TRIBOLIUM INFORMATION BULLETIN

NUMBER 2

MARCH, 1959

Editor’s Notes 2

Selected Bibliography on Grain Insects 3

Effect of Ether on Fecundity of T. castaneum 35

A Technique for Censusing Large Populations of T. castaneum 36

Safe Periods of Etherization of T. castaneum 37

Equipment for Transferring Individual Beetles and Pupae 39

RESEARCH NOTES

Sokoloff, A. Effect of ether on fecundity of T. castaneum.

Chicago wild-type beetles two months old were subdivided into lots of 10 males and 10 females and subjected to etherization for 0, 1, 2, 3 and 5 minutes. After observing the process of recovery, the beetles were placed in creamers containing 4 grams of flour, and the cultures introduced into an incubator maintained at 29º C and 70% relative humidity. Beetles were transferred almost daily to fresh medium, except that on the sixth day the adults were left in the same creamer for a period of 18 days. The number of progeny in each creamer was recorded as larvae at the end of 19-24 days. The data are summarized in the following table. It is assumed that ether has no effect on the viability of the eggs and that all eggs survive to hatching.

Number of Larvae Produced by Beetles Exposed to Ether

Days Elapsed Time of Exposure to Ether (Min.)  
After Etherization 0 1 2 3 5   
\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
 1 6 4 3 2 1

2 27 23 33 35 7

3 47 41 65 79 42

4 74 64 63 69 89

6-24 127 120 122 116 69  
\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_   
  
It is evident that no appreciable effect is produced when beetles are exposed to ether for a short time: Beetles exposed for one, two, or three minutes lay about as many eggs (as early as 24 or 48 hours after exposure) as the controls (column two). However, if beetles are exposed to ether for five minutes, the effects are longer lasting: At the end of 48 hours these beetles have laid about as many eggs as females exposed to ether for a shorter period had laid for the first 24 hours after etherization.

Beetles exposed for five minutes apparently recover fully by the end of 48 hours, so that by the end of the third day they have laid as many eggs as the controls.

Other data not included in the table suggest that exposure to ether for a longer time will have longer lasting effects: Beetles etherized for 10 minutes will not lay any eggs the first 48 hours, very few by the end of the third day, and achieve normal egg laying by the sixth day. A 15-minute exposure will postpone egg laying 24 hours over the preceding period: Egg laying begins on the fifth day, and may decrease sharply on the sixth day. A 20-minute exposure will delay egg laying about as much as the preceding period, but females that survive this prolonged exposure may recover full egg production by the sixth day. A 25-minute exposure will prevent females from laying for a full five days, and few eggs are produced on the sixth. A pair surviving etherization for 30 minutes had not produced any progeny by the end of 17 days. Beetles subjected to ether for 45 minutes may recover, but they subsequently die without leaving progeny.

The sudden rise in egg production on the second and third day by beetles exposed to ether for two or three minutes requires further investigation.

Sokoloff, A. A technique for censusing large populations of T. castaneum.

Populations started with 50 pairs of beetles in 100 g of flour yield very large numbers of offspring in one generation. A method for handling large populations has been developed in our laboratory which greatly shortens the time spent in censusing a single population. The following procedure takes advantage of the clinging ability of larvae and adults, and the tendency, on the part of the adults, to seek shelter in a dark corner (negative phototactism).

Place a porcelain pie plate with scalloped edge in a 200 mm nesting dish. Prop one end of the plate. (A creamer lying on its side will give the plate the proper angle). Sift the culture through #2 silk bolting cloth, placing the sifted material (adults, larvae, pupae and frass) on the plate. Resift the flour through #5 silk bolting cloth to separate eggs and small larvae from the flour. Spread this material thinly over black construction paper and spill the frass onto another piece of black construction paper. Larvae will cling to the first piece of paper, and they can be dropped into the dish by tapping it vigorously. Repeat the process five times. Over 99% of the small larvae will thus be recovered from the frass. Since eggs and fecal pellets are of about the same size, the eggs are sacrificed and discarded with the frass.

The nesting dish is coded to identify the population and oriented so that the uptilted edge of the plate faces the strongest source of light. The adults climb the edge of the plate and fall into the dish. In this manner about 95% of the adults separate themselves out of the frass. The rest of the beetles have to be separated with a brush: Aliquots from the plate are transferred to a clean dish. The live adults are grouped together. The dead adults (including pieces of beetles) are placed in a separate dish. The frass is blown off gently, and the larvae and pupae counted and returned to a bottle containing fresh medium.

Since in this particular “infection” experiment the eye phenotype is essential, the whole adult population is etherized for three minutes, placed on a white glass plate between guide lines imprinted with a wax pencil, and counted under the dissecting microscope.

Once the flour is sifted and the adults sorted out, it takes half an hour to census as many as 2500 adults in a culture.

