INNOVATIONS

Classroom Modified Split-Root Technique and Its Application in a Plant Habitat Selection Experiment at the College Level

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Abstract: The split-root technique produces a plant with two equal root masses. Traditionally, the two root masses of the single plant are cultivated in adjacent pots with or without roots from competitors for the purpose of elucidating habitat preferences. We have tailored this technology for the classroom, adjusting protocols to match resources and time periods characteristic of undergraduate teaching laboratories. Our classroom modified split-root technique is presented here through detailed instructions as part of an eight-week college laboratory experience. Adapted from the literature on root competition, this exercise also enables students to determine how Sugar Ann English pea plants allocate their root masses when experiencing competition, and more specifically, the applicable habitat selection model. This novel laboratory experience offers hands-on activities for students to learn more about the structure and development of roots, root competition, the crucial role of roots in plant survival, and plant cultivation.

Key Words: Split-Root technique, root competition, habitat selection models, root development, plant ecology, plant physiology, Hotelling's T^2 test

INTRODUCTION

Studying how plants respond to their environment enables growers to increase production of specific organs. For example, if an agricultural crop such as the Kenyan bean is found to overproduce roots when experiencing competition, much energy will be transferred away from fruit production. To increase the fruit yield, the goal is to breed cultivars that do not over-proliferate their roots when experiencing competition. The study of root processes, including root competition, to maximize the production of a more valuable component of the plant, usually the fruit, is a central objective of agriculture and of interest to those studying plant ecology and physiology (Maina, Brown, & Gersani, 2002).

Roots function in anchorage, hormone production, water and mineral absorption, water and mineral conduction, and in water, mineral, and food storage; therefore, they are crucial to the survival of the plant. However, since roots are usually below the ground, out of view, the average observer often discounts them. Thus, experiments designed to elucidate root processes provide the student with concrete evidence of their essential role. In addition, when conducting such experiments, students experience scientific techniques and data evaluations used in research, plus an introduction to reviewing current scientific literature. The split-root technique offers a unique opportunity to accomplish all of these goals.
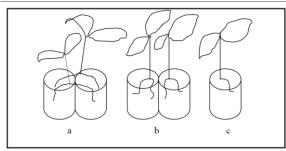
Although the split-root technique was developed over 100 years ago (Bohm, 1979), its recent resurrection provides a unique tool for investigating environmental effects on root development and root-shoot interactions. Germlings are manipulated during development to form two equal or twin root masses (Figure 1). For experimental purposes, a split-root plant is positioned on the fence with its root halves in separate pots to create a "fence-sitter," while another split-root plant has its twin roots in a single pot,

Fig. 1. A mature split-root pea plant.

called an "owner" (Maina et al., 2002, Figures 2a-b). The removal of half the root mass from a split-root plant forms a "single-root" plant, also useful in examining root-shoot responses (Figure 2c).

Originally, the split-root technique was employed to test nutrition effects on root growth

Fig. 2. Drawing of two fence-sitters (a), two owners (b), and a single-root plant (c).

(Bohm, 1979). For example, the control received water, while the experimental plants received water with dissolved nutrients usually formulated as Hoagland's medium (Hoagland & Arnon, 1950). Depending on the species, generally the larger root mass was developed by plants experiencing a higher nutrient concentration. Beginning in the 1990's, new experimental designs using split-root plants investigated topics such as impact of competitors, root discrimination, and root habitat selection models (Fallik, Reides, Gersani, & Novoplansky, 2003; Maina et al., 2002; Gersani, Brown, O'Brien, Maina, & Abramsky, 2001; Gersani, Abramsky, & Fallik, 1998; and Gersani & Sachs, 1992).

