A leaf of a pitcher plant may be considered as a microcosm or minature community. The presence of two species so similar in their niche requirements, namely, detritus feeders in pitcher plant leaves, confined together in a small circumscribed space with a finite amount of common food, raises serious problems for the ecologist. The traditional view of competition embodied in Gause's Rule or the competitive exclusion principle leaves no doubt concerning the problem. Put in its baldest terms it simply states that two species requiring the same resources cannot coexist (Pimentel *et al.*, 1965). Some contemporary definitions of the niche permit competition, for example Margalef (1968) defines the niche in such a way that two species utilizing the same resource would have the same niche if there is a non-negative feedback loop between them resulting from the parallel negative feedbacks of each species with the resource. No matter how defined, the essential question here is in what way is there niche segregation between the midge and the mosquito to permit mutual survival in a common circumscribed space with a finite food supply utilized by both. A similar question was asked of two higher Diptera by Pimentel *et al.* (1965).

Since both of these species are obligate inhabitants of pitcher plants and both are detritus feeders, they both can be said to be utilizing the same resource. It appears to be the differential utilization of this resource which provides the niche segregation permitting cohabitation. Larvae of *W. smithii* depend to a large extent on filter feeding in their utilization of the detritus for food by passing water across their mouth by the vibration of the brushes. *M. knabi* on the other hand crawls over and among the plant fragments and other debris and feeds on adhering particles by chewing movements of the mandibles. We might consider the food particles in the pitchers' water as going through different stages of ecological succession with *M. knabi* utilizing the early stages of succession, namely, the large particles and their adherents, and *W. smithii* using the later successional stages where the material is further broken down by bacterial action and plant enzymes to smaller particle size. Although they are using the same food resource, at any given moment they are using portions of the resource which are of different ecological age. This sufficiently segregates their requirements so that their niches can be said to have a sufficient but essential area of non-overlap.

Another aspect of considering the pitcher as a dynamic community is that of species diversity. Of course, in the usual case *W. smithii* is the sole species in the community. In this case the diversity of species is obviously minimal. The diversity per individual (Brillouin, 1960; Pielou, 1966) expressed as $H=\frac{1}{N}\log_2\frac{N!}{N_1!\,N_2!\ldots N_s!}$, where there are N_1 individuals in s species and $\Sigma N_1=N$, will be zero since $N_1=N$ and the $\log_2$ of 1 is zero. Therefore the addition of *M. knabi* to this dynamic system of necessity results in increased species diversity irrespective of the relative abundance of either species. Such an increase in diversity suggests a more mature system, one with greater complexity and stability. Of more interest it suggests a more efficient system where the limited resources of the pitcher plant microcosm are more efficiently exploited than they would be if *W. smithii* were the sole inhabitant.

The cohabitation of this limited ecosystem by these two species throws some interesting light on two problem areas which are of extreme interest to today's ecology.

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A CONTAINER FOR USE IN FIELD STUDIES OF SOME PATHOGENS AND PARASITES OF MOSQUITOES[1]

H. C. CHAPMAN, D. B. WOODARD, T. B. CLARK AND F. E. GLENN, JR.

Entomology Research Division, Agr. Res. Serv., U. S. Department of Agriculture
Lake Charles, Louisiana 70601

More than once we have wanted to know whether a pathogen was active in a particular pond, especially when its mosquito hosts were absent or only a few were present. Also, we have wondered whether some mosquito species might be adequate hosts of a particular pathogen though they seldom or never occurred naturally in a given infected pond. Additionally, we have been interested in obtaining large numbers of larvae infected with specific pathogens when naturally infected populations were low or absent.

[1] In cooperation with McNeese State College, Lake Charles, Louisiana 70601.

Since many pathogens of mosquitoes, especially the Microsporidia and fungal species of *Coelomomyces*, are difficult or impossible to maintain or transmit in the laboratory, we can only obtain such information by taking the mosquito into the field where the pathogens are active. However, if we release numbers of early instar mosquitoes into the ponds, only a small percentage or none are recovered. Moreover, unless these released larvae are tagged in some way, we do not know whether the larvae we capture are the native or the released populations. Therefore, a container was needed that would allow pathogens to contact the potential hosts, would confine the potential hosts, and would exclude any predators.

Yellow plastic containers 4.5 in. in diameter and 8.5 in. tall and clear plastic containers 4.5 in. in diameter and 5.5 in. tall were modified by making windows of 80-mesh screen along the lower sides and bottom (Fig. 1). Then first-instar larvae from laboratory colonies of mosquitoes or from egg batches or rafts deposited by adult females collected in the field were placed in the containers with a small amount of high protein pellets. Also, a piece of cheesecloth was placed on top of the taller container and held with a rubber band, and the original top of the smaller container was put in place. In addition, floats made of polystyrene foam were sometimes attached to the middle of the containers so they could be recovered after a heavy rain. After the larvae had been exposed in the containers for several days to a week, depending on the prevailing temperatures, the containers were returned to the laboratory and the mosquitoes were checked periodically to determine whether they had acquired infections of the particular pathogen being investigated. Adequate controls were used when they were needed.

