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Comparative Transcriptome analysis of *Ambrosia artemisiifolia* L. pollen after exposing the plants to elevated ozone during a whole vegetation period

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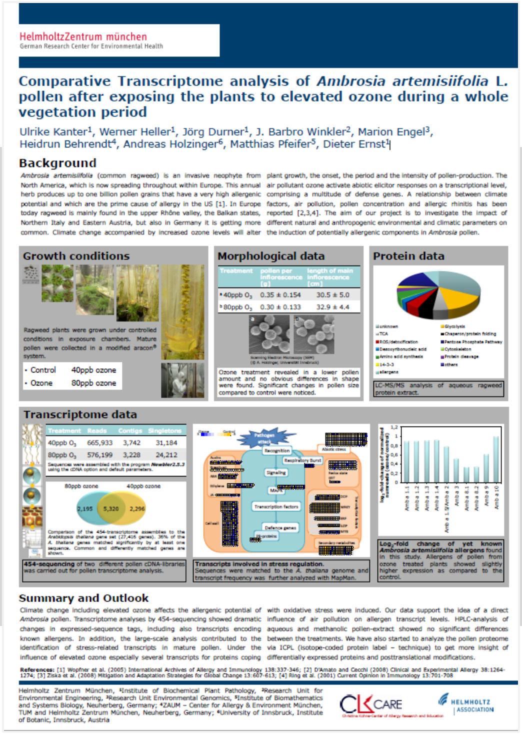

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Comparative Transcriptome analysis of *Ambrosia artemisiifolia* L. pollen after exposing the plants to elevated ozone during a whole vegetation period

Ulrike Kanter[1], Werner Heller[1], Jörg Durner[1], J. Barbro Winkler[2], Marion Engel[3], Heidrun Behrendt[4], Andreas Holzinger[6], Matthias Pfeifer[5], Dieter Ernst[1]

Background

Ambrosia artemisiifolia (common ragweed) is an invasive neophyte from North America, which is now spreading throughout within Europe. This annual herb produces up to one billion pollen grains that have a very high allergenic potential and which are the prime cause of allergy in the US [1]. In Europe today ragweed is mainly found in the upper Rhône valley, the Balkan states, Northern Italy and Eastern Austria, but also in Germany it is getting more common. Climate change accompanied by increased ozone levels will alter plant growth, the onset, the period and the intensity of pollen-production. The air pollutant ozone activate abiotic elicitor responses on a transcriptional level, comprising a multitude of defense genes. A relationship between climate factors, air pollution, pollen concentration and allergic rhinitis has been reported [2,3,4]. The aim of our project is to investigate the impact of different natural and anthropogenic environmental and climatic parameters on the induction of potentially allergenic components in *Ambrosia* pollen.

Growth conditions

Ragweed plants were grown under controlled conditions in exposure chambers. Mature pollen were collected in a modified aracon® system.

- Control 40ppb ozone
- Ozone 80ppb ozone

Morphological data

Treatment	pollen per inflorescence [g]	length of main inflorescence [cm]
a 40ppb O_3	0.35 ± 0.154	30.5 ± 5.0
b 80ppb O_3	0.30 ± 0.133	32.9 ± 4.4

Ozone treatment revealed in a lower pollen amount and no obvious differences in shape were found. Significant changes in pollen size compared to control were noticed.

Protein data

LC-MS/MS analysis of aqueous ragweed protein extract.

Transcriptome data

Treatment	Reads	Contigs	Singletons
40ppb O_3	665,933	3,742	31,184
80ppb O_3	576,199	3,228	24,212

Sequences were assembled with the program ***Newbler2.5.3*** using the cDNA option and default parameters.

Comparison of the 454-transcriptome assemblies to the *Arabidopsis thaliana* gene set (27,416 genes). 38% of the *A. thaliana* genes matched significantly by at least one sequence. Common and differently matched genes are shown.

454-sequencing of two different pollen cDNA-libraries was carried out for pollen transcriptome analysis.

Transcripts involved in stress regulation. Sequences were matched to the *A. thaliana* genome and transcript frequency was further analyzed with MapMan.

Log_2-fold change of yet known *Ambrosia artemisiifolia* allergens found in this study. Allergens of pollen from ozone treated plants showed slightly higher expression as compared to the control.

Summary and Outlook

Climate change including elevated ozone affects the allergenic potential of *Ambrosia* pollen. Transcriptome analyses by 454-sequencing showed dramatic changes in expressed-sequence tags, including also transcripts encoding known allergens. In addition, the large-scale analysis contributed to the identification of stress-related transcripts in mature pollen. Under the influence of elevated ozone especially several transcripts for proteins coping with oxidative stress were induced. Our data support the idea of a direct influence of air pollution on allergen transcript levels. HPLC-analysis of aqueous and methanolic pollen-extract showed no significant differences between the treatments. We have also started to analyze the pollen proteome via ICPL (isotope-coded protein label – technique) to get more insight of differentially expressed proteins and posttranslational modifications.

