European Corn Borer Rearing Manual

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PREPARATION OF DIETS

Diets are food, and should be treated similarly to human foods in hospital settings. It is very important to reduce the possibility that disease inoculum will enter the diets during preparation. Diet preparation should be done in an isolated area used only for this purpose. Only the individual preparing diet should enter this area to keep the potential for disease inoculum to a minimum. All preparation should be done with rubber gloves. Surface disinfect all work surfaces, utensils, and containers before beginning diet preparation. Appendix A is a list of diet ingredients and our current lowest-cost provider.

Larval Diet

European corn borer larval diet ingredients are listed in Table 1. Several antibiotics are used to suppress disease and undesired microbial growth. Fumidil B can be used to suppress *Nosema pyrausta* (Lewis 1970). The concentration of Fumidil B can be varied from 500 ppm (6.9 g) to 2000 ppm (27.6 g). Under typical circumstances, we will use either 0 ppm or 500 ppm. Alternate generations receiving a diet containing Fumidil B, thereby reducing the selection pressure on *Nosema pyrausta* to develop resistance to Fumidil B. Molds are controlled by methyl-p-hydroxybenzoate, sorbic acid and the proprionic-phosphoric acid mixture. *Aspergillus niger*, *A. flavus*, and *Cladosporium avellaneum* are the primary molds associated with ECB cultures. A 14 percent concentration of aureomycin (chlortetracycline) is used to control bacteria. Higher concentrations may cause sterility in adult moths. All of these antibiotics have detrimental effects on ECB and they should be used sparingly. We have found that aureomycin can be eliminated and proprionic acid reduced by 50% without increasing microbial growth on the diet Andow and Stodola 2001).

Bt diet ingredients are listed in Table 2.

Procedure for Making Larval Diet

1) Water and agar (part A) are combined in the cooker. With continuous heat and constant stirring this mixture is brought to 90°C. This takes approximately 30 minutes in our large Groen cooker.

2) The cooker is then turned off. Cold water (part B) is added to the agar mixture, followed by the addition of wheat germ. Allow the mixture to cool to 54°C. This takes about 1 hour. Placing the mixture in a cold bath speeds this process. The agar will remain a liquid at this temperature, but will not be so hot that it is difficult to work with. Most importantly, this temperature is cool enough that it will prevent destruction of critical ingredients in part C.

3) The water in part C is measured and added to blender, followed by the dry components, and finally the proprionic-phosphoric acid solution. Blend on high for three minutes. When the agar mixture in the cooker has cooled to 54°C add the blended ingredients (part C). Allow the solution in the cooker to mix for three minutes.

4) Pour part of the prepared diet into blender. Take care that the diet is evenly mixed so that each blender batch is the same. Blend on high for one minute. Pour the hot solution in diet containers to a depth of two cm, continuing into successive diet dishes. We primarily use 7 1/8 inch diameter by 3 inches high plastic dishes, and other dishes we use are 1 pint wide-mouth canning jars (pour to 2 cm, approximately 65 grams) and bioassay trays (amount of diet varies based on purpose). Place a solid cover on the dish, off-setting the cover to allow for the escape of heat.

5) When diet has cooled to room temperature, remove the lids, and expose to UV sterilization for 1 hour. The UV light will surface-sterilize the diet and dishes. After the light has turned off, replace and secure lids, label all dishes with date and number of the order poured with a wax pencil or a piece of masking tape, and store the diet at 4°C in the refrigerator set aside for diet (near room 241C). Diet can be stored for fourteen days at 4°C, but it is better to use it within a week.

6) A diet log is kept with the date, type and number of dishes poured, and the concentrations of aureomycin and Fumidil B. The individual who prepared the diet should initial the log. The lot number for each component used should be tabulated as well. This will allow one to determine if variation in the colony is due to components in the diet (i.e., different lot number, expired components, etc.).

7) Clean diet room thoroughly. A clean-up checklist is provided in Appendix B.

Note: It is best not to use dishes on the same day that they were prepared. In the diet literature it is reported that vapors from the mold inhibitors may kill the eggs; however, we no longer use formaldehyde, which was the primary toxic vapor, and we use half the proprionic acid mix, which is the other volatile mold inhibitor. So, although it is best to wait one day, we have infested diet the same day it was prepared, and have not noticed higher egg or neonate mortality.

Table 1. Full Batch - Meridic Diet

Component	Volume	% Weight	Instructions
Part A			
Deionized Water	7.5 liters	51.7	Combine in cooker and heat to 90 °C
Agar	280 grams	1.9	
Part B			
Cold Deionized Water	3.25 liter	22.4	Add to above, and allow to cook for 1 minute. Remove from double boiler, and cool to 54 °C.
Wheat Germ	520 grams	3.6	
Part C			
Deionized Water	1.625 liters	11.2	
Dextrose	400 grams	2.8	
Casein	440 grams	3.0	Combine ingredients in blender while parts A and B are cooling. Blend for 3 minutes just before those parts get to 54 °C. Add to parts A and B, mix for 1 minute. Pour back into blender (in batches), blend for 1 minute on high speed, and pour to depth of 2 cm in larval dishes.
Cholesterol	32 grams	0.22	
Salt Mixture XIII	144 grams	1.0	
Vitamin Supplement	92 grams	0.63	
Ascorbic Acid	120 grams	0.83	
Fumidil B (500 ppm)	0 or 6.9 grams	0.05	
Methyl-p- hydroxybenzoate	21 grams	0.14	
Sorbic Acid	8 grams	0.06	
Aureomycin	0 or 42.5 grams	0.29	
Proprionic Acid Mixture*	21.5 mls	0.15	

***Proprionic-Phosphoric Acid Mixture**

Proprionic Acid	418 mls
Phosphoric Acid	42 mls

Bring total volume to 500 ml with distilled water.

Table 2. Bt Diets - 1/3 Batch Regular Diet with Modifications

Modified Guthrie's wheat germ diet (Guthrie, et al. 1972, Reed 1972). For use in F2 screen, *Bt* bioassays/resistance studies.

Component		Volume	% Weight	Instructions
Part A				
Deionized Water		2.5 liters		Combine in cooker and heat to 90 °C
Agar		97 grams		
Part B				
Cold Deionized Water		1.08 liter		Add to above, and allow to cook for 1 minute. Remove from double boiler, and cool to 54 °C.
Wheat Germ		180 grams		
Casein		153 grams		
Part C				
Distilled Water		0.63 liters	11.2	Combine ingredients and blend for 3 minutes while parts A and B are cooling; when they reach 54 °C, mix parts together, add Bt mixture, and mix well. Blend (in batches), for 1 minute on high speed, and pour to depth into larval dishes.
Dextrose		139 grams	2.8	
Cholesterol		11 grams	0.22	
Salt Mixture	XIII	50 grams	1.0	
Vitamin Supplement		92 grams	0.63	
Ascorbic Acid		21 grams	0.83	
Fumidil B (500 ppm)		0 or 6.9 grams	0.05	
Part D				
Dextrose		139 grams		Combine in blender, add water.
Cholesterol		11 grams		
Salt Mixture		50 grams		
Vitamin Supplement		48 grams		
Ascorbic Acid		21 grams		
dd Water		0.63 liters		

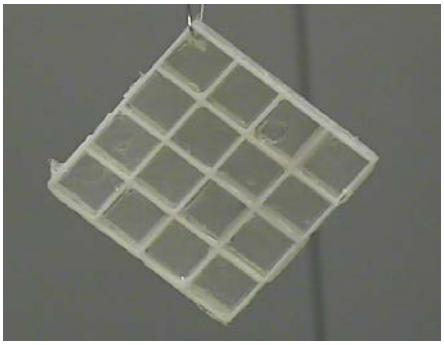
Bt product (concentration dependent)

6 μg/mL => 2.14 mL *Bt* product

3 μg/mL => 1.07 mL *Bt* product

1.5 μg/mL => 0.535 mL *Bt* product, *etc.*

Adult Diet



Results from experiments conducted in our lab show that an adult food increases adult longevity nearly two-fold, and that more total eggs and egg masses are oviposited by females receiving the food (Leahy and Andow 1994).

