MSc BOOST, Mentor Project 2022 Plant -Oomycete Interactions

Aurélien Boisson-Dernier

Plant – Oomycete Interactions

- **Oomycetes: Filamentous, fungus-like pathogens**
  - Responsable for important Crop losses
  - Major constraint for conventional and soil-less agriculture

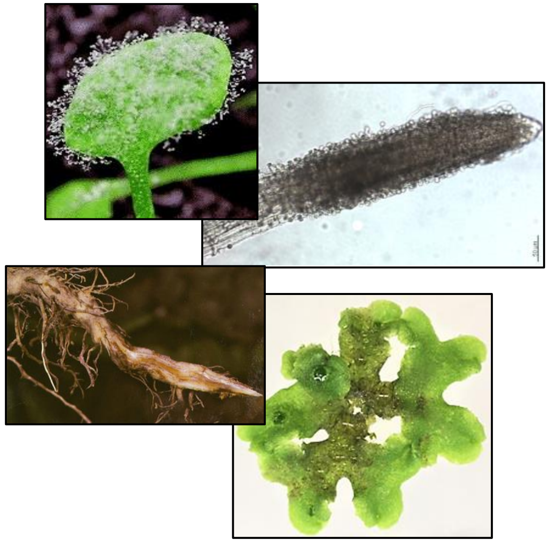
  → **Need for new methods of control that are adapted and environmentally friendly.**

- **The IPO team objective :**
  Better understand the plant – oomycete interactions
  - Study both the **plant** and the **pathogen**
  - Characterize the molecular mechanisms and molecular dialog established throughout infection.
  - Models :

Arabidopsis thaliana — ***Hyaloperonospora arabidopsidis*** (specialist, downy mildew, leaves)

Arabidopsis thaliana, Tomato, *Marchantia polymorpha* — ***Phytophthora parasitica*** (polyphagous, roots and leaves rot)

Phytophthora palmivora (polyphagous, roots, leaves and fruits rot)



The IPO Team


INRAe
Harald
Celso
Valérie
Carlotta
Marie-Line
Agnès
Enora
Cathy

IPO MENTOR DAYS FOR MSc BOOST FEBRUARY 2023

Group	Group Size	Firstname	Lastname	email
Group A	6			
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Group B	6			
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Biocontrol Solutions for Plant Health

IPO MENTOR DAYS FOR MSc BOOST FEBRUARY 2022/2023

16 M1 students (+2 visiting) students divided in 3 groups A, B, C;
14 workpackages, 3 days (D1,…,D4,D5)

Monday 13th + Thursday 16th and Friday 17th

14 Workpackages

1-Arabidopsis seeds sterilization and sowing.

2-Marchantia observation, gemmae propagation.

3- Preparation for Agrobacteria infiltration in Nicotiana. 3bis- Infiltration.

4-Phytophthora palmivora/parasitica -Observing hyphae, sporangia, fixed zoospores… Propagation by transferring agar blocs in V8 media under laminar flow hood.

5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution.

6-Arabidopsis seedling inoculation with P. parasitica.

7-Marchantia thalli inoculation with P. palmivora.

8-Observe swimming zoospore and attraction.

9-Image aggregation on Arabidopsis roots, analyze/quantify area with ImageJ.

10-Embedding/sectioning of infected Marchantia thalli.

11-Observe fluorescent P. palmivora infecting Marchantia at the confocal microscope.

cancelled

12-Phenotyping disease symptoms on Marchantia (area quantification with ImageJ).

13-Observation/phenotyping of infiltrated Nicotiana leaves.

14-Observation of Arabidopsis (WT vs pskr1) cotyledons infected w/ Hyaloperonospora arabidopsidis. Aniline blue staining of hyphae/haustoria + observation.

IPO MENTOR DAYS FOR MSc BOOST FEBRUARY 2023

16 M1 students (+2 visiting) students divided in 3 groups A, B, C.
Over 3 days, Monday 13th + Thursday 16th and Friday 17th February

A — 1, 2, 5, 6, 13

Monday 13th

- *1-Arabidopsis seeds sterilization and sowing.*
  2-Marchantia observation, gemmae propagation.
- *5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution.*
- *6-Arabidopsis seedling inoculation with P. palmivora.*

Thursday 16th

- **Article Discussion/Free time Thursday Morning**
- *13-Observation/Phenotyping of infiltrated Nicotiana leaves.*

Friday 17th

- *cancelled*
- *Article Restitution*

B — 3, 5, 7, 3bis, 13, 12

Monday 13th

- *3- Preparation for Agrobacteria infiltration in Nicotiana.*
- *5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution.*
- *7-Marchantia thalli inoculation with P. palmivora.*
- *3bis- Agrobacteria infiltration.*

