A Handbook on Bean Beetles, *Callosobruchus maculatus*

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Acknowledgments:

Figure 3, Dorsal view of male and female *Callosobruchus maculatus*, was reprinted with permission from, Brown, L. and J.F. Downhower. 1988. *Analyses in Behavioral Ecology: A Manual for Lab and Field*. Sinauer Associates, 194 pages.

Photographs were taken by L. Blumer.

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Introduction

This handbook provides basic information about raising, handling, and maintaining cultures of bean beetles, *Callosobruchus maculatus*. Our intended audience is the faculty and staff who teach or coordinate undergraduate laboratory courses. The information provided here is based on our own experiences working with this insect in undergraduate laboratory courses, and information available in the research literature. Our references to specific commercial vendors for supplies are intended to assist you in finding the types of supplies we have used, but these references are not an endorsement of these particular vendors. Comments to the authors with corrections or suggestions for additional information to include in future versions of this handbook would be much appreciated.

Background Information

Natural History

Bean beetles, *Callosobruchus maculatus* (Coleoptera: Bruchidae), are agricultural pest insects of Africa and Asia that presently range throughout the tropical and subtropical world. This species also is known as the southern cowpea weevil. The larvae of this species feed and develop exclusively on the seed of legumes (Fabaceae) hence the name bean beetle. The adults do not require food or water and spend their limited lifespan (one - two weeks) mating and laying eggs on beans. The systematic placement of bean beetles is as follows: *Callosobruchus* is one of the genera in the family Bruchidae (seed weevils) that is in the superfamily Chrysomeloidea. This group is part of the order of beetles, Coleoptera (from Greek "sheath-winged" referring the stiff outer, first pair of wings (elytra) that protect the membranous second pair of flight wings). The Coleoptera is largest of the orders that comprise the class Insecta. Insects are the largest and most diverse (750,000 described species) of all the animal classes that are found in all but marine environments. Insects are protostomous animals and are thus more closely related to mollusks and crustacea than to the deuterstomous vertebrate classes. See the Tree of Life webpage on the Chrysomeloidea for more information on the systematic placement of *Callosobruchus* (Tree of Life Web Project 1995) or start from Animals page (Tree of Life Web Project 2002) and work your way down to the level of family Bruchidae. Systematic information also can be found on the Animal Diversity Web (Myers et al. 2006).

Bean beetles exhibit two adult forms (morphs), a sedentary (flightless) form and a dispersal (flying) form. The dispersal morph is induced by high larval density in stored beans or laboratory cultures, and is caused by density dependent micro-habitat temperature increases (Utida 1956, 1972). Induction of the dispersal morph allows individuals to move to new, higher quality habitats. These two morphs have very different life history characteristics such as longer adult lifespan in the dispersal morph and significantly reduced fecundity compared to the sedentary morph (Utida 1956, 1972). In the sedentary form, the sexes are highly dimorphic and readily distinguished but sex differences are very subtle in the dispersal form. Thus, it is essential to maintain laboratory cultures at low density (one or two larvae per bean) and temperatures no greater than 30°C, if individual beetles need to be unambiguously identified by sex (see **Identifying the Sexes**).

We are frequently asked, by students and even fellow academics, what is the purpose of bean beetles? A very short adult life span and a larval stage in which most or all life-time feeding occurs is not unusual in insects (for example the Order Ephemeroptera, mayflies), but this life cycle seems strange compared to the dominance of adult stages in familiar birds and mammals. Ultimately, in evolutionary terms, the purpose of bean beetles is the same as in all other living things, reproduce and leave descendants. Ecologically, bean beetles are herbivores that have specialized on seed consumption. They are a part of food webs in that eggs and larvae are prey for parasitoid wasp species (Boeke et al. 2003), and adults may be prey for birds, reptiles and amphibians, so they do have the purpose of providing food for other organisms.

Life Cycle

Once inseminated, adult females will lay (oviposit) single fertilized eggs on the external surface of a bean. Individual eggs (0.75mm long) are oval or spindle shaped, clear, shiny and firmly glued to the bean surface (Figure 1a). The larva that hatches from the egg burrows from the egg through the seed coat and into the bean endosperm without moving outside the protection of the egg. Once the larva burrows into the bean, the remaining egg (shell) becomes opaque white (Figure 1a) or mottled as it fills with frass (feces) from the larva. The larva (Figure 1b) burrows and feeds on the bean endosperm and embryo, undergoes a series of molts, and burrows to a position just underneath the seed coat prior to pupation. Although the seed coat of the bean is still intact, a round 1-2mm window is apparent at the location where the beetle is pupating (Figure 1c). Pupation (Figures 1d) is the complete metamorphosis of the larval maggot to a winged adult. The adult that results from pupation chews through the seed coat and emerges from the bean (Figure 1e and 1f). The adults are fully mature 24 to 36 hours after emergence. Males seek females to inseminate (see **Mating Beetles**) and females store viable sperm in their spermatheca (a structure in the female reproductive tract for storing sperm). Neither male nor female adults require food or water during their short adult lifetime (10-14 days).