The split-root technique holds promise for meaningful, constructive learning experiences involving seed germination, plant development, nutrition, nutrient absorption, competition, plant culture, and habitat selection. However, the procedure for creating split-root plants in the literature is time-consuming and was not yet adapted for the classroom schedule. For example, the split-root technique involves carefully removing planted seeds and manipulating them several times in one week. The person or persons performing the literature-based split-root technique must have exceptional hand coordination and extra time during the initial week. A modified split-root technique using the Sugar Ann English pea (*Pisum sativum* var. Sugar Ann English) was developed for the classroom (Figure 3) and is presented here as part of an eight-week laboratory experience adapted from the literature (Maina et al., 2002) that focuses on determining the habitat selection model employed by the pea (Elliott, 2007). This exercise was designed, taught, and analyzed in Medicinal Botany and Plant Biotechnology Laboratory at the University of West Florida (UWF) as part of a Doctor of Education/ Biology Education Specialization degree.

Habitat Selection

Plants cannot simply get up and move to a better location when resources are diminishing. Instead, a plant compensates by distributing energy to its organs in an appropriate way to sustain life (Gersani, et al., 1998). For example, if neighboring competition is fierce, a plant may partition its growth to certain areas such as increased root or shoot growth. The way in which plants assess and respond to their surroundings is called habitat selection. Three models explaining how plants distribute their roots are reported in the literature: (a) inter-plant avoidance response, (b) resource matching response, and (c) intra-plant avoidance response (Maina et al., 2002). Each of these models predicts how a plant should allocate root mass and energy under a variety of hazards and opportunities.

Inter-plant avoidance response presumes that plants prefer to proliferate roots in the absence of another plant (Maina et al., 2002). Plants employ different strategies to try to segregate their roots from the roots of other individuals. For instance, some plants produce a zone of depletion around their roots, which discourages other plants from foraging in this nutrient deficient environment. Other plants proliferate roots in order to physically hinder the invasion of other roots. In addition, some plants secrete allelopathic chemicals from their roots that inhibit root growth of other plants (D'Antonio & Mahall, 1991).

Resource matching response assumes that root proliferation matches the available nutrients within the soil (Maina et al., 2002). It is not the presence or absence of competitors near the plant that affects root growth, but the opportunity for nutrient uptake. If nutrients are highly accessible, then root production is substantial. If nutrients are limited, then growth is slow. Additionally, root mass produced prior to alterations in available nutrients is a factor. In general, the plant produces enough roots to take in as much nutrients as possible. Resource matching response is based upon the ideal free distribution principle that predicts that plants invest resources to equalize average returns.

Intra-plant avoidance response indicates that plants avoid proliferating roots among themselves (Maina et al., 2002). Under this approach, plants establish roots among neighbors to try to maximize whole-plant fitness. Plants seem to operate on the premise that it is better to 'steal' resources from a neighbor than from oneself. However, if plants overproduce roots in an attempt to take all the nutrients in the environment, they are engaging in a response called the "tragedy of the commons" (Gersani et al, 2001). Hence, the collective yield or, perhaps, the fruit of the plant is sacrificed because excessive energy goes into root production.

MATERIAL AND METHODS

The habitat selection model employed by the pea can be determined through this eight-week classroom modified experiment. During the first three weeks, each student group creates split-root pea plants, then arranges them into fence-sitter and owner scenarios (Figures 2a-b). The roots of the plants in the fence-sitter scenario contact the roots of a neighbor, while the roots of each plant in the owner scenario isolate themselves. Each pot cavity contains the inert potting material, vermiculite, which does not have inherent nutrients. Each plant, whether in the fence-sitter or owner scenario, receives equal amounts of nutrients (0.5 strength Hoagland's medium) applied twice a week.

For four weeks following the initial set-up, students take observational data on the above-ground portion of the plants. After the growth phase, each student group harvests the plants and places them into pre-dried and weighed crucibles. Since vermiculite is utilized, precautions, such as masks worn over the nose and mouth, must be taken to avoid breathing vermiculite dust. After the harvested plants have been in an oven (75 °C) for a week, students determine the individual weights of the root, shoot, and flowers/fruit of each scenario and utilize the data for statistical analysis employing the Hotelling's T^2 test. The null hypothesis of this experiment is that the fence-sitters and owners are not different in regards to the mass of the roots, shoots, and flowers/fruits. Conversely, the alternative hypothesis is that the fence-sitters and owners are different in at least one of the organ masses. Each student group compares the root production for the fence-sitter and owner scenarios to reveal the habitat selection model of peas (Table 1). The Sugar Ann English pea plant utilizes the resource matching habitat selection model as determined from the literature (Gersani et al., 1998) and from our developmental phase experiments and two classroom trials.