Thursday 16th

- *13-Observation/Phenotyping of infiltrated Nicotiana leaves.*
- **Article Discussion/Free time Thursday Afternoon**

Friday 17th

- *12-Observation/Phenotyping disease symptoms on Marchantia (area quantification with ImageJ).*
- *Article Restitution*

C — 4, 5, 8, 6, 9, 14

Monday 13th

- *4-Phytophthora palmivora, observing hyphae, propagation by transferring agar blocs.*
- **Article Discussion/ Free time Monday Morning**

Thursday 16th

- *5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution.*
- *8-Observe swimming zoospore and attraction.*
- *6-Arabidopsis seedling inoculation with P. palmivora.*
- *9-Image aggregation on Arabidopsis roots, analyze/quantify area with ImageJ.*

Friday 17th

- *14-Observation of Arabidopsis (WT vs pskr1) infected w/ H. arabidopsidis. Aniline blue staining and observation.*
- *Article Restitution*

Aurélien | Marie-Line | Carlotta | Agnès | Enora/Valérie

BOOST
Biocontrol Solutions for Plant Health

Aurélien | Marie-Line | Carlotta | Agnès | Enora/Valérie

DAY 1

lecture

(Aurélien) Plant model organisms, evolutionary developmental biology… — D206

9h30-12h

Transgénèse
- *1-Arabidopsis seeds sterilization and sowing.*
- *2-Marchantia observation, gemmae propagation.* — A

D216?
- *3- Preparation for Agrobacteria infiltration in Nicotiana.* — B

D218
- *4-Phytophthora palmivora, observing hyphae, propagation by transferring agar blocs.* — C

Lunch break 12h-13h30

lecture

D206 — **(Agnès)** Introduction to Oomycete life cycle, classification, host plant variety, symptoms…

14h-17h

D218
- *5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution.* — A, B

and

Transgénèse
- *6-Arabidopsis seedling inoculation with P. palmivora.* — A

or

Transgénèse
- *7-Marchantia thalli inoculation with P. palmivora.* — B
- *3bis- Agrobacteria infiltration in Nicotiana.*

DAY 4

lecture

(Agnès) Signaling during the first events of infection : from zoospore attraction to penetration. — D206

9h30-12h

D218
- *5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution.*
- *8-Observe swimming zoospore and attraction.* — C
- *6-Arabidopsis seedling inoculation with P. palmivora.* — C
- *13-Observation/phenotyping of infiltrated Nicotiana leaves.* — B — Zeiss Confocal

Lunch break 12h-13h30

lecture

(Carlotta) Zoospores: biology of a swimming cell. — D206

14h-17h

- *9-Image aggregation on Arabidopsis roots, analyze/quantify area with ImageJ.* — C

Zeiss confocal
- *13-Observation/Phenotyping of infiltrated Nicotiana leaves.* — A

DAY 5

lecture

cancelled — D206

9h30-12h

Transgénèse
- *12-Observation/Phenotyping disease symptoms on Marchantia (area quantification with ImageJ).* — B
- *cancelled* — A — Zeiss Confocal
- *14-Observation of Arabidopsis (WT vs pskr1) cotyledons infected w/ H. arabidopsidis. Aniline blue staining and observation.* — C — D216 + Axioplan

Lunch break 12h-14h

14h-17h

D206 — A, B, C

Article Restitution

BOOST

Biocontrol Solutions for Plant Health

Each group chooses 1 article from the following:

1- A Phytophthora Effector Suppresses Trans-Kingdom RNAi to Promote Disease Susceptibility
https://doi.org/10.1016/j.chom.2018.11.007

2C-The Phytophthora capsici RxLR effector CRISIS2 triggers cell death via suppressing plasma membrane H+-ATPase in the host plant
https://doi.org/10.1093/jxb/erac500

3A- Elicitin recognition confers enhanced resistance to Phytophthora infestans in potato
https://www.nature.com/articles/nplants201534

4B- Conserved Biochemical Defenses Underpin Host Responses to Oomycete Infection in an Early-Divergent Land Plant Lineage
https://doi.org/10.1016/j.cub.2019.05.078

