Axenic Rearing of the Oriental Fruit Fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae)[1]

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ABSTRACT

Axenically rearing the oriental fruit fly, *Dacus dorsalis* Hendel, through many continuous generations revealed that microbes were not essential for development. Absence of microbes did not affect the incubation period of the eggs, percent egg hatch, larval period, percent pupation, pupal period, adult emergence or the preovipositional period of the adults. However, axenic rearing had a highly significant effect on fecundity. Axenically reared flies laid significantly fewer eggs than xenically reared flies. Ovarian development was reduced in axenically reared females. Fecundity was not increased even when the axenically reared adults were fed food which increased fecundity in xenically reared adults. There were no differences in the fertility of the eggs laid by xenically or axenically reared flies.

KEY WORDS: Insecta, axenic rearing, fruit flies, *Dacus dorsalis*.

There have been many attempts to rear fruit flies under axenic (Dougherty 1959) or sterile conditions. Most of these were made in conjunction with studies of bacteria associated with tephritids to elucidate their roles in development, nutrition, fecundity, fertility, longevity, or host finding (Maeda et al. 1953; Hagen 1966; Girolami 1973; Luthy et al. 1983; Fitt and O'Brien 1985; Lloyd et al. 1986; Drew and Lloyd 1987; Howard and Bush 1989).

These studies showed that bacteria can play important roles in the development and reproductive biology of fruit flies by providing nutrients or by serving directly as food (Drew et al. 1983). However, these conclusions were based on experiments which involved the axenic rearing of just the larvae and pupae. Eggs obtained from xenic adults were chemically sterilized and placed on sterile media or media containing anti-microbial agents. The larvae were grown under axenic conditions and the mature larvae were allowed to pupate in sterile pupation chambers. Axenic rearing ended at this point. The adults were exposed to microbes.

An axenic system, where continuous generations of fruit flies can be reared, is required to accurately quantify the effects of the microbes on adult reproductive parameters. This requires that the adults also be kept sterile. The system must permit axenic production of eggs from axenically

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reared adults through several generations. With a completely axenic rearing system, a single species of microbe may be introduced at any stage of the life cycle to assess its effect.

This study was undertaken to develop a completely axenic rearing system for the oriental fruit fly (OFF), *Dacus dorsalis* Hendel, and to evaluate the effects of rearing several continuous generations of the flies under axenic conditions on growth, development, fertility and fecundity.

MATERIAL AND METHODS

The medium used to rear *D. dorsalis* was similar to that used by the USDA's Tropical Fruit and Vegetable Research Laboratory (Tanaka et al. 1969). The basic medium consisted of wheat mill feed, torula yeast, sugar, hydrochloric acid, sodium benzoate, methyl-p-hydroxybenzoate and distilled water. Sixteen grams of the medium were placed in 50 ml medicine bottles. An additional 5 ml of distilled water was added before the medium was autoclaved because autoclaving for 30 min at 120° C and 15 psi desiccated the medium. Media used for both axenic and xenic rearing were autoclaved.

For axenic rearing, the microbial inhibitors, sodium benzoate, and methyl-p-hydroxybenzoate, and the HCl were not included in the medium. The microbial inhibitors were not necessary since the medium was sterilized by autoclaving and no microbes were introduced during the rearing process. For xenic rearing, however, the microbial inhibitors were required. Without the microbial inhibitors, the xenic medium was overgrown in one to three days by fungi and bacteria introduced with the unsterilized eggs. The microbial inhibitors, at the concentrations used, do not affect the growth or development of the fruit flies (Maeda et al. 1953, Vargas et al. 1984).

The initial batch of eggs was obtained from the USDA's Tropical Fruit and Vegetable Research Laboratory. For axenic rearing, 50 sterile eggs were layered on a piece of organdy and placed on the medium. These were sterilized by immersion in a solution containing 10% formaldehyde and 0.2% HCl for 10 min. The eggs were periodically shaken in the sterilant. The pH of this sterilant was approximately 4.2. After 10 min, the eggs were quadruple rinsed with sterile, double distilled water. Over 90% of the eggs subjected to this treatment hatched. This hatching percentage did not differ from that of untreated eggs. With subsequent generations, egg sterilization was not required since eggs were obtained from axenically reared adults. For xenic rearing, 50 non-sterile eggs were placed on the medium.

Eggs were held in a bottle in a laboratory which averaged 21.0 ± 0.5° C and 64.6 ± 2.0% relative humidity during maturation and larval development. When the larvae reached maturity the bottle was connected to a pupation chamber, which allowed the larvae to egress directly from the bottle into the pupation chamber.

The pupation chamber consisted of a 236.6 ml cardboard ice cream carton with approximately 4 mm of sterile vermiculite as the pupation

medium. A hole approximately the size of the mouth of the larval rearing bottle was cut into the side of the carton. The bottle was then inserted into the hole and held at a slight angle to allow the larvae to "pop" directly into the vermiculite (Figure 1).

