A Method for Rearing Agrotis (Scotia) segetum Schiff. and Agrotis (Scotia) exclamationis L. (Lep., Noctuidae) on an Artificial Diet

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A method is described for rearing *Agrotis segetum* and *A. exclamationis* on an artificial diet. A year round culture is necessary so that insects can be provided for microbiological control studies. Adults are kept in an ovipositional cage covered with paper toweling on which eggs are laid. Newly hatched larvae are placed in small plastic grids on a tray with artificial diet. They are moved to a larger tray and grid arrangement after about 10 days. When ready for pupation larvae are placed in a double grid set-up, the lower grid filled with soil-like substrate. Mortality is high during the first instar and just before pupation. Reasons for mortality and improvements on the method are discussed.

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Introduction

During the past few years the search for alternative pest control measures has become increasingly intensive. In Sweden, two species of cutworm, *Agrotis segetum* and *Agrotis exclamationis*, which can cause considerable economic damage in potatoes and garden crops have been the objects of biological control studies. The investigations have centered around microbiological control methods using a virus disease. A year round supply of larvae is necessary in order to provide live material for growing virus and for tissue culture studies. This supply can be provided by a laboratory culture of the pests. The use of an artificial diet can simplify laboratory procedures and decrease the risk of bacterial and fungal infections. For the reasons outlined above a laboratory culture of *A. segetum* and *A exclamationis* was started at the Department of Zoology, University of Lund.

Görnitz (1951) reared *A. segetum* on leaves and root vegetables. A culture of *A. exclamationis* was raised by Kowalska (1962) in Poland. Khlistorskii and Uspenskaya (1969) reared *A. segetum* on a semi-synthetic diet in the U.S.S.R., the diet was modified by Uspenskaya and Kozhaeva in 1974. In the G.D.R. a method for mass rearing of *A. segetum* on lettuce leaves and carrots was developed by Schwartz (1971). A culture on semi-synthetic diet was also raised in the G.D.R. by Fischer and Otto (1976). Both *A. segetum* and *A. exclamationis* have been reared in France on an artificial diet by Poitout and Bues (1974).

Artificial Diet

A diet developed by Claude E. Rivers has been used for the rearing of *A. segetum* and *A. exclamationis*. The diet is a general medium for the rearing of Lepidoptera. Two ingredients from the original recipe have been deleted as they were found to be unnecessary. A list of the ingredients minus the deleted items, dried leaf and sinigrin, follows:

Agar	30.0 grams
Water (distilled)	900.0 ml

The water is added to the agar and they are brought to a boil in a saucepan. They are then removed from the heat.

Casein	52.8 grams
Wheat Germ	112.2 grams
Wesson Salts	15.0 grams
Cholesterol	1.5 grams
Dried Yeast Powder	22.8 grams

Methyl p-Hydroxy Benzoate	1.5 grams
Sugar	44.4 grams
Sorbic Acid	2.4 grams

The dry ingredients are mixed together thoroughly in a mixing bowl.

Water (distilled)	480.0 ml
KOH (4 molar)	7.5 ml

The water and KOH are added to the dry ingredients and blended in thoroughly.

Linseed oil	3.0 ml
Formaldehyde (10 %)	6.6 ml

These are added just before pouring in the agar which has cooled to 80°C. When the diet plus agar have been mixed and have cooled to 70°C the antibiotic and vitamin mixture is added and blended into the diet. The antibiotic and vitamin mixture is as follows:

Nicotinic Acid	5 grams
Calcium pantothenate	5 grams
Riboflavine (B_2)	2.5 grams
Aneurine hydrochloride (B_1)	1.25 grams
Pyridoxine hydrochloride (B_5)	1.25 grams
Folic Acid	1.25 grams
D-biotin	0.1 grams
Cyanocobalamine	0.01 grams

1 gram of the vitamin mixture is then taken and added to:

Streptomycin	2 grams
Aureomycin	18 grams
Ascorbic Acid	40 grams

(This mixture can be stored in the refrigerator.)

When the diet is ready the mixture is poured into plastic cups for freezing or onto trays for use during the same week. Freezing the diet allows us to make large batches for storage.

