig. 3), then gradually declining to 1.60 x 10^5-1.11 x 10^6 cells/ml on day 7 posttreatment (day 20 post-flooding), when the test was terminated. Bacterial densities in the bottom water were higher than those in the top water on every sampling day. Their numbers increased from 1.90-4.20 x 10^5 cells/ml at three hours after enriching and filling the tubs to peak populations of 1.99-2.32 x 10^6 cells/ml on day 3 post-flooding, then declining progressively to 2.50 x 10^4-1.27 x 10^5 cells/ml on day 7 posttreatment.
Figure 2. Mean number of total bacteria (CFU x 10^5/ml) (A), and spore-forming bacteria (CFU x 10^2/ml) (B) present in top water of tubs enriched and unenriched with organic matter. Asterisks indicated significant differences between unenriched and enriched tubs by 1-factor ANOVA (Scheffé F test) for repeated measurements at the 0.05 level.

Figure 3. Mean number of total bacteria (CFU x 10^5/ml) present in top water of tubs enriched with organic matter and treated with VectoBac G formulation. *Unshared letters indicate significant differences among control and treatments by 1-factor ANOVA (Scheffé F test) for repeated measurements at the 0.05 level.
Marked differences in declining rates of bacterial populations were noted between the tubs with mosquito larvae and without larvae. After reaching peak populations (2.05 x 10^9 cells/ml) on day 3 post-flooding, in top water of check tubs without larvae (check 2 w/o larvae), the bacterial counts declined to 1.45 x 10^7, 7.80 x 10^7, and 2.68 x 10^7 cells/ml on days 7, 13, and 14 post-flooding (day 1 posttreatment), respectively. For these three intervals, the bacteria in the check tubs without larvae declined by 29, 62, and 87%, respectively from their original highs (Fig. 4A). In the check tubs with mosquito larvae (check 1 w/larvae), however, bacterial densities in top water declined rapidly from peak populations of 2.12 x 10^9 cells/ml on day 3 post-flooding to 6.20 x 10^4, 8.70 x 10^4, and 2.80 x 10^4 cells/ml on days 7, 13, and 14 post-flooding, respectively. The magnitude of bacterial reduction for these three intervals was 71, 96, and 99% on days 7, 13, and 14 post-flooding, respectively, a much greater reduction than that in check tubs without larvae. The declines in bacterial numbers in the top and bottom water taken from the tubs without larvae were very similar for all days sampled (Fig. 4A, B). On the other hand, in the check tubs with larvae, bacterial decline in the top water was higher (71%) than in bottom water (51%) on day 7 post-flooding (Fig. 4A, B).

In top water of B.t.i. treated tubs, the growth and decline trends of bacterial abundance were similar to those in the check tubs with larvae (check 1 w/larvae) from three hours after enriching and filling the tubs up to day 1 posttreatment. However, on days 3 and 7 posttreatment with B.t.i. formulation, bacterial levels in the check 1 tubs was slightly but significantly lower than in the treated tubs (Fig. 3). The bacterial counts in top water of check 1 tubs were 1.70 and 1.60 x 10^4 cells/ml, while in treated tubs their numbers were 8.00 and 7.10 x 10^4 cells/ml (at the applied rate of 5.3 lb/ac), and 9.20 and 8.10 x 10^4 cells/ml (at the rate of 10.6 lb/ac) on days 3 and 7 posttreatment, respectively. The reductions of bacterial abundance in bottom water were similar to those in top water, but the reduction was greater in top water on day 7 posttreatment (Fig. 4A, B).

In the various treatment regimens, spore forming bacterial densities in top water increased slightly from 1.50-2.50 x 10^2 cells/ml at three hours after enriching and filling the tubs to 2.00-6.00 x 10^2 and 2.0-4.0 x 10^2 cells/ml on days 3 and 7 post-flooding, respectively. These declined slightly to 1.50-2.50 x 10^2 cells/ml on day 13 post-flooding (Fig. 5A). However, after treatment with VectoBac G, the numbers of spore-formers in treated tubs sharply increased due to the addition of B.t.i. spores, while in check tubs, spore-forming bacterial counts remained low (Fig. 5A).

Due to the application of VectoBac G formulation, B.t.i. spores were recovered from water of treated tubs at every sampling time following treatment. No B.t.i. spores were detected in check water (Check 1) on any of the sampling days (Fig. 5B). In top water of tubs treated with VectoBac G at rates of 5.3 and 10.6 lb/ac, B.t.i.

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**Figure 4.** Reduction of total bacterial densities from their peak populations on day 3 post-flooding in water of tubs enriched with organic matter and treated with VectoBac G formulation.
spores were detected in relatively high numbers, reaching 5.3 and 10.0 x 10^3 spores/ml, respectively, three hours after treatment, then rapidly declining to 4.00 and 5.50 x 10^3 spores/ml, respectively, on day 7 posttreatment. In tubs treated with VectoBac G at the high rate of 10.6 lb/ac, B.t.i. spore counts in top water were significantly higher than in water treated at the low rate of 5.3 lb/ac and day 3 posttreatment (Fig. 5B).

As in the top water, B.t.i. spores in bottom water were found in high numbers of 7.75 and 10.0 x10^3 spores/ml at three hours after treatment with VectoBac G at the rates of 5.3 and 10.6 lb/ac, respectively, then gradually declining to 6.30 x 10^2 and 1.36 x 10^3 spores/ml, respectively, on day 7 posttreatment. The treatment using the high rate showed significantly higher B.t.i. spore counts than the low rate at three hours, and three and seven days posttreatment (Fig. 5B).

**Mosquito Production and Control with B.t.i.**

Mosquito oviposition activity was noted on day 1 after enriching and filling the tubs and reached a peak on day 7 post-flooding (Fig. 6A), then progressively declined to a low level on day 3 posttreatment. However, on day 7 posttreatment, a minor peak of oviposition activity occurred in both check and treated tubs.

Early instar (1st and 2nd) larvae were noted on day 7 post-flooding in assigned check and treated tubs. Larval densities reached averages of 105.5-135.0 total larvae/dip at a time of treatment in check and treated tubs. The two rates of VectoBac G yielded equally good control up to seven days (Fig. 6B). The reduction in larval population was 80, 93, and 73% for the low rate of 5.3 lb/ac and 94, 93, and 86% for the high rate of 10.6 lb/ac on days 1, 3, and 7 posttreatment, respectively.

The densities of late instar (3rd and 4th) larvae were 39-49 larvae/dip at the time of treatment in check and treated tubs. The two rates of VectoBac G yielded equally good control up to seven days (Fig. 6C). On days 1, 3, and 7 posttreatment, VectoBac G reduced the populations of late instar larvae by 67, 95, and 91% at 5.3 lb/ac and 91, 95, and 99% at 10.6 lb/ac, respectively.

As to the changes in species composition in check tubs during sampling period as indicated by identification of late instar larvae, Culex stigmatosoma kept a relatively stable proportion of 45.3-40.8%, while Cx. quinquefasciatus decreased progressively posttreatment from 33.8% to 15.8%. Culex tarsalis, however, increased from 14.9% on treatment day to 35.0% on day 7 posttreatment. Culiseta incisens and Cs. inonata were found in low proportions (2.0-5.8%) during the test period.

**DISCUSSION**

The results of test 1 showed a natural pattern of growth and decline of bacterial populations in outdoor tubs both unenriched and enriched with organic matter in the winter season. The irrigation water contained a very low number of total bacterial cells per milliliter, while the rabbit pellets used for enrichment of the tubs contained a large number of bacteria per gram. The bacterial populations in enriched water at three hours after enriching and filling the tubs were 11-fold higher than those in unenriched water. The bacteria preexisting in rabbit pellets constituted an important source of bacterial abundance in the enriched regimen. These bacteria, along with those coming from irrigation water, reproduced in much higher densities in the enriched tubs when the rabbit pellets were present. The peak populations of bacteria on day 3 post-flooding in the enriched regimen were 25-fold higher than in unenriched water. The organic matter required for bacterial growth and propagation was in low quantity in unenriched water, while in enriched water, the bacteria decomposed and used the ample supply of rabbit pellets for metabolic activity, growth, and reproduction. After reaching the peak populations, bacterial densities in unenriched and enriched regimens gradually declined by 83 and 91% at the end of the test on day 22 post-flooding, respectively. Due to lack of adequate mosquito production in cool weather in test 1, these bacterial declines were natural when the source of required nutrients in the environment were gradually exhausted.

Analysis of the relationship between bacterial abundance and dissolved oxygen values indicates that the high bacterial counts were directly related to the low oxygen concentration in the enriched regimen. In the unenriched water, the DO values were significantly higher than in the enriched water soon after flooding. The DO in the enriched water decreased to a very low level which inversely coincided with peak bacterial populations. The great reduction in oxygen concentration at the peak time of bacterial population in enriched water indicates strong biological demand for oxygen (BOD) by the bacteria during the logarithmic division phase. The occurrence of low DO and high bacterial populations concomitantly was also true in test 2.

The results of test 2 clearly showed the influence of mosquito larval presence in high numbers on bacterial abundance in water enriched with organic matter. Bacterial densities in enriched water devoid of mosquito larvae as well as with larvae and treated with B.t.i. granular formulation progressively declined to low numbers at the end of the test. However, the natural decline of bacterial abundance from peak populations in
Figure 5. Mean number (CFU x 10^3/ml) of spore-forming bacteria including *B.t.i.* (A), and *B.t.i.* spores (B) present in water enriched with organic matter and treated with VectoBac G formulation. *Unshared letters indicate significant differences among control and treatments by 1-factor ANOVA (Scheffé F test) for repeated measurements at the 0.05 level.
Figure 6. Mosquito oviposition (A), total larvae (B), and late instar larvae (C) developing in water of tubs enriched with organic matter and treated with VectoBac G formulation. *Unshared letters indicate significant differences among control and treatments by 1-factor ANOVA (Scheffé F test) for repeated measurements at the 0.05 level.
enriched water without mosquito larvae was much lower than in water with larvae, which was brought about by the feeding activity of mosquito larvae. The marked decline in bacterial numbers in the top and bottom water with larvae was noted on day 7 post-flooding, with the magnitude of decline higher in the top water than in the bottom water. This difference in bacterial declines is probably due in part to more larval feeding activity in surface water than in the bottom water. The influence of mosquito abundance on bacterial populations was further evidenced by the greater reduction in bacterial densities in untreated tubs as compared with the treated tubs, where B.t.i. treatments caused significant reductions in larval numbers.

The treatment with the B.t.i. formulation contributed very little to the total bacterial population. This was because of the application of relatively small quantities of the product necessary for the control of mosquito larvae. However, B.t.i. spores contributed significantly after treatment to the population of spore-forming bacteria, which prevailed in very low numbers as compared with the total bacterial population. The rapid decline in B.t.i. spore numbers in treated tubs after treatment could be the result of larval feeding activity and settling-out of bacterial spores.

There probably are other factors which influence the population trends of bacteria in microcosms enriched with organic matter. Bacterial densities and growth could be affected by weather, background bacterial populations in incoming water and enrichment substrates, and availability of organic matter. These factors could likewise influence mosquito larval abundance and survival. We have noted that the decline in bacterial numbers was much faster and greater in the presence of larvae than the natural bacterial decline in the tubs without larval breeding. In the absence of larvae, the declines in bacterial numbers in the top and bottom water were quite similar. However, the decline in bacterial numbers in the top water layer was greater than that in the bottom water in the presence of larvae. Therefore, it is evident that mosquito larvae play an important role in the decline of bacterial populations by their feeding activity.