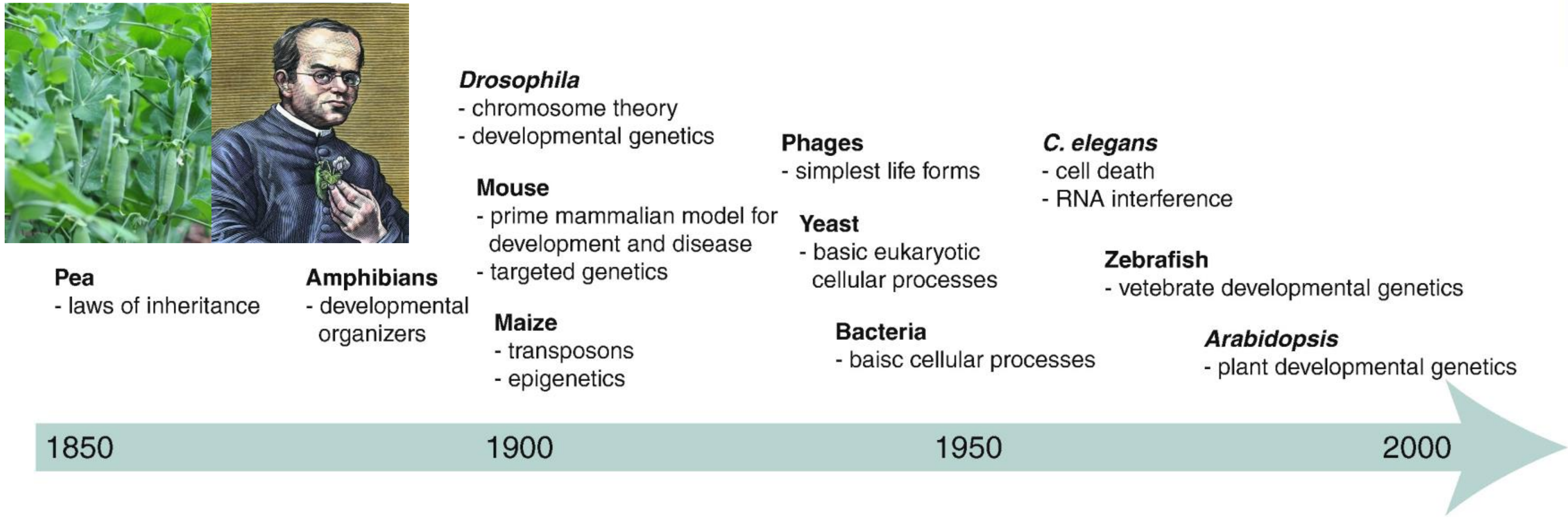
5-Developmental Modulation of Root Cell Wall Architecture Confers Resistance to an Oomycete Pathogen
https://doi.org/10.1016/j.cub.2020.08.011

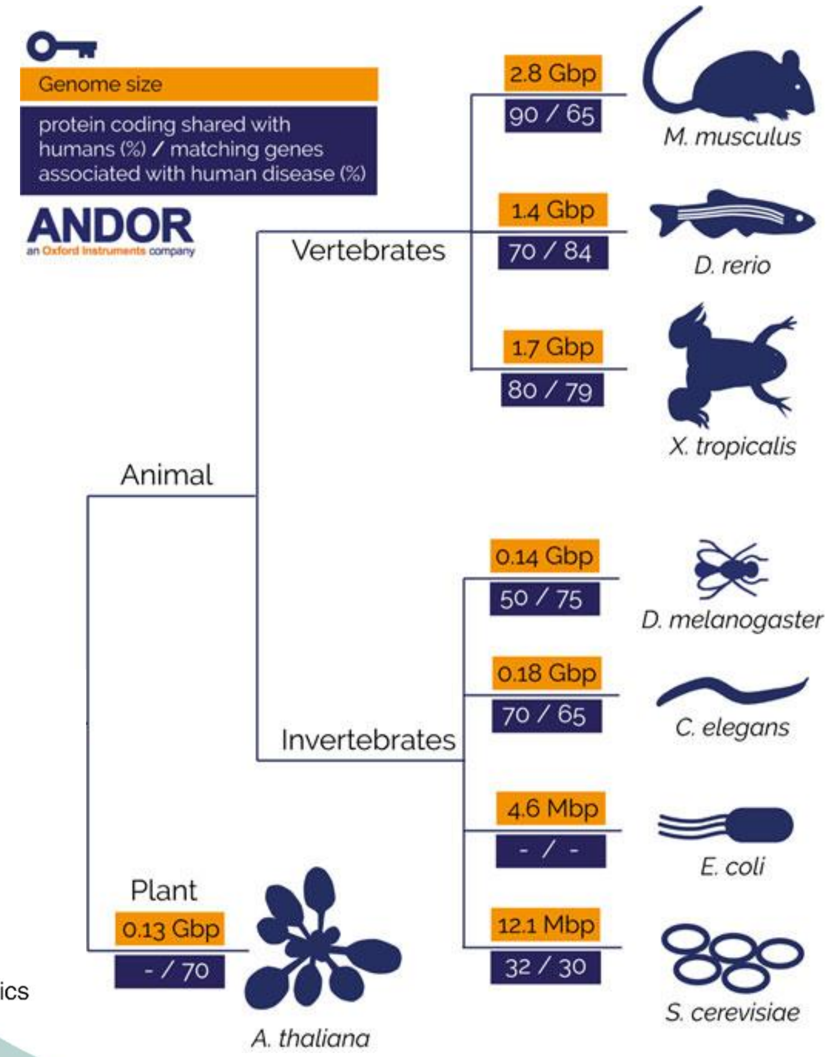
	EVALUATION	Points (/20)	
Individual	Participation to the Scientific Activities (including punctuality, respect, curiosity, understanding, communication…)	4	
Collective (by group)	Overall quality of the oral presentation -Quality and clarity of the oral presentation. -Attitude towards the audience (gestures, tone, expression, …). -Quality of illustrations. -Respect of the speaking time.	4	
	Introduction -The background information to understand the paper has been well delivered. -The main QUESTION the authors set out to answer in the published work was well introduced.	4	
	Results -The main findings are clearly presented at the level of scientific goals and experimental strategy. -Routine techniques are briefly exposed. -New techniques are sufficiently exposed without getting lost with details. -Clear & Correct conclusions are drawn from the presented data.	4	
	Global Comprehension Understanding the scientific context and interest of the study, the limitations as well.	4	

