



STATION
BIOLOGIQUE
ROSCOFF



Bioassay methods to study induced defenses and resistance in seaweeds

Philippe POTIN

Marine plants and Biomolecules

UMR 7139 (CNRS/UPMC-Paris6)

Station Biologique, Roscoff, Brittany, France

MARBEF TRAINING COURSE ON BIOASSAY METHODS IN MARINE CHEMICAL ECOLOGY

September 9-14, 2006

**Exploration of ecologically
relevant biotic interactions
in seaweeds
using molecular and
bio-chemical methods**

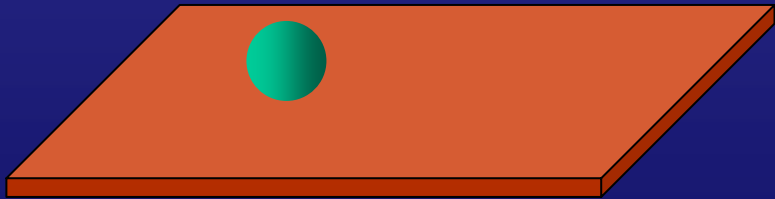
Host-parasite (pathogen)

Algae-grazers

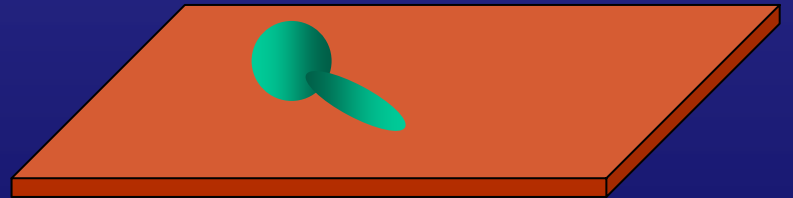
Defense against epi- and endophytes

Host-pathogen recognition

- Mechanical Signals (?)
- Chemical Signals (?)



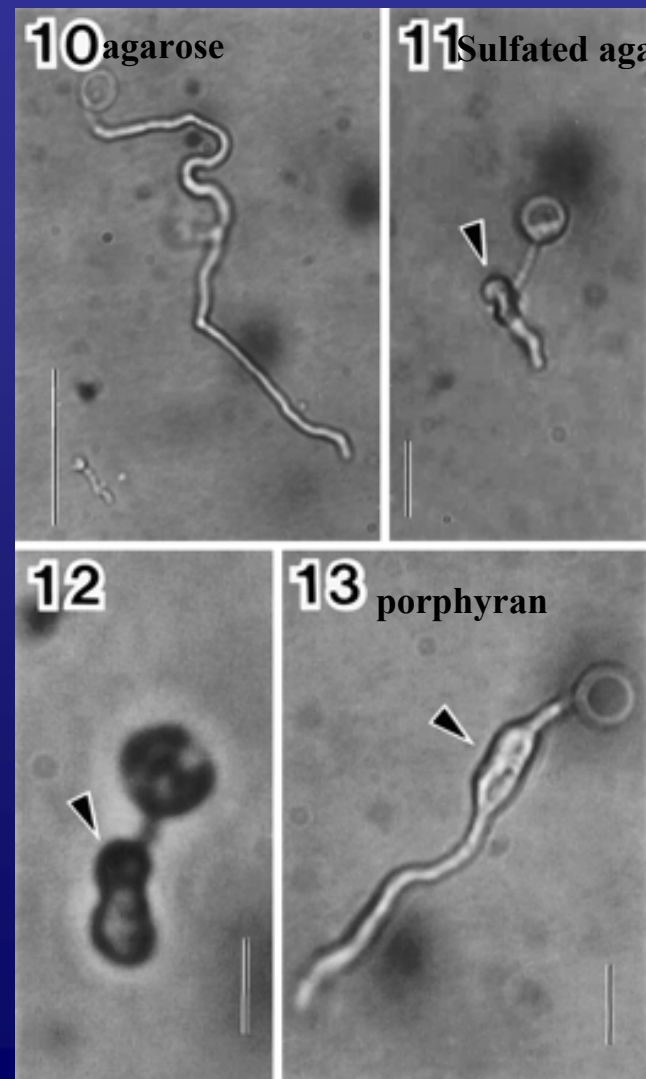
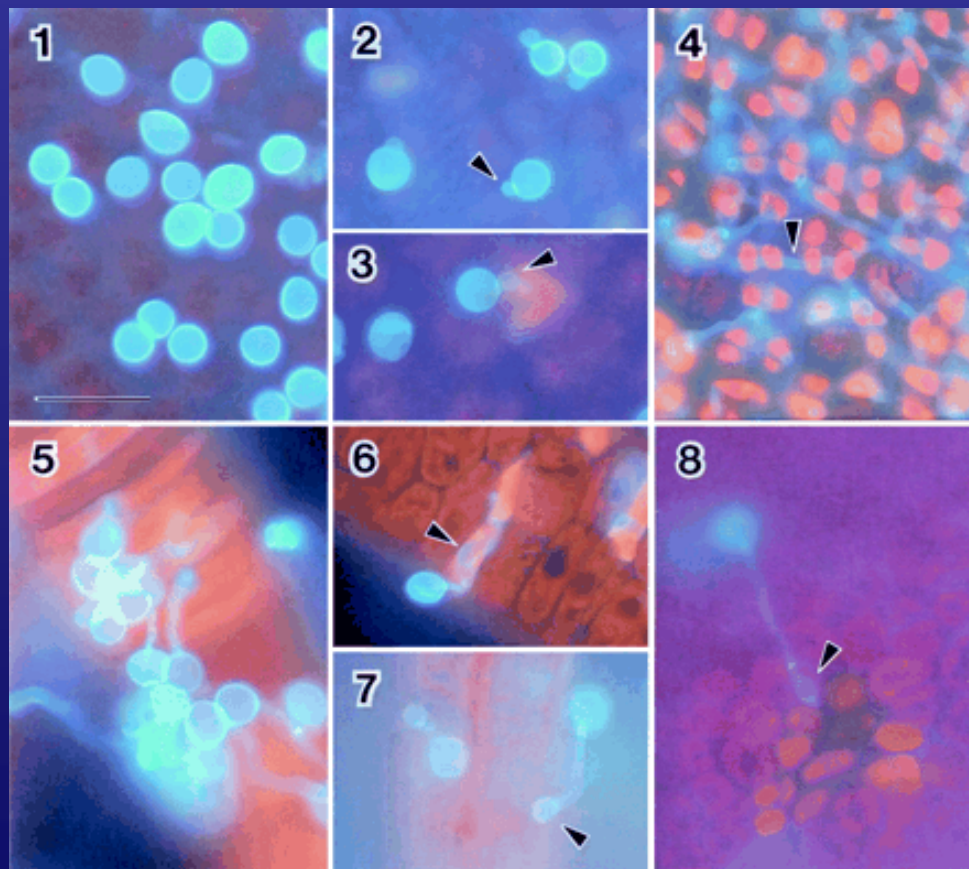
Attachment



Penetration



Porphyra yezoensis/ Pythium porphyrae



appressoria formation is mediated by the perception of sulfated agar located in host cell walls

Defense mechanisms in seaweeds

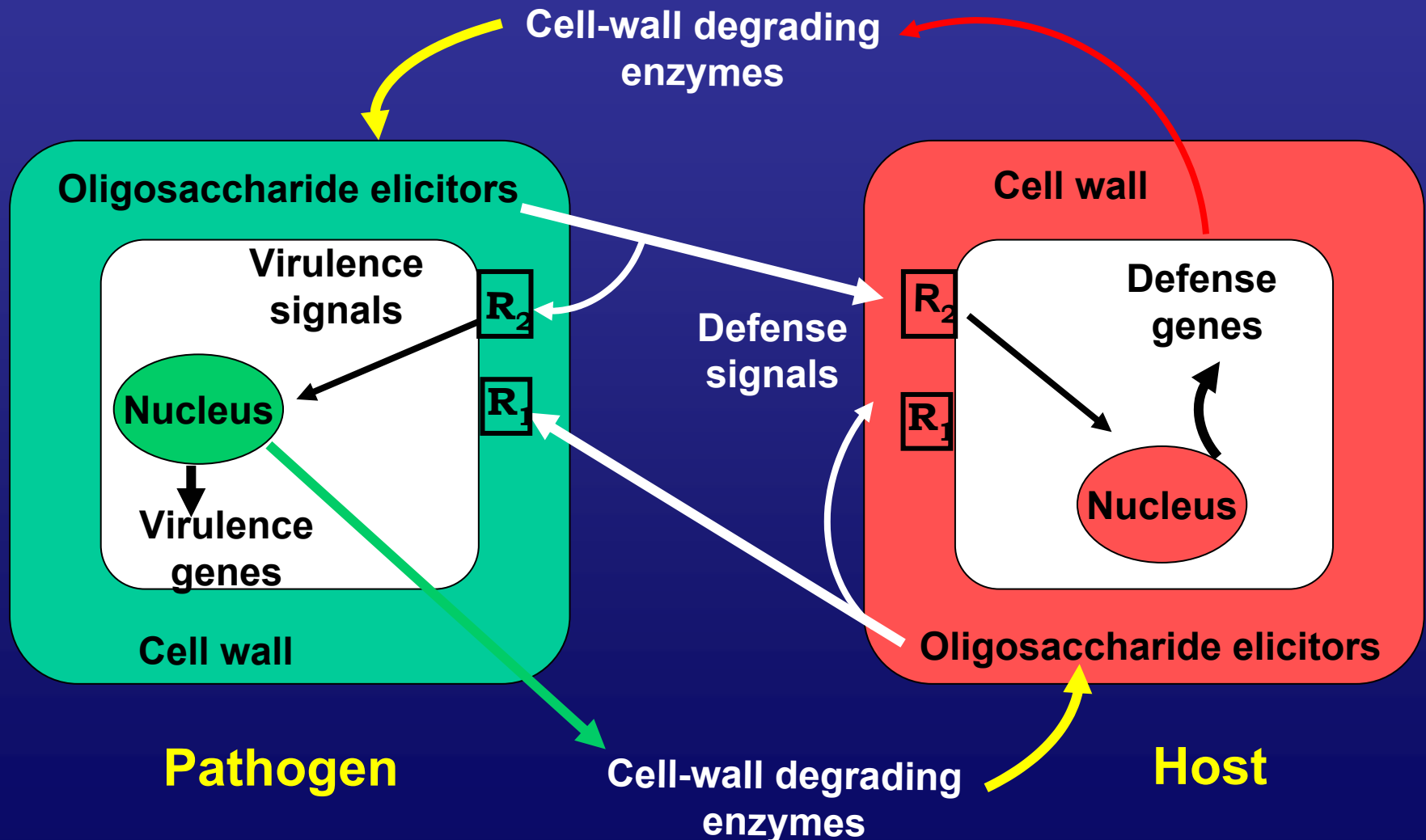
- **Oligosaccharides as signalling compounds**
- **The importance of halides**
- **Oxylipins as secondary signals in regulation processes**

An underwater photograph of a kelp forest. Large, dark green kelp blades are visible, with a prominent, lighter green blade in the foreground. A small, brown, segmented sea slug is crawling on the blade. The background is dark and slightly out of focus.

