Project report:
Allelopathy experiment with Estonian trees

Nora Tomsová, Axel Bergeon, Sophie Baur, Ingmar Pappel

Supervisors: Virginie Baldy, Ilja Reiter, Jordane Gavinet

Erasmus - Soil and Water - 2017

Introduction

Allelopathy is a chemical interaction between plants, and between plants and microorganisms. Plants produce allelochemicals (secondary metabolites), which are released into the environment. These secondary metabolites have influence on growth, germination, reproduction, distribution of vegetation.

In our experiment, we have been observing allelopathy of 3 common Estonian plant species: *Acer platanoides*, *Picea abies* and *Quercus robur.* From scientific articles, we know that allelopathy of boreal shrubs has been evidenced, but we have low knowledge about allelopathy of Estonian trees *(Gallet 1994).*

We raised 2 main hypotheses:

- H1: Macerate of leaves or needles of Estonian common trees (*Acer platanoides, Picea abies, Quercus robur*) have allelopathic effect on germination of *Lactuca sativa*, *Lepidium sativum*.

- H2: allelopathic effect of young needles of *Picea abies* is higher compared to old needles of *Picea abies.*

Our experiment was mainly done by germination tests.

Material and method

1. First experimentation

The first experimentation is carried out to observe the potential allelopathic effect of the macerates of needles of *Picea abies* and of leaves of *Acer platanoides.*

i. Preparation of macerates

We put 20g (fresh weight) of needles of *Picea abies* or leaves of *Acer platanoides* in a glass with 100ml of distilled water. After 17 hours we filtered the macerate, this was the mother solution at 10% (mass/volume). Another solution of 5% was realized by mixing 50ml of the solution at 10% with 50ml of distilled water.
A control was also realized with only distilled water (0%).
6 treatments were tested: 2 species (*P. abies* and *A. platanoides*) at 3 concentrations (5%, 10% and control at 0%).

ii. Germination test

For the germination test we used Petri dishes with a substrate of filter paper. In this filter paper, we put 25 seeds of the target species: *Lepidium sativum*.
There were 24 Petri dishes: 6 treatments and 4 replicates per treatment.
We watered each Petri dish with 2ml of one solution (distilled water as 0% control, 5% or 10% of macerate of each species).
48 hours after watered the Petri dishes, we counted the number of germinated seeds, and the germination stage, according to the Figure 1.".

Figure 1: the 4 germination stages considered for the experiment. 1: no germination, 2: germinated without leaves, 3: germinated with yellow leaves, 4: germinated with green leaves.

Data analysis was done with Chi^2 tests.

2. Second experiment

The first experiment demonstrated no significant inhibition effect of the tested treatments. We performed a second experiment, with the same protocol but we used 2 target species (*Lepidium sativum* and *Lactuca sativa)*, and we selected two tree species *Picea abies* and *Quercus robur* and two phenological stages for *Picea abies* (young and old needles).

i. Preparation of macerate

We put 20g (fresh weight) of leaves or needles of *Quercus robur* and young or old needles of *Picea abies* in a glass with 100ml of distilled water. After 17 hours, we filtered the macerates.
A control for the macerate is also realized with only distilled water (0%).
8 treatments were tested: 3 macerates (young or old needles of *Picea abies* or leaves of *Quercus robur*) at 10% and 1 control (0%), with 2 target species (*Lepidium sativum* and *Lactuca sativa*).

ii. Germination test

For the germination test we used Petri dishes with a substrate of filter paper. In this filter paper, we put 25 seeds of the target species: *Lepidium sativum* and *Lactuca sativa*.
There were 24 Petri dishes: 8 treatments and 3 replicates per treatment.
We watered each Petri dish with 2ml of one solution (distilled water as control or macerate).
48 hours after watered the Petri dishes, we counted the number of germinated seed, and the germination stage, according to the Figure **1.**

Data analysis was done by using Chi² tests.