Model Organisms, what are they for?

•**Model organisms** are non-human species used in the lab to **help us understand a range of biological phenomena**, **with the hope that data, models and theories generated will be applicable to other organisms**, particularly those that are in some way more complex than the original.

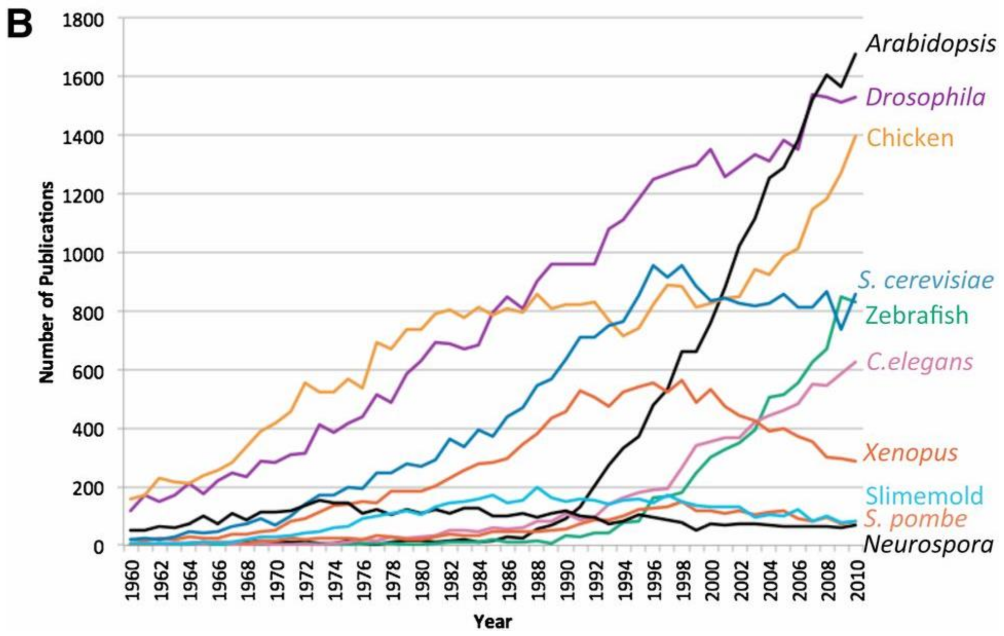
•They should display particular **experimental advantages**.
For example, they have **small physical and genomic** sizes; **low costs** to breed, maintain and transport; **short generation times and life cycles**; **high fertility rates**; and high mutation rates or high susceptibility to techniques for **genetic modification**. They may also occupy a **pivotal position in the evolutionary tree**…





Arabidopsis thaliana (Thale Cress): a weed, yes, but the Reference Plant Model of the last 4 decades!