*Laminaria digitata/
Laminariocolax tomentosoides*

Frithjof Küpper 2001

Cell-cell recognition in host-pathogen interactions



The life cycle of *Laminaria digitata* (Phaeophyceae)

sporophytes: diploid

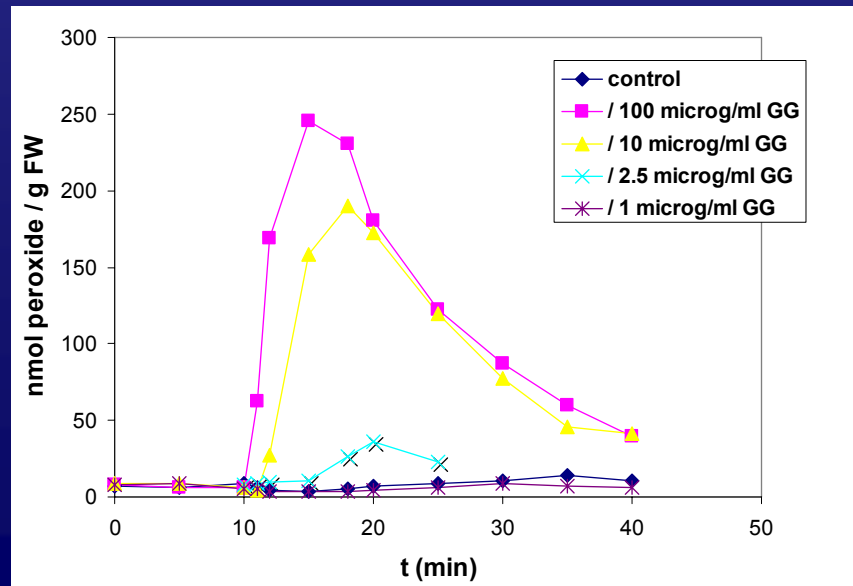
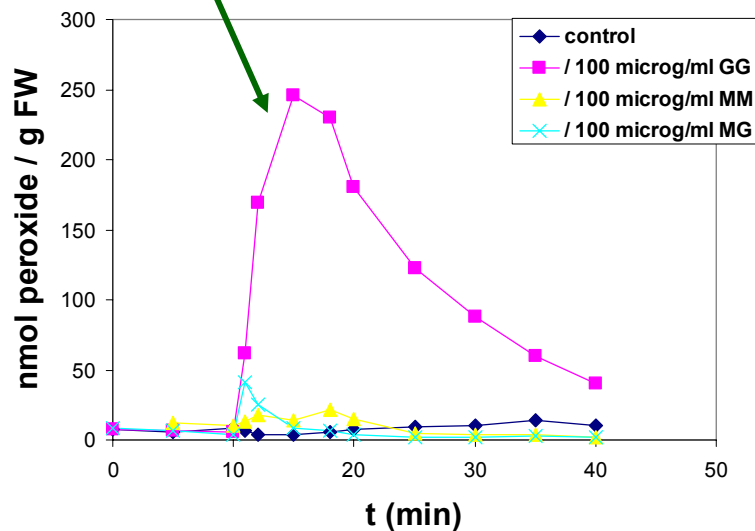
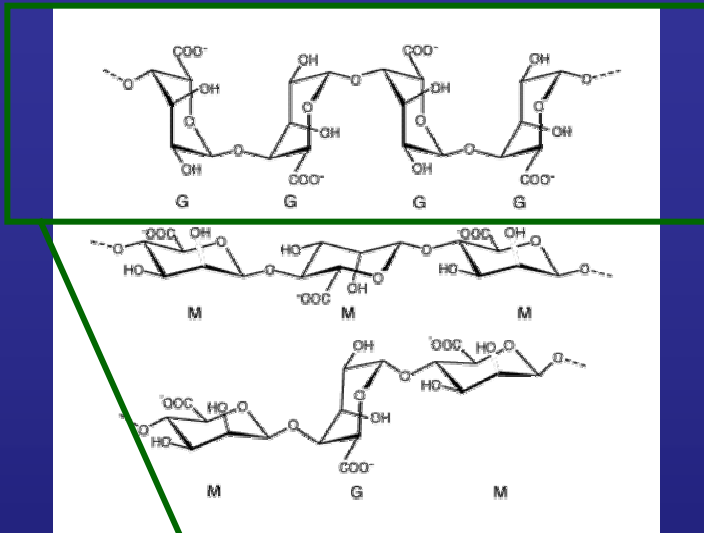
gametophytes: haploid

fertilization

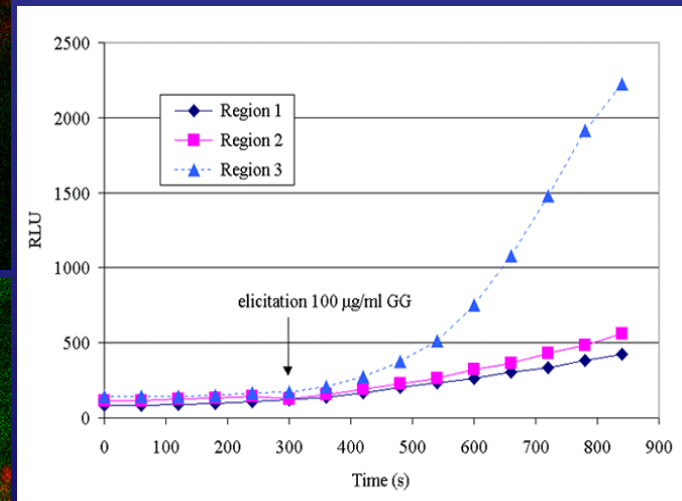
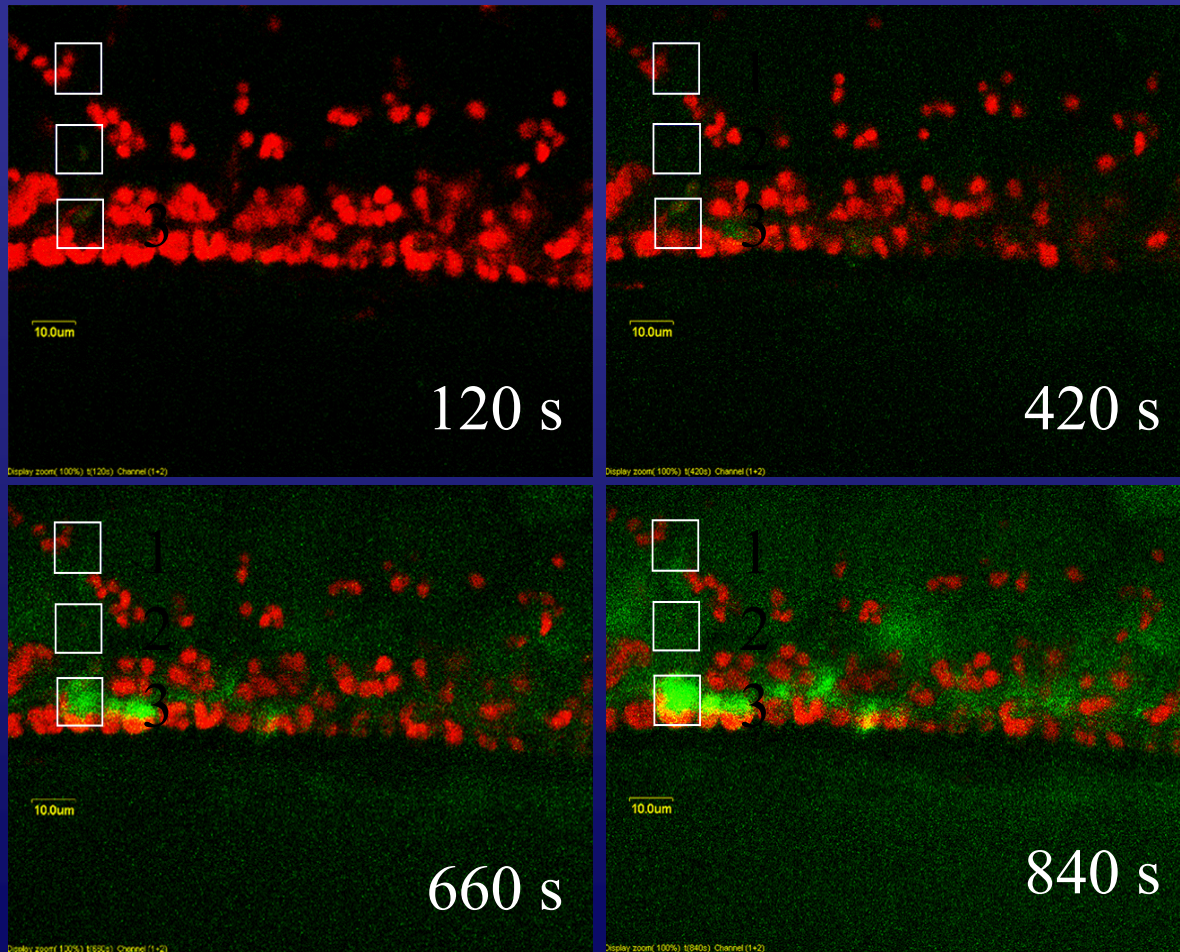


meiosis

Oligoguluronates induce an oxidative burst in the sporophytes of *Laminaria digitata*



The oxidative burst in the apoplast of *Laminaria*, visualized by DCFH-DA and confocal microscopy



