BIOL266: PLANT PHYSIOLOGY LAB MANUAL

INTRODUCTION TO EXPERIMENTAL DESIGN, REPLICATION, DATA COLLECTION AND ANALYSIS

EXPERIMENTAL DESIGN

Experimental design starts with a research question(s). We then choose a system in which to investigate our question and consider various ways we could treat or alter a system to find the answer. Usually only one variable is manipulated at a time because manipulating multiple variables can make results difficult to interpret.

Ways to investigate a biological system:

- add or remove a component
- change a variable (e.g., growth temperature)
- compare the responses of variants of the system (e.g., cultivars of pea) to the same treatment or responses of a single system (e.g., single cultivar) to various treatments

We also consider:

- what responses we wish to measure
- how best to measure responses
- how to analyse the data collected

Frequently there are many treatments, measurements and variables we can choose from and, while we might like to try all possibilities, time and resources will dictate limitations. For example, let's say we discovered a new growth factor that in preliminary studies appears to increase the growth rates of cultured mammalian cells and we want to test its effect on whole organisms.

Question: Does feeding 8-week-old mice growth factor X influence their growth?

System: We choose mice because we already know a lot about their growth and development from previous studies (Bryda, E.C. 2013 Mo Med. 110(3): 207–211). We can use a wild type (WT) mouse line (no genetic manipulations) or a transgenic mouse line (see Figure 1.)

Hypothesis: Addition of growth factor into diet of WT mice increases biomass.

What would the null hypothesis be in this case?

Independent variable: Growth factor

Plant genetics note:

A **wild type** phenotype is the phenotype most commonly seen naturally. Most agronomically important crops are not wild type because humans have selected for the mutant features that suit us.

A **cultivar** is a true breeding plant strain; for example, if you cross a particular dwarf plant with other plants of the same cultivar, the offspring will all be dwarf as well. When a trait breeds true it means that the trait is genetically determined and that the organisms being breed are homozygous for all alleles that determine this trait.

Dependent variable: Biomass

A simple experimental design would involve a WT system (healthy with no genetic variations, of a typical weight and size) with a single manipulation: plus/minus growth factor. Condition with growth factor added would be considered the "treatment" while condition without the growth factor is the "control"

Measurements: mass, length, brain mass (final only, since requires culling of system). One other measurement we could add to this list is skull circumference because rapid growth can cause brain damage if the skull growth does not keep pace. Such decisions are made following a review of the literature on the topic.

In a more complex study design, we can use a wild type and a poor growing mouse line (likely a knock-out mouse line) to see if our growth factor could enhance normal growth and/or help the poor growing mouse line.

Try generating your own hypothesis and map out an experimental design for the more complex example above. Contact us plantphys@uregina.ca with questions!

Hint: Variables include two mouse lines, basic (control) and growth hormone supplemented (treatment) feed. For each mouse line, feed **half the mice*** the control formula and the other half the supplemented formulas.

* Why can this study not be done on a single mouse per treatment?

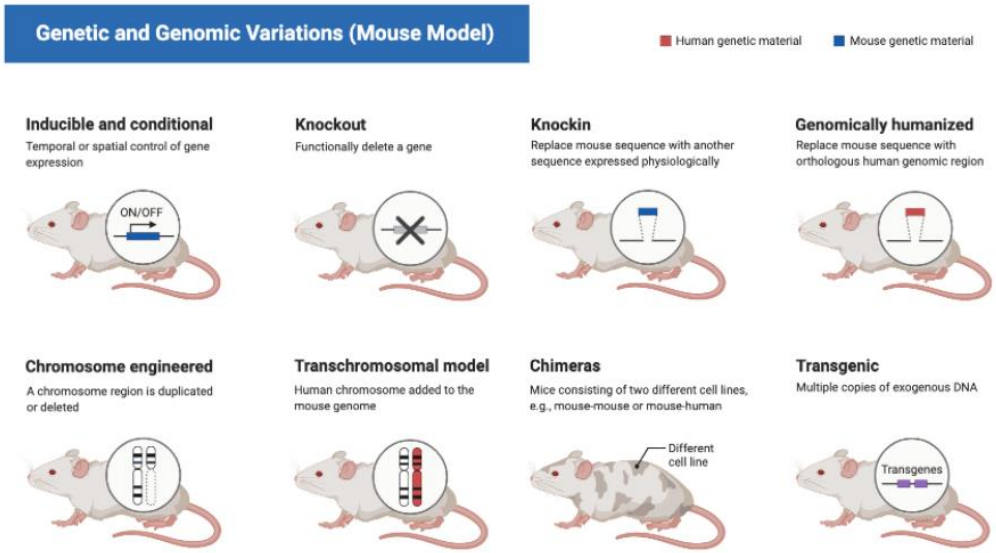

Figure 1. Genetic and Genomic Variations (Mouse Model). BioRender (2021). Retrieved from https://app.biorender.com/biorender-templates/figures/5c95180bc2753f33003dacd3/t-5fcac9b55933f000ae610650-genetic-and-genomic-variations-mouse-model

REPLICATION

Replication of an experiment is one of the important foundations of the scientific method. Replication gives us an idea of the variation in response to a treatment. When performing experiments with living organisms it is essential that both technical and biological replicates be performed in order to appropriately interpret the results.

Technical replication: the experimental steps (technique) are repeated using the same sample in order to accurately measure technical variation (user or instrument variation) to account for this variation during analysis

Biological replication: different biological samples that have undergone the same experimental treatment (e.g. addition of growth hormone to diet) are used to measure the biological variation between samples

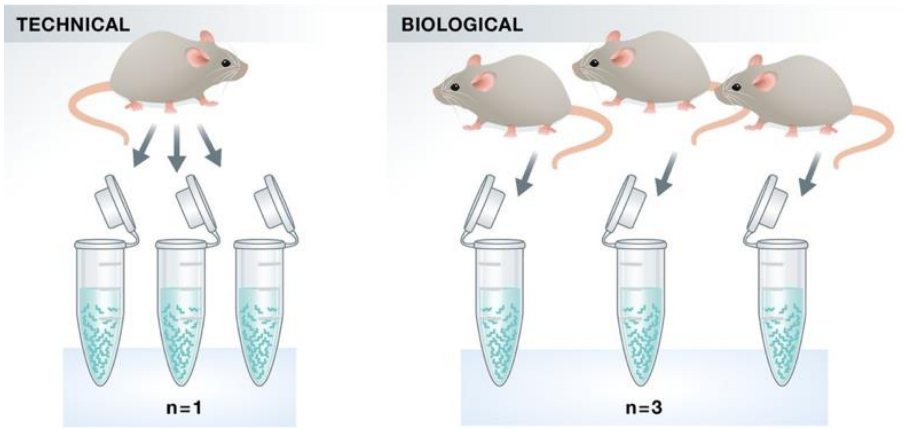

Figure 2. Visual showing difference between technical and biological replication. In the left panel, the same individual is sampled multiple times to test variation in sampling technique, while in the right panel, multiple individuals are sampled to us a better understanding of the biological variation. Reproduced from Klaus B., EMBO J (2015) 34: 2727-2730

In our study all the plants in a single treatment can be considered biological replicates (individual plants all treated exactly the same way). Replicates are important in all experiments as they give us an idea of the variation observed in your experiments; they are even more important when studying living organisms, as biological systems are inherently variable. For example, even though all plants were pre-germinated and sowed on the same day, we expect variability in plant height, even if the plants are genetically identical.

KEEPING A RESEARCH JOURNAL

Keeping a research journal or notebook is a requisite for performing Science. This may be the first time you are keeping a formal laboratory notebook/ research journal. Keeping a proper notebook is essential to performing good science. A lab notebook is a detailed account of what you did in lab, and more importantly, why you did it. A lab notebook can be considered a journal that contains all the details of your experiment, as well as your conclusions and future directions at the end of each experimental entry, leading you to the purpose of the following experiment.

As novices, you are learning the habits that will help you keep a good lab notebook. Start an ***<experimental entry>*** each time you perform an activity relating to your lab, even if you are only thinking about the experiment or performing a relevant literature review. Each experiment/entry should contain the following (appropriately labelled) sections as required:

1) Entry number, date and a descriptive title;
2) Purpose statement;
3) Protocols/Experimental procedures with risk assessment and mitigation measures
4) Data/results and interpretation
5) Reflection/Discussion

Each entry should always end with 5) metacognitive self-reflection on the activities performed, and your learning through this process. Please refer to the keeping a lab notebook handout on UR courses for more information.

DATA COLLECTION

Data collection is an important activity you will perform as a scientist. Ensuring that you are collecting and storing data in a way that is easily accessible and understood by others is essential for reproduceable science. You will learn more about data collection practices though the course of this lab. Please ensure you are following the data collection guidelines and templates provided to you.

ANALYSING EXPERIMENTAL DATA

Analysing experimental data involves methodically looking for relationships between treatments and outcomes. Data analysis can be a complex and intimidating process for students and professional scientists alike. When a large data set is staring you down, it is easy to panic and start randomly making graphs. However, if your analysis is not driven by the questions you wish to answer, you may find yourself wasting time investigating the wrong relationships. Instead, take some time to consider what approach will identify important relationships, that is, the relationships that are most likely to help address the central purpose.

Data analysis is planned when the study is designed. You should be able to determine, from the central purpose and the study design, what relationships can be examined. In other words, what comparisons can be made based on the study design and background information provided. We will do this using the statistical language R in the user interface Rstudio.cloud (web-based service; you will need to create an account to use this service). Tutorials to help you create your account and training for using R and Rstudio.clould are available on UR Courses.