Materials and Equipment Needed

Week 1: Seed for Sugar Ann English peas, Bleach, Paper towels (autoclaved), Containers (jars or beakers), Plastic wrap, Cold room (15 °C), Sterile distilled water

Week 2: Paper towels (autoclaved), Razor blades (autoclaved, if possible), Plastic wrap, Cold Room (15°C)

Week 3: Razor blades (autoclaved, if possible), Masks, Planting trays, Vermiculite, Skewers (bamboo), Velcro strips, 0.5 strength Hoagland's medium, Light bank of broad spectrum fluorescent lamps

Weeks 4-7: String, Ruler, Velcro strips , 0.5 strength Hoagland's medium, Light bank

Week 7: 48 oven-dried crucibles, Crucible tongs, Razor blades, Balance to 3rd decimal, Masks, Washing baths, Drying oven

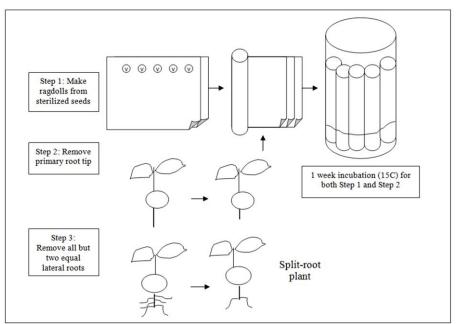*Week 8:* Crucible tongs, Balance with mg accuracy

Laboratory Instructions

The following instructions are intended for students to perform in groups and with minimal before-class preparation by the instructor. To minimize student downtime during the first week, the instructor should surface sterilize the seeds by washing them in a bleach solution of 5-10% for 20 minutes and then rinsing 3 times for 5 minutes each with sterile distilled water. This can be performed prior to the start of class or during lecture introduction to habitat selection models.

Week 1: Planting seeds in ragdolls
1. Obtain 150 surface sterilized pea seeds.
2. For each ragdoll (Figure 3), layer 2 autoclaved paper towels and place them on a clean surface. Squirt sterile distilled water using a water bottle in a line 2/3 of the way from the bottom of the towels.
3. Starting 3 cm from the left side of the paper towels, equally space 5 seeds along the water line (Figure 3). Make sure that the radicles, which look a "V," are pointing down. Add a third paper towel on top of the other two.
4. Roll the 3 paper towels horizontally beginning with the void space at the left.
5. Stand each tube-shaped ragdoll vertically with the peas near the top in a container, such as a 10 inch battery jar, that has 3-4 inches of sterile, distilled water.
6. Repeat steps 2-6 until all 150 seeds are in 30 ragdolls. Label the container(s) then cover with plastic wrap.

Table 1. Root production predicted for each plant habitat selection response.

Response	Root Production
Inter-plant avoidance	Fence-sitter < Owner
Resource matching	Fence-sitter = Owner
Intra-plant avoidance	Fence-sitter > Owner

Fig. 3. Ragdolls and modified split-root technique for the classroom. Ragdolls are prepared during week 1 (Step 1). Small, vertical ovals represent pea seeds (notice radicals pointing down) and rectangles are paper towels. The paper towels plus seeds are rolled to form a tube, subsequently placed vertically in a jar with distilled water. During week 2, about 0.5 cm of primary root tip are removed (Step 2) and germlings are incubated for another week in ragdolls. Split-root plants are formed in week 3 when all but 2 lateral roots are removed (Step 3).

7. Incubate the container(s) under a grow light in a cold room at 15 °C, which will provide the necessary cold hours for flower development.

