Inquiry-based Investigation in Biology Laboratories: Does Neem Provide Bioprotection Against Bean Beetles?

Amy R. Pearce[1*], Amanda Lovelace Sale[2], Malathi Srivatsan[2], Christopher W. Beck[3], Lawrence S. Blumer[4], and Anne A. Grippo[2]

[1] Department of Psychology, Arkansas State University, State University, AR 72467, [2]Department of Biological Sciences, Arkansas State University, State University, AR 72467, [3]Emory University, Atlanta, GA 30322, [4]Morehouse College, Atlanta, GA 30314

*Corresponding Author: apearce@astate.edu

Abstract: We developed an inquiry-based biology laboratory exercise in which undergraduate students designed experiments addressing whether material from the neem tree (*Azadirachta indica*) altered bean beetle (*Callosobruchus maculatus*) movements and oviposition. Students were introduced to the bean beetle life cycle, experimental design, data collection, and reporting practices. At the end of the semester students exhibited increases in specific areas such as self-confidence in science process skills related to the design and implementation of experiments, as well as scientific reasoning skills.

Key words: Bean beetles; neem; inquiry skills; experiment design; biology; instructional strategies; inquiry based learning

INTRODUCTION

Inquiry-based teaching methods reportedly inspire students to develop curiosity, investigative abilities, and teamwork skills. The inquiry-based method provides students with opportunities to actively engage in problem solving rather than simply applying memorized concepts to preconceived experiments (Rehorek, 2004). In inquiry-based studies, students usually work in teams to explore an issue and develop a scientific question that is solved by applying the scientific method. These activities allow students to learn by active, rather than passive, teaching methods. In the final step, students present the experimental rationale, design, and results to their peers, and respond to feedback and questions. This active form of learning is thought to enhance depth of understanding; studies have shown that students taught using an inquiry-based method score significantly higher on exams than those taught using the so-called cookbook method (Luckie, 2004; Rissing, 2009). Because of the positive impact of inquiry-based approaches on student learning, these approaches are recommended to be foundational to national changes in the undergraduate biology curriculum (AAAS, 2011).

Inquiry-based instruction, like many approaches in science teaching, is not without its challenges. Kirschner et al. (2006) contend that learning in a minimally guided environment is less effective than direct instructional guidance and maintain that changes in long-term memory are required for effective learning and that only guided instruction results in such changes. According to Kirschner et al., a guided-inquiry approach, in which students are presented with the scientific question, are tasked to develop an experiment to test the question, and are then led as a class to an effective experimental design through leading questions, provides a more structured inquiry process than a truly open-ended inquiry. Furthermore, our experience and that of others supports the idea that undergraduate students may be capable of learning scientific content knowledge, but have little to no experience in the actual scientific process (Campbell et al., 2012), thus advocating for a guided inquiry-based approach.

As affiliates of the Bean Beetle Curriculum Development Workshop supported by the National Science Foundation (NSF), two of the authors of this study participated in a training workshop in which groundwork was laid for the development of a guided inquiry-based learning exercise using neem (*Azadirachta indica*) and bean beetles (*Callosobruchus maculatus*). The goal of the above learning exercise would be to help undergraduate students improve their understanding of the nature of science and their scientific reasoning skills. Upon our return to our home institutions, we shared our newfound knowledge with peers and formed a team to implement our guided inquiry-based activities over the course of one academic year. As part of the process we formally assessed students in the courses that included the exercise, the primary purpose being to evaluate the effectiveness of the approach. The exercise reported here can be easily adapted to students of different levels, does not require costly equipment, and results in important student gains in scientific reasoning skills and self-confidence in science process skills.


Fig. 1. Female bean beetle on bean.

Bean Beetles

Callosobruchus maculatus is an agricultural pest insect of Africa and Asia that presently ranges throughout the tropical and subtropical world (Figure 1). The larvae of this species feed and develop exclusively within the seeds of legumes (Fabaceae); hence the name bean beetle. The sexually dimorphic adults do not require food or water and spend their one to two week adult lifespan mating and laying eggs on beans. Generation times are as short as three to four weeks in a 30°C incubator. Bean beetles are widely used in research in evolutionary ecology, host-parasite relations, and sexual selection (Messina and Fox, 2011; Messina and Jones, 2011; Tuda, 2011; also see Blumer and Beck, 2011), and the bean beetle is gaining popularity as a model organism for inquiry-based undergraduate laboratory activities in ecology, evolution, animal behavior, and physiology (Blumer and Beck, 2011).