Cut plastic light diffusion panels (1/4 inch x 1/4 inch) into three inch square grids. Seal grids with Parafilm. Place on a surface disinfected tray. Adult diet components are listed in Table 3.

Procedure for adult diet.

1) Heat agar and water to 90 °C (part A).

2) Prepare grids by covering one side with parafilm and placing parafilm-side down on a surface disinfected tray.

3) Add the sucrose to the agar solution. Stir with sanitized spatula.

4) Cool the mixture to 54°C – if one is pressed for time, place in a cold water bath, and stir constantly. The agar will remain a liquid at this temperature, but will not be so hot that it is difficult to work with. If it is hotter than this temperature it will melt the parafilm. Pour mixture into prepared grids.

5) Let set. The gel will be loose until set. When cool, cover with parafilm. Store at 4 °C in closed container in refrigerator.

6) A diet log is kept with the date and batch number of the adult diet. The individual who prepared the diet should initial the log.

7) Clean any mess that has been made in the diet room.

Adult diet can be stored for 14 days. One liter is sufficient to prepare approximately 18 grids.

Table 3. Adult diet components to make 1 liter.

Component	Volume	Instructions
Part A		
Agar	10 grams	Combine and heat to 90°C.
Distilled Water	710 mls	
Part B		
Sucrose	280 grams	Stir and cool to 54 °C.

GENERAL FLOW OF WORK

Each day the work on the colony should flow from “clean” work to “dirty” work. Clean work involves those operations that must be kept disease-free and have a low potential for becoming contaminated. Dirty work involves those operations that might harbor or spread disease. Clean work includes handling egg masses and setting up larval dishes. Work that has both clean and dirty parts to it is setting up oviposition cages. The clean part is setting up the cage. The dirty part is handling the spent larval diet. Dirty work includes taking down oviposition cages and cleaning larval diet dishes and oviposition cages.

In addition, it is important to minimize time exposed to the most allergenic parts of the colony, which is moth scales. The oviposition chamber and oviposition cage cleaning are the two times that exposure could be high. Moreover, it is essential to clean oviposition cages at the earliest possible time, to minimize the scale concentration in the rearing areas.

One reasonable flow of work is to conduct it in the following order.

1) Care for egg masses.
2) Change sheets in oviposition chamber.
3) Start making larval diet (if necessary).
4) Set-up larval dishes.
5) Continue making diet (if necessary).
6) Set-up oviposition cages.
7) Take down oviposition cages.
8) Clean up and finish making diet (if necessary).
9) Clean diet dishes and oviposition cages.
10) Clean up and then care for the egg masses before leaving work for the day.

CARING FOR EGG MASSES

European corn borer egg masses go through several stages of development. When freshly laid, they are a milky white color and no discernable embryo can be seen under a dissecting scope. Within a day, the eggs turn to a cream color, and by 24 hours at 27 °C an embryo can be observed as a light crescent near the bottom of the egg (see adjacent picture). Then larval cuticle begins to form and the head capsule begins to darken. Within a day of hatching, the head capsule becomes quite dark, a stage called the blackhead stage (see picture to lower right). Within a few hours of hatching, the larva is completely formed and sometimes can be observed to move inside the egg. Hatching (eclosion) occurs shortly thereafter.

The stages of the egg have different sensitivities to cold. The mature blackhead stage is the most resistant to periods of cold, and egg masses in this stage can be held at 10 °C for 9 days without any obvious loss of vigor. Prolonged cold exposure at earlier stages of development can harm the developing larvae.

The primary objective of egg handling is to enable the eggs to develop to the blackhead stage with minimal disruption, and then store them at 10 °C for later use.

25 °C and 10 °C chambers

After changing sheets, egg masses should be hung in the 25 °C chamber until they blackhead. When they blackhead they should be moved to the 10 °C chamber. Until enough experience has been accumulated, egg masses should be examined at least once a day. After some time, when the developmental pattern of the eggs is better understood and greater confidence is gained, they can be examined less frequently. The development time of eggs is given in Table 4. Care should be taken to hang egg mass sheets at all times. Friction from the sheets can damage the egg chorion, leaving an entry point for pathogens.

Procedure

1) Wash hands.

2) First thing in the morning, egg masses should be checked. If they are blackheaded, they should be moved to the 10 °C chamber. If they are not, leave in the 25 °C chamber.

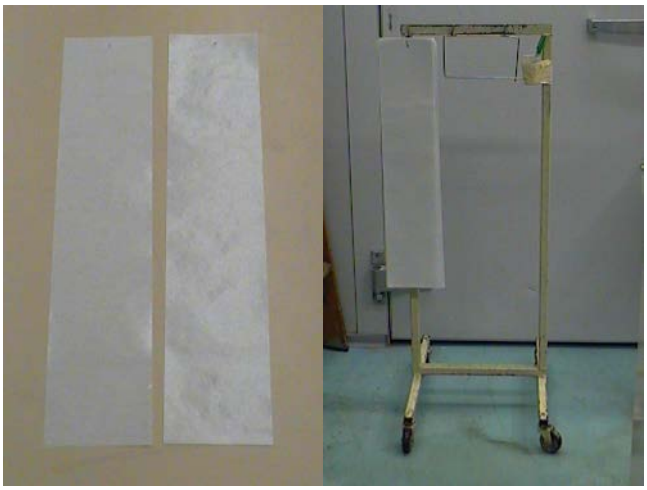
3) Check the egg masses again, just before leaving. Hands should be washed. If the eggs are blackheaded, move them to the 10 °C chamber. If the eggs are not quite blackheaded, inform supervisor. If supervisor is not around, move the eggs to the 10 °C chamber, making a note on the daily work sheet.

Table 4. Typical European corn borer egg development times.

Temperature (°C)	Days to Hatch
8	no hatch
10	no hatch
15	10
20	6
25	4
27	3
30	2.75

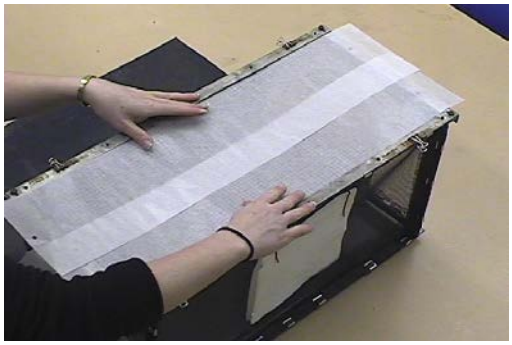
CHANGING SHEETS

Oviposition cages are maintained in the oviposition chamber. Each cage is supplied with two waxed paper sheets, on which ECB will oviposit. Sheets should be changed every day at the same time of day, unless the eggs are not needed for several days in a row. A sheet changing stand is available to facilitate this process. Sheets should be moved to the 25 °C reach-in chamber at the same time each day for maturation.