INTRODUCTION TO CONCEPTS YOU WILL WORK WITH IN THIS LABORATORY

You will learn more about these concepts in lecture. Here are short summaries to get you started on thinking about your experimental design and hypotheses.

PLANT GROWTH AND DEVELOPMENT

Plant growth and development is different from animal growth and development in many ways, but for our purposes the simplified key points are as follows:

- Plants grow more or less indeterminately from their ends, the root meristem and the shoot apical meristem.
- Plants grow primarily by increasing their cell volumes. Unlike animal cells, plant cells can expand as much as 10,000 times. However, this does not mean that cells expand indefinitely. Most cells will eventually stop growing and changes in the cell wall will make their size and shape less malleable.
- Plant cells can grow by expansion, characterized as growth in all three dimensions, or by elongation, characterized by growth in only two directions, or sometimes by a combination of the two.
- Stems get longer primarily by cell elongation. New cells are created in the apical meristem and then, as the meristem moves away the cells will elongate.
- Plant cells can differentiate into specialized structures thought their lives. For example, as a plant gets taller, it will need to produce side shoots and leaves. In angiosperms, the first step in production of new side shoots is the formation of nodes.
- The balance between elongation and differentiation in a plant's stem will determine its overall shape. Rapid elongation with little cell differentiation (aka no new nodes) will create a tall, thin plant while slower elongation accompanied by more differentiation (aka more nodes) will result in a more compact, bushier plant.

One thing that is not different between animal and plant growth is the interplay between genetics and environment. A plant's genome will dictate its overall growth and development potential, but interactions with the environment will also play a critical role. If you have ever visited the west coast, you probably noticed that plants that grow in both Regina and Vancouver look very different on the coast!

PLANT GROWTH AND AGRICULTURE

One might think that the goal of agriculture is to produce a bigger plant. However, keep in mind that we generally only consume/use a portion of the plant. A bigger cherry tree is of no use to us unless it produces proportionally more cherries. Even if a bigger tree produced more cherries, if the inputs required (in agriculture inputs are anything the grower must supply that

cost time and/or money) are more costly than the added value of the additional cherries, or if the bigger cherry trees are more susceptible to environmental damage, than a grower will stick with the smaller trees.

The concept of inputs applies to natural selection as well, the only difference being that naturally selected plants have no growers to supply them with needed inputs. Again, a bigger plant has some competitive advantages over a smaller plant, but if these are outweighed by negative consequences, the plants will not likely evolve to be taller.

NITROGEN AND PLANT GROWTH

Nitrogen (N) is the #1 macronutrient for plants, and soil nitrogen management is an important aspect of agriculture. Proteins represent by far the largest nitrogen pool in plants, but nitrogen is also found in many other plant components including chlorophyll, nucleic acids (RNA and DNA) and in minor amounts in two plant hormones (auxins, cytokinins). As well, photosynthesis is a nitrogen-intensive process, with the various enzymes (e.g. Calvin cycle and sucrose synthesis) and photosynthetic electron transport chain multi-protein complexes all representing large amounts of nitrogen as biomass.

Plants take up most nutrients (including nitrogen) from the soil solution via specific transport systems in the roots. Roots can transport various forms of inorganic nitrogen from soil, including nitrate (NO_3^-) and ammonium (NH_4^+). Soil nitrogen is ultimately derived from atmospheric nitrogen (N_2) via the processes of biological and industrial N_2 fixation over long time scales. Biological N_2 fixation converts N_2 into 2 molecules of NH_4^+ (simplified view of the reaction) and is mediated by the prokaryotic enzyme nitrogenase. Plants do not possess nitrogenase, but some plants (legumes) form symbiotic associations with bacterial species that exhibit nitrogenase activity.

Legumes house N_2-fixing bacteria in root nodules (i.e. the nodules are the site of the symbiosis). The plants supply reduced carbon (C) to the bacteria (= energy source), while the bacteria release NH_4^+ to the plants (the NH_4^+ is rapidly assimilated into organic N in the roots). In natural settings, legume species are often pioneer plants that grow in low N soils. In agricultural settings legumes can be used to increase soil N in addition to being harvested as a crop.

Biological N_2 fixation occurs in various types of symbioses (not only legumes, but also some lichens, water ferns [*Azolla*], some cycads, various non-legume plant species) and by free-living soil and aquatic prokaryotes (Bacteria and Archaea), including many species of cyanobacteria. While most prokaryotes do not possess nitrogenase activity, prokaryotic biological N_2 fixation has nonetheless been the major input of biologically available N to both terrestrial and aquatic systems over geological time scales. In the last few decades, industrial N_2 fixation (which also

converts N_2 -> 2 NH_4^+, as part of N fertilizer production) has greatly increased in magnitude and has had major effects on the global N cycle.

Biological N_2 fixation is usually viewed as a response to nitrogen limitation/deficiency. The complete nitrogenase-mediated N_2 fixation reaction is:

$N_2 + 10\ H^+ + 8\ Fd_R + 16\ ATP \rightarrow 2\ NH_4^+ + H_2 + 8\ Fd_O + 16\ ADP + 16\ P_i$

Thus, the cost for each NH_4^+ is 8 ATP and 4 high energy electrons (Fd_R); this is energetically "expensive" N compared to the costs of transporting NO_3^- or NH_4^+ from the soil solution. If less expensive N sources are readily available, the rate of biological N_2 fixation is often much lower than in the absence of readily available N (i.e. organisms will use the energetically cheaper stuff). For example, high soil nitrate levels may decrease the degree of legume root nodulation and/or decrease nitrogenase activity of legume nodules.

GIBBERELLINS AND PLANT GROWTH

Gibberellins, also known as gibberellic acids (GAs), are a well-characterized class of plant hormones. GAs influence numerous aspects of plant growth and development, including seed germination, stem elongation, flowering, bud break, timing of fruit production, and chlorophyll breakdown. One of the best-known responses to exogenous GA application is enhanced stem elongation. Depending on the species and condition of the plant, the increase in stem elongation may be accompanied by an increase in leaf surface area. However, GA can affect plant morphology without altering plant biomass.

The complex metabolic pathways responsible for GA biosynthesis and action have been elucidated mainly by studying flowering plants (angiosperms) with mutations in GA metabolism, including in the model plant system *Arabidopsis thaliana*. Mutations in GA metabolism cause plants to either be shorter and stockier (commonly called dwarf plants) than wild type plants, or to be taller and spindlier. Two general classes of GA dwarf mutants are well known: mutants that are deficient in endogenous GA synthesis (due to a defect in an enzyme of the GA biosynthetic pathway), and GA-insensitive mutants that are do not respond to GA (due to a defect in the GA receptor or in a component of the GA signal transduction pathway; reviewed in Hedden 2003). A third class of mutants causes hypersensitivity to GA and results in tall spindly plants.

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EXPERIMENT 1. THE EFFECT OF VARYING CONCENTRATIONS OF MIRACLE GRO® ON THE GROWTH AND NODULATION IN PEA (*PISUM SATIVUM*)

LEARNING OUTCOMES

- Investigate how "Miracle Gro®" (fertilizer rich in N) influences plant growth and root nodulation
- Generate a hypothesis and make experimental predictions
- Create appropriate and meaningful visuals that highlight important data trends
- Identify, highlight (using visuals) and discuss important data trends
- Describe how nodulation affects plant wet weight
- Identify any differences in the response to treatments
- Write a scientific report to communicate results to a general audience

INTRODUCTION

Nitrogen is an essential macronutrient required for plant growth and survival. Nitrogen in its elemental form (N_2) makes up 78% of the earth atmosphere. However gaseous N_2 is inaccessible to plants. Plants acquire nitrogen required for growth and metabolism from the soil, in the form of inorganic nitrogen-containing ions; nitrate (NO_3^-) and ammonium (NH_4^+). Elemental N_2 is made available to the plant through the process of N_2 fixation.

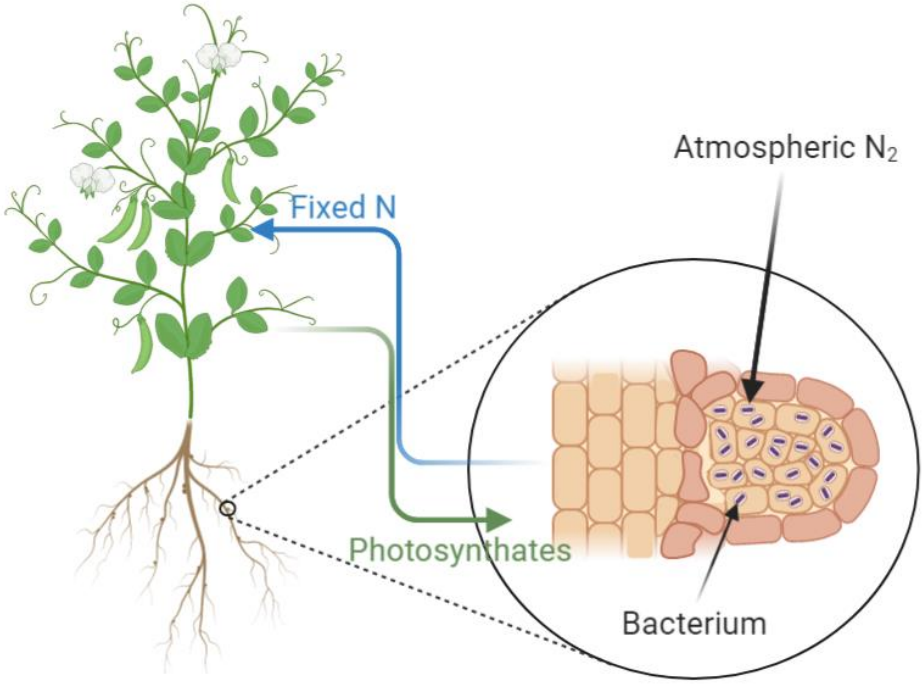

Figure 3. Simplified visual showing the symbiotic relationship between the plant and nitrogen-fixing bacteria. Visual created on BioRender.com

Biological N_2 fixation in leguminous plants occurs in root nodules, which are outgrowths on plant roots (see Figure 3) housing bacteria (several different species collectively called "rhizobia") that convert atmospheric N_2 into ammonia (NH_4^+), a form that can be used by the plant to make the macromolecules required for life (amino acids and nucleotides). In turn the plant provides the bacteria with a stable environment abundant in malate, a carbon source (and product of photosynthesis) that can be readily used by the bacteria. The legumes (family Fabaceae/Leguminosae) are well-known for their root symbiotic associations with N_2-fixing bacteria. The legume family includes peas, lentils, beans, alfalfa and many other wild and domesticated species. There are approximately 20,000 legume species, making this group the third largest plant family.