#**The popularity of *A. thaliana*** took off in the late **1980s**, when researchers began to **combine genetics with powerful molecular biology** methods.
#The Arabidopsis **reference genome sequence** was published as the first nuclear genome of a flowering plant **in 2000**.
Fostered **enormous fundamental progress** in our knowledge of the molecular principles of **plant development, cell biology, metabolism, physiology, biotic and abiotic stress responses, genetics and epigenetics.**



#Its uses continue to **expand** in the fields of **systems biology and adaptation of molecular functions** in natural environments.

#**But it is only one plant species among 400,000! Can not apprehend alone the biodiversity of land plants** in terms of ecosystems, architecture, biochemistry, reproductive systems…

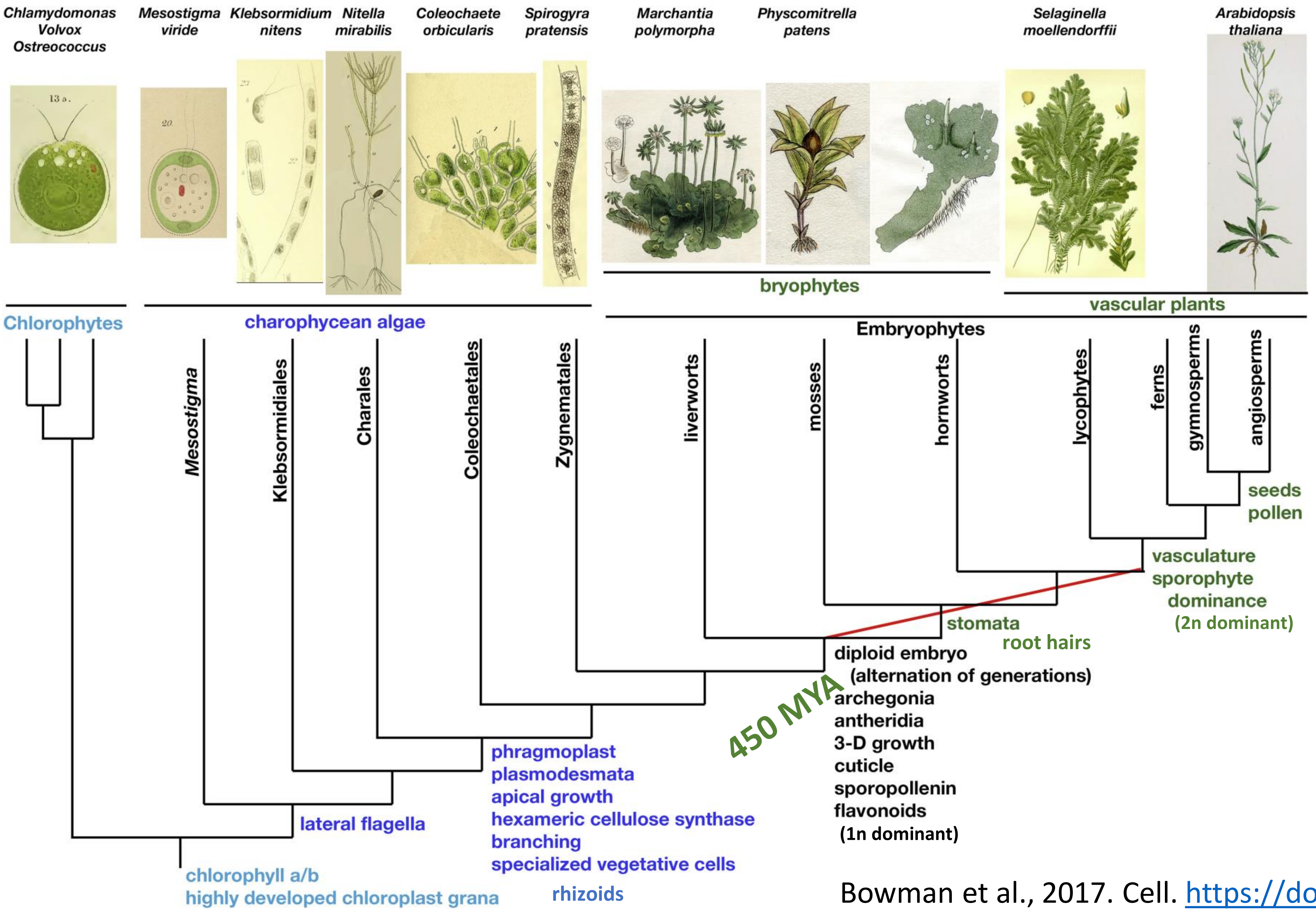
Dietrich et al., 2014 Genetics. https://doi.org/10.1534/genetics.114.169714
Krämer, 2015. Elife. https://doi.org/10.7554/eLife.06100
Chang et al., 2016. Cell. https://doi.org/10.1016/j.cell.2016.08.031

