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Artificial Rearing Substrata of Chironomid Larvae (Diptera, Chironomidae)[1,2)]

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Abstract Three kinds of rearing substrata (smashed filter paper, tissue paper, rinsed tissue paper) were tested for the rearing of *Chironomus yoshimatsui* and *C. kiiensis* larvae in comparison with sifted and heated paddy soil. Under the same rearing conditions (temperature, photoperiod, food, aeration and rearing density), percentage emergence, sex ratio, number of ovarian eggs and wing length were not significantly different among these four substrata, but developmental period was significantly different between paper and soil substrata. Chemical treatment to remove impurities and boiling the paper substrata as done by previous authors is not necessary with this method.

Key words: Paper rearing substrata; *Chironomus yoshimatsui*; *Chironomus kiiensis*; Chironomidae.

Introduction

Several artificial media used in place of soils for rearing chironomid larvae under laboratory conditions have been reported (Table 1). These media developed to maintain cultures for mass rearing for cytological or physiological studies were composed of substrata and foods (e.g., filter paper plus nettles), or the material itself was utilized by the larvae both for rearing substrata and as food (e.g., alder leaves). There are, however, problems in using these media, one of which is the difficulty in evaluating the food effect on development since exact nutritional benefit or food amount cannot be determined.

We have been rearing chironomid larvae on media comprised of heated soil as pure non-organic substrata and foods which can be exactly weighed or measured. However, this medium cannot be used repeatedly since the soil substratum has already been mixed with food and frass of the larvae, and soil processing is not easy. We therefore tested artificial rearing substrata made from paper products for use in our biological studies of the Chironomidae in paddy fields.

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Table 1. Rearing media for chironomid larvae made of paper or plants described by previous authors.

Media	Species	Developmental data[1)]	Reference
Paper pulp+dried nettle, yeast	*Chironomus thummi*	0	STRENZKE, 1959, 1960
Paper towel+fish food, dog food	*C. decorus*	0	MAIER *et al.*, 1990
Paper towel+fish food	*C. tentans*	X	BATAC-CATALAN & WHITE, 1982
Paper towel+dog food	*Glyptotendipes barbipes*	0	MEIER & TORRES, 1978
Cellulose+nettle	*Chironomus thummi*	X	KROEGER, 1964
Filter paper+nettle leaves	*C. tentans*	X	ENGLEMANN & SHAPPIRIO, 1965
Plant levaes (nettle, alder)	*C. tentans*	X	CLEVER, 1961, 1962 a, b
Algae+fish food	*C. riparius*	X	CREDLAND, 1973
Nettle powder	*C. thummi*	X	LAUFER & NAKASE, 1965

1) 0: data available, X: data are lacking.

Table 2. Substrata used for *Chironomus yoshimatsui* and *C. kiiensis*.

Substratum	Treatment	pH of medium[1)] (25°C)		
		0	7	14 (days)
Filter paper[2)]	Smashed by mixer	6.0	7.1	6.8
Tissue paper[3)]	Smashed by mixer	6.3	7.2	6.5
Rinsed tissue paper	Rinsed by running tap water for 30 min. before smashing	6.2	7.1	6.5
Soil	Heated at 500°C for 2 hrs	5.9	7.2	7.6

1) Substratum+fish food+chironomid eggs, larvae.
2) Toyo Roshi Co., No. 2.
3) Kami Shoji Co., "Natural".

Materials and Methods

Substrata

The materials of the substrata tested in this study were filter paper, tissue paper and rinsed tissue paper as shown in Table 2. For rearings, square plastic cases (12.5×12.5 cm and 10 cm deep) were used, and 5 g of filter or tissue papers and 750 ml of distilled water per case were smashed in a mixer for 5 minutes. Rinsed tissue paper was prepared after rinsing tissue paper by running tap water for 30 minutes before smashing.

As a control rearing substratum, paddy soils were sifted and heated at 500°C for 2 hours to eliminate the organic matter. The soil was then placed in cases about 1 cm deep with 750 ml of distilled water.

The percentages of organic carbon of filter and tissue papers were 41.6% and

41.7%, respectively. The pH of the media comprising substrata, food and chironomids was counted, and it was slightly higher a week after the rearings started. This was probably due to ammonium and associated micro-organisms occurring from fish food and the frass of the larvae.

As stated, the term substrata and media are distinguished in this paper. The rearing material without food is termed substrata, and the term media is used when food was added to the substrata.

Rearing method

One hundred eggs of *Chironomus yoshimatsui* MARTIN et SUBLETTE were placed into each rearing case, and 100 mg of golden fish food (Tetra Fin) were given everyday after hatching. Rearing cases were aerated and kept at 25°C under a photoperiod of 16L: 8D. Four rearing cases were used for each substratum to obtain developmental data.