Table 1: Summary table of both experiments:

	First experimentation	Second experimentation
Number of 0% control (distilled water)	8	3
Donor plants	2 (*Picea abies* and *Acer platanoides*)	3 (young and old needles of *Picea abies* and leaves of *Quercus robur*)
Concentrations	5% and 10%	10%
Target species	1 (*Lepidium sativum*)	2 (*Lepidium sativum* and *Lactuca sativa*)
Replicates	4	3

Results

1. First experiment

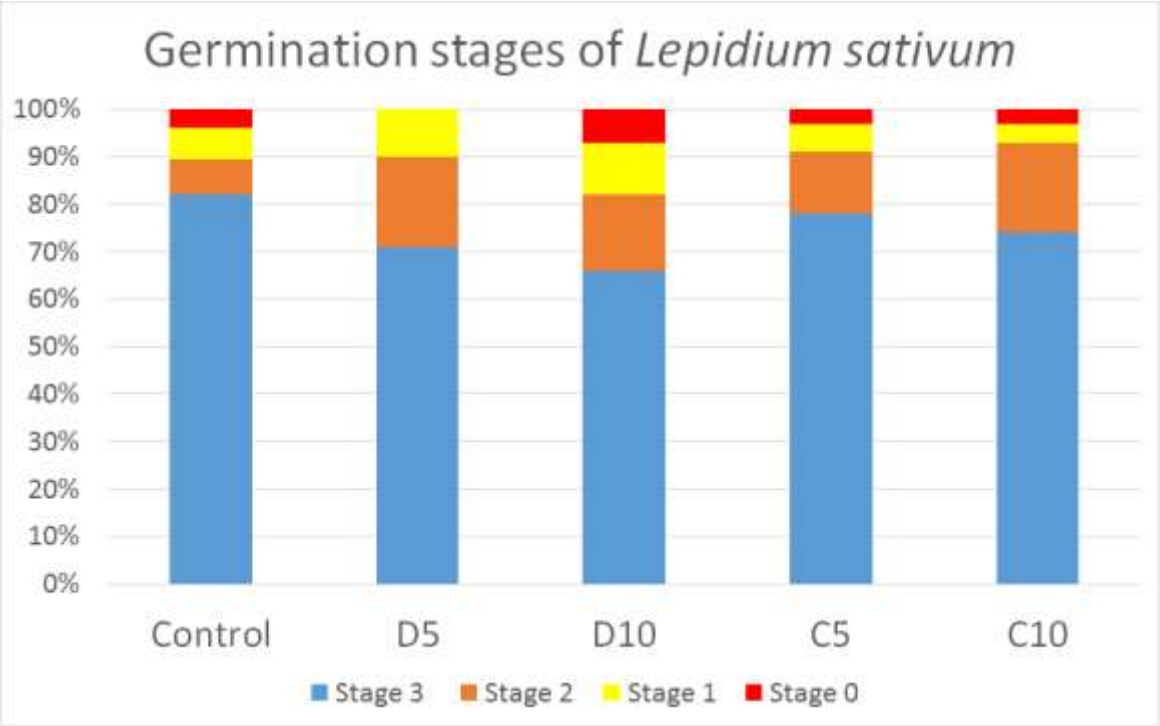

Figure 2: Germination rates of *Lepidium sativum* with 0% control with distilled water, with 5 and 10% macerates for the two donor plant species (C=conifer=*Picea abies*, *D=deciduous=Acer platanoides*).

Results showed that most of the seeds germinated (Figure 2). All treatments lead to all the germination stages, except D5 (*Acer* 5%), which had no stage 0 (all seeds have germinated). D10 (*Acer* 10%) treatment showed the highest proportion of non-germinated seeds. Both *Picea* macerates concentrations (C5, C10) showed comparable results.
The statistical test Chi^2 realized gave 6.5 and showed no significant difference between the treatments, reporting no allelopathic effect of donor plants on target plants.

2. Second experiment

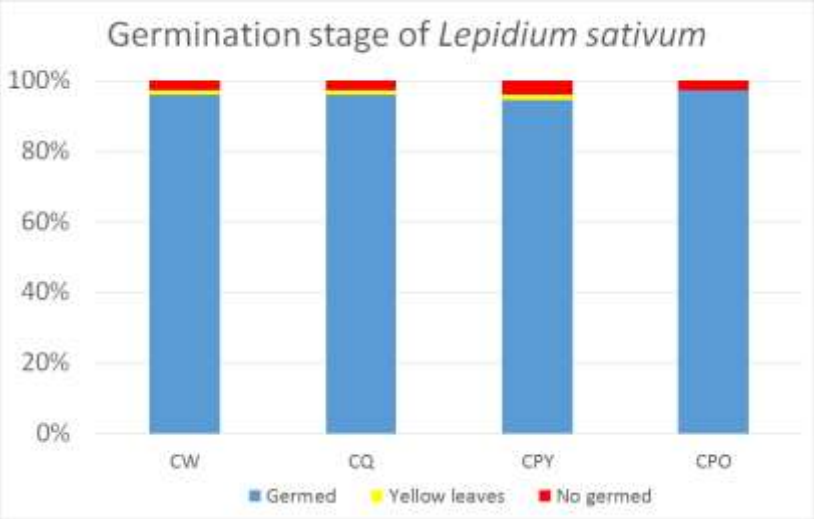

Figure 3: Germination rates of *Lepidium sativum*. with 0% distilled water (CW) and 10% macerates of two species, *Quercus robur* (CQ) and *Picea abies* (CPY=young needles, CPO=old needles).