Sokoloff, A. Safe periods of etherization of T. castaneum.

In contrast to the characteristic posture exhibited by overetherized Drosophila, it is difficult to tell from the appearance of Tribolium whether it has been overetherized. Because etherization is necessary in many phases of work with flour beetles (e.g., sexing of adults, identification to phenotype, etc.) it is desirable to know the tolerance of Tribolium to this narcotic. Two month old Chicago wild-type beetles were subdivided into lots of 10 males and 10 females and exposed to ether fumes for different periods. The behavior of beetles following narcosis was observed (to obtain a reliable criterion on when beetles should be re-etherized), and the number of beetles surviving the various periods of exposure was recorded. The room temperature during the beginning of the experiment was 780 F. An etherizer of the type described by Muller (DIS 6:55, 1936) was used throughout the experiment. The data are summarized in the following table.

Time exposed Survival Approximate time elapsed before  
to ether (min.) % beetles begin to walk (hours)  
\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
 1 100 1/3  
 2 100 2/3  
 3 100 1-1/2  
 5 100 2  
 10 100 2-3/4  
 15 45 6  
 20 25 Probably 72  
 25 10 Probably 72 or more  
 30 55 Undetermined  
 45 20 Undetermined  
 60 0 --  
 90 0 --

It is evident that if beetles are etherized for as long as 10 minutes all will recover. Following longer exposures to ether beetles may walk, but they may die a few days later without leaving progeny. Those that survive may have long-lasting effects (see accompanying article on the effect of ether on fecundity).

A few remarks on the behavior of beetles following short periods of etherization may help other investigators to plan their work.

1. Ether for 1 minute

When observed immediately after removal from the etherizer, the beetles are  
 not completely narcotized: legs and antennae are moving, and those on their  
 feet attempt feeble walking movements.

5 min. later – Those on their legs attempt to walk, but some may fall on their  
 backs.

20 min. later – Beetles will walk and stay on their feet if righted.

32 min. later – Beetles can cling to bristles showing coordinated movement.  
 If poked, will take a few steps back.

40 min. later – Righted beetles walk, back, forward or in circles.

60 min. later – About half of the beetles walk.

70 min. later – About 90% of the beetles walk.

2. Ether for 3 minutes

No movement at all when observed immediately after removal from   
 etherizing bottle.

6 min. later – Tarsi and antennae twitch.

13 min. later – Movement of the head ventrally.

15 min. later – Ovipositors may be extruded. Eggs may be laid by  
 gravid females.

25 min. later – If beetle is poked, the legs move faster.

60 min. later – If beetle is righted, it will fall on its back.

80 min. later – If beetle is righted, it will stay on its feet.  
 95 min. later – Beetles begin to walk.  
 120 min. later – Feeble walking, with no fixed direction.

3. Longer exposures to ether

The “waking up” pattern is the same, but movement of beetles is greatly   
 delayed: If etherized for five minutes, most beetles will be motionless at  
 the end of 25 minutes and will fall on their backs after attempting to crawl even   
 at the end of 100 minutes. If etherized for 10 minutes, some may crawl if righted   
 at the end of 150 minutes. Half of the beetles will walk normally 5-1/2 hours   
 after treatment, and 90% will be fully recovered at the end of 23 hours.

With 15 minute exposure to ether, beetles begin to die. Those that recover may take 90 minutes before tarsi and antennae begin to move and nearly four hours before they begin to crawl if righted. Full recovery may not occur until 50 hours have elapsed.

It is of interest that if beetles receive too much etherization, the color of the soft membranes between sclerites or tergites may change, acquiring a maroon color, which thus differs from the characteristic chestnut color of the normal beetle. This is particularly noticeable in the extruded ovipositor or in the soft parts between head and thorax. This change in color has also been observed in eggs and larvae, some becoming a very deep purple. If beetles are starved too long before etherization, some maroon or purplish material is observed in the rectum of the beetle when it is squeezed with forceps to extrude the ovipositor or the penis. Whether this material is produced following etherization or produced in the course of the beetle’s normal metabolism and the nature of the material itself has not been determined.

Since mature females straining to turn themselves upright often lay one or two ripe eggs, etherization may be the solution to obtain flour-free eggs.

Bywaters, James H. Equipment for transferring individual beetles and pupae.  
 (Reference authorized).

If you are having difficulty transferring adult beetles or pupae from one container to another, you might try using about twelve inches of flexible rubber or plastic tubing to one end of which has been attached a common blowpipe and to the other a common pipe stem (some find the curved stem more satisfactory). With a small amount of practice one can soon learn to pick up these forms without difficulty. Also, the reverse procedure (blowing) can be very helpful in uncovering beetles in flour or frass. The basic idea for this was picked up at the Purdue Laboratory from Dr. Grady Martin.