# Allelopathy as a way to control pests and weeds

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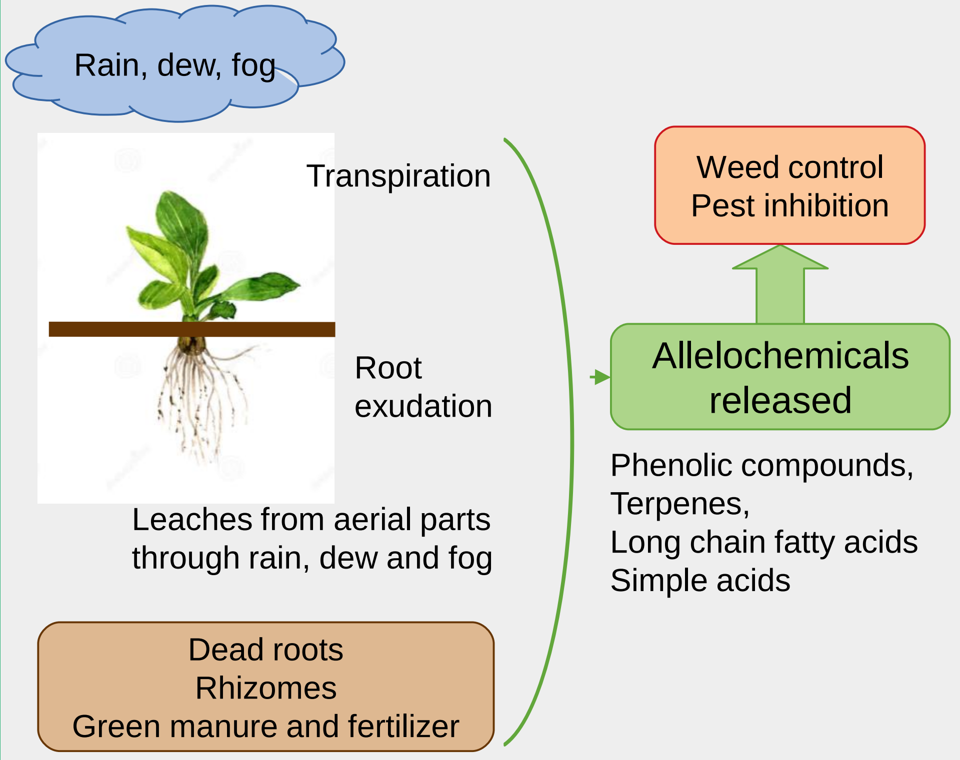
# What is allelopathy ?

Definition : all direct or indirect and positive or negative effect of a plant on another plant via bicochemicals components released in the envrionment [1]

Conditions impacting allelopathy efficiency [2]

- Aerial factors
  - Precipitations
  - Temperature
  - Light intensity
  - Wind
- Soil factors
  - Amount of water
  - pH
  - Quantity of organic matter
  - Type of soil
- Biologic factors
  - Development stage
  - Physiology and biochemistry of the plants




Schema of allelopathy mechanisms [2]

Different ways of action [3]

Weed control and plant enhancing

- Growth regulation
  - Hormones (ex : cytokinines)
- Seed germination inhibition
  - DIMBOA

Pest control :

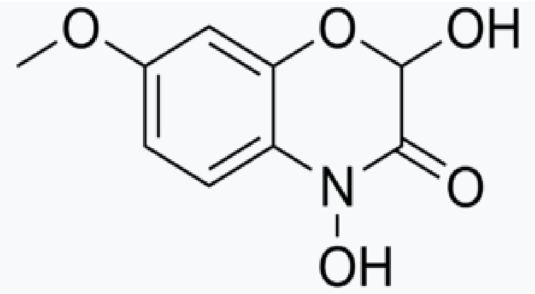
- Insecticides and nematicides :
  - Inhibit larval emergence
  - Inhibit post-embryonic development
- Fungicides

Nutrition

- Nutrition stimulators (oxalic and citric acids)
  - Enhance nutrition
- Inhibition of nitrification or mycorhizae germination [4]
  - Inhibit nutrition

