INVOLVEMENT OF ANTIOXIDANT ENZYMES IN THE RESPONSE OF QUERCUS ROBUR LEAVES TO ERYsipHE ALPHITOides INFECTION

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Oak powdery mildew is caused by a species of fungus - *Erysiphe alphitoides*. Fungi which cause powdery mildew belong to a division of ascomycetes (*Ascomycotina*).

Infected plants display white powdery spots on leaves and stems which are characteristic features of the disease. It is created by colorless fungal hyphae and conidia.
CONTAINER NURSERY

- more controlled cultivation conditions
- covered root system
AIM OF THE STUDY:

How do the basic mechanisms function related to the regulation of redox in oak leaves response to biotic stress caused by the fungal pathogen?

- Histochemical detection and biochemical analysis of hydrogen peroxide
- Determination of enzymatic antioxidants in response of oak leaves to biotic stress, caused by the fungal pathogen
- Analysis of the isoenzymes patterns after native PAGE
PLANT RESPONSE TO STRESS

OVERPRODUCTION OF ROS: $\text{H}_2\text{O}_2$

THE CHANGES IN THE ENZYMES ACTIVITY, WHICH ARE INVOLVED IN THEIR METABOLISM: POX, CAT, SOD

ASCORBATE – GLUTATHIONE CYCLE

MECHANICAL DAMAGES

PATHOGENS ATTACK
PLANT RESPONSE TO STRESS

OVERPRODUCTION OF ROS: $\text{H}_2\text{O}_2$

THE CHANGES IN THE ENZYMES ACTIVITY, WHICH ARE INVOLVED IN THEIR METABOLISM:

POX, CAT, SOD

ASCORBATE – GLUTATHIONE CYCLE

PATHOGENS ATTACK

MECHANICAL DAMAGES
MATERIAL OF THE STUDY

ONE YEAR-OLD SEEDLINGS OF PÆDUNCULATE OAK

CONTROL AND INFECTED LEAVES (<5%, 12-15%, 25% OF INFECTION AREA)

CONTAINER NURSERY OF FOREST DISTRICT GIDLE
RESULTS – HYDROGEN PEROXIDE

H$_2$O$_2$ concentration [nmol g$^{-1}$ FW]

- Jul
- Aug
- Sep

C
<5% Inf
12-15% Inf
25% Inf

* September, 2016, Riga, Latvia

12th Annual Meeting of the Northern European Network for Wood Science and Engineering WSE
HISTOCHEMICAL DETECTION OF HYDROGEN PEROXIDE

CONTROL  <5% INFECTION  12-15% INFECTION  25% INFECTION

[Images of leaf samples with varying degrees of infection]
RESULTS – POX ACTIVITY

POX activity [U mg⁻¹ protein]

- C
- <5% Inf
- 12-15% Inf
- 25% Inf

POX activity over time:
- Jul
- Aug
- Sep

* indicates significant differences.
RESULTS – LIGNIN CONTENT

LIGNIN DECOMPOSED IN VASCULAR BUNDLES OF CONTROL AND INFECTED OAK LEAVES
RESULTS – CAT ACTIVITY

CAT activity [U mg⁻¹ protein]

Jul Aug Sep

C <5% Inf 12-15% Inf 25% Inf

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CAT 1 ➔
RESULTS – SOD ACTIVITY

SOD activity [U mg⁻¹ protein]

- Jul
- Aug
- Sep

C
<5% Inf
12-15% Inf
25% Inf

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ELECTROPHORESIS OF SOD

INHIBITORS

5mM H₂O₂

2mM KCN

C  <5%  12-15%  25%  C  <5%  12-15%  25%  C  <5%  12-15%  25%

Mn-SOD
Cu/Zn-SOD1
Cu/Zn-SOD2
Cu/Zn-SOD3
Cu/Zn-SOD4

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AA and DHA

Concentration of AA [mg g\(^{-1}\) FW]

July
August
September

C 0.5% Inf
12-15% Inf
25% Inf

DHA
AA
RESULTS – APX ACTIVITY

[Graph showing APX activity over time from July to September 2016 in Riga, Latvia]

- **APX activity** [U mg\(^{-1}\) protein]
- **Axes:** X-axis = Months (Jul, Aug, Sep), Y-axis = APX activity
- **Legend:**
  - C
  - <5% Inf
  - 12-15% Inf
  - 25% Inf

- **Graph Notes:**
  - Significant differences indicated by asterisks (*)
  - Data from the 12th Annual Meeting of the Northern European Network for Wood Science and Engineering (WSE)
RESULTS – DHAR ACTIVITY

DHAR activity [U mg⁻¹ protein]

- C
- <5% Inf
- 12-15% Inf
- 25% Inf

Jul  | Aug  | Sep
---  | ---  | ---
0.40 | 0.20 | 0.60

* indicates significant difference.
The significant increase in the concentration of H$_2$O$_2$ after *Erysiphe alphitoides* infection confirms intense reaction of plant response to contact with biotrophic pathogen and activation of a rapid response in order to stop the infection.

Increase in activity of POX and CAT after pathogen infection, in the powdery mildew-infected leaves may indicate the induction of additional mechanisms that protect cells from oxidative stress.

The highest content of lignins was observed in the leaves where the area of infection was 12-15% that was confirmed by microscopic analysis.
SUMMARY

We observed the differentiation of isoforms of POX and SOD in the investigated leaves.
Reducing the number of Cu/Zn-SOD forms in infected oak leaves may indicate a change in the metabolism of the host plant cells after contact with a biotrophic pathogen.

We observed increase in the activity of APX in infected oak leaves that may indicate disturbed functioning of the ascorbate-glutathione cycle during pathogenesis.

The increase in AA concentrations after pathogen infection and the changes in the DHAR activity are related to regeneration of reduced form of AA.