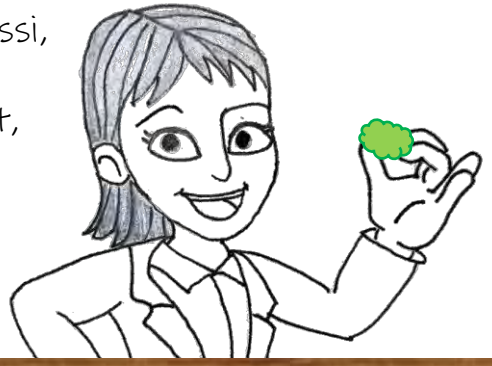
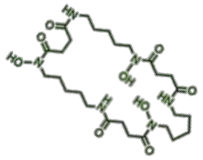
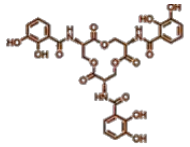
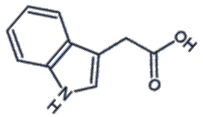
Nicephore Avlessi,
Hélène Joie,
Salomé Joubert,
Pius Otto

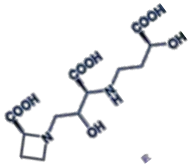


The PGPR's story

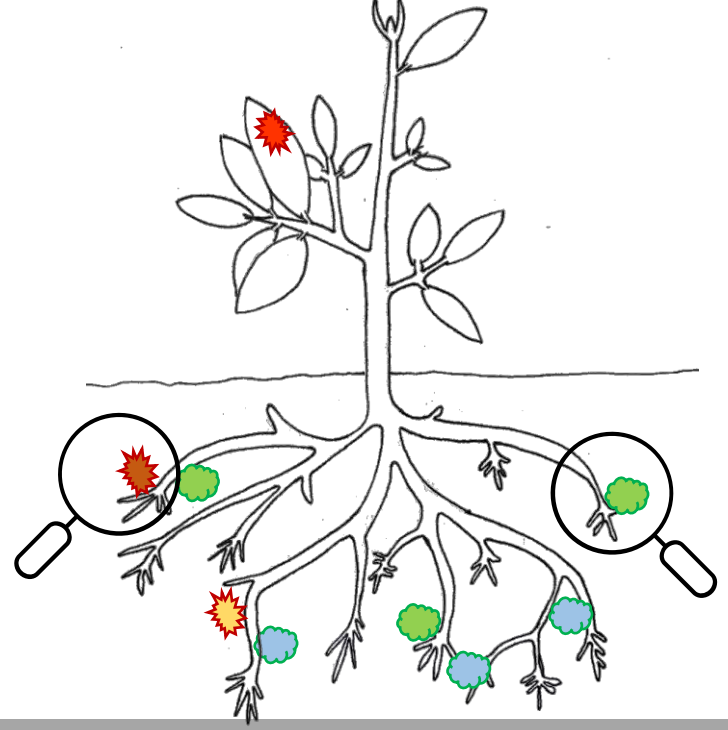








Plant is interacting with beneficial and pathogen microorganisms throughout its life. Let's take a look at them!

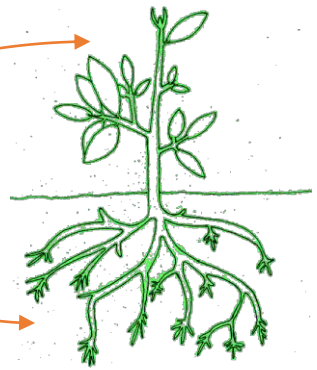


Meet **Patrick**, it is part of the **pathogens organisms** attacking plants. They develop in the plant by using it's resources.

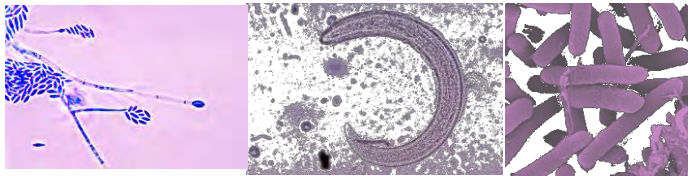
Diner time!

Air borne

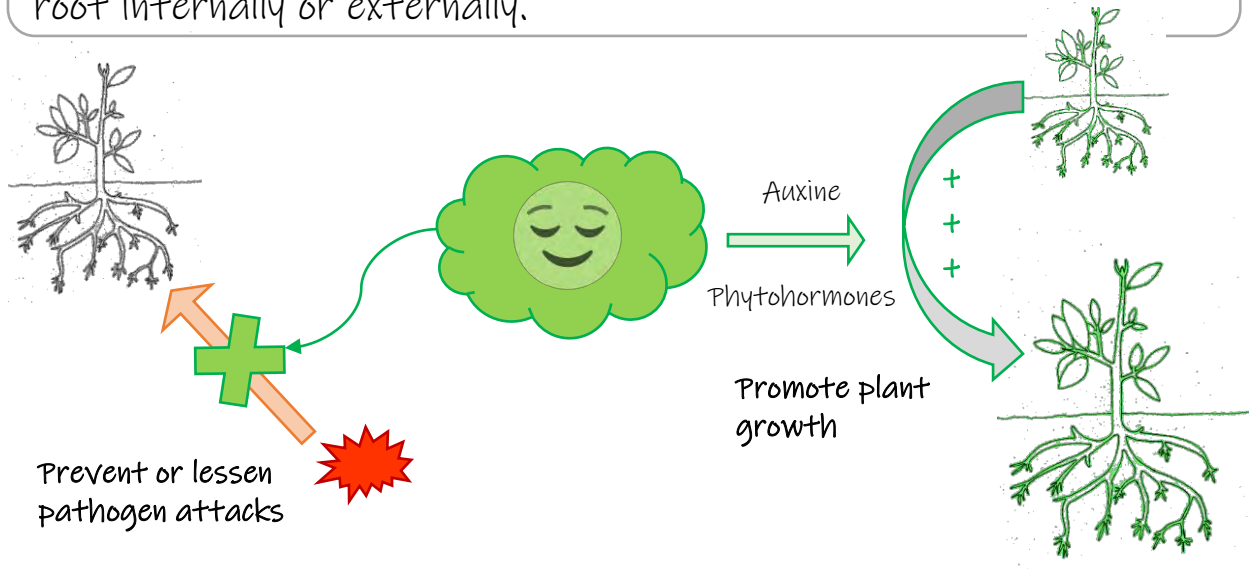
Soil borne



Fungi Nematodes Bacteria



And meet **Brad**, it is part of the beneficial organisms called the **PGPR: Plant Growth Promoting Rhizobacteria.** They colonize the root internally or externally.



