ROOTS AS FORAGERS:
STUDENTS CAN EXPERIENCE PLANTS AS RESPONSIVE ORGANISMS

Stanley A. Rice
Professor Emeritus of Biological Sciences
Southeastern Oklahoma State University
1405 N. Fourth Ave. Box 4027
Durant OK 74701
srice@se.edu
www.stanleyrice.com
Phone 580-745-2688
FAX 580-745-7459

Roots as foragers: Students can experience plants as responsive organisms

Abstract

In this project, students can experience plants as responsive rather than passive organisms. Roots forage through heterogeneous media and proliferate in portions of the soil that have abundant nutrients. Students can see and measure this growth. Students also get to address issues of experimental design such as the sequence effect.

Introduction

Many students in high school and college biology classes assume that plants are motionless, passive organisms, quite unlike active and intelligent animals. For a long time, scientists and science educators have tried to reverse the passive image of plants. Charles Darwin demonstrated that plant shoots actively search for the brightest spots in which to grow (Darwin 1880). With the advent of time-lapse photography, it has been possible to actually see aboveground plant movements such as circumnutation and nyctinasty. More recently, scientists have discussed plant responses in terms of intelligence (Trewavas 2005).

While it may be too much to expect students to consider plants to be intelligent, it is clear that their appreciation of plants will be enhanced if they can observe active plant responses to environmental cues. I describe in this article a laboratory activity that will allow students to see and measure an active plant response: root growth in response to spatial heterogeneity of the growing medium. The students observe roots growing differentially in vertical layers of soil and perlite. That is, they see that roots don't just grow; they forage.

I begin by having the students consider in which medium roots would proliferate the most. They invariably choose soil, because perlite contains few nutrients. Perhaps without realizing it, they have attributed an "animal" property to plants. This is not necessarily intelligence, but may simply be a physiological acclimatization response (Fransen et al. 1999; Kembel and Cahill 2005; McNickle et al. 2009; Ruffel et al. 2011). Assuming soil and perlite to have similar water content and physical properties, the roots must respond to nutrient content by producing more branch roots.

This thought process allows the students to make these predictions:

- *Hypothesis:* Roots will proliferate in portions of the growth medium that have more nutrients.
- *Null hypothesis:* Roots will grow the same amount in each growth medium regardless of nutrient content.

Methods

Since the purpose of the project is to promote scientific thinking, I provide minimal instructions for setup. Materials available to them are:

- Seeds
- Soil
- Perlite
- Glass cylinders
- Drinking straws and tape

The choice of seeds is crucial. The first year in which we used this project, we used bean seeds. The second year, we used sunflower seeds, which produced entirely different results. The perlite must be plain perlite, without added nutrients, a commodity that is increasingly difficult to find. The glass cylinders that we used were soil sedimentation columns, which had been donated to our university by a USDA soil analysis laboratory when it closed down.

I tell the student groups to add vertical layers of soil and perlite to the glass cylinders, water the layers, and plant seeds on top. The students immediately encounter problems of experimental design, to which I alert them without giving a solution.

- The first problem is the sequence effect (Christensen 2007). If the roots begin growing in soil, then enter a layer of perlite, they may grow differently than they would if they begin growing in perlite, then enter a layer of soil. The student groups solve this problem by having two kinds of columns: one with soil as the top layer, another with perlite as the top layer.
- The second problem involves germination. If the top layer is perlite, the seeds may germinate abnormally, or may die of fungus infestation before completing germination. I invite the students to think about why seeds do not usually mold when germinating in soil. Some of the students will realize, and share with their groups, that the diversity of soil microbes inhibits the growth of any one kind of fungus, either through competition or the production of antifungal compounds.

They solve this problem by having a very thin layer of soil on top of the perlite layer if it is the top layer in the column.

