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Myricaria germanica

Experiments regarding seed germination & water stress

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Natural scientific term paper within the project "Integrales Flussgebietsmanagement"

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1. Abstract

As a contribution to the project "Integrales Flussgebietsmanagement" the following two experiments with the threatened and ecologically valuable riparian plant species *Myricaria germanica* were implemented:

In a first experiment we investigated how well seeds of *M. germanica* germinate and grow on different substrates, including sand, gravel and soil.

In a second experiment the germination performance and the growth of *M. germanica* seeds with a different genetic background were analyzed. For this experiment individuals of different populations (see figure 4.1) with different pollination treatments (see figure 4.2) were used. When the majority of the individuals reached a certain growth state the young seedlings were water stressed.

As a main result we can say that *M. germanica* is able to germinate quite fast after the first contact with water. Furthermore a strong preference for sandy substrates for the germination process was found. However, after the germination process the plants did not grow very fast. It took about one month for most of the individuals to produce secondary leaves. Evidence was found that an enhanced investment into root development can be the cause for this slow growth.

Myricaria germanica further seems to be very resistant to water stress for about two weeks. Overall, one can say that individuals from both regions and from all pollination treatments react similar towards water stress.

There are some differences in germinating performance, growth behavior and water stress susceptibility among individuals from different regions and pollination treatments. Individuals from the river Rhone germinated better and faster than the individuals from the river Inn. There is an indication that the Rhone individuals dried out faster as soon as water stress occurred. The pollination treatment also seems to have some influence on the germination and water stress performance of some individual plants. Especially one inbred individual from the canton Valais seemed to have a higher germination and growth rate than all other individuals.

2. Introduction

The following term paper has been worked out within the scope of the project "Integrales Flussgebietsmanagment". This project's goal is to close knowledge gaps in the field of water protection and to investigate interactions between habitat diversity of a river system and of flood control. One essential aspect of this project is the research of lateral and longitudinal cross-linkage and their impact on biodiversity (Schleiss et al., 2008).

If certain areas do not display sufficient connectivity, the occurrence of a species may be threatened. This problem occurs mainly for rare and ecologically valuable species. Caused by inbreeding their fitness will diminish over a certain period of time. On the other hand artificial linkage of areas may cause unfavorable gene flow that will lead to outbreeding and thus might erase local adaptions (Hamilton, 2009).

To investigate the effects of cross-linkage as well as to analyze the demands of riparian species concerning their habitat, several model organisms were chosen for the project "Integrales Flussgebietsmanagement". *Myricaria germanica* was selected as a model organism for an ecologically important riparian plant species with good dispersal abilities. *Myricaria germanica* belongs to the family of *Tamaricaceae* and is a characteristic pioneer plant. *Myricaria germanica* can colonise an area very quickly and is reliant upon dynamic floods (Kammerer, 2003).

To be able to protect other species as endangered and ecologically valuable like *M. germanica,* it is important to investigate their characteristics. Of enhanced interest and importance are traits that influence geographic dispersal such as development, dispersal abilities and germination condition demands (Chen, 2007).

So far there have been only few scientific investigations concerning the germination behavior of *Tamaricaceae*. Chen (2007) was able to show during germinations experiments with *Myricaria laxiflora* that the seeds loose germination capacity after about one week. As soon as the substrate gets moist, germination starts within 24 hours with very high germination rates. In the first few hours of the germination, the seeds of *M. laxiflora* are able to adsorb large amounts of water that protect them against dry conditions for a short period of time. Furthermore, the germination is strongly dependent on the water content of the growing substrate. *M. laxiflora* favors saturated and sandy substrates.

More studies have been performed for different species of poplars and willows, which can establish quite well on gravel banks. Such studies have shown that germination, establishment of seedlings and growth of gravel bank vegetation are mainly determined by the characteristics of a gravel bank and of fluvial processes. The germination and the early establishment of the young seedlings are influenced by the particle size of the substrate while the growth and viability of the plants in a later state depend mostly on the presence or absence of water stress (McBride, 1984).

According to Karrenberg (2002), *Salicaceae* are capable of germinating within 24 hours in optimal conditions. However, if water stress does occur, the germination rate will be significantly reduced. The seedlings of some species like *Populus deltoides* or *Salix nigra* are even able to germinate while being submerged without taking any damage. The longevity can vary quite a lot between seedlings. It has been shown that most of the established seedlings do not survive the first year in pristine environments.

The germination process of *Populus nigra* has been studied more accurately by Guilloy-Froget (2002). The seedlings of this species adsorb water with fine hairs right after the germination. The speed of root development is very slow at the beginning and will not increase until the first month is over. Thus *P.nigra* is very delicate towards water stress during this time. Germination experiments have proven that germination rates on clay are higher than on gravel substrates. Furthermore it has been shown that seeds do grow much better if the substrate is flooded instead of being just humid or dry, though then there is no more difference between substrates. Moreover, the moment of seed gathering seems to be vital in these experiments as the germination capacity depends on this (Guilloy-Froget, 2002).

In defiance of existing studies concerning germination behavior and water stress resistance of *Salicaceae* and one *Tamaricaceae* there is not enough awareness to characterize those attributes for *M. germanica* yet. To better specify the habitat demands of this model species and also to contribute to the investigations about longitudinal and lateral cross-linkage in aquatic systems, experiments regarding germination and water stress were preformed with *M. germanica* for this term paper. The following questions have been asked respecting these experiments:

(1) How does the germination capacity of *M. germanica* vary while growing on different substrates?

(2) How fast and to which degree do established seedlings of *M. germanica* react against water stress?

(3) Will artificially pollinated seeds originating from different regions perform differently on the subject of question (1) and (2)?

3. General Traits of *Myricaria germanica*

3.1 Importance and Danger

Due to the fact that *M. germanica* is the only representative of the family *Tamaricaceae* in Middle Europe, the species is therefore very important for Middle Europe (Kammerer, 2003). As mentioned in the Introduction, because of her properties *M. germanica* is used as a model organism with high dispersal ability within the project "Integrales Flussgebietsmanagement" (Schleiss et al., 2008). *Myricaria germanica* is one of the first colonizers of the alluvium. The plant can most commonly be found on open gravel- or sandbanks which are strongly influenced by temporarily floods and droughts. Because the species is weak in competition there is a strong need of recurring disturbances for establishment. The long roots are sometimes connected with the groundwater so that *M. germanica* can settle on coarse gravel substrates. Furthermore the ability to store water in its xeromorphic leaves thus is an adaption to over come droughts (Kammerer, 2003). The dynamic river bed habitats where *M. germanica* can be found were largely destroyed during the river bed constructions in the 18th and 19th centuries. As a result *M. germanica* became rare and can today only be found in small isolated populations (see map chapter 4).