Wax sheets and sheet changing stand

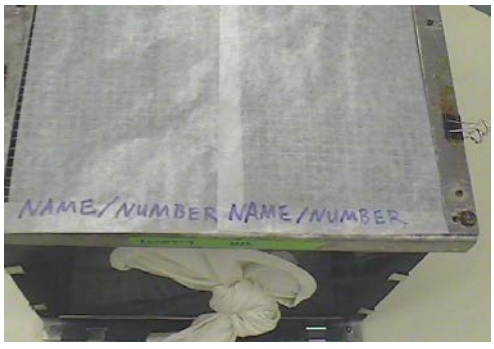
When sheets are changed, they should be changed rapidly to prevent the escape of moths from the cages. Rapid changing requires practice, and is accomplished by removing both sheets from the top of the cage with one hand, and replacing the two new sheets on the cage with the other hand. A short demonstration video of changing the sheets is provided in QuickTime or RealOne Player. (Videos are uploaded separate from this manual).



The new sheets are adjusted to cover the top uniformly, all moths that escaped but are caught under the new sheets should be crushed (they will escape), and the black mat is replaced. The black mat provides enough weight so that moths cannot force their way out of the cage underneath the wax paper sheets.

All sheets should be labeled with the cage number from which they originate by writing the cage number on the sheets.

Several weights of waxed paper are available to purchase. All sheets are wet wax bleached paper sheets, which we purchase from Anchor Paper, St. Paul, MN. The thin sheets (20/35#) are easiest for popping eggs off the sheet, and the thick sheets (40/60#) are easier to punch with a compressed air puncher.



After changing sheets it is critical to water the cages, kill all escaped moths, and clean up the chamber. A monthly

cleaning checklist for the Oviposition Chamber is provided at Appendix D.

Procedure for Changing Oviposition Sheets

1) Assemble sheet changing stand with a sheet rack, new sheets with a single hole punched in them, and a marking pen.

2) Change sheets on all oviposition cages.

a) Remove black mat, taking care not to disturb sheets or cage, or to let water drain onto the sheets. Hold sheets down at the front of the cage when removing black mat.
b) Hold two new sheets in one hand, and in a single motion, remove the sheets from the cage and replace with new sheets.
c) Arrange new sheets on cage so moths will not escape.
d) Hang old sheets on stand.
e) Arrange new sheets carefully on the cage, so that all surface of the ¼ inch hardware cloth is covered and the sheets extend on both ends of the cage.
f) Squish moths under sheets.
g) Cover with black mat.
h) Label new sheets with cage number.
i) Repeat for all cages.
j) Take sheet changing stand out of chamber.

3) Water the teri-cloth on the sides of the cages.

4) Kill all escaped moths in the chamber using a flyswatter or vacuum.

5) Collect any escaped larvae and dispose of them.

6) Sweep out chamber floor.

7) Squeegee and mop up any water that has escaped the chamber to remove puddles.

8) Record oviposition rates for each cage on the colony data log sheets. Record the date of first oviposition for cages that have just started to oviposit.

SETTING UP DISHES

Waxed Ring Preparation

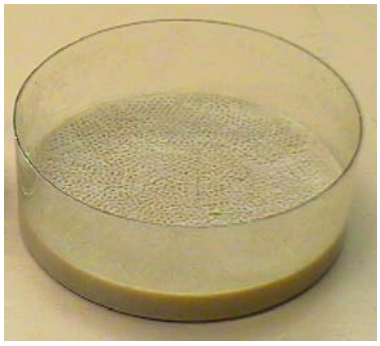
Waxed rings are used in larval rearing dishes for pupation sites. Described below is a method for making the rings. At this time, we purchase rings from Custom Bio-Products (Bruce Lang) in Iowa (515-387-8883). Waxed rings should be used to reduce deterioration and mold growth. We use two sizes, 7" diameter for our regular colony dishes, and 3" diameter for single egg mass (60 egg) dishes.

"A flute" special seal corrugated paper is purchased in 36 inch by 250 foot rolls. A 13.5 foot section is cut. This is tightly rolled around a four inch (in diameter) aluminum vent pipe, glued into place with a 3:1 solution of white glue (water to Elmer's glue), and secured on the ends with straight pins. The vent pipe is then removed. Each roll is set aside to dry. The 36" roll is then cut into 1" sections. The individual 1" rings are placed back onto the vent pipe, enclosed in a paper bag, and autoclaved for 45 minutes at 121°C. Paraffin wax is melted. Three percent (by weight) sorbic acid is added to the hot wax, to inhibit mold. A fume hood should be used for this procedure. The sterile corrugated rings are dipped into the hot wax/sorbic acid solution. Excess wax is removed by hitting the ring against a section of ¼ inch metal hardware cloth. The rings are set on a plastic sheet to air dry. The waxed rings are stored in plastic bags until needed.

Preparation of the Work Area

Wash hands before beginning. Disinfect work surface, counter top, compressed air puncher (including the area under the puncher, the punch and the concave surface under the punch itself), and the screen used to catch the waxed paper disks with egg masses on them.

Preparation of the Diet



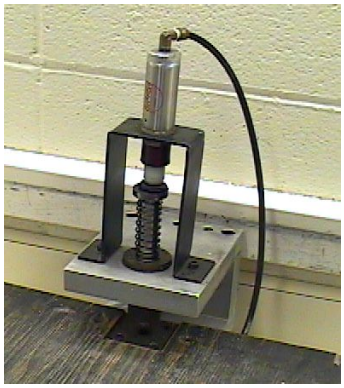
Remove the diet that is stored in the refrigerator and allow it to reach room temperature. Excess moisture should be wiped from the surface and sides of the dish with a sterilized paper towel (neonates are trapped by the surface tension of the moisture). The diet is perforated with a "curry-comb" (which is stored in

0.5% bleach, rinse before using). The perforations enable neonates to penetrate the surface of the diet for good establishment.

Selection of Egg Masses

Blackheaded egg masses are used to set up dishes. Three to six day old eggs are optimal. Egg masses stored for more than nine days should be discarded. It is important to use only egg masses that are even in appearance and development, showing no damage to the eggs. All egg masses should be the same age (from the same day of oviposition) to ensure synchronous development within a dish. Egg masses from at least two cages (more cages is better) should be used to maintain genetic diversity in the colony.

Punching Egg Masses



A compressed-air punch is used to punch egg masses on small wax paper disks. The air compressor should remain plugged in at all times. The power switch is located on the side of the black plastic box just inside of the handle. This "on/auto" lever should be switched on to the on/auto position (up). The air compressor will run only when pressure is low, and it will start automatically and pump up to a pressure of approximately 125 lbs. and then shut off. It will usually not go on when you turn on the switch. It is very noisy and may startle you when it does go on.

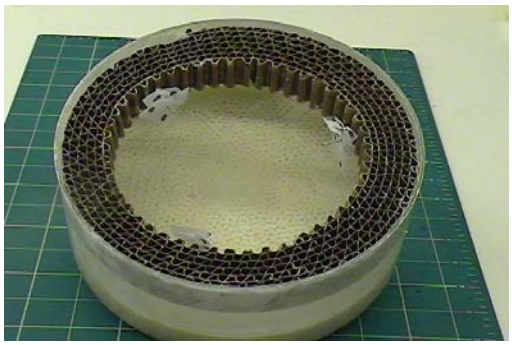
The air value is located directly below the handle. After pressurizing, the value should be opened in the horizontal position. This will provide air pressure to the punch so that it can be operated.

