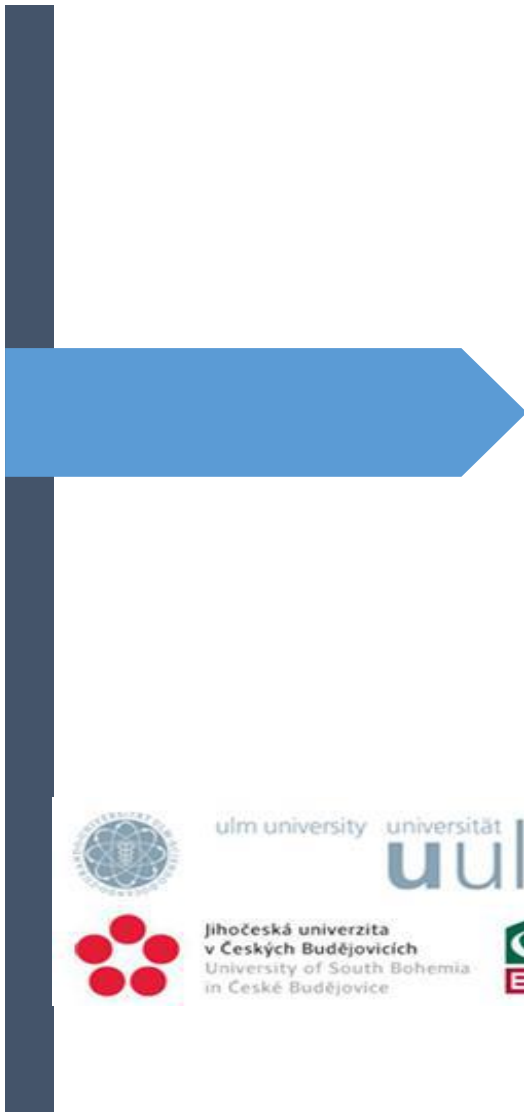


Mesofauna & allelopathy in Mediterranean ecosystems

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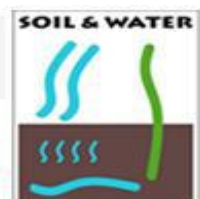


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Introduction

The Mediterranean climate is characterized by hot and dry summers, with violent climatic events such as storms. Regional models predict an increase in both temperature and drought conditions in the Mediterranean region during the present century (IPCC 2013; Polade et al. 2014). These changes are expected to result in increased frequency, intensity and duration of drought, especially during the hot season. Moreover, since the early twentieth century, there is an important rural exodus and consecutively a great reforestation in agricultural abandonment lands. Reforestation is currently advancing large-scale in the north of the Mediterranean and represents a direct threat on habitat mosaics with high biodiversity value in this region. This phenomenon occurs in a process called secondary succession where in absence of disturbance, re-colonization takes place in a progressive dynamic from herbaceous fallows to the forest formations. Particularly observed the expansion of very competitor's forest species as Aleppo pine (*Pinus halepensis* Mill.). In addition, *P. halepensis* present a high diversity of secondary metabolites. The activity of many allelochemicals is still little known; nevertheless it is known that some phenolics can inhibit the germination. These dynamics bring a homogenization of floristic communities and biodiversity loss in which the allelopathic process may have a role. So, our first experiment is focused on these allelopathic processes of *P. halepensis*: our hypotheses are that the germination rate of target plant species is affected differently according to different phenological stages of *P. halepensis* needles and the different allelochemical concentrations as well.

Decomposition of plant material is a key ecosystem function determining the global carbon cycle and nutrient recycling to a great extent (Bardgett. 2005). This process is governed by environmental conditions (humidity and temperature), litter quality (chemical and physical characteristics of litter) and the decomposers community (composition and activity) (Hättenschwiler et al. 2005). Soil mesofauna, mainly constituted by Collembola and Acari, plays a crucial role in the decomposition process: detritivorous mesofauna directly affects the decomposition process through litter fragmentation and transport and distribution of compounds released from litter, but also indirectly by controlling abundance, diversity, activity and dispersion of microbial communities (Seastedt 1984). Predatory mesofauna affects indirectly the decomposition process through the predatory pressure exerted on detritivorous mesofauna (Coleman et al. 2004). As soil moisture has been reported to be the most limiting factor for soil mesofauna in Mediterranean ecosystems (Tsiafouli et al.; Kardol et al. 2011; Moron-Rios et al. 2010), it appears crucial to investigate response of these key organisms to future environmental conditions (increase of temperature and/or increase of the summer drought period). Thus, our hypothesis for the mesofauna experiment is that drought has a negative impact on mesofauna abundance.

