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LABORATORY REARING OF THE PREDATORY COCCINELLID *CLEOBORA MELLYI* [*COL. : COCCINELLIDAE*] FOR BIOLOGICAL CONTROL OF *PAROPSIS CHARYBDIS* [*COL. : CHRYSOMELIDAE*] IN NEW ZEALAND

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Cleobora mellyi Mulsant has been introduced into New Zealand in an attempt to control *Paropsis charybdis* Stål. Artificial diets and a practical method for rearing the predatory Australian ladybird, *C. mellyi*, are described.

The adults and larvae of the eucalyptus tortoise beetle, *Paropsis charybdis* Stål, are the most important defoliators of *Eucalyptus* spp. in New Zealand forests. Defoliation of some species, e.g. *Eucalyptus viminalis* Labillardière and *E. nitens* Maiden, is so severe and repeated that trees may die (**Bain**, 1977). *Cleobora mellyi* Mulsant adults and larvae feed on eggs and 1st instar larvae of *P. charybdis* and other paropsines. Predation by *C. mellyi* can result in up to 80 % mortality of paropsine eggs in the field (**H.J. Elliott**, pers. comm.).

Adults of *C. mellyi* were first introduced into New Zealand by the Forest Research Institute from Tasmania, Australia, in 1977. A few 2nd generation adults were produced from these in the laboratory using *P. charybdis* eggs and small larvae as food for the developing stages (**New Zealand Forest Service**, 1978). Further consignments arrived in New Zealand in 1979 and a rearing method was developed using psyllids (*Psylla acaciaebaileyanae* Froggatt) and *P. charybdis* eggs (**New Zealand Forest Service**, 1979). Unfortunately, the survival of *C. mellyi* larvae in the laboratory was low. Losses from cannibalism were high but the main disadvantage of this rearing method was that it was extremely labour intensive and dependent upon the amount of natural host material available.

In order to produce sufficient numbers for a biological control programme, it was necessary to develop a practical rearing method for *C. mellyi*. Several species of predatory ladybirds have been successfully reared on artificial diets (**Matsuka** *et al.*, 1972 ; **Hukusima & Takeda**, 1975 ; **Singh**, 1977 review ; **Okada & Niijima**, 1977 ; **Kariluoto**, 1980 ; **Racioppi** *et al.*, 1981) but there have been difficulties in formulating diets with adequate phagostimulatory properties and nutritional completeness. This paper reports practical artificial diets and a rearing method for *C. mellyi*.

MATERIALS AND METHODS

COMPOSITION OF ARTIFICIAL DIETS

Several artificial diets were originally formulated. The composition of the 3 best larval diets and the best adult diet is shown in tables 1 and 2.

TABLE 1

Composition of artificial larval diets for Cleobora mellyi

Ingredient	(g)		
	PL	PT	HG
Freeze-dried pork liver (PL)	8.0	–	–
Freeze-dried potato tuberworm larvae (PT)	–	8.5	–
Freeze-dried huhu grub (HG)	–	–	10.0
Sucrose	4.0	1.0	1.0
Yeast hydrolysate (Yeast Products Inc., 25 Styertowne Rd, Clifton, NJ, USA)	2.0	0.5	–
Casein hydrolysate (ICN Pharmaceuticals, Cleveland, Ohio, USA)	0.5	–	–
Liver extract (BioServ Inc., PO Box BS, Frenchtown, NJ, USA)	1.0	–	–
Vanderzant vitamin mix (ICN Pharmaceuticals, Cleveland, Ohio, USA)	0.2	–	–
Vitamin A (Koch-Light Laboratories, Colnbrook, Bucks, UK)	–	0.01	–
Vitamin C (Pharmaceutical Sales and Marketing, PO Box 40079 Glenfield, Auckland)	0.1	0.025	–
Vitamin E acetate (Sigma Chemical Co., St Louis, MO, USA)	–	0.01	–
Cholesterol (Sigma Chemical Co., St Louis, MO, USA)	0.05	–	–

TABLE 2

Composition of artificial adult diet for Cleobora mellyi

Ingredient	Amount
Agar (Davis Gelatine (NZ) Ltd., Christchurch, NZ)	1.5 g
Sucrose	16.0 g
Honey (Sanitarium Health Foods, Private Bag, Auckland, NZ)	6.0 g
Casein (NZ Coop Dairy Co., PO Box 459, Hamilton, NZ)	3.5 g
Bacto-peptone (Difco Laboratories, Detroit, MI, USA)	1.5 g
Vitamin solution (Berger, 1963)	5.0 ml
Water	100.0 ml
Oxo (R) (Beef extract) (Oxo (NZ) Ltd., Christchurch, NZ) (1 cube)	6.0 g

PREPARATION OF ARTIFICIAL DIETS

Pork liver diet (PL)

Fresh pork liver was cut into 5 — 10 g pieces, frozen, placed in a vacuum flask, freeze-dried for 48 h at a water vapour vacuum of approximately 3.2×10^{-1} mbar in a Virtis or an Edwards EF6 freeze-drier, then powdered in a Waring Blender. The sucrose and yeast hydrolysate were ground to a fine powder in a large mortar and pestle. The vitamins, cholesterol, liver extract, casein and pork liver were then added, thoroughly ground and mixed in.