Bean beetle infestation affects stored beans causing losses in food resources and infestation is especially problematic in developing countries where beans are the main source of nutrients for the population. For example, in some parts of Africa, over 90% of stored cowpeas (*Vigna unguiculata*) are infested by bean beetles (Sallam, 2008). Therefore, preventing beetles from laying eggs on the beans while maintaining the quality of beans has been of great interest to researchers and economists.

Neem

Azadirachta indica, or the neem tree, is native to Southeast Asia where parts of the plant have found varied uses, including use as medicine, food, dental hygiene products, and as an insect repellant (Lowery and Isman, 1994; Ruckin, 1992). The active insect-repelling ingredient in the neem tree is believed to be azadirachtin, a chemical found in the highest concentration in the leaves, fruit, and seeds. Azadirachtin is considered harmless to humans (Miller and Uetz, 1998; HDRA, 1998), and neem, as well as neem products, is widely available to people whose food supplies are threatened by insects.

Although neem is widely reported as a bioprotectant, to our knowledge no scientific studies have systematically addressed the following questions: (1) Is bean beetle behavior, e.g., movement patterns, altered by powdered neem leaves? (2) Does neem extract or powder prevent female bean beetles from laying eggs on beans? (3) If neem extract does in fact prevent egg laying, are other neem products such as neem oil as effective? (4) If eggs are laid on neem-treated beans, is the viability of the eggs affected? (5) At what concentration, if any, is the neem product maximally effective? Using a guided inquiry-based approach in an undergraduate biology lab, the experiments described here were used to preliminarily address these questions.

METHODS

Participants

The laboratory experimental protocol and the student survey methods were approved by the Institutional Review Board of Arkansas State University (#125182-1) and classified as exempt by the Institutional Review Board of Emory University. Participants included students from three sections of a sophomore-level cell biology course, (n=18, n=17, n=2), students from one section of a senior-level cell biology course (n=25; taught by the same instructor as the one teaching the sophomore-level course), and students from a sophomore-level Psychology as Science and a Profession course taught by a different instructor (n=13). The numbers provided reflect only the subsamples of students who completed the online assessments. Additionally, another subsample of these students (n=10) participated in a focus group session at the end of the semester. Due to the small number of students in each course, we combined the data from all of the courses for analysis.

Laboratory Approach

Prior to the first lab, students (n=75, representing several lab sections) took on-line pretests assessing their self-confidence in science process skills, understanding of the nature of science, and scientific reasoning skills. Students were also given material to read that provided a brief background on neem, bean beetles, and experimental design (Blumer and Beck, 2011). Each instructor gave a short pre-lab presentation on bean beetles, neem, and the methods of studying bean beetle behavior and reproduction. Students were informed that they would be gathering new information, and that the effect of neem on bean beetles had not been tested scientifically. Students were tasked with formulating one or more hypotheses that could be tested during the semester and with drafting an experiment to test the hypothesis. Additionally, students were asked to predict the outcomes for their experiments, identify and list variables and controls, and list the types of data they would need to collect to determine whether their predictions were supported.

The students' experimental objectives for this activity were:

- To determine whether the presence of neem acutely alters bean beetle movement patterns
- To determine whether the presence of neem deters oviposition (egg laying) by female bean beetles
- To test the effect of neem on egg viability

The materials needed for this exercise per student group were: male and female bean beetles (a minimum of three of each sex; initial stock cultures are available from Carolina Biological), neem leaf powder or neem oil (Organneem LLC) , spray bottles and liquid detergent (if using neem oil), a magnifier or dissecting microscope, cardboard, mung or other desirable dry beans, such as black-eye peas and adzuki beans (~40 g per group; these may be purchased from local food stores), white adhesive tape, sorting brushes (Drosophila sorting brush, Carolina Biological Supply Company), plastic zipper bags, permanent markers, a digital balance, and petri dishes [3 large (150 mm) and 1 small (35 mm) per group]. An incubator (28^{o}C), although optional, is recommended. Three to four weeks before beginning the activity, bean beetles must be cultured for sufficient numbers to be available; for detailed instructions and sample student results from the weekly activities see: *Bioprotection from Beetles: Investigating the Untapped Secrets of the Neem Tree (Azadirachta indica)*, available at http://www.beanbeetles.org/protocols/bioprotection.