Week 2: Cutting the distal ends of the radicles
1. Remove the ragdolls from the plastic wrapped container(s).
2. Lay a ragdoll flat on the lab bench, carefully unroll it, and remove the top paper towel. Observe that most peas have germinated.
3. If a pea seedling has a radicle over 2 cm, make a straight, horizontal cut with a razor blade to carefully remove the distal end of the root until only 1-1.5 cm remains. If a radicle is not present or too short, remove the pea from the ragdoll and discard.
4. Note: If the ragdoll or seed has any evidence of fungal or bacterial growth, discard the infected material properly.
5. Repeat steps 2-4 until all eligible seeds have their radicles cut.
6. Form new ragdolls following the same process as Week 1 but using the germinated pea plants with cut radicles instead of pea seeds. Stand the new ragdolls vertically in the container(s) with fresh, sterile distilled water.
7. Cover the container(s) with plastic wrap and return to the cold room outfitted with grow lights for another week.

Week 3: Fashioning split-root plants
1. Obtain the container with treated ragdolls.

2. Carefully unroll the ragdolls and remove the top paper towel.
3. Observe that lateral roots have formed along the entire length of the radicle.
4. Using a razor blade or your clean fingers, remove all lateral roots except two lateral roots that are approximately the same length. Do not cut the roots to make equal lengths.
5. Select split-root plants so roots are roughly the same size as the other split-root plants.
6. Repeat this process until 16 split-root plants are formed.
7. Obtain 8 small planting trays with potting cavities opposite each other such as annual flowering plants small potting trays. In a well-ventilated area, preferably outside and using a mask, fill 2 opposing cavities ¾ full with vermiculite, an inert potting mix, in each tray. Wet the vermiculite with equal amounts of distilled water.
8. To each tray, add 2 bamboo skewers on the inside of the cavities (Figure 4).
9. For each fence-sitter scenario, stake a split-root pea to each skewer using thin Velcro strips, and then place one lateral root in each pot cavity (Figure 4). Gently add vermiculite to both cavities until full and re-wet the vermiculite to ensure roots are in a moist environment.
10. Repeat steps 8-9 to obtain four fence-sitter setups, and label as fence-sitter 1 a/b, fence-

sitter 2 a/b, fence-sitter 3 a/b, and fence-sitter 4 a/b.

11. Use the other 4 trays for owner scenarios and stake a split-root pea to each of the 2 skewers using thin Velcro strips. Place both lateral roots of each plant into only one cavity (Figure 4). Gently add vermiculite to both cavities, and re-wet the vermiculite to sufficiently moisten roots.

12. Repeat steps 9 and 11 to obtain 4 trays with owner setups, and label as owner 1 a/b, owner 2 a/b, owner 3 a/b, and owner 4 a/b.

13. Carefully transport the labeled fence-sitter and owner scenarios to a continuous grow-light apparatus consisting of 4, 30-Watt plant growth fluorescent bulbs elevated 0.5 m above the pot and exposed to room temperature, about 24 °C.

14. Twice per week, saturate the plants with approximately 40 ml of half strength Hoagland's medium per cavity. Once per week, saturate the plants with approximately 40 ml of distilled water to prevent accumulation of ions in the vermiculite.

Weeks 4-7: Collecting observational data and harvesting

1. For each plant, record the number of compound leaves, number of tendrils, and plant height during weeks 4-7. Stretch string between stem base and tip and then measure string length with a ruler for plant height.

2. Also, note if individual plants have developed flowers and/or fruit. If present, record the numbers of each reproductive structure, if present.

3. Document other observations such as wilting, chlorosis, or necrosis.

4. Calculate means for number of leaves, tendrils, height, flowers, and fruits of each scenario.

Week 6:

1. After taking measurements and making observations, number 48 crucibles while wearing gloves to avoid oils from hands affecting weight.

2. Using crucible tongs, place crucibles in a drying oven of 75 °C for one week.

Week 7:

1. After taking measurements and making observations, weigh and record the dry weight of 48 crucibles.

2. Harvest the root, shoot, and flower/fruit separately for each plant.

3. While wearing masks, add the roots plus vermiculite from each cavity to a wash bath to help remove the vermiculite. Gently shake the roots to remove the vermiculite and/or use forceps. Cut roots to fit within the assigned crucibles if necessary.