The regulator and gauge (gold in color) are also located below the handle. The gauge reads from 0-160 lbs. It should read 60 lbs., which is the pressure that operates the punch. This is pre-set and should not require adjusting.

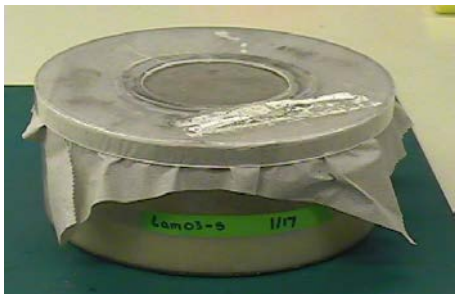
Turning the air value off (vertical) and on/auto lever off (down) turns off the punch. Press the foot pedal several times to release the pressurized air in the lines; this will prevent inadvertent punching during clean-up.

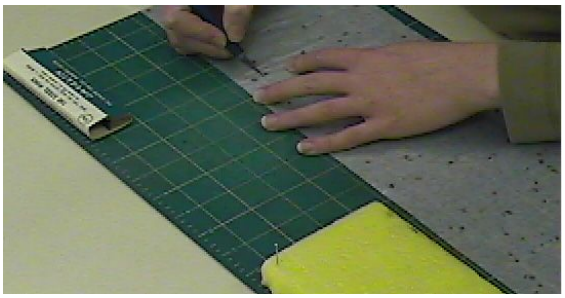
Place a screen under the punch and punch out the necessary egg masses. A total of approximately 700 eggs (18-24 egg masses) are used for each dish.

Disks of egg masses are pinned on straight pins, eight egg masses to a pin. Care should be taken to ensure that no egg mass is covered by another disk of waxed paper so that larvae can easily emerge from the egg masses. The



pins with egg masses are pinned to the interior of a waxed ring. The waxed ring is placed on the top of the dish, and secured so that it does not touch the diet. The purchased rings fit the dished snugly and usually do not require any additional efforts to secure them. If necessary, they can be secured using small pieces of waxed ring to create a snug fit. The dish is covered with a clean paper towel, which helps seal the dish and keeping larvae from escaping. This is then covered with a clean screen lid. Sheets with unused egg masses should be returned to the 10 °C chamber.





Alternatively, when relatively few dishes are being setup, the egg masses can be cut out of the waxed sheets with a utility knife and a cutting mat. The egg masses should be chosen and pinned in the same way.

Clean-up of the work area

All equipment should be cleaned (compressor, punch, counters), and the floor swept of any debris. A monthly cleaning checklist for the Work Room is provided in Appendix E. The 'curry-comb' and solid lids should be washed immediately. The curry-comb should be returned to its storage container after washing. It is stored in a 5% bleach solution (1 part bleach to 19 parts water). The lids should be sent through the dishwashing system (see Dishwashing Procedure). Wash all surfaces, and surface disinfect all countertops, punch surfaces, and screen. Clean the punch thoroughly. Scrape off the wax build-up if necessary. Acetone can be used to remove wax if there are particularly hard areas to clean the wax build-up.

Procedure for Larval Dish Setup

1) Wash hands.

2) Make sure air compressor is off and pressure to puncher is discharged.

3) Disinfect all work surfaces.

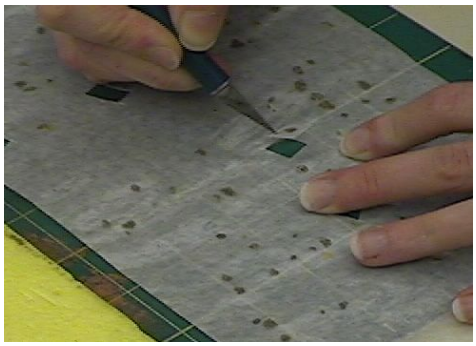
4) Get diet dishes, and prepare them
   a) Wipe off moisture with a paper towel.
   b) Perforate with curry comb.

c) Wash curry comb and replace in bleach bath.

5) Get egg masses from 10°C chamber.

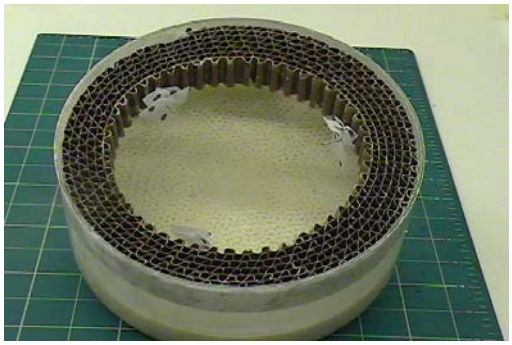
6) Turn on air compressor.

7) Punch egg masses OR cut out egg masses. Take care to mix egg masses from at least 2 cages for each dish. Select egg masses carefully for uniform development, and viability (i.e., not desiccated).
   a) Make sure there are no egg masses in the punch.
   b) Place screen under punch.
   c) Select and punch egg masses.
   d) When finished with a set of egg masses, make sure none are stuck in the punch.
   e) Repeat as needed.



8) Turn off compressor and discharge pressure in the punch by stepping on the foot pedal.

9) Pin the egg masses and attach to inside of waxed rings.



10) Secure waxed rings in top of dishes above the diet.

11) Cover dish with a clean paper towel.

12) Cover with a screen-lid. Check lid for cracks and for the screen pulling away from the lid. Do not use cracked lids. When the screen comes loose, it needs to be re-melted onto the dish before that cover is used again. Since the larvae will chew through tape and glue, these methods of fixing a loose screen are not desirable.

13) Label dish with colony name and date.

14) Record information in data log.

15) Take unused egg masses back to the 10°C chamber.

16) Clean up work area.
   a) Put away all materials (pins, paper towels, etc.)
   b) Clean compressor, punch, and counters.
   c) Sweep the floor of any debris.
   d) Surface disinfect counters, punch, and screens.
   e) Take lids to dishwashing.

INCUBATION OF DISHES

Dishes should be incubated at 27 °C (81 °F), 40-50% relative humidity and continuous low light. Air should circulate freely through the chamber and around the dishes. It is important to adjust the access vent in the walk-in chamber to let in enough fresh air to allow the diet to dry properly, which encourages larvae to crawl from the diet into the rings for pupation. If many dishes are in the chamber, the vent should be wide open and a fan may be needed to circulate the air faster. During the winter, it may be necessary to reduce the size of the vent. Too much light will distract the larvae, and lead to poor establishment in the diet. However, too little (especially lights off periods) will lead to larvae diapausing.

Larvae reach 3rd instar 7-9 days from the date the dish was started, provided all eggs hatch immediately. Larvae will begin to pupate around 13-15 days. A ring with approximately 80% fill will contain 550 to 600 pupae, which appears to be an optimum number of adults for maximum egg production. Rings are set into oviposition cages at 19 days; if it takes longer to fill a ring this could signal a problem and should be noted.

Male moths will begin to emerge the first evening; females will begin to emerge over the next two days. The first eggs are usually laid the 4th evening. The cage should remain productive for approximately 21 days after being set up. Maximum production can be sustained for 10 consecutive days.

Guthrie et al. (1974) observed that ECB cultures reared continuously on a meridic diet can have reduced survivorship on corn plants when artificially infested in the field, but we have not observed this phenomenon. Because we start with about 700 eggs and produce about 550-600 pupae, there is relatively low selection in the meridic diet and our ECB have maintained their vigor on plants in the field. However, larvae reared at these densities tend to result in smaller adults compared to wild EBC, so we are experimenting with lower larvae to diet concentrations. We have found this to result in fewer, but larger, adults that produce a similar number of egg masses to adults from more densely crowded dishes. Consequently, it is very important to carefully control the number of eggs in a dish and to monitor the fill rate of the waxed rings.