Briefly, although experiments varied, during the first week of the laboratory exercises the students were generally instructed on how to prepare plates to observe bean beetle avoidance behavior. Then students were divided into groups, asked to propose specific experiments, and agree on a final experimental approach. Groups were given 20 minutes to outline their basic methods before discussing their proposed experiment with course instructors to ensure no two groups had proposed the same experiment. Once each experiment and its methods were approved, each group gave a brief presentation to their classmates for additional feedback.

Each group was then provided with three male and three female bean beetles placed in the center in a small culture dish (35 mm) within a large cell culture dish (100 mm). The large dish was divided into two to four equivalent regions depending on each group's experimental design, and each region was filled with beans, with type again depending on each design. Neem was introduced to the beans in one or more regions of each plate and avoidance behavior was observed and recorded: student groups divided themselves into observers for each region, timer, and recorder, and beetle movement between regions was determined. Beetles, eggs and neem treatments in each region were transferred to fresh dishes, and placed in the incubator until the next lab session. During week two, students addressed whether neem influenced oviposition in the plates prepared the week previous by observing each bean for the presence and number of beetle eggs. During subsequent weeks, students tested the effect of neem on egg viability by counting and recording the number of emergent beetles.

Examples of experiments proposed included testing: (a) neem oil on varied bean types (pinto, lima, kidney), (b) varied neem oil application methods (neem oil applied to beans by spraying vs. beans soaked directly in neem oil), (c) the presence of filter paper vs. its absence under the beans, (d) neem oil vs. powdered neem leaves, (e) various concentrations of neem oil, and (f) neem leaf powder on various bean types (mung, pinto, black-eyed peas). Students in the upper level class presented their overall findings regarding their experiment to their peers, and reported the acceptance or rejection of their hypotheses. In the lower-level classes, students discussed their experiments and results then presented their data and interpretations within a group lab report which included descriptive statistics.

The duration of the activities can vary widely depending on the approach taken and, after the second week, these activities may be conducted concurrently with other lab exercises.

Student Assessments

We assessed the effect of inquiry-based laboratory courses incorporating the bean beetle exercise on self-confidence in process skills related to the design and implementation of experiments (Champagne, 1989), understanding of the nature of science (Murcia and Schibeci, 1999), and scientific reasoning skills of the students (Lawson et al., 2000). The student self-confidence assessment consisted of 12 statements scored on a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). It addressed students' self-perceived confidence in a variety of domains including, but not limited to, their ability to answer a scientific question through experimentation, to assess methodologies, to read and understand articles and graphs on science, and to describe natural phenomena. The nature of science assessment was composed of 15 statements covering topics on experimental design, personal beliefs and attitudes about science, ethics, and professionalism that students evaluated as either correct, incorrect, or 'don't know'. Finally, the scientific reasoning assessment presented three different scientific scenarios in which students predicted outcomes and provided a rationale for their predictions. Students were given these pre-tests at the beginning of the semester. They were then given the same questions as post-tests at the end of the semester. The pre-tests also surveyed students on their previous university-level laboratory courses and on basic demographic information (i.e., gender, year in school, race/ethnicity).

Statistical Methods and Software

To determine if student self-confidence, understanding of the nature of science, and scientific reasoning skills improved over the semester, we compared pre-test scores with post-test scores using Wilcoxon signed rank tests. In addition, we determined if any of the demographic variables or past experience in previous university-level laboratory courses influenced learning gains (post-test score minus pre-test score) for each assessment with generalized linear models that included pre-test score as a covariate. Finally, we examined the relationship between student performance on the three assessments with Spearman rank correlations independently for pre-test scores, post-test scores, and learning gains. All analyses were carried out using SPSS 20 with statistical significance at $P<0.05$.

