REARING NON-DIAPAUSING WESTERN SPRUCE BUDWORM ON PRE-MIXED ARTIFICIAL DIET

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ABSTRACT

The western spruce budworm, *Choristoneura occidentalis* Freeman, was reared on pre-mixed artificial diet in the laboratory without diapause. The colony was maintained indefinitely with a generation time of 38 to 40 days. Females deposited an average of 307 eggs of which about 91% survived. The rearing technique provided a steady and reliable supply of the insects for other basic research. The supply of insects could be adjusted according to need at any particular time.

Sanitation is essential to successful rearing, because contamination of diet or rearing facilities produces an unsuitable environment for the survival and development of newly-hatched larvae.

INTRODUCTION

The continuing need for basic research often calls for a large and constant supply of insects and thus for practical mass rearing techniques. Clearly, it is important to have field populations available for specific research projects, but dependence on wild populations in a seasonal climate can create problems. Among these are an irregular supply of the numbers needed in the proper stages and the difficult and time-consuming effort to collect them. Rearing in the laboratory circumvents these difficulties.

Many insect species have been reared to order in the laboratory using artificial diet and natural food sources, especially for research on pest problems in forestry (Wellington 1949, Bergold 1951, Stehr 1954, Heron 1961). In 1965, McMorran described an artificial diet mixture which was suitable for spruce budworm. Several workers have since modified the diet and improved the rearing techniques as well as adapting these to the rearing of several other closely related species (Allen *et al.* 1968, Grisdale 1973). Still further changes in the diet and improvements on the rearing techniques by Lyon *et al.* (1972) and Robertson (1979), made possible the rearing of spruce budworm as diapausing or diapause-free colonies.

This paper reports on the rearing and performance of a non-diapausing colony of the western spruce budworm, *Choristoneura occidentalis* Freeman, on a pre-mixed artificial diet. The rearing was intended to provide a large and constant supply of larvae for research.

MATERIALS AND METHODS

Stock Colony

Pupae of a non-diapausing colony were obtained originally from Dr. J. L. Robertson of the Pacific Southwest Forest and Range Experimental Station, Forest Service, Berkeley, California.

Rearing Environment

The rearing techniques were based on those of Lyon *et al.* (1972) and Robertson (1979). The larvae were reared on ready-mixed artificial diet (Table 1, Bio-Mix #9769, obtained from Bio-Serv, Inc., Frenchtown, New Jersey) in 210-cc clear polystyrene specimen containers with fitted paper covers, the latter being pervious to water vapours (Lab-Tek Product, Division of Miles Laboratories, Inc., Naperville, Illinois). The containers allowed easy observation of the insects' development, and prevented moisture from condensing on the container walls by allowing water loss through the cover. Moisture condensed on container walls could easily drown newly-hatched larvae, and it promoted fungal growth on the diet.

All the rearing stages were held at 26° ± 1°C and 30 to 40% RH, in a room with a 16-h photoperiod, provided by two fluorescent 40-W tubes. The rearing containers were spaced so as to allow light to reach them easily.

Preparation of Diet

The diet was prepared according to instructions

TABLE 1. Spruce Budworm Diet, BIO-MIX #9769, and Mixing Instructions as Provided by the Commercial Supplier*.

Part	Ingredient
A	Agar
	Distilled Water
B**	Casein
	Fiber
	Wesson Salt Mix
	Toasted Wheat Germ
	Methyl Parahydroxybenzoate
	Aureomycin
	Ascorbic Acid
	Choline Chloride
	Sucrose
	Linseed Oil
C**	Vitamin Mixture #722
D**	4M KOH Solution
	Formaldehyde

Mixing Instructions for 1 l of Diet.

1. Add 25.3 g of Part A (agar) to 835 ml of water.
2. While stirring agar solution constantly, bring to a full boil for 1 min.
3. Transfer agar solution to blender. Cool to 65C to 70C, add 135.2 g of Part B, 10 g of Part C, and 5.6 ml of Part D.
4. Blend for 1 minute or until mixed thoroughly.
5. Dispense immediately.

