A Technique for Rearing the Limabean Pod Borer, *Etiella zinckenella* TREITSHKE (Lepidoptera: Pyralidae) in Large Scale

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Rearing of the limabean pod borer, *Etiella zinckenella*, in large scale has been practically impossible without a technique to reduce cannibalism. This problem was resolved by isolating larvae on a diet with a multicellular unit.

The rearing procedure is as follows: Newly hatched larvae were placed on the roughened surface of diet in each cell of the unit. The top of the multicellular unit was covered with rough papers and pressed with a magazine. The tray was then closed with a lid, its windows covered with net, and placed in darkness for three days. Afterwards, it was transferred to a constant temperature cabinet at 25°C under a 16-photoperiod. Ten days after the larval infestation, the magazine was removed. During the period from 12 to 16 days after infestation, the mature larvae were transferred daily to a plastic pot (9.5 cm diam.) with nonabsorbent cotton. After 20 days, pupae were collected from the cotton. By this method, 51.5% of the larvae introduced into the tray were recovered as moths.

Eggs were collected by providing the cage in which the moths were released with a soybean twig covered with cheesecloth as an oviposition site.

INTRODUCTION

Rearing of the limabean pod borer, *Etiella zinckenella*, without using fresh pods of leguminous plants was first attempted by NAITO (1959). The water-soaked dry soybean seeds in a 200 ml Erlenmeyer flask were infested with about 20 eggs under aseptic conditions. The rate of adult emergence, however, was only 20% or less. Recently, HATTORI and SATO (1980) reported that the limabean pod borer can be reared on artificial diet with a higher survival rate when reared individually in glass vials. However, for mass rearing of this insect, a labor-saving device to eliminate larval cannibalism should be developed. The present report deals with a rearing technique for *E. zinckenella* on artificial diet using a multicellular unit to eliminate larval cannibalism, and with an efficient method for egg collection. Some experiments were also conducted to improve the recovery of mature larvae.

MATERIALS AND METHODS

Diet preparation and rearing devices. The constituents of the diet used in the present studies are almost the same as the soybean-wheat germ diet reported earlier by HATTORI

and SATO (1980) except for a few minor modifications (Table 1). The dried soybean seeds (60 g) were soaked in water for 12 hr at room temperature. These soaked soybeans (145 g) were added to agar solution (5 g/300 ml) and brought to a boil. After cooling below 60°C, they were homogenized in a mixer. The other ingredients were then added to the homogenate and blended for a few minutes. The hot mixture was poured into a plastic tray (19×30×7 cm) to a depth of about 8 mm. The surface of the diet was roughened with a rubber spatula. A polystyrene multicellular unit with about 200 cells of 1.5×1.5 cm (light diffusion grid, 18×29×1.5 cm) was inserted into the diet before it solidified. The tray was closed with a lid and stored in a refrigerator at about 7°C until use.

General rearing procedure. Newly hatched larvae were individually placed in each cell of the grid with a fine brush whose tip was washed in 70% ethanol. Two sheets of rough paper were placed on top of the grid to prevent their escape and excessive drying out of the diet. The tray was then covered with a lid having two Saran® net windows (3.5 cm-diam.) and kept in a constant temperature cabinet at 25°C under a 16-hr photophase (Fig. 1).

Table 1. Composition of artificial diet of *E. zinckenella*

Ingredient	Grams for 1 tray
Soaked soybeans	145
(Dried soybeans	60)
Wheat germ	16
Dried brewer's yeast	4
(Ebios®)	
Ascorbic acid	1
Sorbic acid	0.4
Methyl- <i>p</i> -hydrobenzoate	0.4
Chloramphenicol	0.2
Agar	2.5
Water	160

Fig. 1. Rearing device for *E. zinckenella*.

About 12 to 18 days after the larval infestation, mature larvae were transferred daily to a plastic container with nonabsorbent cotton. Twenty days after the infestation pupae were collected from the cotton and sexed.

All equipment was sterilized with 0.1% NaClO solution for one night before use. Used grid were immersed in 0.3% NaClO solution for 2 days to remove remaining diet particles and any silk webbing. All procedures involving preparation of the diet were conducted in a clean laboratory.

Egg collection. About 20 pairs of newly emerged moths were confined in an oviposition cage (30×30×40 cm). A 10% sucrose solution in a 50 ml plastic cup with a wick of rolled filter paper was served as food for the adults. A detached soybean twig (about 25 cm long) with a few compound leaves held on a 200 ml Erlenmeyer flask containing water and covered with a sheet of cheesecloth was placed in the cage. Eggs deposited on the cheesecloth were collected.

Effects of weight and darkness after infestation with newly hatched larvae on the recovery of mature larvae. After the rough papers were placed on the unit the papers were pressed with a magazine for 10 days primarily to prevent the movement of larvae from cell to cell. Also, to allow the larvae to settle down and start feeding, the trays were kept in darkness for 1, 3, and 14 days before transferring to 16hr-photophase under two different conditions, with and without the weight of the magazines. The number of mature larvae that were no longer cannibalistic in this stage were counted until 16 days after the infestation. Each treatment was replicated 3 times.