RESULTS

Laboratory Approach

To the students' surprise, the overarching hypothesis that neem provides bioprotection against bean beetles was not supported. Most of the experiments designed and executed by the student groups did not reveal any consistent or striking difference between the compartments that contained beans treated with neem and untreated beans in deterring or attracting the bean beetles (data not shown). The experiments likewise did not reveal an influence of neem on oviposition behavior (data not shown). However, mung beans exposed to 0.75 g of neem powder showed some possible deterrent to movement that could be explored further (Figure 2). Also, after failed attempts to expose beetles to beans coated with oil, students realized that properties of the oil itself were a deterrent simply because the beetles would get stuck in the oil. Students determined that to use the oil, beans needed to be soaked and allowed to dry, or needed to be sprayed and the remaining residue removed. Beyond this, our students did not systematically study the application of neem oil, although this method of neem application may be more promising as a bioprotectant than the method of applying neem as a powder.

It should be noted that our undergraduate students had no prior compulsory statistical training; for this reason we allowed them to calculate and report totals and percentages pooled from the entire class, which were then presented descriptively. If one were to adapt this exercise for more advanced students, Chi-Square tests for the frequency data are recommended.

Student Assessments

A comparison of pre-test and post-test data showed a significant increase in self-confidence in science process skills and in scientific reasoning skills, but not in an understanding of the nature of science (Table 1). Further pre- vs. post-test analysis of the individual statements comprising the overall confidence assessment revealed student-perceived gains in areas such as stating a testable hypothesis, providing scientific explanations for natural processes, assessing methodologies, designing experiments, describing natural phenomena, challenging authority, and providing an instance of how scientific discovery has affected society. However, they did not express increased confidence in their ability to read science, to understand science, or to interpret scientific articles and graphs.

For all three assessments, learning gains were significantly negatively related to pre-test scores. In other words, students who performed better on the pre-test showed lower gains than students who had lower scores on the pre-test. Gains in self-confidence were unrelated to previous experience in laboratory courses and demographic variables. In contrast, gains in scientific reasoning skills were significantly greater for students who had *not* previously designed experiments in their university-level laboratory courses ($P=0.021$) and for students who had taken more university-level laboratory courses ($P=0.008$). Overall, students did not show an increase in their understanding of the nature of science, as scores decreased for some students and increased for other students. Scores were significantly more likely to decrease for male students ($P=0.014$), for students who had taken more university-level laboratory courses ($P=0.041$), and students who were not seniors ($P=0.013$). However, these changes in student understanding of the nature of science should be interpreted with caution, as the assessment showed

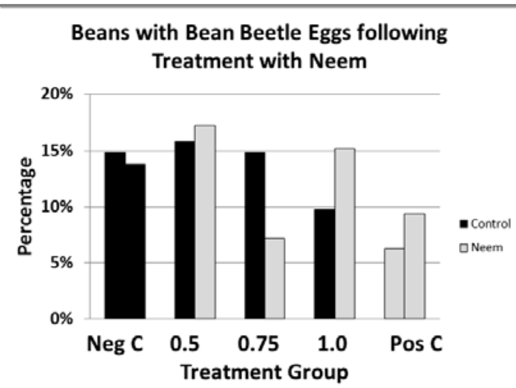

Fig. 2. Sample student-generated graph showing percentages of beans with eggs following exposure of beans to various amounts of neem powder. Beetles were placed in partitioned dishes with no neem powder on beans in either side of the partition (Neg C); varied amounts of neem powder on beans on one side of the partition (0.5, 0.75, 1.0 g) and no neem on the other side; or 1.0 g neem on beans on both sides of the partition (Pos C).

Table 1. Student learning gains in inquiry-based laboratory courses. Values are means ± SE.

Measure	Pre-test	Post-test	Wilcoxon Signed-Rank
Self-confidence	3.22±0.10	3.72±0.11	-5.198**
Nature of Science	0.66±0.02	0.65±0.02	-0.452
Scientific Reasoning	0.47±0.03	0.53±0.03	-2.011*

Note: Self-confidence was measured on a 5-point Likert scale with 5 being the most confident. Values for nature of science and scientific reasoning are the proportion of correct answers. *P<0.05, **P<0.01

low internal reliability (Cronbach's alpha = 0.51), suggesting that it might not be a good instrument for measuring student understanding of the nature of science.

