INQUIRY & INVESTIGATION

Plants in Your Ants: Using Ant Mounds to Test Basic Ecological Principles

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ABSTRACT

Urban students often have limited access to field sites for ecological studies. Ubiquitous ants and their mounds can be used to study and test ecology-based questions. We describe how soil collected from ant mounds can be used to investigate how biotic factors (ants) can affect abiotic factors in the soil that can, in turn, influence plant growth.

Key Words: *Ecology; ant mounds; macronutrients; micronutrients; plant growth;* Brassica rapa.

Students in urban schools often have limited access to field sites for ecological studies, but there are numerous ant species that thrive in disturbed habitats and are easy to obtain and study. Many Americans who live in southern states are familiar with the distinct mounds built by red imported fire ants, *Solenopsis invicta* (Figure 1). Native to South America, *S. invicta* was accidentally introduced into the United States in the 1930s (Callcott & Collins, 1996) and has become established in 13 states in just 70 years (Callcott, 2002). Predictions now show that its spread has not abated (Morrison et al., 2005). Fire ants use excavated soil to build a chambered mound in which the colony lives. Workers continually build and maintain tunnels, which can lead to soil modifications such as increased aeration and turnover. In addition, soil occupied by fire ants has lower moisture content and pH but higher concentration of nutrients (Lafleur et al., 2005). These modifications can occur when the mound is established and continue as the colony grows. Here, we explain how the use and study of soil collected from ant mounds can clearly show that biotic factors (ants) can affect abiotic factors in the soil that can, in turn, influence plant growth. We describe how students can compare germination success and development of plants growing in active ant mounds, abandoned ones, and adjacent, nonmound soil. By conducting hands-on experiments, students can experience the scientific method, which should lead into a discussion of ecological principles, including

> *[The] use and study of soil collected from ant mounds can clearly show that biotic factors (ants) can affect abiotic factors in the soil that can, in turn, influence plant growth.*

- Experimental design and the scientific method
- Ecological effects of invasive species
- Decomposition and nutrient cycles
- Macro- and micronutrient use and deficiencies in plants
- Differentiating between ecotypes and phenotype plasticity

Although we describe the use of fire ants here, the nests of any ant species that constructs mounds in soil can be used.

○ Safety Considerations

Some ants, and most notably fire ants, have the ability to inflict injury. Fire ants get their common name from their sting, which contains venom that causes a burning sensation and ultimately produces a characteristic reddened welt topped with a pustule. For the vast majority of people, these stings are not serious, but in hypersensitive individuals, an anaphylactic reaction is possible (1.9 per 10,000 individuals; Caldwell et al., 1999). Accordingly, students who have experienced allergies to insect stings should avoid handling live ants. Instructors can consider collecting mound soil, freezing it to kill any ants, and then dispensing it to students for use in the lab.

○ Pre-Lab Preparation: Soil Collection

Three types of soil should be collected for this project: (1) soil from active ant mounds, (2) soil from abandoned ant mounds, and (3) soil from unoccupied ground nearby. Instructors and students can minimize exposure to ant venom by taking several precautions while in the field. Most ant species are less active when ambient temperatures are below 21°C (70°F), and this is the ideal time to collect their soil while minimizing the risk of being stung. Soil can be collected and stored frozen in a standard household freezer for several months prior to use. If, instead, soil is collected during warm weather, when ants are most active, using the following items will simplify the collecting process:

- Shovel
- Three 5-gallon (18.9 L) buckets

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Figure 1. Characteristic mound of red imported fire ants.

- Fluon (BioQuip Products, Rancho Dominguez, CA)
- Gallon (3.8 L) sealable storage bags

Fluon (also known as Insect-a-Slip) can be applied to the inner rim of each bucket to form a slick barrier that will prevent ants from crawling out of the containers. Once soil is collected, it can be placed into bags and frozen solid to kill any ants. To standardize the treatment of soils, all soil types should be kept frozen until use.

○ Materials for Each Lab Group

- Potting soil that is enriched with fertilizer (e.g., Miracle-Gro® Potting Mix)
- Three collected soils (active, abandoned, and nonmound soil)
- 4-inch (10.2-cm) plant pots
- Labeling tape or pot labels
- Permanent marker
- Seeds
- Light source (fluorescent bulbs are ideal)
- Deionized or distilled water
- A ruler
- An artist's paint brush with natural bristles
- Digital balance scale (recommended)

We recommend seeds of rapidly cycling *Brassica rapa* (Wisconsin Fast Plants®) purchased from Carolina Biological Supply Company (Burlington, NC). Inexpensive packages of radish, lettuce, or marigold seeds can be substituted if necessary. However, consider that leguminous plants form symbiotic relationships with nitrogen-fixing bacteria. Therefore, the association between plant growth and soil type may not be as distinct in legumes.