A monthly cleaning checklist for the Growth Chamber is provided at Appendix C.

Procedure

1) Two-three days after dish set-up, the dishes should be checked to determine the percent egg hatch. This should be recorded on the colony log sheets.

2) Dishes should be spaced out on shelves to allow air flow around them. This allows the diet to dry sufficiently so that larvae will leave the diet to pupate in the cardboard ring.

OVIPOSITION CAGES AND CHAMBER

Oviposition Cages

Individual cages

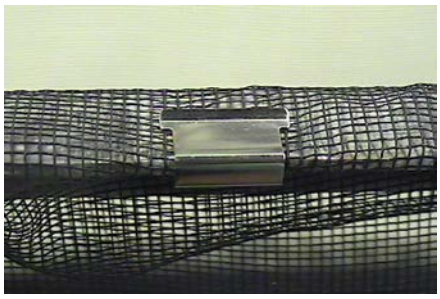
The old version of individual oviposition cages are constructed of ¼ inch hardware cloth, plastic and aluminum window screen, wire, and a canning jar. A 6¼ inch x 7¾ inch section of ¼ inch

hardware cloth is rolled into a cylinder. This is secured using aluminum pop rivets, one on each end. The bottom and top of the cylinder are fit into a standard-sized canning jar lid band, with the band oriented away from the cylinder. Both the bottom and top band can be screwed into a canning jar. A thin wire is attached near the top of the cylinder, stretched across the diameter of the cylinder, and attached to the other side. This wire is used to suspend adult food and a cotton wick inside the cage. A 3" square of plastic window screen is used for the bottom of the cage. The screen is placed over a ½ pint canning jar and the bottom of the cage is screwed into the jar, forming a well-sealed bottom. A cotton wick is attached to the wire, threaded through the window screen into the jar. A disk of aluminum window screen is cut to fit in the lid band on the top. A paper clip can be used as a handle to open and close the cage on the top. The jar is filled with water to supply water into the cage and create stability for the cage. A 1" square grid of adult diet can be suspended from the top of the cage. Wax sheets are held to the cylinder with rubber bands.

A design that is simpler, but does not allow for feeding by the adults is to form a piece of ¼ inch hardware cloth into a cylinder that fits inside a wide-mouth canning jar lid band. The canning jar lid bands are fitted with disks of aluminum window screen and are secured to the cylinder facing in. A rubber band can secure the cage, and waxed paper held around the hardware cloth.

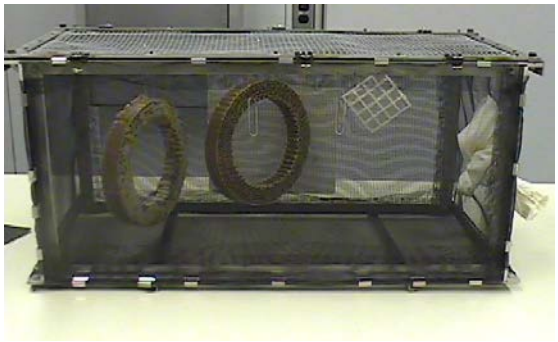
Currently (see picture above), we use individual female cages consisting of a circle of ¼ inch hardware cloth, with a hole in the center just big enough to fit a corn borer adult through, and a circle of metal window screen, both cut to fit inside of 1 pint wide-mouth canning jar lids, where they are glued in place (3 3/8 inch diameter). A two inch wide, 11 inch long strip of metal window screen fastened into a cylinder, and is fit between the two canning lids, where it is glued in place. A square of waxed paper is fit over the hardware cloth surface of the cage, and the cage is topped with a plastic petri dish cover to hold the piece of waxed paper in place.

Mass rearing cages



Mass rearing cages are constructed of aluminum rail frames. The sides, end, and bottom of each cage consist of 16 to 18 mesh plastic window screen, which is secured to the

aluminum frame with “clam clips.” The top of the cage is ¼ inch hardware cloth. It is important not to allow any smooth surfaces to be exposed inside the cage, because moths will oviposit on any smooth surface. The cage dimensions are designed so that a single ring with 80% fill will provide the number of moths to optimize oviposition.



A cage that is set up (procedure) will have one waxed ring with pupae, a nylon net, adult food, cotton sheets for water, and waxed paper on top as an oviposition substrate. An unfolded paper clip is used to suspend the waxed ring from the top of the cage. The top of the cage is secured with binder clips.

If mold overgrows the ring during the life of the cage, it should be removed from the cage. Two weeks after the first night of oviposition the ring should be removed from the cage. Nylon net, approximately 10 inches by 12 inches is suspended from the top of the cage to provide additional surface area for the resting moths. A grid of adult diet is also suspended from the top of the cage near the cloth access port. The grid should be changed when dry, noticeable fungal growth occurs, or after 7 days.

Cotton "Teri" sheets (Kimberly-Clark, Teri wipers #34762); are attached to the outside on each side of the cage with paper clips. The sheets retain water for the moths to imbibe. Waxed paper sheets are placed on outside on the top of the hardware cloth and a rubber mat holds the sheets in place and keeps water off the sheets.

After oviposition declines (no longer than 21 days), the cage is removed from the chamber. The adult food, the "Teri" sheets, and the ring are removed. The remaining moths should die within 2 days. After they die, the moths are vacuumed from the cage. All parts of the cage are washed and rinsed well (see Dishwashing Procedure). Although a 5% solution of Roccal can be used to inhibit sporulation of fungal spores (Roccal has been shown to have no significant effect on egg laying (Guthrie 1990)), it is expensive. We prefer to use a Sanibet solution. The effect of Sanibet on ECB has not been tested, but we have not seen any obvious adverse affects.

Procedure for Oviposition Cage Setup

1. Locate materials needed for oviposition cage setup:

a. Mass rearing cage
b. Hardware cloth top
c. Black mat
h. 3 Paper clips
i. Clam clips
j. Labeling tape

d. Waxed ring with pupae
e. Adult diet
f. Cotton sheets
g. Waxed paper sheets
k. Sharpie
l. Pencil
m. ECB Maintenance Log Book

2. Examine cage for holes or loose netting. Repair when necessary.

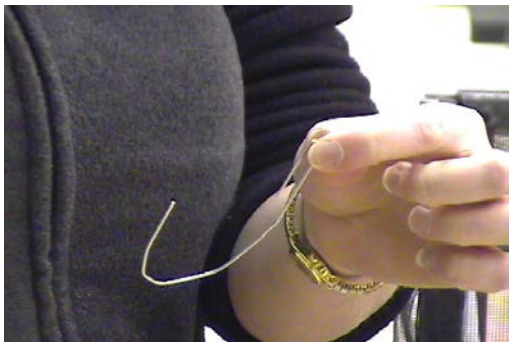
3. Fold cotton sheets into quarters.

4. Attach a towel to each side of the oviposition cage using either bobby pins or paper clips for a water source for the moths.

5. Remove waxed ring from larval dish, removing paper towel remnants, any attached frass and all live larvae.

6. Estimate the proportion of the waxed ring holes that are occupied by pupae. An easy way to do this is to hold it up to allow light to pass through unoccupied holes. Record this data.