As might be expected, pre-test scores were significantly positively correlated for all three assessments (P<0.05 in all cases). Similarly, post-test scores were significantly positively correlated (P<0.03) with the exception of the correlation between self-confidence and understanding of the nature of science (P=0.21). Interestingly, gains in the three assessments were not significantly correlated (P>0.35 in all cases). However, Beck and Blumer (2012) found a similar result for the relationship between self-confidence and scientific reasoning skills using the same instruments.

DISCUSSION

Although none of the neem concentrations tested here had a measurable and direct effect on bean beetle deterrence, oviposition, or adult emergence, we found that the inquiry-based activity was constructive for students in several ways. For instance, students who participated in the focus group reported that the inquiry-based lab was more interesting, yet more challenging, than traditional "cookbook" labs performed in the course during the same semester. These students also shared that they were surprised when no significant differences in attraction or repulsion of bean beetles by neem occurred. They stated that they expected the instructors had known that neem would be a deterrent and were surprised to learn that the experimental outcomes were indeed completely unknown, and thus perhaps more realistic. Focus group students also commented that the exercise was beneficial in that they learned to postulate hypotheses as they creatively designed experiments. They felt comfortable presenting their ideas to peers for feedback, and believed that their critical thinking skills were enhanced as they critiqued others' ideas. Students took ownership of their experimental designs, leading to the completion of data collection and the writing of lab reports; thus increasing the students' opportunity to strengthen their scientific writing abilities. Furthermore, through their lab reports, all students were exposed to accepted scientific reporting practices using their own data.

Whereas our activity was conceived as guided inquiry-based, most existing university science lab activities accommodate the traditional approach. Considering the growing body of studies showing the benefits of inquiry-based instruction, and given the recommended national changes in the undergraduate biology curriculum (AAAS, 2011), institutions may feel the need to convert some traditional, so-called "cookbook" labs, to inquiry-based ones (Volkmann and Abell, 2003). A limitation of the present study is that we did not compare a traditional activity using bean beetles to this inquiry-based lab to determine which method improved their learning. However, based on our own experiences in teaching both types of labs, we prefer to offer inquiry based labs once students have learned some basic skills from traditional labs so that they may more constructively devote the full lab period to discussing and designing experiments using inquiry-based methods.

It was not surprising that our students with more lab experience, as well as our upper class-level students, scored better on assessments of scientific reasoning. It was also not surprising that our students reported gains in self-confidence in science, because other studies have reported gains in student confidence in communicating science using inquiry-based approaches (Brickman et al., 2009). However, it would be interesting to further explore the meaning behind some of the other student assessment data. For instance, why were gains greater for students with less lab experience? And why weren't gains in self-confidence in science process skills correlated with gains in scientific reasoning skills?

In conclusion, although unanswered questions remain, our experience supports the idea that bean beetles are an organism amenable to use in biology laboratories. We contend that using bean beetles as a model organism allows for the exploration of various scientific questions. Bean beetles are well suited for creating inquiry-based learning opportunities, especially in departments that have limited resources. Furthermore, we conclude that this laboratory exercise using neem fully engages students in the construction of experimental designs and in collaborating with other students. The laboratory exercise described here has the potential to be adapted for use in both high school and college biology departments and can provide a positive and effective learning experience for students.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Bean Beetle Curriculum Development Network, supported by grants (DUE-0814373 and DUE-0815135) from the

National Science Foundation. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessary reflect the views of the National Science Foundation.

REFERENCES

ALEXANDRE, D., LINHARES, R.T., QUEIROZ, B., FONTOURA, L., AND A.F. UCHÔA, et al. 2011. Vicilin-derived peptides are transferred from males to females as seminal nuptial gift in the seed-feeding beetle *Callosobruchus maculatus*. *Journal of Insect Physiology* 57(6): 801–08.

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. 2011. Vision and change in undergraduate biology education: A call to action. http://visionandchange.org/finalreport on 3 Sept 2012.