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ABSTRACT: When late 3rd or early 4th-instar larvae of Culex tarsalis Coquillett and Culex quinquefasciatus Say mosquitoes were treated with sublethal dosages of neem insecticide until pupation, the blood-feeding activity of the resulting adults was essentially the same as that of untreated controls. In contrast, blood-feeding activity was suppressed when newly emerged adults were fed continuously on 10 parts per million (ppm) or 50 ppm azadirachtin (AZ) in 10% sucrose solution for seven days. Fecundity was also reduced by the various neem treatments. When late 3rd or early 4th-instar larvae were treated with 0.010 ppm AZ to pupation, the resultant females had a lower rate of oviposition than did the untreated controls after a full blood meal. When late instar larvae were treated at 0.005 ppm and 0.010 ppm AZ, the resultant females produced smaller egg rafts after a full blood meal, as compared to the controls, but egg viability was not affected. In newly emerged adults feeding continuously on 10 ppm and 50 ppm AZ in 10% sucrose for seven days (before blood feeding), the oviposition rate, size of egg raft, and hatching rate of the eggs after a full blood meal were all reduced. When newly blood-fed adults were fed continuously on 10 ppm and 50 ppm AZ in 10% sucrose for five days, their oviposition rate was lower than controls in most cases, but the egg raft size and viability of eggs were not affected. In freshly blood-fed females topically treated with AZ with 1 or 5 μg/female, the oviposition rate and size of egg rafts were generally reduced. The females receiving topical treatment laid eggs and their hatching was not affected. The longevity of adult females feeding continuously on 10 ppm and 50 ppm AZ in 10% sucrose solution after emergence was reduced, whereas, the longevity of males was only affected at the higher concentration.

INTRODUCTION

Due to environmental concerns and the development of resistance to synthetic chemical insecticides, researchers have focused attention on the development of alternative pesticides, such as biopesticides possessing a wider margin of safety to the environment and nontarget biota. As renewable resources, botanical components possessing pesticidal properties constitute a major category of biopesticides. Among these entities, the bioactive preparations from the neem tree (Azadirachta indica A. Juss) containing the tetrtrantriperenoid azadirachtin (AZ) have received much attention. Neem extracts exhibit multiple effects on various insect species, such as feeding deterrence, growth regulation, fecundity suppression, and even blockage of development of vector-borne pathogens (Schmutterer 1990, Ascher 1993, Mordue and Blackwell 1993, Mull and Su 1999). With regard to mosquitoes, it has been known that at least 344 species of plants including the neem tree are known to contain bioactive materials that show some mosquitocidal activity (Sukumar et al. 1991). Neem products serve primarily as larvicides against mosquitoes (Su and Mull 1998a, Mull and Su 1999), even though technical AZ and experimental formulations of AZ have also shown ovicidal capability (Su and Mull 1998b), and crude preparations of neem seed or technical AZ exerted effects on reproductive events of adult mosquitoes (Dhar et al. 1996, Ludlum and Sieber 1988). Because neem products adversely affect different systems of test insects at certain dosages (Schmutterer 1990, Ascher 1993, Mordue and Blackwell 1993), it is likely that adult mosquitoes might be equally affected by exposure to neem products. As part of our ongoing...
research on the activity and biological effects of neem products against mosquitoes, the current study was initiated to elucidate the impact of experimental neem formulations on blood feeding, fecundity, and survivorship of adult Culex tarsalis Coquillett and Cx. quinquefasciatus Say. We examined delayed biological effects of neem products on adults resulting from treatment of larvae at sublethal concentrations as well as adults treated directly with neem products either per os or via topical applications.

MATERIALS AND METHODS

Mosquito Culture

The test species were Cx. tarsalis and Cx. quinquefasciatus. Larvae were reared in two separate culture rooms at 26 ± 1°C, 35-45% RH and a 16:8 (L:D) hour photoperiod with 1 hour dawn and 1 hour dusk. To maintain the colony, 4 to 5 egg rafts were placed in an enamel pan (40 x 24 x 6 cm) containing 3 liters distilled water. Rabbit pellets (Brookhurst Mill, Riverside, CA., crude protein ≥17%) were added as larval food to the rearing pans at the rate of 5 g/pan when the egg rafts were set up and then again when the larvae reached the early 3rd-instar. Water was added to the pans every other day to replenish lost due to evaporation. Pupae were removed from the pans and placed in 240-ml waxed paper cups each containing 200 ml of distilled water and placed in screened cages (23 x 23 x 32 cm) where the adults emerged. Adults were provided continuously with 10% sucrose solution in a jar with a cotton wick. On day 5 postemergence, the adults were deprived of sugar feeding for 12 hours, then provided with restrained 1-week-old chicks overnight for blood feeding (Animal Use Protocol No. A-S 9901002-1, University of California, Riverside). The late 3rd or early 4th-instar larvae, newly emerged adults or newly blood-fed females were used for different experimental purposes.

Neem Products and Presuspensions

The test neem formulations were Azatin WP4.5 (WP) and Azatin EC4.5 (EC) (Thermotriology Corp., Columbia, MD, USA). Both formulations contained 4.5% of the active ingredient (AZ). Stock suspensions of the formulated products were prepared in distilled water at the concentrations of 10 ppm and 1,000 ppm AZ, which were used for larval treatment and adult treatment, respectively. For each test, stock suspensions were freshly prepared. All the tests were conducted in an insectary maintained at 26 ± 1°C, 35-45% RH and a 16:8 (L:D) hour photoperiod with 1 hour dawn and 1 hour dusk.

Effects on Blood Feeding

Delayed effects from larval treatment: In order to elucidate any delayed effects of the treatment with neem formulation applied to the larval stage on blood feeding of the resultant adults, about 1,000 late 3rd or early 4th-instar larvae from the colonies of two species were transferred to distilled water containing the sublethal dosages of 0.005 ppm and 0.010 ppm AZ of both formulations, which were made by adding 1 ml and 2 ml of the 10 ppm stock suspension to 2 liters water in an enamel pan, respectively. The larvae were provided with 5 g rabbit pellets as food in each pan, and exposed to the treatment until pupation. Controls consisted of late 3rd or early 4th-instar larvae from the same stocks as treated larvae in both test species were transferred to the same amount of distilled water in the same size pans, and fed on the same amount of rabbit pellets as in treated larvae. The pupae from treated and control pans were collected as needed. About 200-300 pupae were transferred to each of two 240-ml waxed paper cups containing 200 ml distilled water and placed in a screened cage. Newly emerged adults of both treatments and control were provided continuously with 10% sucrose solution. On day 5 postemergence, the adults were deprived of sugar feeding for 12 hours and offered a one-week-old chick overnight for blood. The following morning, the total number of blood fed and unfed females was counted and blood-feeding rate (%) was calculated as number of blood fed/total number of females multiplied by 100. Three cages of adults were used for each formulation at each AZ concentration against each test species. The differences in blood-feeding rate between control and treatment were determined by a Chi square test.

Adult treatment: About 200-300 pupae from the regular colonies of the two test species were put in a screened cage. Newly emerged adults were allowed to feed continuously on a 10% sucrose solution containing 10 ppm and 50 ppm AZ, which were made by adding 1 ml and 5 ml of 1,000 ppm AZ stock suspension to 99 ml and 95 ml distilled water held in a 200-ml plastic jar with a cotton wick. After seven days continuous feeding on the neem-treated sugar solution, the adults were used for blood-feeding tests. The control adults were from the same batch of pupae, but fed on 10% sucrose solution after emergence for the same period (7 days) without AZ. The adults of control and treatment were deprived of feeding on sugar solution or sugar solution containing neem for 12 hours before offered the host overnight for blood feeding. The same methods were used as in the previous larval treatment tests to determine blood-feeding rates. Three replicates were made in each test and a Chi square test was employed to determine...
significant differences in blood-feeding rates between control and treatments.

**Effects on Fecundity**

In order to explore the effects of various types of exposures in the larval stage or direct treatment of adults either orally or topically by neem products on fecundity of adult mosquitoes, such as oviposition rate, size of egg rafts, and viability of eggs, the mosquitoes were treated as follows.

*Delayed effects from larval treatment*: Approximately 1,000 late 3rd or early 4th-instar larvae were exposed to sublethal dosages of neem at 0.005 ppm and 0.010 ppm AZ until pupation. The resulting pupae were collected, 200-300 of which were placed in a screened cage. Adults emerging from these pupae were fed continuously on 10% sucrose solution for five days. They were deprived of sugar feeding for 12 hours, then given a chick overnight for blood feeding. The adults from the colonies of both species reared without exposures to neem were used as controls and handled in an identical manner. The fully blood-engorged females from controls and treatments were removed with an aspirator, where 20 females were placed in each of five cages and provided with 10% sucrose solution for five days. The gravid females were then provided with an oviposition cup containing 100 ml distilled water. The number of egg rafts and number of eggs in each raft were counted the following morning. The oviposition rate (%) was calculated from the total egg rafts collected from five cages (total of 100 females) and subjected to a Chi square test to explore the impact of neem treatments. The average number of eggs in an egg raft was also calculated and subjected to a z test to determine if there were any effects of neem treatment on the size of egg rafts. To ascertain hatching rates of eggs, five egg rafts selected at random from the egg raft collection were placed individually in 120-ml waxed paper cups with 100 ml distilled water. The hatching was assessed three days later and the larvae from each egg raft were counted. Hatching rate was calculated as the total number of larvae hatched/total number of eggs in five egg rafts observed, multiplied by 100. The hatching rates of controls and treatments were subjected to a Chi square test to evaluate the possible impact of neem treatments on egg viability.

*Adult/oral treatment postemergence (before blood feeding)*: About 200-300 pupae from the colonies of each species were placed in a screened cage and newly emerged adults were allowed to feed continuously for seven days either on a 10% sucrose solution (controls), or on a 10% sucrose solution containing 10 ppm or 50 ppm of AZ of each test formulation. The adults of control and treatment groups were deprived of feeding on sugar in controls or sugar and neem in treatments for 12 hours before blood feeding. After overnight blood feeding, 20 fully engorged females were removed to other cages and 10% sucrose solution was provided continuously. Five cages were set up for each control or treatment. The handling procedures for obtaining oviposition, egg raft, and individual egg counting and hatching, as well as the statistical methods used were the same as those in the previous test of “larval treatment.”

*Adult/oral treatment after blood meal*: About 200-300 pupae from the colonies of each species were placed in a screened cage and the newly emerged adults were allowed to feed continuously on 10% sucrose solution for five days. After starvation for 12 hours by removing the sugar cup from the cage, the adults were blood fed with a chick host overnight. After blood feeding, 20 fully blood-engorged females were aspirated into each cage, using five cages for each treatment and control. The blood-fed females were fed on 10% sucrose solution for five days as controls, and the other group fed on 10% sucrose solution containing 10 ppm or 50 ppm AZ for five days as treatments. On day 5 after blood feeding, gravid females were provided with oviposition cups. The handling procedures for oviposition setting, egg raft, and egg counting and hatching test, as well as statistical methods were the same as those in the previous test of “larval treatment.”

*Adult/topical treatment after blood meal*: About 200-300 pupae from each colony were placed in a cage, and newly emerged adults were fed continuously on 10% sucrose solution for five days. Before a host was provided for an overnight blood meal, the adults were deprived of sugar feeding for 12 hours. The following morning, the fully blood-fed ones were aspirated and immobilized with CO₂ and treated topically on the abdomen with 1 or 5 µg AZ/female using a 5-µl micropipette, in the form of 1 µl/female and 5 µl/female of the 1,000 ppm AZ suspension of the test formulations. After treatment, 20 females were transferred to each of five cages and provided with 10% sucrose solution for five days. Control adults were handled in the same manner but treated topically with equal volumes of distilled water. Five cages were set up for control and treatment in each case. On day 5 after blood feeding, gravid females were provided with oviposition cups. The handling procedures for oviposition setting, egg raft, and individual egg counting and hatching test, as well as statistical procedures were the same as those in the previous test of “larval treatment.”