7. Unfold one loop of a paper clip into a hook, and slide through an unoccupied hole of the waxed ring.



8. Hang the waxed ring from the hardware cloth grid towards the back of the cage not to letting it touch the sides of the cage.

9. Remove parafilm from an adult diet grid and hang towards the front of the cage in a same way as the waxed ring.

10. Secure hardware cloth top with at least 4 binder clips making sure there are no spaces large enough for moths to escape.

12. Place two wax sheets on top of hardware grid labeled with cage name and number.

13. Place black mat on top of cage to hold the wax sheets in place and to prevent moths from escaping.

14. Label cage with date, cage name and number

15. Tie cloth sleeve into a knot.

16. Record data in ECB Maintenance Log Book.

17. Clean up work area.
a) Put away all materials
b) Discard remaining larval diet and larvae from dish into sealed plastic bag

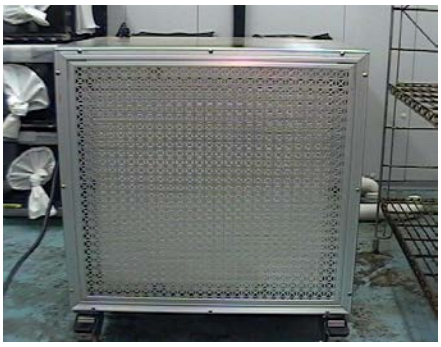
c) Sweep the floor of any debris.
d) Surface disinfect counters.

Oviposition Chamber

The oviposition chamber is maintained with a day-night cycle of 16 hours day, 8 hours night. Daytime (photophase) temperature is 27°C and the nighttime (scotophase) temperature is 18°C. (The relative humidity is set for 80%, but it actually fluctuates with the temperature, being drier during the night.) The photophase and scotophase are offset from the real time so that oviposition occurs during the late afternoon. European corn borers lay their eggs in response to condensation that occurs when the temperature drops. Oviposition usually will begin within two hours after the temperature has reached 18°C. Oviposition can be regulated in this manner. For example, we have found that by reversing the day-night periods, we can harvest freshly laid eggs during the day.

A small fresh air vent may be necessary to prevent pheromone buildup, which may inhibit oviposition. We have not found this to be a problem.

To provide adequate air circulation, oviposition cages are placed on two sections of 2” diameter PVC pipe, connected with 90 degree elbow joints. The elbows give the pipe stability, preventing the pipe from rolling out from under the cages.



After sheets have been changed and "Teri" towels moistened, the chamber floor is rinsed down with water, and mopped. The chamber should be cleaned thoroughly and disinfected monthly. The rough filter on the HEPA filter, that removes scales from the air, should be washed monthly and replaced.

A monthly cleaning checklist for the Oviposition Chamber is provided in Appendix D.

DISHWASHING PROCEDURE

General Guidelines

Dishwashing should be done after the standard colony work is completed, so that the possibility of disease transmission is reduced. Dishwashing should be done immediately. Dirty dishes should not be allowed to accumulate. After cleaning, the dishwashing area should be cleaned. This area should be maintained as clean as the diet room.

Three solutions are necessary, and each is in a large waste container.

1) double deionized (dd) water
2) bleach solution (~0.2% sodium hypochlorite or ~5% bleach)
3) Sanibet solution

Plastic and glass containers should be handled separately from containers with any metal on them. Metal will deactivate bleach (and bleach will corrode metal).

Disinfecting Plastic and Glass

Dirty dishes should be washed immediately. They can be soaked for 30 minutes (not overnight) before washing.

1) First wash: Wash thoroughly with water. All dishes should be put through this first wash without delay.

2) Second wash: Fill up one side of sink with water and add ½ cup of bleach and dishwashing soap. Place dishes in bleach solution and scrub thoroughly. Rinse dishes well. All soap residue must be rinsed from the dishes, because soap will interfere with the disinfecting process. Drain and clean the sink. Glass should be put through an autoclaving process to complete the disinfecting process, for plastics, continue with the following steps.

3) Bleach soak: Let dishes soak in bleach for 1 hour.

4) Third wash: Wash dishes in distilled water as follows. Remove dishes from the bleach solution, draining the bleach solution from the dishes to minimize the amount of bleach on the dishes. Rinse the dishes in the distilled water container. All bleach must be removed from the dishes at this time, because bleach will deactivate the Sanibet.

5) Sanibet soak: Place thoroughly rinsed dishes in the Sanibet solution. Soak for at least 2 hours (OK to soak overnight).

6) Drying: Remove dishes from Sanibet solution, and place on wooden racks to dry.

7) Clean up. Clean up entire area (wash sink, wipe down surfaces, mop floor).

A plastic rake is available to aid in easy access of dishes in the waste containers.

Disinfecting Metal

All items with metal on them should be washed separately from the plastic. The tops of the oviposition cages should be washed with this method.

1) Soak all items in a soap and bleach solution for at least 15 minutes. Fill up one side of sink with water and add ½ cup of bleach and dishwashing soap.

2) Wash all items in the same soap and bleach solution. Be sure to remove all traces of larval silk from the metal dish tops. Wash them again if the silks are not removed.

3) Rinse well in tap water. All soap must be removed.

4) Rinse again in the distilled water container.

5) Sanibet soak. Place thoroughly rinsed items in the Sanibet solution. Soak for at least 15 minutes.

6) Remove from Sanibet solution and place on a rack to dry.

7) Clean up. Clean up entire area (wash sink, wipe down surfaces, mop floor).

Washing the Bottoms of the Oviposition Cages

The bottoms of the oviposition cages are filled with moth scales. Even though they have been vacuumed out, they still have lots of moth scales. It is important to remove the scales during cleaning before the cages are put in the disinfecting solutions.

1) Rinse bottom of cage with pressure sprayer to remove most of the loose scales. This helps keep the dishwater cleaner.

2) Fill the sink with water and soap and wash all the cages.

3) Drain and clean sink. Refill with water and soap and wash all the cages again.

4) Repeat water and soap wash if wash water is still dirty.

5) Soak all items in a soap and bleach solution for at least 15 minutes. Fill up one side of sink with water and add ½ cup of bleach and dishwashing soap.

6) Wash all items in the same soap and bleach solution. Be sure to remove all moth scales. Wash them again if the scales are not removed.

7) Rinse well in tap water. All soap and scales must be removed.

8) Rinse again in the distilled water container.

9) Sanibet soak. Place thoroughly rinsed items in the Sanibet solution. Soak for at least 15 minutes.

10) Remove from Sanibet solution and place on rack to dry.

11) Clean up. Clean up entire area (wash sink, wipe down surfaces, mop floor).

Washing without Disinfecting

All field items can be washed without disinfecting. Dirty items should be washed immediately. They can be soaked for 30 minutes (not overnight) before washing.

1) Soak all items in a soap and bleach solution for at least 15 minutes. Fill up one side of sink with water and add ½ cup of bleach and dishwashing soap.

2) Wash all items in the same soap and bleach solution. Second wash: Fill up one side of sink with water and add ½ cup of bleach and dishwashing soap. Set washed items in the other side of the sink. Drain and clean the sink. Rinse items well, and place in bleach solution.

3) Clean up. Clean up entire area (wash sink, wipe down surfaces, mop floor).

Preparation of Solutions

Bleach: One gallon bleach in the waste container. Fill the rest to the top of the fill line (marked with a piece of masking tape) with distilled water.

Sanibet solution: Ten ounces (1¼ cup) of Sanibet in the waste container. Fill the rest to the top of the fill line (marked with a piece of masking tape) with distilled water.