Mechanics of PGPR

[1, 2, 3, 4]

Direct mechanics

Nutrient solubilisation and uptake facilitation e.g Phosphates

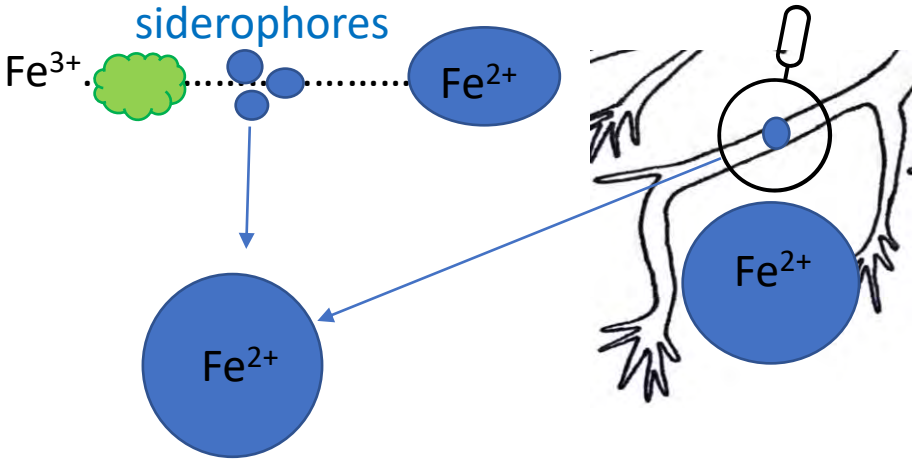
Fe/Al/Ca-Phosphorus complex

H_2PO_4 ⟷ Available to plants

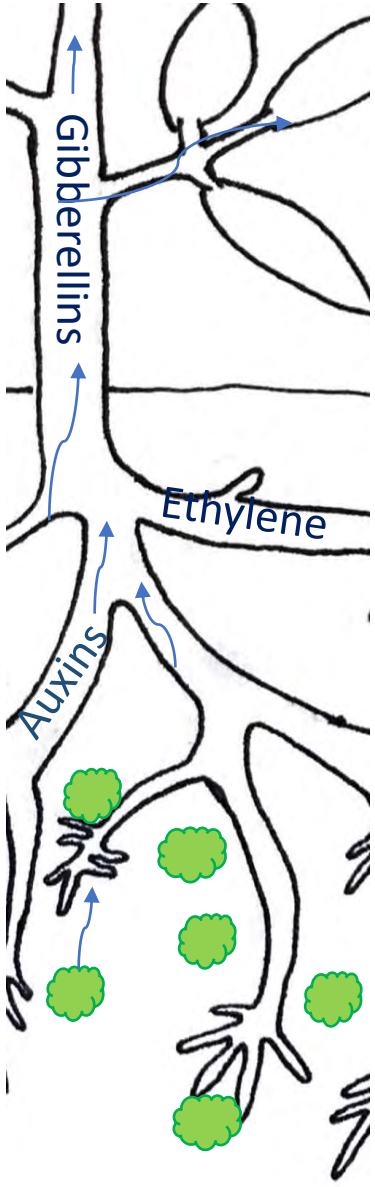
Biological N_2 fixation

N_2 → NO^-_3

Siderophores production; Iron acquisition



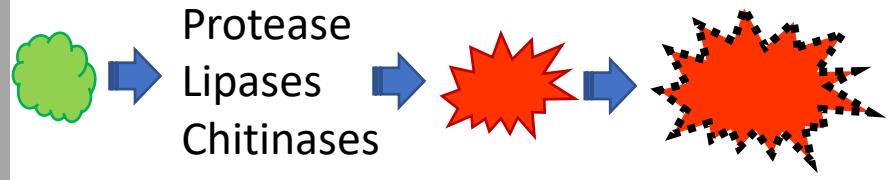
Phytohormone production



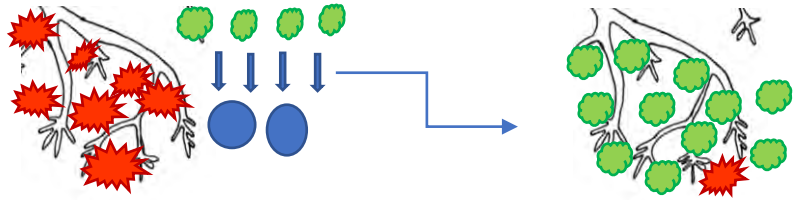
Indirect mechanics

Antibiosis

Lytic enzymes

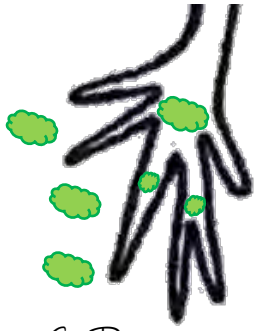


Siderophores production; resulting into competition, Niche exclusion and limiting microbes of iron



ISR (Induced systemic resistance)

How does PGPR communicate with plants to tell them they are friends ? [5, 6, 7]



extracellular PGPR

intracellular PGPR

→ **Rhizobium** : symbiotic PGPR, in nodule, can fix N_2 from air

Before the symbiosis...

Secretion of flavonoids by the plant induces **three different responses** by the PGPR

ROOTS

Flavonoids

NF

PGPR

LCO

T3SS protein

Nops

EPS
LPS

... During the process of symbiosis...