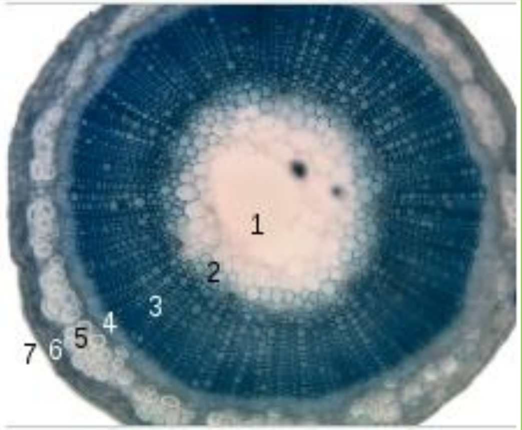
# EXEMPLE OF ALLELOPATHY:
# 2,4-dihydroxy-7-metoxy-1,4-benzoxazine-3,(4*H*)-one (DIMBOA)

**What is DIMBOA ?**




DIMBOA is a phytoanticipin benzoxanoid.
The maximum concentration occurs when the root is 2 weeks old [5].
The benzoxazinoid glucosides are stored in the vacuole as inactive phytoanticipins and the specific glucosidase is deposited in the maize chloroplast .
When pathogen or herbivore attack destroys the integrity of the cell organelles, glucoside (glucose derived molecole) and glucosidase are combined and the toxic aglycone is produced. In maize, the enzymatic release of DIMBOA after wounding is completed within 30 min [6].

**Prerequisites:**
"phytoalexins are low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms"
"**phytoanticipins** are low molecular weight, antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting
**Benzoxazinoids** are secondary metabolites that are effective in defence and allelopathy. [7]



