

# **Apical Dominance and the Shoot Branching Dogma**

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### **Background**

Apical dominance is a term used to describe the mechanism by which the apex of a shoot inhibits the outgrowth of secondary, or lateral, shoots. It is best demonstrated via decapitation (i.e. shoot tip removal), which leads to the development of lateral shoots. Lateral shoots do not randomly materialize, but rather emerge from tiny buds that are located along the main stem in the axis of the leaves. This region where the leaf and bud are attached to the stem is called the node.

Often, the ability to form lateral shoots via bud outgrowth is critical for the plant to complete its life cycle. Thus, plants may have evolved this developmental process to ensure their survival following the potentially devastating consequences of decapitation events that can result from animal grazing or physical damage, etc. However, branching requires energy for growth and development, thus the process is tightly regulated by the plant as a means of optimizing resources.

Although the process has long been recognized, to date the mechanism(s) orchestrating apical dominance remain largely unknown. It has been suggested that the phytohormone (i.e. plant hormone) auxin is involved in the process. This is supported by findings that high levels of the bioactive form, indole-3-acetic acid (IAA), are produced in the apex and transported basipetally (i.e. downwards) along the stem, where the lateral shoot buds reside. Decapitation removes the apical source of IAA and stimulates lateral bud outgrowth in less than 24 hours. This branching phenotype (i.e. appearance) can be prevented by exogenously (i.e. externally) applying IAA to the decapitated shoot, thus maintaining the apical dominance phenotype. Despite this evidence, there is considerable uncertainty over the precise role of auxin in shoot branching.

### **Hypotheses**

Currently, three somewhat different hypotheses continue to arise regarding the role of auxin derived from the shoot tip in apical dominance: **1)** the classical hypothesis states that auxin acts to regulate shoot branching in conjunction with secondary messengers, such as cytokinin (another class of phytohormone), **2)** the auxin transport hypothesis proposes that regulatory control is exerted by the rate, volume or capacity of auxin moving from the apex down the shoot, as opposed to the actual level of auxin in a given tissue and **3)** the bud transition hypothesis postulates that the bud enters different developmental stages that have varying degrees of sensitivity or responses to long-distance signals, including auxin.

We propose that several components of each of the hypotheses can be incorporated into one model of shoot branching. In this workshop, we will address these hypotheses in a super-exciting, fun-filled presentation. But there's more! We will also employ a variety

of techniques to test the mechanism of apical dominance control and to investigate the roles of IAA in the process.

## Materials and Methods

You will be provided with a whole bunch of stuff, including: plants, rulers, blades, paper and pens, pipette, wax, required chemicals (e.g. NPA and IAA), needles and syringes, BlueTac, computers.

### *Morning*

The session will start with a preparatory presentation reiterating the basic principals of apical dominance and introducing the basic terms and concepts involved in the process. Using a hands-on approach, we will then test aspects of the apical dominance hypotheses by investigating long-distance signaling in bud outgrowth, with a focus on IAA. This will include employing techniques such as **1) decapitation**, **2) IAA application**, **3) naphthylphthalamic acid (NPA) application** and **4) stem girdling**. Bud outgrowth will then be assessed (using plants treated by us the week prior) by measuring the size of the buds/lateral shoots at each node along the stem.

For your studies, you will be using *Pisum sativum* (pea) plants having at least 5 fully expanded leaves (i.e. 5 nodes).

- 1) Decapitation:** using a razor blade (please don't cut yourself, or others!), cut perpendicularly through the stem above the upper-most expanded leaf. The portion that you have removed contains the apex, which is situated between the developing stipules of the emerging leaf. You can observe the apex by gently pulling back the stipule layers. This excised tissue can then be discarded.
- 2) IAA application:** using a syringe containing a previously made-up mixture of IAA in lanolin, apply a small ring of the mixture around the stem in a location similar to that outlined above for decapitation. Using this method, exogenous IAA diffuses into the plant at the application site, increasing the level of IAA in the IAA transport stream.
- 3) NPA application:** as above for IAA application, only using a syringe containing NPA in lanolin. NPA acts to block IAA transport. It is thought to do so by competitively binding to proteins that transfer IAA to other proteins located in the plasma membrane, which act to pump IAA out of cells (called efflux carriers). Thus, by applying NPA, IAA is prevented from moving cell-to-cell and the IAA transport stream is "blocked". This subsequently causes a depletion in the transport and level of IAA below the site of NPA application.
- 4) Stem Girdling:** form a cup out of BlueTac around a region of the stem located between 2 nodes. This tissue is called an internode. Using a pipette, transfer approximately 1 ml of hot wax into the cup (wax will be heated to 100°C using a hot plate, so please be careful). The heat of the wax will kill all of the living tissue it contacts. Like many signals, IAA is actively transported via efflux

carriers. These carriers require living tissue, hence girdling prevents them from working, which inhibits the transport stream of IAA. However, xylem, which acropetally (upwards) transports water and nutrients from the roots to the shoot, is comprised of dead cells and hence is not affected by girdling. This allows for the persistence of the apical region of the plant, making girdling unique from decapitation, in that the apex remains, as does the continued flow of upwardly moving signals.

### *Tea Break*

Coffee and tea to be provided, margaritas are not.

### *Afternoon*

To finish the session, you will enter your results into the computer, and perform a statistical analysis using your data. This will allow you to create graphs to impress your friends with.

Throughout the workshop, we will be interacting with you and entertaining any questions that you might have. If desired, we can also summarize the results and ideas at the end of the day.

### **Expected Results**

1. decapitation
  - significant bud outgrowth should occur
2. decapitation and IAA
  - significant bud outgrowth should **not** occur
3. NPA treatment
  - significant bud outgrowth should **not** occur
4. stem girdling up high
  - significant bud outgrowth should occur below the girdle, **not** above
5. stem girdling down low
  - significant bud outgrowth should **not** occur

### **Questions:**

- Did you observe bud outgrowth in any of the treated plants compared to untreated, control plants?
- If so, do you see any trends in the outgrowth pattern?
- Do all of the buds within a plant behave in the same way (i.e. grow out at equal rates)?
- Is the amount of bud outgrowth conserved between treated plants exhibiting outgrowth (i.e. do similarly treated plants branch in a similar manner)?
- What other techniques could you use to investigate apical dominance?
- What happens when you combine the techniques (e.g. girdle an internode, and decapitate above, or girdle an internode and add IAA below)?
- What would happen if you remove the buds?
- How do you explain plants that form branches without being decapitated?