**Effects on Adult Survivorship**

About 350-400 pupae from each colony of the test
species were collected in two 240-ml waxed paper cups, each containing 200 ml distilled water, and introduced into a screened cage for emergence. The newly emerged adults were allowed to feed continuously either on 10% sucrose solution (controls) or on sugar solution containing 10 ppm or 50 ppm AZ of the WP or the EC formulations (treatments). To insure the continuous availability of sugar solution or the sugar solution containing AZ, a quantity of 150 ml of the feeding solutions was placed in a 200-ml plastic cup with lid, through which a cotton wick was placed to absorb the liquid for feeding access of the adult mosquitoes. The feeding solution and cotton wick were replaced every three days with fresh preparations. The dead female and male mosquitoes were separately collected and counted every three days. The test was conducted until the last adult mosquito died. Three replicates were made in both control and each treatment. The cumulative mortality and lethal time for 50% mortality (LT$_{50}$) with 95% confidential limits were determined and analyzed using a probit regression line (Finney 1971, Abacus Concepts, Inc. 1987).

RESULTS

Effects on Blood Feeding

The neem exposure tests were carried out by exposing both larval and adult stages. When the late 3rd or early 4th-instar larvae of the test species were treated with sublethal dosages of AZ (0.005 and 0.010 ppm AZ in WP and EC formulations), the blood-feeding activity of the adults was not affected, except in the case of Cx. tarsalis treated with the EC, where the blood-feeding rate in the treatment group was significantly lower than that of the control (Fig. 1). In another experiment where the newly emerged adults were fed on 10 ppm and 50 ppm AZ for seven days, the blood-feeding activity in resulting females was adversely affected, showing a significant reduction in feeding rate compared with the controls, regardless of species and formulations. Suppression of blood-feeding activity was dose-dependent in both species, with greater suppression occurring in the higher dose (50 ppm AZ) treatment (Fig. 1).

Effects on Fecundity

Oviposition rate: In the initial tests, low dosages of AZ were applied in larval and adult treatments. When the late 3rd or early 4th-instar larvae were treated by 0.005 ppm AZ of two neem formulations, the oviposition of resulting adults of both test species after a full blood meal was comparable to the controls. In adults treated orally, when the cohorts (both females and males) were fed continuously on 10 ppm AZ in 10% sucrose solution for seven days, the oviposition rate in treated females was significantly reduced after a full blood meal. Another group of adults was treated po after ingestion of a full blood meal with 10 ppm AZ in 10% sucrose solution for five days. In these tests, only Cx. quinquefasciatus exhibited lower oviposition rate than the controls. Topical application of AZ at 1 μg/female on the abdomen of newly blood-engorged females also significantly reduced oviposition rate with both neem formulations in both species (Fig. 2).

In further tests, a doubled AZ concentration (0.010 ppm) was applied in larval treatment. In adult feeding tests, either postemergence for seven days or after blood feeding for five days, five times the AZ concentrations (50 ppm) were added to 10% sucrose solution. In topical application tests, the dosage was increased to 5 μg/ female. In these tests using high doses, the oviposition rate was significantly reduced in all tests, indicating a dose-dependent action (Fig. 2).

Average size of egg rafts: The number of eggs in all the egg rafts collected from the above oviposition tests was counted and the averages calculated and analyzed. In the tests using low AZ concentration, the fully engorged females resulting from larval treatment using 0.005 ppm AZ produced significantly smaller egg rafts than the controls. In adult treatment tests, when the cohorts were allowed to feed on 10 ppm AZ for seven days postemergence before blood feeding, fully engorged females also laid significantly smaller egg rafts than did controls. The treatment of females by feeding on AZ after blood feeding, however, did not affect egg raft size. As to the post-blood feeding treatment by topical application at 1 μg/female on the abdomen, only the treatments with the EC formulation caused a reduction in egg raft size in both test species (Fig. 3).

In further tests, a doubled dosage of AZ (0.010 ppm) was used for larval treatment and the high concentration of 50 ppm of AZ was used for adult treatment by feeding (postemergence or after ingestion of blood meal) and 5 μg/female for topical treatment. All these treatments, except AZ feeding after ingestion of blood meal, reduced the average egg raft size (Fig. 3).

Hatching of egg rafts: Five egg rafts were selected at random from the egg raft collection in the tests for oviposition rate, and transferred individually to 100 ml distilled water in a 120-ml waxed paper cup for hatching. In the low dosage treatments, i.e., 0.005 ppm AZ for larval treatment, 10 ppm AZ for adult feeding postemergence or after ingestion of blood meal, and 1 μg/female for topical application, only the egg rafts laid by the females fed on AZ for seven days after emergence.
Figure 1. Blood-feeding rates of mosquitoes treated with neem formulations Azatin WP4.5 or Azatin EC4.5. Upper: Late 3rd or early 4th instar larvae were treated at 0.005 ppm and 0.010 ppm AZ until pupation; Lower: Newly emerged adults were treated by feeding on 10 ppm and 50 ppm AZ in 10% sucrose for seven days after emergence. *Indicates a significant difference between control and treatment in each comparison by Chi square test at the 0.05 level.
Figure 2. Oviposition rate of mosquitoes treated with neem formulations Azatin WP4.5 or Azatin EC4.5. Upper: L-late 3rd or early 4th-instar larvae treated at 0.005 ppm until pupation; O/PE-oral, postemergence for seven days, at 10 ppm; O/PBF-oral, post blood feeding for five days, at 10 ppm; T/PBF-topical, post blood feeding at 1 µg/female. Lower: L-late 3rd or early 4th-instar larvae treated at 0.010 ppm till pupation; O/PE-oral, postemergence for seven days, at 50 ppm; O/PBF-oral, post blood feeding for five days, at 50 ppm; T/PBF-topical, post blood feeding at 5 µg/female. *Indicates a significant difference between control and treatment in each comparison by Chi square test at the 0.05 level.
Figure 3. Size of the egg rafts laid by mosquitoes treated with neem formulations Azatin WP4.5 or Azatin EC4.5. Upper: L-late 3rd or early 4th instar larvae treated at 0.005 ppm until pupation; O/PE-oral, postemergence for seven days, at 10 ppm; O/PBF-oral, post blood feeding for five days, at 10 ppm; T/PBF-topical, post blood feeding at 1 μg/female. Lower: L-late 3rd or early 4th instar larvae treated at 0.010 ppm until pupation; O/PE-oral, postemergence for seven days, at 50 ppm; O/PBF-oral, post blood feeding for five days, at 50 ppm; T/PBF-topical, post blood feeding at 5 μg/female. * Indicates a significant difference between control and treatment in each comparison by t test at the 0.05 level.
(before blood feeding) exhibited a significantly lower hatching rate than the control, regardless of species and formulations. All other treatments failed to reduce the hatching of the resultant eggs laid by treated females, with the exception of *Cx. tarsalis* treated topically with the EC (Fig. 4).

In further tests, the high doses of AZ, i.e., 0.010 ppm AZ for larval treatment, and 50 ppm AZ for adult feeding (postemergence or after ingestion of blood meal) and 5 μg/female for topical application were used. The treated females feeding on neem postemergence for seven days laid eggs with lowered viability. The eggs laid by the females treated topically by AZ after ingestion of a blood meal also showed lower viability than controls, except in the case of *Cx. tarsalis* treated with the WP. Other treatment regimens did not have any adverse effects on egg viability (Fig. 4).

**Effects on Survivorship**

*Cumulative mortality*: In each case, the mortality of males was higher than that of females. Feeding on both the WP and EC formulations in 10% sucrose solutions increased the mortality of both females and males. This effect was more obvious when they were fed on 50 ppm AZ than on 10 ppm AZ (Figs. 5, 6).

*Lethal time for 50% mortality* (LT₅₀): The probit analysis of the mortality data of the females and males feeding on neem formulations indicated that 10 ppm AZ alone reduced the longevity of females, while 50 ppm AZ was detrimental to the survivorship of both females and males (Fig. 7).

**DISCUSSION**

In the current study, the commercial formulations developed for agricultural pest control were employed to investigate effects on blood feeding, fecundity, and survivorship of adult mosquitoes, *Cx. tarsalis* and *Cx. quinquefasciatus*. In most cases, larval treatment using sublethal doses of AZ did not have any delayed effects on blood feeding of the adult females. The blood-feeding activity of both test species, however, was impaired when the newly emerged cohorts were allowed to feed continuously on 10 ppm and 50 ppm AZ of both neem formulations. It is well known that the initiation of blood-feeding behavior in mosquitoes is hormonally modulated. Juvenile hormone (JH) III secreted by corpora allata in *Culex* mosquitoes is responsible for the initiation of blood-feeding behavior after emergence or after oviposition (Meola and Readio 1988). In other insect species, one of the modes of action of AZ is to reduce hemolymph JH titer by inhibiting the release of allatotropins from the brain-corpus cardiacum complex (Mordue and Blackwell 1993, Ascher 1993, Rembold 1995). If this is also the case in mosquitoes, it may account for the negative impact of AZ in the initiation process of blood feeding in test species.

As an extended effect of larval treatment, the resulting adults exhibited a lower oviposition rate after a complete blood meal when using 0.010 ppm AZ for larval treatment. The resultant females laid smaller egg rafts at both 0.005 ppm and 0.010 ppm AZ in larval treatments. Treatment of larvae with neem usually yielded less fit adults with small body size observed qualitatively, which showed a lower reproduction capacity (Takken et al. 1998).

The fecundity of females was reduced by various AZ treatment regimens applied directly to the adult stage. In particular, continuous feeding on 10 ppm and 50 ppm AZ in neem products in 10% sucrose for seven days after emergence (before blood feeding), significantly reduced oviposition rate and size of egg rafts after a full blood meal. The viability of eggs resulting from this treatment was also reduced. This phenomenon was seen for both neem formulations and test species. From the previous studies, as mentioned earlier, the dominant mode of action of AZ against target insects is to reduce hemolymph JH titers (Mordue and Blackwell 1993, Ascher 1993, Rembold 1995). It has been well known that in mosquitoes, JH stimulates previtellogenic development of the follicles (Meola and Readio 1988, Klodwen 1997). We suggested that ingestion of AZ after emergence prior to blood feeding inhibits the previtellogenic development of the ovaries by reducing JH titers, which could be one of the reasons for reduced fecundity. The vitellogenic stage of follicle development (see below) may also be affected by the residual effect of ingested AZ before blood feeding. As to the reduced viability of the eggs laid by the females feeding on AZ, three factors could be involved. First, JH mediates the development of female receptivity to males by acting on the terminal abdominal ganglion that regulates mating behavior (Klodwen 1997). If it is also the case in our test species, ingested AZ might reduce female receptivity to males through suppressed JH production. Secondly, from the available evidence in other insect species, response to sexual pheromones (Dorn et al. 1987), spermatogenesis in males (Shimizu 1988), and transmission of spermatozoa to the females (Schmutterer and Rembold 1995) can all be affected by the AZ treatment. We assume that continuous ingestion of AZ after emergence until blood feeding could have some subtle effects on mating and the insemination process. Finally, the low hatching rate of the eggs laid by treated females was partially related to the interference of applied AZ with both previtellogenic and vitellogenic...
Figure 4. Hatching rate of eggs laid by mosquitoes treated with neem formulations Azatin WP4.5 or Azatin EC4.5. Upper: L-late 3rd or early 4th-instar larva treated at 0.005 ppm until pupation; O/PE-oral, postemergence for seven days, at 10 ppm; O/PBF-oral, post blood feeding for five days, at 10 ppm; T/PBF-topical, post blood feeding at 1 µg/female. Lower: L-late 3rd or early 4th-instar larva treated at 0.010 ppm until pupation; O/PE-oral, postemergence for seven days, at 50 ppm; O/PBF-oral, post blood feeding for five days, at 50 ppm; T/PBF-topical, post blood feeding at 5 µg/female. *Indicates a significant difference between control and treatment in each comparison by Chi square test at the 0.05 level.
Figure 5. Cumulative mortality of newly emerged adult mosquitoes feeding on 10 ppm AZ of Azatin WP4.5 or Azatin EC 4.5 in 10% sucrose solution.
Figure 6 Cumulative mortality of newly emerged adult mosquitoes fed on 50 ppm AZ of Azatin WP4.5 or Azatin EC4.5 in 10% sucrose solution.
Figure 7. Lethal time for 50% mortality (LT_{50}) with 95% confidence limits of mosquitoes feeding on 10 ppm (upper) and 50 ppm (lower) AZ of Azatin WP4.5 or Azatin EC4.5 in 10% sucrose solution after emergence. *Indicates a significant difference in LT_{50} between control and treatment in each comparison by probit analysis at the 0.05 level.
ovary development, yielding eggs with low viability (see below).

