DECIPHERING *Brassica napus*-microbiome associations in interaction with root herbivorous insect *Delia radicum*: a feedback loop in the rhizosphere

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General context

Chemical compounds
(Reviewed in Nishida, 2014)
General context

Defenses (Reviewed in Chen, 2008)

Secondary metabolites (Reviewed in Fürstenberg-Hägg et al., 2013)
Alcaloids, phenolics, glucosinolates

Plant-insect interactions induce defense production: physical or chemical defenses (food supply, nutrient value, damages)
Plants recruit microorganisms using their chemistry
- Mutualist microorganisms promote plant growth and health
General context

- Above or belowground
- 1 species

Chemical compounds

DUAL INTERACTIONS

HERBIVORES

- 1 species or strain
- 1 plant compartment

SYMBIONTS
**General context**

- **Chemical compounds**
  - Above or belowground
  - 1 species

- **Dual interactions**
  - Above or belowground
  - 1 species or strain
  - 1 plant compartment

- **Herbivores**
  - Above and belowground
  - Multiple species, populations, communities

- **Symbionts**
  - Communities
  - Several plant compartments

- **Integrative interactions**
  - Communities
  - Several plant compartments
Through manipulation, soil microbiota can modify plant chemistry and insect performance (Hol et al., 2010)

General context

HERBIVORES

SYMBIONTS

Defenses

Chemical compounds

INTEGRATIVE INTERACTIONS

Chemical compounds

DUAL INTERACTIONS

Chemical compounds
Aboveground herbivory modified the rhizosphere microbial communities (Kong et al., 2016)
The plant is a key biological hub, influenced by both above (herbivory) and belowground (soil symbionts) factors.
Studying the tripartite interaction between a root herbivore, a crop plant and soil microorganisms
What are the effects of selected soil microbiota on the plant chemistry and the herbivore development?

Cabbage root fly (Delia radicum) Root herbivore as a larva

Oilseed rape (Brassica napus)

Chemical compounds Defenses

Soil microbial communities

Studying the tripartite interaction between a root herbivore, a crop plant and soil microorganisms
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Hypotheses

■ Soil microbial diversity influences the herbivore life history traits.
■ Fly phenotype change can be explained by plant chemistry modulated by soil microbial diversity.
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- Fly phenotype change can be explained by plant chemistry modulated by soil microbial diversity.

Materials and methods

→ Experimental approach to manipulate soil microbiota
  - Soil inoculum resuspended in water
  - Series of dilutions: 3 dilutions selected
Hypotheses

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→ Experimental approach to manipulate soil microbiota
  - Soil inoculum resuspended in water
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  - Inoculation in the same soil matrix sterilized by gamma radiation
Hypotheses

- Soil microbial diversity influences the herbivore life history traits.
- Fly phenotype change can be explained by plant chemistry modulated by soil microbial diversity.

Materials and methods

→ Experimental approach to manipulate soil microbiota
  - Soil inoculum resuspended in water
  - Series of dilutions: 3 dilutions selected
  - Inoculation in the same soil matrix sterilized by gamma radiation
  - Incubation for 2 months to reach similar microbial density
Hypotheses

- Soil microbial diversity influences the herbivore life history traits.
- Fly phenotype change can be explained by plant chemistry modulated by soil microbial diversity.

Materials and methods
Hypotheses
- Soil microbial diversity influences the herbivore life history traits.
- Fly phenotype change can be explained by plant chemistry modulated by soil microbial diversity.

Materials and methods

Initial microbial diversity
- Bacterial and fungal communities (16S/18S)

Materials and methods

Infestation

5 week-old plant

1 DAI
1st instar larva

35 DAI emergence

Development traits
- Emergence rate
- Hind tibia length (fitness proxy)

Leaf and root amino acids, sugars, glucosinolates

Effects of initial soil microbial diversity

Biological models and objectives

General context

Effects of initial soil microbial diversity
Analysis of initial soil microbial diversity – diversity indices

- Bacterial alpha-diversity (richness and diversity): High > Medium > Low (P = 0.001)
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- Bacterial alpha-diversity (richness and diversity): High > Medium > Low (P = 0.001)
- Bacterial beta-diversity (community structure): 3 significant different profiles (P = 0.001)
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- Bacterial beta-diversity (community structure): 3 significant different profiles (P = 0.001)

Similar results for fungi

Validation of 3 microbial diversities obtained through the dilution to extinction method in the same soil matrix
Analysis of *D. radicum* life history traits

- **Oviposition:** no significant differences for the egg number
Analysis of *D. radicum* life history traits

- **Oviposition**: no significant differences for the egg number
- **Emergence rate**: medium > high = low
Analysis of *D. radicum* life history traits

- **Oviposition**: no significant differences for the egg number
- **Emergence rate**: medium > high = low
- **Fitness**: similar tibia length between soil microbial diversity

<table>
<thead>
<tr>
<th>Initial soil microbial diversities</th>
<th>Mean number of eggs (± se)</th>
<th>Mean emergence rate (± se)</th>
<th>Mean hind tibia length (mm ± se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>40</td>
<td>0.6</td>
<td>1.84</td>
</tr>
<tr>
<td>Medium</td>
<td>40</td>
<td>0.8</td>
<td>1.82</td>
</tr>
<tr>
<td>Low</td>
<td>40</td>
<td>0.2</td>
<td>1.80</td>
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Analysis of *D. radicum* life history traits

Does medium diversity increase larval survival? Or, on the contrary, do the other 2 modalities decrease it?

