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Diet and Metabolism in Bean Beetles

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Here we describe a lab exercise to determine which nutritive molecules influence adult bean beetle (*Callosobruchus maculates*) metabolism. The project as described requires approximately two hours for setup and one, two-three hour lab session for introduction, experimentation, discussion, and analysis. Primary objectives of the project were to have students test hypotheses related to the influence of different nutritive molecules on CO_2 production in bean beetles and use the resulting data to generate a novel follow-up experiment(s). Ideally, this lab would follow lectures in which students had learned about different macromolecules, their constituents, and the primary metabolic functions of each.

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Introduction

Although bean beetle adults do not require additional nutritional intake following pupation, they are capable of ingestion. Here we describe a lab exercise where students conduct an experiment to determine which, if any, potentially nutritive molecules influence adult bean beetle metabolism. Metabolic activity was measured indirectly via CO_2 output and O_2 consumption. Following the completion of the experiment, data from all groups were analyzed to produce class conclusions that served as background for additional experimentation. In our course, this activity precedes the development of group projects designed by the students themselves. However, the activity could easily be modified for other situations or used as a stand-alone exercise. Ideally, this lab would follow lectures in which students had learned about different macromolecules, their constituents, and the primary metabolic functions of each. Additionally, students should have a working knowledge of the scientific method and a general understanding of the bean beetle life cycle prior to this lab. This project was originally developed through our participation in the Bean Beetle Curriculum Development Network Workshop (**www.beanbeetles.org**).

Student Outline

Objectives

- **Test hypotheses related to the influence of different nutritive molecules on CO_2 and O_2 production in bean beetles**
- **Use the resulting data to generate a novel follow-up experiment for your group project**

Introduction

Energy is required by all living organisms in order to sustain most cellular reactions. The catabolic process of cellular respiration provides much of this energy from the breakdown of various nutritive molecules. For many, but not all, animals, these molecules are ingested at regular intervals throughout their life. Today we will examine an interesting variation on this theme *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae), the bean beetle. Native to Africa and Asia, but currently found throughout the tropics, these insects present a major economic threat to grain-legume agriculture with up to 30% annual crop losses attributed to their actions (Yadav, 1997). As you will remember from class, adult bean beetles do not require food or water following pupation (Beck and Blumer, 2011). Most of their nutrition is derived from consumption of the endosperm of a legume prior to emergence. However, as adults they are capable of extending their lifespan through ingestion of food, water, and (in the case of females) ejaculate obtained during mating (Ursprun et al., 2009). In this lab you will test the hypotheses you generated last class period as you determine whether consumption of various nutritive molecules alters CO_2 production during aerobic respiration.

Aerobic Respiration in Bean Beetles

As you will recall, cellular respiration involves the three processes of glycolysis, the Krebs cycle, and the electron transport chain (ETC). The first process (glycolysis) can occur aerobically and anaerobically since the reaction is not dependent upon the presence of oxygen. However, if oxygen is present, the two molecules of pyruvic acid (the end product of glycolysis) will be taken into the mitochondria as acetyl coenzyme-A, where they are further processed through the reactions of the Kreb Cycle and ETC. The Krebs cycle produces only one ATP molecule directly (per acetyl co-A), but produces two other products (NADH and $FADH_2$) which are further processed for up to an additional 34 ATPs via the ETC. Aerobic respiration uses oxygen and produces carbon dioxide as a by-product (see equation for glucose metabolism below).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP$$

As it would be difficult to directly measure the consumption of nutrients or the production of ATP, today we will indirectly determine metabolic activity by measuring changes in the levels of CO_2 and O_2 within a closed container.

Materials

In addition to the normal computers and Logger Pro® interface, each table has CO_2 and O_2 sensors (the former has a cheesecloth covering), 50 ml Falcon tubes, Parafilm, forceps, and a test tube rack to hold the assembled containers. Last period the class decided on four different nutritive compounds to test and generated hypotheses related to these solutions. A 5% solution (w/v in water) of each compound was prepared 48 hours ago and ~50 beetles were transferred to new culture jars containing moist cotton squares saturated with of one of the solutions. Additionally, two control jars were prepared: one with distilled water and one with beetles alone (no feeding solution). Each table has one of these jars to work with today. At the end of the lab, we will analyze data from each table as a class. As we work through the lab, be thinking about questions you would like to pursue with your group project. Today's data will provide some background information that will help you frame your group's questions and hypotheses.