* BIO-SERV, Inc., Frenchtown, New Jersey.

** Premixed by supplier.

from the suppliers (Table 1), and poured immediately into 150 x 25 mm sterilized plastic Petri-dishes, to a depth of about 2 cm, and allowed to cool and set. After 1 to 2h, the diet gel was cut into cubes of about 2 x 2 x 2 cm. Each rearing cup was half-filled with these cubes. If the diet was not used immediately, it was kept uncut in the Petri-dishes at 4°C for up to two weeks.

Propagation of Colony

Pupae and Adults. Pupae were collected from the rearing cups about twice weekly and sexed according to the number of abdominal segments visible on the venter as described by Robertson (1979). The pupae were then placed in brown paper bags of about 20 x 23 x 39 cm with six strips of Scotch wax paper loosely tossed in, each strip 2 to 4 cm wide and 30 cm long. The wax paper provided the newly emerged moths with a support they could grasp during mating and oviposition. Fifty male and 50 female pupae were placed in each bag and held on shelves in the described rearing room.

Eggs. After 7 to 10 days, depending on the age of the pupae collected, the bags were opened and strips of wax paper with egg masses adhering to them were collected. The adult moths were transferred to new bags, prepared as before, for another oviposition. The eggs were collected from these new bags after three days. The procedure was repeated for one more time before the adults were disposed of by deep freezing at -12°C. The strips of wax paper were cut into pieces to separate each egg mass. The eggs and the pieces of paper to which

TABLE 2. Development Time for Non-Diapausing Colony of Western Spruce Budworm, *C. occidentalis*.

Stage of Development	Mean Number of Days[1] Males[2] Mean ± S.D.		Females[3] Mean ± S.D.	
Eggs[4]	7.00	0	7.00	0
Larvae				
1	3.04	0.20	3.40	0.51
2	3.13	0.34	3.13	0.35
3	3.17	0.38	3.20	0.41
4	3.33	0.48	3.33	0.49
5	3.58	0.50	3.60	0.51
6	5.29	0.55	5.87	0.35
Sub-total, Larvae:	21.54		22.53	
Pre-Pupae	0.50	0	.50	0
Pupae	7.88	0.74	7.80	0.68
Adults	11.79	1.38	11.60	1.06
Total:	48.71		49.43	

[1] Number observed was 43, but 3 died as larvae and 1 as a pupa; these were not included for computing the means. The sex was determined at the pupal stage.

[2] n = 24. [3] n = 15. [4] n = 43.

TABLE 3. Fecundity of 10 Female Western Spruce Budworms*, *C. occidentalis:* Number of Eggs per Egg Mass.

	Day of Oviposition**						Total Egg Production per Female
	1	2	3	4	5	6	
	80	81	81	47	56	-	345
	78	78	70	42	46	-	314
	44	63	32	22	62	31	254
	123	65	40	35	31	31	325
	38	37	43	85	28	27	258
	61	72	66	39	45	-	283
	66	52	41	48	33	34	274
	74	104	26	44	44	62	354
	53	98	63	74	51	30	369
	111	35	32	35	35	47	295
Σ	728	685	494	471	431	262	3,071

* Of 12 females observed individually, two did not oviposit.

** Egg masses were laid on successive days, beginning approximately one-half day after mating.

they adhered were immediately surface-disinfected to avoid later contamination of the diet, especially by saprophytic fungi. The disinfectant was 10% formaldehyde solution mixed with a drop of wetting agent, polyethylene sorbitan monolaurate (Tween 20) (Sigma Chemical Company, St. Louis, Missouri). The eggs were stirred gently in the solution for 10 min using a No. 3 camel's hair brush. Disinfectant was followed by two 10-minute washes in distilled water. The egg masses were allowed to dry for 1 to 2h on Whatman filter paper in Petri-dishes.

