Underground Battle - Cress attacks roots with chemistry

Class 4G Kantonsschule Zug

Introduction about roots in general and about our Experiment

The main function of the root is warranty of water and ion absorption of the soil (via active transport in opposition to the concentration gradient). The root also stores assimilates[1]. The plant is detained in the ground by the root, which therefore is a stabilizer. Near the root top are the hair roots, which divide in many directions. In many countries roots are important comestibles because different spices can be gained from some of them (*E.g. Angelica, Sassafras* and *Sarsaparilla*). Another interesting fact in terms of economics is, that yam roots are used to produce birth control pills because they contain estrogen. Roots are the source of two of the most important parts of a plant (phloem and xylem), which with the help of transpiration and the osmosis in the root tissue pump water and nutrients to the branches [1]. The xylem contains a watery solution that consists of organic as well as inorganic substances[2]. In case of no or decreased transpiration and root pressure plants stimulate a biologically very interesting process of adaptation named guttation[3]. This process is especially present in humid climates. During this process, xylem is excreted by the tips of the leaf[2]. The guttation is therefore responsible for extracting the surplus water. The hydathodes are responsible for the passive excretion, which depends on the pressure. On the other hand the trichome hydathodes are responsible for the active excretion, which consumes energy[3]. In the ground roots constantly fight for space and ressources. Therefore roots send out chemicals, which attack the neigbouring roots. The mutual influence of roots with biochemical substances (secondary metabolites) is called an allelopathic effect[4]. Especially walnuts are well known for this effect. The walnut determines the growth of other roots with juglone. In our experiment we want to determine, if cress has an allelopathic effect on roots when it gets in touch with them. Furthermore, benzylisothiocyanate causes the sharp taste of cress.

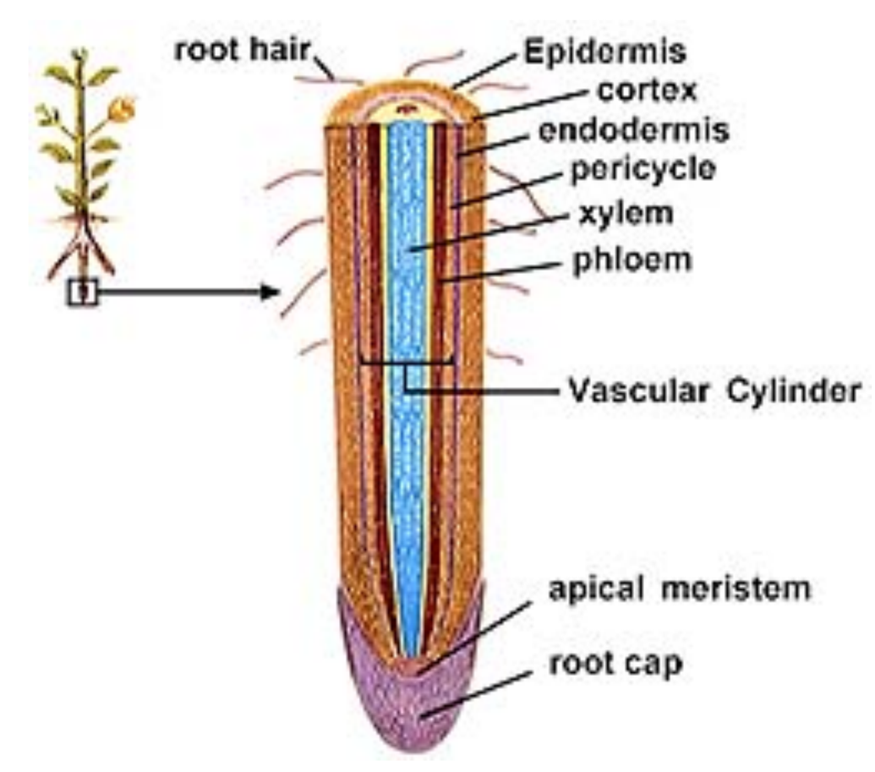

Figure 1: Schematic structure of a root

Defining the problem and selecting variables

Does cress (*Lepidium sativum*) inhibit the growth of roots and has therefore an allelopathic effect?