RHIZOSPHERE

binding

LCO-LysM

ROOT CELL

MAMP

transport

Cascade of reactions

interaction

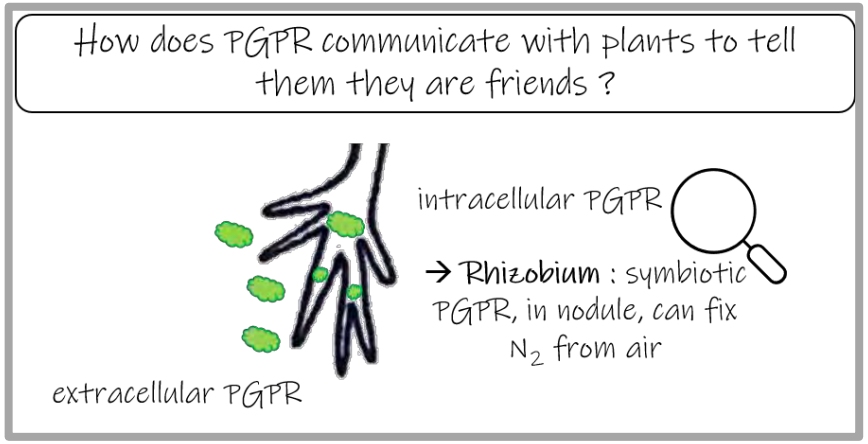
Suppression of plant immunity

These **three pathways** lead to several reactions that induce the **suppression of the plant immunity**

... Symbiosis !

Intracellular PGPR : in nodule for Rhizobium

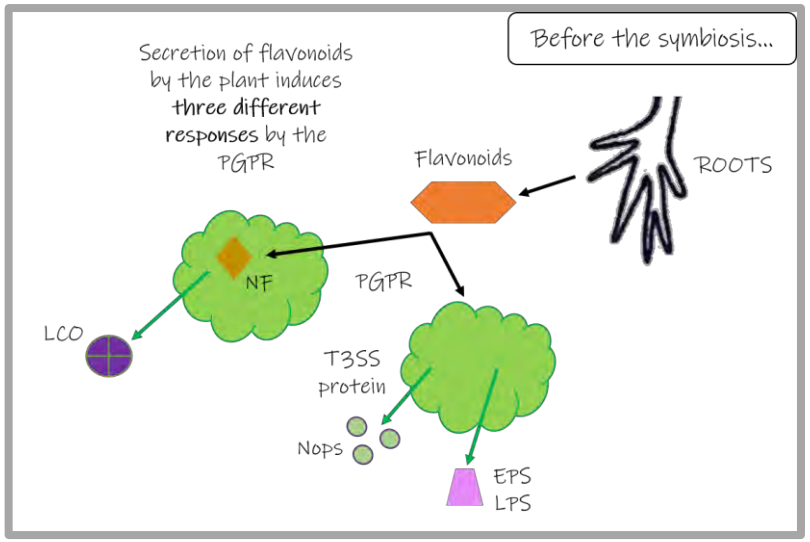
Legend :
EPS = exopolysaccharides
LCO = lipo-chitooligosaccharides
LPS = lipopolysaccharides
LysM = LysM receptors = lysine motif receptors = LCO receptors
NF = Nod Factor
Nops = Nodulating outer proteins
T3SS = type III secretion system



Now, we're going to talk about how plants and PGPR communicate and specifically how PGPR « tell » to plants they are friends.
First, we need a little reminder on PGPR because they can be divided into 2 categories :

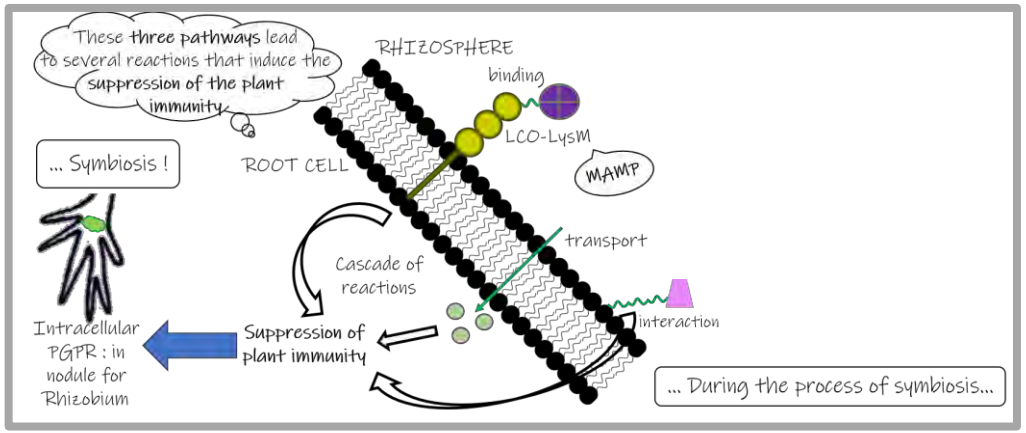
- **extracellular** PGPR : they stay in the rhizosphere
- **intracellular** PGPR : they enter into the plant and stay in the spaces between the cells of the root, or stay in specialized structures as nodules.

For the next part, we'll focus on the example of *Rhizobium*, a symbiotic intracellular PGPR that can fix N_2 when it is in the plant.



When PGPR are still in the rhizosphere, they are influenced by the root exudates, especially the **flavonoids**. In fact, the secretion of flavonoids induces three different responses in the PGPR :

* The first pathway is dependent to the **Nod factors (NF)** : the flavonoids induce the transcription of rhizobia NF that produce lipo-chitooligosaccharides also called **LCO**.
* The second pathway is NF-independent but don't exist for all PGPR (on the contrary, the NF-dependent pathway exists for all PGPR). This pathway is called rhizobia **type III secretion systems (T3SS)** and allows, thanks to T3SS protein, to transport effectors proteins called **Nops** into the rhizosphere.
* In response to flavonoids, the PGPR release polysacharides as **EPS** and **LPS**.