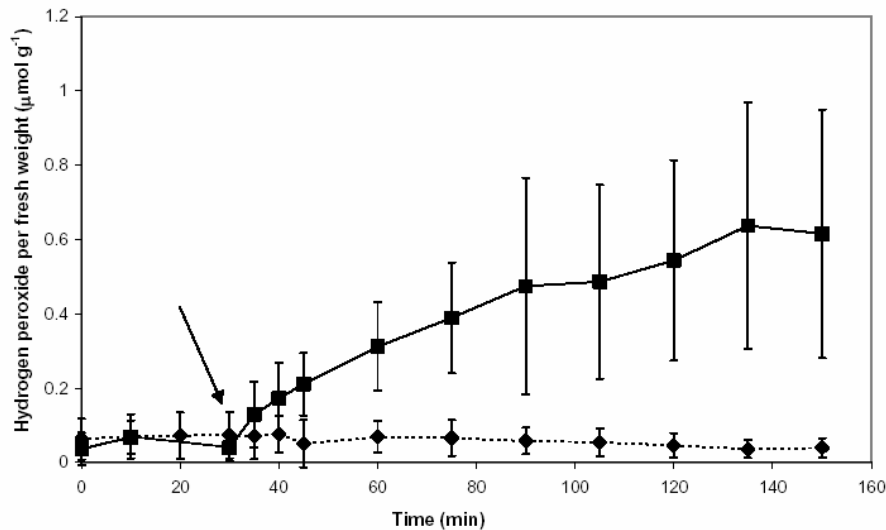
DCFH-DA :
dichlorohydrofluorescein
diacetate

Elicitors of defense responses in *Laminaria* sporophytes

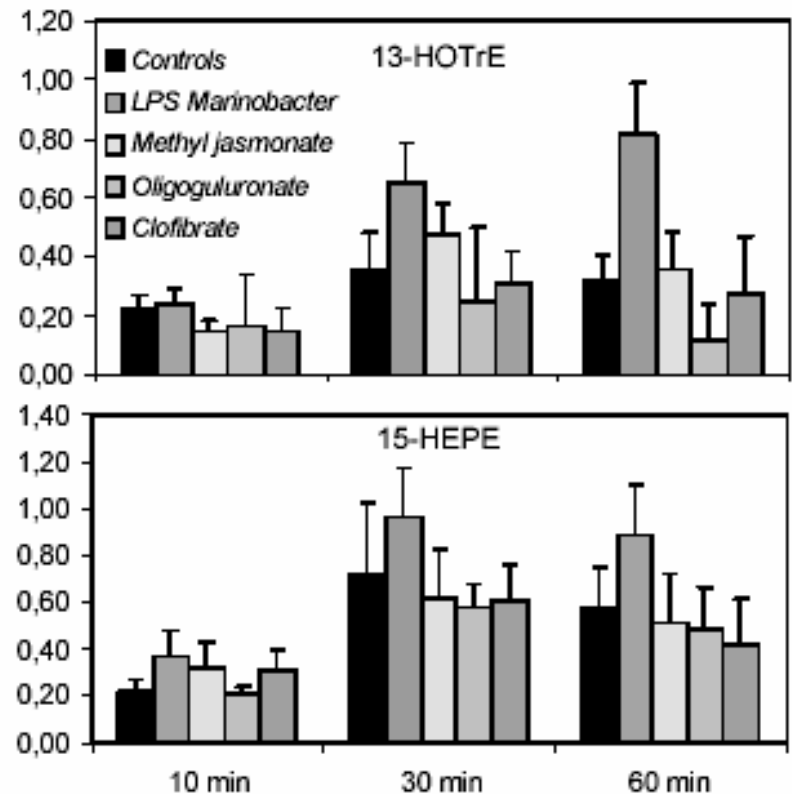
- oligo-guluronates
- bacterial endotoxins
- arachidonic acid
- methyl jasmonate

Gametophytes do not respond!

Early events in the perception of lipopolysaccharides in the brown alga *Laminaria digitata* include an oxidative burst and activation of fatty acid oxidation cascades



Strong H_2O_2 emission



Production of oxylipins.

Bioassay to test induction of resistance or susceptibility of *L. dig.* against *L. tomentosoides*

L. digitata

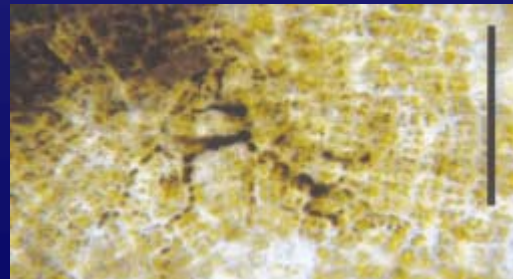


Pre treatment with
oligoguluronates over
different time series



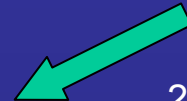
Provasoli ES (50 ml)

Data recording

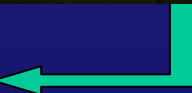


plantlet
L. digitata

2–20 mm in thallus length



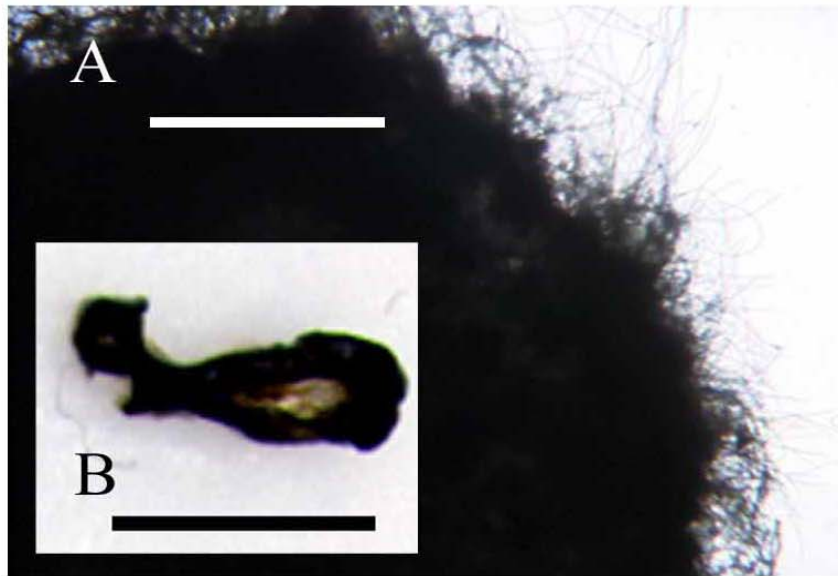
3 days
Co-cultures



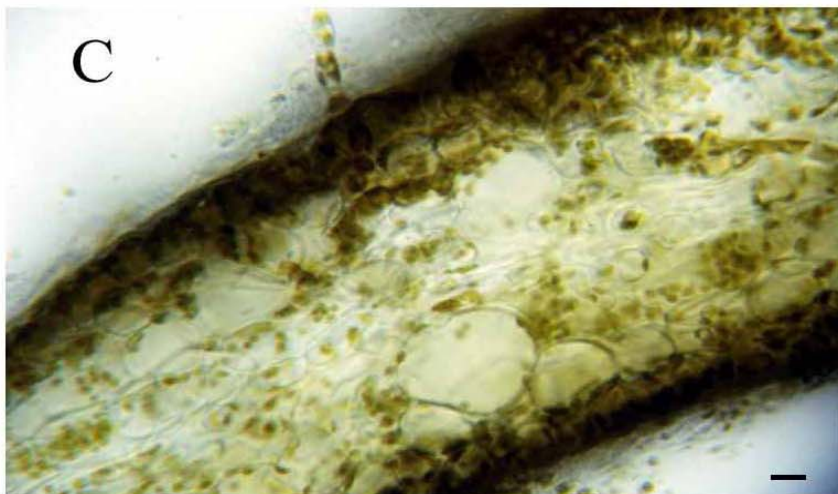
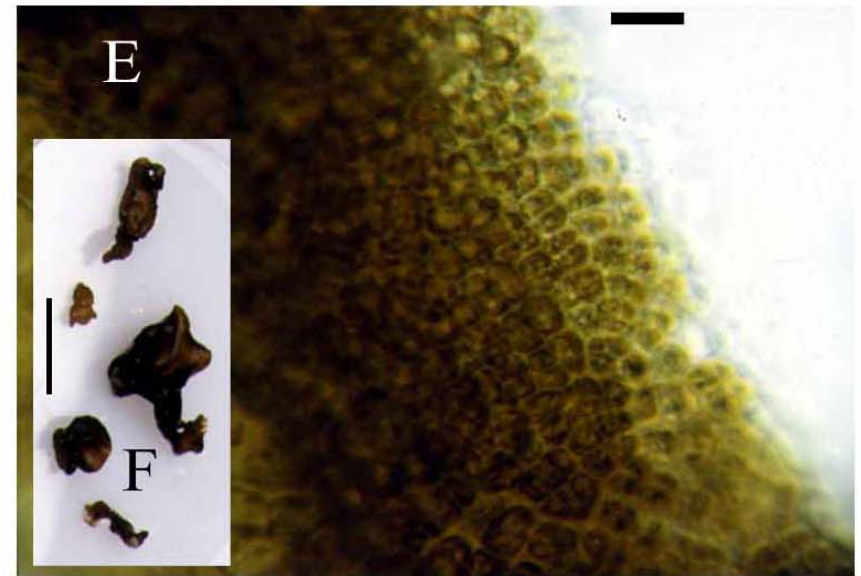
3 weeks
2 Medium
changes/week

Oligoguluronates induce resistance against *Laminariocolax tomentosoides* in *Laminaria digitata*