In most cases of AZ exposures after blood feeding, either orally for five days or topically at 1 and 5 μg/ female application, the parameters of oviposition rate and average size of egg raft were affected. Hatching rate of the eggs was reduced in some cases of topical treatment. In these treatments, the females have undergone the previtellogenic stage in ovarian development before feeding. Therefore, lower fecundities were the results of partially impaired vitellogenic process due to AZ. In anautogenous mosquitoes, vitellogenesis is initiated after blood feeding under the control of endocrine system, where ecdysone is secreted by ovaries stimulates the fat body to synthesize vitellogenin using the nutrients from blood meal digestion (Klowden 1997). Some studies indicated that JH may also participate in regulation of vitellogenin synthesis in conjunction with ecdysone (Kelly et al. 1987). JH increases the competence of follicles to ecdysosteroidogenic hormone (OEH) to synthesize ecdysone (Shapiro and Hagedorn 1982), and competence of the fat body to ecdysone to synthesize vitellogenin (Flanagan et al. 1989). Reduced fecundity in females feeding on or treated topically with AZ after blood feeding could be partially attributable to the negative effects of AZ on vitellogenesis through interference with hormone system. Circumstantial evidence is available in other insects, where the mode of action of AZ includes a reduction of hemolymph JH and ecdysteroid hormone titers by inhibiting the release of allatotropins and prothoracotrophic hormone (PTTH) from the brain-corpus cardiaca complex (Mordue and Blackwell 1993, Ascher 1993, Rembold 1995). In most cases of topical treatment using 5 μg/female after a blood meal, the eggs laid by treated females exhibited lower hatching than the controls, which implied that poor viability of the eggs was related to the detrimental effect of AZ on follicle development. Because no neem exposures occurred before blood feeding, the supposed interference of AZ with mating and/or insemination could be excluded.

In some comparable studies conducted on insects of medical and veterinary importance, uptake of AZ through the cuticle or via ingestion negatively affected reproductive events of the adults (Mulla and Su 1999). In mosquitoes, the gonotrophic events of female Anopheles stephensi and An. culicifacies were impaired by neem exposures (Dhar et al. 1996). In Ae. aegypti, significant transient retardation of oocyte growth was observed for up to 72 hours after feeding on 100 ng AZ/μl blood meal. In a decapitation test, immature oocytes were observed in 86% of AZ-fed females (100 ng/μl blood meal) decapitated 10 hours after a blood meal, whereas, 96% of decapitated control females contained maturing oocytes (Ludlum and Sieber 1988). The latter studies suggested that AZ delayed the release of one or more factors from the brain that regulates oogenesis. Our findings and interpretations in the current investigation agree with this study. For explanation of the observed negative impact of various neem exposures on blood feeding and fecundity in this study, general metabolic disturbance by applied AZ could be another reason, in addition to assumed endocrine disturbance.

AZ has detrimental effects on a variety of tissues and cells, especially those with rapid mitosis, e.g., epidermal cells, midgut epithelial cells, ovary and testis etc., which results in an overall toxic syndrome after exposure. Biological fitness of many insect species is substantially reduced even at neem concentrations and doses below those interfering with the molting process. Changes in biological fitness in test insects can be manifested as reduced lifespan (Wilps 1989), high mortality (Dorn et al. 1987), loss of flying ability (Wilps 1989), low absorption of nutrients (Wilps 1989), immunodepression (Azambuja et al. 1991), and enzyme inhibition (Naqvi 1987). In the current study, continuous AZ feeding after emergence increased mortality and reduced longevity, which implied an extensive impact of AZ treatment on the target species. It seemed that females were more vulnerable than males to AZ treatment.

When neem products are used as larvicides against mosquitoes, delayed effects of larval treatment on reproductive capacity in resultant adults may occur. To date a number of neem products have been manufactured, commercialized, and registered to control pests of ornamentals, landscape plants, and food crops (Immaraju 1998). Natural mosquito populations will have the chance to ingest and contact neem constituents during nectar feeding or resting on treated foliage. This exposure could have marked effects on blood feeding, fecundity, and survivorship of the adult mosquitoes. Neem products also have the potential to be used as larvicides against mosquitoes. As established in our current study, various modes of contact and exposure induce a variety of behavioral and biological effects in mosquitoes.

Acknowledgments

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REFERENCES CITED


Compatibility of *Bacillus thuringiensis* serovar *israelensis* and Chemical Insecticides for the Control of *Aedes* Mosquitoes

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**ABSTRACT:** The compatibility of the commercial aqueous *Bacillus thuringiensis* serovar *israelensis* (*B.t.i.*) formulation, Vectobac 12AS®, with the chemical insecticides Actellic 50EC®, Aqua Resigen®, Resigen®, and Fendona SC®, for the simultaneous control of *Aedes* larvae and adults was studied by dispersing nine different formulations using a portable mist blower, in single story half-brick houses. The effectiveness of the treatment was evaluated by measuring the larval mortality, adult mortality, and droplet analysis at varying distances from the sprayer. Persistence of the larvicidal activity of the chemical insecticides and *B.t.i.* was also determined by measuring the larval mortality in the test samples 7 days posttreatment. The sprayed particles in all the trials were 50-60μm in size, indicating that the particles were those of mist spray. Test samples placed within 3 m from the sprayer gave the maximum larval and adult mortality. Chemical insecticides exhibited maximum larval mortality in the 1 h posttreatment test samples and it was comparable to the larvicidal activity of *B.t.i.* The larvicidal toxins of *B.t.i.* were more stable and were able to affect sufficient larval mortality for 7 days posttreatment. The larvicidal activity of the mixtures, i.e., chemical insecticides with *B.t.i.*, in the 1 h posttreatment test samples was not significantly different from the larvicidal activity of the chemical insecticides and it was comparable to the larvicidal activity of *B.t.i.* alone. However, the larvicidal activity of the mixtures was significantly more than the chemical insecticides alone in the 7 days posttreatment test samples except for the Actellic 50EC® and Vectobac 12AS® mixture. In all the trials, with or without *B.t.i.*, there was no significant difference in adult mortality, indicating that this *B.t.i.* formulation, Vectobac 12AS®, was not antagonistic to the adulticidal activity of the chemical insecticides. From this study, it can be concluded that chemical insecticides can be used effectively for both adult and larval control, but the chemical insecticides do not exhibit residual larvicidal activity. Hence, for an effective control of both *Aedes* larvae and adults, it is advisable to add *B.t.i.* to the chemical insecticides, as *B.t.i.* is specifically larvicidal and is also able to affect extended residual larvicidal activity.

**Keyword Index:** *Aedes*, control, *Bacillus thuringiensis* serovar *israelensis*, chemical insecticides.

**INTRODUCTION**

In Malaysia, dengue fever (DF) and dengue haemorrhagic fever (DHF) continue to pose a serious public health problem. The two common vector species are *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse), which are largely indoor and outdoor container breeders, respectively. Attempts to control dengue currently include anti-larval and anti-adult activities together with health education and community participation. Operations against adult mosquitoes involve treatment with malathion of outbreak areas within a radius of 1 km from where a suspected dengue case is reported. The chemical is dispersed by either ultra-low-volume (ULV) or thermal fogging, and repeat spraying is carried out 7-10 days after the first treatment. Residents in the affected areas are also encouraged to apply Abate® (temephos) in all water-storing containers as larvicides (Tham 1997).

There has been an increase in the number of reported dengue cases since 1989, with 24,681 reported cases in 1998 (Tham 1998). The dengue problem has been perpetuated due to inadequate public compliance towards prevention and control of *Aedes* breeding sites and the lack of manpower to conduct a complete inspection of the breeding sites.
An effective and efficient control of *Aedes* mosquitoes can be achieved if the health authorities are able to implement a system that can control both adults and larvae, simultaneously in the same operation. *Bacillus thuringiensis* serovar *israelensis* (*B. t.i*) is an effective microbial control agent against mosquito larvae that is highly specific and toxic to mosquito larvae on ingestion. An effective usage of *B. t.i* in a mosquito vector control system would be in combination with chemical insecticides that are being predominantly used as adulticides. This paper reports on the compatibility of dispersing *B. t.i* simultaneously with chemical insecticides for the control of both *Aedes* larvae and adults.

**MATERIALS AND METHODS**

The field studies were conducted in three unoccupied single story half-brick houses. Each house was 4.5 m × 10.6 m × 3.0 m (width × length × height) in size. The area and volume of each house was 47.7 m² and 143.1 m³, respectively. To determine the effectiveness of the treatment in relation to the distance from the sprayer, each house was divided into three sections: 3 m, 6 m, and 9 m from the sprayer.

**Insecticides**

The trials were conducted using the following insecticides: a commercial aqueous suspension of *B. t.i* formulation, Vectobac 12AS® (Abbott Laboratories) containing 1200 ITU/mg against *Ae. aegypti* larvae; Actellic 50EC® (Zeneca, UK) containing pirimiphos-methyl 48.1% (w/w); AquaResigen® (AgrEvo Environmental Health) containing s-bioallethrin 0.14% (w/w), permethrin 10.26% (w/v), and piperonyl butoxide 9.79% (w/v); Resigen® (AgrEvo Environmental Health) containing s-bioallethrin 0.8% (w/w), permethrin 18.7% (w/w), and piperonyl butoxide 16.8% (w/w); and Fendona SC® (Cyanamid) containing alphacypermethrin 1.47% (w/w). The dosages of the insecticides used in the trials were based on the manufacturers' recommendations for ULV indoor application, i.e., Vectobac 12AS® - 500 ml/ha; Aqua Resigen® - 1:10 mixture, 100 ml mixture/2000 ml³; Resigen® - 1:10 mixture, 500 ml mixture/2000 ml³; Fendona SC® - 1:10 mixture, 1000 ml mixture/2000 ml³; and Actellic 50EC® - 500 ml/ha. The chemical tank of the sprayer requires a minimum volume of 2000 ml, so all formulations were prepared to a total volume of 2000 or 2200 ml. In each trial the spraying time (seconds/house) was determined, ensuring that the recommended dosage of the insecticides was achieved in each house.