This study design is based on comparing the effect of two treatments of N-fertilizer on wild type Pea cultivar. Our goal in this experiment is to determine how nitrogen influences pea plant growth and root nodulation.

AT-HOME LAB KIT

You will conduct this experiment at-home over 6 weeks using "at-home kit" available for pick up from the University of Regina, main campus. Please contact us if you need the kit mailed to you.

At-home lab kit contains:

- 21 seeds of 1 pea cultivar (Little Marvel cultivar, purchased from Lindenberg Seeds Limited) in a petri dish for germination
- 2 packets of Miracle Gro® All-Purpose Water-Soluble Plant Food (24-8-16), 1 tsp to make up 1x and 1tbsp to make up the 3x treatments
- 1x and 3x SDS labels for treatment solutions
- Two STERILE microfuge tubes for nodule collection. Please drop these off in the provided envelope at UR Stores for other students to use in their research.
    - NOTE: Only open tubes once nodules have been collected to maintain sterility

If you are unable to come to campus to collect your kit, please contact us as soon as possible via plantphys@uregina.ca. We may be able to mail you the pea seeds (depending on location). You will need to source other materials independently. Save 900 g yogurt (or similarly sized) containers from your recycling or purchase 6 inch pots from your local hardware store. Please make sure to label all materials clearly, especially treatment solutions and keep them out of reach of children and pets. Collect sand or gravel for drainage. Miracle Gro® All-Purpose Water-Soluble Plant Food (24-8-16) is available at Walmart: (https://www.walmart.ca/en/ip/miracle-gro-water-soluble-all-purpose-plant-food-24-8-16/6000198904462) and Canadian Tire (

https://www.canadiantire.ca/en/pdp/miracle-gro-all-purpose-water-soluble-plant-food-0592210p.html#srp)

Materials you will need source

- Soil: Collect enough local Saskatchewan soil (ideal to get wild rhizobial species) to fill three 6-inch pots (around 11 metric cups). Mix gravel or sand with the soil to create a homogenous mixture. This will increase the porosity for root aeration.
    - You might also want to mix the soil with small pebbles, gravel or sand depending on the texture of your soil. For more information on soil texture, see Appendix A
- Pots: Collect 900 g yogurt containers (or other similarly sized containers) from recycling or purchase 6 inch plastic pots from your local hardware store (an option: https://www.canadiantire.ca/en/pdp/grower-planter-green-0594435p.html?rrec=true#spc)
- Treatment solution storage: Two 1L pop/soda bottle with all labels removed

PROCEDURE

Create a hypothesis and map out the experimental design to study the impact of Miracle Gro® All-Purpose Water-Soluble Plant Food (24-8-16) on pea plant growth, health and root nodulation in local Saskatchewan soil types. This experiment will last 5 – 6 weeks from germination to harvest.

Treatments:

- Make-up your treatment solutions as follows in an appropriately re-labelled recycled pop/soda bottle
    - 0X : 1000 L tap water (No Miracle Gro®)
    - 1X: 1 teaspoon dissolved in 1000 mL (4 cups) tap water
    - 3X: 1 tablespoon dissolved in 1000 mL (4 cups) tap water

NOTE: Please remove all existing labels (including any marking on caps) on treatment solution storage; re-label as 0% and 1% using the SDS labels provided in the “at-home kit”. Please ensure the solutions are stored out of reach of children and pets.

SEED GERMINATION:

- Place a piece of paper towel below and above seeds in a covered container
- Add water just until the paper towel is moist
- Keep covered in the dark for up to 5 days until they sprout (check daily to ensure paper towel stays moist)

Soil Gathering and Preparation:

- For tips and best practices, see the Soil Guide (Appendix A)
- Collect approximately 11 metric cups of soil (enough to fill the 6-inch pots about an inch from the top)
- Mix in pebbles, soil, or gravel if needed. This depends on the texture of soil collected – see the Soil Guide (Appendix A) for more information.

Sowing:

- Use 6 pre-germinated seeds per pot, these will serve as your biological replicates
- Use a pencil or a stake to make 1-inch equidistant depressions in the soil surface
- Place seed in the depression
- Cover with soil
- Water to moisten soil if needed
- Label each pot with one level of treatment (either 0x, 1x of 3x)

Growth conditions:

- Record the following in your notebook/research journal during each data collection

  - # hours in day (photoperiod) and night

  - Temperature

  - Relative humidity (if available)

  - Soil type and collection location (GPS coordinates if available)

  - Frequency of watering

Day of Emergence (Day 1):

- Note the day that all seedlings emerge from the soil. This is day 1.

Watering Plants:

- Check soil moisture and water as necessary (see Appendix B)
- Ensure all plants get the same total volume of liquid

Treatment Levels (start Day 7):

- Treat plants once every 7 days for 3 weeks after the day of emergence (Days 7, 14, and 21).

- Plants will be given the following volume of pre-made Miracle Gro® concentrations:
    - ½ cup (125 mL) 0x (tap water)
    - ½ cup (125 mL) 1x Miracle Gro® solution
    - ½ cup (125 mL) 3x Miracle Gro® solution
- Use additional water as necessary, please ensure all plants get the same total volume of liquid

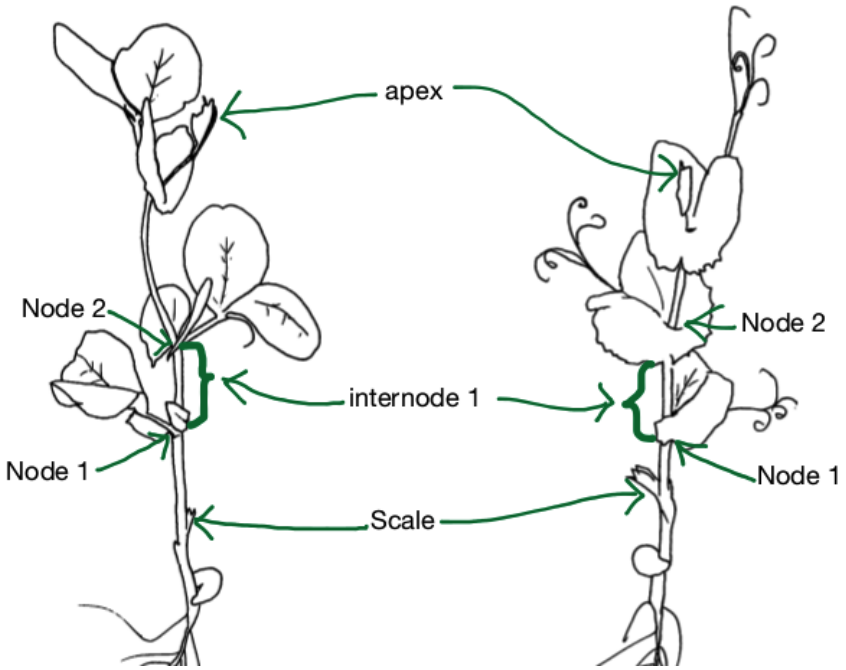

Figure 4. Illustration of Pea shoots. The leaf-like structure near the soil line is called the scale or bract. We do not consider this a node. Nodes will have stipules (Leaf-like structure at the node). *Notice that the apex is not necessarily the highest point on the plant. See plant photos posted on UR courses for further clarification.*

OBSERVATIONS AND MEASUREMENTS (DAY 7-28):

Notes:

◊ 2 times per week – minimum, starting day 7
◊ Use data collection template provided (google sheets), example on page 31
◊ You will also need to stake and add twist tie hooks to support plants as they grow, see tips video
◊ Refer to Appendix C for tips on making observations and measurements

- Record Day
- Record growth conditions

- Record the following in the data collection sheet

1. Measure height of plant in mm (from base of soil to apical meristem).
    a. Note: this is not the tallest tip of the plant, see Figure 3 and tip sheet
2. Count Number of internodes
3. Assess color and overall health of the plant (see Figure 5):
    - 90% of leaves are green and plant growing well 5
    - Some of leaves slightly chlorotic (yellow) 3
    - 90% of leaves very chlorotic (yellow) 1

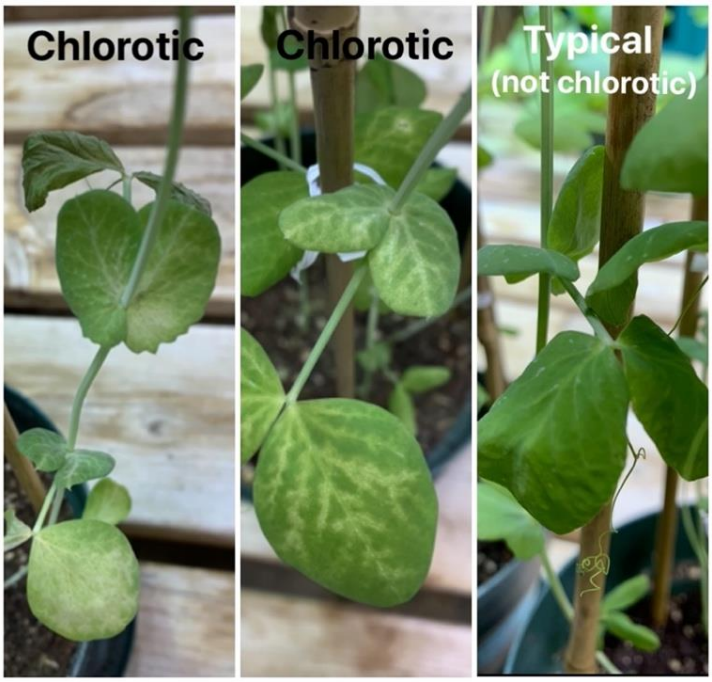

Figure 5: Example of plant colour (Photo: L Schnell) Left panel: Leaves are chlorotic (yellow), Middle Panel: Leaves are slightly chlorotic, Right panel: Plant is green and growing well.

