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**AN ALTERNATIVE TO USING FRESH ALFALFA FOR
CULTURING *COLIAS PHILODICE EURYTHEME*
BOISDUVAL (LEPIDOPTERA: PIERIDAE)¹**

L. J. Crain, S. J. Roberts, and E. J. Armbrust^{2,3}

ABSTRACT

Various artificial materials were implemented at each life stage of the alfalfa caterpillar, *Colias philodice eurytheme* Boisduval, to provide effective and economical replacements for alfalfa. Alfalfa substitutes were used for oviposition and feeding. We also developed a favorable laboratory mating environment. Also, the development of a method for removing disease organisms from eggs without increasing egg mortality was an essential facet of this work. The performance of individuals on artificial surfaces was compared with similar individuals reared on greenhouse alfalfa.

Green tape oriented at the top of oviposition cages or near some object which could be held on to while ovipositing was the most successful surface. Egg yields on green tape were comparable to those on alfalfa. A dilute bleach wash followed by two water washes required little time, yet provided adequate disease control, and all larval instars thrived on artificial diet. Methods for handling pupae and new adults and the favorable mating environment reduced colony maintenance time yet survival rates insured propagation of the colony.

INTRODUCTION

Conventional laboratory rearing methods for the alfalfa caterpillar, *Colias philodice eurytheme* Boisduval, require the use of fresh alfalfa (Floyd, 1940; Stern, 1960; and Martignoni, 1961), and this has several drawbacks: (1) alfalfa must be frequently added to rearing containers and this time consuming process makes large colonies impractical, (2) alfalfa must be available throughout the winter, necessitating substantial greenhouse space, and (3) larvae crowded on a few plants increase the chance of disease epizootics mentioned by Martignoni (1961).

Previous methods used in our lab involved rearing larvae on alfalfa until third instar, then transferring them to individual 1 oz medicine cups containing the alfalfa powder diet recommended by Henneberg (1966). Tanada (1969) raised all instars on a pinto bean diet which decomposed too quickly for our purposes.

This research was designed to find an effective, economical, and disease-free alternative to alfalfa for rearing the alfalfa caterpillar. The requirements of this system were as follows: (1) a surface which will induce significant levels of oviposition, (2) an egg retrieval process which is not overly time consuming and removes disease organisms from the eggs, (3) an artificial diet which permits 1st and 2nd instar survival, and (4) an environment which is conducive to mating.