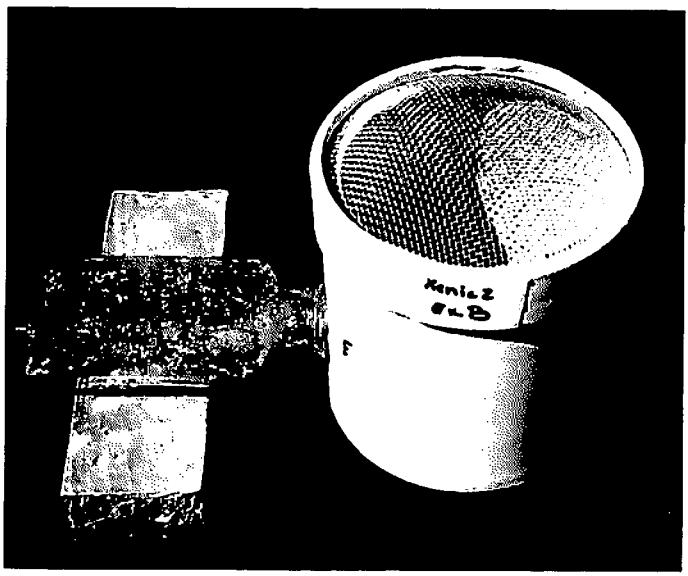

FIGURE 1. Larval rearing container inserted into sterile pupation chamber. Larvae egressed directly into sterile vermiculite for pupation.

Several days after pupation, pupae were sieved from the vermiculite using a sterile sieve and collected in Petri dishes. The Petri dishes were placed in a cage for adult emergence. The cage consisted of a piece of shade screen rolled into a cylinder with a plastic Petri dish at the bottom. The lid was made from the cover of a 236.6 ml ice cream carton which had the center removed and replaced with wire screen (Figure 2).

A yeast hydrolysate-sugar mixture (1:3 ratio) and two cubes of sugar were autoclaved, and placed in the cage as food for the adults. Sterile water was provided in a dilute agar gel that was placed on top of the cage. Water and food were provided *ad libidum*. For axenic rearing, the adult cages were held in a laminar flow hood to maintain the sterile environment.

The adults were regularly checked to ascertain when oviposition started and to count the number of eggs laid. A small plastic container, which contained a sponge soaked with guava juice, served as the ovipositional "fruit". The container had small holes through which the flies could oviposit. The containers were removed after 24 h exposure and the eggs were harvested and counted.

FIGURE 2. Cages for sterile adults. These cages were held in a laminar flow hood and used for studies of oviposition, fertility and fecundity of axenically reared oriental fruit flies.

Throughout the entire rearing process periodic checks were made to insure sterility. External sterility was ascertained by dropping randomly selected eggs, larvae, pupae and adults into fluid thioglycollate medium. The results were read after 24 h. If the specimen was externally sterile, it was crushed in the thioglycollate and incubated an additional 24 h to ascertain internal sterility. If any specimen from a container showed contamination, the entire container was destroyed. Sterility was maintained in more than 98% of the containers.

Laboratory equipment and materials used were sterilized by autoclaving for those items that were heat stable, and by immersion in 10 percent formaldehyde for those that could not be autoclaved.

Both xenic and axenic flies were reared in the same laboratory until pupation, but the xenic adults were moved to another laboratory for the observations on fecundity. This was necessary to prevent contamination of the axenic flies by escapees from the xenic cages. It was difficult to completely prevent the adults from escaping since the cages had to be opened to place and remove the ovipositional "fruit". Unfortunately, there were differences in the temperatures and humidities in the two laboratories which may have affected ovipositional rates. The xenic laboratory averaged 23.5

± 0.4° C with an average relative humidity of 73.9 ± 1.9% while the axenic lab averaged 21.0 ± 0.5° C and 64.6 ± 2.0% relative humidity.

The data were subjected to ANOVA and means separated by the DUNCAN option, or the means were compared using a *t* test (SAS 1985).

RESULTS AND DISCUSSION

Surface sterilization of eggs confirmed that bacteria normally associated with the oriental fruit fly were transmitted on the surface of the eggs. Internally, the eggs were sterile. The bacterial species and the frequency at which they were isolated from samples taken on 15 different dates were: *Cedacea* sp. 3 (1), *Citrobacter freundii* (1), *Enterobacter aerogenes* (1), *E. cloacae* (5), *E. gergoviae* (1), *Klebsiella pneumoniae* (1), *Providencia rettgeri* (1), *Pseudomonas fluorescens* (1), *Serratia liquefaciens* (4), and *S. marcescens* (4). Jang (personal communication) also isolated *Cedacea* spp., *Enterobacter cloacae,* and *Providencia rettgeri,* along with several other species, from flies reared in a laboratory on the island of Hawaii. These three bacterial species, therefore, were found in both rearing laboratories.