In 1967, the containers were used in two ponds (Chloe 2 and Chloe 3) intermittently over a period of many months: some stocked with first-instar larvae of *Anopheles bradleyi* King, *A. crucians* Wiedemann, *A. quadrimaculatus* Say, and *A. punctipennis* (Say) were placed in the pond with a known high incidence of *Coelomomyces punctatus* Couch and Dodge; some stocked with first-instar larvae of *Culiseta inornata* (Williston) were placed in the pond that had a fair incidence of *Coelomomyces* near *psorophorae* Couch. Table 1 gives the results obtained over a 13-week period. Only larvae of *A. punctipennis* were not invaded

FIG. 1.—Types of containers used in pathogen studies.

TABLE 1.—Summary of tests in which first instar mosquito larvae were exposed in screened containers in ponds with a known high incidence of *Coelomomyces* ssp. over a 13-week period in 1967.

Species	No. of tests	Total no. of containers vs. no. of positive containers	Total no. larvae in positive containers	Mean % infection in positive containers
		Chloe No. 3[1]		
Anopheles				
bradleyi	12	52/38	8711	26
crucians	7	11/8	1110	19
quadrimaculatus	17	49/22	3865	7
punctipennis	8	13/0	....	..
		Chloe No. 2[2]		
Culiseta				
inornata	15	48/9	2095	19

[1] Known to contain *Coelomomyces punctatus.*
[2] Known to contain *Coelomomyces* near *psorophorae.*

by the pathogen, but only about ¼th of the containers stocked with larvae of *A. quadrimaculatus* yielded infected larvae, and the mean level of infection in the positive containers was only 7 percent. In the positive containers stocked with larvae of *A. crucians* and *A. bradleyi* mean levels of infection were 19 and 27 percent, respectively, and ¾ths of these containers yielded infected larvae. In contrast, the natural infection in larvae of *A. crucians* during the same period was 59 percent based on the results of weekly dipping in the pond. However, the number of larvae collected was often low, less than 50, and no more than 116 infected larvae were ever collected at one time. Therefore, the containers which sometimes produced as many as 1,264 infected larvae, often provided us with much larger numbers of infected larvae for our studies.

Since both *A. crucians* and *A. quadrimaculatus* are reported hosts of *C. punctatus* (Couch and Dodge 1947) and *A. punctipennis* is not, our failure to infect *A. punctipennis* while the other *Anopheles* spp. were acquiring infections again suggests that it is not a host of this fungal species. In contrast, about ⅓th the containers stocked with larvae of *C. inornata* were infected with the fungus, and the mean level of infection for 15 tests was 19 percent. We also demonstrated activity of *Coelomomyces* in a pond that had failed to breed *C. inornata* during one season.

The same containers were used in a cypress swamp that was producing larval populations of *Culex peccator* Dyar and Knab which were 75 percent or more infected with *Coelomomyces pentangulatus* Couch. First-instar larvae from our laboratory colony of *C. peccator* placed in the containers ultimately acquired a substantial infection of the pathogen.

Also, first-instar larvae from a laboratory colony of *C. inornata* were exposed in nine containers for a week in another pond in April, 1967, about 2–4 weeks after *C. inornata* had ceased breeding in the field. All nine containers were found to have larvae infected with *Thelohania inimica* Kellen and Wills (8 percent mean level of infection). The only other *per os* transmission of *Thelohania* which has been achieved in the field was that reported by Kellen *et al.* (1966) for *Thelohania campbelli* Kellen and Wills in *Culiseta incidens* (Thomson) (the plastic containers they used in that study were the basis for our use of containers). In addition, in 1967, before we achieved a laboratory culture of the nematode *Romanomermis* sp., we periodically placed early instar larvae of many species of mosquitoes in the containers in a pond with a high incidence of the nematode (Petersen *et al.*, 1968). The levels of infection of these larvae were not high (1–40 percent), but some *Anopheles bradleyi, A. quadrimaculatus, Aedes vexans* (Meigen), *Culiseta inornata, Psorophora confinnis* (Lynch-Arribálzaga), and *P. cyanescens* (Coquillett) were infected. During the same period, the natural level of infection of the nematode in larvae of *P. confinnis* in the pond was 87 percent. Because we thought that the preparasitic stage of the nematode might be having difficulty entering the 80-mesh screen windows, we tried some containers with 50-mesh screen windows, but unfortunately, the first instar larvae of many species of mosquitoes are able to escape through the larger mesh.