Distilled water: Fill to the top of the fill line (marked with a piece of masking tape) with distilled water.

All solutions should be changed at least once a month or more frequently as needed. Distilled water rinse probably should be changed more frequently (2-4 weeks). Solutions can be emptied using a siphon or using a bucket (the bucket method is faster).

SAFETY EQUIPMENT

Safety and cleanliness

The primary safety concern in the lab is exposure to insect allergens. The main source of allergenic material is moth scales. Consequently, every effort should be taken to minimize exposure to moth scales. Specifically, the cardinal rule of the lab is that no one is allowed in the rearing room (241C Hodson Hall) without a mask or respirator and protective clothing.

VERY IMPORTANT: Anyone seen without a mask or respirator in the room, for no matter how short a period of time, will suffer consequences. A student worker or other temporary worker will be dismissed immediately without question. A graduate student in the lab will have their stipend cut for one pay period. An undergraduate or graduate student in another lab will have use privileges suspended for at least 3 months. A civil service employee will be issued a formal reprimand that could lead to dismissal and will be required to take a two day leave without pay.

Cleanliness will also help to maintain equipment for longer periods of time. By working safely and cleanly, the lab will have a better work environment.

Weekly cleaning of reach-in chambers.

All reach-in chambers should be washed with a soapy solution. All surfaces, including the wire shelving should be washed. All larvae and larval silks must be removed.

Weekly cleaning of walk-in chambers.

All walk-in chambers should be cleaned weekly. Cleaning should focus on 3 things. First clean whatever appears dirty in the chambers. Note on daily worksheet if nothing seems dirty. Second the floors should be cleaned and disinfected. Sweep up all moths and other debris and dispose. Using a soap solution mop the floor to clean it of dirt and debris. Rinse floor thoroughly (at least twice) to remove soap. Squeegee the water down the floor drain. Dispose of soapy water. Mop the floor with a Sanibet solution (2 oz. in 4 gallons). Remove excess water down the floor drain. Third, clean the outside of the Hepa filter.

Monthly cleaning of rearing room and walk-in chambers (241C Hodson Hall).

This a major cleaning that involves 3 parts, the oviposition chamber, the larval chamber, and the work room.

Oviposition chamber. 1) Clean walls and ceiling with soapy water. Use a dilute bleach solution if necessary. Don't use bleach extensively on the ceiling, because it might drip on you. Rinse thoroughly with water. 2) Clean humidifier with sponge and water. Turn off water feed and turn down the humidifier control before working on it so that it does not turn itself on while you are working. Remove and replace the water in the reservoir, and wipe all parts, inside and out. 3) Vacuum the evaporator exhaust fins. 4) Replace filters on evaporator intake. 5) Clean Hepa

filter unit. Unplug the unit and remove it from the chamber. Remove roughing filter and wash out (spray water from the inside of the filter to the outside. Vacuum out the inside of the filter, if there are any particles in it. Wipe out the inside with a sponge and water. Wash the outside with soapy water and rinse thoroughly. Be careful not to let anything (including dirty or soapy water) fall inside the filter unit. Replace roughing filter and let the roughing filter dry (~30 minutes). Return Hepa filter to oviposition chamber and plug it back in. 6) Clean the floor. Sweep up all moths and other debris and dispose. Using a soap solution mop the floor to clean it of dirt and debris. Rinse floor thoroughly (at least twice) to remove soap. Squeegee the water down the floor drain. Dispose of soapy water. Mop the floor with a Sanibet solution (2 oz. in 4 gallons). Remove excess water down the floor drain.

Larval chamber. 1) Clean walls and ceiling with soapy water. Use a dilute bleach solution if necessary. Don't use bleach extensively on the ceiling, because it might drip on you. Rinse thoroughly with water. 2) Clean fluorescent light fixtures. Remove covers and clean them. Damp-wipe fixtures if they are dirty. 3) Clean wire racks, removing all larval silks. 4) Clean all outlets and fixtures in chamber. 5) Clean humidifier and controls. 6) Clean the portable fan. 7) Clean the floor. Sweep up all moths and other debris and dispose. Using a soap solution mop the floor to clean it of dirt and debris. Rinse floor thoroughly (at least twice) to remove soap. Squeegee the water down the floor drain. Dispose of soapy water. Mop the floor with a Sanibet solution (2 oz. in 4 gallons). Remove excess water down the floor drain.

Work room. 1) Clean puncher, if needed. 2) Clean all counter surfaces, using whatever cleaners are needed. Surface disinfect with Sanibet spray when done. 3) Clean the air compressor. 4) Clean the outside of the Nilfisk vacuum and all of the attachments, using whatever cleaners are needed. 5) Clean all cabinet surfaces. 6) Clean outside of Hepa filter unit. Be careful not to get the Hepa filter wet, because it is not waterproof. 7) Replace roughing filters on Hepa unit. Vacuum out the inside if there is any dirt or debris. 8) Clean all tables. Disinfect with Sanibet spray when done. 9) Clean the floor. Sweep up all moths and other debris and dispose. Using a soap solution mop the floor to clean it of dirt and debris. Rinse floor thoroughly (at least twice) to remove soap. Squeegee the water down the floor drain. Using the special disinfectant solution (in purple-colored bottle) disinfect the work room floor. Using an appropriate strength of Lime-away, clean floor to remove lime deposits. Rinse twice with water.

Disinfecting sprays

Disinfecting sprays are in the plastic spray bottles with green labels. This is a Sanibet solution, mixed at ½ ounce Sanibet per quart of distilled water. This is equivalent to 12.5 ml (2½ tsp.) Sanibet to the quart. These sprays can be used for general disinfecting of work surfaces. At this concentration Sanibet is applied in human food service applications without rinsing, so it is likely to be safe for insects.

Disinfecting solutions

Disinfecting solutions should never be put in spray bottles. They can be mixed and put into squirt bottles, or preferably mixed in solutions in a bucket, used and disposed. They have many disinfectants in them, and are corrosive. If they are sprayed into an aerosol and inhaled for lengthy periods of time, they might be damaging to the user.

Cleaning sprays

Cleaning sprays should be in their original bottles. These include Windex, and other typical household cleaners. They can be used for spot cleaning. More serious cleaning should be done using a bucket, brush, and cleaning solutions.

ESTABLISHING A NEW COLONY

Collecting Feral Adults

During one of the moth flights (second flight is usually more convenient) feral, mated females should be collected. Live catches from black light traps are often most convenient, but sweep netting in action sites, or other methods can be used. Grassy (foxtail) weed patches near corn fields are reported to be favored by the moths during the day and make good collection sites for sweep netting (Showers et al., 1976).

Collect egg masses from individual feral females using small oviposition cages (see section on oviposition cages) for two or three days. Dissect females, examining their reproductive tract and hemolymph for presence of *Nosema pyrausta* spores. *Nosema* is transovarily passed to the egg.

From individuals without detectable levels of *Nosema* spores, set up individual dishes on Fumidil free diet (see section on infesting dishes). After pupation, select individuals to pair-mate and rear in small oviposition cages. Collect egg masses and dissect for presence of *Nosema*. Repeat for three generations.

Randomly pair-mate adults, collect egg masses and screen for the presence of *Nosema*. At this time, if free from all pathogens, the main colony is started.