Variables

Independent variable: Ingredients oft the added liquid
Dependent variable: Growth of the roots

Measurement of the dependant variable: Length of the roots [mm]
Controlled variable: Amount of liquid, medium, temperature, light

Material and Methods

We bought the cress (*Lepidium sativum*) as fully grown plants in a department store (Coop). We shredded cress together with tap water and filtered the liquid. We made two control experiments, one with water as substrate and one with a salad-water substrate (we took garden salad (*Lactuca sativa*) from a neighboring farm) to determine the influence of a plant, which doesn't have an allelopathic effect. The salad was also shredded and mixed. Then we used different extraction-water ratios. The experiment was performed with the following substrates: Water, salad/water 1:10, cress/water 1:5,1:10 and 1:50. We gave our substrates to sunflower seeds (to see the impact on other plants) and to cress seeds (to see if the cress affects the growing of their own species). We took sunflower seeds (from a department store), because their roots grow fast. Each substrate was poured over 48 cress seeds and 48 sunflower seeds. The seeds got arranged in petri dishes (16 seeds per plate) on filter paper with the exact same distance to each other. Four cotton pads were put underneath the filter paper. The substrate was put over the seeds with a micropipette daily. According to that all seeds got cultivated under the same circumstances. After four days, we cut off the roots from the seed and measured the length with graph paper. In a side experiment (because of a lack of space it's really briefly described) we searched for the evidence that cress roots send out benzylisothiocyanate. To show that cress roots send out benzylisothiocyanate, intact white roots were immersed in a solution of colorless silver(I) nitrate and ammonia[5]. If there is benzylisothiocyanate around the roots (the substances can't get into the roots), the silver ions react with the benzylisothiocyanate within a few minutes to black silver(I) sulfide.

Data processing

The box-and-whiskers-plot shows not only the median but also the outliers and how good the data accumulates. We made our boxplots with the program excel. To make the boxplot we calculated the maximum, upper quartile, median, lower quartile and the minimum (table two, see below). The number on top shows how many values the boxplot contains.

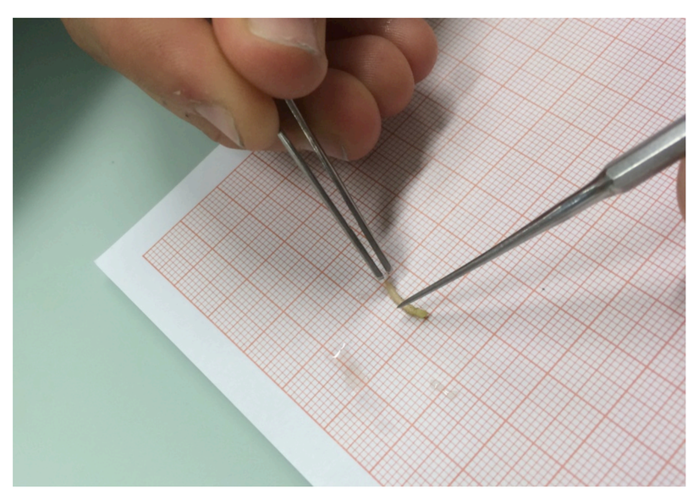

Figure 2: Measurement of the roots on a graphpaper with 1 mm² squares.

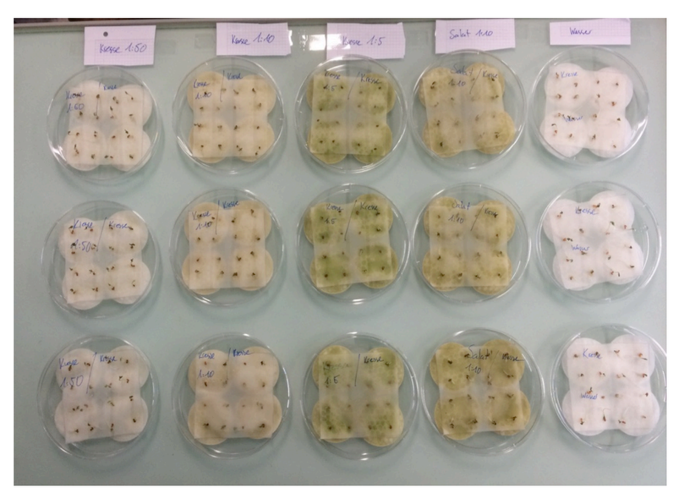

Figure 3: The setup of the cress seeds with substrates. In total we cultivated 15 plates with cress- and 15 plates with sunflower seeds, in one plate were 16 roots, which makes in total 480 roots/seeds.