2. Methods and materials

2.1. Study site

Needles of *P. halepensis* (green and senescent) and mesofauna organisms were collected on the site of the OH₃P (Oak Observatory at the Haute-Provence astronomical Observatory). It is located in the south of France in the Luberon Natural Regional Park, SE France. Climate is Mediterranean with a pronounced dry season in summer. The average annual precipitations varies between 600 and 900 mm and mean annual temperature is around 11.5°C to 14°C with January (mean temperature from 4°C to 6°C) and August (mean temperature from 21°C to 25 °C) as the coldest and warmest months respectively (Luberon Natural Regional Park).

The Mediterranean region is interesting for the study of allelopathic process because it contains plant species adapted to stresses during the dry summer. Indeed, these species can synthesize secondary metabolites as a defense when environmental conditions are unfavorable particularly during drought periods (Fernandez et al. 2013).

2.2. Material collection

P. halepensis was chosen for the study because it is rich in secondary metabolite (phenolic acids and terpenoids). This plant is useful for study plant-plant interaction through allelopathic process. Thus, the plant material was collected on the site and placed in plastic bags. We used only the needles of *P. halepensis* because they contain a higher amount of terpenoids than roots (Fernandez et al., 2009).

To test and understand the effect and the allelopathic potential of *P. halepensis*, *Laetluca sativa* was used as target species. Our choice fell on this species for its germination speed and its ability to react quickly to environmental changes. Furthermore, Fernandez et al. (2006) have already shown that *L. sativa* can be affected by *P. halepensis*'s allelochemicals.

2.3. Allelopathy bioassay

For this study, the aqueous extracts of needles were tested using two phenological stages (green and senescent needles). Leaching appears sufficient to measure the effect of allelopathic compounds of *P. halepensis* on germination because the soluble compounds are most involved (Vyvian 2002). Moreover, we used foliar leachates because of higher phenolics and terpenoids diversity than roots (Fernandez et al. 2009). We used two concentrations of *P. halepensis* extracts (2.5% and 10%) and a control with distilled water. For each concentration, we used two different phenological stages (green and senescent needles) to measure the effect of phenology on allelochemicals production. To prepare the aqueous solutions, 50 g of each needle

types were placed in glass container and added to 500ml of distilled water (10% of dry weight). The glass containers were covered with a plastic bag and left for 24 hours in darkness.

Then, the 2.5% solution was prepared at room temperature, diluting with distilled water, from the mother/ or stock solution (10% of dry weight).

The seeds of *L. sativa* were put on two sheets of Whatman® n°4 filter which were placed in glass Petri dishes (diameter 9cm). Each glass Petri dish containing 25 seeds respectively was watered with 2ml of different concentrations of aqueous extracts of *P. halepensis* or with distilled water (control) using a 5ml syringe. We watered twice between the beginning (12/09/15) and the end (15/09/15) of the experiment.

2.4. Experimental Design of allelopathy process

For the experiment of allelopathy process, we performed 4 replicates per concentration of aqueous extracts of *P. halepensis* (including 0% for control) and per phenological stage (green and senescent needles) for a total of 6 tested modalities. This corresponds to 100 seeds of *L. sativa* (4X25) by treatment and 24 Petri dishes.

We know that the light factor in the room could affect seeds germination, so the Petri dishes were randomised in order that the 4 replicates of each modality integrate this hypothetical light heterogeneity.



Fig.1: Experimental design with circles represented the 24 Petri dishes which containing respectively 25 seeds of *L. sativa*. For each Petri dish, 2 concentrations (2.5 % or 10 % of needles dry weight) of aqueous extracts of *P. halepensis* needles and one control (distilled water) were put. Black circles correspond to senescent needles of *P. halepensis* and green circles to green needles of *P. halepensis*.

2.5. Measured parameters

After 4 days of treatments, we observed the seeds of *L. sativa* and calculated germination rates by using the following formula:

$$\text{Germination (\%)} = (\text{number of seeds germination in a treatment} / \text{number total of seeds}) \times 100$$

2.6. Mesofauna extraction

The samples of mesofauna organisms were collected from the O₃HP site: 10 litter samples on control plot and 10 on rain exclusion plot in order to measure the effect of the decrease of precipitation on the abundance of Collembola and Acari associated to decomposing leaves. Organisms belonging to mesofauna have been extracted during 10 days by using the Tullgren funnel method (Berlès 1905). Mesofauna is captured using a funnel in which animals may avoid the dryness and so fall in a container (Diameter 5cm). Then mesofauna was stored in 95% ethanol which enabled us to conserve it, before the determination. We determined only the abundance of Collembola and Acari by using a binocular magnifier.