Plant chemistry can influence insect phenotype (van Leur *et al.*, 2008): investigation of amino acids, sugars and glucosinolates in the leaves and roots.
Analysis of *B. napus* root metabolites – RDA

Plant treatment and initial soil microbial diversity

- Healthy plants (1 DAI) – Red
- Infested plants (1 DAI) – Medium
- Infested plants (35 DAI) – Low

No effect of initial soil microbial diversity on root metabolites analyzed ($P = 0.285$)
Analysis of *B. napus* root metabolites – RDA

But 3 different profiles according to the treatment ($P = 0.001$)

*Infestation effect >> initial soil microbial diversity effect*
Analysis of *B. napus* root metabolites – RDA

Infested plants: decrease of most metabolites except for glucosinolates (Hopkins *et al.*, 2009)
Infestation coupled to the time factor is a strong driver of root chemistry.

What is the impact of root herbivory on plant chemistry and microbial community dynamics?
Hypotheses

- Root herbivory modifies plant physiology
- As consequences, microbial communities are influenced
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- Root herbivory modifies plant physiology
- As consequences, microbial communities are influenced

Materials and methods

Initial microbial diversity
- High
- Low

6 week-old plant

Infestation

1 DAI hatching - 1\textsuperscript{st} instar larva

14 DAI 3\textsuperscript{rd} instar larva

42 DAI end of emergence

Root amino acids, sugars, glucosinolates and chemical elements

Root and rhizosphere bacterial and fungal communities (16S/18S)
Hypotheses

- Root herbivory modifies plant physiology
- As consequences, microbial communities are influenced

Materials and methods

- Infestation
- 6 week-old plant
- 1 DAI: hatching - 1st instar larva
- 14 DAI: 3rd instar larva
- 42 DAI: end of emergence
- Herbivory intensity
- Plant defense accumulation
Results (DIABLO) – Root chemistry analysis

Herbivory influenced the root metabolomics and elemental profiles
Herbivory influenced the root metabolomics and elemental profiles

- Most compounds decreased
- But: increase of
  - Trehalose and neoglucobrassicin
  - Nitrogen
Results (DIABLO) – Microbial communities analysis

Herbivory influenced:
The root bacterial communities
  o Increase of *Pseudomonas* and
    *Stenotrophomonas* abundances (γ-proteobacteria)
  o Increase of *Clostridium* and *Paenibacillus*
    abundances (Firmicutes)

But fungal communities were impacted to a lesser extent.
Results (DIABLO) – Relationship between root chemistry and microbial communities

At the peak of herbivory, chemical modifications can be associated to changes in microbial communities (Hervé et al., 2018: Singh et al., 2018)
Potential scenario

Trehalose, plant defense (Singh et al., 2011)
Neoglucobrassicin (van Dam & Raaijmakers, 2006)
Potential scenario

Neoglucobrassicin
(van Dam & Raaijmakers, 2006)

Trehalose, plant defense
(Singh et al., 2011)

Consume

Pseudomonas
(De La Fuente et al., 2007)

Produce

Stenotrophomonas
(Wolf, 2002)
Against herbivory, plants produce defensive metabolites but also attract and recruit microorganisms with chemical compounds to maintain their defenses.
Take-home message

Effects of initial soil microbial diversity
- Modulation of the fly phenotype
- No link to chemical changes

Effects of root herbivory
- Influence of root chemistry
- Modification of the plant microbial communities
Perspectives

- Determine the drivers responsible for the fly phenotype changes and microbial community modifications.

Measure other plant defenses

**well-documented**: chemical compounds, root physical defenses

(Reviewed in Chen, 2008)
Perspectives

Determine the drivers responsible for the fly phenotype changes and microbial community modifications.

Measure other plant defenses

well-documented: chemical compounds, root physical defenses  
(Reviewed in Chen, 2008)

Differentiate plant chemical compounds from microbial ones
Perspectives

- Determine the drivers responsible for the fly phenotype changes and microbial community modifications.

Measure other plant defenses well-documented: chemical compounds, root physical defenses

(Reviewed in Chen, 2008)

Differentiate plant chemical compounds from microbial ones

Identify functions and assess gene expression associated to each actor of this tripartite interaction.
Acknowledgements

Insect team

Lachaise*, Ourry* et al., Insect Science (2017).
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Microbiota team

Chemical analysis platforms

Sequencing platform

INRA PhytoMic network
THANK YOU FOR YOUR ATTENTION

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