**Spraying Trials**

A portable mist-blower, Mist Blower MD 300, Maruyama Mfg. Co., Inc.™ with ULV attachments was used to disperse the insecticides. The discharge rate of the insecticides was maintained at 60 ml per minute by setting the volume switch dial at 3. The throttle lever was maintained at 4 during spraying operations.

The spraying trials were conducted on nine different days, dispersing nine different formulations. The insecticides were mixed at the field site prior to their application. The operator of the sprayer stood at the front door of the house, pointing and rotating the nozzle towards its interior. The effectiveness of each trial was evaluated by measuring three different parameters: larval mortality, adult mortality, and ULV droplet analysis.

**Larval Mortality**

In trials 1-9 the larval mortality was measured by placing two sets of cups, containing 200 ml distilled water, in each section of the house. Both sets were collected an hour after spraying. To one set of the cups, 20 laboratory bred *Ae. aegypti* larvae (L3/L4) were added per cup. The larval mortality was scored 24 h and 48 h after exposure. The second set of the cups was left at ambient temperature (28-30°C) and relative humidity of about 85% for 7 days to determine the persistence of the insecticides in the test water 7 days posttreatment. On the 7th day, 20 laboratory bred *Ae. aegypti* larvae (L3/L4) were added into the cup. The larval mortality was scored 24 h and 48 h after exposure. Two additional cups per test containing distilled water were used in the laboratory as control replicates. The statistical significance of the larval mortality among trials was analyzed using a Student t-test.

**Adult Mortality**

In trial Nos. 2-9, the adult mortality was determined by placing cages of 25 sucrose-fed, < 7 days old, laboratory reared adult females of *Ae. aegypti* in each section of the house. For trial Nos. 8 and 9, an hour after spraying the treated mosquitoes were transferred into paper cups and fed with 10% sucrose solution in a cotton pad. Adult mortality was recorded 24 h posttreatment. Resigen®, Aqua Resigen®, and Fendona SC® are pyrethroids known to cause rapid knockdown of mosquitoes (Elliott et al. 1978). Therefore, for trial Nos. 2-7, knockdown of adult mosquitoes was recorded 5 min, 1 h, and 3 h post fogging. All the mosquitoes were then transferred into paper cups and were fed 10% sucrose solution in a cotton pad. Adult mortality was recorded 24 h post spraying.
Droplet Analysis

The distribution and size of sprayed particles were monitored through the use of magnesium oxide (MgO) coated slides. A slide was placed amidst the cups holding the larvae. Droplet diameter was measured for an average of 30 droplets for each MgO coated slide using a calibrated micrometer. The data were analyzed using the ULV droplet analysis program of Sofield and Kent (1984). In any spray the droplets are divided into two equal parts by volume. Droplets with large volume are represented by volume median diameter (vmd) while the small volume droplets are represented by number median diameter (nmd). A uniform size of droplets, when the ratio of vmd to nmd is near to 1, is preferred for an efficient spray (Mount 1985).

RESULTS

The droplet analysis and larval and adult mortality for each trial are expressed as the arithmetic mean of the samples from the treated sites at each distance. The larval and adult mortality results are shown together with the standard deviation for the mean in TABLES 1 and 2, respectively.

Trial 1: Commercial Aqueous B.t.i Formulation (Vectobac 12AS®)

The vmd and nmd of the sprayed particles was 57.9 ± 4.1 μm and 45.6 ± 2.1 μm, respectively, with a ratio of 1.3. Vectobac 12AS® achieved a 86.7-95.6% and 95.6-100% mortality for Ae. aegypti larvae in the 1 h post treatment samples for 24 h and 48 h exposure, respectively. A larval mortality of 100% was achieved in the 7 days posttreatment samples (24 h and 48 h exposure).

Trial 2: Aqua Resigen®

The vmd and nmd of the sprayed particles was 51.1 ± 5.4 μm and 32.2 ± 5.1 μm, respectively, with a ratio of 1.6. Aqua Resigen® achieved 100% larval mortality in 1 h posttreatment test samples placed within 3 m from the sprayer (24 and 48 h exposure). In the test samples placed beyond 3 m, 55-60% larval mortality was obtained on 24 h exposure and this was further reduced to 41.7-46.7% on 48 h exposure. This could be because Aqua Resigen®, as a pyrethroid, initially caused a rapid larval knockdown during the first 24 h exposure but was insufficient to cause larval mortality. Thus, the larvae recovered within the next 24 h, reducing the larval mortality (48 h exposure). In the 7 days posttreatment test samples, larval mortality was only observed in samples placed within 3 m from the sprayer. Larval mortality was not observed in samples placed beyond 3 m from the sprayer, thus, indicating the breakdown of sprayed Aqua Resigen® within 7 days of fogging. In comparison to trial 1, there is no significant difference in the larval mortality for 1 h posttreatment test samples (p > 0.05), but the larval mortality was significantly less in the 7 days posttreatment test samples (p < 0.05). In trial 2, complete adult mortality was observed in all the cages within 3 h post spraying.

Trial 3: Vectobac 12AS® and Aqua Resigen® Mixture

The vmd and nmd of the sprayed particles was 62.8 ± 7.0 μm and 48.2 ± 8.5 μm, respectively, with a ratio of 1.3. Vectobac 12AS®, and Aqua Resigen® mixture provided 100% larval mortality in 1 h post spray for test samples placed within 3 m from the sprayer (24 and 48 h exposure). In the test samples placed beyond 3 m, a larval mortality of 48.3-70.0% was obtained on 24 h exposure and this was further increased to 66.7-86.7% on 48 h exposure. In this trial the knockdown and recovery of larvae was not observed in the 1 h posttreatment samples as in trial 2. This could be due to the presence of B.t.i. toxins that are specifically larvicidal. As for the 7 days posttreatment samples, larval mortality was observed in all the test samples, 25.0-98.0% and 30.0-100.0% mortality on 24 and 48 h exposure, respectively. In comparison to trial 1, there is no significant difference in the larval mortality for 1 h posttreatment test samples (p > 0.05) but it was significantly less in the 7 days posttreatment test samples (p < 0.05). In comparison to trial 2, there is no significant difference in the larval mortality for 1 h posttreatment test samples (p > 0.05), but it was significantly more in the 7 days posttreatment test samples (p < 0.05).

In trial 3, complete adult mortality was observed in all the cages within 3 h of spraying, thus, indicating that Vectobac 12AS® does not have any adverse effects on the adulticidal activity of Aqua Resigen®.

Trial 4: Resigen®

The vmd and nmd of the sprayed particles was 58.1± 3.8 μm and 42.6 ± 3.5 μm, respectively, with a ratio of 1.4. Resigen® achieved 100% larval mortality in 1 h posttreatment test samples placed within 3 m from the sprayer (24 h and 48 h exposure). In test samples placed beyond 3 m, 41.7-70.0% larval mortality was obtained for 24 h exposure and this was further reduced to 40.0 - 65.0% on 48 h exposure. This could be for the same reasons as for the pyrethroid Aqua Resigen® formulation. As for the 7 days posttreatment, a very low larval mortality was observed in samples placed within 3 m from the sprayer. Larval mortality was not observed in samples placed beyond 3 m from the sprayer, indicating the breakdown of sprayed Resigen® within 7 days of
TABLE 1. Percent larval mortality expressed as the arithmetic mean of the samples from the treated sites at each distance.

<table>
<thead>
<tr>
<th>Distance From Sprayer (m)</th>
<th>1 Hour Post Spray</th>
<th>7 Days Post Spray</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Hours</td>
<td>48 Hours</td>
</tr>
<tr>
<td><strong>Trial 1: Insecticide Formulation: Vectobac 12AS®</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>95.6 ± 7.7</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>6</td>
<td>86.7 ± 17.6</td>
<td>95.6 ± 7.7</td>
</tr>
<tr>
<td>9</td>
<td>95.6 ± 7.7</td>
<td>100 ± 0</td>
</tr>
<tr>
<td><strong>Trial 2: Insecticide Formulation: Aqua Resigen®</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>6</td>
<td>60.0 ± 52.9</td>
<td>41.7 ± 52.0</td>
</tr>
<tr>
<td>9</td>
<td>55.0 ± 50.8</td>
<td>46.7 ± 50.3</td>
</tr>
<tr>
<td><strong>Trial 3: Insecticide Formulation: Aqua Resigen® + Vectobac 12AS®</strong></td>
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<tr>
<td>3</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>6</td>
<td>48.3 ± 20.2</td>
<td>66.7 ± 28.9</td>
</tr>
<tr>
<td>9</td>
<td>70.0 ± 5.0</td>
<td>86.7 ± 15.3</td>
</tr>
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<td><strong>Trial 4: Insecticide Formulation: Resigen®</strong></td>
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</tr>
<tr>
<td>3</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
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<tr>
<td>6</td>
<td>41.7 ± 28.9</td>
<td>40.0 ± 26.0</td>
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<td>9</td>
<td>70.0 ± 18.0</td>
<td>65.0 ± 13.2</td>
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<td><strong>Trial 5: Insecticide Formulation: Resigen® + Vectobac 12AS®</strong></td>
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<tr>
<td>3</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
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<tr>
<td>6</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
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<tr>
<td>9</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
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<tr>
<td><strong>Trial 6: Insecticide Formulation: Fendona SC®</strong></td>
<td></td>
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<tr>
<td>3</td>
<td>93.3 ± 11.6</td>
<td>96.7 ± 5.8</td>
</tr>
<tr>
<td>6</td>
<td>80.0 ± 17.3</td>
<td>90.0 ± 10.0</td>
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<td>9</td>
<td>91.7 ± 7.6</td>
<td>91.7 ± 14.4</td>
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<td><strong>Trial 7: Insecticide Formulation: Fendona SC® + Vectobac 12AS®</strong></td>
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<tr>
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<td>100 ± 0</td>
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<td>63.3 ± 12.6</td>
<td>83.3 ± 2.9</td>
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<td>70.0 ± 18.0</td>
<td>83.3 ± 20.8</td>
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<td><strong>Trial 8: Insecticide Formulation: Actellic 50EC®</strong></td>
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<td>6</td>
<td>48.3 ± 41.9</td>
<td>61.7 ± 53.9</td>
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<td>21.7 ± 18.9</td>
<td>30.0 ± 30.0</td>
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<td><strong>Trial 9: Insecticide Formulation: Actellic 50EC® + Vectobac 12AS®</strong></td>
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<td>100 ± 0</td>
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<td>6</td>
<td>68.3 ± 7.6</td>
<td>91.7 ± 7.6</td>
</tr>
<tr>
<td>9</td>
<td>56.7 ± 15.3</td>
<td>90.0 ± 17.3</td>
</tr>
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TABLE 2 Percent adult mortality expressed as the arithmetic mean of the samples from the treated sites at each distance.

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<tr>
<th>Distance from Sprayer (m)</th>
<th>5 Minutes Post Spray</th>
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<td>17.8 ± 20.4</td>
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<tr>
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<td>80.0 ± 24.1</td>
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<td>9</td>
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<td>93.3 ± 6.7</td>
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<td>Trial 3: Insecticide Formulation: Aqua Resigen* + Vectobac 12AS*</td>
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<td>57.2 ± 40.1</td>
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<td>88.7 ± 10.6</td>
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<td>31.0 ± 15.7</td>
<td>86.5 ± 13.6</td>
<td>100 ± 0</td>
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<tr>
<td>Trial 7: Insecticide Formulation: Fendona SC* + Vectobac 12 AS*</td>
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<td>0</td>
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<td>Trial 8: Insecticide Formulation: Actellic 50EC*</td>
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<td>24 Hours Post Spray</td>
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<td>3</td>
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<td>6</td>
<td>89.7 ± 10.5</td>
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<td>85.7 ± 12.7</td>
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<tr>
<td>9</td>
<td>73.3 ± 24.1</td>
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<td>67.0 ± 21.7</td>
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</table>
spraying. In comparison to trial 1, there was no significant difference in the larval mortality for 1 h posttreatment test samples \((p > 0.05)\), but the larval mortality was significantly less in the 7 days posttreatment test samples \((p < 0.05)\). In this trial 100% adult mortality was observed in all the cages within 3 h of spraying.