Data collection, processing and presenting

Recording raw data

You can see the recorded raw data in this table :

[mm]	Sunflower roots															
water	12	14	10	7	10	11	15	10	9	11	13	10	13	11	5	10
salad 1:10	8	13	10	9	9	5	11	10	11	12	12	7	11	10	11	0
cress 1:5	4	4	4	4	5	3.5	3.5	4	5	4	3	4	4.5	5	0	0
cress 1:10	7	10	8	8	6	9	8	7	7	8	7	6	6	6	7	9
cress 1:50	10	9	13	16	12	14	12	10	10	6	14	10	13	13	0	0
[mm]	Cress roots															
water	23	28	18	22	19	21	15	23	14	22	22	17	25	17	22	12
salad 1:10	8	9	6	7	6	6	8	8	9	11	3	3	9	2	0	0
cress 1:5	0.5	5	1	1	3	3	0	0	0	0	0	0	0	0	0	0
cress 1:10	3	3	3	5	5	3	4	5	6	3	2	4	4	4	0	0
cress 1:50	15	15	14	14	11	10	15	12	17	13	8	15	7	14	11	10

[mm]																
water	12	10	12.5	14	13	8	11	12	10	12	11	8	11	13	11	10
salad 1:10	11	9	7	11	10	10	10	10	7.5	10	10	11	8	6	7.5	6
cress 1:5	3.5	3.5	6	5	3	4	4	3.5	7	4	5.5	4	4	5	5	5
cress 1:10	7.5	4	7	5	7	7	5	7.5	8	3	7.5	5	7	7	8	7
cress 1:50	11	9	9	8	9	11	11	9	12	11	10	14	10	6	10	9.5
[mm]																
water	15	16	5	4	16	17	17	9	12	0.5	14	13	6	9	12.5	6
salad 1:10	6	2	10	7	0.5	10	7	6	6	6	4	2	9	7	3	8
cress 1:5	2	3.5	4	3	0.5	0.5	0.5	0.5	1	1	0.5	0.5	0.5	1	3	2
cress 1:10	4	3	5	7	3	4	4	4	3	5	4	4	3	5	2	4
cress 1:50	12	13	14	14	16	20	20	16	19	7	7	18	8	11	0	0

[mm]																
water	12	7	17	12	10	5	9	13	15	8	9	11	8	13	10	0
salad 1:10	12	10	10	8	9	9	9	8	10.5	7	8	10	13	4	7.5	3.5
cress 1:5	4	5	4.5	3	4	3.5	4	4	3.5	5	4	3	4	4	3	4
cress 1:10	5.5	7	8	8	9	7	7.5	6	5	7	6	6	10	8	6	4
cress 1:50	9	9	8	10	11	13	10	10	9	13	15	9	5	11	14	10
[mm]																
water	2	20.5	19	11	17	18	10	22	9	23	18	16	21	16	9	11
salad 1:10	11	7	8	11	7	9	7.5	8	9	8	11	10	7	8	8	10
cress 1:5	4	5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0	0	0	0	0
cress 1:10	5	4	6	7	4	4	4	4	3.5	4	4	5	4	3	4	0
cress 1:50	21	11	11	15	4	3	12	6	7	13	18	6	18	14	15	12

Table 1: Our collected raw data (root length in millimeter).

Sunflower roots [mm]	water	salad 1:10	cress 1:5	cress 1:10	cress 1:50	Cress roots [mm]	water	salad 1:10	cress 1:5	cress 1:10	cress 1:50
maximum	17.0	13.0	7.0	10.0	16.0	maximum	28.0	11.0	5.0	7.0	21.0
upper quartile	12.0	10.5	4.5	8.0	12.0	upper quartile	20.5	9.0	1.0	4.5	15.0
median	11.0	10.0	4.0	7.0	10.0	median	16.0	7.5	0.5	4.0	13.0
lower quartile	10.0	8.0	3.5	6.0	9.0	lower quartile	11.0	6.0	0.0	3.0	9.5
minimum	0.0	0.0	0.0	3.0	0.0	minimum	0.5	0.0	0.0	0.0	0.0

Table 2: Our processed data (root length in milimeter).