Finally, we calculated the ratio of Acari/Collembola for each samples.

2.7. Statistical analyses

For allelopathy experiment and for each treatment corresponding to the 2 studied factors (concentration and phenological stage) we compared the germination rate by using Kruskal Wallis tests. For mesofauna, we used Kruskal Wallis test to compare the ratio Acari/Collembola between both conditions in control and rain exclusion plots. For the statistical tests, we used Minitab statistics software.

3. Results

3.1. Allelopathy bioassay

We found that the concentrations of *P. halepensis* extracts have a significant effect on the germination rate of *L. sativa* (Kruskal-Wallis Test; $P < 0.05$) (Fig.2).

However, there is no significant difference between the germination rate according to the 2 phenological stages (green and senescent needles) (Kruskal-Wallis Test; $P > 0.05$).

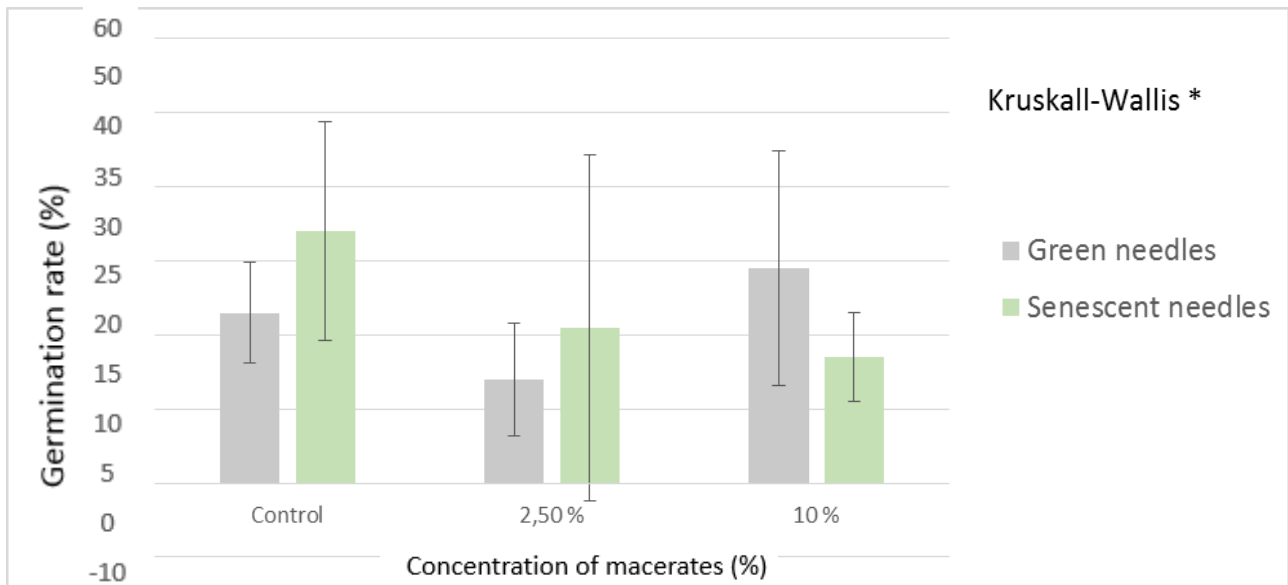


Fig. 2: Mean ratio of germination rate (\pm standard error) as a function of *Pinus halepensis* needles extract dose (high vs. low). Kruskal-Wallis test results are given (P-value: * significant at 0.05; ** significant at 0.01; ***: significant at 0.001). Values sharing the same letter are not different at $\alpha=5\%$.

3.2. Mesofauna experiment

Results showed a significant difference in Acari/Collembola ratio between the control and the rain exclusion plots (Kruskal-Wallis Test; $P < 0.01$).

We observed an increase of Acari/Collembola ratio and a decrease of the number of Collembola individuals with the decrease of precipitation (in the rain exclusion plot).