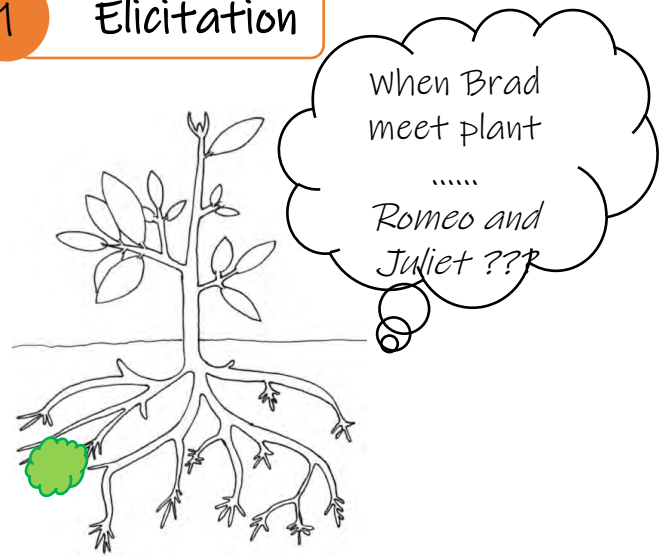


After all the **rhizobacteria molecules interact with the root cell**, a cascade of signaling events and reactions occurs into the plant and lead to the **suppression of the plant's immune system with the root cell**. This suppression allow the entry and the infection of a rhizobacteria thread, which allow the establishment of the **symbiosis in the root cell**.
For *Rhizobium*, it leads to the creation of **nodules** in the root cell, in which the PGPR will settle.

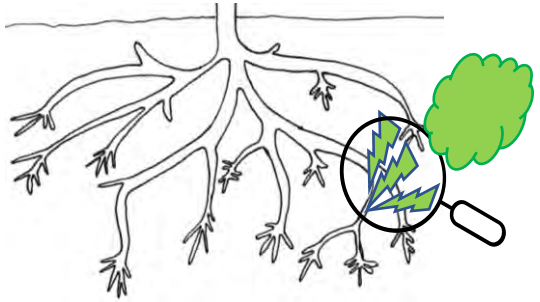
[5, 6, 7]

How PGPR is involved in ISR,....

1 Elicitation

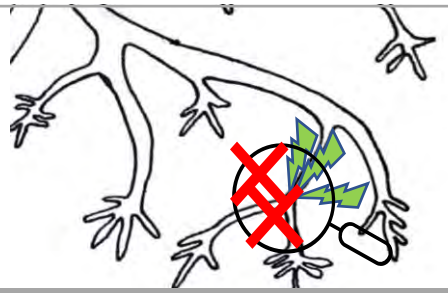


Brad produce elicitors that are perceived by plant :
LPS, flagellin, VOCs, Antibiotics, DAPG, pyocyanin, LPP, Siderophores [8]
Like a good Romeo it wants to please .....



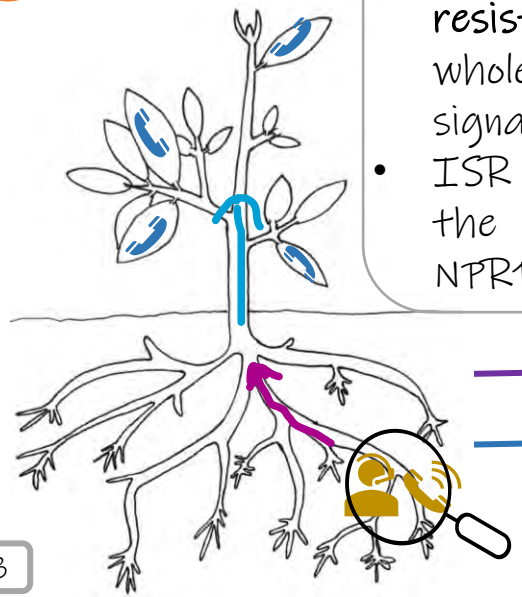
Activation of the plant's immune system after elicitor perception at cellular level
This leads to 2 cellular responses

- a local burst of reactive oxygen species (ROS),
- Ion (H+ and Ca2+) fluxes across the plasma membrane, [9, 10]



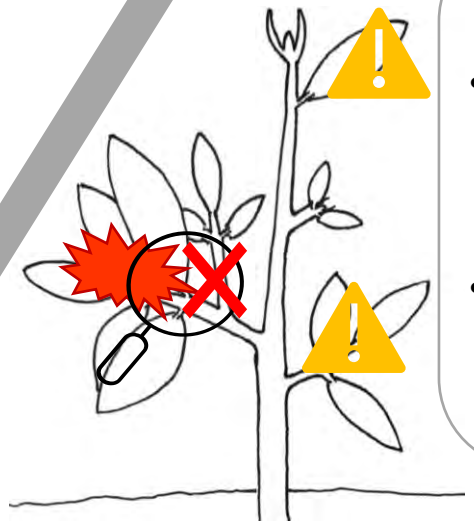
2 Transduction

- Triggering of the **Induced systemic resistance (ISR)** at the scale of the whole using plant (**JA**) and (**ET**) signaling pathways [11,12]
- ISR transduction pathway linked to the activation of a protein factor, NPR1. [13]



→ JA-dependent pathway
→ ET-dependent pathway

3 Defense mechanism



- Some elicitors can trigger a process called **priming**
- It prepares the plant for a faster, efficient and stronger resistance only when a subsequent pathogen attack occurs [10 14]
- **Defense = Stomacal closure + Callose formation** [8]

Primed state

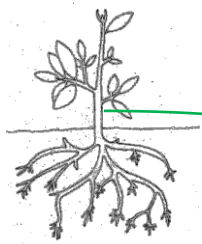
Real love story, to be continued...

Let's take the example of the PGPR Paenibacillus strain B2 (PB2) in wheat against *Mycosphaepella graminicola* [15]

PGPR specificity and interactions

Source!

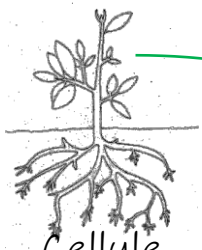
Depending on the genotype of the cultivar, the transcriptional changes induced by PB2 will not be the same



Alixan

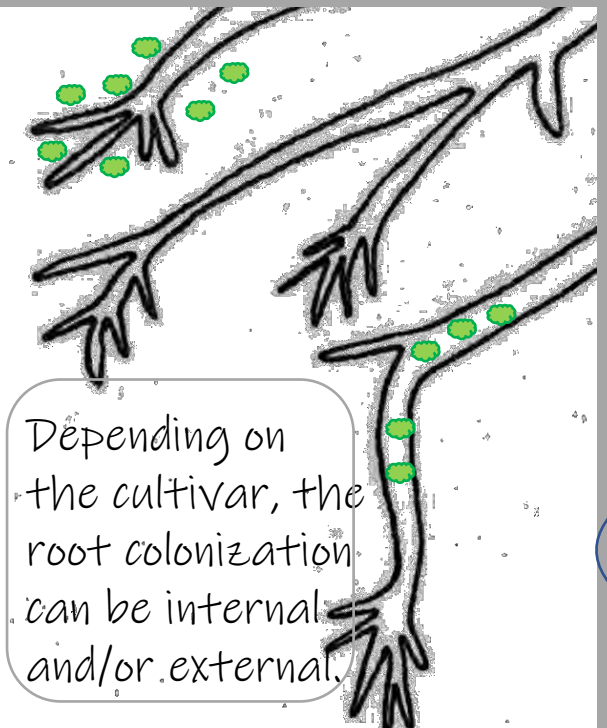
PR protein
JA signalling
Phenylpropanoids & phytoalexins
ROS

Upregulation



Cellule

PR protein
Phenylpropanoids & phytoalexins
ROS



Depending on the cultivar, the root colonization can be internal and/or external.