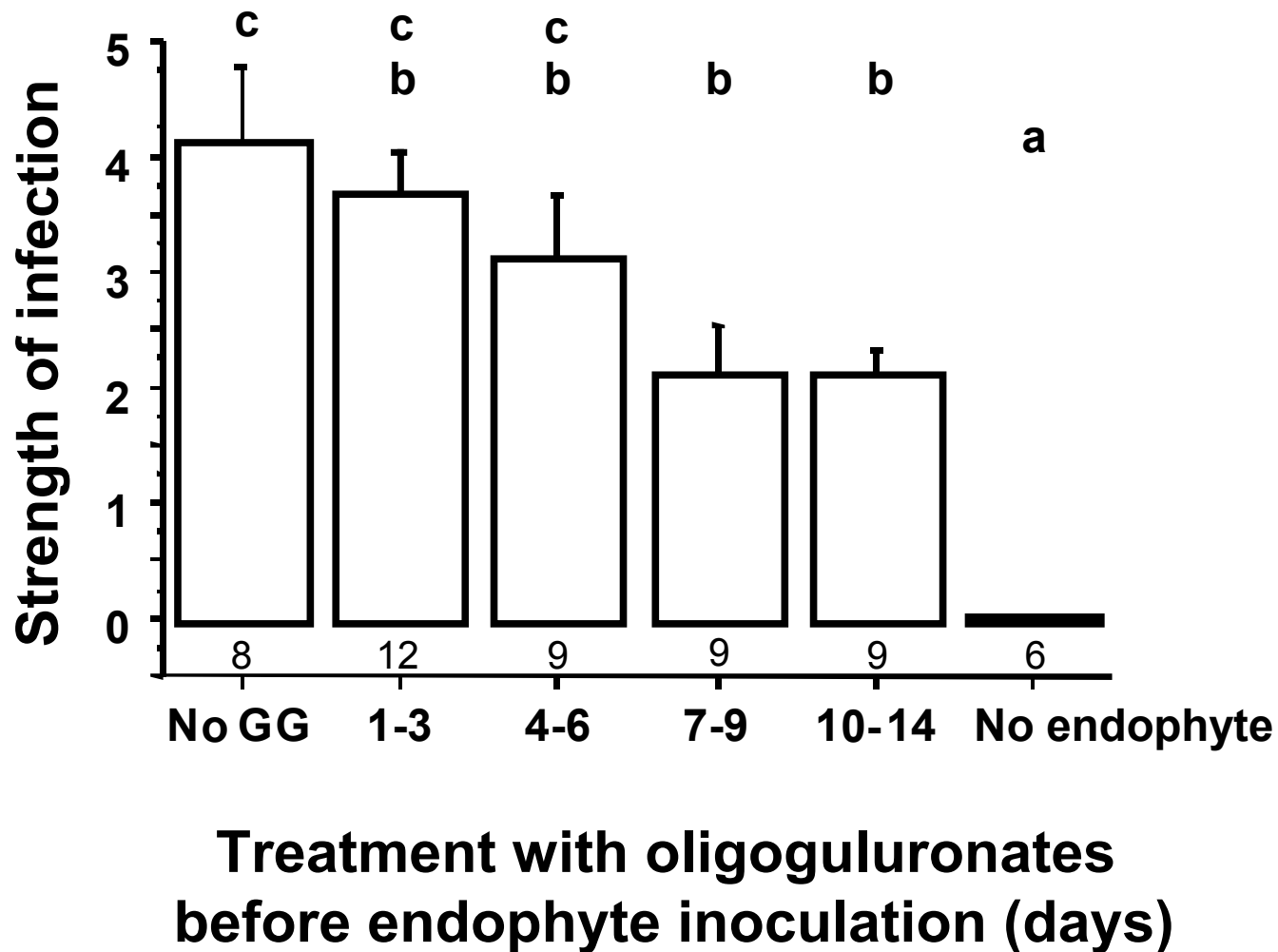
Control



Elicited

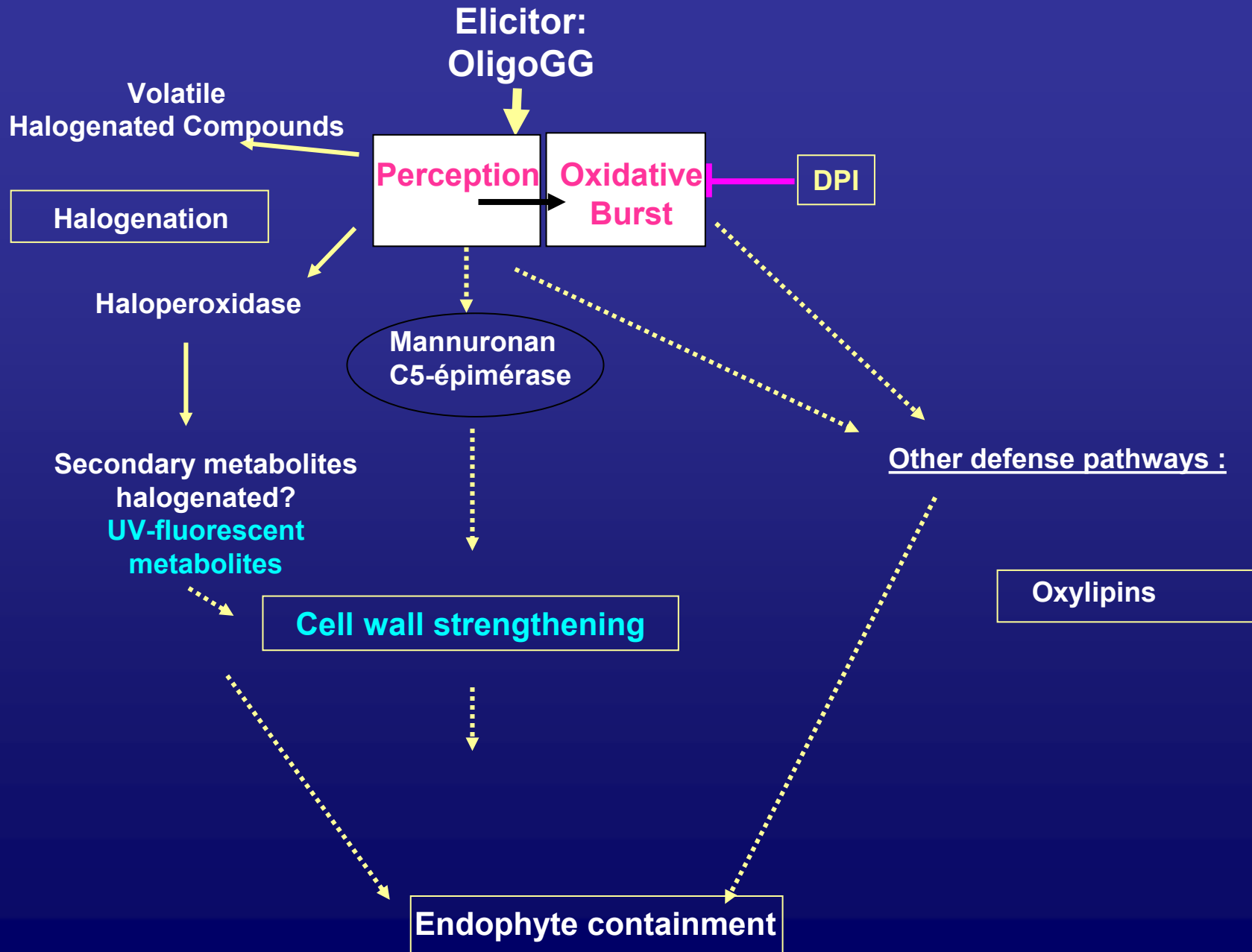


Establishment of the oligoguluronate-induced resistance in *Laminaria digitata*.

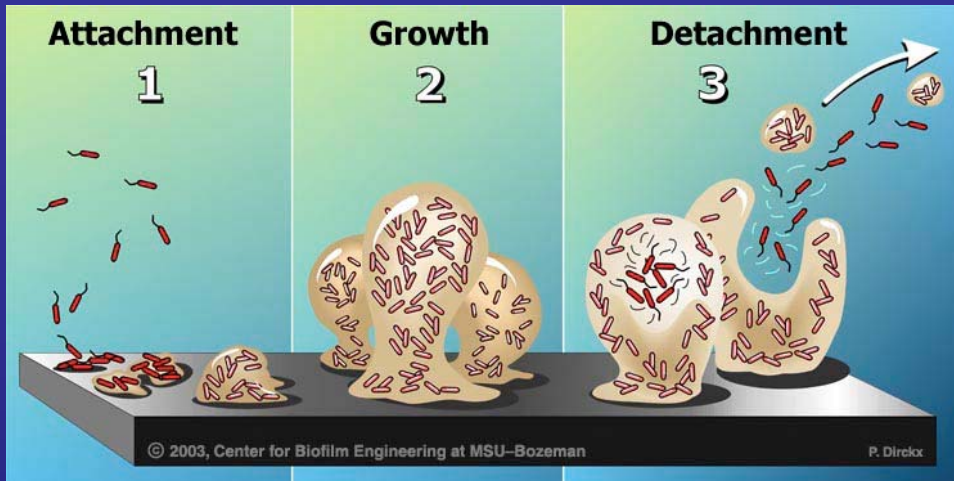


0, no symptoms of pathogen infection nor unusual necroses;
1, infections and/or necroses only very marginal;
2, few infections or necroses: overall plants in good shape;
3, strong infections or necroses in at least one plant, but other plants of the same batch in good shape;
4, strong infections and necroses in several plants;
5, strong infections, at least one plant dead; and
6, massive infections, all plants dead.

Metabolic pathways involved in the natural resistance of *L. digitata*



Control of Biofilm dynamics



Direct effect of algal-generated oxidants and metabolites on the communication signals of Gram- and possibly Gram+ bacteria

Kelp-herbivore interactions



- Functional Biodiversity
- Population structure
- Analysis of Food webs

EKOKEP

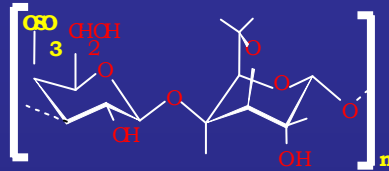
***Chondrus crispus/
Acrochaete operculata***

PhDs Bouarab 2000
Gaquerel 2005

The interaction between the red *Chondrus crispus* and the green algal endophyte *Acrochaete operculata*

GAMETOPHYTE

(n)

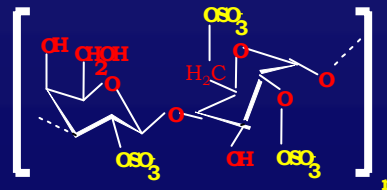


ou
κ-carrageenan

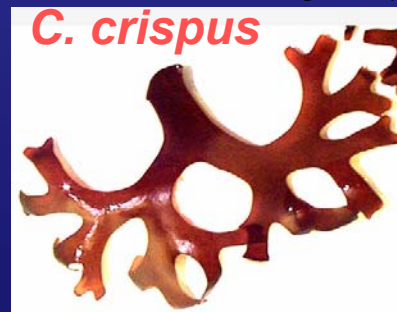
(2n)



λ-carragenan

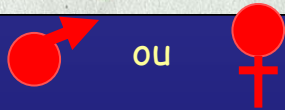


SPOROPHYTE



Only tetrasporophytic strains of *Chondrus crispus* are successfully invaded by *Acrochaete operculata*

(n)



κ-carrageenan

(2n)