Starvation test

Two series of starvation tests (Ser. A and B) were made to check whether or not the paper substrata were utilized by *Chironomus kiiensis* TOKUNAGA larvae as food.

Series A: Fifty eggs in each substratum were placed in petri dishes (5.5 cm diameter and 8.5 cm deep), and larvae hatched the same day. They were reared without food for 1, 2, 3, 4 and 7 days. After each starvation period, 50 g of golden fish food was supplied daily, and numbers of larvae surviving 10 days after the supply began were counted. This experiment was conducted at 25°C.

Series B: Fifty eggs in each substratum were placed in square plastic cases (4×4 cm and 1.2 cm deep), and larvae hatched the same day. They were reared without food through the rearing period, and the number surviving was counted every day until all larvae had died. This experiment was done in August 1992 at room temperature.

Normal rearings with food for both paper and soil substrata were made in Series A as control. Rearings in distilled water without any substrata or food were carried out in both series.

Chironomus yoshimatsui used for the developmental study mentioned was not available for this starvation test owing to the stock culture problem. It should be expected that the present two *Chironomus* species are used together for both developmental and starvation studies to obtain more generalized data than those of the present study.

Results and Discussion

Percentage emergence, wing length (male), sex ratio and number of ovarian eggs of *C. yoshimatsui* reared in different substrata are shown in Tables 3, 4 and 5.

Table 3. Percentage emergence and developmental period of *C. yoshimatsui* reared on artificial substrata[1].

Substratum	No. eggs	No. adults emerged	% emergence	Developmental period (days)
Filter paper	400	328	82.00± 2.73 a	15.90±2.21 a
Tissue paper	400	319	79.75±10.78 a	15.25±1.02 a
Rinsed t-paper	400	310	77.50±15.59 a	15.77±1.14 a
Soil	400	322	80.50±10.50 a	13.26±0.67 b

1) Numbers in column followed by the same letter are not significantly different (P= 0.05; DUNCAN's new multiple range test).

Table 4. Wing length (male) of *C. yoshimatsui* reared on artificial substrata[1].

Substratum	No. males examined	Wing length (mm)
Filter paper	154	3.25±0.048 a
Tissue paper	158	3.26±0.075 a
Rinsed t-paper	153	3.21±0.094 a
Soil	162	3.30±0.065 a

1) Numbers in column followed by the same letter are not significantly different (P=0.05; DUNCAN's new multiple range test).

Table 5. Sex ratio (female %) and number of ovarian eggs of *C. yoshimatsui* reared on artificial substrata[1].

Substratum	No. adults examined	Sex ratio (%)	No. females examined	No. ovarian eggs
Filter paper	328	49.35 a	48	408.06 a
Tissue paper	319	49.54 a	40	395.18 a
Rinsed t-paper	310	50.21 a	39	327.08 a
Soil	322	48.63 a	31	441.84 a

1) Numbers in column followed by the same letter are not significantly different (P= 0.05; DUNCAN's new multiple range test).

There were no significant differences in these characteristics between the each substratum.

Developmental period of *C. yoshimatsui* reared in these substrata is shown in Table 3 and Fig. 1. Those reared in paper substrata required 15.25–15.90 days while those in soil 13.26 days. There is thus significant differences between the paper substrata and soils. As seen in Fig. 1, adult emergences occurred simultaneously in soil substratum in contrast to the emergence in paper substrata.

Survival of *C. kiiensis* larvae reared on the paper substrata without food for different periods is shown in Table 6 a. The percentage survival decreased drastically between larvae starved for 2 days and those starved for 3 days. When the