FINAL MEASUREMENTS AND NODULE COUNTING AND COLLECTION (DAY 28):

- Qualitatively Assess Nodulation and Nitrogen Fixation adapted from the Saskatchewan Field Assessment Guide
- Measure height, internodes, and health as above in the weekly measurements' spreadsheet.

Harvest (Refer to Appendix D for tips and help videos/photos)

- Find a large container or perform the following outside your house
- Gently remove all contents from pots and separate the replicate plants roots from soil

- Optional measurements: fresh weight using kitchen scale
- Cut plants near the first bract (where the shoot emerged from the soil) and collectively weigh the shoots by treatment (all 0% shoots together then do all 1% shoots together). See Appendix D.
- Gently wash the roots (see Appendix D) and weigh them by treatment
- Record measurements in sheet 2 (sheet name: HarvestData) in the spreadsheet
- Count the number of nodules for each replicate

Classify by size if clusters are present using the guide below:

Colour and Abundance of Nodules

- Three to five cluster groups of mostly pink nodules 2
- Less than three clusters of nodules OR white or green nodules 1
- No nodules 0

Nodule Position

- Both crown and lateral nodulation 3
- Mostly crown nodulation only 2
- Mostly lateral nodulation only 1
- No nodulation 0

Total qualitative assessment score

⇒ 4 – 5 = Effective Nodulation
⇒ 2 – 3 = Nodulation less effective
⇒ 0 – 1 = Poor Nodulation

DATA ANALYSIS

Analysing experimental data involves methodically looking for relationships between treatments and outcomes. We will do this using the statistical language R in the user interface R studio. Once your data has been submitted to your instructor, tutorials on how to analyse your data will be made available via UR Courses. Visual representation of data in the form of figures and tables are a great way to compare the effects of multiple treatments. By this time, you should have already completed data analysis on practice data sets. When you begin your data analysis, focus on figuring out what visual best helps you answer the central question posed in your hypothesis. You may need to create more than one visual before you find the best one; do not be afraid to play with the data. In the data analysis tutorials, we will help you get started

with manipulating your data and creating visuals. As you conduct the analysis, make sure to **note anything interesting or unexpected**, even if it is not directly related to your stated purpose. A good scientist is always on the lookout for unanticipated results because these results often lead to interesting discoveries.

(ALTERNATIVE) EXPERIMENT 2. THE EFFECT OF THE PLANT HORMONE GIBBERELLIN ON THE GROWTH OF INTACT PLANTS

LEARNING OUTCOMES

- Investigate how gibberellins influence plant growth
- Identify how a particular experimental design allows us to answer specific questions
- Generate a hypothesis and make experimental predictions
- Identify, highlight (using visuals) and discuss important data trends
- Create appropriate and meaningful visuals that highlight important data trends
- Describe how the B9 (Daminozide) affects pea plant growth
- Identify differences in the physiological response of two pea cultivars to GA manipulation
- Identify the importance of the use mutant plants to explore wild type (WT) plant physiology
- Write a scientific report to present analysed results and answer specific scientific questions.

Note: You will not be conducting this experiment, but you will be using data collected in prior years to perform your first data analysis exercise. Therefore, it is important to understand the background, experimental design and method used in this experiment.

INTRODUCTION

Plant hormones (like animal hormones) are chemicals produced within the organism that influence various physiological processes. However, unlike in animals, the location of hormone production in plants is not localized to specific glands or tissues (although younger tissues tend to the source of many hormones in plants). As well, while some plant hormones will act at a distance from their synthesis location, other hormones may act within the tissue or even within the cell where they are produced.

Gibberellins are a class of plant hormones that were first discovered and extracted from the fungus *Gibberella fujikuroi* in Japan in the 1930's (reviewed in Stowe & Yamaki 1957). This fungus causes a rice disease called "bakanae", or "foolish seedling disease" that is characterized by excessive height growth. The fungus causes this excessive growth by producing and secreting gibberellins onto the rice plant.

There are approximately 100-characterized gibberellins (see http://www.plant-hormones.info/occurrence_of_gas_in_plants.htm). Gibberellins are named GA (gibberellic acid)

with a different subscript to distinguish them ($GA_{\#}$). GA_3, the first highly active gibberellin isolated, is often referred to simply as "gibberellic acid". Only a few of the characterized gibberellins are physiologically active; most are deactivation products or intermediates in the biosynthesis of endogenous active form(s). In most species, only one or two GAs are physiologically active in each plant organ.

This study design is based on comparing two pea plant cultivars, a wild type and a dwarf mutant. Our main goal in this experiment is to determine how GA influences pea plant growth via application of exogenous GA and Daminozide (B-9). B-9 (N–dimethylamino Succinic acid) is a GA antagonist (Brown et al. 1997). It most likely prevents GA synthesis by competitively inhibiting the late stages of GA synthesis (reviewed in Rademacher, 2000). It competes with the cofactor 2-oxoglutaric acid. Using GA and a GA antagonist, you will try to tease apart the type of mutation that results in the dwarf phenotype of the mutant cultivar.

Since GA causes an increase in plant height, there are two possibilities of the type of mutation that could result in a dwarf phenotype. The cultivar may be GA insensitive, i.e. the dwarf cultivar produces the appropriate amount of GA but lacks the GA receptors that activate gene expression leading to increase in plant height. Or the cultivar is deficient in GA production, i.e. lacks the enzymes in the GA synthesis pathway. Now think about the type of questions the following procedure will allow you to answer.

MATERIALS & SUPPLIERS

- Gibberellic Acid (GA) - Sigma Aldrich
- Daminozide (B-9) – MacDermid Agricultural Solutions Canada Company
- Ethanol - Fisher Scientific
- Peas - Lindenberg Seeds Limited
- Peat - Altwin Distributors
- Vermiculite - Early's Garden Centre
- Rice hulls - Prarie Brew Supply

PROCEDURE

Create a hypothesis and map out the experimental design to study the impact of plant hormone gibberellic acid on pea plant growth and biomass. This experiment will last 5 – 6 weeks from germination to harvest.

You will work in groups for the duration on this experiment. Each group of students will work with either the mutant or the wild-type cultivar. At the end of the experiment, you will receive

data for the other cultivar from another group. It is a good idea to regularly observe both cultivars and compare their visible responses to the experimental treatments. You can also watch time lapse video of this experiment available on UR Courses

Refer to Handling Pea Plants guide available on UR Courses and Appendix B, Appendix C & Appendix E for tips on observations you can make about your experimental plants

TREATMENTS:

all solutions are prepared for you and stored in the growth-room refrigerator

- Control: dH2O + 0.5% (v/v) EtOH
- 10 µM GA (in 0.5% (v/v) EtOH)
- 75 µM GA (in 0.5% (v/v) EtOH)
- 0.5% (w/v) Daminozide (in 0.5% (v/v) EtOH)

SEED GERMINATION:

- Place a piece of paper towel below and above seeds in a covered container
- Add water just until the paper towel is moist
- Keep covered in the dark for up to 5 days until they sprout (check daily to ensure paper towel stays moist)

SOIL GATHERING AND PREPARATION:

- Soil mixture: 2:1:1 :: peat: vermiculite: rice hulls

SOWING:

- Clearly label each pot with one level of treatment
- Use 8 pre-germinated seeds per pot, these will serve as your biological replicates
- Use a pencil or a stake to make 1 to 2-inch equidistant depressions in the soil surface around the periphery of the pot
- Place germinated seed in the depression
- Cover with soil
- Water to moisten soil, as needed

GROWTH CONDITIONS:

- Record the following in your notebook/research journal during each data collection

- # hours in day (photoperiod) and night: 16 h photoperiod, full sunlight conditions

- Temperature: 25 °C in growth room

- Relative humidity (if available): check growth room detector

- Soil type and location: loam; purchased

- Soil pH

- Frequency of watering

DAY OF EMERGENCE (DAY 1):

- Note the day that all seedlings emerge from the soil. This is day 0.
- If all eight planted seeds come up, let them grow until first day of the experiment
- Stake plants, add twist tie hooks to support plants as they grow (see Appendix C)

WATERING PLANTS:

- Check soil moisture and water as necessary, (see Appendix B)
- Ensure all plants get the same total volume of liquid

PREPARING THE PLANTS FOR THE EXPERIMENT (DAY 7):

Be careful not to damage the fragile plant stemsSee Appendix C for tips

- Each group will start with 4 pots each containing 6 – 8 pea seedlings.
- Choose and stake 6 plants in each pot.
    - If your pot has more than 6 plants, choose 6 that look similar in height.
    - If the plants are tall enough, attach them to the stake using the twist tie hooks (see Appendix C)
- Number the stakes from 1 – 6 (use the same pattern for all four pots as this it will make it easier to keep track of the plants later).
- If a stem breaks, remove the plant by pinching it off at the soil level and stake one of the other plants (you should have a couple of extras in each pot).
- You can also try and save the plant – ask us how
- Label each pot with your group name/number and the treatment

MEASUREMENTS (GETTING STARTED ON DAY 7):

Measure and record the following for each plant in you research journal

Please share data with your group and the add it to the lab data tracking sheet (link)

Always measure first and treat second – the process of measuring might dislodge the solution droplet.