Larvae. Each egg mass was placed in a rearing cup with 6-10 cubes of diet. The cup was covered with the paper cover and wrapped with aluminum foil around the top-half. Pin-sized holes were punctured through the foil to allow moisture to escape. The cup was then placed on a shelf for the eggs to hatch and the larvae to develop. The aluminum foil wrapping was necessary in order to keep light from the top-half of the cup. Having the bottom-half of the cup illuminated, attracted the positively phototactic, newly-hatched larvae to the diet. Without the foil, young larvae migrated to the tops of the cups, away from the diet, and then diapaused.

After about 15 days each cup was opened and three or four fresh cubes of the diet were added to the partially dried old cubes. The cover was replaced and the cup was returned to the shelf for further development of the larvae.

Life Cycle. One mass of 43 eggs was reared separately. The larvae were separated after hatching and reared individually in 30-cc clear polystyrene cups to determine details of this insect's life cycle on hand of individual insects.

Fecundity. Twelve pairs of newly-emerged male and female moths were put into separate larval rearing cups, containing strips of wax paper, and

placed in a brown paper bag. The insects were observed daily and egg masses removed for counting of the eggs.

RESULTS AND DISCUSSION

Reared as described, the insects reproduced successfully and an ample supply of larvae was available for research. The colony size could be regulated according to need at any time during the course of the reaearch.

The times the insects required to complete each stage of their life-cycle are shown in Table 2. Of 43 insects started, three died as larvae and one as a pupa. The insects lived 48 to 50 days from oviposition to the death of the adults, or 38 to 40 days for a complete generation from egg to egg. It was seen that each of the first five instars required a period of 3 to 3.6 days for their development, whereas the sixth and last instar required 5 to 6 days. The female had the longer period of larval development, and thus, the overall period for completion of the generation. This is consistent with the general observatin that, in the colony, the females normally pupate one or two days later than the males.

Table 3 shows the fecundity of the spruce budworm. The moths mated within one-half day of their emergence. The first egg mass was laid within about one-half day after mating. Five or six egg masses were laid by each female on successive days. The egg masses contained from 22 to 123 eggs. The total number of eggs laid by each female ranged from 254 to 369, with a mean of 307. Most of the eggs were laid during the first two days, with a mean of 707/day. For days 3, 4, and 5, the mean/day dropped to 465; on the 6th day, the number of eggs laid dropped sharply to 262. Two of the observed 12 females did not oviposit, although the mating of one pair was observed.

According to Bio-Serv, Inc., the ready-mixed artificial diet was developed by McMorran (1965) and Grisdale (1973). The synthetic diet has been used successfully for the rearing of several closely related species such as the eastern spruce budworm, Douglas-fir tussock moth, corn earworm, soybean and cabbage loopers. Lyon *et al.* (1972) were successful in rearing colonies of diapausing and non-diapausing western spruce budworm on a slightly modified diet.

Comparing this colony of *C. occidentalis* with those reared in the laboratory by Lyon *et al.* (1972) and Robertson (1979), the following general observations were made: the hatching of the eggs within seven days of oviposition was in complete agreement; larval development in our colony was 7 to 9 days shorter but the pupal stage was longer by 5 to 7 days. The shorter larval period was caused mainly by shorter first and second instars. They lasted 3 to 3.5 days, whereas the reported periods were 9 and 5 days, respectively. The generation time of 38 to 40 days from egg to egg, was shorter here than those reported by Robertson (1979) and Lyon *et al.* (1972), by 3 to 4 days and 9 to 11 days, respectively.

These differences could have been caused by differences in the rearing environment such as the smaller range and higher temperature of 26° ± 1°C and the relative humidity of 30 to 40% used in this work. By contrast, temperatures of 23 to 26°C and relative humidities of 30 to 50% were reported by Lyon *et al.* (1972) and Robertson (1979). In the field, temperature changes have been shown to affect larval development significantly, especially that of first and second instars (Shephard, 1961). According to Peterson (1953) temperature and humidity are probably the most important environmental factors in the habitats where the insects breed. Bursell (1964) stated that within the limits of tolerance the velocity of insect development is greatly affected by temperature. Therefore, the higher temperature used here could be the influential factor, especially in the development of first- and second-instar larvae.