- ❖ The third problem involves the vertical movement of nutrients. Nutrients from a soil layer above a perlite layer can percolate down into the perlite layer, providing nutrients to it. It does not take long for the students to figure out how to deal with this, when they see the drinking straws. They tape straws together to form watering tubes that penetrate down to just the layer they want to water. There is a separate straw tube for each layer of substrate except for the top. As they maintain the experiment, they pour water into the tubes and onto the top layer, but just enough to water that layer. This process minimizes the downward movement of nutrients (Figure 1).
- ❖ The fourth problem is that roots, even when growing in uniform substrate, may have vertical patterns of growth. The students established some control columns—either pure soil or pure perlite—and treated the columns as three layers of substrate.
- ❖ The fifth problem is that different individual plants may respond differently to the experimental conditions. I simply assured them that the statistical analysis would take this into account. When analyzing the results, I included "plant" as an independent variable.


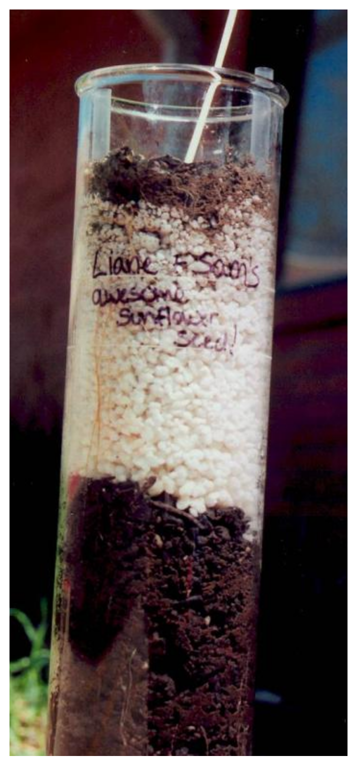

Figure 1 (a and b). Glass cylinders with layers of growth medium (soil vs. perlite). In some, the soil layer is on top; in others, the perlite layer is on top, with a thin layer of soil to prevent seed mold. Control cylinders have only soil or only perlite. Students figured out how to use straws to provide water to just a single layer of growth medium.

For the next several weeks, the students watched the growth of the roots whenever they water the columns. They took visible pride of ownership in their plants. The number of weeks of growth depends on the plant species and conditions (the beans in the first year grew faster than the sunflowers in the second).

Once the roots have proliferated in the bottom layer, it is time to harvest the plants, in order to avoid having excessive growth in the bottom layer, which will occur regardless of the type of medium. The students measure root growth in two ways:

- Root length of intact root systems. Using map wheels, students measure the total length of root in each layer, as visible from the outside (Figure 2).
- Root weight. The students carefully remove the root systems and cut them so that the roots in each soil or perlite layer are separate (Figure 3). They remove as much soil as possible before weighing the roots. We used fresh root weight because dry root weight would be too small for typical laboratory balances. We did not wash the roots, because a single drop of water can weigh as much as the entire root system.

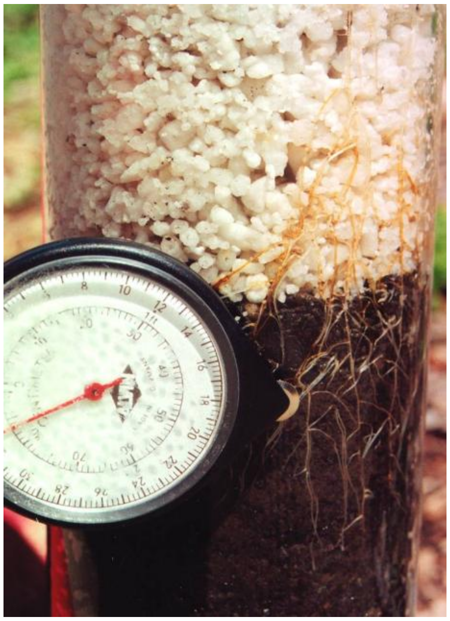

Figure 2. Map wheels allow the measurement of root length that is visible against the glass of the cylinder.

Figure 3. After careful removal of the roots, students divided the roots into layers for each growth medium.

The students provided the data to me. I analyzed it using JMP analysis of variance, available from SAS Inc., and provided the results to them. A more advanced class may be able to do this analysis themselves. I had to explain the meanings of basic statistical parameters, especially *p*. It is counterintuitive to think of that a low *p* value confirms a hypothesis.