In ancient medicine *M. germanica* was applied for various therapies, especially for spleen diseases. For problems with organs such as lung, liver, kidney and bladder but also for toothache and many other diseases *M. germanica* was thought to help (Madaus, 1938). The green compressed leaves and the out boiled roots of *M. germanica* were used as medicine and the oil of the tamarisk was used for inhalation and embrocating (Kiem, 1992). Presently the importance of *M. germanica* as a possible medical plant has been forgotten. If an effect to human health can be proven then maybe also today's medicine might find a use for *M. germanica*.

Because of the possible medical use and the singularity for middle Europe it is very important to find an appropriate strategy to prevent *M. germanica* from extinction.

3.2 Germination and Growth Traits

From literature it is known that seeds of *M. germanica* can only germinate during a really short time. They lose germination capacity quite quickly. The germination itself starts immediately after the contact with a wet substrate. On the contrary a slow growth during the first vegetation period was observed (Kammerer, 2003). For best germination conditions Bachmann 1997 and Petutsching 1994 assumed a good water imbued substrate. Such conditions are best fulfilled with a fine and silt containing sediment (Kammerer, 2003). Morphology and lifecycle of *M. germanica* is described in figure 3.1.

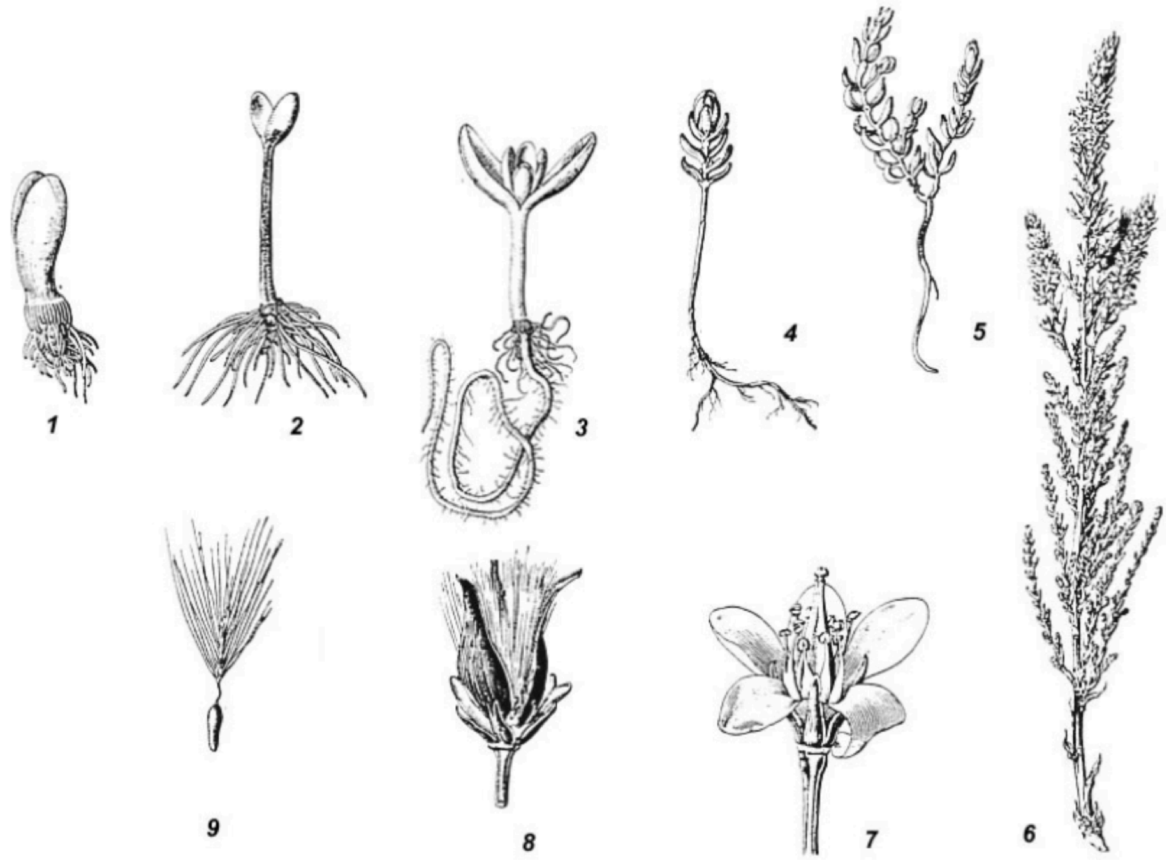

Figure 3.1: Morphology and Lifecycle of M. germanica: Growth of seedling (1-5), flowering shoot of a young plant (6), single blossom (7), mature open boll (8), seed (9), Figure from Kammerer (2003).

4. Methods

4.1 Individuals and Pollination

For the germination experiments seeds from individuals originating from the three rivers Inn, Rhine and Rhone in Switzerland were used. First two populations from the river Inn in the canton of Grisons were used. Individuals from one population near Pontresina and one from Val Bever were collected. Furthermore, two populations from the river Rhine, also located in the canton Grisons, one near Zizers and one from Bonaduz were gathered. Finally two populations from the river Rhone in the canton of Valais, one population from Zinal and one near Salgesch were supplied (see table 4.1 and figure 4.1).