Experimental Design

Turn on the computer, check that the Logger Pro® interface is connected (power and USB cables) and the gas sensors are connected to channels 1 and 2. Then open the Logger Pro® software program. The software should automatically detect the probes, if this doesn't happen ask for help. Open the program *Bean Beetle Metabolism*. **Do not press the start button at this time.**

You will need to transfer 25 beetles to each Falcon tube. As the sex of the beetles was not a factor included in our original hypotheses, it doesn't matter which beetles (male or female) you select today. However, sex differences might be interesting to examine through your group project. To prevent escapees, we will anesthetize the beetles **BEFORE** opening the jar. To do this, place the entire jar in the lab freezer for 90 seconds. Check the beetles at this point. If any are still moving, return to freezer for another 30 seconds. Repeat until all beetles are anesthetized.

NOTE: Frozen beetles will not respire. Do not forget your beetles and leave them in the freezer!

Once beetles are completely anesthetized, return the jar to your table, open the lid, and transfer 25 beetles to the falcon tube using the forceps. You should take care to gently pick up the beetles to avoid harming them. As you are transferring the beetles, loosely cap the tube using the blue lid. This will prevent the awakening beetles from escaping. Once you have transferred 25 beetles, remove the blue lid and insert the CO_2 probe (with cheesecloth cover) into the tube and seal with Parafilm. Repeat this process with the second Falcon tube and the O_2 probe. Once assembled, raise your hand so that I can check your set up and verify that the probes are calibrated correctly. Click the "Start" button on the Logger Pro® program (green button at upper right of screen). This program will collect data at three minute intervals for 60 minutes. Note that the units on these two measures are not the same. You will need to use the correct units when you graph your data at the end of the lab.

Once you have finished collecting your data, open Excel and transfer your results to a new spreadsheet. As a group, prepare graphs of your data and calculate the rate of CO_2 production and O_2 consumption (slope of a trend line is a reasonable approximation). You will also need to enter your data in the class spreadsheet on the computer at the front of the lab. While you wait for the rest of the class, discuss the data. Does it support or refute your hypotheses? What additional questions do these data bring to mind? Are there variations on the experiment that you would like to perform as your group project? What additional hypotheses would you like to test? Use this time to begin designing your experiment. Once all groups are finished we will analyze the data as a class. Copies of the class spreadsheet will be posted on Blackboard.

Notes for the Instructor

Experimental Design

Pilot trials of this lab focused on the following questions:

- Will adult bean beetles consume liquid nutritive solutions?
- If so, how could you determine (directly and indirectly) the effect on metabolic activity?
- Which solution will have the greatest effect on CO_2 production and/or O_2 consumption?

Follow-up questions that were generated by our students after the lab:

- Is there a sex difference in metabolic activity of adult bean beetles?
- Are males/females more likely to consume a given solution?
- Is the change in metabolic activity dependent upon the concentration of a given solution?

The **Bean Beetle Handbook** contains a wealth of information related to the life cycle and culturing techniques for these critters and, as such, we won't discuss such details here. You will want to allow plenty of time to culture an adequate number of beetles for your class. This lab requires 40-50 beetles per group as described. Variations on the described method (i.e. using the same beetles for both CO_2 and O_2) can reduce this number. You should also plan to lose a small fraction of your beetles as your students will likely crush a few while transferring them with the forceps. When beetles are properly anesthetized, you should not have a problem with escapees. However, students need to be reminded to work quickly and be vigilant as the beetles warm and become active. At the end of the lab, all beetles should be disposed of properly by either returning them to the culture containers for reuse or frozen for at least 72 hours.

In our course, this lab follows lectures on both cellular respiration and an introduction to bean beetles. In the latter, students discuss the life cycle of bean beetles and are assigned portions of the **Bean Beetle Handbook** as background reading. As most students have never worked with any insect model, it is important to spend adequate time introducing the bean beetles. Students typically find their life cycle interesting and we often field questions related to reproduction, life span, and influence of the nutrient content of legumes on development. These can either be addressed as part of a lecture/discussion or turned back on the students as a pre-lab assignment.