ASSHOFF, R., AND O. ROTH. 2011. Fostering students' inquiry skills: Developmental time and offspring rates of flour beetles. *The American Biology Teacher* 73: 232-7.

BECK, C. W., AND L. S. BLUMER. 2012. Inquiry-based ecology laboratory courses improve student confidence and scientific reasoning skills. *Ecosphere* 3(12):112. http://dx.doi.org/10.1890/ES12-00280.1

BLUMER, L.S., AND C.W. BECK. 2011. Bean Beetles: A model organism for inquiry-based undergraduate laboratories. A Handbook on Bean Beetles, *Callosobruchus maculatus.* http://www.beanbeetles.org/handbook/handbook.pdf on 3 Sept 2012.

BRICKMAN, P., GORMALLY, C., ARMSTRONG, N., AND B. HALLAR. 2009. Effects of inquiry-based learning of students' science literacy skills and confidence. *International Journal for the Scholarship of Teaching and Learning* 3(2): 1-22.

CAMPBELL, T., DER, J.P., WOLF, P.G., PACKENHAM, E., AND N.H. ABD-HAMID. 2012. Scientific inquiry in the genetics laboratory: Biologists and university science educators collaborating to increase engagement in science processes. *Journal of College Science Teaching* 41(3), 74-81.

HDRA, THE ORGANIC ORGANISATION. 1998. The Neem Tree. http://www.infonet-biovision.org/res/res/files/494.hdra%20NeemTree.pdf on 3 Sept 2012.

KIRSCHNER, P.A. 2006. Why minimal guidance during instruction does not work: An analysis of the failure of Constructivist, Discover, Problem-Based, Experiential, and Inquiry-Based Teaching. *Educational Psychologist* 41(2): 75-86.

KOLAWOLE, A.O., AJELE, J.O., AND R. SIRDESHMUHK. 2011. Studies on glutathione transferase of cowpea storage bruchid, *Callosobrochus maculatus F*. *Pesticide Biochemistry and Physiology* 100(3): 212–220.

LOWERY, D.T., AND M.B. ISMAN. 1994. *Effects of neem and azadirachtin on aphids and their natural enemies.* In P.A. Hedin (ed). 1994. *Bioregulators for Crop Protection and Pest Control*. American Chemical Society, Washington, D.C. Ch. 7, pp 78-91.

LUCKIE, J.B. 2004. Infusion of collaborative inquiry throughout a biology curriculum increases student learning: a four-year study of "Teams and Streams". *Advances in Physiology Education* 287: 199-209.

MESSINA, F.J., AND C.W. FOX. 2011. Egg-dumping behavior is not correlated with wider host acceptance in the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). *Annals of the Entomological Society of America* 104: 850–856.

MESSINA, F.J., AND J.C. JONES. 2011. Inheritance of traits mediating a major host shift by a seed beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). *Annals of the Entomological Society of America* 104: 808–815.

MILLER, F., AND S. UETZ. 1998. Evaluating biorational pesticides for controlling arthropod pests and their phytotoxic effects on greenhouse crops. *Hort Technology* 8(2): 185-192.

REHOREK, S.J. 2004. Inquiry-based teaching: An example of descriptive science in action. *The American Biology Teacher* 66(7): 493-499.

RISSING, S.W. 2009. Can an inquiry approach improve college student learning in a teaching laboratory? *CBE-Life Sciences Education* 8: 55-61.

RUCKIN, F.R. (ed). 1992. *Neem, A Tree for Solving Global Problems.* National Academy Press. Washington, D.C. pp 39-50.

TSENG, H.-F., YSNG, R.-L., LIN, C., AND S.-B. HORNG. 2007. The function of multiple mating in oviposition and egg maturation in the seed beetle *Callosobruchus maculatus*. *Physiological Entomology* 32: 150–156.

TUDA, M. 2011. Evolutionary diversification of bruchine beetles: Climate-dependent traits and development associated with pest status. *Bulletin of Entomological Research* 101: 415–422.

VOLKMANN, M.J., AND S.K. ABELL. 2003. Rethinking laboratories: Tools for converting cookbook labs into inquiry. *The Science Teacher* September: 38-41.