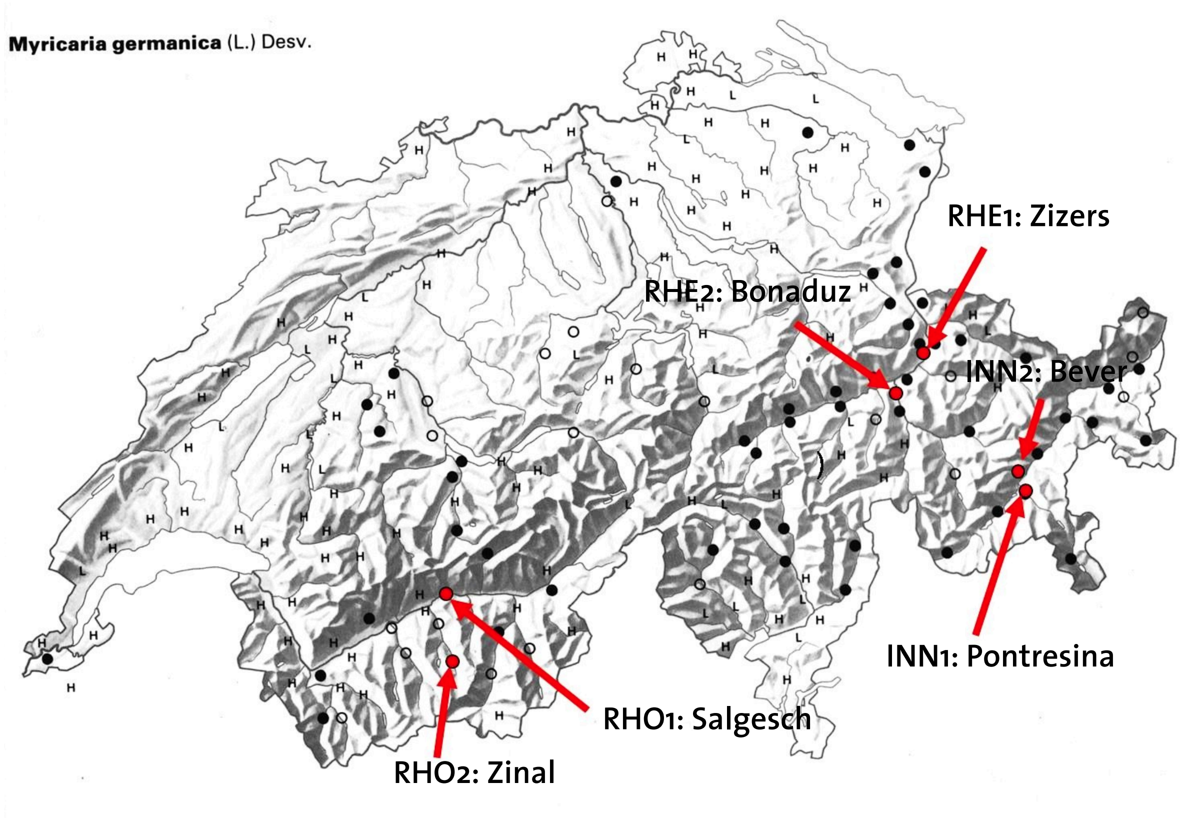

Figure 4.1: Map of different M. germanica Populations. Red circle locations of: Populations used for the experiments (Source: Silke Werth)

Table 4.1: Population numbers, altitudes and municipalities of the populations used for the experiments.

Population name	Altitude (m)	Municipality
INN 1	1824	Pontresina
INN 2	1752	Val Bever
RHE 1	522	Zizers
RHE 2	561	Bonaduz
RHO 1	544	Salgesch
RHO 2	1674	Zinal

The Individuals from all these populations were first raised at WSL and then used for different pollinations. In the first experiment, where we tested the different substrates, we used seeds from blossoms which flowered before they could be packed into nets preventing pollination. The pollination in such cases was therefore random for all individuals, populations and regions. For the second experiment seeds from controlled pollinated blossoms were used. Three main types of pollination were applied (see figure 4.2). First individuals from different rivers were crossed (REG), furthermore individuals within one population were crossed and finally each individual was self-pollinated (SELF).

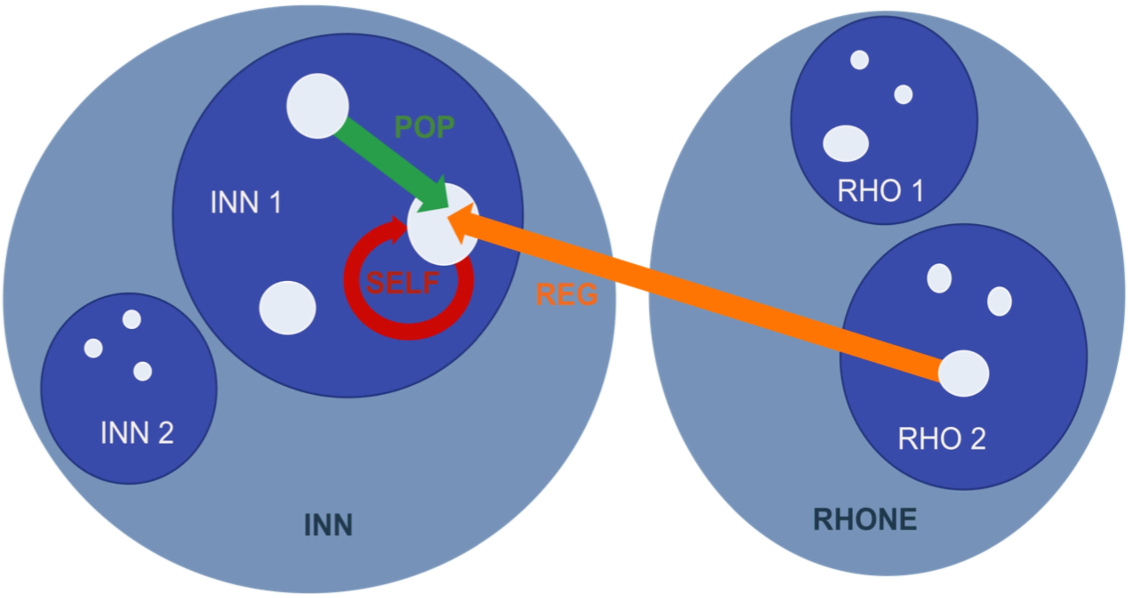

Figure 4.2: Pollination scheme for an individual from INN 1 Population. POP: Cross within the Population, REG: Cross between two different River Regions, SELF: Self-pollination

To ensure that we would have enough seeds for the second experiment (> 500 seeds per pollinated blossom), only individuals from the RHO 2, INN 1 and INN 2 populations were applied. First from the RHO 2 population the individuals 10, 16 and 20, furthermore from the INN 1 population the individual 19 and finally from the INN 2 population the individuals 01 and 12 were chosen.

4.2 Substrate Experiment

In the first experiment the germination capacity of *M. germanica* seeds was investigated using a specially designed pot experiment. In a greenhouse with regulated climate conditions seeds were germinated on five different substrates (see Figure 4.3). As substrate mixtures of round gravel (8-16mm), round sand (0-4mm) and potting soil (WSL "Topfsubstrat", wide appendix) were used. The pots that have been used have a volume of 5 liters and were each placed on a bottom plate. Before and during the experiment the bottom plates were filled with water to make sure that the substrates were always saturated. During the experiment the substrate B which only contained gravel never had a really humid surface. Moreover after the second day of this experiment on the gravel substrate hardly any seeds were found anymore.