- Total plant height from soil surface to the base of the shoot apex, in mm.
    - Note that in pea plants the shoot apex is usually not the highest point on the plant. Refer to Handling Pea Plants guide on UR courses
- Record the number of internodes on each plant (start from the bottom).
    - Note that pea plants often have a leaf-like scale near the soil; this is not a node. Please check help images provided, or ask your lab supervisor
- Make any additional qualitative observations about plants/ pots.

Once your plants are numbered, staked and the measurements recorded, pinch off any extra plants at the soil level.

TREATMENTS (DAY 7-28):

NOTES:

◊ Treat plants twice every week for 3 weeks after the day of emergence (schedule will be provided)

◊ This experiment will run for 3 weeks. All treatments and measurements following day 7 will be carried out in the Plant Growth Room on the main floor (LB 151). You will need to sign out the key for this room from the Biology Dept. Office (LB 244).

◊ All groups must record their weekly data collection on the physical data sheet (your notebook) AND they must enter their data on the digital spreadsheet (accessed through UR Courses).

Levels of Treatments:

- all solutions are prepared for you and stored in the growth-room refrigerator
    - Control: dH2O + 0.5% (v/v) EtOH
    - 10 µM GA (in 0.5% (v/v) EtOH)
    - 75 µM GA (in 0.5% (v/v) EtOH)
    - 0.5% (w/v) Daminozide (in 0.5% (v/v) EtOH)
- Each treatment will be applied to all 6 plants in a single pot (see how below)
- Please ensure you are wearing all PPE

Treatment method: Using a Pasteur pipette, place a single drop of the appropriate solution on the apical meristem (growing tip) of each plant.

- ◊ This meristem is often enclosed with a leaf-like structure (developing leaves) that must be very gently opened to expose the apex (see Appendix C).
- ◊ See help video available on UR courses
- ◊ Avoid dripping the solutions on other plant parts as this could influence the results. Use a different Pasteur pipette for each treatment solution. Seedlings break easily so handle with care.

OBSERVATIONS AND MEASUREMENTS (POST DAY 7):

- Check moisture level of the soil, water plants if necessary
- Measure plants once per week on days 14, 21, and 28 as described above in "Measurements (Getting Started on Day 7)"
- *Reproductive structures*: record the date you first observe a reproductive structure for each plant; also make note of the type of reproductive structure
    - Look carefully at each plant each time you are in the growth room. Depending on the timing of your checks you may first see a bud, or you might miss it and first observe a flower or a pod.

FINAL MEASUREMENTS AND NODULE COUNTING AND COLLECTION (DAY 28):

- Measure and record the following for each plant (data sheets shared on URcourses, hard copy (back-up) will be provided in lab- to be housed in the lab)
    - Record all lengths in mm
- Total plant height from soil surface to apical meristem
- Total number of internodes per plant
- Fresh weight (following removal all reproductive structures)
    - Harvest one plant by cutting it at the soil level. Quickly weigh the plant (you will need to fold the plant to get the weight).
    - Harvest and weigh the remaining plants one at a time to prevent water loss via evaporation.

DATA ANALYSIS

You will use cohort-collected data to understand how manipulating GA concentration in the apical meristem did or did not influence plant growth in your experiment. Tutorials on how to analyse your data will be made available via UR Courses. By this time, you should have already completed data analysis on practice data sets. To get a full understanding of the effects of GA on plant growth, you will need to create visual representations of the data. You will do so using the statistical programming language R in the user interface R studio. Analysing experimental data involves methodically looking for relationships between treatments and outcomes. Visual representation of data in the form of figures and tables are a great way to compare the effects of multiple treatments. When you begin your data analysis, focus on figuring out what visual best helps you answer the central question posed in your hypothesis. You may need to create more than one visual before you find the best one; do not be afraid to play with the data. In the data analysis tutorials, we will help you get started with manipulating your data and creating visuals. As you conduct the analysis, make sure to **note anything interesting or unexpected**, even if it is not directly related to your stated purpose. A good scientist is always on the lookout for unanticipated results because these results often lead to interesting discoveries.

COMMUNICATING YOUR RESULTS IN A RESEARCH PAPER

AUDIENCE

As with all writing, it is important to consider the audience you are writing for. For example, deciding what background information to include in your introduction or what details to explain in your methods depends on what you expect your audience to already know. For this report, your audience is biology or biochemistry students with basic plant physiology knowledge like your own, but who have not performed the experiment.

GENERAL STRUCTURE

For this lab, follow the structure below, feel free to add subsections as necessary.

Title

Introduction

Methods

Results and Discussion

Conclusion

References

Visuals (Figures and Tables)

INTRODUCTION

Prepare your audience to understand and appreciate your study. Below are some points you may want to address in your Introduction; note that this is not an exhaustive list.

- What is root nodulation and why is it important?
- Why is it important to investigate the impact of root nodulation on plant growth?
- What was the central purpose of your study?
- Very briefly, what strategy did we use to address your central purpose (keep details for the methods)?

MATERIALS AND METHODS

- Briefly and clearly describe how plants were maintained, how (and what) treatments were carried out and how the data were collected and analysed.

- When writing the Methods, you can reference the lab manual for details of a particular method. For example, “treatments were administered to the apical meristem of each plant, as described in Davis et al. (2020)”.
- Organize the Methods section so that each method is described only once. You will find organizing easier if you use subtitles (i.e. organize by method, but this may not always be chronological in terms of how you performed it in Lab).

VISUALS (INCLUDING FIGURES AND TABLES)

Following your data analysis, you will need to choose the figure and tables that will be included in your final report. When performing data analysis, try to generate as many visual representations of the data as possible. These can be repetitive showing the same data and trends in multiple ways. You need to then decide (based on the main trends you decide to highlight in the following sections) which figures and tables you will include in your report. Make sure all visuals are self-contained.

Self-contained visuals: All the information the reader needs to be able to understand the figure or table should be present in the figure caption or table title. If the reader cannot interpret the visual without referring to the main text of the lab report, the visual is not self-contained.

RESULTS AND DISCUSSION

In this paper you will be combining the traditional Results and Discussion sections into a single combined section (Scitable, Nature.com).

Present your analysed results in a manner that allows you to easily highlight the important trends. In addition to visuals (figures, tables), you need to clearly state the important trends evident in your results. Besides addressing the proposed question/hypotheses/predictions you posed in the Introduction, make note of any interesting or unexpected results, especially if you want to discuss them in this section. You must first state a result before you can go on to discuss it.

Use self-contained visuals (figures and tables) that show the important trends you want to discuss in the Results and Discussion section. Note that your figures and tables are required to be included after the Reference list (see “Style Requirements in Rubric”). While it is perfectly okay to have more than one visual, do not present the same data in multiple ways (for example a graph and a table). Instead, pick the visuals that make it easiest for you to show your audience the trends/answers you found.

IDENTIFYING AND PRESENTING IMPORTANT TRENDS

The important trends are those that (a) answer your proposed question or (b) show interesting or unexpected results. Do not fall into the trap of simply repeating what a figure shows without answering the question.

A good strategy when writing this section is to start each paragraph by stating an actual finding. Below are some examples using the hypothetical mouse growth factor experiment. In example 1 below, I state my important trend in the first sentence and then back up my statement with a quantitative measurement from the analysed data and refer the reader to the figure that shows this result. I made my important trend more compelling by comparing how dwarf and wild type mice responded.

> Example 1: Both dwarf and wild type mice fed growth factor (GF) enriched formula were longer than their respective controls (Figure 1). However, the increase in length was more pronounced in the dwarfs, as dwarf mice fed GF enriched formula were 30% longer than the untreated dwarfs (Figure 1) while treated wild type were only 5% longer than their controls (Figure 2). Furthermore, by day 14 the GF treated dwarf mice were only 2% shorter than non-treated wild type mice (Figure 1 and 2).

A common problem we see in results sections is one does not want to make any clear statements about the important trends in the data, and instead describe the data ad nauseam. Here is an example of how not to write your results:

> Example 2: Wild type mice fed regular formula were 100 mm long on day 0, 120 mm long on day 7, 150 mm long on day 14, and 200 mm long on day 21. Dwarf mice fed growth hormone were 50 mm long on day 0, 118 mm on day 7, 145 mm long... (you get the picture)

The problem with example 2 is that the writer is not describing an important trend but instead is simply stating results and leaving the reader to interpret them.

Slightly better but still problematic is the following:

> Example 3: Both the wild type and dwarf mice fed growth hormone supplemented diets grew longer than those fed regular formula. After 21 days the hormone treated wild type mice were 50 mm longer than the controls and the dwarf mice fed hormone were 100 mm longer than the dwarfs that were fed normal formula.

Example 3 is better because an important trend is described with and summarized results. However, they have not indicated the proportionality of the length change and they have not told us if the dwarf responded differently from the wild type (presumably the dwarf was

shorter at the start of the experiment, but it is hard to know if 50 mm longer for the wild type is proportional to 100 mm longer for the dwarf).