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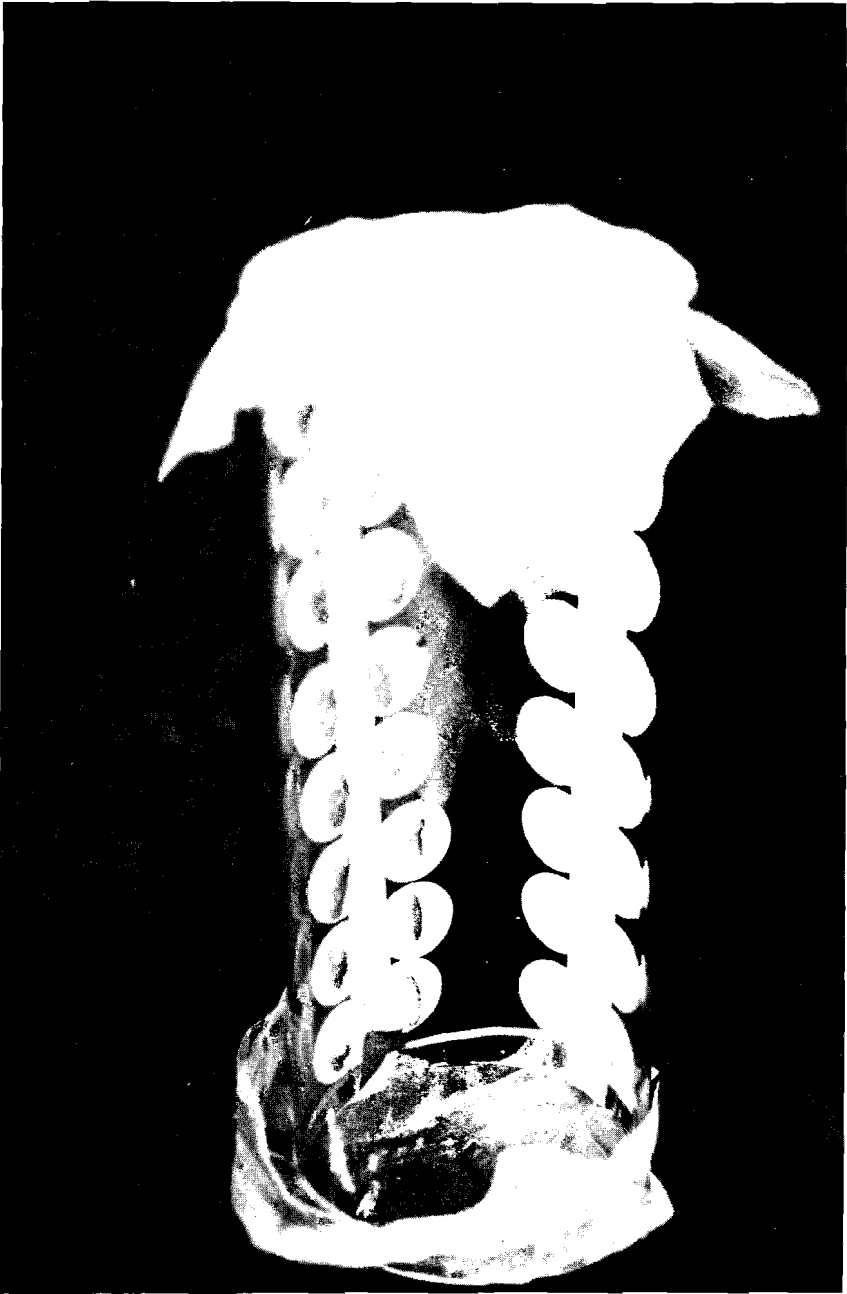


Fig. 1. Pupal emergence cylinder. For purposes of illustration artificial pupae were used.

METHODS

Oviposition cages were 1 pt cardboard Fonda[®] cartons which included a wire mesh top, filter paper on the bottom, and a hole cut in the side to fit a rubber stopper for controlled access. A sucrose solution (33gm/100ml H₂O) was placed in a plastic nectar cup pushed through another hole in the side of the carton. Each of four cages (replicated two times) contained a different artificial oviposition surface. The following surfaces were used: wax paper, paper towel, green tape,⁴ and microscope slides covered with green tape. The wax paper and paper towel were cut into strips ca 1.5 cm wide and 8 cm long. The strips were fitted securely into small vials, then were folded into branching shapes resembling alfalfa. The green tape was placed on the wall of the cage along the rim. The tape-bearing microscope slides rested against the inside wall of the cage.

The surfaces were selected to provide variety in texture, color, and configuration. Many of these criteria were used by Pedigo (1971) for oviposition studies with the green cloverworm, *Plathypena scabra* (Fabricius). As a control, eight additional cages contained fresh bouquets of three to five stems of alfalfa in vials of water. Five mated females were placed in each cage. All cages were placed in constant temperature cabinets and maintained at 27 ± 5°C with a 12 hr photophase and 65 ± 10% relative humidity. Dead females were removed and eggs were counted daily.

After we determined that green tape provided the most successful surface, all eggs for larval rearing were obtained from this surface. Egg-bearing tape was peeled from the cages and washed in a solution of Chlorox[®] bleach and Triton X-100[®] (Leppla, 1974) to minimize the presence of disease. Following the bleach wash, the eggs were rinsed twice for 15-30 seconds in small bottles of water. The tape strips were then stuck to the inside wall of a ½ pt Fonda carton for hatching. A moistened dental wick was added to prevent dessication during incubation. The cartons were checked daily for new larvae. Larvae were placed in individual 1 oz plastic cups containing artificial diet.

After about 18 days, larvae usually attached themselves to the underside of the cardboard diet cup lids and pupated. Lids were then stuck to long strips of tape and hung in clear plastic emergence cylinders (Fig. 1). Those pupae not attached to lids were placed directly on tape strips. Fine cloth mesh was placed over each end of the cylinders to prevent escape of newly emerged adults.