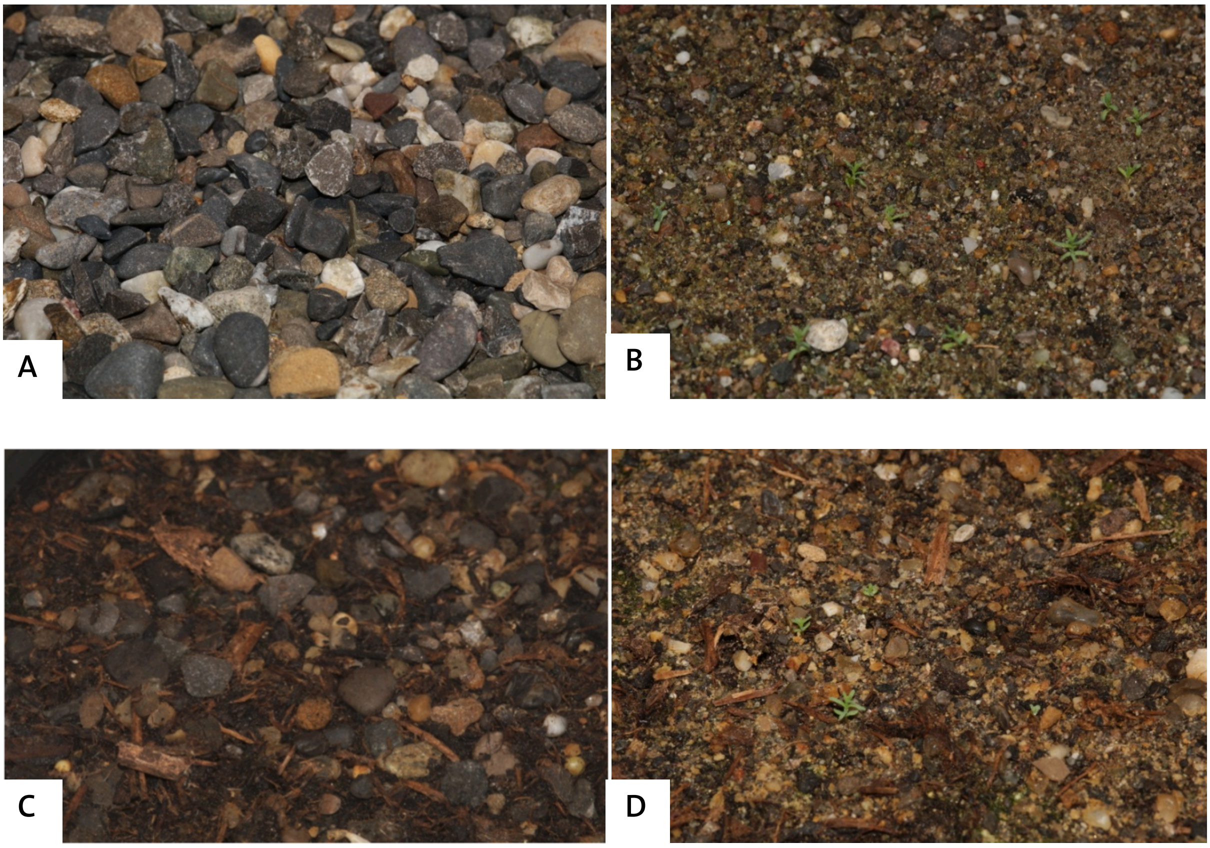

Figure 4.3: Different substrates used for the experiment:

A: gravel
B: sand
C: soil gravel
D: soil sand
E: sand gravel

Seeds from randomly pollinated blossoms were collected and mixed. Directly after the collection on the May 19 2009 twenty seeds were put in each pot. There were four replicate pots for each substrate. The duration of the whole seeding process took about four hours and was done as randomly as possible. During the first two days of the experiment the pots were covered with a fleece to make sure that conditions on the substrate surface were humid enough to allow the seeds to germinate. The fleece was removed after the second day of the experiment.

During the experiment we counted the number of seedlings every day from May 19 to May 26. Additionally we counted the number of seedlings on May 28 and June 8. This experiment ended on June 18. We also examined the growth of the seedlings, allowing us to define four different states of growth for the seedlings (see figure 4.4). “K” means that the plant has just germinated, at the “KB” state it developped cotyledons, and at “PB” and “SB” primary leaves and secondary leaves respectively. Because the seedlings were really small at the beginning of this experiment they were hard to detect. So on fourth day toothpicks were put into the substrates to better find the seedlings again.

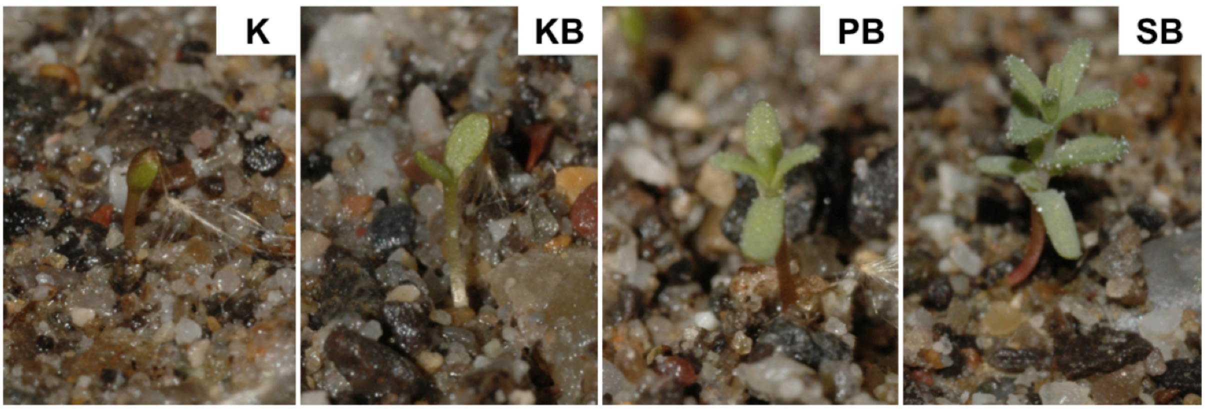

Figure 4.4: Different states of growth defined for both experiments. On “K” the seed germinated, on “KB” it showed cotyledons, on “PB” primary, and on “SB” secondary leaves

4.3 Germination and Water Stress Experiment

The second experiment did start similar as the first - with the preparation of the involved flowerpots. Tough unlike in the first experiment we only utilized two different types of substrate. From the 28 pots we prepared we filled 14 with 100% sand (0-4mm grain size) and 14 with gravel (75% round gravel, 8-16mm grain size and 25% sand, 0-4mm grain size). We had seven pots of both substrates that were stressed and as a control group seven without stress. The pots were exactly the same as the ones already used in substrate experiment, having five liter volume.

With separators made of PET sheets, we split the growth area of each pot into six even sections (see figure 4.5). Half the part of the pot (three of the sections) contained plants from Rhone, the other half from the Inn. Each pot contained two diagonally opposite sections with one of the three crossings (POP, REG or SELF; see figure 4.2).