INTERPRETING RESULTS

A good interpretation addresses 4 key tasks:

1) Clearly answers the central question(s) posed in the introduction.
2) Notes interesting or unexpected findings and discusses biological insights and implications.
3) Relates results to the general understanding in the field.
4) Makes meaningful suggestions for further studies

Tasks 2, 3 and 4 do not need to be addressed in any order and in some cases, you will address them simultaneously.

Interesting or unexpected findings: Were the effects of the different concentrations of nitrogen (supplied as Miracle Gro®) as you predicted? Where they different from the effects observed in other studies? Consider not only how additional nitrogen altered growth of the individual parameters, but the relationship between the parameters. For example, if additional nitrogen influenced plant height, did it have a proportional effect on plant biomass? Was the effect on plant height same as the effect on internode number, and what does this observation tell you? Did you notice something interesting that was not measured in our study?

General understanding: How do your results add to what we already know about root nodulation and plant growth? Compare and contrast your results with findings in other studies, particularly if your results support a conclusion that is contrary to what you found in the primary literature. Keep in mind that it is NOT our goal to support previously published results and if your results are different from previously published results, do NOT declare that what you found is incorrect. Instead, give reasons why your results are different, for example, is there a difference in the methods used in the published study that might explain the difference?

CONCLUSIONS

The Conclusion section captures the focus of the research paper. Here you can summarize the main aspects of the results and connect them to the purpose outlined in your Introduction. You should also provide recommendations and meaningful suggestions for how the study could be improved. The Conclusion includes a final poignant comment or insight that inspires the reader to continue thinking about your research.

Meaningful suggestion: Suggest a future study that would clarify or extend this experiment. If something in our study was inconclusive, how could we improve the study to get more conclusive results? If some of our results are different from what was found in other studies, what new study might help us determine why? Make sure suggestions are specific and that you clearly explain what new knowledge your proposed study would provide.

REFERENCES

The full form of the citations that will become the reference list will primarily come from the Introduction and in the Results and Discussion sections (and some from your Methods section). In the Introduction you should cite the background information, and in the Results and Discussion you cite other studies that you are comparing or contrasting your results with. References can simply follow the results and discussion (i.e. do not need to be on a separate page). Self-contained visuals follow the References, starting on a new page. Note that you cannot "cite" your own ideas or conclusions; instead, support these with your results. You should use as many references as you think necessary, however, we expect you to use at least five primary peer-reviewed sources.

Format: Canadian Science Publishing reference format (e.g. Canadian Journal of Plant Science CJPS)

For more information on referencing, please watch the video on "Where Do I Begin" in the Tips for Scientific Writing section on UR Courses.

To make citation organization more efficient as you write, edit, and review your report, use a citation manager like Zotero or Mendeley (help videos in "Tips for Scientific Writing" on UR Courses).

How to cite your manual: cite manual as a book with an editor.

Excerpt from Author guideline for CJPS:

"This journal uses the Harvard citation style. In-text citations take the form (Author Year). References must be listed in alphabetical order according to the last name of the first author and should not be numbered. References with the same first author are listed in the following order:

- papers with one author only are listed first in chronological order, beginning with the earliest paper
- papers with dual authorship follow and are listed in alphabetical order by the last name of the second author
- papers with three or more authors appear after dual-authored papers and are arranged chronologically.

Include DOIs and hyperlinks whenever possible."

For more information on writing scientific papers, visit: https://www.nature.com/scitable/topicpage/scientific-papers-13815490/

DATA COLLECTION TEMPLATE

Please use link on UR courses to create a copy of the template

Key (*please do not change any heading, simply input your data into you data sheet*)

- treatment - Levels of treatment
- plant id - replicate # given to each plant
- ha - health
- ht - height (in mm)
- in - number of internodes
- in:**#** - **#** is day of experiment

treatment	plantid	ha:7	ht:7	in:7	ha:10	ht:10	in:10	ha:14	ht:14	in:14	ha:17	ht:17	in:17	ha:21	ht:21	in:21
control	1															
control	2															
control	3															
control	4															
control	5															
control	6															
1X	1															
1X	2															
1X	3															
1X	4															
1X	5															
1X	6															
3X	1															
3X	2															
3X	3															
3X	4															
3X	5															
3X	6															

APPENDIX A – SOIL GUIDE

Soil can be thought of as a solution of weathered bedrock or surface stone, gravel or sand deposits mixed with water, organic materials in varying levels of decay, and nutrients. Soils are classified based on their composition, which vary greatly by location and climate differences (Agriculture and Agri-Food Canada, 2009). The Canadian system of soil taxonomy defines soils in orders, further grouped into great orders (sometimes called zones) and categorized by horizons (layer of a particular soil type by depth and composition) (Soil Classification Working Group 1998). There are also agricultural classifications based on crop suitability (Agricultural Capability - Soils of Saskatchewan).

Texture is an important consideration when collecting soil for your experiment (Agriculture and Agri-Food Canada et al., 2009). Soil texture is defined by particle size. Sandy soils contain the largest particles, silts/loams in the middle, and clays with the smallest particle size. Clay-sized particles have a diameter smaller than 0.002 mm (Agriculture and Agri-Food Canada et al., 2009) . This means that clay particles can pack together tightly and prevent water from adequately draining from your experimental pots. Regina and closely surrounding areas tend to have vertisolic soil, an order that has a high (>60%) percentage of clay-sized particles (Cerkowniak, 2021; Agriculture and Agri-Food Canada et al., 2009) .

Tips to increase water drainage:

- Collect small pebbles, sand, or gravel to mix in for drainage
- Dry clay particles can sometimes feel sandy: wet your soil while collecting it to see how it behaves when wet
- Don't dig too deeply to get your soil – lower horizons have less organic matter and generally smaller particles

If you live in Saskatchewan, the SKSIS database can help you assess the soil zone of your area.

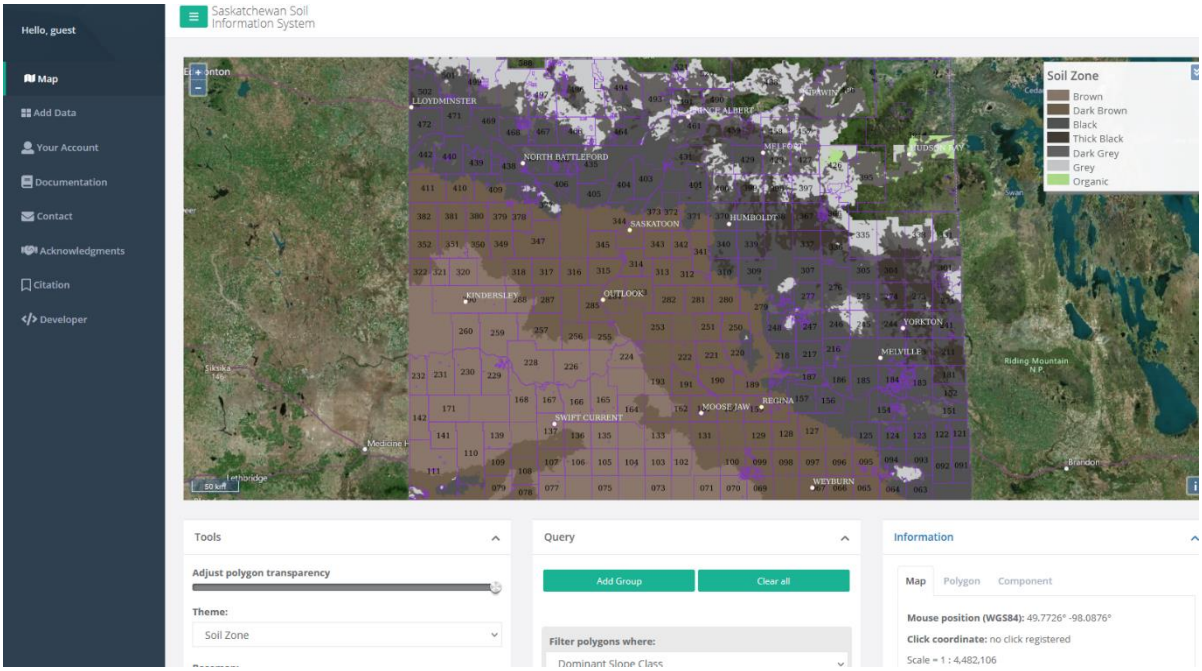

Figure A1. Screenshot of the Saskatchewan Soil Information System (SKSIS) website. SKSIS is a database that shares Saskatchewan soil information based on soil surveys done by the Canadian Soil Information Service (CanSIS) as well as user-uploaded data.

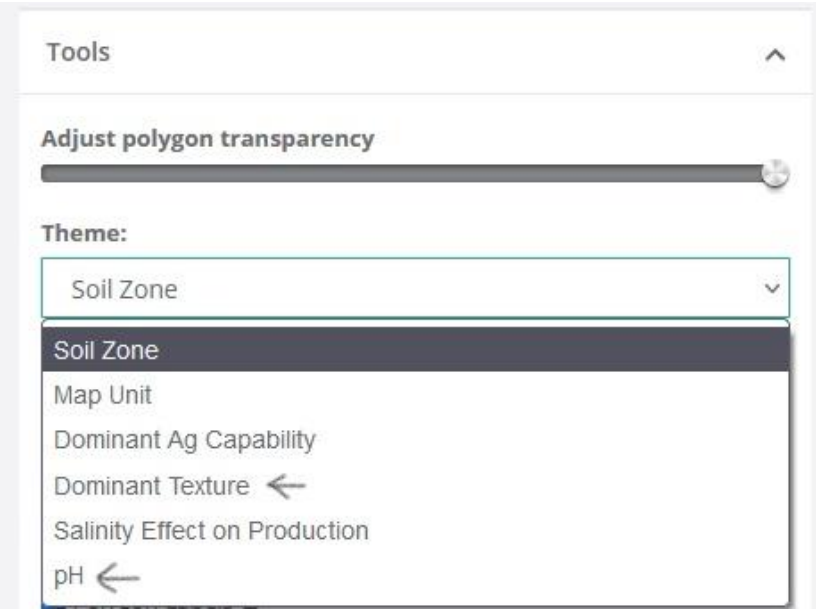

Figure A2. The SKSIS Theme drop down menu options.