Potato tuberworm diet (PT)

Potato tuberworm larvae were reared in the laboratory according to the method described by **Finney** *et al.* (1947). Larvae were coddled at 55°C for 5 mn in water with 3 % Chlorodux([1]) added to dissolve the silk cocoons. They were then air-dried on paper towelling for an hour, freeze-dried and powdered in a blender. The sugar was finely ground with the vitamins and yeast hydrolysate in a mortar and pestle, then the potato tuberworm powder was added and mixed in.

Huhu grub diet (HG)

Huhu grubs (larvae of *Prionoplus reticularis* White (*Col. : Cerambycidae*)) were collected from logging residues in *Pinus radiata* D. Don plantations that were thinned to waste 2-3 years previously. Larvae were freeze-dried, blended and powdered. Excess hexane was added to extract surplus fats and oils. After filtering, the solid residue was dried and mixed with 10 % by weight of finely ground sugar. Further information on the biology of *P. reticularis* can be found in **Hosking** (1978).

Adult diet

The agar and water were heated to 120°C to dissolve the agar. The Oxo cube (beef extract) was ground to a powder with the sucrose, casein and Bacto-peptone. The agar solution was cooled to 60°C before adding the Oxo mixture. Finally the honey and vitamin solutions were mixed in thoroughly.

All artificial diets can be kept for several months when stored below 4°C.

REARING

C. mellyi were reared at 25°C, 50-60 % RH under a 16 h photoperiod in a controlled environment room with continuous fresh air circulation. The development on 3 artificial larval diets and on eggs of *P. charybdis* plus psyllids was compared.

Two types of containers were used to rear *C. mellyi* from egg to adult. Container A (fig. 1) consisted of a 95 (80) diameter × 65 mm high unwaxed (i.e. made of unwaxed paper) 8 oz Dixie (R) cup with a transparent lid (No. 2188, American Can Co., USA). A 60 (50) mm clear plastic cup containing water and with a soft plastic lid was positioned beneath the Dixie cup. Holes 23 mm in diameter were made with a cork borer in the bottom centre of the Dixie cup and the lid centre of the plastic cup. A piece of 50 mm long clear plastic hose of 23 mm external diameter passed through the holes with a snug fit. Two 150 mm cotton dental roll wicks, doubled over, had previously been placed inside the plastic tube. These were kept moist with water from the bottom cup.

([1]) Chlor-o-gene Supplies Ltd, Petone, New Zealand.

Container B (fig. 2) consisted of 2 opaque plastic cups, one within the other (UEB Industries NZ Ltd., Auckland, Nos 395SP and 535SP). The lower cup held water. A cellosene wick was positioned between the top and bottom cups through a 15 mm PVC electrical conduit plain-to-screw connector. The lid was a clear plastic 9 cm Petri dish with a gauze-covered, 25 mm diameter hole for ventilation.

Both containers were provided with a piece of concertina-shaped paper towel or a circle (held by a staple) of paper towel to reduce cannibalism by providing a greater surface area for the larvae. About 0.5 g of diet was then sprinkled over the bottom of the rearing cup. Ten neonate larvae were placed on the bottom of the containers with a fine camelhair brush. Diet was replenished as necessary during larval development. Pupation and adult emergence occurred in the same cup.

Adults oviposited on paper towelling in container A or in 9 cm glass Petri dishes. Oviposition was initiated by feeding adult beetles with *P. charybdis* eggs from a laboratory colony and *Psylla acaciaebaileyanae* collected from acacia trees (*Acacia* spp.). Eggs were collected daily. Each wick, cup and plastic hose was changed weekly to minimise bacterial contamination. When *P. charybdis* eggs were in short supply or unavailable the adult diet was used as a substitute.

RESULTS

The 3 larval diets were all suitable for rearing *C. mellyi* to adult. The developmental periods of *C. mellyi* immature stages reared in the laboratory are shown in table 3.