In each section we placed three lines of seeds, each line consisting of five seeds and representing one of the six different ancestors (Inn1-01, Inn2-19, Inn1-12, Rho2-10, Rho2-16, Rho2-20). This time the seeds have not been pollinated randomly but controlled. We did exactly know which seed descended from which ancestor. To recognize which seeds belonged to which ancestor, crossing or region, we used the following color-code (see figure 4.5 and 4.6 , tables 4.3 and 4.4):

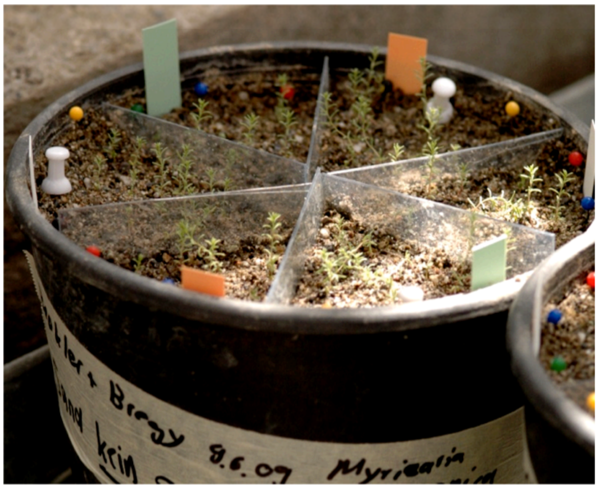

Figure 4.5: Picture of a pot

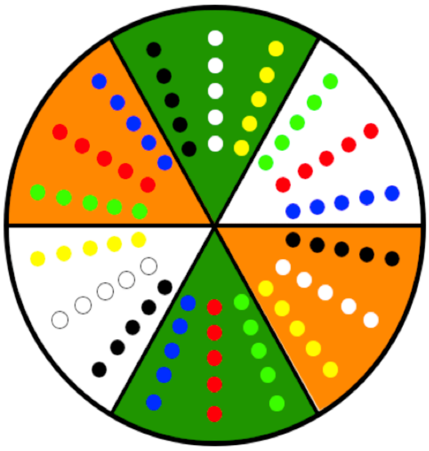

Figure 4.6: Scheme of a pot

Table 4.3: Legend for the pollination treatments

Section color	Section type (crossing)
Green	REG (within regions)
White	SELF (self-pollinated)
Orange	POP (within populations)

Table 4.4: Legend for the individuals

Line color	Line type (ancestor)
Green	Inn1-01
Red	Inn1-12
Blue	Inn2-19
Black	Rho2-10
White	Rho2-16
Yellow	Rho2-20

On the 10th of June we placed a total of 90 seeds in each pot - a total of 1260 on the sand and 1260 on the gravel substrate. Then we placed the pots on their according bottom plate, watered them by the same method as described in the first experiment.

From then on we observed the plants and collected data, at first every other day. After two weeks we increased the intervals as things started to change slower. Then we gathered data only every third or fourth day. To quantify their growth state we operated with the same four growth levels as described in the substrate picture (see figure 4.4). During this period that lasted 31 days, the plant pots always contained water and have been refilled once every few days. However the surface of the pots containing the gravel substrate often seemed to be rather dry after several days without rainfall. During the first section of the experiment without water stress, we wet them cautiously with a water dispenser when we collected the data.

31 days after the start of the experiment, on the 16th of July, we removed the bottom plates from half of the flowerpots and thus set them on stress. The control group continued to get watered as usual. From the 16th of July we only measured the stress level and did no more pay attention on the growth states, because all plants either reached growth level four or were stuck on their respective level since weeks. To quantify the stress-states of the plants we defined four stress levels, similar to the four growth levels used already before (see figure 4.7). Some days after the start of the dry stress experiment, during which nothing in particular happened, we again started to observe them every second day to be sure to catch the beginning of the stress period. After 22 days of water stress all plants were completely dried out and dead. This indicated the end of the experiment on the 7th of August.

Figure 4.7: Different levels of water stress (S1: the lowest quarter dried, S2: the lower half of the plant dried, S3: the lower three quarters dried, S4, the complete plant dried)

Caused by a lack of time, this study will not include a detailed statistical analysis. We mostly interpreted plots derived out of our collected data. To provide a basic level of estimation, we calculated standard errors of our data which are shown as error bars in all displayed plots. Therefore, our results show tendencies and observations. A fundamental statistical analysis has yet to be done - maybe in a following project.

5. Results

5.1 Substrate Experiment

For the substrate experiment the mean number of seedlings over the whole duration of the experiment (19.05.09 – 18.06.09) is shown in the figure 5.1. The sand and the sand gravel substrate show the best germination rates (about 50%). As the mean of seedlings in the states KB to SB indicates, also the growth was best on these two substrates. The soil substrates were less successful, but also within these, the sand substrate preformed better than the gravel substrate. On the gravel substrate no seedlings were found.

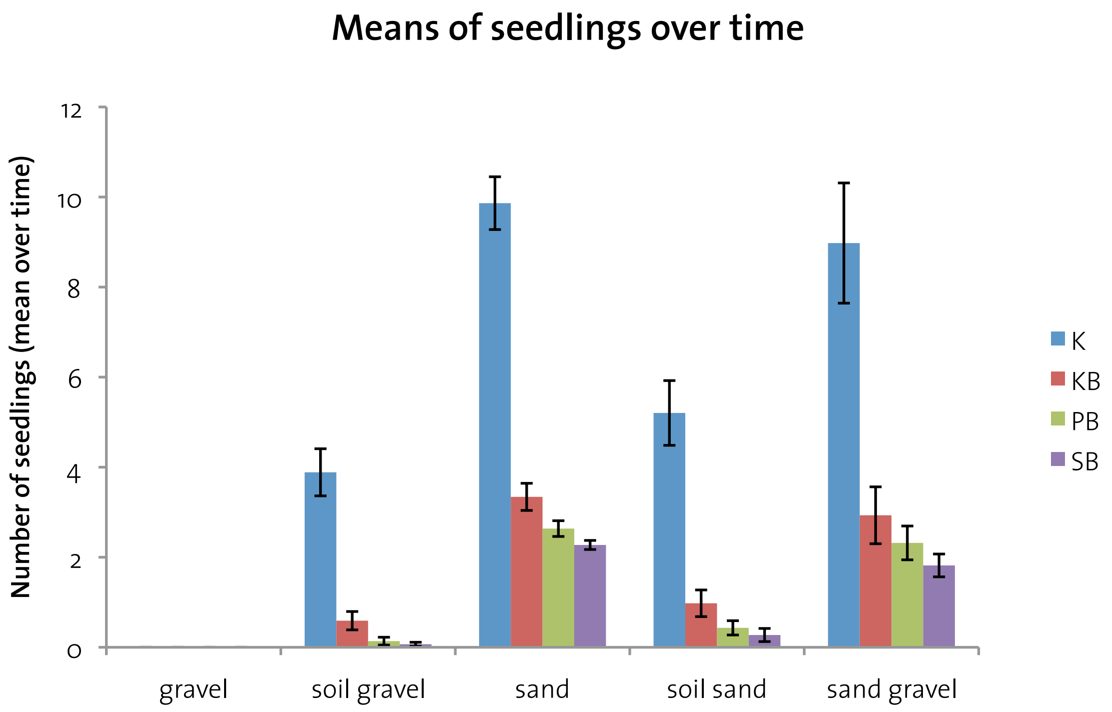

Figure 5.1: Means and standard errors of seedlings over time for the four different growth states and the five different substrates (K = germinated, KB = cotyledons, PB = primary leaves, SB = secondary leaves)

A more detailed development for the single growth states for the period of time of the whole experiment (19.05.09 – 18.06.09) is shown in the figure 5.2. In this figure the growth state K, which illustrates the total number of germinated seedlings over time, shows a fast germination within the first five days. Only a few seeds germinated after this first fast germination phase. The two substrates with sand and sand gravel had the best germination and growth performance over the whole time span of the experiment. Almost no seedlings died on the sand and sand gravel substrates. The two soil substrates first showed a good germination performance. Conversely to the sand and sand gravel substrates seedlings on the soil substrates mostly died after about one week. The few surviving seedlings grew then with a similar rate as on the other substrates. On the gravel substrate no seedlings were found during the whole time span of the experiment. The behavior of the three growth states KB, PB and SB was quite similar. They all show that the two sand substrates (sand and sand gravel) showed the best growth performance.