**Trial 5: Vectobac 12AS® and Resigen® Mixture**

The vmd and nmd of the sprayed particles was 58.3 ± 12.2 µm and 36.2 ± 17.1 µm, respectively, with a ratio of 1.6. Vectobac 12AS® and Resigen® mixture produced 100% larval mortality in 1 h posttreatment test samples placed within 9 m from the sprayer (24 h and 48 h exposure). In this trial the knockdown and recovery of larvae was not observed in the 1 h posttreatment samples as in trial 4, and this could be due to the presence of *B. t. i.* toxins, which are specifically larvicidal. As for the 7 days posttreatment test samples, larval mortality was observed in all the test samples, 67.7-95.0% and 88.3-100% mortality on 24 and 48 h exposure, respectively. In comparison to trial 1, there was no significant difference in the larval mortality at 1 h and 7 days posttreatment test samples \((p > 0.05)\). In comparison to trial 4, the larval mortality was significantly greater in the 1 h and 7 days posttreatment test samples \((p < 0.05)\).

In trial 5, 100% adult mortality was observed in all the cages within 1 h of posttreatment, thus, indicating that Vectobac 12AS® does not have any adverse effects on the adulticidal activity of Resigen®.

**Trial 6: Fendona SC®**

The vmd and nmd of the sprayed particles was 56.6 ± 1.2 µm and 43.8 ± 4.4 µm, respectively, with a ratio of 1.3. In the 1 h posttreatment test samples, Fendona SC® produced 80.0-93.3% and 90.0-96.7% larval mortality on 24 and 48 h exposure, respectively. This larval mortality was reduced in the 7 days posttreatment samples, thus indicating the breakdown of sprayed Fendona SC® within 7 days of spraying. In comparison to trial 1, there was no significant difference in the larval mortality for 1 h posttreatment test samples \((p > 0.05)\), but it was significantly less in the 7 days posttreatment test samples \((p < 0.05)\).

In trial 6, complete adult mortality was observed within 3 h of posttreatment, in the cages placed 3 m from the spraying machine. In cages placed beyond 3 m complete adult mortality was only observed within 24 h of posttreatment.

**Trial 7: Vectobac 12AS® and Fendona SC® Mixture**

The vmd and nmd of the sprayed particles was 55.4 ± 5.2 µm and 40.5 ± 1.1 µm, respectively, with a ratio of 1.4. Vectobac 12AS® and Fendona SC® mixture achieved 100% larval mortality in the 1 h posttreatment test samples placed within 3 m from the sprayer (24 and 48 h exposure). In the samples placed beyond 3 m a larval mortality of 63.3-70.0% and 83.3% was achieved for 24 h and 48 h exposure, respectively. In the 7 days posttreatment test samples, a larval mortality of 86.7-96.7% and 90.0-96.7% was observed for 24 and 48 h exposure, respectively. In comparison to trial 1, there is no significant difference in the larval mortality in 1 h and 7 days posttreatment \((p > 0.05)\). In comparison to trial 6, there is no significant difference in the larval mortality at 1 h posttreatment \((p > 0.05)\), but it was significantly more than at 7 days posttreatment \((p < 0.05)\).

In this trial a 100% adult mortality was observed in all the cages within 3 h of spraying, indicating that Vectobac 12AS® does not have any adverse effects on the adulticidal activity of Fendona SC®.

**Trial 8: Actellic 50EC®**

The vmd and nmd of the sprayed particles was 54.3 ± 6.4 µm and 42.5 ± 3.1 µm, respectively, with a ratio of 1.3. In the 1 h posttreatment test samples, Actellic 50EC® produced a 100% larval mortality in samples placed 3 m from the sprayer (24 h and 48 h exposure). However, in samples placed beyond 3 m it only achieved a 21.7-48.3% and 30.0-61.7% larval mortality on 24 and 48 h exposure, respectively. This larval mortality was further reduced in the 7 days posttreatment test samples, thus indicating the breakdown of Actellic 50EC® within 7 days of spraying. In comparison to trial 1, there is no significant difference in the larval mortality for 1 h posttreatment test samples \((p > 0.05)\), but it was significantly less in the 7 days posttreatment test samples \((p < 0.05)\).

In trial 8, complete adult mortality was observed within 24 h of posttreatment in the cages placed 3 m from the sprayer. In cages placed beyond 3 m, 73.3-89.7% adult mortality was observed.

**Trial 9: Vectobac 12AS® and Actellic 50EC® Mixture**

The vmd and nmd of the sprayed particles was 52.5 ± 5.2 µm and 32.3 ± 4.3 µm, respectively, with a ratio of 1.6. Vectobac 12AS® and Actellic 50EC® mixture produced 100% larval mortality in 1 h posttreatment samples placed within 3 m from the sprayer (24 and 48 h exposure). In the samples placed beyond 3 m, a larval mortality of 56.7-68.3% and 90.0-91.7% was achieved for 24 h and 48 h exposure, respectively. In the 7 days posttreatment samples, complete mortality was only observed in samples placed 3 m from the sprayer (24 h exposure).
and 48 h exposure). In the 7 days posttreatment samples that were placed beyond 3 m from the sprayer, the larval mortality was reduced by more than 50% in comparison to 1 h posttreatment samples. In comparison to trial 1, there is no significant difference in the larval mortality for 1 h posttreatment samples (p > 0.05), but it was significantly less in the 7 days posttreatment samples (p < 0.05). In comparison to trial 8 there is no significant difference in the larval mortality for 1 h and 7 days posttreatment test samples (p > 0.05).

In trial 9, complete adult mortality was observed within 24 h of posttreatment in the cages placed 3 m from the sprayer. In cages placed beyond 3 m, 67.0-85.7% adult mortality was observed.

DISCUSSION

The portable mist-blower, equipped with the ULV attachment, successfully dispersed the nine different formulations that were tested. All formulations flowed smoothly through the sprayer nozzle. The volume median diameter (vmd) of the sprayed particles for all the trials was 50-60 μm. This indicated that the particles were not of ULV spray but of mist spray (Matthews 1985). The number of sprayed particles decreased with increasing distance from the sprayer. Therefore, test samples placed within 3 m from the sprayer gave the maximum larval and adult mortality.

The chemical insecticides tested in this study are generally used as adulticides. However, this study has shown that these insecticides used at the manufacturer’s recommended dosage also exhibit mosquito larvicidal activity. The larvicidal activity of the insecticides in the 1 h posttreatment samples is comparable to the larvicidal activity of the commercial aqueous B.t.i. formulation, Vectobac 12AS®, as no significant difference was observed in the larval mortality (p > 0.05). However, the larvicidal activity of the chemical insecticides was significantly less than Vectobac 12AS® in the 7 days posttreatment test samples (p < 0.05). This could be due to the degradation of larvicidal activity in the chemical insecticides. The larvicidal toxins of Vectobac 12AS® are more stable and are able to effect sufficient larval mortality for a duration of 7 days posttreatment.

All the chemical insecticides mixed homogenously with Vectobac 12AS®, thus flowing smoothly without clogging the nozzle. The larvicidal activity of the mixtures in the 1 h posttreatment samples was not significantly different from the larvicidal activity of the chemical insecticides (p > 0.05). However, the larvicidal activity of the mixtures was significantly greater from the chemical insecticides in the 7 days post-spray test samples except for the Actellic 50EC® and Vectobac 12AS® mixture (p < 0.05). The significant difference in the larvicidal activity for the 7 days posttreatment samples could be due to the larvicidal activity of the chemical insecticides degrading over time, thus, effecting a far lesser larval mortality than the formulations mixed with Vectobac 12AS®. This also indicates that the larvicidal activity of the mixtures in the 7 days posttreatment samples was mainly contributed by Vectobac 12AS®.

In the Actellic 50EC® and Vectobac 12AS® mixture, the larvicidal activity of the Vectobac 12AS® was not exhibited as in other mixtures and this could be due to the antagonism of Actellic 50EC® to the larvicidal toxins of Vectobac 12AS®.

The larvicidal activity of the mixtures in the 1 h posttreatment samples was not significantly different from the larvicidal activity of Vectobac 12AS® (p > 0.05). However, it was significantly less in the 7 days posttreatment samples of the Aqua Resigen® and Vectobac 12AS® mixture and Actellic 50EC® and Vectobac 12AS® mixture (p < 0.05), suggesting that Aqua Resigen® and Actellic 50EC® might be antagonistic to the larvicidal toxins of Vectobac 12AS® in the mixtures. On the other hand, the larvicidal activity of Resigen® and Vectobac 12AS® mixture and Fendona SC® and Vectobac 12AS® mixture was comparable to the larvicidal toxins of Vectobac 12AS® in the 1 h and 7 days posttreatment test samples.

Overall, the Resigen® and Vectobac 12AS® mixture showed significantly better larvicidal activity than Aqua Resigen® and Vectobac 12AS®, Actellic 50EC® and Vectobac 12AS®, and Fendona SC® and Vectobac 12AS® mixtures in the 1 h posttreatment test samples (p < 0.05). This could be because the amount of active ingredient in Resigen® was 2.6-9.1 times greater than in Aqua Resigen®, Actellic 50EC®, and Fendona SC®.

In all the trials, with and without Vectobac 12AS®, there was no significant difference in adult mortality (p > 0.05). Thus, it can be concluded that Vectobac 12AS® is not antagonistic to the adulticidal activity of chemical insecticides. From this study it can be concluded that chemical insecticides Actellic 50EC®, Aqua Resigen®, Resigen®, and Fendona SC® can be used effectively for both adult and larval control. However, these chemical insecticides do not have significant residual larvicidal activity and are thus a disadvantage in Aedes control, as the second application in dengue endemic areas is carried out 7-10 days after the first spraying (Tham 1997). Within this period, Aedes larvae can emerge and
transmit dengue virus. Therefore, for simultaneous effective control of *Aedes* larvae and adults, it is thus advisable to add Vectobac 12AS® to the chemical insecticides, as it is specifically larvicidal and also able to give residual larvicidal activity for 7 days after spraying.

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Ticks of South Carolina (Acari: Ixodoidea)

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ABSTRACT: County and host records are reported for 19 species of ticks from South Carolina: Amblyomma americanum, Amblyomma maculatum, Amblyomma tuberculatum, Aponomma latum, Boophilus annulatus, Boophilus microplus, Dermacentor albipictus, Dermacentor variabilis, Haemaphysalis leporispalustris, Ixodes affinis, Ixodes brunneus, Ixodes cookei, Ixodes marxi, Ixodes minor, Ixodes scapularis, Ixodes texanus, Ixodes woodi, Rhipicephalus sanguineus, and Ornithodoros capensis. Ixodes woodi is recorded from South Carolina for the first time. Boophilus annulatus and Boophilus microplus probably no longer exist in South Carolina, and Aponomma latum is an exotic species that is not established in South Carolina. Brief notes follow each species.

Keyword Index: Ticks, South Carolina.