References: [1] Wopfner et al. (2005) International Archives of Allergy and Immunology 138:337-346; [2] D'Amato and Cecchi (2008) Clinical and Experimental Allergy 38:1264-1274; [3] Ziska et al. (2008) Mitigation and Adaptation Strategies for Global Change 13:607-613; [4] Ring et al. (2001) Current Opinion in Immunology 13:701-708

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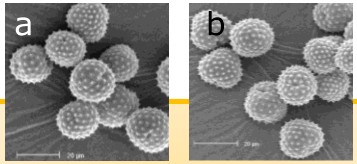
Growth of plants under controlled conditions

Treatments:

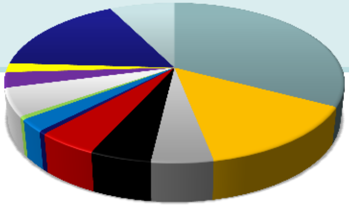
- Control 40ppb ozone
- Ozone 80ppb ozone



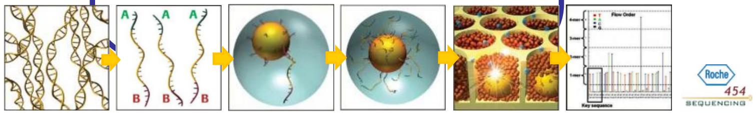
Pollen analysis

Treatment	Pollen[g]	Inflorescence [cm]
[a] 40ppb O_3	0.35 ± 0.154	30.5 ± 5.0
[b] 80ppb O_3	0.30 ± 0.133	32.9 ± 4.4

Proteome analysis (LC-MS/MS; ICPL)



Transcriptome analysis via 454-sequencing



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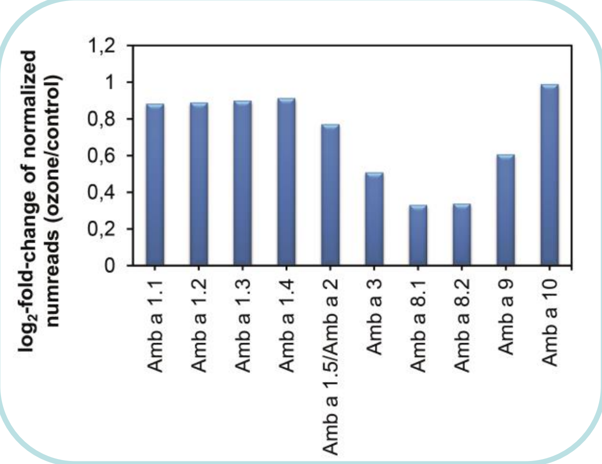
40ppb O_3	665,933	3,742	31,184
80ppb O_3	576,199	3,228	24,212

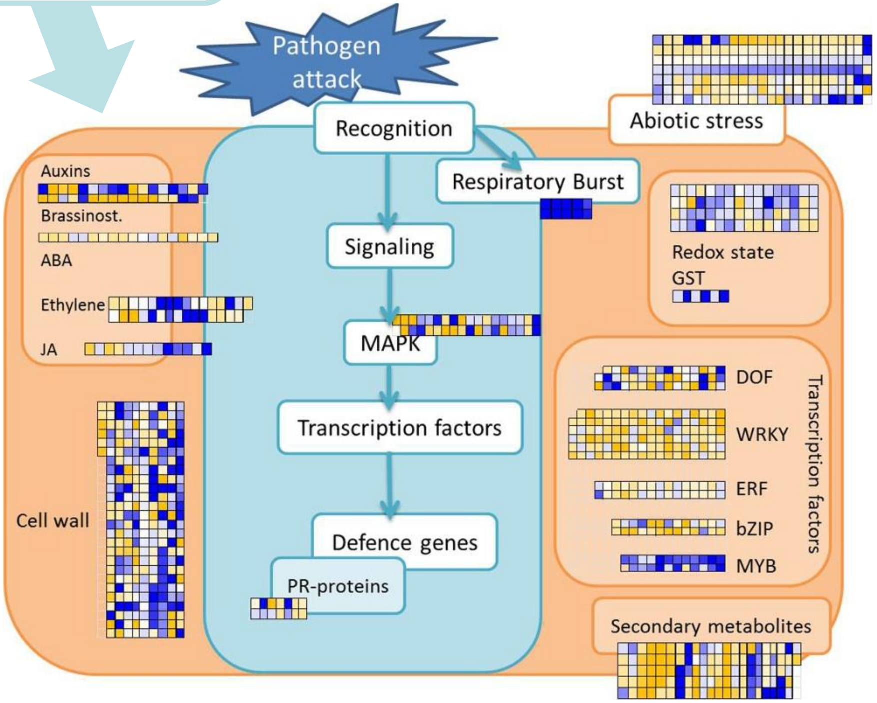
Transcriptome analysis via 454-sequencing

Sequence assembly
Newbler2.5.3

BLASTX
Matched on
non-red. Protein



BLASTX
Matched on
Arabidopsis thaliana



- Stress regulated transcripts are induced due to ozone treatment
- Yet known allergens are slightly up-regulated

People involved in the project:

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