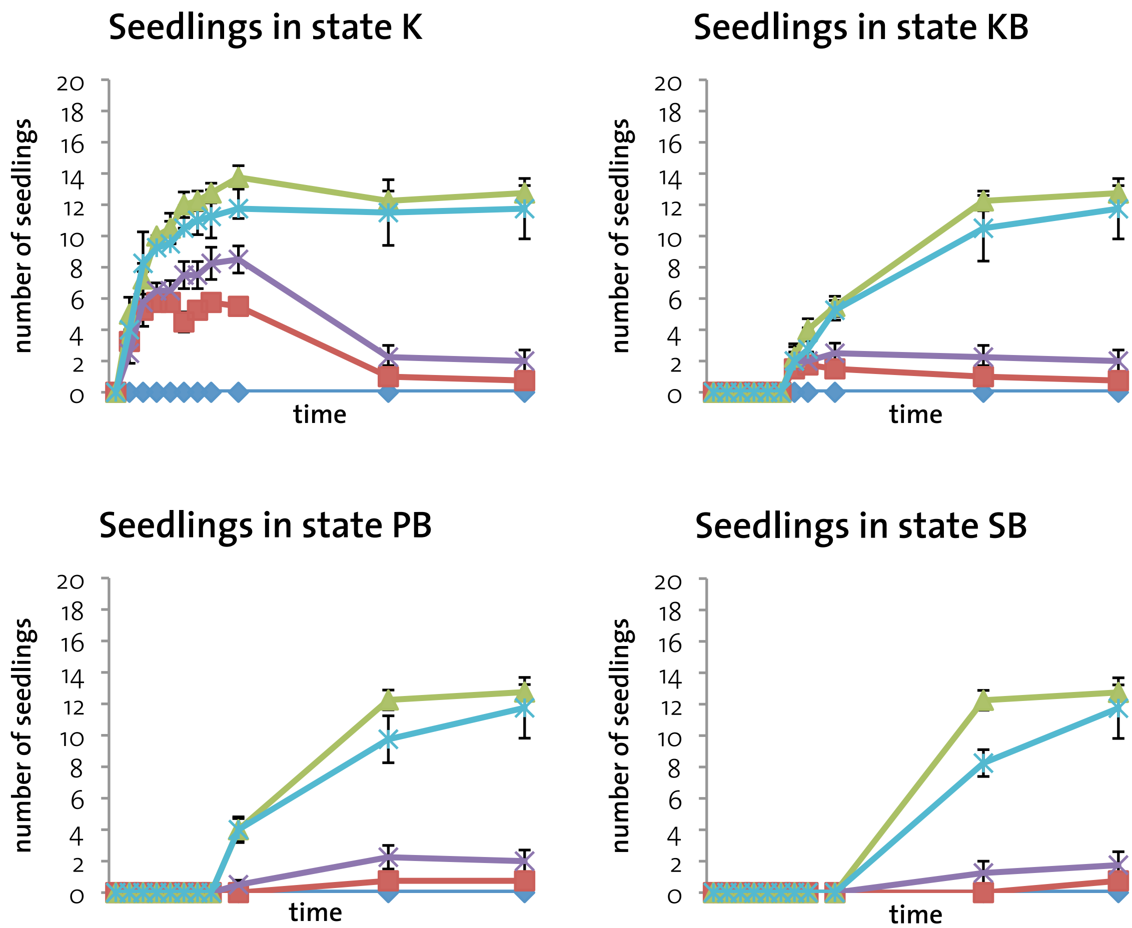

Figure 5.2: Each Diagram shows the mean of the number of seedlings for a growth state (K till SB) for the five different substrates (gravel, soil gravel, sand, soil sand, sand gravel).

5.2 Germination and Water Stress Experiment

In the figure 5.3 the mean and the standard errors of germination rates over the time span of the experiment without dry stress were calculated for two different substrates. Therefore the mean number of seedlings in the growth state K was taken into account. In this calculation a germination rate of 100% can be reached if all the five seeds germinated and survived. The calculation summarizes all individuals from one region with the same pollination. The Rhone individuals showed in all pollination treatments higher germination rates than the Inn individuals. Germination on the gravel substrate was much lower than on the sand substrate. It was so low, that these data couldn't be used for further analyzes. Differentiation between the pollination treatments is not really obvious for both the Rhone and the Inn Individuals. It seems like the self pollinated Rhone Individuals have a little higher germination rate and that the Rhone population treatment had the lowest germination rate. A completely opposed behavior can be seen within the Inn Individuals.