Again, most of the seeds germinated (Figure 3). In this experiment, only a few proportion of seeds germinated until the stage « yellow leaves ». For CPO, no seeds reached this stage. The statistical test Chi² realized was 0.45 and showed no significant difference between the treatments, reporting no allelopathic effect.

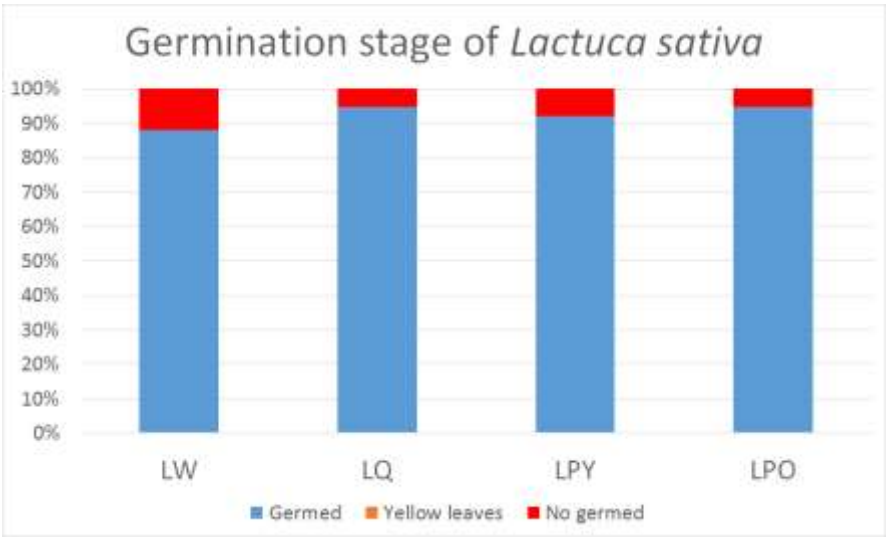

Figure 4: Germination rates of *Lactuca sativa* with 0% distilled water (LW) and 10% macerates of two species, *Quercus robur* (LQ) and *Picea abies* (LPY=young needles, LPO=old needles).

As it was observed previously, most of the seeds germinated (Figure 3). However, we did not observe any « yellow leaves » germination stage except a really low proportion with *Quercus*. The statistical test Chi^2 realized was 1.05 and showed no significant difference between the treatments, indicating no allelopathic effect.

Discussion

We reported no allelopathic effects during these experiments. We hypothesized that donor plants produced not enough allelochemicals for inhibiting germination rates of target species.

Possible reasons, why there was not enough allelochemicals could be raised:

- if plants are not under stress then they produce less secondary metabolites;
- allelochemicals were emitted by using different ways (e.g. root exudates, litter decomposition) than leaf or needles leachates;
- target species used in these experiments are not competitors for trees and trees do not release allelochemicals harmful for these target species.

Possibilities to improve the method:

- repeat experiments with needles and leaves from trees under environmental stress - bogs, sandy areas etc.,
- let more time for macerates (standard is 24 hours)
- use soil instead of distilled water in Petri dishes
- use native Estonian species as targets in germination tests
- count rate of germination earlier (12 or 24 hours)
- use fallen needles under the Picea
- use target species which grow in Estonian spruce or oak forests.

Conclusion

Our main conclusion is that we have not observed any allelopathy in leaves or needles leachates. The reasons why we have not observed any allelopathic effect can be different. Firstly, there is no allelopathy of the donor plants used on these target species (*Lactuca sativa, Lepidium sativum*). These species do not growth in Estonian ecosystem and, they are very resistant species. Secondly, we have not observed allelopathy, because these species in natural environment do not grow together, so there is no competition between them, because they live in different ecological niches.

Bibliography

Gallet, C. (1994) Allelopathic potential in bilberry-spruce forests, influence of phenolic compounds on spruce seedlings. J. Chem. Ecol. 20, 1009–1024.