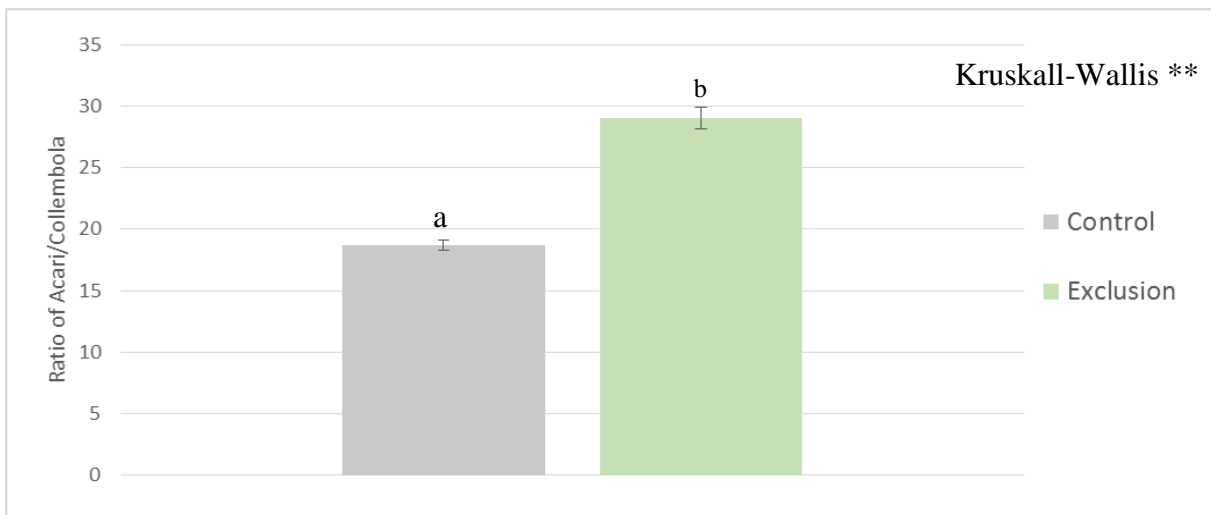


Fig. 1: Mean ratio of Acari/Collembola (\pm standard error) as a function of both treatments plots (control plot and rain exclusion plot). Kruskal-Wallis test results are given (P-value: * significant at 0.05; ** significant at 0.01; *** : significant at 0.001). Values sharing the same letter are not different at $\alpha=5\%$.

4. Discussion/Conclusion

4.1. Allelopathy discussion

There is no significant differences between phenological stages of needles but there is significant difference among the different concentrations though the trend does not meet our expectation. We expect the germination rate goes down as the concentration rises, because the secondary metabolites of *P. halepensis* should inhibit the germination of Lactus seeds. However, according to data, the lowest germination rate the 25%, the middle one, instead of the highest concentration rate. Moreover, we expect the metabolites extracted from the younger *P. halepensis* have more effects on reducing the seeds germination rate but there is no difference. In this experiment, the growing period of the seeds were only five days, so a longer time would have provided a better profile of the germination. It could be interesting to measure the impact of allelochemicals on the germination speed also. We used only four replicates per treatment, so with more replicates (at least ten), the results may have been significant in statistics. Moreover, we focused the experiment only on *P. halepensis* compounds but it would be better to try on other Mediterranean plant species. Maybe the phenomena of allelopathy is affected by other plants.

4.2. Mesofauna discussion

Our results show that the ratios of Acari to Collembola in control and drought condition are significantly different. With the higher ratio in the drought condition, we can deduce that Collembola escape from desiccation, more than Acari. So, they are more sensitive to drought. According to the cuticle structure [1], the variation between species [2] can explain this difference. The cuticles of Collembola are robust and anti-adhesive allowing cuticular respiration under humid conditions in the soil environment. The interaction between different species and conditions are still a mystery which waits to be explored, because we expect a drier environment as global climate changes. Following former researches, we choose two different kinds of animals which are sensitive to desiccation as the targets. Collembola act as an important role in the food web and the decomposition process of soils, so do on the entire ecosystem functioning. It would be interesting to keep working on this topic: how will react the Mediterranean food web and the nutrients cycle to the increase of drought period in the future?

Table of figures

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Fig.2. Mean ratio of germination rate (\pm standard error) as a function of *Pinus halepensis* needles extract dose (high vs. low). Kruskal-Wallis test results are given (P-value: * significant at 0.05 ; ** significant at 0.01 ; *** :significant at 0.001). Values sharing the same letter are not different at $\alpha=5\%$6

Fig.3. Mean ratio of Acari/Collembola (\pm standard error) as a function of both treatments plots (control plot and rain exclusion plot). Kruskal-Wallis test results are given (P-value: * significant at 0.05; ** significant at 0.01; *** significant at 0.001). Values sharing the same letter are not different at $\alpha=5\%$).6

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