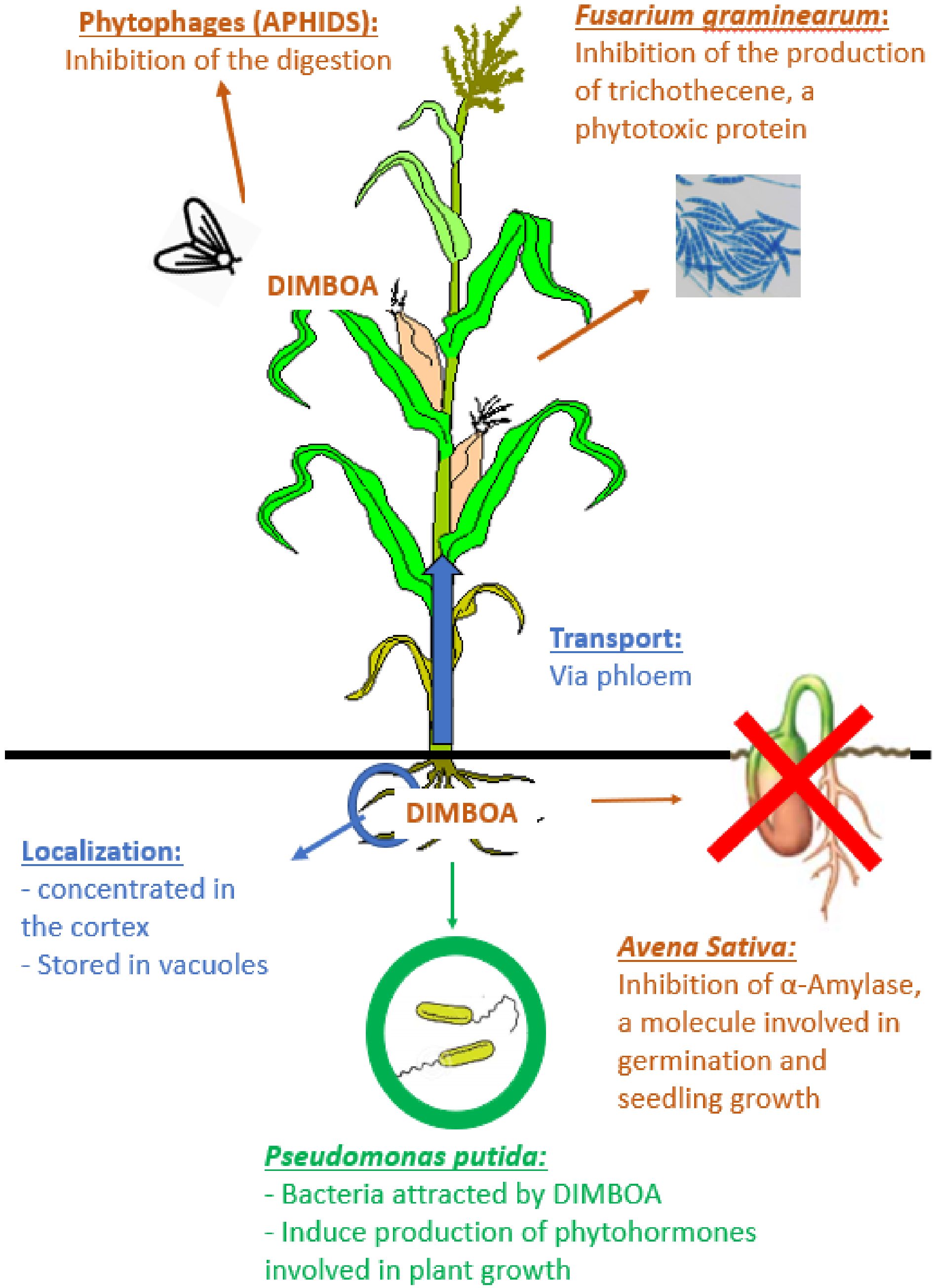

**<u>Figure 1: Cross section of a root</u>**
1- Pith
2- Protoxylem,
3- Xylem
4- Phloem
5- Sclerenchyma
6- Cortex,
7- Epidermis

# Examples of DIMBOA's effects

**ON PHYTOPHAGES** (*Aphids, Ostrinia nubilalis* and others)
DIMBOA in leaves inhibit the development of about 25% of *Ostrinia nubilalis* by the same means [8].
DIMBOA can be transformed in MBOA by some enzymes presents in some insects intestine.
DIMBOA acts primarily as a digestive toxin and that the effects of MBOA occurred after digestion.
DIMBOA causes a decrease in the approximate digestibility (AD) and the efficiency of conversion of ingested food (ECI) and has no effect on the efficiency of conversion of digested food (ECD).
MBOA causes a decrease in both ECI and ECD but has no effect on AD [9].

**LOCALIZATION**
Mostly concentrated in the cortex of all parts of some Maize and others Grasses roots. DIMBOA gets out actively of the cell via the multidrug and toxin extrusion (MATE) transporters and/or passively through the membrane. [10]

**On *Pseudomonas putida***
Benzoxazinoids in root exudates of maize attract *P. putida* to the rhizosphere [11].
This rhizobacteria enhance the presence of encoding genes involved in pathways linked to plant growth promoting properties, such as the production of phytohormones. It also enhance defense responses of maize after wounding [8].




**On *Fusarium graminearum***
DIMBOA strongly affects the expression of Tri6, encoding a major transcriptional regulator of several genes of the trichothecene biosynthesis pathway. DIMBOA also repressed expression of Tri5, encoding trichodiene synthase, the first enzyme in the trichothecene biosynthesis pathway [12].

Trichothecenes are mycotoxins predominantly produced by the phytopathogenic *Fusarium* fungus, and act in plants as virulence factors aiding the spread of the fungus during disease development. Known for their inhibitory effect on eukaryotic protein synthesis, trichothecenes also induce oxidative stress, DNA damage and cell cycle arrest and affect cell membrane integrity and function in eukaryotic cells [13].

**On *Avena sativa***
DIMBOA would inhibit the germination and seedling growth of *A. sativa* by inhibiting the gibberellin-induced induction of $\alpha$-amylase production [14].
$\alpha$-Amylase plays a major role in the degradation of reserve carbohydrate to soluble sugars during germination.
Therefore, the induction of $\alpha$-amylase is a requirement for not only seed germination, but also for subsequent seedling growth until photosynthesis is sufficient to support growth [15].