By changing the “theme” drop-down menu under tools, you can see a map based on texture (particle size) and pH (which gives a general label). Record this information in your laboratory notebook and compare with the soil you find and pH you get when you measure.

Finding soil containing wild rhizobia

There will likely be more wild rhizobia in areas where legumes had been planted previously. For example, a garden that had grown pea plants in recent years. There are also wild Fabaceae (legume family) plants that grow in Saskatchewan that you could seek out: purple prairie clover *(Dalea purpurea)*, silvery lupin (*Lupinus argenteus*), and alfalfa (*Medicago sativa*). If you can source soil from a field that you've seen these plants bloom in, it would likely have a higher proportion of wild rhizobia.

Figure A3. Example photos of purple prairie clover *(Dalea purpurea)* (left) and silvery lupin (*Lupinus argenteus*) (right). Photo: G. Lee for www.saskwildflower.ca

Soil collection tips:

Your first choice will likely be gardens or flowerbeds. If you do not have access to soil from one of those options, here are some other ideas:

- Reach out to Regina Public Interest Research Group (RPIRG).

RPIRG has a community garden on campus and would be willing to trade some soil for volunteer hours. < https://rpirg.org/events-projects-paren/greenpatch/>

- Mix some local soil with potting mix

If you can get 2 or more cups of local soil but not enough to fill all experimental pots, mix the local soil thoroughly with potting soil. The rhizobia likely in the local soil should be able to colonize the potting soil and still form associations with your plants.

If you cannot source any soil at all, contact us at plantphys@uregina.ca and we can help you.

Citations and Further Readings:

Agricultural Capability - Soils of Saskatchewan Available at: https://soilsofsask.ca/soil-survey-soil-characteristics/agricultural-capability.php [Accessed July 23, 2021].

Agriculture and Agri-Food Canada, Saskatoon Research Centre, and Saskatchewan Land Resource Unit (2009). Saskatchewan Soil Resource Database User's Manual for SKSIDv4. *Soils of Saskatchewan*. Available at: https://soilsofsask.ca/documents/sksid_usermanual.pdf [Accessed July 23, 2021].

Bedard-Haughn, A., Bentham, M., Krug, P., Walters, K., Jamsrandorj, U., and Kiss, J. (2018). Saskatchewan Soil Information System – SKSIS. *SKSIS Work. Gr.* Available at: https://app.sksis.ca/map [Accessed July 23, 2021].

Cerkowniak, D. Vertisolic - Soils of Saskatchewan. *Dep. Soil Sci. Univ. Saskatchewan*. Available at: https://soilsofsask.ca/soil-classification/vertisolic-soils.php [Accessed July 26, 2021].

Pennock, D. (2005). Field Handbook for Saskatchewan Soils. *Dep. Soil Sci. Univ. Saskatchewan*.

Soil Classification Working Group (1998). The Canadian System of Soil Classification. *Agric. Agri-Food Canada*. Available at: http://sis.agr.gc.ca/cansis/publications/manuals/1998-cssc-ed3/cssc3_manual.pdf [Accessed July 23, 2021].

APPENDIX B – WATERING GUIDE

Plant-water relations are fundamental to plant health and a topic you will cover more in lecture. Although we do not discuss matrix potential (ψ_m) in detail, it plays an important role in plant physiology. Regina and surrounding areas have clay soils, which have the highest water holding capacity (O'Green 2013) . This means that less water will drain and evaporate from clay textured soil when compared to sand or loam textured soils (see Appendix A for more on soil texture). Water in soil is not necessarily biologically available to the plant (this is determined largely by ψ_m) and can lead to root rot if soil remains saturated for too long. Here are some watering tips to help you maintain a healthy soil to water ratio.

- Err on the side of caution – it is easier to add water to soil than take it away.
- When dry, water each of your experimental pots with approx. 4 tablespoons of water and see how they do.
    - On treatment days if pots are quite dry, add 2 tablespoons water to the Miracle Gro® pots before adding your 2 tablespoons of treatment. This will give the pot extra water without risking flushing the Miracle Gro® out of the soil or to the bottom of the pot.
    - Alternatively, treat in the morning and water more later in the day if needed
- Assess dryness by checking the top 2 inches of soil in the pot either using your finger or a chopstick/pencil.
- Wait until the plant leaves start to droop. *Pisum sativum* will bounce back quickly from a loss of turgor when water is added (Figure B1).

Figure B1. Pea (*Pisum sativum)* plants with loss of turgor appear droopy. Photo: L. Schnell

If soil is over-saturated and/or seems to be quite damp for several days, follow these tips:

- gently squeeze the cup so that the surface of the soil gets broken up (alternatively, gently poke holes into the soil). This increases soil surface area and should allow for more evaporation
- move the experimental pot temporarily to a place with increased air flow to help increase evaporation

Citations and further readings:

O'Green, A.T. 2013. Soil Water Dynamics. [Online] Available: https://www.nature.com/scitable/knowledge/library/soil-water-dynamics-103089121/ [2021 Jul. 27].

Appendix C – Planting Tips

- Make six depressions in the soil surface equidistant apart, about an inch deep with either your finger or a chopstick/pencil
- When you put the stakes into the soil, add twist ties all the way up them. When the plant grows tall enough, they can be gently guided into the hook you already made. This reduces the chance of plant damage.
- Tape the top of your stakes together for stability
- If all of the planted pea sprouts emerge from the soil, pull the extra replicates gently from the soil so that the root comes out

Handling plants:

- Be mindful of how you are moving around/between the plants as you measure and treat them.
- Keep space between the experimental pots so you have more room to measure and treat or move them to a table/space that allows easy measuring
- Measure from behind the plants so it is easier to see the measuring tape read and where it lines up with the apical meristem (Figure C1)

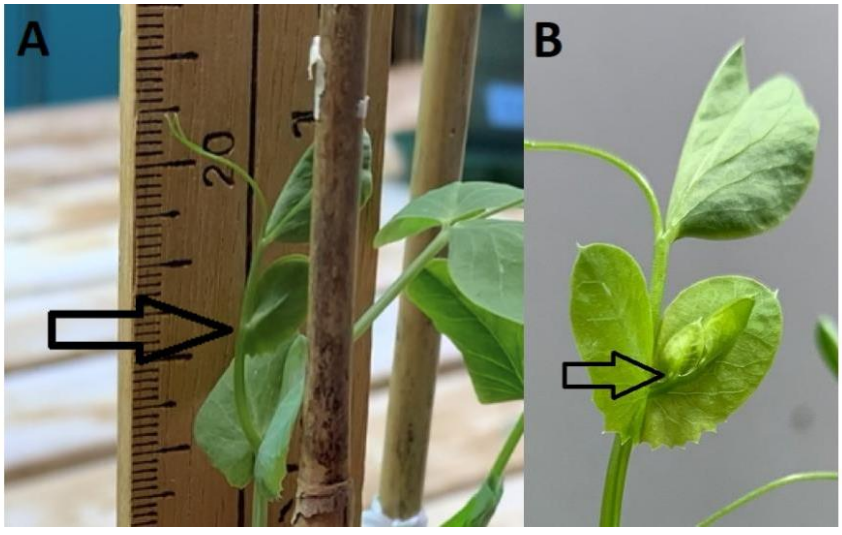

Figure C1. A) Example of measuring from behind the plant. Measure from the top of the soil to the newest or youngest node (closest to the apical meristem) carefully to avoid damaging the apical meristem. B) The youngest node on another plant. Apical meristem is inside the small leaves at node shown (arrow). Photo: L. Schnell

APPENDIX D – HARVEST TIPS

- Undo the twist ties from the bottom to the top and lay the plants over the side of the cup.
- If your soil is more sandy (textured), do not water for 2-3 days before your planned harvest as dry soil is easier to get out of the cup and crumble away from the roots.
- Let the roots soak in rinse water for a few minutes before trying to get the last of the clumps off – this will help soften them.
    - If the soil contains a high proportion of clay particles root damage may occur as dry soil breaks apart. If the soil is densely packed in the cup and you are unable to get the soil out, or if it won't crumble once out, submerge the entire cup in water.
- Any clumps of soil still stuck to the roots can be squished gently and will fall off thereafter.
- Cut the plants for weighing where the shoot emerged from the soil. See Figure D1 for an example.

Figure D1. Cut the plants at the arrow, where the shoot emerged from the soil. Photo: L. Schnell

APPENDIX E – HEALTH GUIDE

Wilting:

- Lower leaves on the Alaska Tall cultivar will occasionally wilt, shrivel, and dry. This does not harm the overall health of the plant and is something to be noted but not worried over. This will look different than wilting from loss of turgor.

Figure E1. A) Lower leaf death on Alaska Tall *Pisum sativum* plants. Note how dry (crumbly) the leaves appear. B) Loss of turgor in Alaska Tall *Pisum sativum* plants (see leaves should look healthy and turgid in a couple hour after watering). Photos: L. Schnell

Plant Decapitation:

- Breakage happens: if the apical meristem of a plant gets broken off, pinch off all buds on lower nodes so only the highest remains. A new meristem will grow from there. Record this in your notebook.