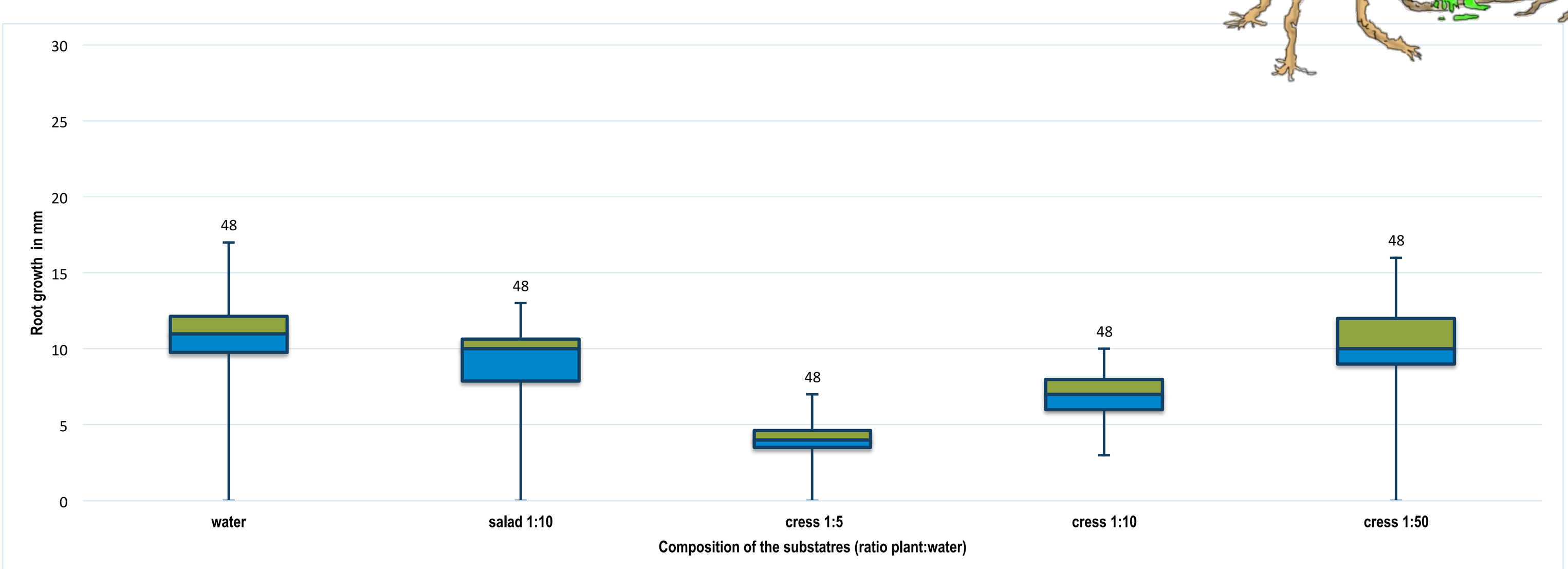

Figure 4: Sunflower root growth

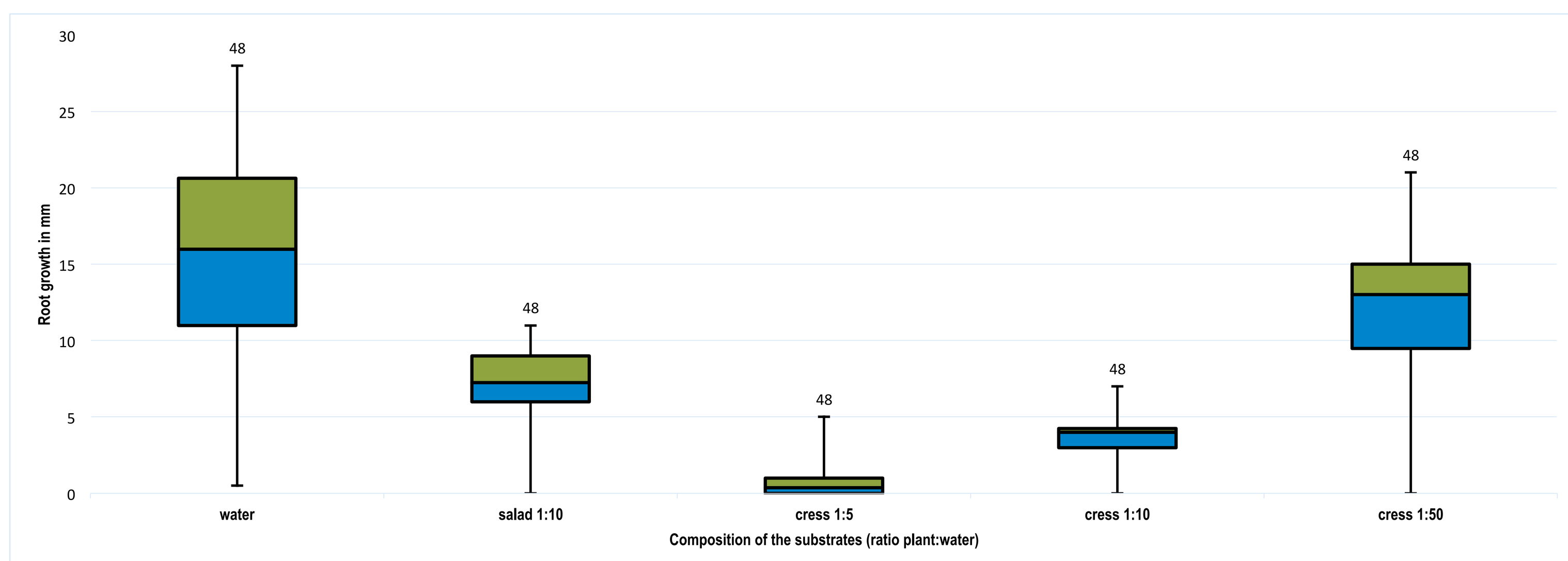

Figure 5: Cress root growth.