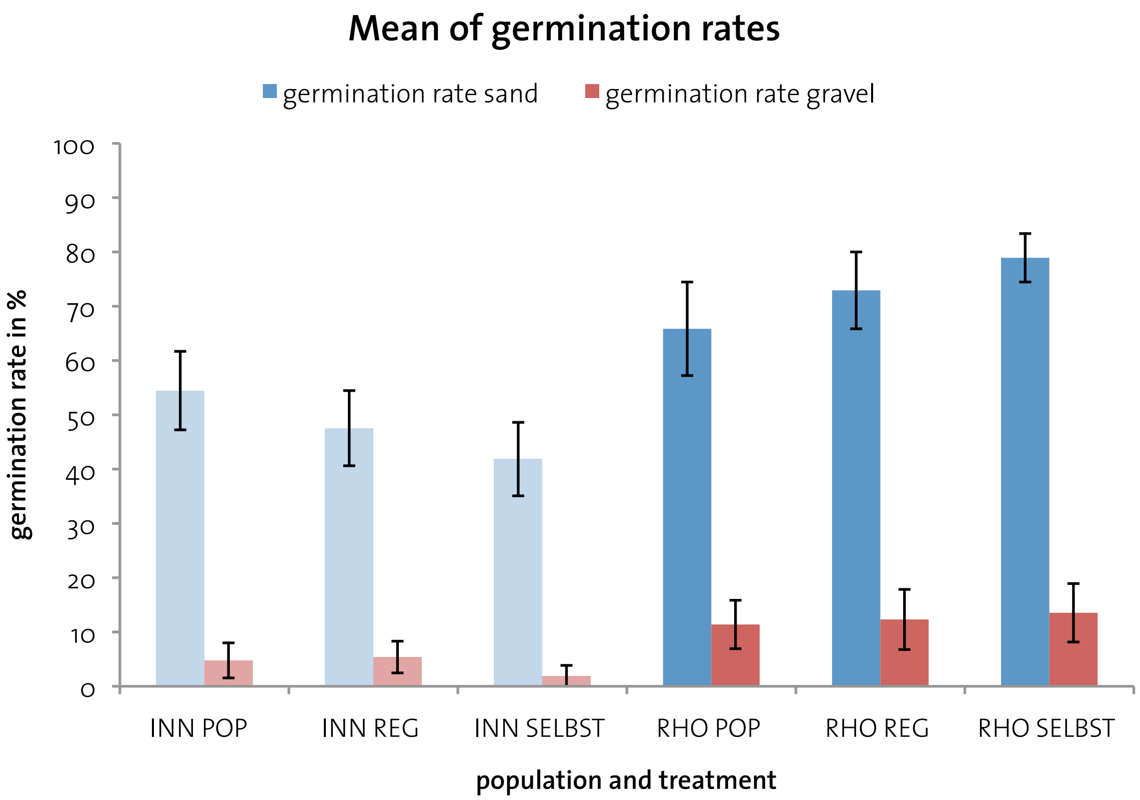

Figure 5.3: Mean of germination rates and standard errors over the time of the experiment without dry stress for two different substrates (gravel and sand).

This behavior can also be seen in diagram 5.4, were the mean number of seedlings in growth state K is shown for all pollination treatments for the whole period of time of the experiment. Most of the seeds germinated in the first three days. After the germination the number of seedlings was more or less constant for all pollination treatments.

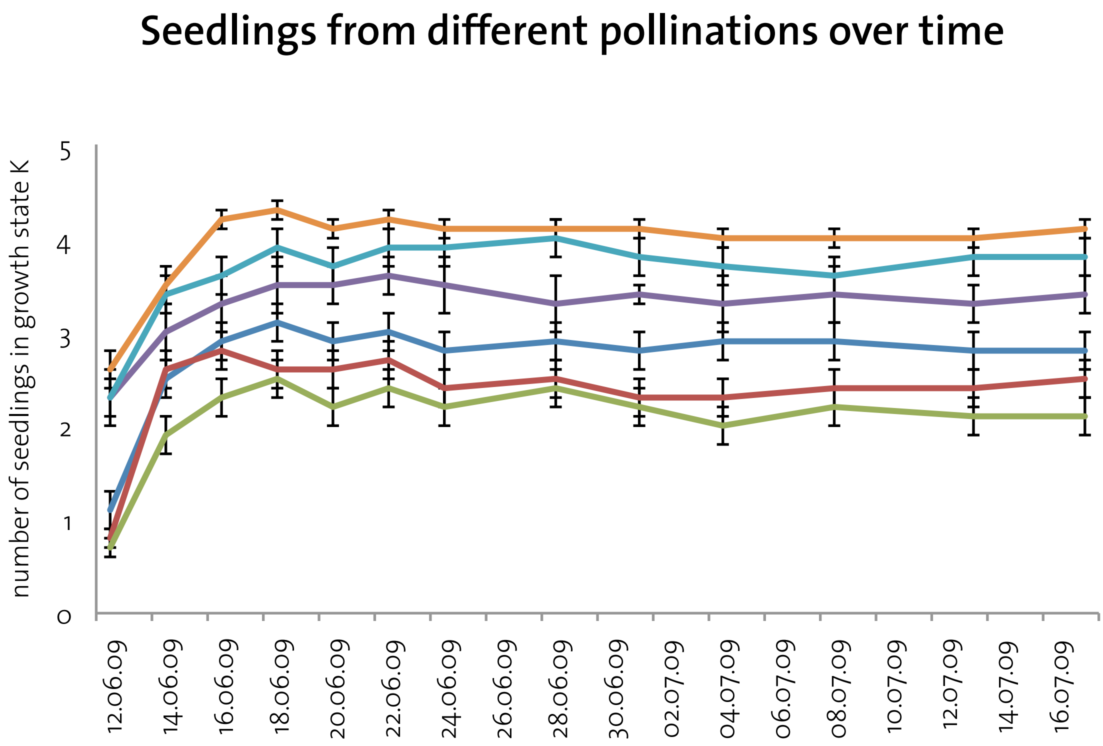

Figure 5.4: The mean number of seedlings in the growth state K and the standard errors for the different pollination treatments and the two different regions (INN POP INN REG INN SELBST RHO POP RHO REG RHO SELBST).

For all pollination treatments the growth rates were similar. For the treatment RHO SELBST this behavior is shown in figure 5.5. The really fast germination is visible in the growth state K. After two days of the experiment the first seedlings achieved the growth state KB and after one week the first seedlings were producing primary leaves. After four more days the first seedlings achieved the growth sate SB. Growth rates seem to be constant, because as one can see in figure 5.5 the vurves are quite linear. After the fast germination at the beginning of the experiment it took about one month until most of the surviving seedlings produced secondary leaves.

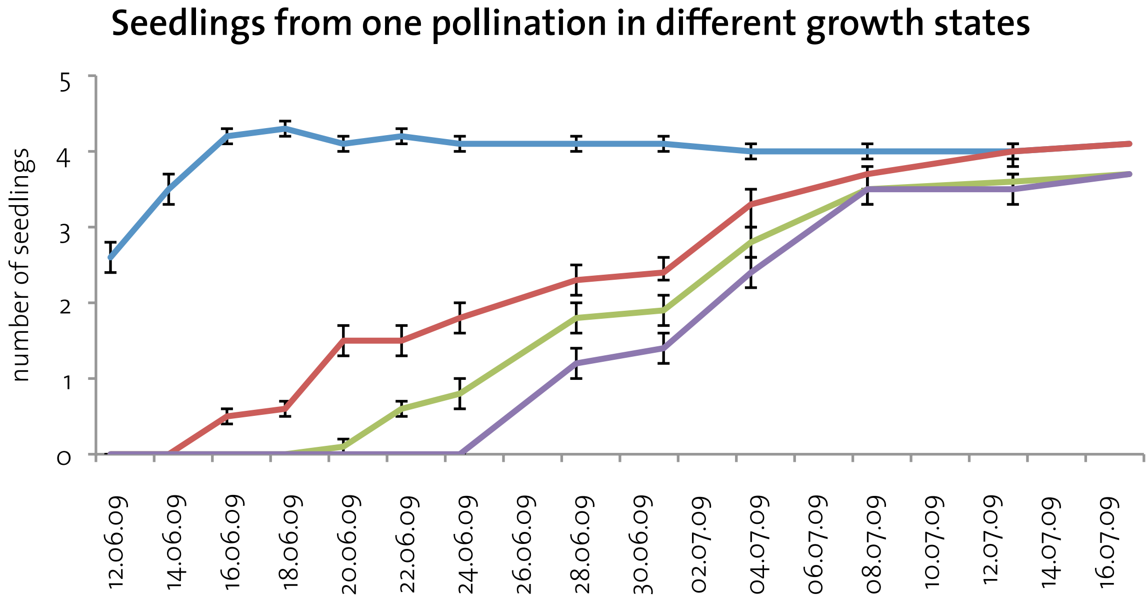

Figure 5.5: The mean number of seedlings and the standard error for all four growth sates (K KB PB SB) for the self-pollination Rhone (RHO SELBST).