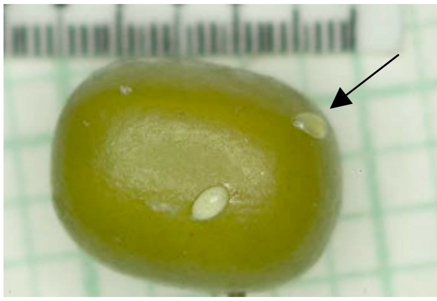

Figure 1a. Single newly laid egg (upper right arrow) and an old egg (center) on mung bean. The graph paper squares are 1mm.

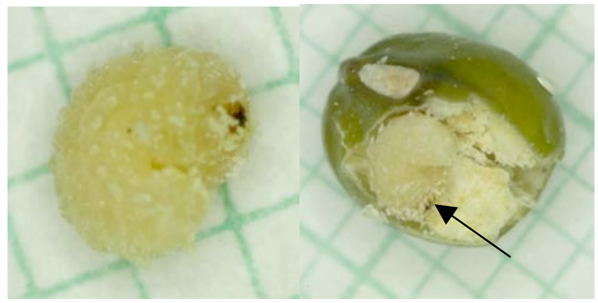

Figure 1b. Larval bean beetle. The dark area at the upper right of the isolated larva is the mouth. A larva in a mung bean is at the arrow. The graph paper squares are 1mm.

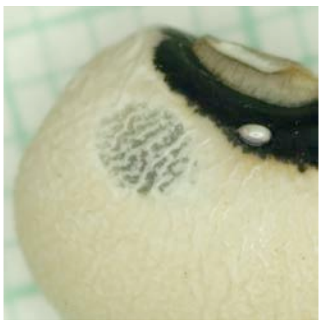

Figure 1c. "Window" in seed coat of cowpea at the location of a pupating beetle.

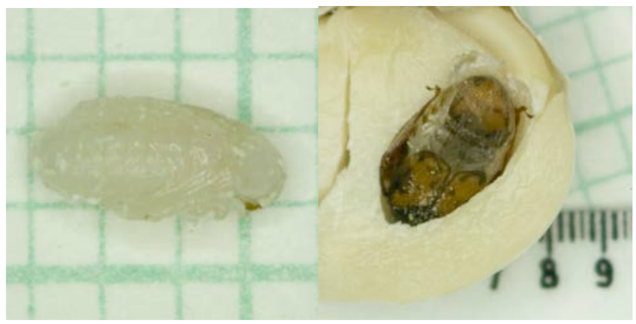

Figure 1d. Pupa of bean beetle. A young pupa (left) and an older pupa (right) in a head down position. The graph paper squares are 1mm.

Figure 1e. Adult bean beetle. An adult female, sedentary morph, on a mung bean.

Figure 1f. Adult emergence holes. These holes are the result of an adult bean beetle emerging after pupation. The graph paper squares are 1mm.