Following this introduction, the class as a whole breaks into small groups to discuss which nutritive molecules are likely relevant given the bean beetles' life cycle. We then ask the groups to share their comments and talk about considerations in experimental design (duration of data collection, replication, number of specimens per container, what makes for an adequate control, etc). Although we typically let the student start the conversation, active moderating of the experimental design is necessary to ensure that the desired learning objectives are met. Once the class has decided on the specific nutritive solutions to be tested, each group generates hypotheses (generally two per group) that will be tested in the subsequent lab. We typically require our students to use an "if/then" format for their hypotheses as this allows them to clearly state the idea to be tested. As described, this lab is meant to spark additional questions in the participating students that will then be pursued in a longer, independent group project later in the semester. For example, we intentionally do not compare differences between sexes and do not allow students to vary the concentration of the feeding solutions. Often students will catch on to these "errors" during the analysis and discussion phase, which then becomes an opportunity to close-the-loop of the scientific method.

Once the class has decided on the solutions to be tested, feeding containers (jars in our case) were prepared as described below. During our pilot testing, 48 hours of exposure to the feeding pad saturated with sucrose or glucose produced significantly elevated level of CO_2 production (relative to beetles exposed to water) that persisted for at least 24 hours after the pads had been removed ($F(2, 57) = 26.64$, $p < .00001$). It should be noted that variations on this feeding schedule have yet to be explored and this feeding scheme may not be optimal.

Equipment and Supplies

Feeding setup (typically 48 hours prior to start of student lab)

One feeding pad made of an absorbent material (square of cotton or thick filter paper) and culture container are needed for each solution to be tested. Depending on the number of beetles required for your class, these containers may range in size from petri dishes (with lids) to larger jars. We used small canning jars capped with a sheet of filter paper or Kimwipes (Fig. 1). Regardless of the container, you will want to provide dry space for the beetles. We found that completely covering the bottom of a container with a moist feeding pad resulted in a large number of drowned beetles.

Figure 1. Sample feeding container consisting of a pint canning jar covered with filter paper.

Nutritive solutions (protein, starch, amino acids, simple and complex sugars, lipids, etc.) and concentrations can vary depending on the specific research questions. The precise volume needed will likely be quite small (<5mL per sample), but it will be dictated by the size of containers and absorbency of the pads. We used 5% (w/v) solutions in distilled water. Depending on the atmospheric conditions in the lab, you may need to rewet the feeding pads after 24 hours to ensure adequate access to the solution.

Bean beetle characteristics (sex, age, etc.) are all dependent upon the specific hypotheses you wish to test. For a 50mL Falcon tube, we obtained good data with 20-25 beetles per tube. If using a container with more volume, you may need to increase the number of beetles or the length of time you collect data. As mentioned above, plan ahead to have sufficient specimens. For a class of 24-30 students, you may easily need several hundred beetles if testing CO_2 and O_2 separately with multiple solutions.

Day of Experiment

Each group of students will need:

- Several pairs of forceps
- Access to a freezer to anesthetize the beetles
- A computer with **Logger Pro** Software, Vernier computer **interface** and **CO_2 /O_2** sensors. The current CO_2 probes (CO_2-BTA) do not fit standard 50mL tubes. If using these probes, you will need to use a larger container such as the 125mL Nalgene bottle (Fig. 2). Probes should be calibrated immediately prior to use per manufacture's instructions. Both old and new (Fig. 2) CO_2 probes have ports large enough for bean beetles to enter the sensor (red arrow). To avoid this, we wrapped the probes with cheesecloth secured with two rubber bands. Alternatively, a Kimwipe taped in place will also work although it isn't as durable and rips easily.
- Two 50mL Falcon tubes (one for each sensor). As mentioned above, if you're using the newer CO_2 probes, a larger container is required. We found that students often fail to fully insert the probes into the container. This was especially true with the new probes that don't have a rubber stopper. We used Parafilm to seal connection between probe and container. If students apply the Parafilm, you will want to make certain the probe is sealed correctly or they will be measuring room air.
- A test-tube rack to hold assembled probes and containers. The assembled apparatus is top heavy and will tip if not supported. A large beaker or ring-stand with adjustable clamps for each container works well.
- Bean beetles. We typically use 20-25 beetles per 50mL Falcon tube. As described in the student handout, we provided each group with one feeding jar from which students anesthetize and transfer beetles to the test containers (Falcon tubes in our case). You will want to remind student to work quickly or their beetles will need to be reanesthetized.

Figure 2. Gas sensors used in the described experiment were covered with cheesecloth or Kimwipes to prevent beetles from entering gas ports (noted by red arrow).

Data Collection

In our course, each group of students (six per class section) was given a single sample to test. Therefore, within a single section, each sample was only tested once. However, as we have six sections of the class running in parallel, it was possible to duplicate tests between sections. Data could then be compiled to generate replicates for a more in-depth analysis. Alternatively, it would also be possible to run multiple trials within a single three hour lab. This would allow for collection of replicate data within a single class section.