It is apparent that many species of bacteria can be introduced into the fruit at the time of oviposition by the oriental fruit fly. The role of these bacteria in the life cycle of the fly has not been completely defined but they did not seem to be essential for the development of the OFF (Table 1).

TABLE 1. Developmental times for eggs, larvae and pupae from four generations of the oriental fruit fly reared xenically and axenically. There were a minimum of three replications for each observation.

Generation	F_0		F_1		F_2		F_6		Mean[2]	
Rearing status[1]	X	A	X	A	X	A	X	A	X	A
Egg (hrs)	48	48	48	24	48	48	48	48	48a	43a
Percent hatch	92	80	74	94	93	88	89	81	87a	86a
Larval period (days)	7	7	6	6	7	7	7	7	7a	7a
Percent pupated	78	89	87	97	*	82	91	77	85a	86a
Pupal period (days)	14	13	14	14	15	14	15	15	14a	14a
Adult emergence (%)	98	99	91	99	90	93	96	90	94a	95a
Preovip. period (days)	11	15	12	16	13	13	12	12	12a	14a

[1]A = axenically reared
X = xenically reared
[2]Means followed by the same letter are not significantly different from each other at $P < 0.05$.
*An unknown number of larvae escaped.

There were no significant differences at $P < 0.05$ in the incubation period, percent hatch of the eggs, larval period, percent pupation, pupal period, adult emergence or in the preovipositional period. None of these developmental characteristics were affected by the absence of microbes.

These results are similar to those obtained by Neilson (1969) who found that the presence or absence of microorganisms in the diet did not affect the growth and development of the larvae of the apple maggot, *Rhagoletis pomonella* (Walsh). Haisch (1968), on the other hand, found that adding dead *Pseudomonas* sp. to the artificial medium for the cherry fruit fly, *Rhagoletis cerasi* L. improved the growth rate. Recently, Howard and Bush (1989) reported that the presence or absence of the bacterium *Klebsiella oxytoca* did not affect the fitness of *R. pomonella.*

In addition, with *D. dorsalis* we also found no significant differences in developmental times among the generations reared axenically or xenically. The rates of development of the first axenic and xenic generations were similar to those of the sixth axenic and xenic generations. Developmental times for both axenically and xenically reared flies from egg to adult were approximately 23 days. These developmental times were in the same range as those found by Vargas et al. (1984), 19 days, when differences in rearing temperatures (21 vs 25° C) are considered.

The xenically reared flies, however, laid significantly more eggs than the axenically reared flies (Table 2). Xenic flies generally laid more than three times the number of eggs produced by axenic flies. However, although fewer in number, the eggs laid by the axenically reared flies were just as fertile as those from xenic adults (Table 1). There were no differences in the percent hatch.

TABLE 2. Mean number of eggs laid per female by 3 generations of xenically (X) and axenically (A) reared oriental fruit flies. A minimum of 3 pairs of adults were held in individual cages.

Oviposition day	Generations 0		1		6	
	X	A	X	A	X	A
1	20	17	60	7	13	1
4	98	31	142	0	57	4
7	88	18	117	0	80	21
10	90	53	121	13	82	14
13	90	31	95	36	71	19
16	95	43	75	6	46	17
19	84	24	85	4	31	4
22	65	27	70	35	51	11
Mean/fly/day[1]	79a	31b	96a	13b	54a	11b
Mean total/fly[1]	630a	244b	765a	101b	431a	91b

[1]Within each generation, means followed by the same letter are not significantly different from each other at $P < 0.05$.

The absence of the microbes, therefore, did not affect the growth of the immature stages but had a highly significant effect on the reproductive capacity of the adults. Dissections of axenically reared adults disclosed that the ovaries were not as well developed as those in xenic flies. The numbers of functional ovarioles were reduced. The reduced fecundity, therefore, appeared to be due to stunted ovarian development. Apparently the microbes altered the medium or provided some nutrient which was required for normal ovarian development. This probably was a quantitative increase rather than a qualitative addition since the ovaries, even in axenic flies, developed sufficiently to produce viable eggs.

Moreover, there was no increase in ovarian development, and the fecundity of the axenic flies did not increase even when they were given food which increased fecundity in xenic adults. All of the adults were fed yeast hydrolysate and sugar, a diet which provided the protein and carbohydrate needed for ovarian development and high fecundity in tephritids (Hagen and Finney 1950; Matsumoto and Nishida 1962).

The relationship between the OFF and the microbes seemed to be commensalism rather than mutualism. The microbes apparently did not provide an essential nutrient which was not already present in the medium. The greater fecundity of xenic adults, however, indicated that microbes provided some nutrient which was quantitatively lacking in the medium.

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