Culture and Handling Methods

<u>Culture Techniques</u>

Starting new cultures requires no more than containers to contain beans and beetles. Virtually any closable containers will work successfully: lidded plastic Petri dishes, screen covered glass jars, snap lid vials, and cotton plugged shell vials are all suitable containers. We prefer to use disposable plastic containers in our teaching laboratories to minimize breakage and to keep cultures relatively small but replicated. Although bean beetles are the easiest of insects to successfully culture, sometimes a culture will fail if adults were very old when introduced to the new culture container or too few adults were introduced for adequate numbers of eggs to be laid. Therefore, we always start stock cultures in pairs (or more if needed) on the same date and we never dispose of old cultures until we see that a new culture has successfully yielded adults. It is always a good practice to check a new culture a few days after it is started to see if numerous eggs have been laid. Plastic Petri dishes 150 x 25 mm (Falcon 351013, Fisher Scientific 08-757-145, VWR 25379-048, Carolina Biological 199279) are ideal containers from which students can easily view cultures and remove selected adults. Although the lids fit loosely on the plates, Petri dishes will confine adults and permit adequate ventilation without any modification. Covering the bottom of a Petri dish with a single layer of beans (approximately 50 ml volume) and introducing 10 adults males and 10 adult females is sufficient to produce a dense culture. Cultures established in this manner will typically sustain two or three sequential generations without adding additional beans and without inducing the production of dispersal morph adults. Cultures older than three generations on the same set of beans should be discarded (see **Disposal of Cultures**). We also have had good results using plastic snap-lid containers (300 ml Corning Snap-Seal Sample Containers, Fisher Scientific 02-540-23) with pin-holes punched in the lid for ventilation. As with the Petri dishes, we use only 50 ml of beans in each 300 ml snap-lid container. The ideal seeds (beans) to use are mung (*Vigna radiata = Phaseolus aureus*), blackeye peas or cowpeas (*Vigna unguiculata*) and adzuki (*Vigna angularis*) (Figure 2). Although bean beetles can be reared successfully on these three species, they differ in nutrient quality (USDA Agricultural Research Service) and secondary compounds (Bisby et al. 1994). We find that raising beetles on blackeye peas is best done in 150 mm petri dishes, which minimizes mold growth on the beans. The successful completion of the bean beetle life cycle on most other bean species is minimal (Janzen 1977). We prefer to use organically grown beans to minimize pesticide problems in our cultures, but it is not essential for successful culturing of beetles. Dry beans and adult beetles in a container that keeps the beetles from escaping are all that you need. Keep the cultures at temperatures between 22° - 30°C (not in direct sunlight and away from radiators).

Figure 2. Beans for culturing bean beetles. Clockwise from the top: adzuki, blackeye peas (cowpeas), and mung beans.

Generation Time

The elapsed time from newly laid eggs to the emergence of adult beetles varies between bean beetle strains and environmental conditions. Previous studies indicate that temperature and relative humidity (Howe and Currie 1964, Schoof

1941) are the most important variables influencing generation times (egg to adult) when beetles are raised on preferred host beans. Within a limited range, increasing temperature will decrease the generation time. In our laboratory, we have observed generation times as short as 3-4 weeks in a 30°C incubator (12:12 day:night light cycle) and ambient humidity (averaging 30% RH and ranging from 20%-40% RH). Cultures raised in a 25°C incubator (12:12 day:night light cycle) and ambient humidity (averaging 50% RH and ranging from 40%-60% RH) had generation times of 5-6 weeks. Cultures maintained on a laboratory bench at room temperature (22°C) with indirect outdoor window lighting and ambient humidity (averaging 50% RH and ranging from 40%-60% RH) had a generation time of a full 7 weeks. Reliably obtaining newly emerged adults for a specific date (a scheduled laboratory class meeting) requires that you grow cultures for a few months under your laboratory conditions so you can predict emergence times. In low humidity locations, or during the winter in North America, when RH is typically low, the emergence rates of beetles may be improved by increasing the RH of an incubator or culture container. Simply placing a tray of water in a temperature controlled incubator may be all that is necessary to bring RH to the 40%-60% range, and improve emergence success rates.

Generation time also depends on the host species of bean you choose to use. We have found longer generation times in adzuki beans compared to either mung or black-eyed peas. At 30°C, it takes seven weeks for emergence from adzuki beans compared to 3-4 weeks from mung beans.

<u>Identifying the Sexes</u>

Male and female bean beetles (of the sedentary morph) are easily distinguished from one another by general appearance. The most distinguishing characteristic is the coloration on the plate covering the end of the abdomen. In the female, the plate is enlarged and is darkly colored on both sides (Figure 3). In the male, the plate is smaller and lacks stripes. In some strains, females are larger in size than males. Also, females are black in coloration and males are brown.

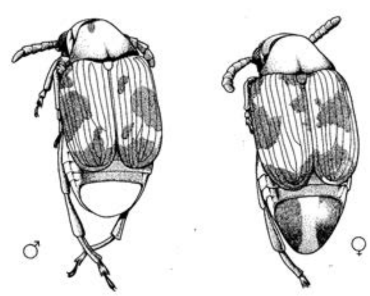

Figure 3. Dorsal view of male and female *Callosobruchus maculatus*. The sex specific coloration of the posterior abdominal plate (pygidium) is shown (Figure from Brown and Downhower, 1988). Photographs of a male and female are at the same scale. The squares are 1mm.

Handling Techniques

Although sedentary morph bean beetles are capable of flying, they rarely do. As a result, they are easy to handle. Beetles can be moved either using *Drosophila* sorting brushes (Carolina Biological 17-3094 or Ward's Natural Science 15 V 3846) or soft forceps (BioquipTM featherweight forceps 4748 or 4750; Ward's Natural Science 14 V 0520).