Wheat: Cultivar \ growth stage	*M. graminicola* Strain IPO323: 3 leaf	Strain IPO323: Flag-leaf	Strain TO256: 3 leaf	Strain TO256: Flag-leaf
Alixan	++	++	+	++
Cellule	+	+	++	++

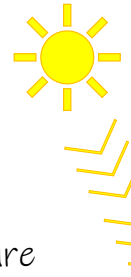
Protection efficacity

At same growth stage for the same pathogen strain the efficacity of PB2 protection depend on the cultivar

For the same cultivar, the protection efficacity of PB2 depends on the pathogen strain (some are able to bypass the PGPR protection)

Limits of the effiiciency of PGPR-based products

No favourable environmental conditions



temperature

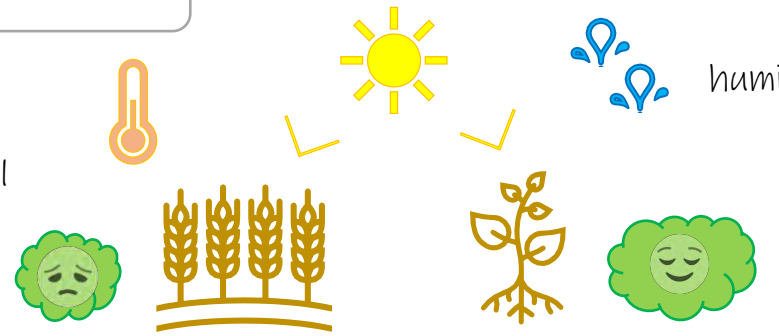
UV

humidity

host

The good association plant /PGPR is not enough to ensure a success! The environmental conditions need to b favourable.

Favourable environmental conditions



humidity

Non-host

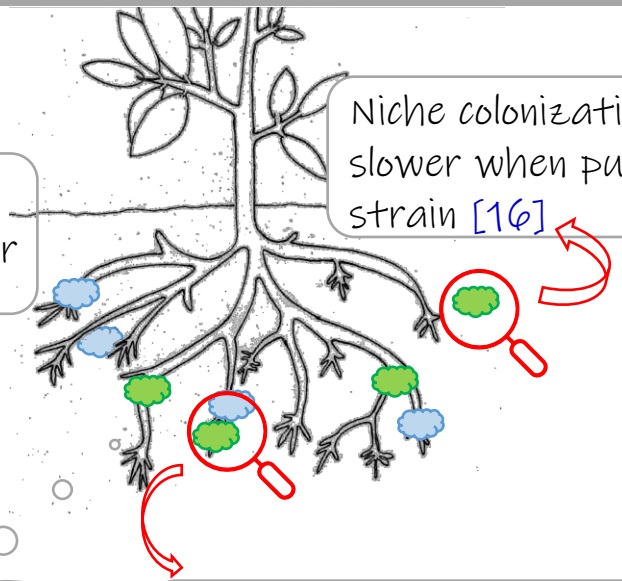
host

"The **best PGPR** products generally consist of **local strains** that are **specific** to the host plant, show good capacity for physiological and genetic **adaptation** and co-evolve with other native strains in a common habitat" [16].

Importance of bioformulation....

Impact of strains mixture, of the carrier

Density > 10^8 UFC /ml

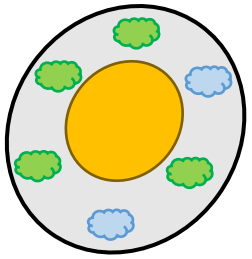


Niche colonization slower when pure strain [16]

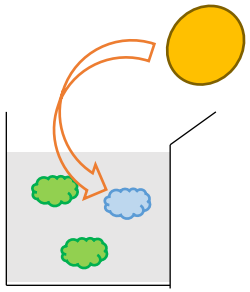
Niche colonization faster than pure PGPR or pathogen due to genetic diversity [6]

.... And application

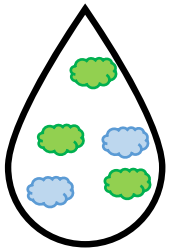
[17]