Rearing Methods

Newly hatched moths are placed in an oviposition cage (Fig. 1 A) which has been described by Knott et al. (1966). Eggs are laid on single ply paper toweling which is removed three times a week. Moths are fed on a liquid diet, cotton soaked with a 10 % sugar solution is placed in a small petri dish. Fresh sugar water is placed in the cage three times a week, at the same time the eggsheets are changed.

During the first year of the culture the moths were held at 20°C, 70–80 % relative humidity, and 16 hour daylight. Due to an insecticide accident the cages had to be removed to a less suitable place. Daylight could be regulated at 18 hours but temperature and humidity could not be held constant.

At first, egg-covered towels were placed just as they were in plastic containers for hatching. Experimentation and experience showed that if a moistened paper towel was placed in the container hatching was better and the larvae seemed healthier. Eggs are now disinfected using 0.1 % sodium hypochloride because of risk for virus infection. The washed eggs are placed on filter paper which is put into the plastic container together with a moist paper towel. Eggs are kept in the same environment as the moths.

The eggs hatch after 3–5 days. When there is an overproduction of eggs we can store them for up to three weeks at 4°C. Newly hatched larvae are placed on artificial diet. A small tray is covered with diet and a plastic grid (Fig. 1 B) is pressed down on the diet. Diet which has been frozen is often very moist and must be dried slightly before use. First instar larvae are then placed in the cells. The tray and grid are covered with Para-film and air holes are punched in the film. After approximately 10 days the larvae have reached the second and third instars and are moved to a larger tray and grid arrangement (Fig. 1 C) with fresh diet. The lid of this set-up is held on by rubber-bands. A week later the larvae are given a fresh bit of diet. When the larvae are almost ready to pupate we move them to a set-up similar to that which Poitout and Bues (1974) have described. Two grids are placed together with a filter paper between them. The bottom grid is filled with sand, vermiculite, or sawdust. In the upper grid the larvae are placed with a bit of food. When the larvae are ready to pupate they eat through the filter paper and go down into the substrate in the lower grid and pupate. When sand or vermiculite are used they are moistened with 0.2 % methyl p-hydroxy benzoate. Sawdust is sterilized in the autoclave. Larvae were kept in a climate chamber at 26°C, 50–80 % R. H. and 19 hours of light. After the insecticide accident they had to be kept for a time with the moths but at somewhat higher temperatures.