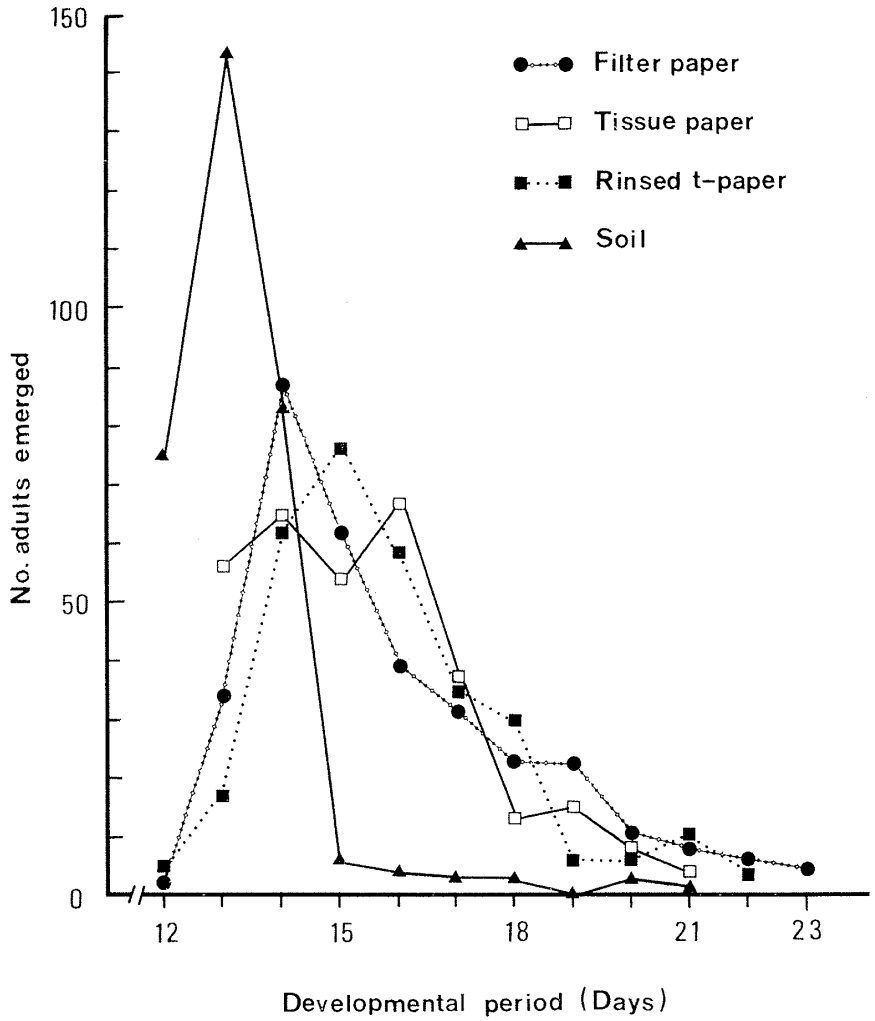

Fig. 1. Developmental period of *C. yoshimatsui* reared on different substrata.

starvation period was 3 days, survival percentage in rinsed tissue paper (28%) and tissue paper (17%) were higher than soil (6%) and filter paper (3%). When the starvation period was 4 days, the percentage was 0 to 3 (rinsed tissue paper). The figures in paper substrata were similar to those of larvae reared in soil, but those in distilled water more distinctly decreased from one day to two days. The data in distilled water may indicate that the larvae find difficulty surviving without substrata to make nests, since food condition is the same as that found in other substrata.

Survival of *C. kiiensis* larvae reared without food until their death is illustrated in Table 6 b. The larvae survived well until 3 days of starvation in paper substrata and then the survival rate distinctly dropped. Two day starvation in distilled water resulted in 28% survival.

From the data, the following conclusions and discussion can be made:

(1) Paper substrata tested are an adequate artificial substitute on which to rear

Table 6-a. Survival of *C. kiiensis* larvae reared on artificial substrata without food.

(A) Food was supplied after starvation[1]

Starvation period (days)	% larvae surviving[2]				
	Filter paper	Tissue paper	Rinsed t-paper	Soil	Distilled water[3]
1	71	63	65	58	94
2	29	34	35	22	10
3	3	17	28	6	0
4	0	0	3	0	0
7	0	0	0	0	0
Control	100	100	100	100	

1) Fifty eggs-larvae were reared on each substratum at 25°C.

2) Number of larvae surviving after 10 days was counted and survival percentages were figured by ABBOTT's formula.

3) Number of larvae surviving was counted on the last day of the respective starvation period.

Table 6-b. Survival of *C. kiiensis* larvae reared on artificial substrata without food.

(B) No food was supplied[1]

Starvation period (days)	% larvae surviving[2]			
	Filter paper	Tissue paper	Rinsed t-paper	Distilled water
1	100	100	100	100
2	100	100	100	28
3	87	93	88	0
4	5	3	3	—
5	3	0	0	—
6	0	0	0	—

1) Fifty eggs-larvae were reared on each substratum at room temperature.

2) Percentage was calculated based on the surviving larvae found.

chironomid larvae, though the developmental period is slightly longer than on soil.

(2) Wing length and number of ovarian eggs were slightly greater in soil substrata than in paper, though the difference was not significant. Speculations possible on these phenomena and developmental period are: the possibility that paper substrata may contain substances which are not beneficial for larval growth; and that soil is physically preferable for larvae for making nests or other living conditions. One or these two factors may be suspected.

(3) As shown by the starvation experiments, larvae live slightly longer in paper substrata than in soil. This may be because they eat some of the paper substrata resulting in slightly lengthening their lives, but many of the nutritional requirements are lacking, and as a rule, they don't consume soil particles.

(4) Some earlier methods using paper towel such as those used by BATAC-CATALAN & WHITE (1982) and MAIER *et al.* (1990) required a acetone procedure to remove impurities. The material was rinsed several times or for several days after acetone treatment, and then boiled to remove the acetone odor. These treatments are not necessary in the present paper substrata.
(5) The process of making the paper substrata is much simpler and easier than that involved in soil substratum which requires painstaking sifting and heating.

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