Seed coating

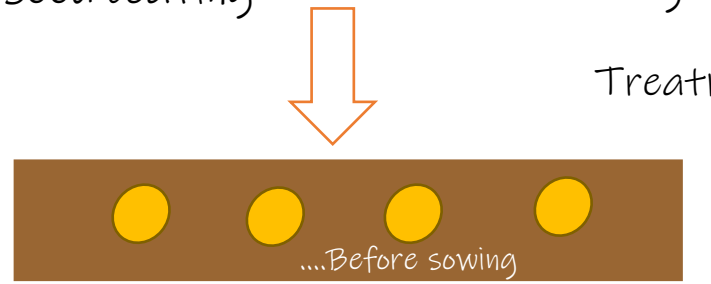


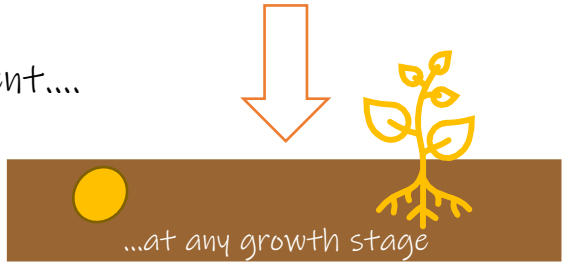
Seed soaking



Soil drench/ leaf spraying

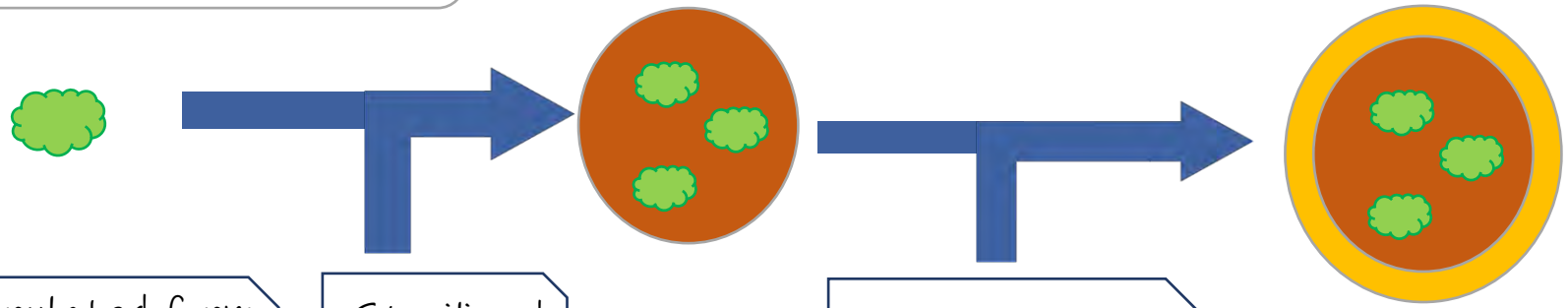
Treatment....





How would we change bioformulation / application?

How we would create our solution



Sporulated form (inactive metabolism)

Sterilized carrier

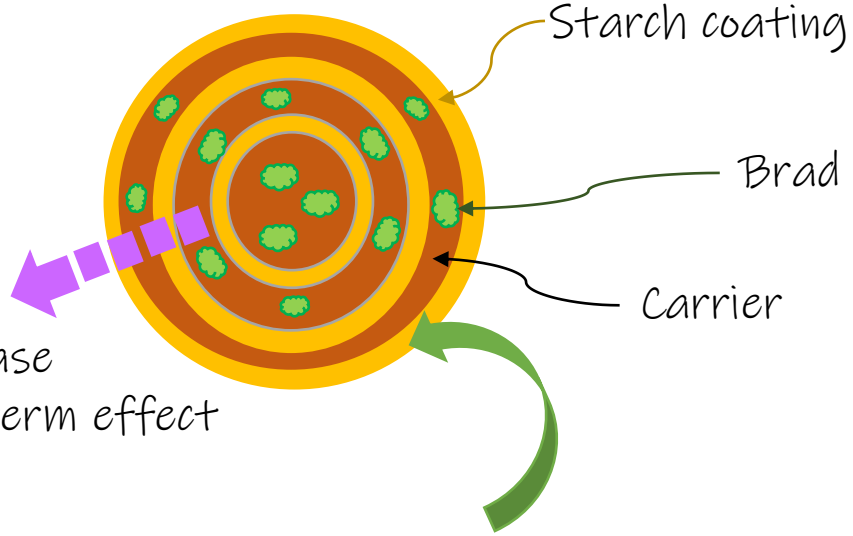
* Ligand and growing media

Coating with starch

* Protection from environment conditions and competition with other microorganisms

Repeated 2 times until....

... we obtain this bead



Slow release
=> Long term effect

Degredation by microorganism or water

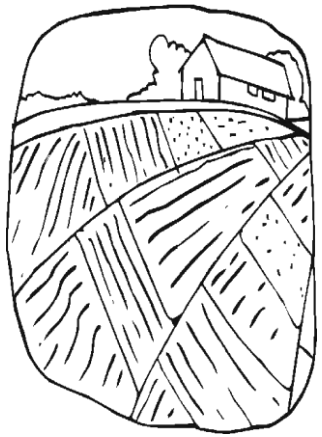
How to experiment:

Variables:

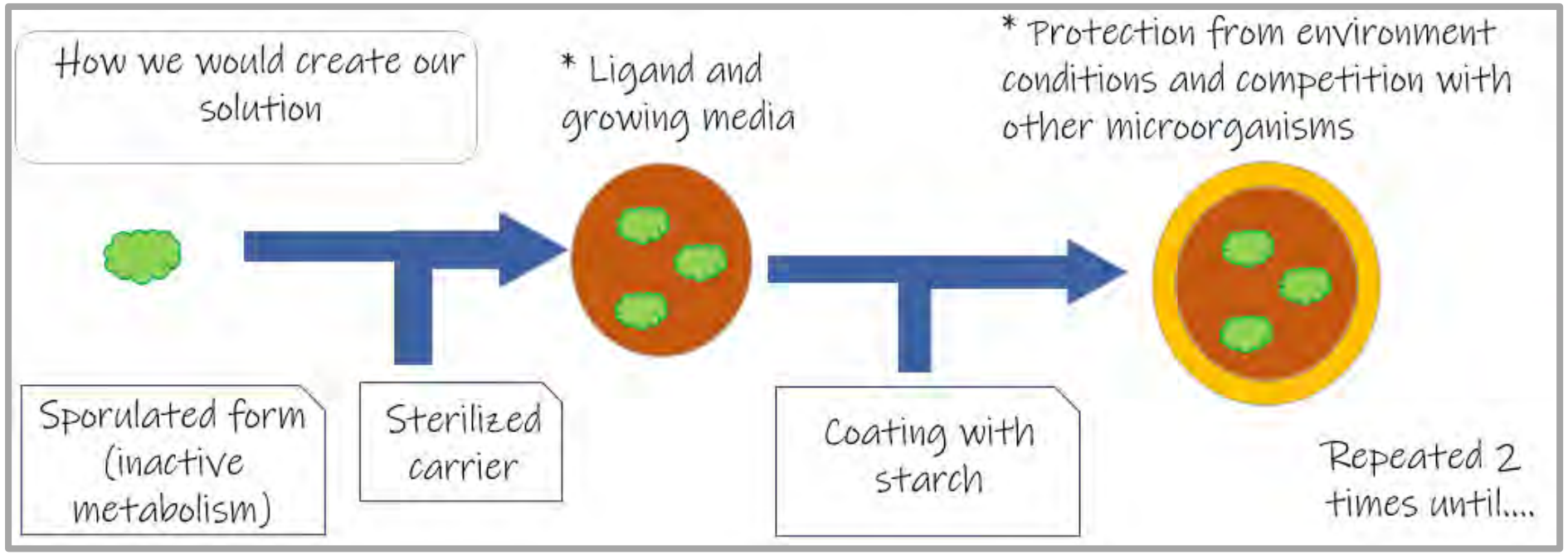
- **Coat thickness** (50-60 µm- urea fertilizer) [18]
- **Origin of the starch** (potato, cassava, maize) -> pore size [19]
- **Carrier**: peat/coir, conditionned cerals [20]
- Concentration of spores in the carrier
- **Number of layers** -> longer/best effect

Experimental conditions:

- host plant
- environmental conditions



How would we change bioformulation / application?