# Limits and ways to improve the use of DIMBOA

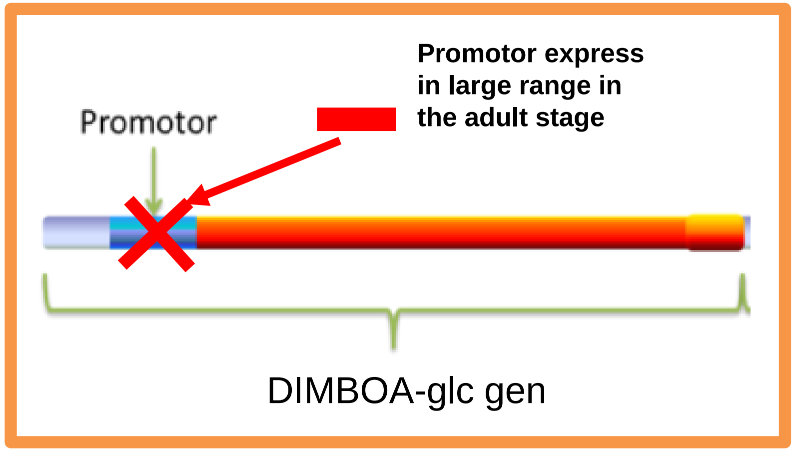
## Change agricultural practices

**Mixture:**
Mix Poaceae with other cultures would permit to control pests, bacterias, fungis and weeds.

**Limits →** Maize producing DIMBOA does not have a lower grain yield [16]. But the farmer needs to be able to produce two different crops instead of one, he would need buy or rent other machines or associate himself with an other farmer.
It has been proven that in association with some crop, maize does not have negative impact on the other plants,[17] but it has not been proven for all the crop.

**Mulch:**
To reduce seed growth and bacterial development, residues (roots especially) of Paceae varieties could be used as mulch in crops (before seedling).
**Limits →** DIMBOA half life is from 30 to 84 hours depending on the concentration, MBOA’s half life is from 4 to 39 days [18].
Consequently, the mulch can be used for short cultures or as a pesticide.

Association of maize and wheat




Schema of the modification of DIMBOA-glc promotor

## Genetic

One of the main factor that limits the use of DIMBOA is its variations with environmental conditions and plant growth stages.

**Variety selection**
The selection of variety with high concentration of DIMBOA and low environment dependence for DIMBOA synthesis could also be a solution for Maize protection. . The locus bx1 is one candidate that can be selected, it explained 12% of DIMBOA concentration variability. [19]

**Express DIMBOA-glc gen in the adult stage**
DIMBOA-glc, the precursor of DIMBOA, has a higher concentration when the plant is young. The biosynthesis stills continue six to ten days after germination and then the concentration drops down due to release, degradation or transformation of the molecule.[20]
The low concentration in adult plant can be an issue for crop protection, especially for aphids attack. Finding a way to reduce the loss of DIMBOA would be a way to improve Poaceae resistance. Maybe by changing the promoter of the gene coding for DIMBOA-glc and choosing a promoter expressed in great quantity in the adult stage.

# Idea of experimentation that could lead to a new use of DIMBOA

## I - Foreword

DIMBOA repress the virulence of *Fusariums* by inhibiting some mychotoxins biosynthesis *Fusarium graminearum* is an Ascomycète that conserve itself on the ground.

## II - Context

Rapeseed is a crop in difficulty today, the pesticides are not effective anymore, no solution in proposed yet. The biggest problem is *Sclerotinia sclerotiorum,* is an Ascomycète that conserve itself on the ground.

## III – Hypothesis 1 That we will test here

DIMBOA could potentially have an effect on S. *sclerotiorum, inhibiting* mycotoxins or by an other way.

## III' – Hypothesis 2 That we will not test here

Maize does not reduce Rapeseed grain yield or less than S. sclerotiorium.

## IV - Protocole to test the Hypothesis

- Sow 4 Brassica napus L. var. Oléor in 20 pots.
- Sow 4 Maize DIMBOA-producing (*Zea mays L var.B73*) in 10 of them and 4 Maize non-DIMBOA-producing (*Zea mays L var. bxbx*) in the 10 left [21].
- Seperate the two pots from the others but kept in the same controlled environment: 20°C, HR=¨60%. Grow them one month.
- Inoculate *Sclerotinia sclerotiorum* in all pots and change the HR to 95% (best *S. sclerotiorium* germination) [22]. Wait two weeks for S. to make several life cycles.

## V - Hoped results

The "bxbx" pots are significantly more affected by S. *sclerotiorium*

## VII - Field association

- Brassica napus L. var. Oléor with *Zea mays L var.B73 in inter-rows.*

## VI - Hoped Conclusions

→ We can suppose that the yield will be lower for the "bxbx".
→ Maize producing DIMBOA could be used to tackle the *S. sclerotiorum* issue on Rapeseed.

## VIII - Perspective

- Test if *DIMBOA* damages *S. sclerotiorum* sclerotes and spores.
- If yes: sow B73 in June, cut it down in August to plant Rapeseed, harvest it in June etc.

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