We also placed larvae from our laboratory colony of *Culex salinarius* Coquillett in the containers in 1968 in a pond that was producing larvae of several species of *Culex* that were infected with a cytoplasmic polyhedrosis virus (CPV) of the gut cells; some of these introduced larvae developed symptoms of CPV in a later instar.

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TRANSFER OF RADIOACTIVITY TO EGGS AND LARVAE BY FEMALE *Culex pipiens quinquefasciatus* SAY TREATED AS LARVAE WITH ^{32}P[1]

B. J. SMITTLE AND R. S. PATTERSON

Entomology Research Division. Agr. Res. Serv., U.S.D.A., Gainesville, Fla. 32601

Patterson *et al.* (1968) reported that male *Culex pipiens quinquefasciatus* Say exposed to ^{32}P as larvae transferred detectable quantities of radioactivity to the females they inseminated. Similar results have been obtained in tests with other species of mosquitoes (Dame and Schmidt, 1964; Quraishi *et al.*, 1966; Smittle *et al.*, 1969). Also Quraishi *et al.* (1966) reported that female *Aedes vexans* (Meigen) exposed to ^{32}P in the larval stage transferred radioactivity to their eggs. This paper presents the results of a study of the relationship between the radioactivity contained in female *C. p. quinquefasciatus* and that found in their eggs and larvae.

MATERIALS AND METHODS. Third and fourth instar larvae of *C. p. quinquefasciatus* from the laboratory colony were placed in distilled water for 4–6 hours and then transferred to distilled water containing 0.25 microcurie of ^{32}P per milliliter of water. The volume of treatment solution ranged from 409 to 512 milliliters and one larva per milliliter was introduced. After 48 hours, the larvae were removed from the solution, placed in distilled water for 10 minutes to remove any external contamination, and transferred to distilled water for pupation. Throughout the exposure and after it, finely ground laboratory chow was fed routinely until all larvae had pupated.

Adults from the treated larvae were allowed to mate and take a blood meal. Then, 3 days later, the females were placed in individual cups covered with nylon net to which hay infusion water had been added as an oviposition medium. Control females were set up in a similar manner. The control and treated females and their egg rafts were assayed for radioactivity. Also, females that had not oviposited were assayed.

The measurements of radioactivity (counts per minute less background) were made with both the G-M and liquid scintillation counters. The G-M counts were obtained with a Nuclear Measurements Corporation DS-1A scaler equipped with a 1.4 milligrams per square centimeter end window G-M tube. The liquid scintillation counts were obtained with a Packard Tri-Carb® liquid scintillation spectrometer (Model 3365D). Twenty milliliter low-potassium glass vials with foil caps were used. The scintillation fluid was composed of 4.0 grams of PPO (2,5-diphenyloxazole) and 0.1 gram of dimethyl POPOP (2,2'-*p*-phenylene-bis(4-methyl-5-phenyloxazole)) per liter of toluene. Also thixotropic gel powder (Cab-O-Sil®) was added at the rate of 30 grams per liter of solution to keep the mosquitoes and egg rafts suspended in the scintillation fluid.

Females and egg rafts were weighed for comparison with radioassays. Some egg rafts were not counted in the liquid scintillation counter, but were allowed to hatch so we could evaluate fertility and radioactivity in the larvae.

RESULTS. Fifteen gravid females that had not oviposited, 25 females that had oviposited, and 25 egg rafts were analyzed for radioactivity with the G-M counter. These same samples (except 15 of the egg rafts) were also analyzed with a liquid scintillation counter. The results were as follows:

	Average Counts Per Minute	
	G-M	Liquid Scintillation
Females not ovipositing	4,451	49,106
Females that oviposited	2,648	30,582
Egg rafts	1,802	21,518

Ovipositing females therefore retained about 60 percent of their radioactivity. The other 40 percent was found in their egg rafts.

Seventeen gravid females weighed an average of 2.62 milligrams per female before oviposition and 1.56 milligrams after oviposition—a difference of 1.06 milligrams. Their egg rafts averaged 1.24 milligrams per raft. Thus, the females lost about 40 percent of their weight during oviposition, and the loss of weight and radioactivity correlated exceptionally well.

Quraishi *et al.* (1966) reported that female *A. vexans* lost only 13 percent of their radioactivity by oviposition. However, this discrepancy between their results and ours may be attributed to the difference in the number of eggs oviposited, as *A. vexans* averaged only 41 eggs per female while *C. p. quinquefasciatus* averaged 155 eggs per female.

No radioactivity was found in the controls or in egg rafts from untreated females mated with radioactive males.

Only 10 of the 25 egg rafts assayed for radioactivity with the G-M counter were assayed in the liquid scintillation counter. The remaining 15 egg rafts were held in hay infusion until the larvae hatched. These eggs had an average hatch of 92.3 percent compared with 98.1 percent

[1] Mention of a proprietary product in this paper is not an endorsement of this product by the U.S.D.A.