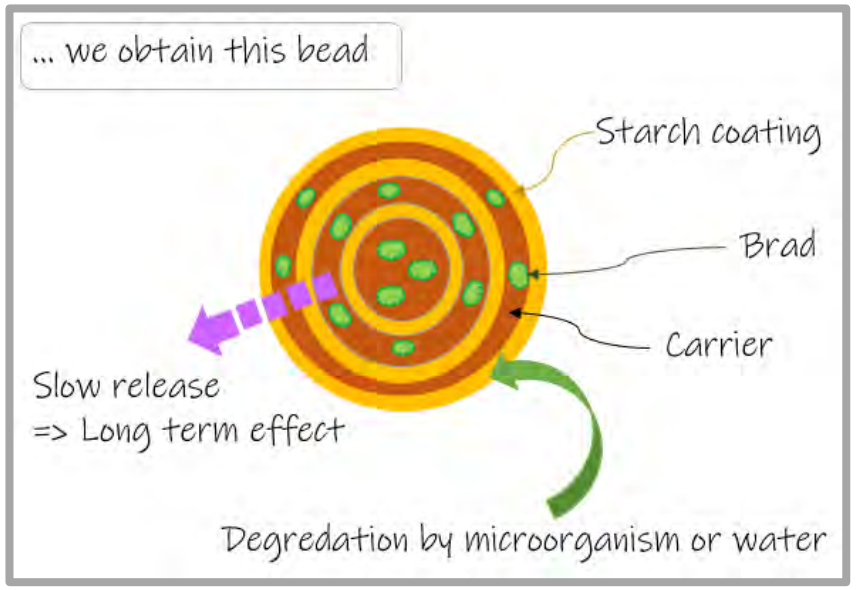
We based the conception of our product on the example of **control release fertilizers** and notably **polymer coated** fertilizers (PCF's). PCF's are like solid granules, with a nutrient core which is coated with a polymer.
Following this example, we choose to make a product based on **sporulated form of PGPR**, which is an inactive form, that we think would be better for storage and product shell-life.

The inactive PGPR would be placed on a **sterilized carrier** (for example : peat, coconut fiber...) to avoid contamination with other microorganisms. Usually a carrier is the growing media or **abiotic substrate** on which bacterial isolates are placed during all the formulation process. Here, the carrier is not really a growing media in itself during the formulation process because we want the PGPR to stay in sporulated form (therefore its uses during the formulation process is to be a **ligand**). In field when the product is applied, by the action of water and/or microorganisms, it's when the **carrier will help to start the growth** of PGPR population.

Then, we choose to coat the mix PGPR/carrier with a polymer that would be **starch**, generally used for PCPF's.

[18, 19, 20]

How would we change bioformulation / application?



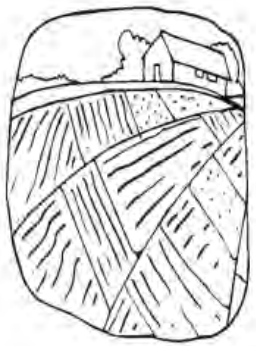
In order to have a **slow release of the PGPR** in field and therefore a long term effect of the product, we choose to make several layers of the pair "core x coat".

In field, the **starch coating** could be degraded either by water or soil microorganisms. Furthermore, it is water that will help the PGPR become active again by activating the spore germination.

How to experiment:

Variables:
- **Coat thickness** (50-60 µm- urea fertilizer) [18]
- **Origin of the starch** (potato, cassava, maize) -> pore size [19]
- **Carrier**: peat/coir, conditionned cerals [20]
- Concentration of spores in the carrier
- **Number of layers** -> longer/best effect

Experimental conditions:
- host plant
- environmental conditions

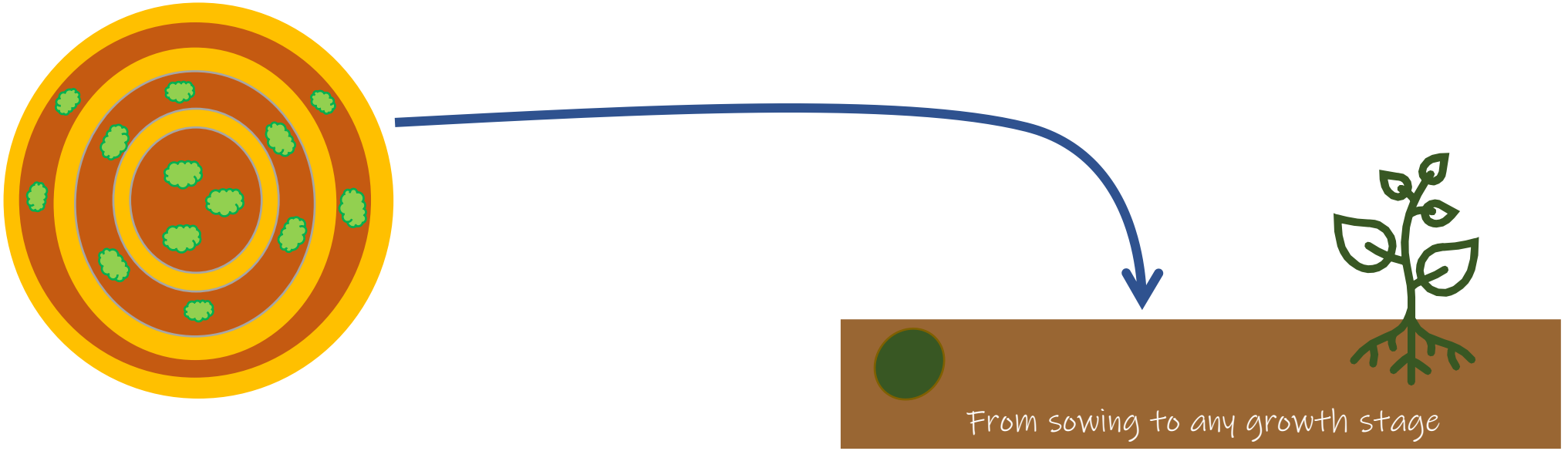


But we'll need **experiments** to be sure of the form of our final product and to be certain of its efficiency.
We'll make experiments to know :