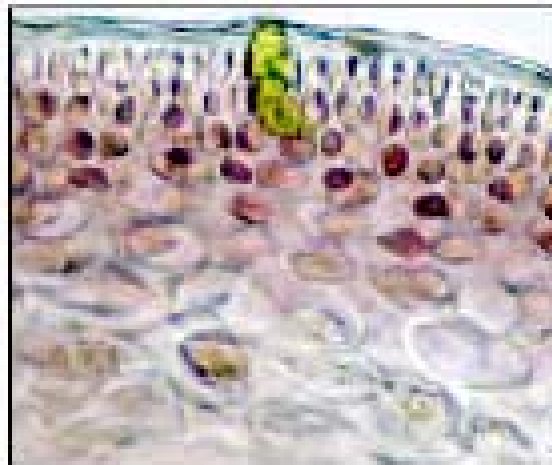
λ-carrageenan

Surface

Cross-section

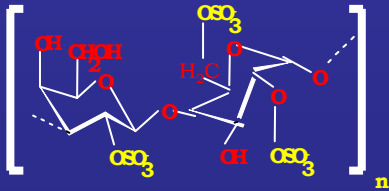
GAMETOPHYTE

SPOROPHYTE

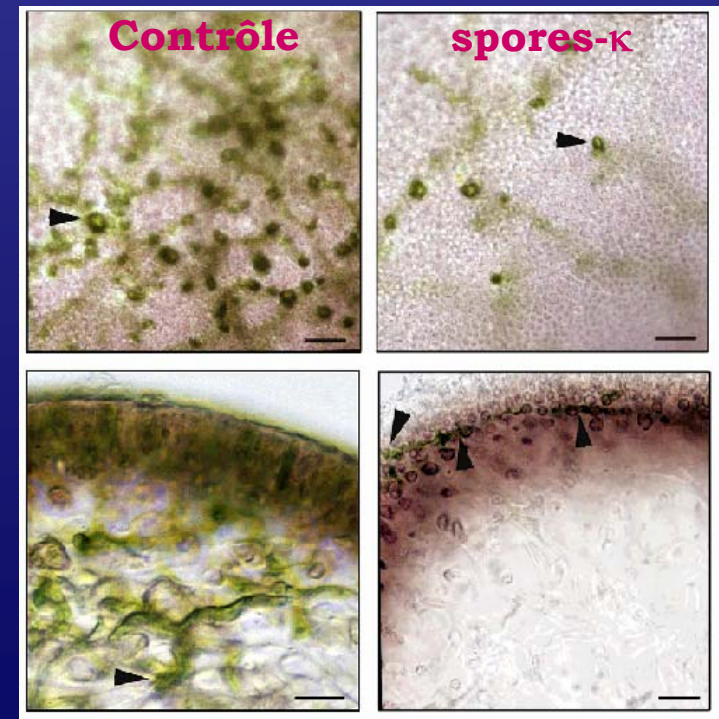
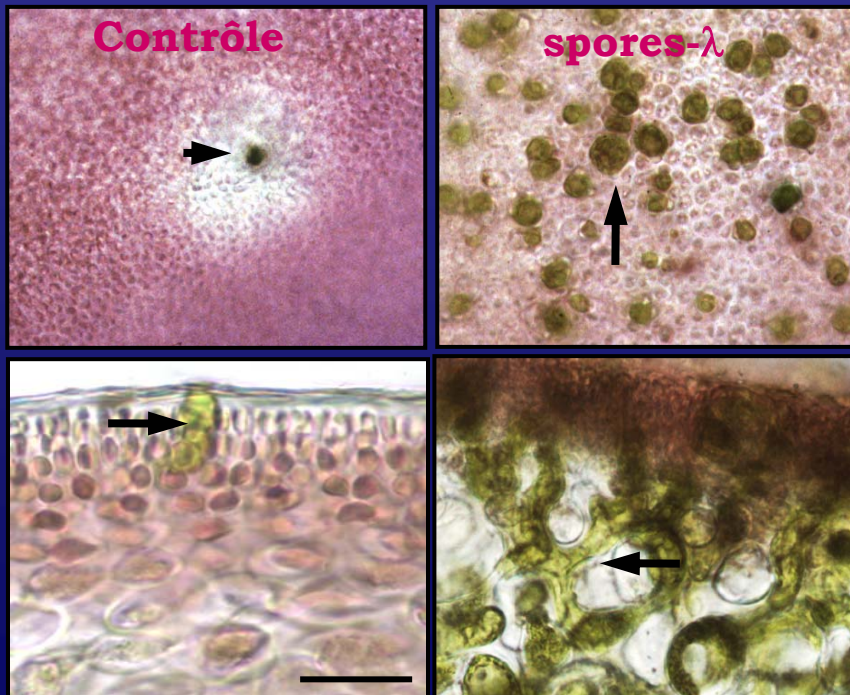


Carrageenan oligosaccharides modulate the interaction between *A. operculata* and *C. crispus*

Oligo- λ -carragenans enhance spore virulence



κ -carragenans inhibit spore virulence



C. crispus gametophytes respond by an oxidative burst to challenge with *A. operculata* cell-free extracts.

Host
C. crispus

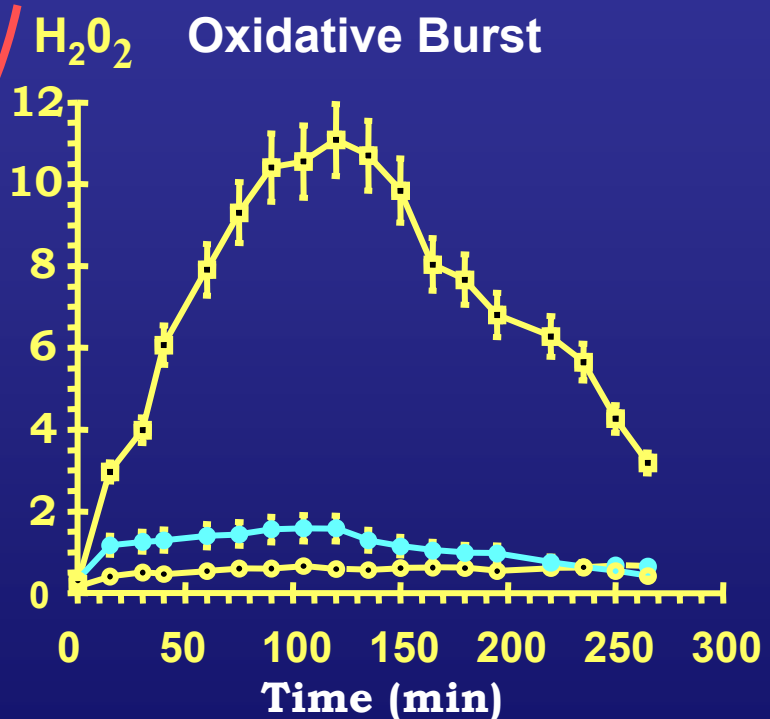
Pathogen
A. operculata

↓
Oxidative burst :
Signal?

Activation of NADPH
oxidase
(EST *Porphyra*
1 cDNA in *Chondrus*)

+

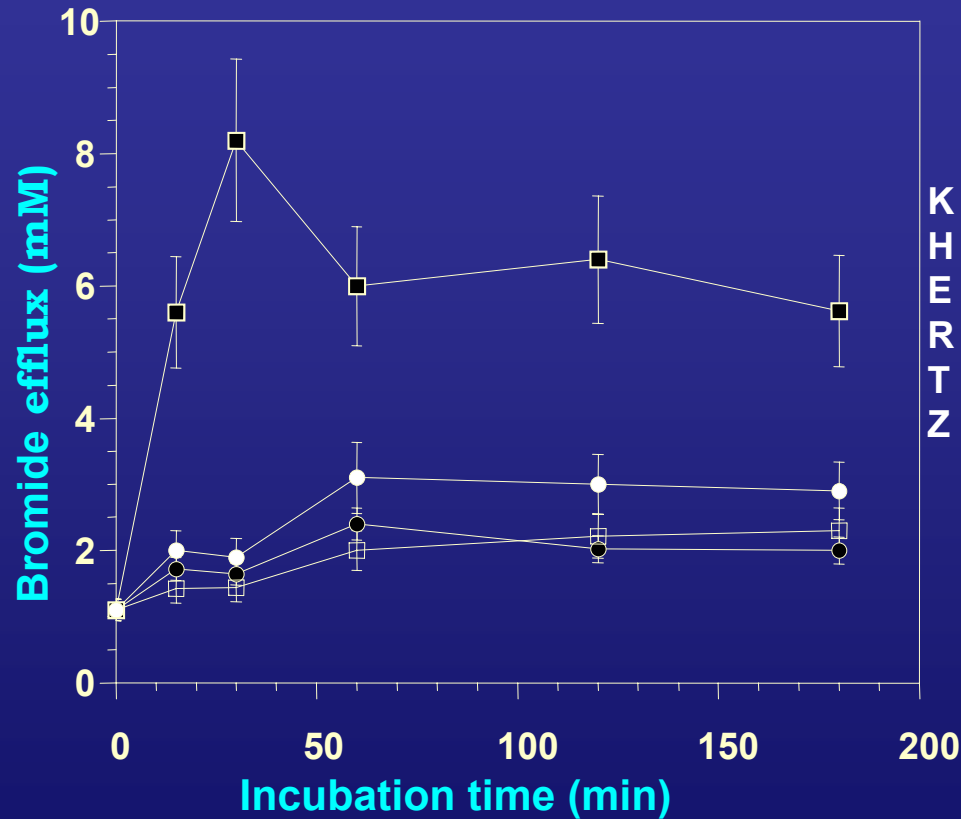
Induced
Defenses



—○— Control
—□— Pathogen extract
—●— 5 μ M DPI/elicitation

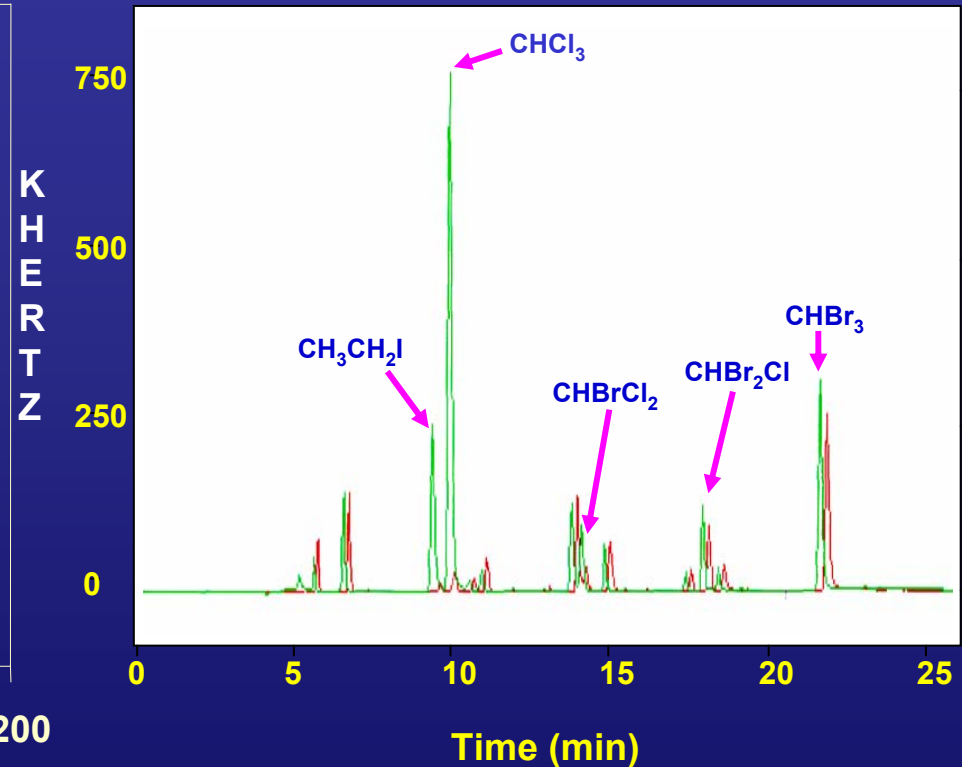
Bouarab et al.
Plant Cell, (1999) 11, 1635
Herve et al.
Curr genet. (2006) 49, 190

***A. operculata* extracts provoke a bromide efflux and halocarbon emission in *C. crispus* gametophytes.**

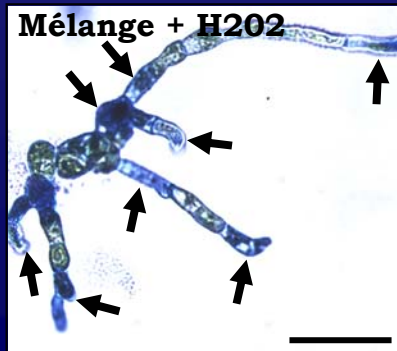
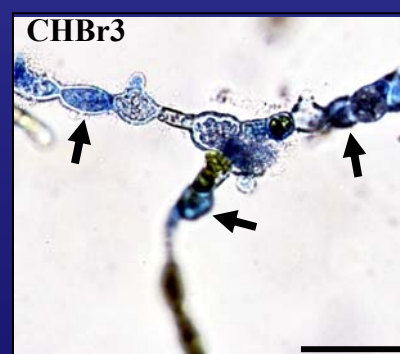
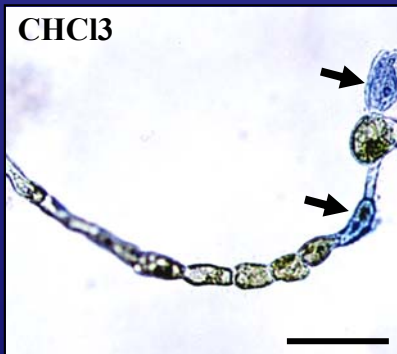
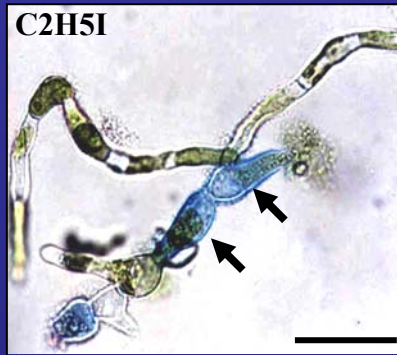
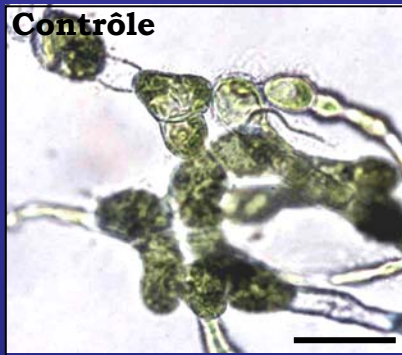


■ Elicitors
● Control

□ LaCl₃ 1 mM/Elicitors
● A-9-C 500 mM/Elicitors



Volatile halocarbons released by *C. crispus* gametophytes are toxic for spores and germlings of *A. operculata*

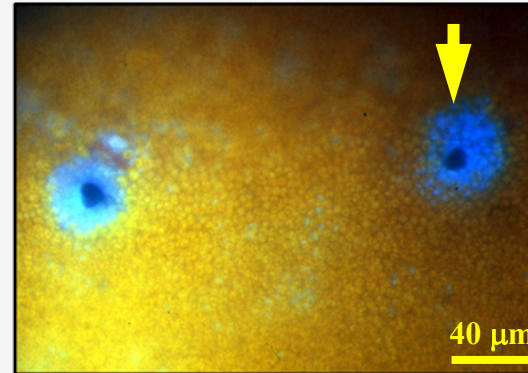
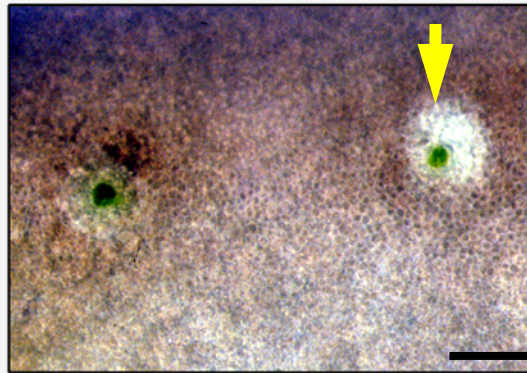


**UV-absorbing compounds accumulate
in *C. crispus* gametophytes at the sites of penetration by
A. operculata zoospores**

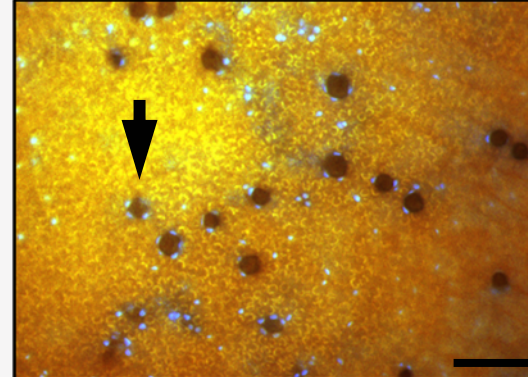
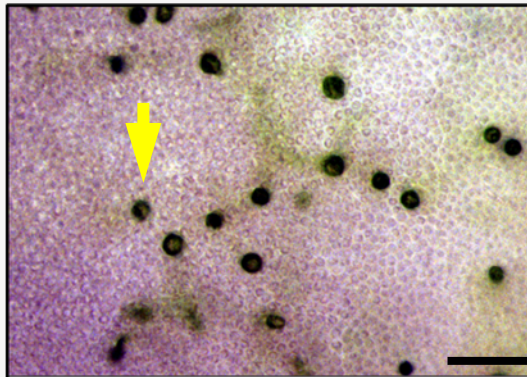
Bright field

UV

Gametophyte

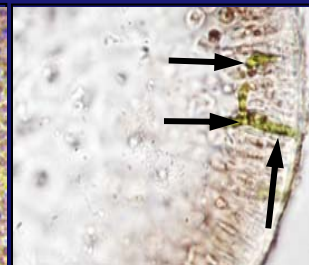
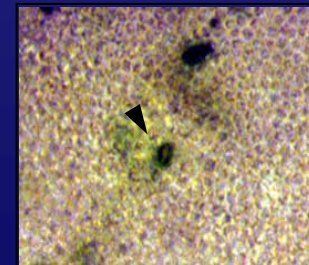
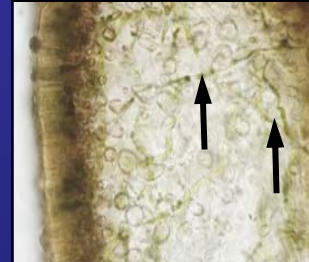
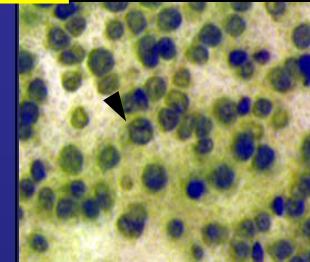


Sporophyte

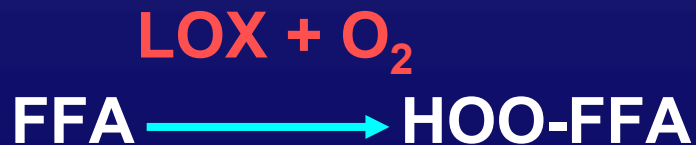
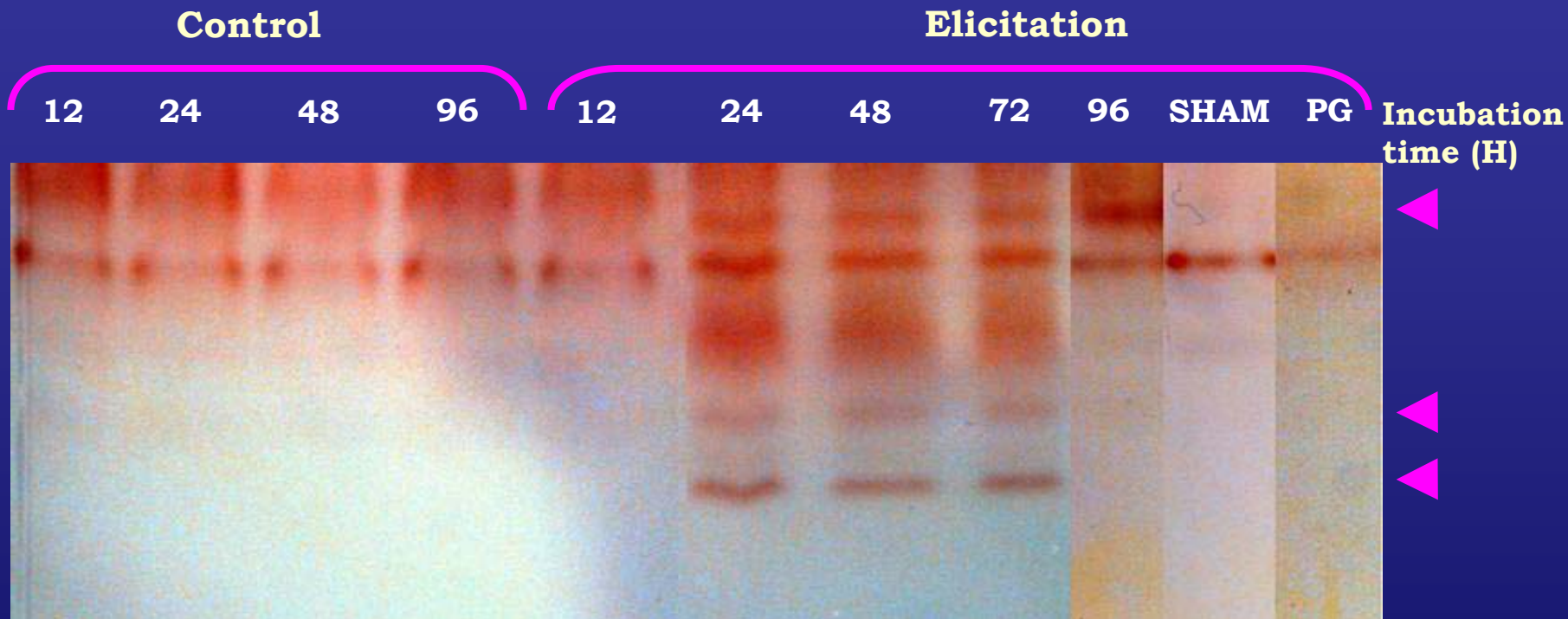


Oxylipins induce resistance against *A. operculata* in *C. crispus* sporophytes

Host	Oxylipin concentrations	Frequency of infection
Control sporophytes		70 ± 12
HPETE-treated sporophytes	30 µM	52 ± 14
	150 µM	24 ± 8
HPOD-treated sporophytes	30 µM	48 ± 10
	150 µM	18 ± 9
MeJ-treated sporophytes	1 µM	68 ± 11
	10 µM	45 ± 13
	100 µM	11 ± 5



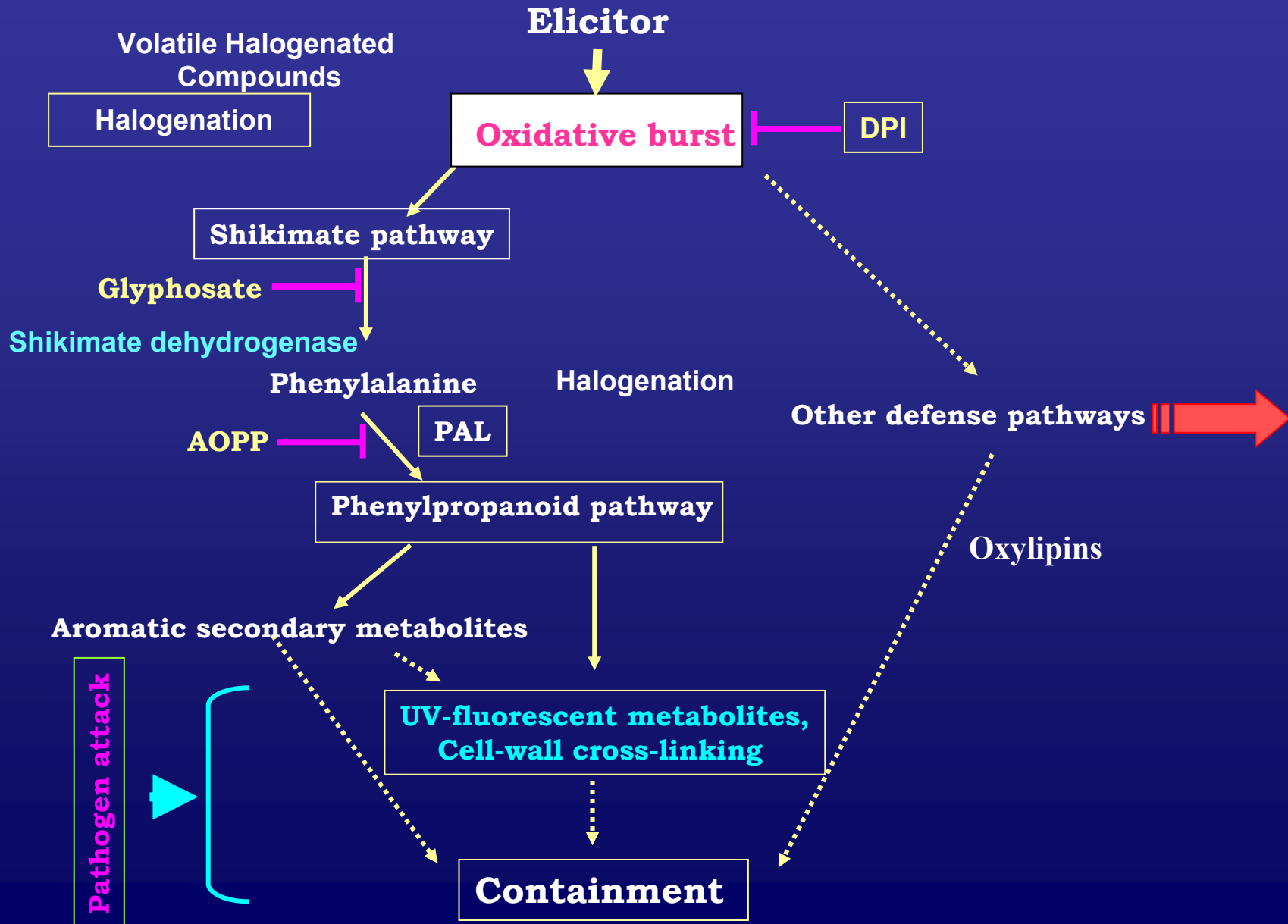
Time course of lipoxygenase activity in *C. crispus* gametophytes challenged by *A. operculata* signal



PG : Propyll-Gallate
SHAM

LOX inhibitors

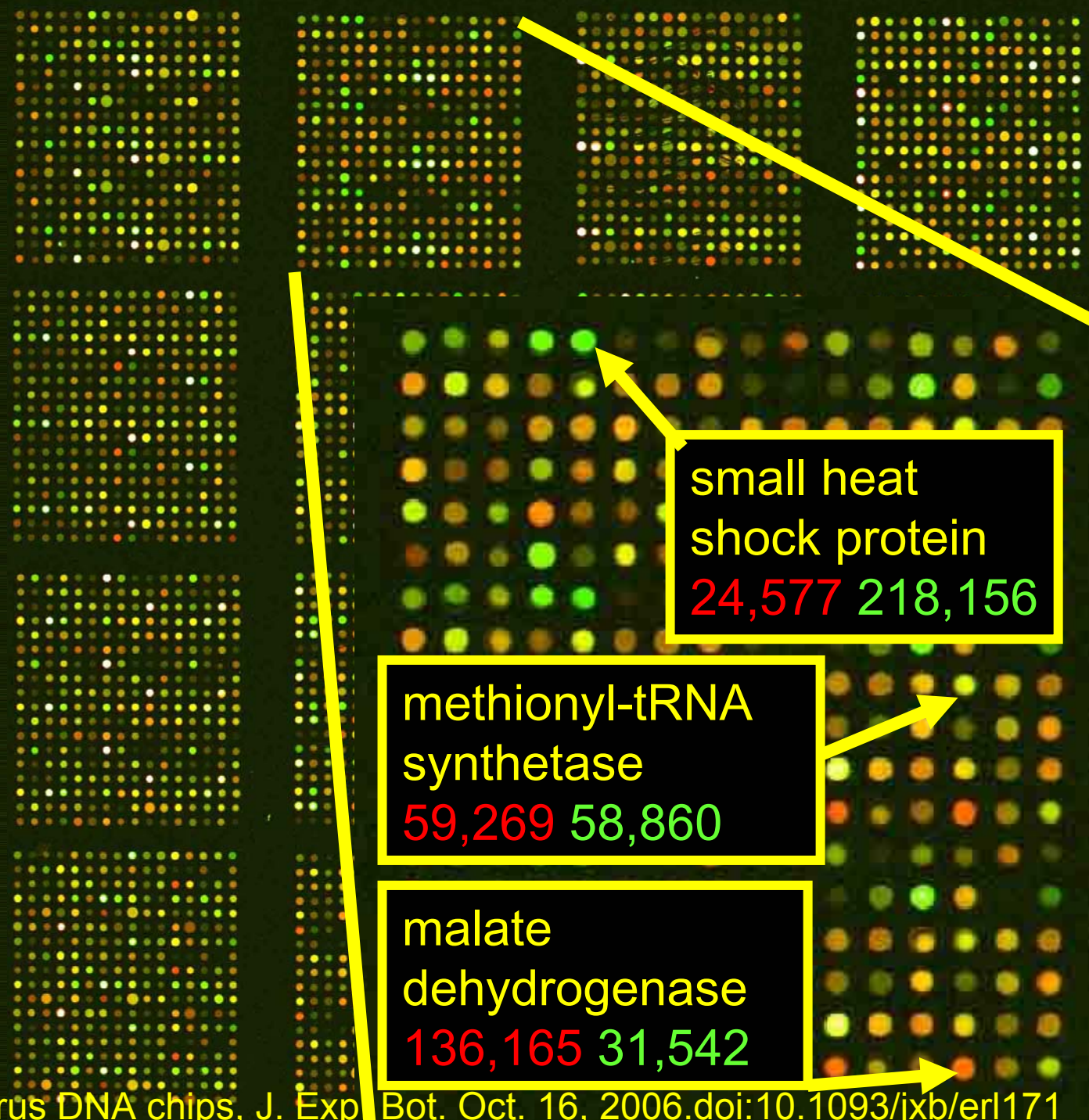
Involvement of the phenylpropanoid pathway in the natural resistance of *C. crispus* gametophytes



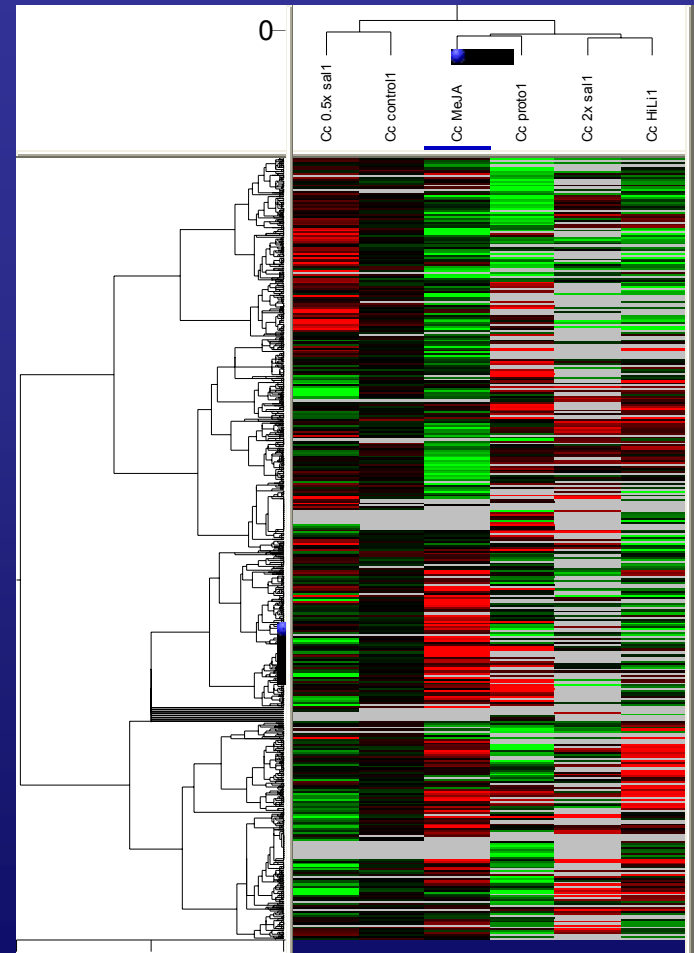
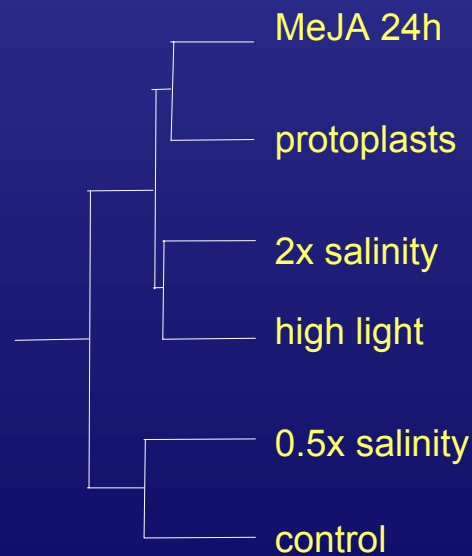
The array

- 1,295 different genes
- 1,920 spotted cDNA
- All spots in triplicate

Stress RNA
marked green
and control RNA
marked red



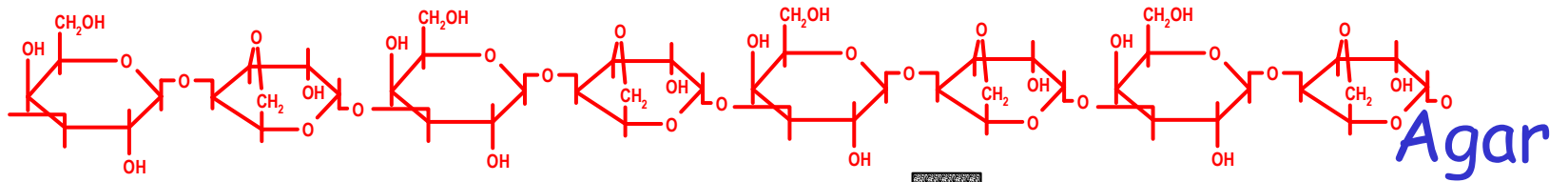
Which stress factors
provide similar
expression patterns?



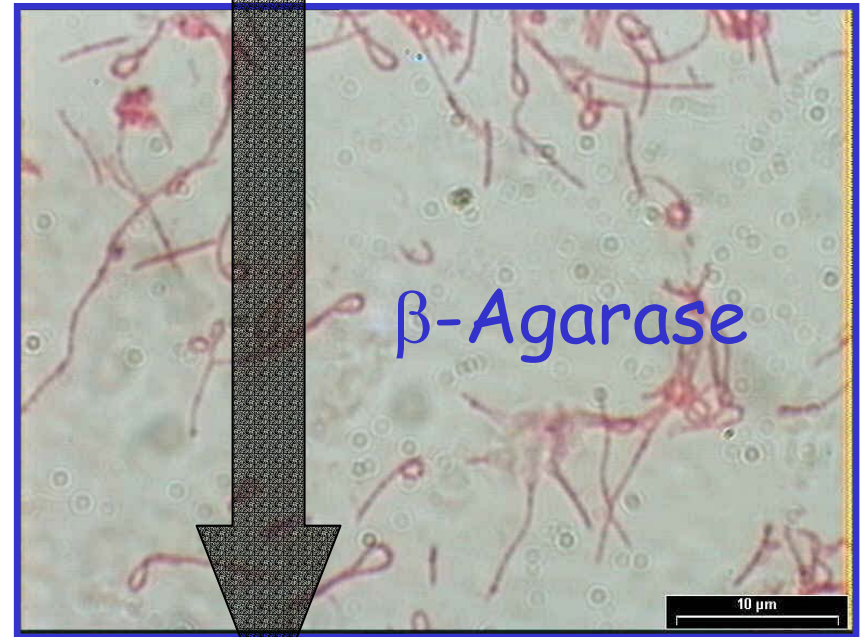
**Integration of transcriptomics and metabolomics for
understanding of global responses to
environmental stresses**

EPIFIGHT: Biochemistry and genetics of resistance to epiphytism in *Gracilaria chilensis*



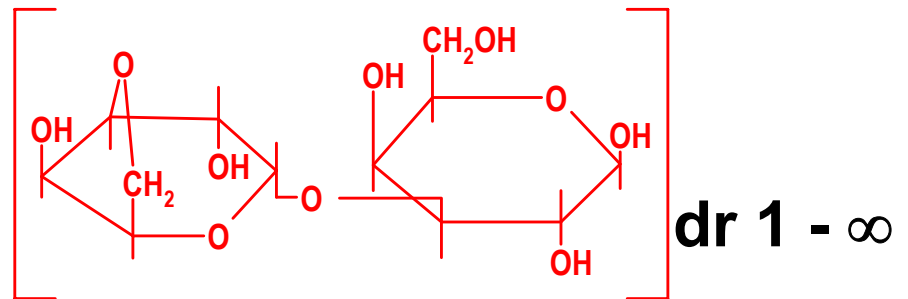


Gracilaria conferta



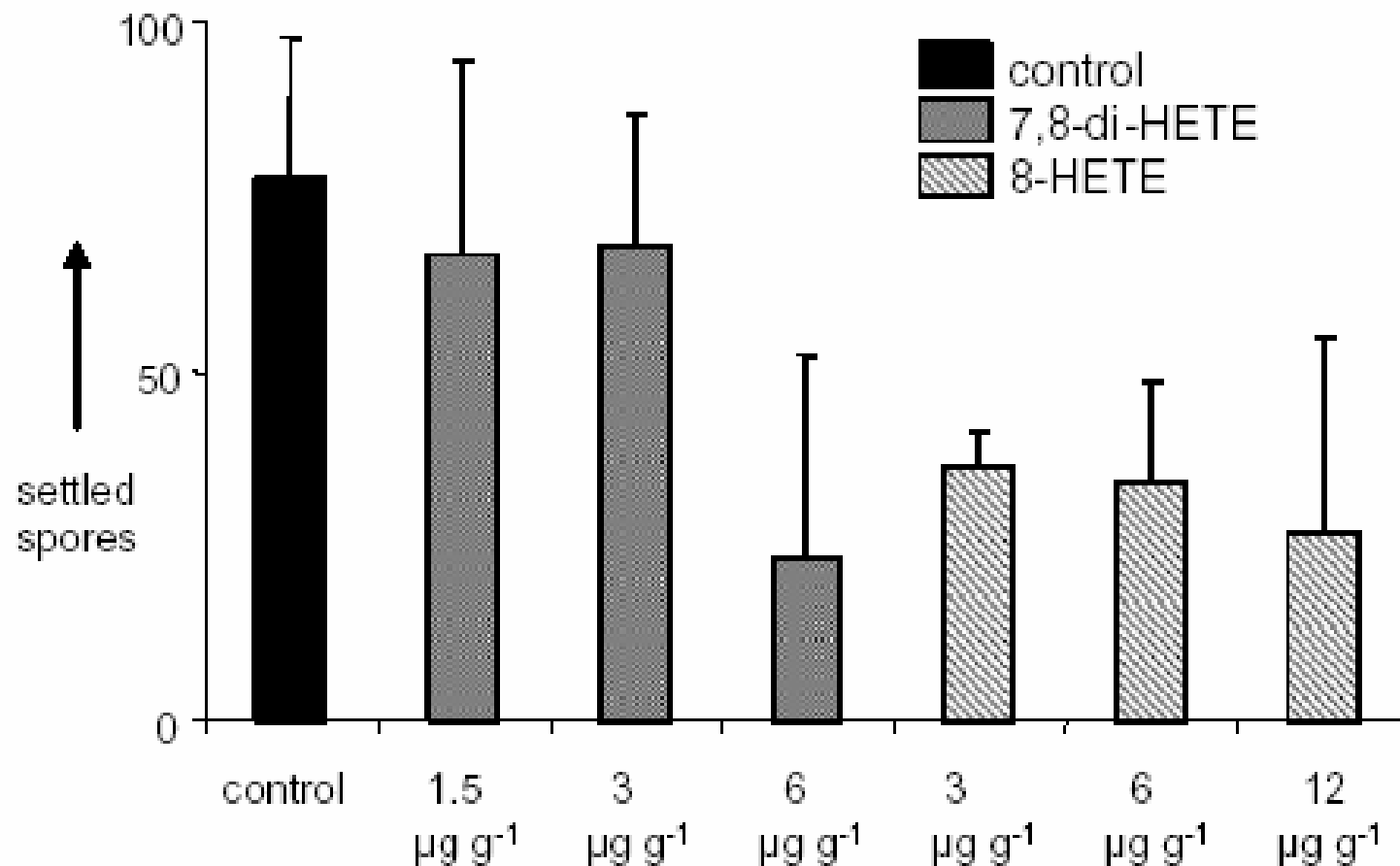
β -Agarase

Elicitation



Neoagararose-oligosaccharides

Two different lipase activities trigger the wound activated defense of *G. chilensis*.



Principal contributions of our research

A better understanding of biotic interactions in the sea

- Chemical signaling and allelopathic interactions
- Defenses/virulence responses may modulate food webs or the whole ecosystem.

A better understanding of the evolution of innate immunity in eukaryotes

- Conservation of the transduction machinery and of metabolism
- Adaptation to the marine environment (halides, aquatic envt)

Potential applications for algal mariculture

- explore the interest of host immunostimulation in land-based aquaculture facilities (nurseries and raceways)
- derive guidelines and tools to select disease-resistant genotypes.

The challenge is to integrate the reductionist approach with the population approach

An aerial photograph of a coastal town, likely Saint-Malo, France. The town is built on a peninsula, with a large harbor filled with many small boats. A large ship is docked at a pier in the background. The town features traditional European architecture with many buildings and a prominent church spire. The water is a deep blue, and the sky is clear.

Thank you

Collaborators

Emmanuel Gaquerel

Cécile Hervé

Kamal Bouarab

Jean-Pierre Salaün

Frithjof Küpper

Florian Weinberger

Ludovic Delage

Bernard Kloareg

Georg Pohnert

Uli Lion

Juan Correa

Jessica Beltran

Dieter Müller

Akira Peters