Over watering issues:

- Increase evaporation in your experimental pot by gently squeezing the sides of the cup so that the surface of the soil gets broken up (alternatively, gently poke holes into the soil with pencils/chopsticks).
- This increases soil surface area and should allow for more evaporation. Temporarily move the experimental pot to an area with increased air flow to help increase evaporation

Spots:

- You may see small yellow or white spots on select leaves (Figure E2). These generally do not spread or kill the plant. Record them in your notebook with photos. If they do spread, email plantphys@uregina.ca

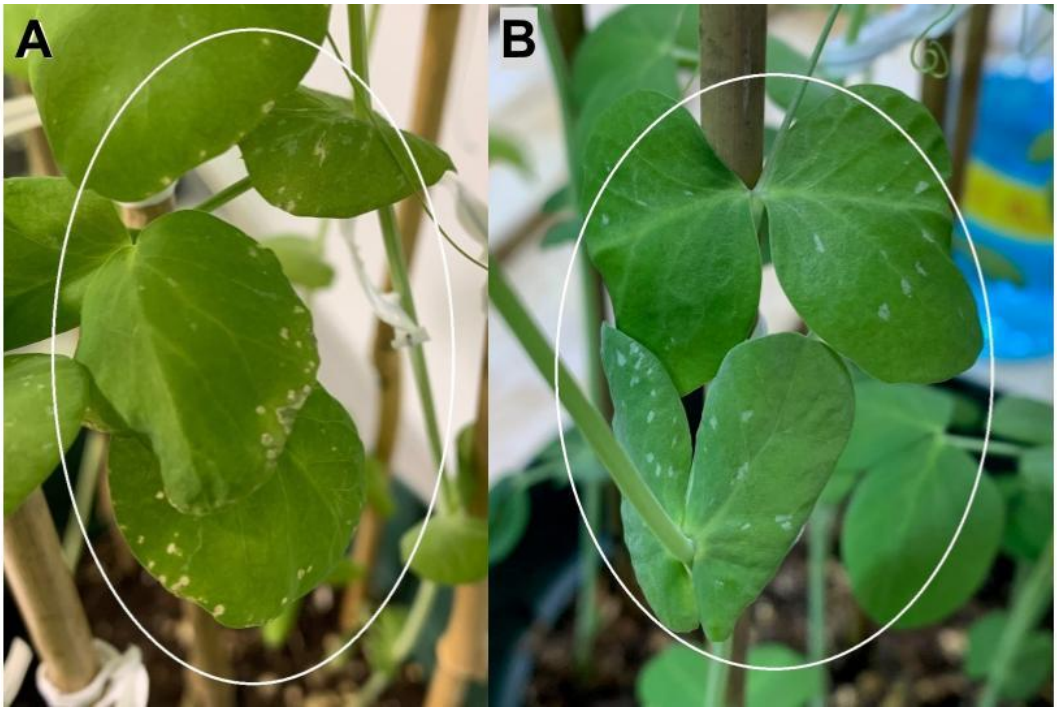

Figure E2. Images depicting spoting that may appears on otherwise healthy looking on Alaska Tall *Pisum sativum* plants. A) Yellow spots on mature plants. B) White spots on mature plants. Photo: L. Schnell

Fungi:

- If you leave a window open, you may have mold issues – if you do not over water the plants, it will be fine.
- Though some fungi can be beneficial or neutral to your plants, others may be harmful and slow growth. Fuzzy white hyphae may grow at the surface of your soil (Figure E3). Scrape this off and discard. Record this in your notebook with photos.

Figure E3. White hyphae clustering around Little Marvel *Pisum sativum* in Fall 2020. Photo: L. Schnell

APPENDIX F – NODULATION ASSESSMENT

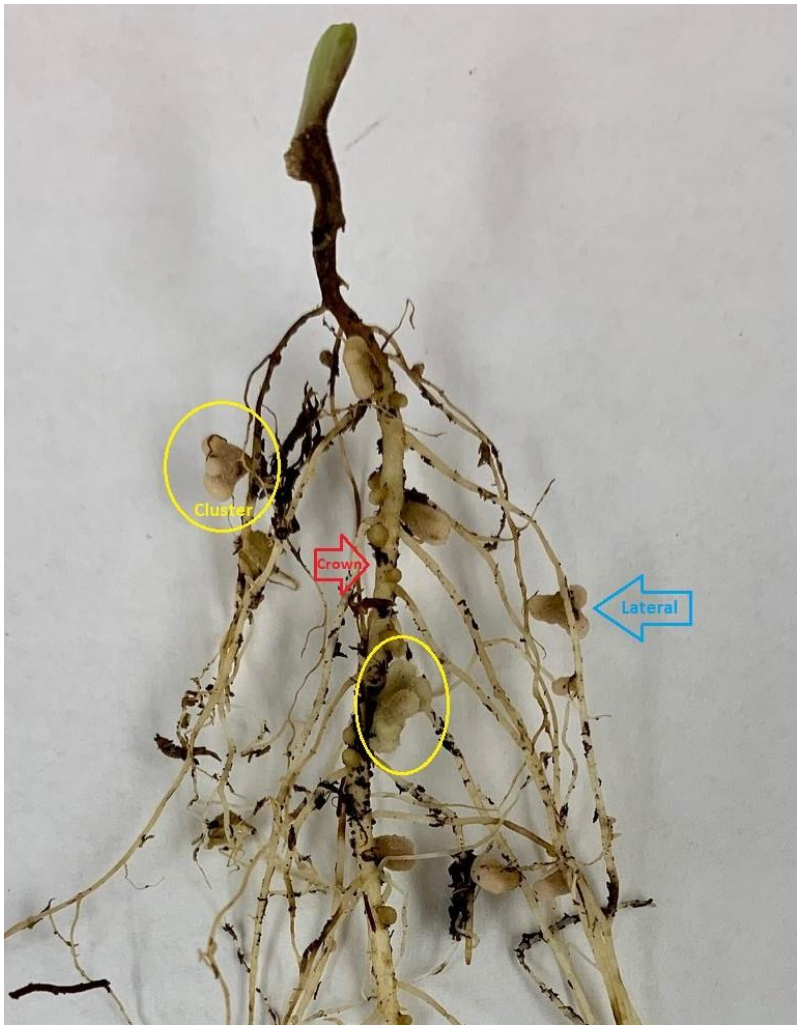

Figure F1. Effective nodulation on Alaska Tall *Pisum sativum* roots. Select clusters (large) highlighted in yellow circles. Crown nodulation indicated with the red arrow while lateral nodulation shown by the blue arrow. As this plant has multiple clusters and large, pink (does not appear in the photo) nodules with both crown and lateral nodulation, we would assign this a total of 5. Photo: L. Schnell

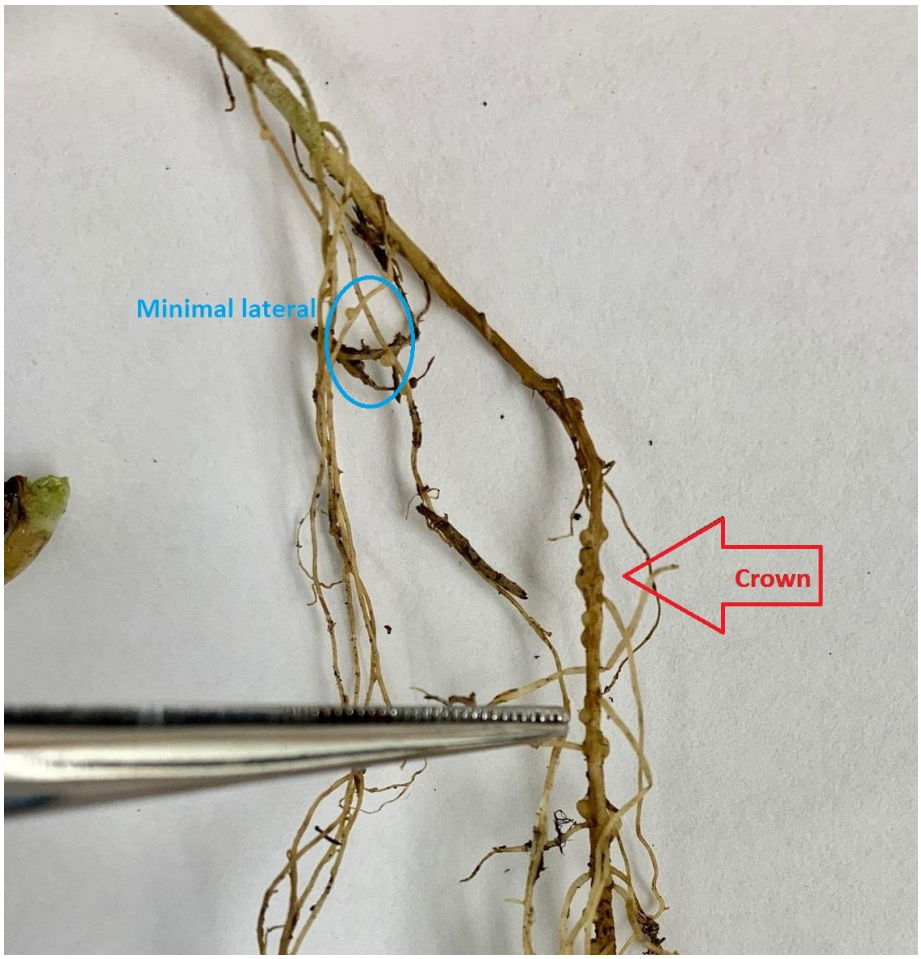

Figure F2. An example of less effective nodulation in Alaska Tall *Pisum sativum* roots. Minimal lateral nodulation is highlighted by a circle while the crown nodulation is highlighted with a red arrow. Because there is mostly crown nodulation and nodules are small and white, this specimen would be given a total score of 3. Photo: L. Schnell