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LABORATORY TECHNIQUES FOR MAINTAINING CULTURES OF THE MONARCH BUTTERFLY.

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INTRODUCTION

THE METHODS DESCRIBED HERE represent the experiences of many years of work carried on at the University of Toronto and University of Delaware. Some of the procedures reported have been developed at both laboratories while others were developed at one particular laboratory.

Our research has sometimes required mass rearing for migration studies, and at other times the rearing of smaller numbers for studies on various research projects such as the study of scent receptors, scent organs, development rates, virus infections, light periodicity, behaviour, and chemoreceptor stimulation by the food plant.

For student laboratory exercises, the Monarch butterfly has proved to be an effective species for illustrating various aspects of invertebrate physiology such as, location of scent receptors, responses to light and temperature, effects of temperature on development rate, flight mechanisms, and receptor-oviposition responses. The Delaware Laboratory has supplied approximately one hundred Elementary School Teachers in many States with viable eggs with which to carry out studies of the Monarch butterfly life cycle.

The present paper outlines the culturing procedures we have followed in one or both laboratories, with a brief discussion of the use of artificial diets in maintaining living specimens of all stages during the winter months.

ADULTS

Flight Cages (60 cm. x 90 cm. x 90 cm.):

These cages, of wood frame and cloth screen construction, have one side left open. The open end is fitted with a hinged, screened door or is covered with cheese cloth; the latter is preferred since it allows easy access to the interior of the cage without liberating any of the actively flying butterflies, because the cheese cloth can be pulled slightly aside allowing the specimen to be captured, at the same time keeping the cloth secured both on the cage and around one's arm as one reaches into the cage. This open end also allows for introducing milkweed plants. By turning the cage so that the open end faces upward, it can also be used for mass rearing (Urquhart, 1960).

Oviposition Cage (30 cm. x 30 cm. x 60 cm.):

This is a smaller cage of the same construction as the flight cage, but it is open at the top and bottom. The cage is placed over a growing milkweed plant and the top covered with a piece of glass or a wood frame covered with plastic sheeting or cloth screen. The removable frame top or glass is pushed to one end thus allowing for removal of the eggs from the milkweed plants without allowing the escape of the occupants. The smaller cage forces the gravid females to come into frequent contact with the milkweed plants, thus stimulating more rapid oviposition.

Cylindrical Cage (90 cm. in height x 50 cm. in diam.):

This type of cage is constructed of wire mesh and equipped with two openings — one near the top and one near the bottom. The openings are closed with wire mesh doors which swing open in or out on rings located at the tops of the doors. No latches are required. A wire mesh bottom keeps the butterflies from coming in contact with the honey solution that may have accumulated on the floor of the cage. With the bottom left open, a smaller form of this cage could be placed over a milkweed plant for oviposition.

Cartons and Envelopes:

We have held adults alive for periods in excess of five months in small cardboard cartons (pint-sized freezer cartons). A small cotton pad, soaked in a four per cent honey solution, placed in the bottom of the carton, maintains a high R.H. and, at the same time, supplies the necessary nourishment. The carton is completely sealed. The specimens are held at 7°C. and removed to 21°C. for two hours once each week which allows sufficient activity for feeding and body movement. Specimens were also held in freezer cartons at 16°C. continuously for four months, the temperature being low enough to decrease activity and still sufficiently high to allow for feeding and body movement. The low temperature decreased the incidence of mould formation and decreased the rate of fermentation of the honey solution — the latter was renewed once a week.

Specimens were held in cellophane envelopes (6 cm. x 12 cm.) for periods in excess of one month. A small piece of cotton, soaked in honey solution, was placed in one corner of the envelope and the butterfly, with wings folded together, was placed in the envelope with its head close to the cotton. The cotton was charged periodically by inserting the needle of a hypodermic syringe through the envelope and into the cotton. The envelope was sealed off by folding the free end and securing it with a paper clip. Specimens were held at 16°C.

Feeding:

Monarch butterflies may be held throughout the winter on a diet of 10% honey-water solution. Among many dispensing methods, the following has proved practical for use with cylindrical cage. Aluminum sheeting 1 mm. thick with 1 mm. holes spaced 1 mm. apart, is bent to fit the outside of the cage and held in place with hooks. The holes in the metal membrane serve as reservoirs for the honey solution. To feed the butterflies, the metal membrane is washed thoroughly and replaced on the cage with the water remaining in the small perforations. It is sometimes necessary to place the specimens in the vicinity of the metal membrane in order to instigate feeding. In doing so, care must be exercised in removing the butterflies from their hold on the cage wire as their tarsi may be broken, making it impossible for them to cling to the cage or to the milkweed plants.

When the butterflies have had a drink of water, honey solution is added to the membrane with a squeeze bottle. It may be necessary to "train" some of the butterflies to use the feeding membrane by first inducing feeding by unrolling the proboscis with the point of a pipette or squeeze bottle and giving the butterfly a small drop of honey solution. They can then be placed on the feeding membrane where they will continue to feed by themselves.