Even if seeds of the species were grown under equal conditions and watered with the same amount of substrate their roots developed different length. Cress roots grown with water had the biggest distribution. Nevertheless with both, cress and sunflower roots, the following trends are evident. 1. The roots with salad and cress substrates are smaller than the roots with water. 2. The higher the cress-concentration in the substrate is, the smaller are the roots. 3. The roots with salad-substrate are slightly longer than the ones with cress substrate of the same ratio. There is also one big differences between cress and sunflower roots: In the water-control the roots of cress are clearly longer than sunflower roots. But in all plant-substrates the sunflower-roots are longer than the cress roots. An other important observation is that sunflower roots form lateral roots with the salad 1:10 substrate and with the cress substrate 1:10 they do not (see figure 8 and 9).

Figure 6: Sunflower root with salad 1:10 substrate.

Figure 7: Sunflower root with cress 1:10 substrate.

Side experiment

As you can see the cress roots which came in touch with the solution of silver(I) nitrate and ammonia were black (figure 8, blue part) whereas the roots which didn't came in touch with the solution were white (figure 8, green part).

Figure 8: Cress roots, green: with nothing. blue: with silver(I) nitrate and ammonia.

Discussion and evaluation

Discussion

We supposed, that the salad extract doesn't have an influence on root growth, because it only contains "normal" plant ingredients. This was wrong. Salad determines the growth of both roots. The salad-substrate only reduces the growth in high concentrations. So we suggest that every plant extract has a small allelopathic effect.

The variation of the growth of roots is a natural phenomenon. The longer the roots averagely are, the bigger are the differences in length among them.

Cress seems to inhibit the growth of roots, depending on its concentration in the substrate. Surprisingly cress roots react more sensitive than sunflower roots. Here are some possible explanations:

- Sunflowers are built stronger than cress what makes them more protected.
- In the struggle of life it is necessary to supress his own fellow species.

We observed that cress-substrate blocks the formation of lateral roots, which are important for the absorption of water and minerals. Salad, in contrast, has no influence on the formation of lateral roots. This underlines the significance of cress as an inhibitor of wood growth and is an indication for his allelopathic effect.

With what substance does cress reduce the growth?: We suggest that the benzylisothiocyanate in cress (which makes the sharp taste of it) is responsible for the allelopathic effect. Benzylisothiocyanate is a toxic substance, which attacks unspecified. We suggest that this benzylisothiocyanate damages parts of the root, but not serious enough to kill the root. There are two ways in which benzylisothiocyanate is excreted into the environment. The first occurs when cress gets injured or dies. In this case glucotropaeolin is transformed into benzylisothiocyanate. Benzylsothiocyanate is excreted by dying cress plants, maybe because of bad conditions. It inhibits or reduces the growth of the roots of other cress plants, until the conditions improve. The second is (as shown in the side experiment), that cress roots actively excrete benzylisothiocyanate. We conclude that cress does that, to reduce the growth of other roots in order to get more space for itself.

Evaluation

A major challenge is the naturally different root growth. Therefore we tried to collect as much data as possible. The measurement of the roots was done as properly as possible. Some roots didn't grow or grew only a little bit. So it was difficult to measure, if a root is 0.25 mm long or 0.5 mm. The measurement of the longer roots was done properly. The difficulty here was, that the roots were bent. So we had to carefully bend them straight. We think that all roots grew under more or less the same circumstances. We poured the substrate carefully and exactly measured with micropipettes over the roots. The substrates, which were given regularly over the roots, were used during four days. We kept them in a refrigerator but we can't exclude a digestion process, which made the substrates different. After all, we can say that under the conditions (time, material) we had, the experiment was performed properly. The side experiment is only a hint, that cress send out benzylisothiocyanate.

Improving the investigation

A higher quantity of data would allow a more precise conclusion. It would probably also be better to measure the influence of cress on many different roots and probably during a longer period of time (that would made the measurement easier). To prove that benzylisothiocyanate is the decisive factor it would be really useful to determine the concentrations of benzylisothiocyanate in the substrates. In a next step it would be useful to complement the experiment with a solution containing only benzylisothiocyanate and water.

Bibliography

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