TABLE 3

Developmental period (days) of Cleobora mellyi *immature stages reared at 25°C, 50-60 % RH, 16 h photoperiod*

Diet	Egg	Larva	Pupa	Total
Pork liver	4.6 ± 0.2	25.8 ± 1.0	7.5 ± 0.2	37.9
Tuberworm	4.6 ± 0.2	10.2 ± 0.2	7.6 ± 0.2	22.4
Huhu	4.6 ± 0.2	25.5 ± 0.4	7.5 ± 0.2	37.6
Natural host	4.6 ± 0.2	16.7 ± 0.4	7.5 ± 0.2	28.8

Development was fastest on tuberworm diet on which the larval period was greatly reduced. The mean egg development time and pupal periods on all diets were similar.

There were no significant differences ($P = .0.05$) in the weights of adults reared on the 3 larval diets (table 4), but 9 % of adults produced from larvae fed liver diet were deformed. The number of deformed adults was insignificant on the other diets.

Adults produced from larvae fed on the diets produced few or no eggs when maintained on the adult diet and/or *Paropsis* eggs. However, oviposition in the adults could be initiated by providing psyllids, scale insects or aphids for 7-8 days. Average egg fertility of adults raised on both natural and artificial diets was relatively low (table 4) and varied from 40-58 %. Adults produced from huhu diet produced the usual complement (cf **Elliott & de Little**, 1980) of

TABLE 4

Fertility, adult emergence and adult weights of Cleobora mellyi

Diet	Egg fertility (%)	Adult emergence from neonate larvae (%)	Mean adult weight (mg)
Pork liver	40.0	58.3	46.0 ± 2.68
Tuberworm	No data	75.5	47.2 ± 1.35
Huhu	55.8 ± 10.8	49.0	45.1 ± 2.2
Natural host	57.9 ± 11.2	43.0	47.2 ± 4.35

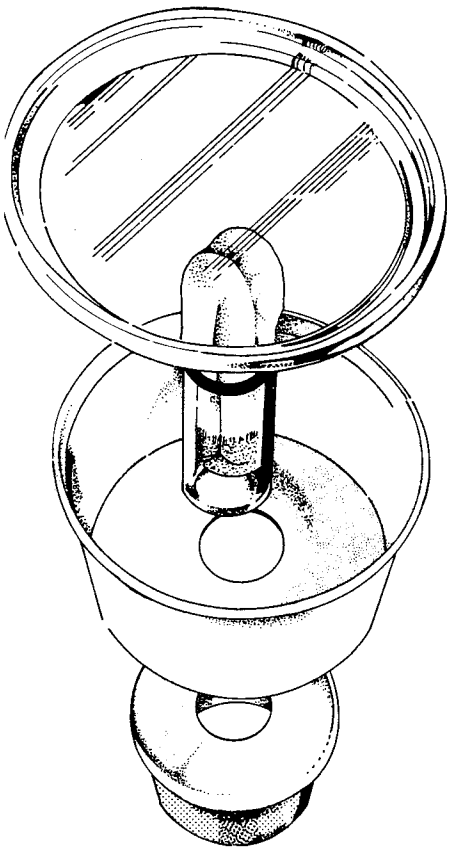

Fig. 1. Rearing container A, used for both larvae and adult.

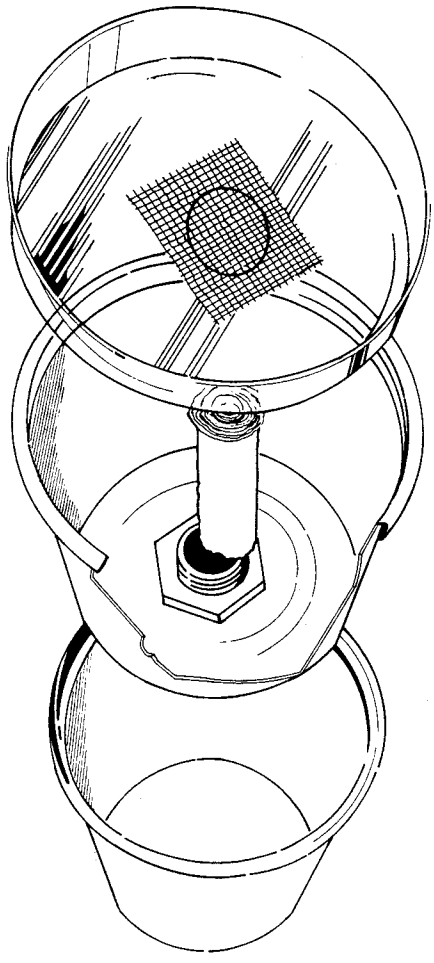

Fig. 2. Rearing container B, used for larvae.

viable eggs in normal-sized batches (15.9 ± 3.1 eggs/batch compared with 16.2 ± 3.1 eggs/batch on natural host). The fecundity of adults produced from the other 2 diets was not recorded. The percentage of neonate larvae reaching the adult stage was highest on potato tuberworm diet. In fact, the production of adults from larvae was greater on all 3 larval diets than on natural host (table 4).