When removing beetles from stock cultures, individual Petri dishes, or well plates, tap the containers lightly on the lab bench before removing the lid to prevent beetles from crawling out immediately. If the lid is left off for more than a minute or so, beetles will escape from the culture dishes.

Especially when kept individually, bean beetles will often "play dead." Don't be fooled! A gentle prod with forceps or brush will cause them to move.

Measuring Beetles

Body Mass – To weigh individual beetles, a 0.1 mg analytical balance, at a minimum, is necessary (for example, Ohaus Analytical Balance Model PA64, Carolina Biological 70-2498, Fisher Scientific S97282). Individual beetles can be placed in the bottom of a 35mm Petri dish (for example, Falcon 351008, Fisher Scientific 08-757-100A or 60mm dish, Carolina Biological 741246) to be weighed.

Linear Measures – Linear measures of body size, such as the length of the elytra (the hard wing covers), may be readily collected on dead adults. Such measurements may be facilitated by using an inexpensive microscope video camera, such as the Moticam 352 (Carolina Biological 591282) attached to the eyepiece of a dissection microscope. This video camera connects directly to a computer (Mac or Windows PC) via the USB port and measurements are made by using image analysis software (included with the camera) to evaluate the length of a line drawn on a body part in a captured image or in a live video image. Dead animals may be sorted by sex and glued to file cards for measurement under a dissection microscope. A free image analysis program (NIH Image J) may be used to make measurements on any digital image, including those captured with a Motic camera.

Mating Beetles

Both virgin and non-virgin beetles will mate readily. However, virgin males may not produce fully formed spermatophores until 24 hours after emergence. In addition, females may not mate for several hours after a previous mating.

To mate beetles, place beetles into a 35mm Petri dish (for example, Falcon 351008, Fisher Scientific 08-757-100A or 60mm dish, Carolina Biological 741246). Males will chase females until they are able to mount and copulate with females (Figure 4). Copulation generally begins within 10-15 minutes, but sometimes may not begin for 30 minutes to an hour.

Figure 4. Bean beetles mating.

To determine if a male transferred a spermatophore successfully during copulation, weigh the male before and after copulation. Males may lose as much as 5% or more of their body mass due to spermatophore transfer. However, spermatophore size will decrease with subsequent mating by a given male.

Isolating Virgins

To isolate virgin beetles, place a mated female in a 35mm Petri dish (for example, Falcon 351008, Fisher Scientific 08-757-100A or 60mm dish, Carolina Biological 741246) with a single layer of beans. The vast majority of females in a stock culture will have mated and are capable of laying fertile eggs. After 12-24 hours, females will begin to lay eggs on beans. With an excess of beans, females will lay only a single egg on each bean. Finding eggs on beans can be facilitated by using a dissection microscope (at 10x total magnification such as Carolina Biological 593290, VWR 15147-844 or Fisher Scientific S94912) or a large magnifying glass (2.0x magnifier Carolina Biological 602106 or 2.5x magnifier Fisher Scientific 14-648-19 or VWR 62379-535). Remove the beans with single eggs (Figure 5) and place each bean in an individual 35mm Petri dish or the well of a 6 or 12-well flat bottom tissue culture well plate (tissue culture plates, Fisher Scientific or Carolina Biological 703466-703467). Replenish the beans as they are removed. A single female can produce more than 100 eggs in her lifetime. In general, the sex ratio is 1:1.

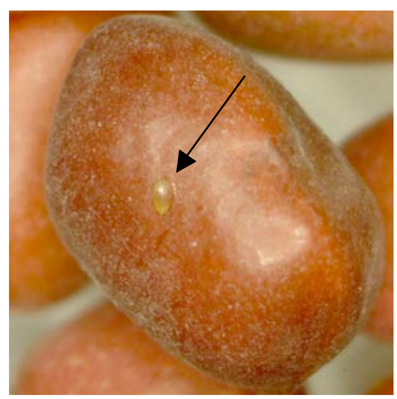

Figure 5. Single egg (arrow) on an adzuki bean.

Disposal of Cultures

Bean beetles are a potential agricultural pest insect that is not distributed throughout the United States and Canada. This species is absent in much of North America because it is intolerant to freezing temperatures and suitable host plant species are not among our native (non-agricultural) flora. None-the-less, it is prudent and appropriate to dispose of living adults, and beans that have had contact with living adults, in a manner that will prevent their release to the natural environment. Placing a live culture (or any beans exposed to adults beetles) in a freezer (0°C) for a minimum of 72 hours prior to disposal will ensure that beetles at every life cycle stage are dead. Then, dispose the frozen culture in the same manner as food waste.

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