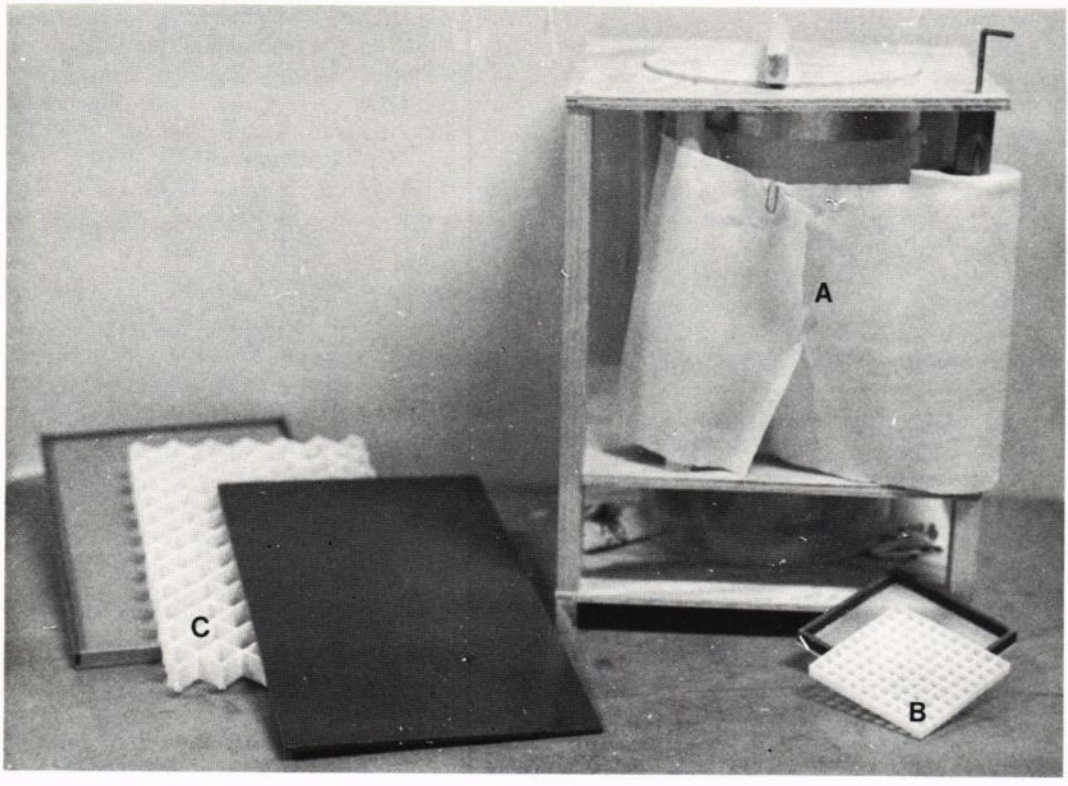

Fig. 1. – A. Oviposition cage. – B. Small tray and grid for first instar larvae. – C. Tray, grid and lid for large larvae.

Äggläggningsbur. – B. Liten bricka plus raster för första larvstadiet. – C. Bricka, raster, och lock för stora larver.

Pupae are collected first after a week in the double grid arrangement and then a second time a week later. They are washed with 10 % formaldehyde and placed in plastic canisters for emergence. Climate conditions for the pupae are the same as for the moths.

Results and Discussion

The *A. exclamationis* culture was lost in the insecticide accident and is now being built up again from recently collected moths. During the time we had this culture about 40 % of the first instar larvae set up pupated and about 30 % became adults.

A. segetum survived the accident somewhat better and the culture could be continued with the help of additions from a Danish culture and field collections. 25–30 % of the first instar larvae placed on diet became pupae. Nearly all pupae hatched.

75 % adult emergence was achieved by Uspenskaya and Kozhaeva (1974). Better results than ours are possible and several weak points can be noted.

Mortality is high at two points. Many larvae are lost during the first week, they do not live past the first instar. The second mortality peak takes place around pupation. Early death has been cut down somewhat with the sterilization and moistening of the eggs. Improvements, however, are needed in rearing procedures. The small larvae seem to try to escape during the first days on artificial diet. They are extremely sensitive to moisture; the diet must not be too wet or too dry. Recently a trial has been made

using low plastic containers with sawdust and bits of diet in them. Small larvae are placed in the containers and then moved to large trays and grids about 10 days later. Results seem promising.

The largest problems with the older larvae are hygiene problems. Rearing localities have not been satisfactory. An infestation of mites and bacterial diseases have greatly reduced pupation percentages. Results have improved recently as the culture has been moved into a more sanitary and isolated room. The problem of keeping the trays and grids sufficiently clean remains, however. Trays and grids simplify handling of the larvae considerably but disposable materials would be preferable.

Live material for virus and bacteria investigations as well as tissue culture experiments are now available in an continuous culture of *A. segetum*. Two generations of *A. exclamationis* have been raised, but the culture is very small and periodic as yet.

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Sammanfattning

En metod beskrivs för odling av jordflyn (*Agrotis segetum* och *A. exclamationis*) på artificiell diet. En odling är nödvändig för kontinuerlig tillgång till insekter för mikrobiologiska försök. Den artificiella maten är gjord efter ett recept som återges i artikeln. Det vuxna stadiet av *Agrotis* spp. hålls i en bur där äggläggningen får ske på hushållspapper. Nykläckta larver placeras på en bricka med artificiell diet och åtskiljs från varandra med hjälp av ett plastraster. Efter ungefär 10 dagar flyttas larverna till en större rasterbricka. Strax innan förpuppningen placeras larverna i en dubbel rasteruppsättning, där det nedre rastret är fylld med sand eller liknande. Många larver dör under det första larvstadiet och vid förpuppning. Några skäl till den höga dödligheten och förbättringar av metoden diskuteras.

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