○ Experimental Design

Because plants often exhibit ideal growth in enriched potting soil, we suggest using this soil treatment as a comparison set. Each student group should sift through the defrosted field-collected soils to remove debris and dead ants. Plant pots should be labeled as "active mound," "abandoned mound," "unoccupied soil," or "potting soil" and then each should be filled halfway with the corresponding sifted soil. (If soil is limited, Petri plates can be substituted for pots to observe early differences in seedling growth; see Figure 2A). By considering space availability, the instructor can determine the number of replicates that each student group can use. Wisconsin Fast Plants® attain maturity and produce seeds within 40 days of planting, which can be perfect for classes that are limited by term length. To ensure adequate germination, six seeds can be planted in each pot, but to prevent crowding, seedlings should be thinned so that only four plants remain. Plants should be watered as needed with deionized or distilled water. Fast Plants grow best when kept at room temperature (18–26°C) with a continuous light source (24-hour photoperiod) using fluorescent bulbs (http://www.fastplants.org).

Students can keep track of plant growth by calculating the percent germination and measuring daily plant height. By calculating and graphing averages, students should see obvious trends in growth patterns within just a few days after germination. We also recommend that students attempt to use statistical tests to determine whether the characteristics (e.g., number of seeds, plant height, and numbers of flowers)

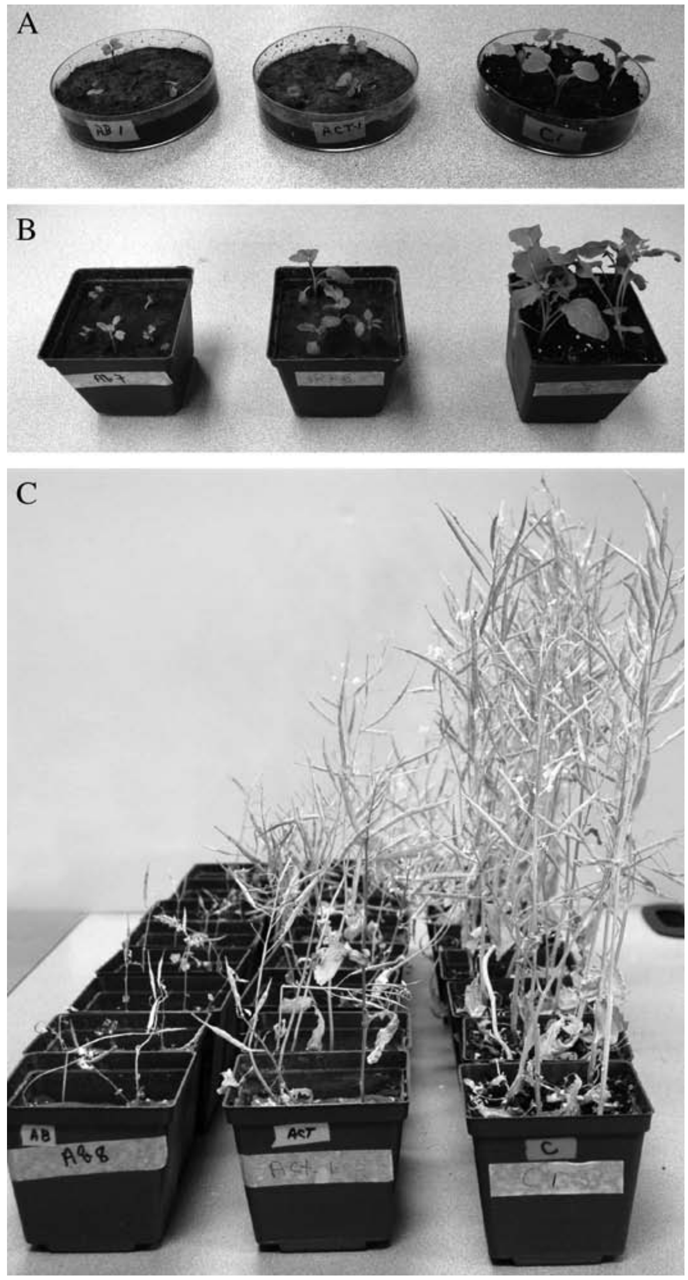

Figure 2. Growth of *Brassica rapa* in soil from (left) abandoned and (middle) active fire ant mounds and (right) potting soil one week after planting in (A) Petri plates and (B) pots. (C) Potted plants after senescence. No plant growth was observed in any pots with unoccupied surrounding soil.

of plants growing in the various soil types show significant differences. In this study, a simple one-way ANOVA (analysis of variance) can be used to compare the plant data from all four soil types. Most spreadsheet programs include basic statistical packages that have this test. If significant differences are detected, more advanced statistical programs may be required for post hoc tests. Although a discussion of this is beyond the scope of this article, instructors are encouraged to review the basic assumptions of any statistical test.