- **What should be the coat thickness**? We know for urea polymer coated fertilizers that the thickness of the coat is between 50 and 60 µm.
- **Which starch should we use**? Should it be a starch coming from potato, cassava, or maize? Indeed, depending of its origin, the pores of the starch coating won't have the same size and structure.
- **Which carrier would be the best**?
- The usual concentration is 10^8 cells/g (or 10^8 UFC/g) but which **optimal spore concentration** should be used? UFC = Unit Forming Colony
- **How many layers would be required to have the best longer effect**? 2,3 or 5 layers ?
- Would there be **interaction between the PGPR and the coating when germination occurs**? Would it be a problem?
- How can we determine / experiment on the **release**? How can we measure the release period? How long would be **durability and longevity** in field?
- For which **environmental conditions and host plant** our product would be the most efficient?

[18, 19, 20]

How would we change bioformulation / application?



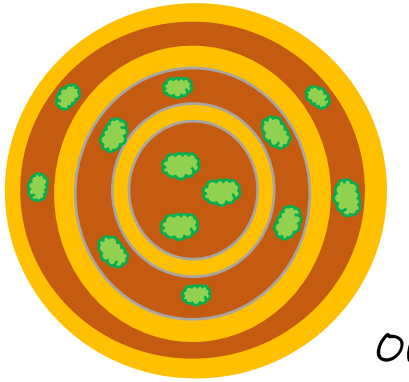
<u>Why</u> is our product relevant:

- Its size will be compatible with **seeding equipment**.
- It can be applied at sowing period but also at **any time** in the plant growth stage → it can be buried in the soil as seeds or it can be put on the surface.
- It could **replace several applications** of foliar spray and **avoids the inevitable losses** (run-off, leaching) because directly at the contact with the soil and close to the root system of plants → one application during sowing and maybe another application during another stage growth should be enough for a good establishment of PGPR in soil.
- **Release** of PGPR is **continuous** through time, one application can ensure several months → there are always PGPR near the plant.
- Potential **long storage and shell-life** of the product, at least one or two years.

[18, 19, 20]

The end

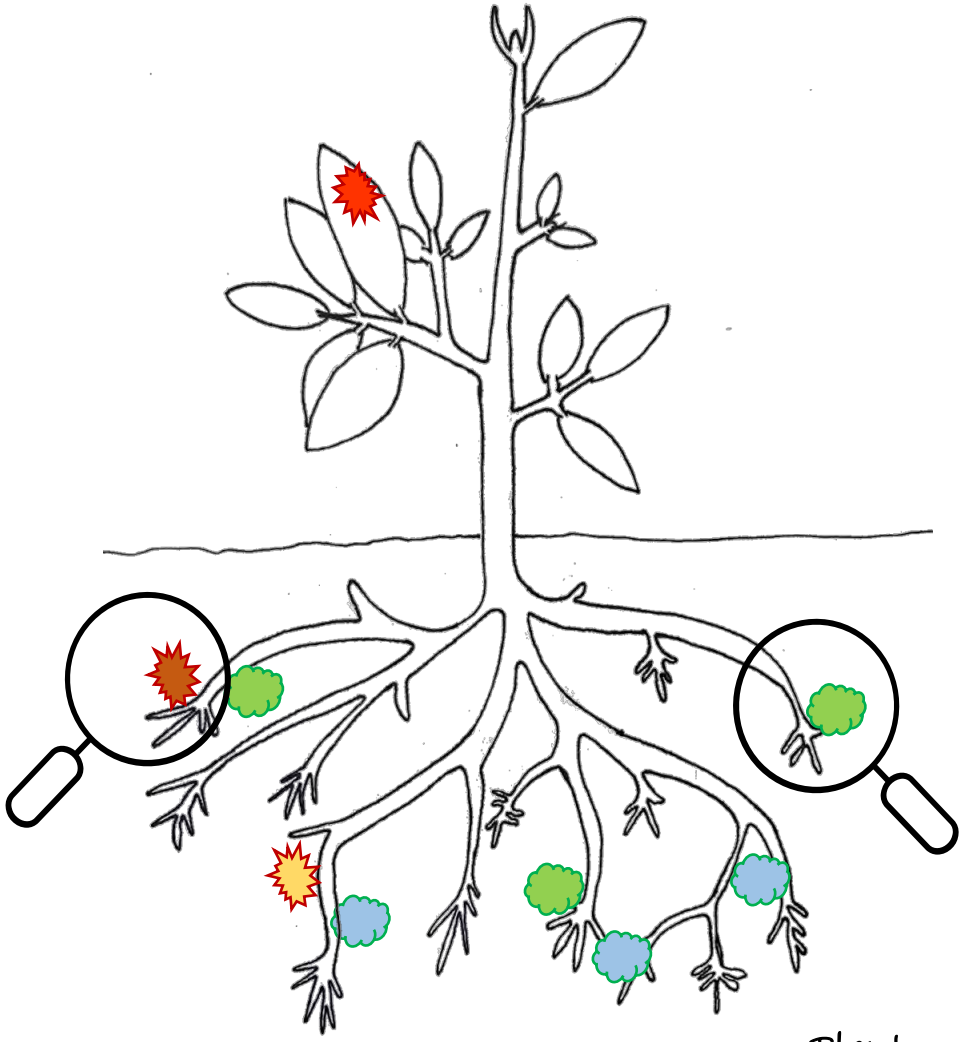
Questions !?!



Our product

Brad

Patrick



Plant

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