Effect of larval density per cell on the recovery of mature larvae. The larvae were placed in cells at a density of 2 or 3 per cell to determine the optimum density for getting a higher recovery of mature larvae per cell. Trays were kept in darkness for 3 days after the infestation. Each treatment was replicated 3 times.

Development, viability, fecundity and adult longevity. Mature larvae collected from the tray were put into another tray containing a layer of nonabsorbent cotton. The larval duration was determined by recording the day of pupation. The pupae were sexed, weighed and held in individual vials until the adults emerged. On the day of emergence, the adults were placed in the oviposition cage described above. Mated pairs were individually transferred to a 200 ml Erlenmeyer flask with a soybean pod and a piece of absorbent cotton moistened with a 10% sucrose solution, in order to examine the number of eggs per gravid female and adult longevity.

RESULTS

Effects of weight and darkness on the recovery of mature larvae

The percentage of mature larvae was not increased by using the weight of magazine in any darkness conditions. On the other hand, the percentage increased as much as 60% with increasing darkness duration of 1 to 3 days (Fig. 2).

Effect of larval density per cell on the recovery of mature larvae

Three experimental plats, in which 1, 2 or 3 newly hatched larvae were placed in a cell, were made. The percentage of larvae which matured in each plat were 59.1, 76.6 and 82.1 respectively. When the above percentages were calculated as the percentage of mature larvae against the initial numbers, they were 59.1, 38.3 and 27.4 respectively (Fig. 3).

Fig. 2. Effect of darkness after larval infestation on the rate of mature larvae under two covering conditions. : paper sheets, : paper sheets and weight*. *Magazine (ca. 1 kg) was placed on paper sheets for the first 10 days.

Fig. 3. Correlation between number of larva(e) introduced per cell and percentage of mature larvae per cell or per larva(e) introduced. : values per cell, : values per infested larva. Trays were kept in darkness for 3 days after infestation and transferred to 16hr-photophase.

Table 2. Development, viabilities of the various stages, fecundity and adult lifespan for *E. zinckenella* reared on an artificial diet

Duration	Egg	(223)	4.8±0.03 days	Egg viability	(93)	93.5%
	Larva	(110)	19.2±0.2 days	Larval survival	(610)	54.4%
	Pupa	(102)	13.7±0.1 days	Pupal survival	(331)	94.8%
	Adult	(44)	10.5±2.0 days	Survival newly hatched larvae to adult emergence	(610)	51.5%
Pupal weight	Female	(50)	37.2±1.2 mg	No. eggs/ gravid female	(20)	85.1±9.5
	Male	(50)	41.1±0.8 mg			

Figures in parenthesis indicate the number of insects examined.

Development, viability, fecundity and adult longevity

Data of rearing *E. zinckenella* on artificial diet with a multicellular unit is shown in Table 2. Durations in larval, pupal and adult stages were not significantly different between male and female. The weight of pupae were 40.1 and 37.1 mg, respectively. Survival rate of newly hatched larvae to adult emergence was 51.5%. The mean total number of eggs laid by a single gravid female was 85.1.

DISCUSSION

Utilization of the multicellular unit has been reported to be useful in mass rearing cannibalistic lepidopterous larvae such as the bollworm, *Heliothis zea* and the tobacco budworm, *Heliothis virescens* (ROBERSON and NOBLE, 1968; RAULSTON and LINGREN,

1969; RAULSTON and SHAVER, 1970). When this unit was utilized for the rearing of the limabean pod borer, some devices were required to prevent the escape of larvae from the cells to obtain a higher recovery of mature larvae. If larvae were distributed onto a diet chilled below 7°C, escape of larvae from the cells was effectively prevented. Also, keeping the tray in darkness for the first 3 days after the infestation promoted their settlement in the cells because the newly hatched larvae do not exhibit phototropic (STONE, 1965) or phototactic (SINGH and DHOORIA, 1971) behavior in this condition. The weight of the magazine may not be significant in preventing larvae escaping from cells through the slit between the paper sheets and the top of grid (Fig. 2); however, it seemed to be useful in controlling the moisture of the diet in the tray.

Although if more than 2 larvae were introduced to one cell, only one could survive due to cannibalism, more cells had mature larvae than those in which only one larva had been placed. However, if one considers the initial numbers of larvae, the rate of mature larvae was not large. This was especially clear with the plat inoculated 3 larvae (Fig. 3).

The pupal duration and the weight of pupae obtained here were similar to those reared individually on an artificial diet in glass vials (HATTORI and SATO, 1980), whereas larval duration was more than 3 days shorter and the percentage of larvae to adult emergence was about 30% lower. These differences may not be due to the slight modification in diet constituents, but to the fact that larvae which were delayed in development were not recovered in the present rearing method. Therefore, recovery could be attained by an improvement in the diet so that the larvae grow faster and more synchronously as on the soybean pod.

The mean total number of eggs laid by a single female in the cage was about 50, fewer than that by the gravid female individually confined in the flask (Table 2). Developing a more efficient method for egg production is also important for mass rearing.

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