Because Fast Plants are obligate outcrossers, they lack the ability to self pollinate. A paintbrush can easily transfer pollen to newly opened flowers. Once pollinated, seeds will develop within pods called "siliques" that can be harvested at plant senescence. At this time, students can determine the collective number and weight of seeds per treatment group to compare the reproductive output of plants growing in the different soil types. In addition, obtaining the dry mass of treatment plants allows overall biomass to be calculated and compared. All collected seeds can be kept in dry storage for future experiments (see below).

○ Investigative Questions: Experiments & Discussions

After receiving some background information, the students can discuss as a group the questions listed below. Answering some of these questions could involve designing additional experiments, which confers an excellent opportunity for students to demonstrate an understanding of the scientific method.

1. Will the abiotic components of mound soil change once ants relocate? Ant colonies frequently abandon mounds and relocate to new locations. Have students consider that inhabited ant mounds can have decreased soil pH (Blust et al., 1982) and increased levels of nitrogen (ammonia), calcium, potassium, and phosphorus (Lafleur et al., 2005). Wagner et al. (2004) found that the nests of harvester ants had 600–800% higher concentrations of mineral nitrogen than unoccupied soil collected nearby. These authors also found that as the colony's nest aged, it developed increased levels of organic matter, orthophosphate, and ammonium. Nutrients that accumulate in ant nests may result from the products that ants bring back to the colony from foraging trips. Likewise, nitrogen in fire ant mounds may originate in their feces, which are primarily composed of uric acid (Chen, 2006). With additional experimentation, students can investigate soil chemistry using inexpensive test kits that are available at home improvement retailers. This project can be a good catalyst for a discussion of soil formation processes such as leaching, weathering, decomposition, and nutrient cycling.
2. What are the effects of macro- and micronutrient deficiencies in plants? Macronutrients such as nitrogen, calcium, potassium, and phosphorus are needed in relatively large amounts for proper plant growth. Micronutrients, like macronutrients, are essential for plant growth but in only small or even trace amounts. In grassland habitats, nitrogen is the single limiting resource (Wilson & Tilman, 1991). Elevated levels of macro- and micronutrients in fire ant mounds may provide enhanced growth to plants located in ant-occupied soils. Students can conduct additional observations to make qualitative assessments of plants grown in the various soils by categorizing and ranking their appearance (e.g., coloration, necrosis, and/or stunting). Cooperative Extension publications are easy to obtain and provide good references for visual symptoms of plants' nutrient deficiencies (e.g., Hoiser & Bradley, 1999; Wong, 2005). Students can also gather quantitative data by taking numerical measurements (e.g., number of reproductive parts, plant height, and dry weight). We recommend that students produce graphs and tables of their results, which will provide a better understanding of the complex ecological interactions between soil nutrients and plant growth patterns.
3. Will adding synthetic fertilizers compensate for the apparent lack of nutrients in abandoned mound soils? Students can do additional studies using various concentrations of fertilizers to compare the growth of plants growing in experimentally enriched mound soils with that of plants in untreated mound soils. Students can follow the procedures outlined by Lee (2003) to use Fast Plants to observe nutrient deficiency symptoms. Hershey (2003) points out problems that may be encountered in Lee's laboratory exercise.
4. Do the soil conditions of the maternal plants influence their seed quality? By using stored seeds harvested from the first experiment, students can determine whether the maternal plants' soil type has a carryover effect on their offspring. For example, in the common plantain, the effects of nutrient enrichment of maternal plants persist over several generations (Miao et al., 1991). We recommend that students plant seeds from the first experiment in containers of potting soil (a common garden experiment) to compare the first generation's seedling growth. Alternatively, if time is limited, seed viability can be investigated by placing seeds from each treatment group on moist filter paper in Petri plates to be checked for germination success. Students should find that the environment (soil type) of the maternal plants affects the growth and reproductive output of their offspring. Ask students to discuss whether these differences are the result of genetic differences or expressions of phenotype plasticity. Students can investigate this question by designing transplant experiments in which the remaining seeds from each group of maternal plants are reciprocally planted in the different soil types. By comparing the growth patterns of several generations of plants from various soil environments, students can determine whether there is a genetic component to differences in populations. The instructor can use this time to discuss ecological terms such as "common garden experiments," "reciprocal transplants," "ecotypes," and "phenotypes."
5. What are the ecological effects of invasive species on community structure? The numbers of fire ant mounds can reach frightening densities, at 470 mounds ha^{-1} (Macom & Porter, 1996). Fire ants are scavengers but are more notorious for their ability to be opportunistic predators. In areas that have been invaded by fire ants, there is a corresponding reduction in native ant and other arthropod populations (Holway et al., 2002). In addition, fire ants have the potential to directly alter plant community structure through several mechanisms, including selective seed consumption (Seaman & Marino, 2003), seedling predation (Drees et al., 1991), and disruptive seed dispersal (Ness, 2004). Because fire ants will never be eradicated, researchers are attempting to control their populations by importing their natural enemies (Flanders & Graham, 2008). This topic is a perfect opportunity for students to discuss ecological terms such as "ecological niche," "competitive release," "competitive exclusion," and "keystone predators."