With disease-free individuals, infest Fumidil-free dishes containing twelve egg masses. Incubate dishes for one week with a photophase of 25°C for 14 hours, and a scotophase of 20°C for 10 hours. The larvae should reach late second instar at the end of the first week. The photophase is then lowered to 25°C for 12 hours and 15°C for a 12 hour scotophase. This procedure initiates diapause. The borers are reared under diapausing conditions for eight to nine weeks. At this time the larvae will have crawled into the ring. Discard diet, switch ring to a clean dish, and cover with a solid lid. Store at 4 °C as long as three to four months. Alternatively, larvae can be reared under non-diapausing conditions and a colony can be started immediately.

Collecting Feral Larvae

To terminate diapause, individual larvae are placed in 30 ml plastic cups containing a agar mixture (Table 5). This mixture provides the water necessary for the larvae to complete pupation. Incubate the larvae at 27°C, 40-50% relative humidity, and continuous light. Monitor for pupation. This may take as long as 30 days.

Table 5. Agar Mixture for post-diapausing Larvae**.**

Components	**Volume**	**Instructions**
Part A		

Distilled Water	980 mls	Combine and
Agar	18.8 grams	bring to 90° C.

Part B

Methyl Paraben	1.6 grams
Sorbic Acid	0.6 grams

Combine with Part A and blend for 30 seconds. Dispense into 30 ml plastic containers to a depth of 1.6 to 1.9 cm. Cool and remove excess moisture before using.

Larvae can also be brought into the laboratory to begin a new colony. Larvae are submerged in a solution of phenylmercuric nitrate (1 gram in 10,000 mls of hot water stirred constantly) to sterilize the cuticle (thus reducing the inoculum potential of *Beauveria brassiana*). Larvae are then placed in individual cups of the agar mixture and diapause is initiated in the previous manner. Screen for *Nosema* disease with the adults produced from these diapausing larvae.

PREPARATION OF LARVAE FOR INFESTATION

Apparatus for Popping Sheets. An open front plexiglass box (61 cm high x 64 cm deep x 91 cm wide) is used to catch and collect egg masses. The top is cut in a deep “U”shape to allow free movement as sheets are passed across a metal bar. A small slot is cut in the back of the box to allow a steel bar (12-inches long by 1/2-inch wide mounted on a perpendicular steel foot) to pass into the box. A three inch (in diameter) hole is cut on the bottom of the box in a front corner. A small petri dish is placed under the hole, which is used to collect popped egg masses.

Collection of Egg Masses. Thin wax sheets (20/30 ww) are used on cages. European corn borer egg masses oviposited during the night are removed the following day. These sheets are allowed to dry approximately four hours before being popped.

The bottom, back, and sides of the inside of the box are lightly dusted with corn starch to prevent egg masses from sticking. Individual sheets are held by the ends, with egg masses away from the bar, and pulled at a sharp angle across the bar. This loosens the egg masses from the sheet. The sheet is then pulled tightly, popping the egg masses from the sheet. This is repeated until all egg masses are removed from the sheet. Egg masses are collected by using a large camel hair brush, "flicking" the egg masses toward the opening in the bottom of the box. A small screen is used to remove excess cornstarch from the egg masses. The egg masses are then weighed and placed in dishes, covered with a paper towel (0.5 ml of distilled water is added to the paper) and the dish is sealed with a solid lid. In our laboratory, 2.5 grams of egg masses mixed with 450 ml of corn cob grits, yield 25 larvae per shot (0.7 ml) with the Davis applicator. European corn borer egg masses are incubated until larvae hatch (Table 4).

Hatched larvae are mixed with 450 ml of 2040 corn cob grits. The grits and larvae are sent through a screen to aid in uniform dispersion of larvae in the grits. The grits and larvae are transferred to one liter bottle (an accessory for the Davis inoculator). Each bottle is then calibrated.

Calibration of the Larvae. The Davis applicator is attached to the bottle. Rotate the bottle gently three or four times. Dispense five shots into a petri-dish. The sixth shot is dispensed to a petri-dish with grid. With this shot the larvae are counted by use of a dissecting microscope. Repeat this step five times to subsample the bottle. Determine the mean and standard error of the sample with these data. We prefer to only use bottles with a standard error less than 1.75 to infest plants in the field. If the SE is larger, the grits and larvae are rescreened to remix them, and then recalibrated.

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APPENDIX A: DIET INGREDIENT INFORMATION

Diet ingredient	Source	Stock #	Unit	Phone #
Agar - Fine Ground	Moorehead and Co., Inc.	700B	20 lbs	877-290-AGAR
Casein	Ustore (ICN)	ICN96012825	25 lbs	773-846-7300
L-Ascobic Acid	Ustore (ICN)	ICN10076991	1 kg	612-624-4878
Cholesterol	Ustore (ICN)	ICN10138001	1 kg	612-624-4878
Dextrose	Ustore (ICN)	ICN90559425	25 kg	612-624-4878
Methyl-p-Hydroxybenzoate	Ustore (ICN)	ICN10234101	1 kg	612-624-4878
Salt Mixture	Ustore (ICN)	ICN90284502	2 kg	612-624-4878
Sorbic Acid	Ustore (ICN)	ICN10293790	0.5 kg	612-624-4878
Vitamin Supplement	Ustore (ICN)	ICN90324401	1 kg	612-624-4878
Wheat Germ	Ustore (ICN)	ICN90328825	25 lbs	612-624-4878
Proprionic Acid	Ustore (Fisher)	A258500	300 ml	612-624-4878
Phosphoric Acid	Ustore (Fisher)	A242500	500 ml	612-624-4878
Fumidil B	B & B Honey Supply	A868	250 g	612-645-8148
Aureomycin	Midwest Veterinary Supply	275.02300.3	6.4 oz	612-894-4350
Sugar	Rainbow or Cub Foods			

Others

Wax Ring	Custom Bio-Products (Bruce Lang)	515-387-8883

APPENDIX B: DIET ROOM (clean after making diet)

_____ Clean all surfaces of cooker (Spray with Sanibet after cleaning)

_____ Clean wall behind cooker

_____ Wipe off top of UV lights

_____ Clean counter (Spray with Sanibet after cleaning)

_____ Clean all shelves and cabinet facing

_____ Clean top of refrigerator and door and handle

_____ Clean blender housing (Spray with Sanibet after cleaning)

_____ Clean doorknobs

_____ Mop floor

Date:

Signed:

APPENDIX C: LARVAL GROWTH CHAMBER

(clean monthly)

_____ Clean all walls and ceiling

_____ Clean fluorescent light fixtures

_____ Clean wire racks

_____ Clean all outlets and fixtures

_____ Clean humidifier and humidifier control

_____ Clean fan

_____ Mop floor

Date:

Signed:

APPENDIX D: OVIPOSITION CHAMBER (clean monthly)

_____ Clean walls and ceiling, removing eggs

_____ Clean humidifier

remove and replace water

wipe all parts, inside and outside

_____ Vacuum evaporator

_____ Replace filters on evaporator

_____ Clean HEPA filter unit (unplug first)

wipe outside

remove roughing filter and rinse out

vacuum inside, if needed

_____ Mop floor (this needs to be modified)

Date:

Signed:

APPENDIX E: WORK ROOM (clean monthly)

_____ Clean puncher (if needed)

scrap and remove wax buildup

clean all surfaces

_____ Clean all counter surfaces

_____ Clean compressor

_____ Clean outside of Nilfisk and clean attachments

_____ Clean all cabinet surfaces

_____ Clean outside of HEPA filter unit

_____ Replace roughing filters on HEPA unit

_____ Clean table

_____ Clean floor with Lime-away

Date:

Signed: