ECFG15
ROME • ITALY 2020

crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Signals in pathogen and host sensing: free fatty acids and oxylipins

Speaker: Valeria Scala

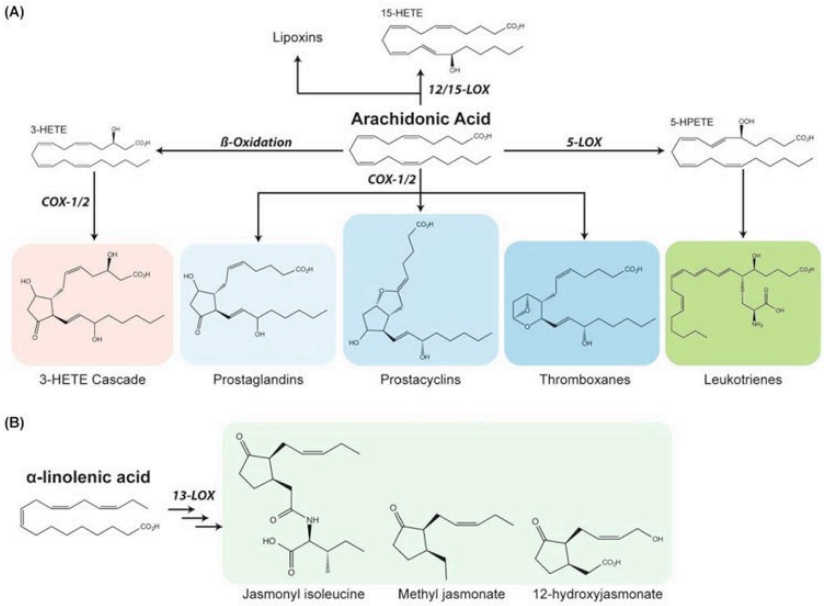
LIPIDS

- Lipids: major constituents of prokaryotic and eukaryotic membranes. The ratio saturated/unsaturated FA contributes to membrane fluidity
- The viscosity of the membrane affects diffusion and movement of proteins and other bio-molecules
- Biological functions in structural components, energy/carbon storage, signal transduction and **stress responses**
- Plants, fungi and bacteria contain a diverse set of lipids (fatty acids, phospholipids, glycolipids, sterol lipids, sphingolipids and waxes)

Fahy E.J Lipid Res2009 https://slideplayer.com/slide/13151606/

LIPIDS IN EUKARYOTES

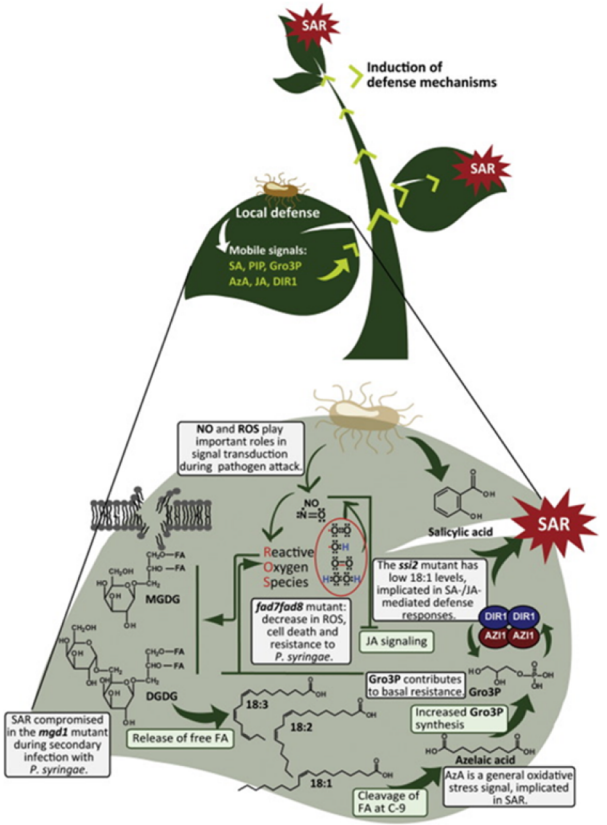
- Higher eukaryotes employ oxylipins to respond to environmental factors
- LOX and COX derived oxylipins play an important role in inflammation, in numerous diseases and adaptive immune responses
- Fungus *Paracoccidioides brasiliensis* inhibits the production of host oxylipins to escape cell recognition
- Yeast *Dipodascopsis uninucleata* and *C. albicans* mimic host oxylipins production



Fischer and Keller 2016

LIPIDS IN PLANT

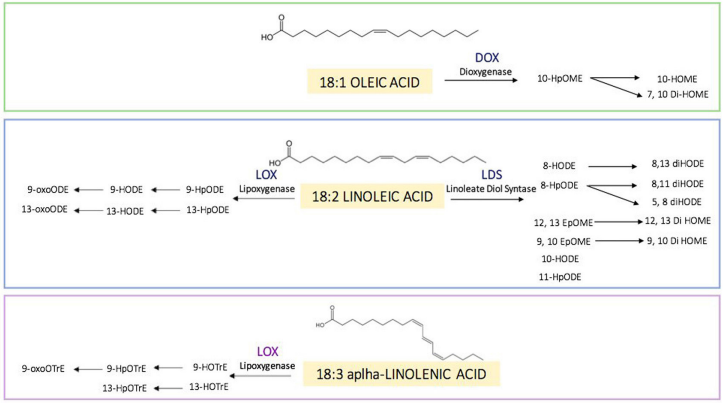
- Lipid metabolism is upregulated after pathogen infection
- Complex lipids are hydrolyzed and contribute to the host defense, inducing the production of phosphatydic acid (PA) and oxylipins such as jasmonates (JA), *inter alia*
- Free fatty acid (e.g. 18:1, 18:2, 18:3) levels affect SA- and JA-mediated defense responses

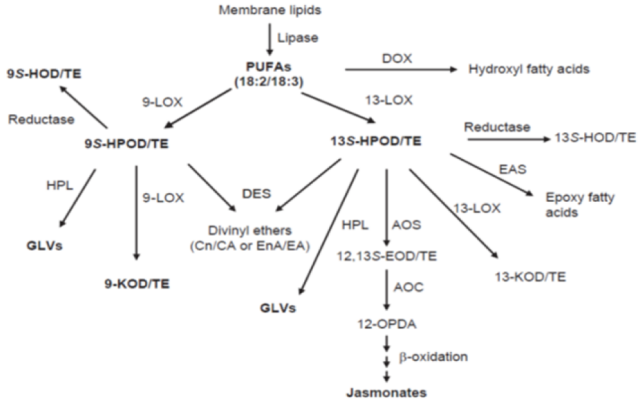


http://dx.doi.org/10.1016/j.bbalip.2016.02.021

OXYLIPINS (OXY)

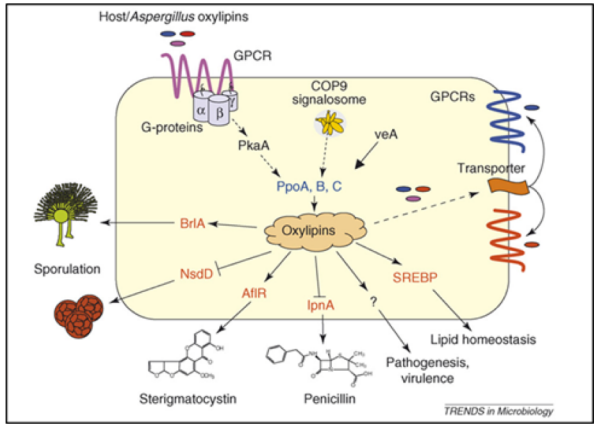
- Oxidized fatty acids produced enzymatically or not
- Signals in stresses condition, programmed cell death (PCD), susceptibility factors or resistance ones (i.e. 9-oxylipins; 13-oxylipins)
- Cross-species signaling between plants and fungi
- Fungi oxylipins mediate important plant immune responses and can impact plant LOX expression
- G-protein coupled receptors and activate cAMP cascade
- Fungal LOXs divided into two groups: a C-terminal isoleucine (Ile-group) or valine (Val-group)



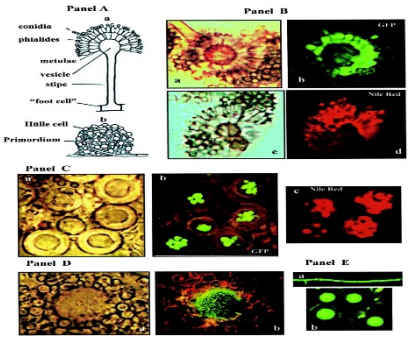


OXY IN FUNGI

- *Magnaporthe oryzae, Cercospora zeae-maydis, Ustilago maydis, Aspergillus flavus, Fusarium verticillioides* and *Trichoderma* spp
- Modulate sexual and asexual sporulation, the quorum sensing, the host colonization and secondary metabolism regulation
- PpoA (oxylipin producing gene) shows a targeting motif to lipid bodies
- The LOX Val-group contains a conserved sequence for an extracellularly secretion



PpoA localizes in lipid bodies of asexual and sexual fruiting bodies (Tsitsigiannis et al. 2004

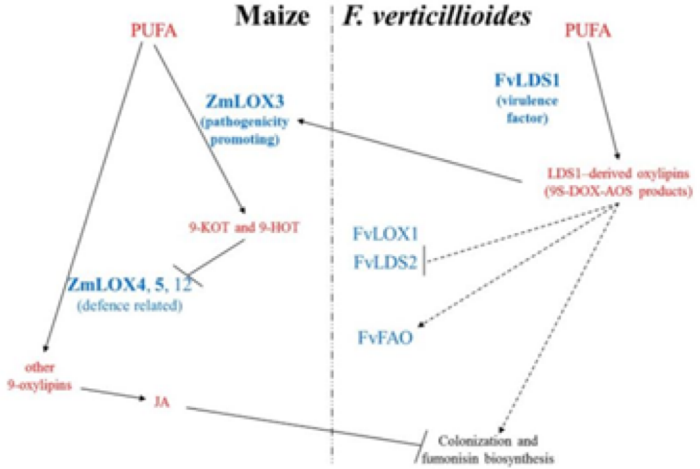


OXY IN *FUSARIUM VERTICILLIOIDES*

- *F. verticillioides lds1* oxylipin-gene influences plant 9-oxylipin accumulation in the host
- Deletion of LDS1 (homologous to *ppoA*) in *F. verticillioides (Fv)* generates more mycotoxins, conidia, reduced production of oxylipins and Fv is more virulent to maize ears

Mol Plant Pathol. 2018 Sep; 19(9): 2162–2176.
Published online 2018 Jul 17. doi: 10.1111/mpp.12690

Figure 8



Model depicting the oxylipin-mediated cross-talk between maize and

- *Fv* LDS1 and host LOX3 oxylipins (9-oxylipins producer) are essential for the normal infection and colonization process
- Host LOX3 is a major susceptibility factor induced by fungal LDS1-oxylipins to suppress JA pathways
- JAs suppress *F. verticillioides* colonization

AOS, allene oxide synthase; DOX, dioxygenase; FAO, fatty acid oxygenase; 9-HOT, 9-hydroxyoctadecatrienoic acid;
JA, jasmonic acid; 9-KOT, 9-ketooctadecatrienoic acid; LDS, linoleate diol synthase; LOX, lipoxygenase

OXY IN *F. verticillioides*

In vitro results «mirror» the field ones?

- 120 ears of commercial hybrids with different FB concentration
- PCA of lipid compounds shows that lipids changed significantly among high FB (HFB) and low FB (LFB) content
- In HFB: 13-HODE decreased whereas 9-HODE increased

In *F. verticillioides* and host oxylipins cross talk we can propose the following model

- PUFAs are oxygenated by maize and Fv
- Host promotes JA-mediated defences
- Fv oxylipins down regulate the parasitic behaviour, favouring an endophite one to colonize the host

FFAs and oxylipins are eliciting molecules and regulate the Fv lifestyle

What happens in other plant pathogen?

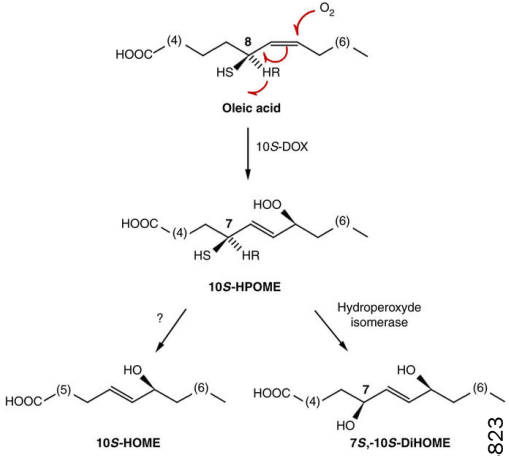
LIPIDS IN BACTERIA

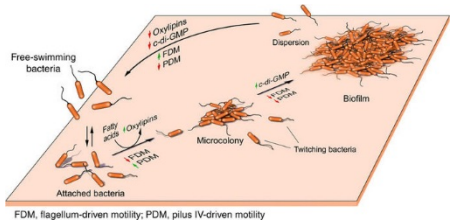
In response to environmental challenges, bacteria remodel their membrane lipid composition to survive.

- The virulence of *Agrobacterium* strictly depends on the presence of PC in the bacterial membranes
- Ornitholipids are elicitors of plant immune response in *Agrobacterium tumefaciens* - host interaction
- Plant 9-LOX affects *Pseudomonas aeruginosa* responsive genes linked to oxidative stress and quorum sensing

Dox-oxylipins in *P. aeruginosa:*

- inhibit flagellum-driven motility
- upregulate twitching motility and organization in microcolonies
- promote biofilms formation *in vitro* and *in vivo*
- promote bacterium virulence in *Drosophila* flies and lettuce





REF: doi:10.1038/ncomms13823

A LESSON FOR FUNGI BY *XYLELLA FASTIDIOSA*

Xylella fastidiosa *causing devastating diseases*

- *in a broad host range*
- *a xylem-limited bacterium*

Corriere della sera May 16th 2014

Olive Quick Decline Syndrome (OQDS)

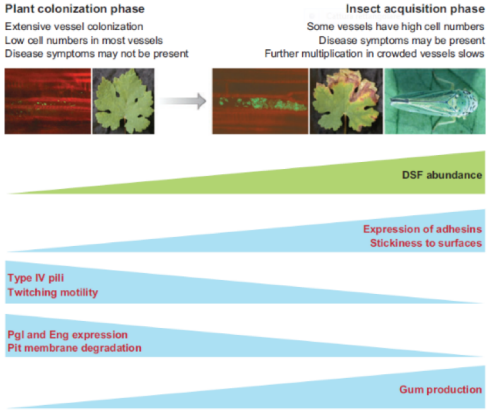
XF ACTORS

Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

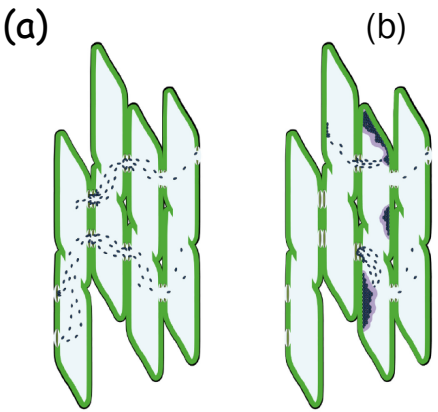
LIPIDS IN *XYLELLA FASTIDIOSA* (Xf)

- Diffusible Signal Factor (DSF) mediate quorum sensing (QS)
- Xf switches to the adherent biofilm phase in high DSF concentrations
- DSF are cis 2-enoic fatty acids (a mix from 12:1 to 19:1)

Xf exhibits self-limiting behaviors during endophytic state



Chatterjee et al 2008

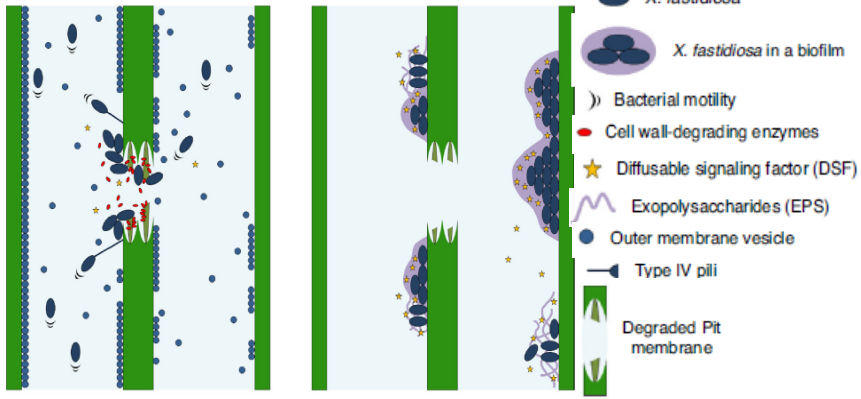


In (a) DSF upregulate in (a) e. g. Type IV pili, cell wall-degrading enzyme,

In (b) DSF downregulates e.g. Type IV pili, wall-degrading enzymes,

(a) Low Cell Density dispersal phase

(b) High Cell Density adherent biofilm phase



Roper et al 2019

Xf– HOST RECOGNITION

Lipopolysaccharide (LPS) O-antigen acts as a shield and delay initial plant recognition during the early phases of infection and allows the bacterium to establish itself in the xylem (Rapicavoli et al., 2018)

- In early stage Xf is not perceived by the plant (plant upregulates genes related to water loss and abiotic stress)
- Later in the infection, the plant detects Xf, initiates an immune response and upregulates the pathways associated with tylose production (despite their ineffectiveness)

Plant kills itself for the extensive vascular blockage that occurs in the xylem (Roper et al., 2019)

Xf IN VITRO

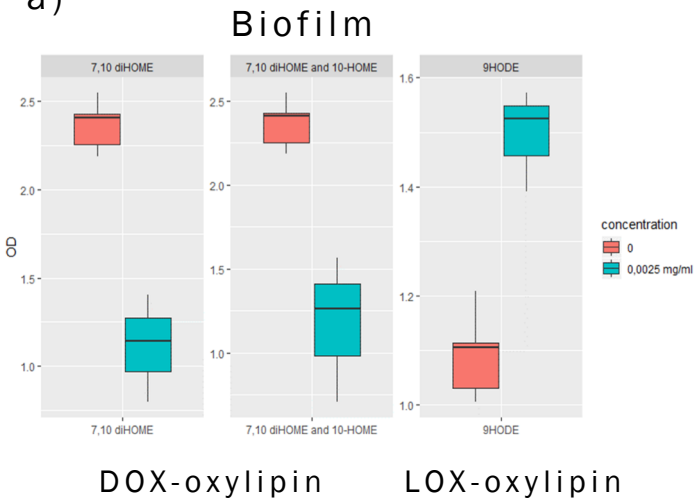
We identify:

- lipids and oxylipins that change in planktonic growth and biofilm formation
- different XfDSFs (18:1 and 18:2) in Xf lifestyle besides XfDSF2 (C16:1) and XfDSF1 (C14:1)

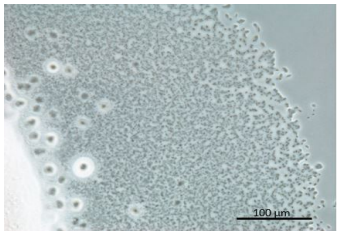
Demonstrate that:

- LOX-9-oxylipins promote biofilm (a)
- DOX-oxylipins and DSF (18:1 and 18:2) inhibit biofilm formation (a) and stimulate planktonic growth
- Xf-Dox deletion inhibits twitching motility (b)

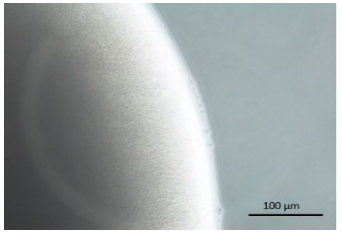
a)



b) wt

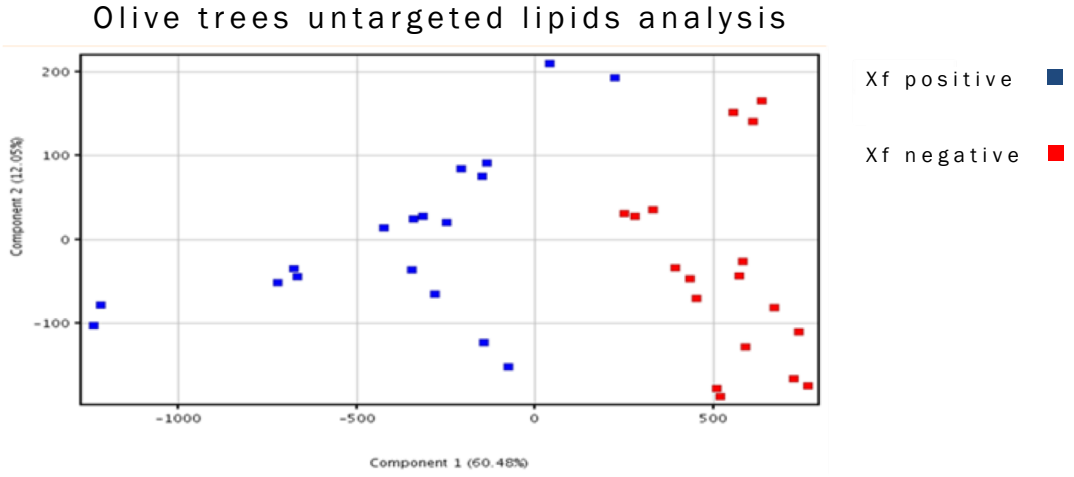


ΔDox-Xf



Xf AND HOST

- Lipid entities vary upon bacterial infection in the host (*Nicotiana tabacum* and *Olea europaea*)



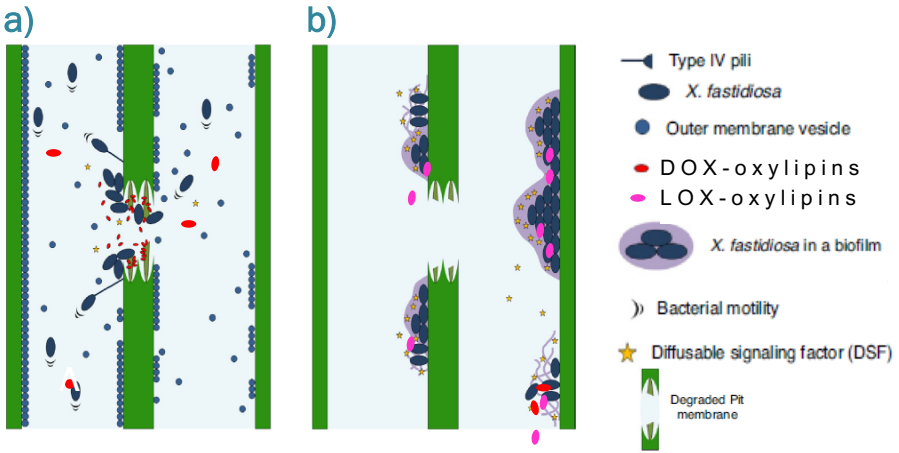
Xf-infected plants accumulate more 9-HODE

We identify lipids hallmarks of Xf infection
in naturally infected *Olea europaea*
and
in *Nicotiana tabacum* artificially inoculated

FFAs/OXY in CROSS TALK Xf-HOST

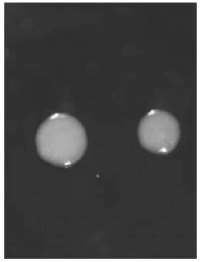
18:1, 18:2 and related oxylipins modulate Xf dual state

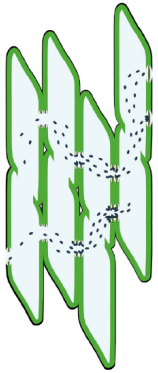
- ✓ Xf coordinates its behaviour trough DSF and DOX-oxylipins to trigger systemic invasion limiting the biofilm formation and acquisition by insect vectors (a)
- ✓ The plant activates defence response. LOX-9-oxylipins triggers the Xf acquisition phase stimulating biofilm formation, vector acquisition and extensive vascular blockage (b)



Conclusions

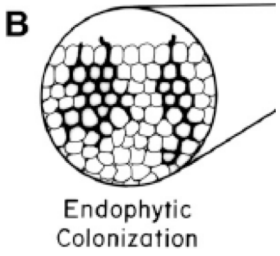
Xf

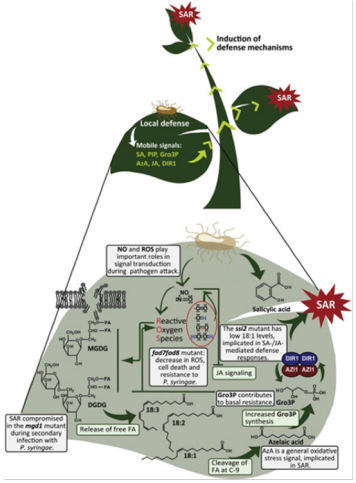




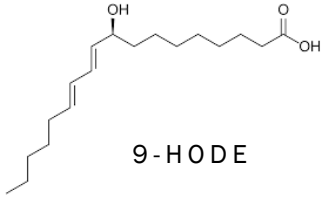
pathogen oxylipins modulate endophytic colonization

Fv





host oxylipins switch the pathogen state

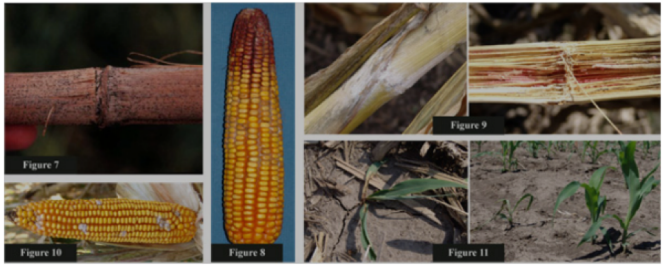


9-HODE

Leaf scorching - Wilting of the foliage – Defoliation - Chlorosis or bronzing of leaf margin and dwarfing

Symptoms

Ear rot - Stalk rot - Seedling Blight – Conidiation and Dispersal



Bleve et al 2016, wattagnet.com, Blacutt et al 2018, Liu et al 2012,

Key Points

- ✓ 9-HODE is a common factor in the pathogen activity
- ✓ FFAs or oxylipins are involved in the switch (e.g. endophytic to parasitic; biotroph to necrotroph; host colonization and evasion)
- ✓ Lipids are common signals in fungi, bacteria and host (mammals, insects and plants)
- ✓ Lipids role should be study in the pathobiome

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HORIZON 2020

XF ACTORS
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REGIONE PUGLIA