INTRODUCTION

Although several tick-borne diseases are endemic to South Carolina, the tick fauna of this state is not well documented. Rocky Mountain spotted fever (RMSF), caused by Rickettsia rickettsii, has been an important human infection in the state for many years. Cases of RMSF actually increased in the 1970s (Burgdorfer et al. 1975, Loving et al. 1978). More recently Lyme disease (LD) and ehrlichiosis (Eh) have been reported from South Carolina (Schuman and Caldwell 1989, Rumpel and Jones 1991, Hawkins 1995). Until recently, little attention has been given to systematic study of distribution, hosts, and ecology of these important arthropods in the state. Despite some recent work (Barton et al. 1992, Keirans et al. 1992, Tedders 1994, Clark et al. 1996, Durden et al. 1997, Clark et al. 1998) there remains a lacuna in the bionomics and distribution of ticks within the state. With the exception of Barton et al. (1992) all of the above mentioned work was conducted in the coastal and piedmont areas of South Carolina (Fig. 1). Much more data are required for surveillance and management of tick-borne diseases.

South Carolina is divided into five physiographic regions (Fig.1). 1) The Mountains are part of the Appalachian system and are only in the extreme northwest corner. 2) The Piedmont extends in a band 161 km wide between the Mountains and the Sandhills. 3) The Sandhills are a narrow northeast to southwest band of hilly sand topography situated between the Piedmont and the Coastal Plain. The Sandhills are thought to be either beaches left over from the Cretaceous period some 130 million years ago or due to erosion of the Appalachian Chain. 4) The Coastal Plain extends almost 200 km from the Sandhills to the Coastal Zone. 5) The Coastal Zone is a narrow band approximately 16 km in width in which the saline and brackish water from the Atlantic Ocean influence the vegetation types. This diverse area provides a variety of soil types, climate, vegetation, and habitats for animals. South Carolina is the southern-most limit for some northern animals and the northern-most limit for some southern animals (Thompson 1982).

Tick records were obtained from veterinarians and the Orangeburg County Society for the Prevention of Cruelty to Animals (SPCA) (mostly in Orangeburg County), our own collections, published literature, and collection records from the U.S. National Tick Collection housed at Georgia Southern University’s Institute of Arthropodology and Parasitology in Statesboro, Georgia. Tick species are listed alphabetically, followed by the county or counties in which they occurred, the host species, and the accession numbers of South Carolina specimens from the U.S. National Tick Collection (appended by the last 2 digits of the year of collection, all made during the 20th century). Finally, a summary of
Figure 1. Map of South Carolina with county borders and names and physiographic regions indicated.
published and personal observations about the species and, where appropriate, a mention of medical importance is included.

Wilson and Baker (1972) listed 25 tick species from Georgia; however, 4 of these were recorded early and are not considered residents at this time. Durden and Kollars (1992) listed 17 species from Tennessee. Although we have no list of ticks from North Carolina, Levine et al. (1989), while doing Lyme disease investigations, reported 6 species from the state.

Here we report 18 species of ixodid ticks and 1 argasid tick from South Carolina as well as the first record of *Ixodes woodi* from this state and the first record of *Amblyomma maculatum* taken from a coyote, *Canis latrans*, in the southeast US.

*Amblyomma americanum* (L.).

**COUNTY RECORDS:** Beaufort, Charleston, Florence, Georgetown, Hampton, Horry, Orangeburg.


**REMARKS:** The lone star tick is abundant along the coastal areas of South Carolina and extends at least as far west as Orangeburg County. It has also been taken from deer in Edgefield and McCormick County (Barton et al. 1992). The adults usually appear in April and disappear by July (Clark et al. 1996, Tedders 1994), although Clark et al. 1998 reports collection of adults from March to September with 1 male collected in January, 1995. One adult was taken from a deer in Georgetown County in November (Barton et al. 1992). These records are similar to observations of this tick in Georgia where adults appeared in March and disappeared by August (Davidson et al. 1994). Tedders collected nymphal *Amblyomma americanum* during every month of the year from Georgetown and Charleston Counties, both coastal counties. He collected larvae in all months except February, March, and December. Clark et al. (1998) collected nymphs from March through October and larvae in March and from August through November. Their collections were also concentrated in coastal counties.

*Amblyomma americanum* uses a variety of wild and domestic animals including birds as hosts. Although this is certainly true in South Carolina as well; surveys have concentrated on wild hosts and little information is available about infestations of domestic animals. Numerous adult and immature *A. americanum* have been observed on feral pigs by one of the authors (DW) at Hobcaw Barony, Georgetown County. Bishopp and Trembley (1945) report that wild turkeys on Bull's Island, Charleston County were observed to be heavily infested with larvae and nymphs of this species.

All stages will attack humans (Felz et al. 1996). Tedders (1994) indicated *A. americanum* as the tick most likely to be encountered by sportsmen, campers, and outdoor workers in the summer months in SC. The long mouthparts of *Amblyomma* spp. make this a difficult tick to remove once embedded. Even without breaking the mouth parts, a bite often results in a local infection or irritation. Bolte et al. (1970) stated "ticks in the genera *Amblyomma*, *Hyalomma*, and *Aponomma* penetrate host tissue during feeding to a greater depth than those of other genera. Sanders and Oliver (1995) evaluated *A. americanum*, *Dermacentor variabilis*, and *Ixodes scapularis* from Georgia as vectors of a Florida strain of *Borrelia burgdorferi*, the agent of Lyme disease. They found *A. americanum* and *D. variabilis* did not transmit this strain. On the other hand, *I. scapularis* was an efficient vector. In Georgia, Lockhart et al. (1995) noticed a temporal association between *A. americanum* and *Ehrlichia chaffeensis*, the agent of human monocytic ehrlichiosis. Loving et al. (1978) detected *R. rickettsii* in this tick in South Carolina. Burgdorfer et al. (1975) showed a higher *R. rickettsii* infection rate in this species in South Carolina (16.6%) as compared to *D. variabilis* (4.9%). *Amblyomma americanum* also is a competent vector of *Francisella tularensis*, the causative agent of tularemia (Hopla and Hopla 1994).

*Amblyomma maculatum* Koch.

**COUNTY RECORDS:** Allendale, Charleston, Chester, Georgetown, Jasper, Newberry, Orangeburg, Sumter.

**Aponomma polyphemus.**

**Amblyomma tuberculatum Marx.**

**Aponomma latum** (Koch).

**Boophilus annulatus** (Say).

**Boophilus microplus** (Canestrini)

**Dermacentor albipictus** (Packard).

**Dermacentor variabilis** (Say).

**REFERENCES:**

**REMARKS:** South Carolina is probably the northernmost extension of the range of the Gulf Coast tick. The adults appear in May and peak in June with a few specimens appearing until mid-October (Clark et al. 1998). This species appears throughout the coastal region of South Carolina and was the second most common species collected in a study in Orangeburg County (Clark et al. 1996). Larvae and nymphs commonly infest birds (Bishop and Trembley 1945). The adults are common on dogs and will readily attack man. Loving et al. (1978) reported *R. rickettsii* from this species in South Carolina.

**Amblyomma tuberculatum Marx.**

**COUNTY RECORD:** Jasper.

**HOST RECORD:** Testudines - *Gopherus polyphemus*.

**ACCESSION NUMBER:** 056913-33.

**REMARKS:** The gopher tortoise tick probably exists throughout the range of its host. In Georgia the larvae are found more frequently on mammals and birds than reptiles (Wilson and Baker 1972). Bishop and Trembley (1945) suggest that adults occur on the host throughout the year. We have one record from the U.S. National Tick Collection of *A. tuberculatum* taken from a gopher tortoise in South Carolina.

**Aponomma latum** (Koch).

**COUNTY RECORD:** Florence.

**HOST RECORD:** Squamata (pet python).

**ACCESSION NUMBER:** 120941-93.

**REMARKS:** The python tick is an exotic species occurring on African snakes and apparently is imported frequently into the U.S. Durden and Kollars (1992) reported this species from a single specimen in Tennessee and reported records from the U.S. National Tick Collection from Connecticut, Illinois, Indiana, New Hampshire, New York, Pennsylvania, and Texas. One female and 1 nymph were identified in this South Carolina record from a pet snake kept in Lake City, SC.

**Boophilus annulatus** (Say).

**COUNTY RECORD:** Georgetown.

**HOST RECORD:** Artiodactyla - Cattle.

**ACCESSION NUMBER:** 115851-11.

**REMARKS:** The cattle fever tick has been eradicated from the U.S. James and Harwood (1970) indicate that this species was the most economically important species in the southern U.S. We have only one record of this species from the U.S. National Tick Collection collected in Georgetown County.

**Boophilus microplus** (Canestrini)

**COUNTY RECORD:** Georgetown.

**HOST RECORD:** Artiodactyla - Cattle.

**ACCESSION NUMBER:** 057164-11.

**REMARKS:** We again have only one record of this tick from cattle in Georgetown County. Becklund (1968) reported that this tick is frequently imported into the U.S. on horses from Cuba and Guatemala as well as on refrigerated beef from Costa Rica and Honduras coming into Florida and on beef from Mexico coming into Ft. Worth, Texas. This species was also collected from cattle hides from Cuba. *Boophilus microplus* is a problem in Australia where it is known, under severe drought, to reproduce by parthenogenesis (Stone 1963). The South Carolina record is from a collection made in 1911.

**Dermacentor albipictus** (Packard).

**COUNTY RECORD:** Abbeville, Beaufort, Charleston, Chester, Chesterfield, Edgefield, Fairfield, Florence, Georgetown, Greenwood, Laurens, McCormick, Newberry, Saluda, Spartanburg, Union.

**HOST RECORD:** Artiodactyla - *Odocoileus virginianus*.

**ACCESSION NUMBER:** 060254-33, 057333-29, 060253-33, 060192-33.

**REFERENCE:** Barton et al. 1992.

**REMARKS:** The winter tick is a one host tick. The species is widespread throughout the U.S. Bishop and Trembley (1945) list horses, cattle, moose, and elk as hosts. Although it probably occurs on white-tailed deer all over the state, Barton et al. (1992) did not collect it on deer in the mountains of South Carolina. This could have been due to the time of year the deer were examined at deer check stations. *Ixodes scapularis* was collected frequently with *D. albipictus* at check stations.

**Dermacentor variabilis** (Say).

**COUNTY RECORDS:** Allendale, Barnwell, Charleston, Chester, Georgetown, Lexington, Oconee, Orangeburg, Richland, Saluda, Spartanburg, Sumter.

floridana, Oryzomys palustris, Peromyscus gossypinus, Peromyscus leucopus.


REMARKS: The American dog tick is considered the most important vector of R. rickettsii in the eastern U.S. (Bishop and Trembley 1945). Loving et al. (1978) studied RMSF in South Carolina. Their studies indicated D. variabilis was the predominant tick found in the Piedmont region, 98% D. variabilis to 2% of A. americanum. However, in the coastal region, collections of A. americanum were higher than those for D. variabilis (62% vs. 38%). Collections from the sandhills were about even for both species. Collections in Orangeburg County (Clark et al. 1996) consisted of 259 adult D. variabilis collected from dogs and 8 from humans, compared to 14 A. americanum during the same time. Amblyomma americanum nymphs were the only immatures taken during the study. Clark et al. 1998 found that Dermacentor variabilis adults usually appeared in early April and were almost all gone by September 1.

Bishop and Trembley (1945) list a large number of hosts for this tick. Lavander and Oliver (1996) list 12 host species from Bullock County, Georgia, and found year round activity. Durden and Kollars (1992) list 29 hosts from Tennessee. We have collected larvae of this species in January from a cotton mouse, Peromyscus gossypinus, in Barnwell County.