○ Conclusion

In studying and discussing the growth of plants in soil from active and abandoned ant mounds, students can quickly see that questions beget questions, and this process is a critical component of the scientific method. It is the unexpected results that can often be the most intriguing, however, and well worth the endeavor.

References

Blust, W.E., Wilson, B.H., Koonce, K.L., Nelson, B.D. & Sedberry, J.E., Jr. (1982). The red imported fire ant, *Solenopsis invicta* Buren: cultural control and effect on hay meadows. *Louisiana State University Agricultural Experiment Station Bulletin*, no. 738.

Caldwell, S.T., Schuman, S.H. & Simpson, W.M., Jr. (1999). Fire ants: a continuing community health threat in South Carolina. *Journal of the South Carolina Medical Association*, *95*, 231–235.

Callcott, A.M.A. (2002). Range expansion of the imported fire ant – 1918–2001. *2002 Annual Imported Fire Ant Research Conference*, Athens, Georgia.

Callcott, A.M.A. & Collins, H.L. (1996). Invasion and range expansion of imported fire ants (Hymenoptera: Formicidae) in North America from 1918–1995. *Florida Entomologist, 79*, 240–251.

Chen, J. (2006). Chemistry of the feces of the red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Meeting Abstract for the IUSSI 2006 Congress*, Washington, DC.

Drees, B.M., Berger, L.A., Cavazos, R. & Vinson, S.B. (1991). Factors affecting sorghum and corn seed production by foraging red imported fire ants (Hymenoptera: Formicidae). *Journal of Economic Entomology*, *84*, 285–289.

Flanders, K. & Graham, L.C. (2008). *Biological control of imported fire ants*. Circular ANR-1149, Alabama Cooperative Extension System.

Hershey, D.R. (2003). Plant nutrient deficiency symptoms. *American Biology Teacher*, *65,* 246.

Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D. & Case, T.J. (2002). The causes and consequences of ant invasions. *Annual Review of Ecology & Systematics*, *33,* 181–233.

Hosier, S. & Bradley, L. (1999). *Guide to symptoms of plant nutrient deficiencies*. Publication AZ1106, University of Arizona Cooperative Extension. Available at http://ag.arizona.edu/pubs/garden/az1106.pdf.

Lafleur, B., Hooper-Bui, L.M., Mumma, E.P. & Geaghan, J.P. (2005). Soil fertility and plant growth in soils from pine forests and plantations: effect of invasive red imported fire ants *Solenopsis invicta* (Buren). *Pedobiologia*, *49*, 415–423.

Lee, C.A. (2003). A learning cycle inquiry into plant nutrition. *American Biology Teacher*, *65*, 136–141.

Macom, T.E. & Porter, S.D. (1996). Comparison of polygyne and monogyne red imported fire ant (Hymenoptera: Formicidae) population densities. *Annals of the Entomological Society of America*, *89*, 535–543.

Miao, S.L., Bazzaz, F.A. & Primack, R.B. (1991). Presistence of maternal nutrient effects in *Plantago major*: the third generation. *Ecology*, *72*, 1634–1642.

Morrison, L.W., Korzukhin, M.D. & Porter, S.D. (2005). Predicted range expansion of the invasive fire ant, *Solenopsis invicta*, in the eastern United States based on the VEMAP global warming scenario. *Diversity and Distributions, 11*, 199–204.

Ness, J.H. (2004). Forest edges and fire ants alter the seed shadow of an ant-dispersed plant. *Oecologia, 138*, 448–454.

Seaman, R.E., & Marino, P.C. (2003). Influence of mound building and selective seed predation by the red imported fire ant (*Solenopsis invicta*) on an old-field plant assemblage. *Journal of the Torrey Botanical Society*, *130*, 193–201.

Wagner, D., Jones, J.B. & Gordon, D.M. (2004). Development of harvester ant colonies alters soil chemistry. *Soil Biology & Biochemistry*, *36,* 797–804.

Wilson, S.D. & Tilman, D. (1991). Components of plant competition along an experimental gradient of nitrogen availability. *Ecology*, *72*, 1050–1065.

Wong, M. (2005). Visual symptoms of plant nutrient deficiencies in nursery and landscape plants. SCM-10, Cooperative Extension Service of the University of Hawai'i, Manoa. Available online at http://www.ctahr.hawaii.edu/oc/freepubs/pdf/SCM-10.pdf.

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