Diversity of Plant Model Organisms

Classification	Family	Species Name	Properties
Angiosperm eudicot	Brassicaceae	**Arabidopsis thaliana (mouse-ear cress)**	predominant plant model system
		Arabidopsis halleri	heavy metal tolerance/accumulation
		Arabidopsis lyrata (lyrate rock cress)	self-incompatible A. thaliana relative
		Cardamine hirsute (bitter cress)	compound leaves
	Fabaceae	Lotus japonicas (birdsfoot trefoil)	nitrogen fixation
		Medicago truncatula (barrel medic)	nitrogen fixation
	Myrtaceae	Eucalyptus globulus (blue gum)	wood, lignification, biofuel
	Phrymaceae	Mimulus guttatus (seep monkeyflower)	ecological studies, flower evolution
	Rosaceae	Fragaria vesca (wild strawberry)	fruit development
		Prunus persica (peach)	fruit tree development
	Salicaceae	**Populus trichocarpa (black cottonwood)**	wood
	Solanaceae	**Solanum lycopersicum (tomato)**	fruit ripening
	Vitaceae	Vitis vinifera (grape)	wine
	Poaceae	**Brachypodium distachyon**	non-crop grass model
		Oryza sativa (rice)	major crop, grass model
		Setaria viridis (green foxtail)	non-crop grass
		Zea mays (maize)	genetics and development, major crop
Basal angiosperm	Amborellaceae	**Amborella trichopoda**	flowering plant evolution
Gymnosperm	Pinaceae	Picea abies/ Picea glauca (spruce)	wood
		Pinus taeda (loblolly pine)	wood
Pteridophyta	Azollaceae	Azolla filiculoides (water fern)	model fern, nitrogen fixation
Lycopodiophyta	Selaginellaceae	**Selaginella moellendorffii (spikemoss)**	clubmoss evolution
Anthocerotophyta	Anthocerotaceae	Antheroceros agresis (field hornwort)	hornwort evolution
	Funariaceae	**Physcomitrella patens**	moss evolution, moss development
Marchantiophyta	Marchantiaceae	**Marchantia polymorpha**	liverwort evolution
Charophyta	Klebsormidiaceae	Klebsormidium flaccidum	filamentous freshwater alga
Chlorophyta	Chlamydomonadaceae	Chlamydomonas reinhardtii	motile unicellular freshwater alga
	Volvocaceae	Volvox carteri	multicellular freshwater alga