One of us (WW) observed a male dog at a veterinary clinic in Orangeburg, South Carolina, that was suffering from tick paralysis due to D. variabilis. Forty-seven D. variabilis were removed from the animal. It was given IV fluids and recovered after 2 days. Sanders and Oliver (1995) showed D. variabilis was unable to transmit a Florida strain of B. burgdorferi. McLean et al. 1985 isolated St. Louis encephalitis virus from adult D. variabilis. This tick is the principal vector in the eastern United States of R. rickettsii and is closely linked to RMSF epidemiology in this region (Felz and Durden, 1998).

Haemaphysalis leporispalustris (Packard).

COUNTY RECORDS: Charleston, Georgetown, McCormick.

HOST RECORDS: Lagomorpha - Sylvilagus aquaticus, Sylvilagus palustris. Avian - Colinus virginianus, Parus bicolor, Thryothorus ludovicianus, Pipilo erythropthalmus, Cardinalis cardinalis, Catharus ustulatus, Zonotrichia albicollis, Dendroica coronata.


REMARKS: This species’ common name is the rabbit tick. Adults are usually only found on rabbits, while immatures parasitize birds or rabbits. Durden et al. (1997) collected nymphs and larvae from several species of birds in coastal South Carolina. Although immatures will also parasitize small mammals, we have no records of this in South Carolina. Strickland et al. (1977) suggested this species played an important role in maintaining RMSF, Q fever, and tularemia in wild mammal populations. It is probably more widespread in South Carolina than records indicate.

Ixodes affinis Neumann.

COUNTY RECORDS: Berkeley, Charleston, Dorchester, Georgetown, Orangeburg, Sumter.


REMARKS: This species was originally reported from Central and South America. It has been, so far, restricted to the southeastern U.S. Gerrish and Ossorio (1965) originally reported it from South Carolina on deer in Dorchester County. Clark et al. (1998) isolated spirochetes that resembled Borrelia burgdorferi from I. affinis collected in the sandhills and coastal zone of South Carolina. They believe that I. affinis is well established in the coastal plain of South Carolina. Clark et al. (1996) collected 1 male from a dog in Orangeburg County. Oliver et al. (1987) list 11 hosts for I. affinis is Georgia.

Ixodes brunneus Koch.

COUNTY RECORDS: Charleston, Cherokee, Dorchester, Georgetown.

HOST RECORDS: Avian - Zonotrichia albicollis, sparrow (not identified to species), Pipilo sp., Toxostoma rufum, Turdus migratorius, Spizella passerina, Dumetella carolinensis, Hylocichla guttata, Lania ludovicianus, Thryothorus ludovicianus. Primate - Homo sapiens.

Ixodes

Ixodes rarely occurs in the Oconee species. The virginiiana tick over removed death will most 058174-30, 058172-31, 058171-31, 058170-24, 058165-30, 057696-33, 057695-31.

REMARKS: This tick is distributed throughout most of the U.S. and is confined to birds as hosts. There will usually only be 1 tick attached to the host, although if the tick is an adult female this is sufficient to cause the death of the host (Bishopp and Trembley 1945). One of the authors (DW) captured a nearly flightless white-throated sparrow, Zonotrichia albicollis, with an engorged female I. Brunneus below one eye. The bird recovered its ability to fly overnight after the tick was removed the previous afternoon. Rickettsia rickettsii has been recorded from this tick in nature (Clifford et al. 1969) raising the possibility of the spread of this pathogen over long distances via the bird host.

Ixodes cookei Packard

COUNTY RECORDS: Charleston, Oconee, Pickens, York.

HOST RECORDS: Marsupialia - Didelphis virginiana. Carnivora - Procyon lotor, Spilogale putorius, Mustella vison.

ACCESSION NUMBERS: 120580-91, 064950-33, 057237-38, 122784-98.


REMARKS: South Carolina records for I. cookei occur from the coast and the mountains and, therefore, it probably also occurs in the areas in between. Medium-sized mammals appear to be the main hosts for this species. Tedders (1994) found this tick to be relatively rare with 2 females and 16 nymphs collected from raccoons and 1 nymph from an opossum out of a total of 3,797 ticks collected during his study. Powasson virus, a serious human pathogen, has been isolated from this species in Canada and the northeast U.S. (Berge 1975). This tick deserves a closer examination as to its distribution and abundance in the southeast U.S.

Ixodes marxi Banks

COUNTY RECORD: Marion.

HOST RECORD: Rodentia - Scirurus carolinensis.


REMARKS: Two South Carolina records exist for this species and both were taken from gray squirrels. Squirrels appear to be a common host for I. marxi. It probably has a wide distribution in South Carolina. However, gray squirrels, although a game animal, are rarely trapped and examined by biologists here.

Ixodes minor Neumann

COUNTY RECORDS: Charleston, Georgetown, Oconee.


ACCESSION NUMBERS: 119984-90, 057696-33, 057632-33, 120050-91, 120004-90, 122313-95, 122312-95, 121717-94, 121641-94.


REMARKS: Georgia was thought to be the northern-most limit for I. minor. However, Tedders et al. (1992) reported this species from coastal South Carolina on roof rats. Ixodes minor was not thought to be a species of public health importance. Recently Durden et al. (1997) isolated B. burgdorferi from a large number of this species suggesting it could be an important enzootic vector of the agent of Lyme disease since it feeds on rodents and birds. Clark et al. (1998) collected I. minor from cotton rats, cotton mice, and eastern wood rats from July through December in Georgetown County, South Carolina. We have one record from a blue jay in Oconee County; because I. minor is a coastal tick, this specimen presumably was taken to this inland county by the bird after attaching to it in a coastal area.

Ixodes scapularis Say

COUNTY RECORDS: Abbeville, Aiken, Allendale, Anderson, Barnwell, Calhoun, Charleston, Chester, Chesterfield, Dorchester, Edgefield, Fairfield, Florence, Georgetown, Greenville, Greenwood, Hampton, Laurens, McCormick, Newberry, Pickens, Richland, Saluda, Spartanburg, Union.


REMARKS: The black-legged tick is the most
widely distributed tick species in SC (Tedders 1994, Barton et al. 1992, Clark et al. 1996, 1998, Felz et al. 1996, Durden et al. 1997). It has a wide range of hosts including humans. It is a frequent parasite of deer in South Carolina (Barton et al. 1992) as well as in Alabama (Durden et al. 1991). Immature stages are common on various reptiles, small mammals, and birds (Bishopp and Trembley 1945, Durden et al. 1997, Clark et al 1998). Tedders (1994) collecting ticks mainly from opossums and raccoons found that *I. scapularis* adults were most abundant on opossums. He also collected adults from gray foxes. Very few immature *I. scapularis* were collected in this study, however, he did record nymphs and larvae on raccoons, opossums, and cotton mice and larvae on house mice and Florida wood rats. Clark et al. (1998) found that immature *I. scapularis* represented only about 3% (n = 52) of all ticks collected from 8 species of rodents. No adults were recovered from rodents. However, among host-seekng ticks, *I. scapularis* was the most abundant species representing 46% of all ticks collected. Studies of infestations of *I. scapularis* on domestic animals in South Carolina are lacking.

In South Carolina adult *I. scapularis* appear first around the middle of September and are usually gone by the middle of May (Clark et al. 1998). Hence, it could be called a winter tick with respect to the adult stage. We have examined specimens from white-tailed deer before they were prepared for consumption and found many ticks to be in copula. They commonly attach to dogs. One of us (WW) walked his black Labrador retrievers during temperatures in the teens and 20s. During these walks *I. scapularis* crawled onto the dogs but sometimes they did not attach for a week to ten days. It appears that the black-legged tick is almost certainly a vector of the agent of Lyme disease (Lane et al. 1991) and probably is one of the main vectors in South Carolina (Durden et al. 1997, Clark et al 1998). However, given its wide distribution and relative abundance within the state, very few reports of nymphal ticks removed from humans exist. Felz et al. (1996) found that in Georgia and South Carolina 3.9% of all tick bites involved *I. scapularis*. Of the 36 *I. scapularis* recovered in their study, all were adult ticks except for one nymph. They suggested that immatures may be feeding on other animals including lizards or that immatures went undetected because of their small size. Although Clark (1996) found *B. burgdorferi* infection levels in cotton mice, cotton rats, and woodrats from the coastal zone of South Carolina (50.0 to 88.8%) to be comparable to those of white-footed mice in the northeastern U.S., he suspected that the enzootic cycle in that area may have been maintained by *I. affinis* and *I. minor* rather than by *I. scapularis* because of the rather low infection rate he found in *I. scapularis*.

*Ixodes texanus* Banks

**COUNTY RECORDS:** Charleston, Georgetown.
**HOST RECORD:** Carnivora - Procyon lotor.
**ACCESSION NUMBERS:** 120244-91, 120243-91, 121110-39.
**REFERENCE:** Tedders 1994.
**REMARKS:** This species has been collected only from raccoons in South Carolina (Tedders 1994). It is probably widespread throughout the state. Durden and Kollars (1992) collected this species from opossum and red fox in Tennessee. It has also been collected from groundhogs and rabbits in Maryland (Clifford et al. 1961). In the western U.S. it has been taken from gray squirrels, martens, skunks, and chipmunks (Furman and Loomis 1984).

*Ixodes woodi* Bishopp

**COUNTY RECORD:** Pickens
**HOST RECORD:** Rodentia - Neotoma floridana haematoreta.
**ACCESSION NUMBER:** RML 122818-99.
**REMARKS:** Three female specimens of *I. woodi* recovered from an eastern woodrat trapped on Salem Top (ca. 1000 m) near McKinney Mountain in Pickens Co. (34°56'04.4"N, 82°52'39.0"W) represent the first records of this tick in South Carolina. It is the easternmost record for this tick in North America although it is only ca. 30 km east of a recent North Carolina record (McCay and Durden 1996). This tick typically inhabits damp montane habitats where it mainly parasitizes woodrats (Robbins and Keirans 1992). Although it is relatively widely distributed in western states, it has only been recorded from Indiana, Alabama, North Carolina, and, now, South Carolina in the eastern United States (McCay and Durden 1996).

*Rhipicephalus sanguineus* (Latreille)

**COUNTY RECORDS:** Greenwood, Richland, Orangeburg.
**HOST RECORDS:** Carnivora - Canis lupus.
**ACCESSION NUMBERS:** 119332-88, 119331-88, 122350-94.
**REFERENCE:** Clark et al. 1996.
**REMARKS:** The brown dog tick is a cosmopolitan species that has traveled with man and his dogs. In the U.S., both adults and larvae are found almost exclusively on dogs (Bishopp and Trembley 1945, Clifford et al. 1961). However, one of us (WW) collected 2 females from a bird nest in Saudi Arabia. This species is found commonly in animal shelters and kennels and is often
carried by dogs into the house where it may become established. They are rarely found outside. Control operators sometimes fail to control this tick since they climb walls in houses, shelters, or kennels. Although we have found only 3 county records, this tick is probably found over much of the state.

Ornithodoros capensis Neumann

COUNTY RECORD: Allendale, Charleston.
ACCESSION NUMBERS: 118625-87, 118572-87.

REMARKS: This species was first reported from South Carolina by Keirans et al. (1992) from Bird Key Stono, a small island bird sanctuary in Charleston County. Brown pelicans had abandoned their nests due to heavy tick infestations. Yunker et al. (1979) isolated 3 strains of Aransas Bay virus from this species. This is the only argasid tick we have recorded from South Carolina. Because O. capensis is a seabird tick, we were surprised to recover a larval specimen from a barred owl in Allendale County.

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