4. Since the roots are too intertwined in the fence-sitter scenario, harvest the roots from a single cavity instead of the roots for one plant and place in a single crucible. Place all roots from the neighboring pot in a second crucible.

5. Remove the reproductive structures (flower/fruit) from each plant for the flower/fruit determination. The shoot determination is the above-ground portion of the plant minus the flower/fruit.

6. Record the crucible number, plant number, and type of contents.

7. Return the crucibles, plus plant material, to the oven for another week so that differences in water content do not affect results for plant mass.

Week 8: Determining dry weights

1. Weigh and record the weight of each crucible plus plant dried material. Use crucible tongs and weigh immediately, so crucibles are exposed to the ambient air for

Fig. 4. Fence-sitter scenario where shoots from each plant are positioned on the pot rim and half the root mass occupies each pot (left picture). Owner scenario where each plant owns a single pot; thus, total root mass for each plant is isolated in a separate pot (right picture).

the shortest possible time.

2. To determine the sample weight, subtract the weight of the crucible taken the previous week from the weight of the crucible plus dried sample.

3. Record and organize the data and apply the Hotelling's T^2 test. Report similarities and significant differences at $\propto=0.05$ level and draw conclusions based on the habitat selection model (Table 1).

RESULTS AND DISCUSSION

During each week of growth, students calculate the means for above-ground parameters: number of leaves, number of tendrils, plant height, number of flowers, and number of fruits. The weekly means for each parameter are entered on a computer spreadsheet and graphed as a function of time (weeks) using Microsoft Excel® or a similar program. Students can compare averages of parameters between fence-sitters and owners. For example, students can determine if there is a difference in the flower/fruit production between scenarios. From our experience through teaching trials, the means for the above-ground parameters are similar between the fence-sitters and owners.

The root growth, while not visible during collection of above-ground data, is assessed as root mass along with shoot and flower/fruit masses at the end of the experiment. The root, shoot, and flower/fruit masses determined during the eighth week for each scenario are compared statistically through the Hotelling's T^2 test (or MANOVA where n=2), which is appropriate for comparing several means between two groups. Individual *t*-tests could be utilized for each individual mass such as root mass but statistical power is lost over multiple comparisons (3 individual tests, for root mass, shoot mass, and flower/fruit mass) instead of one comparison (incorporates all three using Hotelling's T^2 test). Statistical analysis programs such as SPSS can be utilized and offer the instructor an opportunity to discuss terminology such as null and alternative hypotheses, alpha value, *p*-value, and sample size. The results from the literature and from classroom trials for peas showed no statistically significant difference between fence-sitters and owners (each group's *p*-value was above the alpha value set at 0.05); thus, the null hypothesis is **not** rejected, agreeing with the model for resource matching.

While students work in groups, individual student lab reports are necessary to ensure that each student understands the material and the results of the experiment. In addition, writing laboratory reports gives students practice with developing written skills in science. The instructor should present students with a clear outline of the sections or chapters within the report (e.g., Introduction, Materials and Methods, Results, Discussion, and References) and what is required in each area. A rubric describing point allocations for grading reports is useful in further defining expectations.

CONCLUSIONS

The split-root technique employed in the literature has been modified from an intensive week in the research laboratory to three-weekly meetings in the college teaching laboratory. In addition, the classroom modified technique meets important goals of novel laboratory exercises: reasonably inexpensive supply list, limited classroom equipment, short instructor before-class preparation, and numerous opportunities for additional experimental designs. Students can use the split-root technique to determine the habitat selection model of the pea (Weeks 3-8). These laboratory exercises require students to collect, record, and analyze above-ground parameters as well as perform statistical analysis using dry weights. Consequently, students actively participate in the scientific process including drawing conclusions based on data analysis and interpretation while improving laboratory skills.

The application of the modified split-root technique is not limited to determining the habitat selection model of the pea. Student groups can be challenged to develop their own experimental design to test any of the numerous aspects of root development, competition, or nutrition. In their arsenal, students have scenarios such as fence-sitters, owners, and single-root plants (only one lateral root) to use and other plants to investigate.

ACKNOWLEDGEMENTS

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