Cheese cloth wicks, suspended inside the cages from one side of the cage to the other, and with the free ends secured by clip pins to the top of the cage, will hold the solution for a twenty-four hour period or more, depending upon the humidity of the environment. Such wicks can be charged with honey solution by means of a squeeze bottle, applying the solution to the free ends of the wick.

Synthetic sponges, cut into thin strips, can be inserted into longitudinal slits (placed near the top of the cage) made in the cloth screen. These can be recharged without opening the cage.

Continuous feeders, designed after the principle of the intravenous feeder, were found useful when the cages could not be attended to for a few days. This dispenser consists of a gallon jar fitted with a two-holed rubber stopper into which is inserted a glass tube of sufficient length to reach the air space above the liquid when the jar is inverted, and a second glass tube, 10cm. longer than the stopper, protruding a sufficient length to allow for the attachment of a plastic tube approximately two feet long. The jar containing the honey solution, is suspended above the cage in an inverted position. The rate of flow of the solution is controlled by a Hoffman Clamp (screw compressor type). The free end of the wick, suspended from the roof of the cage, passes into a gallon jar.

Plastic vials have been used successfully by G. Grisdale of the Insect Pathology Research Institute, Sault Ste. Marie, Ontario. Shell vials (13 x 35mm) filled with honey solution, are taped together in bundles of six or more. These vial clusters are then suspended from the upper part of the cage.

Humidity:

Humidity is an important physical factor in the successful maintenance of adult butterflies over long periods of time. At the University of Toronto laboratory an R.H. in excess of 90% is maintained in the growth chambers. Adults, collected or reared in late August, have been kept alive until the following April at which time they were successfully mated with resulting viable eggs.

Reproductive colonies are maintained the year round at the University of Delaware in a small laboratory with no special arrangements except the operation of a small humidifier and the maintenance of a temperature between 21° and 27°C. Adults have been kept alive for periods of four months under these conditions.

Oviposition:

Gravid females will readily oviposit on milkweed leaves either as growing plants or as individual leaves suspended in the cage. The plants are placed at the side of the cage that faces the most intense illumination. The butterflies congregate at the side of the cage where the light is most intense thus coming into more frequent contact with the milkweed plants. Scent receptors are located on the mesothoracic legs, (Urquhart, 1960) and contact with the milkweed leaves triggers off the process of oviposition. So long as the tarsi rest upon the leaf, the egg is deposited on any adjacent surface. Thus, eggs may be deposited on a piece of paper placed at the tip of the abdomen. This procedure is especially useful when the eggs are to be shipped by mail. The eggs develop normally on paper.

Females will oviposit on thawed milkweed as well as on a paste of dehydrated milkweed. Females will sometimes oviposit on a cloth soaked in fermented honey solution. It is not unusual to find hundreds of eggs on the feeder wicks as a result of this peculiar reaction.

Females will lay eggs even when held by the wings. Several eggs may be deposited in quick succession followed by a pause. If the specimen is allowed a brief period of flight, or to move its wings while being held, oviposition will take place more rapidly than when the specimen is not permitted such activity. Although when caged, the females may lay dozens of eggs on one plant, in their natural surroundings the eggs are widely scattered – rarely are two eggs found on a milkweed leaf.

The leaves, bearing the eggs, may be placed in a petri dish or small jar until hatching has taken place. Hatching may be delayed by holding the eggs at a low temperature or accelerated by high temperature.

Potted milkweed plants are desirable for oviposition since they can be left in the cage without deterioration and the larvae will have fresh food available when they hatch. Milkweed can be propagated from seeds or from root stalks, the latter being the quicker method. *Ascelpias currasavica* is convenient because it sprouts new shoots readily after the tops have been cut off, and it is easily propagated from stem cuttings.

Since larvae often devour the egg shell upon eclosion they may also devour adjacent eggs. To prevent this, the eggs are separated by cutting the leaves into small fragments, each fragment bearing an egg.

Rearing Containers:

Petri dishes are most suitable for early instars, and glass or plastic jars or tubes for more mature forms. There are many different kinds of containers that are satisfactory for holding large larvae but tight covers are necessary for the smaller ones. When large containers are used, the resulting pupae can be left until the adult butterfly matures. Or, a piece of paper or cardboard can be fastened to the top of the jar and in this way the pupae can be transferred to another jar or cage by simply removing the paper.

When rearing large numbers for migration studies, eggs are placed in wide-mouthed gallon jars with screw tops. When the larvae reach the third instar, they are placed in flight cages. As many as three hundred larvae can be reared in one cage

(Urquhart, 1960). Pupae are formed on the wood frame where they remain until the emergence of the adults.

Flight cages have also been used successfully for rearing large numbers by placing the open end of the cage over a stand of milkweed plants upon the leaves of which a number of larvae have been placed. When the plants have been consumed, the cage is placed over a fresh stand and the larvae transferred.