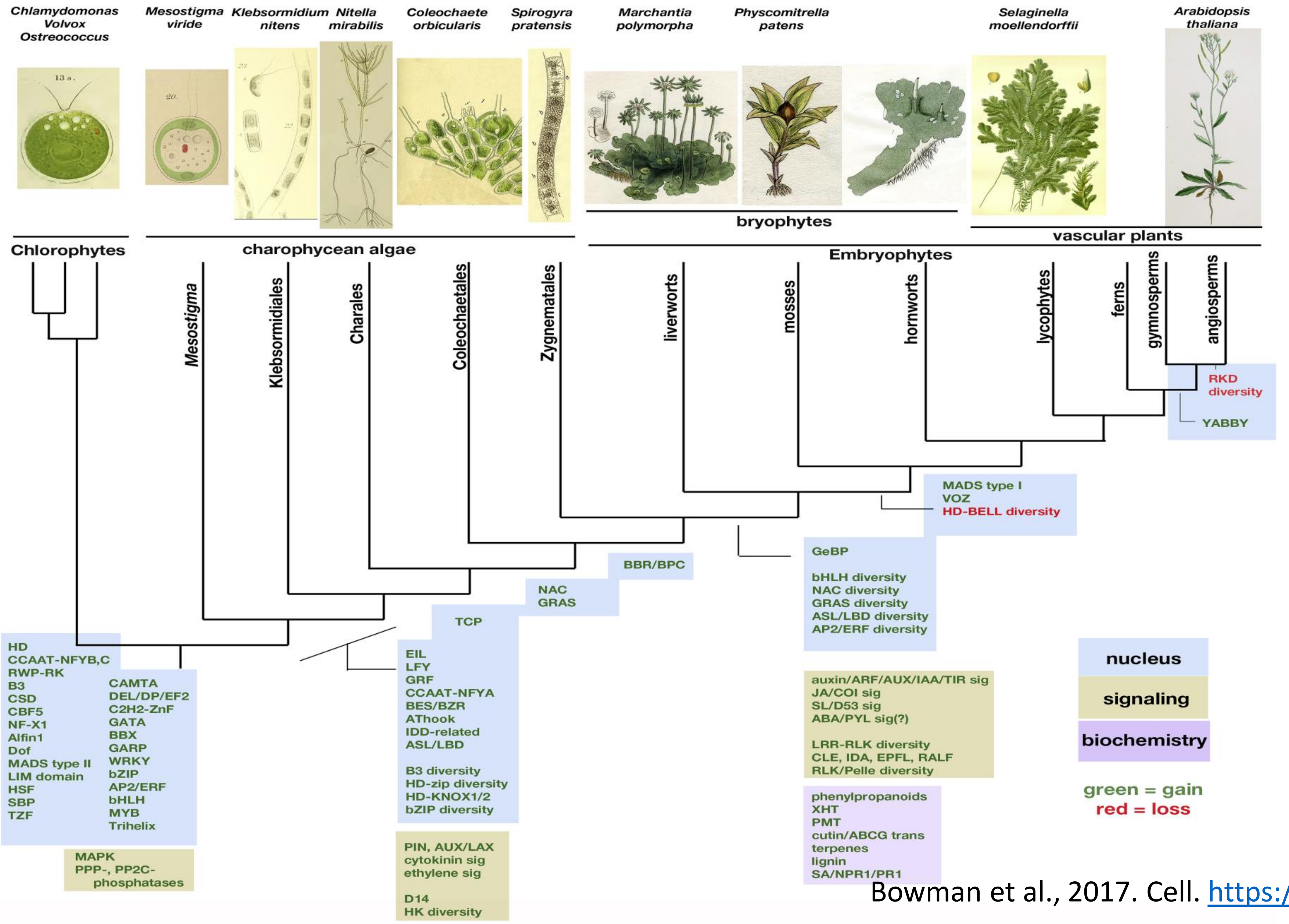
Chang et al., 2016. Cell. https://doi.org/10.1016/j.cell.2016.08.031

Major Evolutionary Innovations of Charophycean Algae and Land Plants



Bowman et al., 2017. Cell. https://doi.org/10.1016/j.cell.2017.09.030

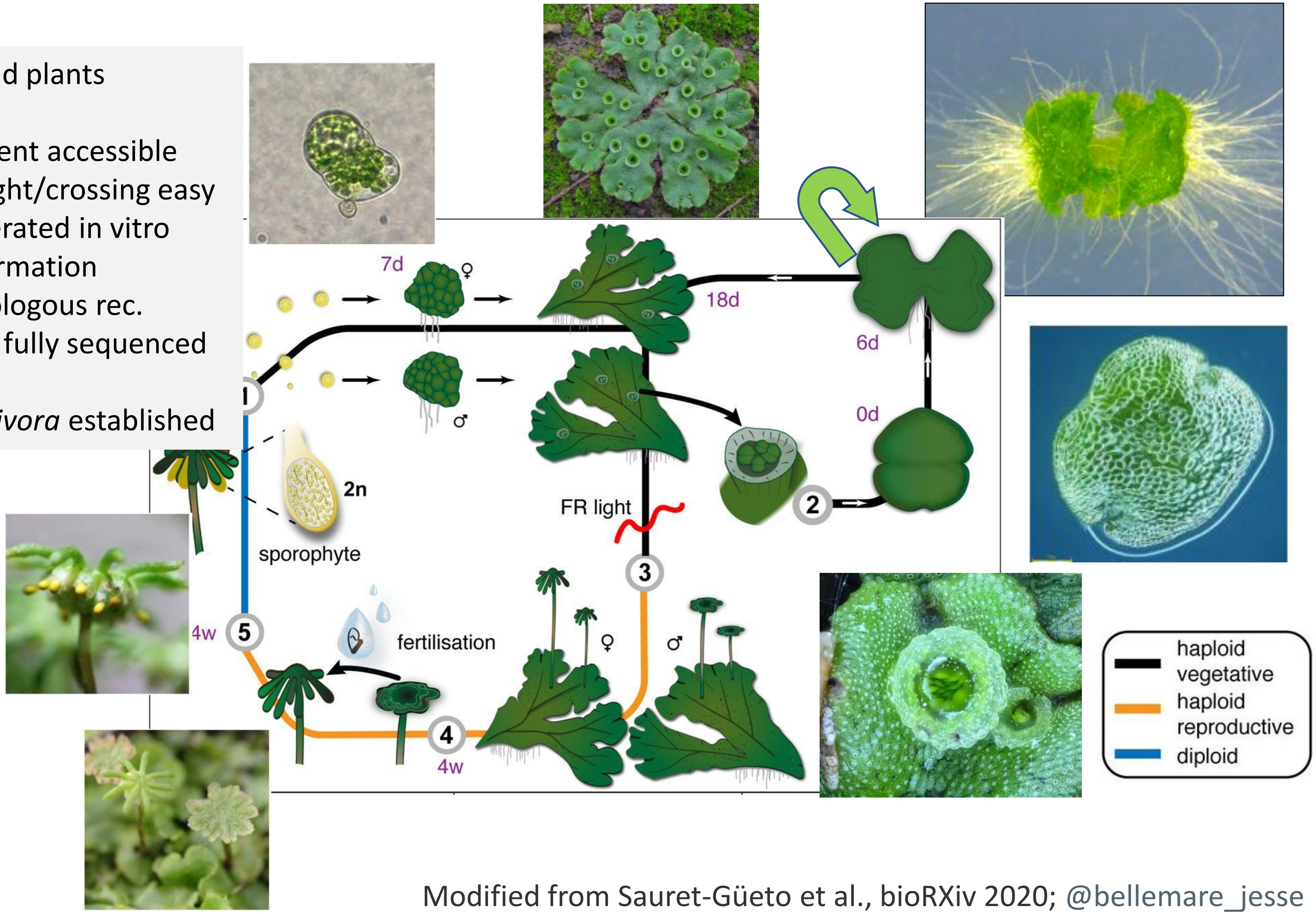
Major Evolutionary Innovations of Charophycean Algae and Land Plants