Interpretation of results

Negative results can be more educational than positive ones. In the first year, there were no statistically significant differences of root growth. That is, the bean roots grew the same in both soil and perlite. I asked the students to think of an explanation. As they had completed lecture and laboratory material about roots, they were able to come up with two explanations:

- Beans are large seeds and may contain enough nutrients in the cotyledons that the soil nutrients may be irrelevant to their growth.
- Bean roots formed symbiotic associations with nitrogen-fixing bacteria, which made nitrogen supply virtually unlimited, although this would not have affected the availability of other nutrients such as phosphorus. The students pointed the nodules out to me before I had seen them myself.

The recommendation of the students in the first year, in the discussion portion of their lab papers, was to use a plant species with smaller seeds and that did not form root nodules.

In the second year, the sunflower roots grew significantly more in soil than in perlite (Table 1). These results included the roots that had grown in the control columns of pure soil and pure perlite. This time, the results were very clear: roots grew more in soil than in perlite. Calculation of means and confidence intervals would easily demonstrate this conclusion. But in order to examine the other factors, an analysis of variance was necessary (Table 2). I asked the students whether some individual plants had more overall root growth than others; the answer was no, because $p = 0.7016$. I asked them whether there was an overall vertical growth pattern, regardless of substrate; the answer was no, because $p = 0.1482$. I asked them whether the roots grew more in soil than in perlite; the answer was yes, because $p < 0.0001$. I asked them whether the sequence effect was important; the answer was no, because $p = 0.6590$ for the interaction term. Some students needed more help than others to interpret these numbers.

Table 1.
Sunflower root growth in soil vs. perlite (averages for 12 plants and 36 layers).

	Root length (cm)	Root weight (g)
Growth in soil	146.2	1.23
Growth in perlite	59.9	0.33

Table 2.
Analysis of variance for sunflower root length.

Source	df	F	p
Model	4	6.56	0.0006
Differences among individual plants	1	0.15	0.7016
Sequence of layers	1	2.20	0.1482
Substrate (soil vs. perlite)	1	21.90	<0.0001
Interaction of layer and substrate	1	0.20	0.6590
Error	31		
Total	35		

I also shared the first year results with the students in the second year. The overall conclusion they drew was that roots proliferate in soil more than in perlite as an immediate response, regardless of the sequence or vertical position of the layers, but that this pattern was true only for species that did not form nodules and whose seeds did not carry large nutrient supplies.

Assessment of student understanding

In both years, I asked the students to answer a list of questions before and after the project as a pre-test and post-test. In one of the questions, I asked them to predict whether roots would grow more in clay soil with leaf litter, or in sandy soil. In another question, I gave them an example of the sequence effect applied to standardized student testing. In another question, I asked them to draw a conclusion from a hypothetical experiment in which plant roots grew better in partially dry than in very wet soil. I expected their understanding of these things to be enhanced by the project. However, most of them answered the questions correctly even before the project. They already knew that roots would grow more in rich than in poor soil; that scores on the last examination given would be lower, regardless of subject, because the students would be tired; and that the results of the hypothetical experiment were due to flooding. Therefore this project appears to enhance student appreciation that plants actively respond to the environment, rather than teaching them that this occurs. In other words, root foraging is something that is obvious once you think about it, but this project forces them to think about it.

In the write-ups, some of the students offered clear and creative summaries. One wrote, "Animals and plants forage in different ways. Animals search an area looking for food based on what they see and what they smell. Plants are not so lucky. Lacking eyes for sight and nares for scent detection they must rely on other methods…One way they could possibly find these nutrients is that the growing section of the root near the tip reacts to higher nutrient levels by growing more." Another wrote, "…plants do not get to choose where they are planted, but they do have a choice in how to expend their energy."

Other students contributed original insights. In one example, a student came up with a human example of acclimatization (and made a Freudian slip): "When we think of the word "forage" we think of an animal seeking out or hunting for food in its environment. Could plants do this by foraging as well? Since plants do not have the ability to "think" we find the possibility of foraging unlikely. However, could they use a response mechanism…that would lead the direction of root growth toward richer soils? A human example of this would be the [production] of red blood cells in a higher elevation…Perhaps plants could do the same think [sic] internally."