Food:

Although fresh milkweed leaves, grown in a green house during the winter months, are most effective, frozen leaves, attached to the stalks, which have been thawed, are used successfully. The leaves should be cut before the seed pods are formed – small, young plants being preferred. The thawed leaves may be supported on a wire-mesh platform or hung on the side of the rearing cage. Thawed leaves must be removed daily since deterioraiton is rapid.

A stand of milkweed can be harvested several times each summer if only the tops of the plants are cut off each time. The old stalk will send out new shoots. If the mature milkweed plants in a field have no insects on them, such as milkweed bugs or beetles, there is a possibility that the field has been sprayed with an insecticide. At least, this possibility should be considered before starting a research project dependent on this source.

To retain the highest nutritive quality in field-cut milkweed, freshly cut stalks, placed in plastic bags, are stored in a field cooler containing dry ice. The bags of milkweed are transferred to a deep freezer refrigerator as soon as possible.

Larvae can be fed on a paste of dried milkweed powder reconstituted with distilled water. Apparently this food is not as stimulating to the appetite or as nutritious as fresh milkweed. The resulting adults, the larvae of which have been so fed, are small, but otherwise normal.

Dale Grisdale (loc. cite) has used an artificial diet, reported by McMorran (1965), with considerable success. Grisdale reports that newly hatched larvae of the Monarch butterfly accepted the food readily and almost one hundred per cent of the larvae established on the food. In order to start the larvae, the artificial food is mixed with powdered milkweed leaves. Without the addition of the powdered leaves, no feeding was evident and all the larvae died during the first instar. He also reports that the adult females were sterile.

Nanavati, working at the University of Delaware, has reared Monarch larvae to adulthood on this medium both with and without the milkweed fraction.

Lyon and Flake (1966) used a similar diet in connection with their studies of the Douglas-fir tussock moth larvae.

E. Alan Cameron of the Division of Entomology, University of California, Berkeley, has used the following diet for rearing the tussock moth. This diet is based on that of Lyon and Flake.

TABLE 1

Composition of Ingredients

Ingredients	*Quantity*
A.	
Caesin-vitamin free	87.5 g
Alphacel	12.5 g
Salt mixture — W	25.0 g
Sucrose	87.5 g
Wheat embryo	75.0 g
Choline Chloride	2.5 g
Ascorbic acid	10.0 g
Methyl parahydroxybenzoate	3.75 g
Aureomycin	0.75 g
Potassium sorbate	5.0 g
B.	
Water, distilled	550.0 ml.
4 M Potassium hydroxide	12.5 ml.
Vitamin solution	25.0 ml.
Formaldehyde (36%)	1.25 mo
C.	
Nutrient agar	62.5 g
Water	1550 ml.

Mix solution "A" and "B" in blender. Add "C" after melting. Pour into wax-paper lined shallow pans or teflon-coated pans.

TABLE 2

Vitamin Solution

Ingredients	*Quantity*
Water	100 ml.
Nicotinic acid (niacin)	100 mg.
Calcium pentothenate	100 mg.
Riboflavin	50 mg.
Thiamine hydrochloride	25 mg.
Pyridoxine hydrochloride	25 mg.
Folic acid	25 mg.
Biotin	2 mg.
Vitamin B-12	0.2 mg.

Disease and Diapause:

Two factors may cause difficulty in rearing the Monarch butterfly successfully throughout the winter months. One is the presence of a virus disease and the other diapause females.

During the past two years ,and commencing three years ago, the natural populations of the Monarch butterfly in North America have been greatly reduced as a result of a polyhedrosis virus epizootic (Urquhart, 1966). Hence, a high mortality has been experienced in our laboratory cultures regardless of feeding methods used. Even careful sterilization methods have proved ineffective in preventing infection.

It is possible to rear continuous generations of the Monarch butterfly during the winter, starting with eggs of gravid females collected in the field during the summer and fall. Monarch females collected in late September at Cape May, New Jersey, readily laid eggs on milkweed when brought into the Delaware laboratory. No special treatment was necessary. However, at the University of Toronto, it was found that although females collected in the spring and early summer produced continuous generations throughout the winter months migrant females collected in late summer and early fall from over-night roosting sites, did not oviposit even when kept for periods of five months. Similarly, over-wintering specimens from Monterey, California collected from over-wintering sites during December and January would not oviposit. Dissected females collected during January and February from such over-wintering sites rarely contained eggs (Urquhart, 1960). In March, however, many females were gravid prior to their spring migration flight.

Francis Munger, of Whittier, California, who has been working with us at the University of Toronto for the past number of years, reared successive generation of Monarch butterflies throughout the winter months. Larval Monarch butterflies have been sent to us by Miss Martha Lussier of Orlando, Florida, in January.

The possible factors involved in the production of a reproductive diapause in the migrating and over-wintering populations are being studied at the laboratories of the University of Toronto.

Note: Monarch eggs, with which to start a breeding colony, are usually available from Dr. Stegner.

Reprints of this article may be obtained by writing to Dr. Stegner.

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