Bowman et al., 2017. Cell. https://doi.org/10.1016/j.cell.2017.09.030

Marchantia polymorpha, the bryophyte model

- #Descendant of earliest land plants
- #Haploid genetics
- #Entire phase of development accessible
- #Gametes induced by FR light/crossing easy
- #Easily propagated /regenerated in vitro
- #High frequency of transformation
- #CRISPR technology /homologous rec.
- #Small and simple genome fully sequenced
- #Less genetic redundancy
- #Pathosystem with *P. palmivora* established



Modified from Sauret-Güeto et al., bioRXiv 2020; @bellemare_jesse

Aurélien | Marie-Line | Carlotta | Agnès | Enora/Valérie

DAY 1

lecture — D206

(Aurélien) Plant model organisms, evolutionary developmental biology…

9h30-12h

- Transgénèse — 1-Arabidopsis seeds sterilization and sowing. 2-Marchantia observation, gemmae propagation. (A)
- D216? — 3- Preparation for Agrobacteria infiltration in Nicotiana. (B)
- D218 — 4-Phytophthora palmivora, observing hyphae, propagation by transferring agar blocs. (C)

Lunch break 12h-13h30

lecture — D206

(Agnès) Introduction to Oomycete life cycle, classification, host plant variety, symptoms…

14h-17h

- D218 — 5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution. (A, B)

and

- Transgénèse — 6-Arabidopsis seedling inoculation with P. palmivora. (A)

or

- Transgénèse — 7-Marchantia thalli inoculation with P. palmivora. (B)
- 3bis- Agrobacteria infiltration in Nicotiana.

DAY 4

lecture — D206

(Agnès) Signaling during the first events of infection : from zoospore attraction to penetration.

9h30-12h

- D218 — 5-Sporulation/release zoospores/Encysting zoospores to count cells; make dilution. 8-Observe swimming zoospore and attraction. (C)
- 6-Arabidopsis seedling inoculation with P. palmivora. (C)
- Zeiss Confocal — 13-Observation/phenotyping of infiltrated Nicotiana leaves. (B)

Lunch break 12h-13h30

lecture — D206

(Carlotta) Zoospore mobility and attraction…

14h-17h

- 9-Image aggregation on Arabidopsis roots, analyze/quantify area with ImageJ. (C)
- Zeiss confocal — 13-Observation/Phenotyping of infiltrated Nicotiana leaves. (A)

DAY 5

lecture — D206

cancelled

9h30-12h

- Transgénèse — 12-Observation/Phenotyping disease symptoms on Marchantia (area quantification with ImageJ). (B)
- Zeiss Confocal — *cancelled* (A)
- D216 + Axioplan — 14-Observation of Arabidopsis (WT vs pskr1) cotyledons infected w/ H. arabidopsidis. Aniline blue staining and observation. (C)

Lunch break 12h-14h

14h-17h

D206 — A B C

Article Restitution

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Biocontrol Solutions for Plant Health