In another example, a student suggested a modification of the experiment: "Use large horizontal tubs instead of cylinders. They should be glass so that roots could be observed. Fill one side of the tub with soil and the other side with perlite. Plant the seed…in the middle of the two soil types. Observe which direction the roots grow. The container is glass so the roots can't grow down. But if the hypothesis of foraging is correct, the plant should grow in one direction or the other 'seeking out' the richer soil. Or the null hypothesis will stand."

Another student pointed out a possible confounding effect that I had not considered: "…perlite would compact less and provide the roots more pore space to forage through…"

This project also allowed fundamental misunderstandings to be identified. One student equated nitrogen with protein and wrote the whole paper in terms of roots foraging for protein. I hope this student's misunderstanding was cleared up before the final exam.

The students seemed to appreciate that the experiment was interesting precisely because it was not a canned lab exercise with a predetermined correct answer. In the first year, when no patterns emerged, one student wrote, "Overall the hypothesis was proved wrong in this test. This does not mean that no roots forage for nutrients, but that in the test we used the roots did not. It would take many more tests to totally disprove the hypothesis. Even though the hypothesis was wrong, the experiment was not a failure. We still learned what didn't happen, and with more experimenting we will learn more."

Toward the end of the semester, I gave the students an example of root foraging that has immediate economic implications: phytoremediation. The roots of certain plants, such as the mustard *Thlaspi caerulescens* (Whiting et al, 2000) or the fern *Pteris vittata* (Mandal, et al., 2012), grow preferentially toward soils contaminated with heavy metals. The roots absorb the metals, which the stems and leaves store in vacuoles. Contaminated soil can be "cleaned up" by harvesting the stems and leaves and disposing of them safely, without disturbing the soil. Genetic engineering can enhance this ability. Examples abound. There is even an *International Journal of Phytoremediation.* The U.S. military is particularly interested in phytoremediation (U.S. Army Corps of Engineers 2012). I regret that I did not think to include this in the writeup instructions for the students. Another thing I wish I had asked them: "Do you think that plants are intelligent? Why or why not?"

This project addresses teaching standards A (inquiry based instruction) and E (developing communities of learners). It addresses content standard A (science as inquiry) and standard C (life science, especially behavior), even though the behavior content standards are discussed in terms of animals (National Academy of Sciences 1996).

References

Christensen, L. B. (2007). *Experimental Methodology,* 10th ed. Boston: Pearson.

Darwin, C. (1880). *The Power of Movement in Plants.* London: John Murray. Available online. URL: http://darwin-online.org.uk/EditorialIntroductions/Freeman_ThePowerofMovementinPlants.html. Accessed July 16, 2012.

Fransen, B., et al. (1999). Disentangling the effects of root foraging and inherent growth rate on plant biomass accumulation in heterogeneous environments: A modeling study. *Annals of Botany* 84 (3): 305-311.

Kembel, S. W., and J. F. Cahill, Jr. (2005). Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs. *American Naturalist* 166 (2): 216-230.

Mandal, A., et al. 2012. Phytoremediation of arsenic contaminated soil by *Pteris vittata* L. I. Influence of phosphatic fertilizers and repeated harvests. *International Journal of Phytoremediation* 14 (10): 978-995.

McNickle, G. G., et al. (2009). Focusing the metaphor: Plant root foraging behaviour. *Trends in Ecology and Evolution* 24 (8): 419-426.

National Academy of Sciences (1996). *National Science Education Standards.* Washington, D. C.: National Academy Press.

Ruffel, S., et al. (2011). Nitrogen economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. *Proceedings of the National Academy of Sciences USA* 108 (45): 18524-18529.

Trewavas, A. (2005). Green plants as intelligent organisms. *Trends in Plant Science* 10 (9): 413-419.

U. S. Army Corps of Engineers. “Phytoremediation research.” Available online. URL: http://el.erdc.usace.army.mil/phyto. Accessed July 16, 2012.

Whiting S. N., et al. (2000). Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* 145 (1): 199-210.