Adults began to emerge in three to five days and were held in a 10°C constant temperature cabinet until large numbers were obtained. Individuals with normal wing development were placed in a large mating cage (122 cm × 122 cm × 183 cm) equipped with a fan and a combination of 'black' and fluorescent lights on a 12 hr photophase (Fig. 2). The adults were also provided with a sucrose solution in a petri dish covered with wire mesh as a food source (Stern and Smith, 1960), and a bouquet of alfalfa which was periodically checked to determine when viable eggs were being laid. Viability was determined by Michelbacher's and Smith's (1943) observation that pink eggs are viable. In our experiments the eggs turned pink 48 hr after being laid.

DISCUSSION AND RESULTS

Of all the artificial surfaces, the females preferred green tape oriented at the top of the cage or near some object which they could hold on to while ovipositing. Tape strips placed along the top of the cage enabled the females to grasp the wire mesh top while touching the tape with their abdomens. In cases where eggs were not laid on tape, they were laid near other projections in the cage, such as around the nectar cups.

Average egg yields were 30.88 per female on alfalfa and 28.74 on green tape. These results show that green tape is an effective substitute for alfalfa, since the tape yielded 93 percent as many eggs as the alfalfa. Microscope slides covered with green tape, although designed to conveniently handle eggs as a unit, were hard to manipulate and only fairly effective in stimulating oviposition. Paper towelling folded in the shape of a fan was also

⁴Professional Tape Co., 144 Town Road, Burr Ridge, IL. Item No. 25421.



Fig. 2. Adult mating cage.

effective, but was not as easy to handle. The brown color of the towels impeded egg counts, and possibly enhanced female mortality by limiting mobility for feeding and/or oviposition. Wax paper did not stimulate oviposition.

The use of green tape as an oviposition surface facilitates transfer of eggs from oviposition cage through the washing process and then to the hatching container. One merely grasps the tape with forceps, dips the eggs into the bleach wash, then into two water washes, blots them on paper towel and places them in the hatching container. Setting tape pieces on alfalfa diet on the floor of the container caused high levels of mortality when larvae crawled to the sticky underside of the tape. It proved impossible to dislodge them intact. A better arrangement would be to stick egg-bearing strips of tape to the walls of a hatching container. Little feeding took place when first instars were promptly transferred to individual diet cups, so diet could be eliminated from the hatching container. The moistened dental wick was necessary to provide adequate moisture. On occasions when the diet dried out and became unacceptable to the larvae they were transferred to new diet to avoid starvation. This problem could be avoided by providing more diet initially.

Table 1. Survival of larvae, pupae and adults* of *Colias philodice eurytheme* Boisduval.

Life Stage	Sample Size	Percent Survival ± 95% Confidence Limits
Larvae		
alfalfa	201	48 < 55 < 61
artificial diet	258	84 < 89 < 93
Pupae		
tape	192	53 < 62 < 69
lids	285	71 < 78 < 83
Adults	323	33 < 38 < 42

*Normal wing development was the criterion used for adult survival.

First to third instar survival on the artificial surfaces (Table 1) was 89.0 percent compared to 54.5 percent on alfalfa. Seventy-one percent of the pupae survived. Those that had to be placed on tape suffered a slightly lower survival rate than those that attached themselves to cardboard lids, 39 and 22 percent respectively. Use of cardboard lids as a pupation site enhanced survival by minimizing handling of pupae. In the adult stage 38 percent had normal wing development. The low yield of adults with normal wings would not hinder the continuation of the colony since each female lays an ample number of eggs. Sample size for survival rates was tested at the 95 percent confidence level using the formula found in Bliss (1967):

$$\frac{y}{y + (n + 1 - y)F_L} < p < \frac{(y + 1)F_U}{n - y + (y + 1)F_U}$$

where n = total sample size, y = amount alive, and F_L and F_U are taken from a table of the 'F' distribution.

These data show that alfalfa caterpillar females will lay eggs on green tape as effectively as they will on alfalfa. When this is used as the oviposition site, and is combined with diluted bleach and water washes, a convenient, efficient, and low mortality system for egg handling is achieved. In addition, first instar larvae did well on artificial diet, thereby enabling the total elimination of fresh alfalfa from the rearing system.

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