Lyon *et al.* (1972) also studied the relationship of adult age to fecundity. Highest fecundity was obtained when mating took place when the adults were less than one day old. The average number of 174 eggs obtained by Lyon *et al.* (1972) was far lower than the average of 307 recorded here with single-pair mating of adults when both were less than one day old. According to Lyon *et al.* (1972) single-pair matings were not so productive as those of the multiple-pair matings. No definite explanation can be given for the higher fecundity, recorded by us, other than the apparent differences in the overall rearing environment. The latter, however, may well have produced generally healtheir, more vigorous, and thus more fertile insects. The adult life-span here was longer than that reported by Robertson (1979), but the average periods for mating and oviposition were about the same as those observed by Lyon *et al.* (1972).

Sanitation is essential to rearing the spruce budworm successfully in the laboratory on artificial diet. Contamination of the diet by saprophytic fungi and bacteria is a common problem. Although these microorganisms are not necessarily pathogenic, insects did not survive on infected artificial diet. Therefore, any contamination, if detected early enough, must be eradicated at once in order to prevent spreading and total loss of the insect culture. It has been found, in this respect, to be a most valuable safeguard to maintain part of the colony in separate quarters. This allows eradication of an infection in one facility, while breeding is continued elsewhere.

It should be noted that establishing a colony for the present project failed until the paramount importance of sanitation and the value of a spare culture were recognized and remedial actions taken. Before that, larvae always died shortly after hatching, and the colony was lost, because the surfaces of the diet cubes, infected with saprophytes, had become slimy and sticky, thus trapping and killing the newly-hatched larvae.

According to Bio-Serv, Inc., the total aerobic

plate count on microbial analysis of the diet as originally purchased was about 10,000 colonies/g. Mixing the diet ingredients with the agar immediately after boiling, did not reduce the infection. Subsequently, at the time of ordering the diet, it was specified that the diet must yield less than 100 colonies/g. Thereafter, rearing of the spruce budworm on the diets complying with these specifications remained largely problem-free. Occasional infections, occurring during processing, were detected early so that such infections could be eradicated.

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REFERENCES

Allen, D. C., F. B. Knight, and J. L. Foltz. 1968. A technique for rearing *Choristoneura pinus* on artificial diet. Ann. Entomol. Soc. Am. 61:362-364.

Bergold, G. H. 1951. The polyhedral disease of the spruce budworm. *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. J. Zool. 29:17-23.

Bursell, E. 1964. Environmental aspects: Temperature. p. 283-321. **In:** Rockstein, M. (Ed.). The Physiology of Insecta. Vol. I. Academic Press. New York and London.

Grisdale, D. 1973. An improved laboratory method for rearing large numbers of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Entomol. 102:1111-1117.

Heron, R. J. 1961. A method for rearing jack-pine and spruce budworm larvae. p. 3-4 **In:** Can. Dept. Forest., Forest Entomol. and Pathol. Br. Bi-mon. Prog. Rep. 17.

Lyon, R. L., C. E. Richmond, J. L. Robertson and B. A. Lucas. 1972. Rearing diapausing and diapause-free western spruce budworm *(Choristoneura occidentalis)* (Lepidoptera: Tortricidae) on artificial diet. Can. Entomol. 104:417-426.

Peterson, A. 1953. A Manual of Entomological Techniques. 7ed. Edward Brothers, Inc., Ann Arbor, Michigan.

Robertson, J. L. 1979. Rearing the Western Spruce Budworm. Canada-US Spruce Budworm Programs. Forest Service. USDA. Washington, D.C. 18p.

Shepherd, R. F. 1961. A comparison of the development rates of one- and two-year cycle spruce budworm. Can. Entomol. 93:764-771.

Stehr, G. W. K. 1954. A laboratory method for rearing the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Entomol. 86:423-428.

Wellington, E. F. 1949. Artificial media for rearing some phytophagous Lepidoptera. Nature 163:574.