In our pilot trials, we opted to collect data every three minutes for one hour. This provided sufficient data for students to prepare a reasonable graph of the increase/decrease in concentration of each gas. However, reducing the time of data collection to 30 minutes would work just as well and would allow for multiple runs during a single lab period. Following the data collection, students were asked to transfer the data to Excel™ for analysis and graphing. As the class was primarily populated with 1st-year and sophomore students, our statistical analysis was minimal. We asked students to create a graph of their data (not simply the one generated by Logger Pro®), and then calculate the mean and rate of change for each gas. This allowed us to discuss various ways to analyze the data. For example, when several students suggested that the mean was the most useful value to compare between groups, we were able to discuss how outliers could skew this measure. If the class has experience with other statistical tests, the data could easily be analyzed more thoroughly using an ANOVA.

Sample Data

The following data table was generated from beetles tested 48 hours after initial exposure to a 5% solution of the indicated nutritive molecules (glucose or sucrose) or water. Logger Pro® was used to measure CO_2 levels (ppm) in three minute intervals. Summary statistics (slopes of trend lines) were calculated and individual data points graphed by each group of students (Fig. 3). Summary data (Fig. 4) were generated by combining data from three lab sections.

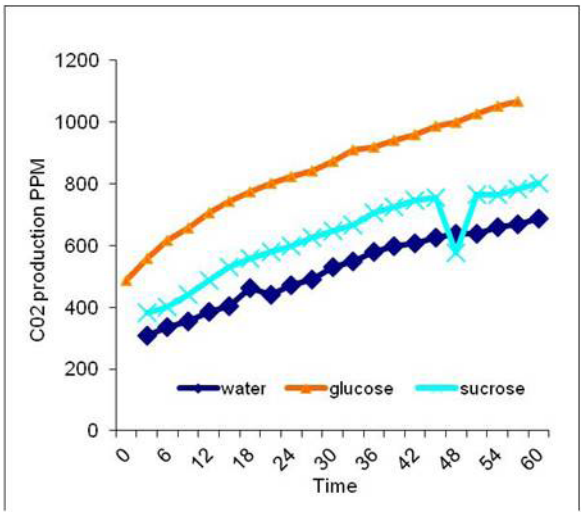

Figure 3. Metabolic responses to nutritional supplements. The carbon dioxide production data were collected by students in one section of BIOL 101 at Centenary College of Louisiana using the described procedures. Measurements were collected at 3-minute intervals for a total of 60 minutes. Data from one such section is presented at right. The initial time point (T=0) served as a calibration point. Each lab group independently graphed their own data. A composite graph of the class data similar to those presented below was prepared for discussion.

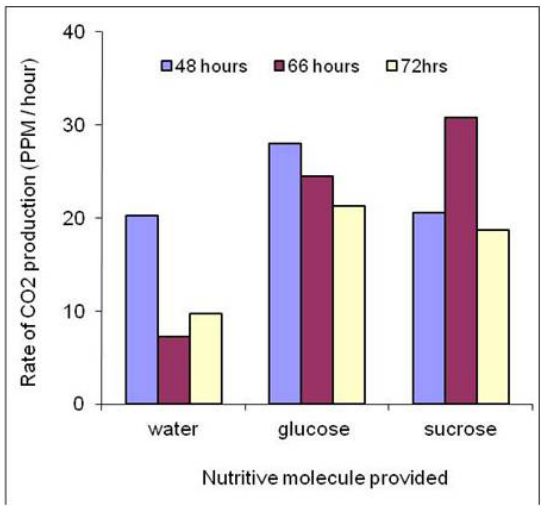

Figure 4. Metabolic rate as a function of nutritional supplements. The metabolic rate of beetles tested at different times after supplemental feeding is shown for each of three treatments: water, glucose and sucrose. Summary class data were shared between sections of the course for additional analysis. In this case, each lab section tested the same beetles which had been exposed to the various solutions for 48 hours. Access to supplemental feeding ended after 48 hours. Metabolic responses were tested at three times after supplemental feeding ended, immediately (blue bars, 48 hours after the start of supplemental feeding), 18 hours after the end of supplemental feeding (red, 66 hours after the start of supplemental feeding), or 24 hours after the end of supplemental feeding (yellow, 72 hours after the start of supplemental feeding).

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