DISCUSSION

The 3 larval diets reported are suitable for rearing *C. mellyi* in sufficient numbers for biological control programmes. However, the developmental periods of the immature stages are different on the 3 diets.

The plastic B-type container was designed and tested for rearing *C. mellyi* in New Zealand because of the unavailability of the unwaxed Dixie container A which is made in the United States. Insects reared in container B had higher larval and pupal mortalities. This container is made completely from plastic and relies on the gauze in the lid for ventilation. The liver and potato tuberworm diets are hygroscopic and mould develops in this container. Container A is considered a better container as its porousness allows continuous exchange of air and mould rarely establishes on the diet.

In New Zealand the hubu grub diet is the most convenient to use. The grubs are always available for collection and the diet has excellent keeping quality because it has a low fat content. Mould is also far less of a problem.

The adoption of the huhu diet on a production basis at the New Zealand Forest Research Institute has resulted in substantial improvements to the rearing programme. The main advantages of the diet are (1) its ease of preparation, (2) larval cannibalism is reduced, (3) it is 4 to 5 times more labour-saving than the use of natural hosts and (4) easier planning for biological control programmes because of reduced dependence on natural hosts.

Adults reared on all 3 artificial diets required psyllids or scale insects for 7-8 days before oviposition occurred, and adults fed exclusively on *P. charybdis* eggs mated and oviposited only infrequently. So even although the larvae of *C. mellyi* develop normally on *P. charybdis* eggs this food must be considered supplementary or alternative (*sensu* **Hodek**, 1973 ; **Mills**, 1983) because it does not promote normal oviposition. In contrast, **Elliott & de Little** (1980) stated that field-collected *C. mellyi* adults mated and oviposited frequently in the laboratory when supplied with *Chrysophtharta bimaculata* (Olivier) eggs.

Irrespective of whether they are reared on natural food or artificial diet most adults must overwinter before they mate and oviposit regularly. Only very few mate and oviposit without doing so and even then only after an extended period of feeding. This is not an obstacle to efficient laboratory rearing as overwintered adults mate and oviposit over an entire season. The greatest impediment to developing a more efficient laboratory rearing programme is the adults' apparent dependence on psyllids, etc. to ensure their fecundity. This is not seen as a difficulty with the field establishment of *C. mellyi* as these sources of food, especially *Ctenarytaina eucalypti* (Maskell), are readily available in eucalyptus plantations.

The pork liver diet was easy and cheap to prepare, and larvae developed well on this diet in A-type containers. B-type containers could be used with only limited success provided the humidity inside the container was maintained around 50 %. The total developmental period was the same on liver diet as on huhu diet. The percent adult emergence from neonate larvae reared on this diet was greater than on huhu when A-type containers were used. Container A used in conjunction with liver diet is recommended for practical rearing in places outside New Zealand unless potato tuberworms are readily available.

Larvae reared on potato tuberworm diet had the shortest developmental period and gave the best yield of adults. They were best reared in A-type containers because of the hygroscopic nature of the diet. A major disadvantage of using this diet is in maintaining the colony of potato tuberworms. It is time-consuming, labour-intensive, and expensive.

Reasons for the low and variable percent egg hatch found in all laboratory-reared adults are unclear, but could be nutritional. **Elliott & de Little** (1980) reported high egg viability in field collected adults fed on eggs of *Chrysophtharta bimaculata* (Olivier), another paropsine eucalyptus defoliator in Tasmania.

In the 1981-82 rearing season, when the diets and techniques were still largely experimental, approximately 3000 *C. mellyi* adults were reared for field release. They were liberated in a *Eucalyptus nitens* plantation in the central North Island of New Zealand which had high *Paropsis* and psyllid populations. Later in the season, eggs, larvae, pupae and teneral adults were found in the field. However, in a 1982-83 survey, there was no evidence of field establishment. Further study of their field establishment is continuing.

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RÉSUMÉ

Élevage en laboratoire de la coccinelle prédatrice, *Cleobora mellyi* [*Col. Coccinellidae*] pour la lutte biologique contre *Paropsis charybdis* [*Col. Chrysomelidae*] en Nouvelle-Zélande

Cleobora mellyi Mulsant a été introduit en Nouvelle-Zélande pour tenter de lutter contre *Paropsis charybdis* Stal, ravageur des forêts d'eucalyptus. Des milieux artificiels et la méthode utilisés pour l'élevage de cette coccinelle australienne prédatrice, sont décrits.

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