Because we could not see any clear germination and growth differences between the pollination treatments on the level of all three individuals from one region, we isolated the data for every individual. Fist we compared the germination and growth performance for all three individuals that were before summarized. On figure 5.6 the results for the three Rhone Individuals and the growth state K is shown (further figures for growth states K and KB in appendix 8.3.). The RHO 10 and the RHO 16 individual behave similar, but the RHO 20 individuals shows a different growth behavior. At the beginning of the experiment the RHO 20 individual had a really low growth, but to the end of the experiment the growth increased. Also within the other growth states this behavior could be detected. Unlike the Rhone individuals the Inn individuals showed more or less a similar behavior. Only in some cases the INN1-01 and the INN1-12 individuals differed lightly from the others (further figures for growth states K and KB see appendix 8.3).

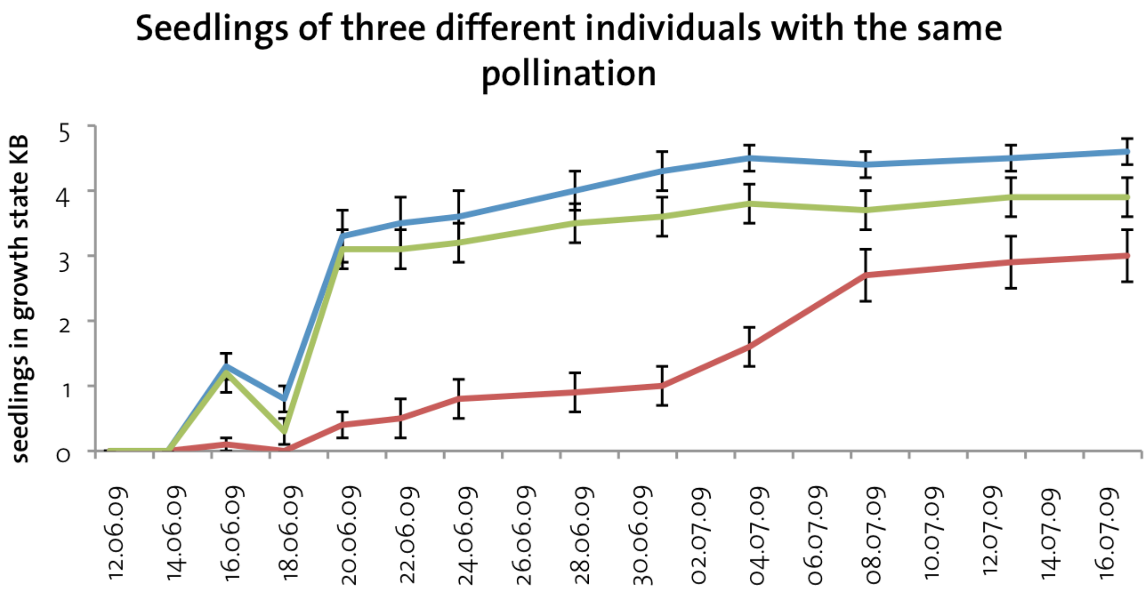

Figure 5.6: Mean number of seedlings in growth state K and standard errors for three different Rhone individuals with the same pollination (RHO 10 REG RHO 20 REG RHO 16 REG).

Of further interest was, if the pollination treatments had an effect on the different individuals. Like in the summarized results for most of the individuals almost no differences could be seen. But for some individuals huge differences appeared. Especially the RHO 20 individual showed throughout the results a special behavior (see figure 5.7). For this individual the self pollination treatment caused an increased germination and growth behavior.

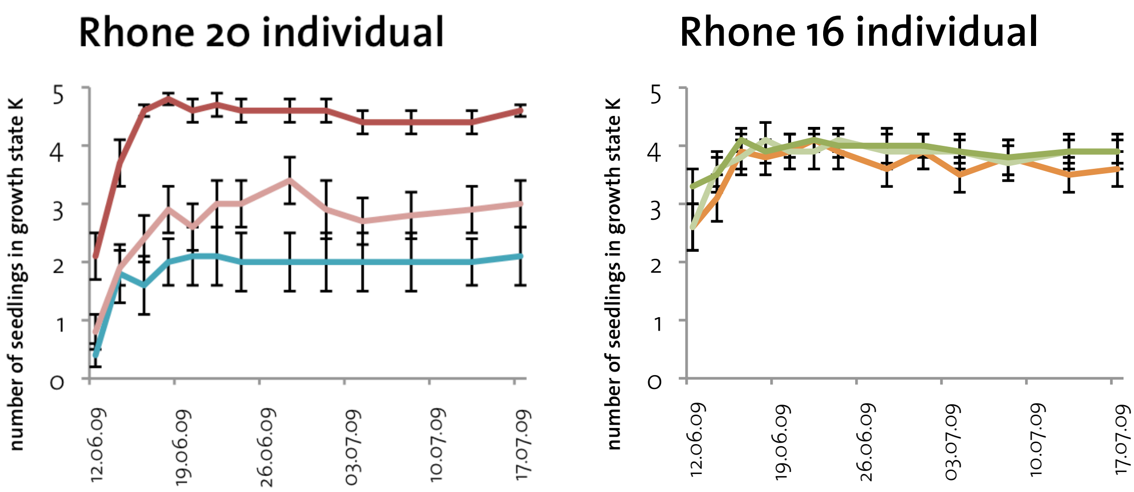

Figure 5.7: Mean number of seedlings in growth state K and standard errors for two different Rhone individuals and three different pollinations (RHO 20 POP RHO 20 REG RHO 20 SELBST RHO 16 POP RHO 16 REG RHO 16 SELBST).

While we noticed quite some differences concerning the germination and growth capacity of different crossing or origins, the response to water stress seemed to be very homogenous. On the 16th of July we imposed water stress on half of our flowerpots. For ten days, until the 26th of July, no single plant reacted in a visible way to the lack of water. Then, on the 1st of August, about 80% of the plants reached stress level one, while higher stress levels were still not achieved. The stress levels S2 and S3 were reached by 80% of the plants on the 3rd and 5th of August respectively. Finally on the 7th of August, all plants reached stress level S4 and were completely dried out. In the following four plots one can clearly see that the curve for all individuals follow a similar progression within the stress levels. Also the standard errors of the curves for stress levels S2 and S3 do look rather short. Looking on these graphs one is tempted to say that the achievement of S1 is logarithmic while the three other stress levels are best approximated by a logistic growth curve, even if there is no statistical analysis available yet to back this theorie.

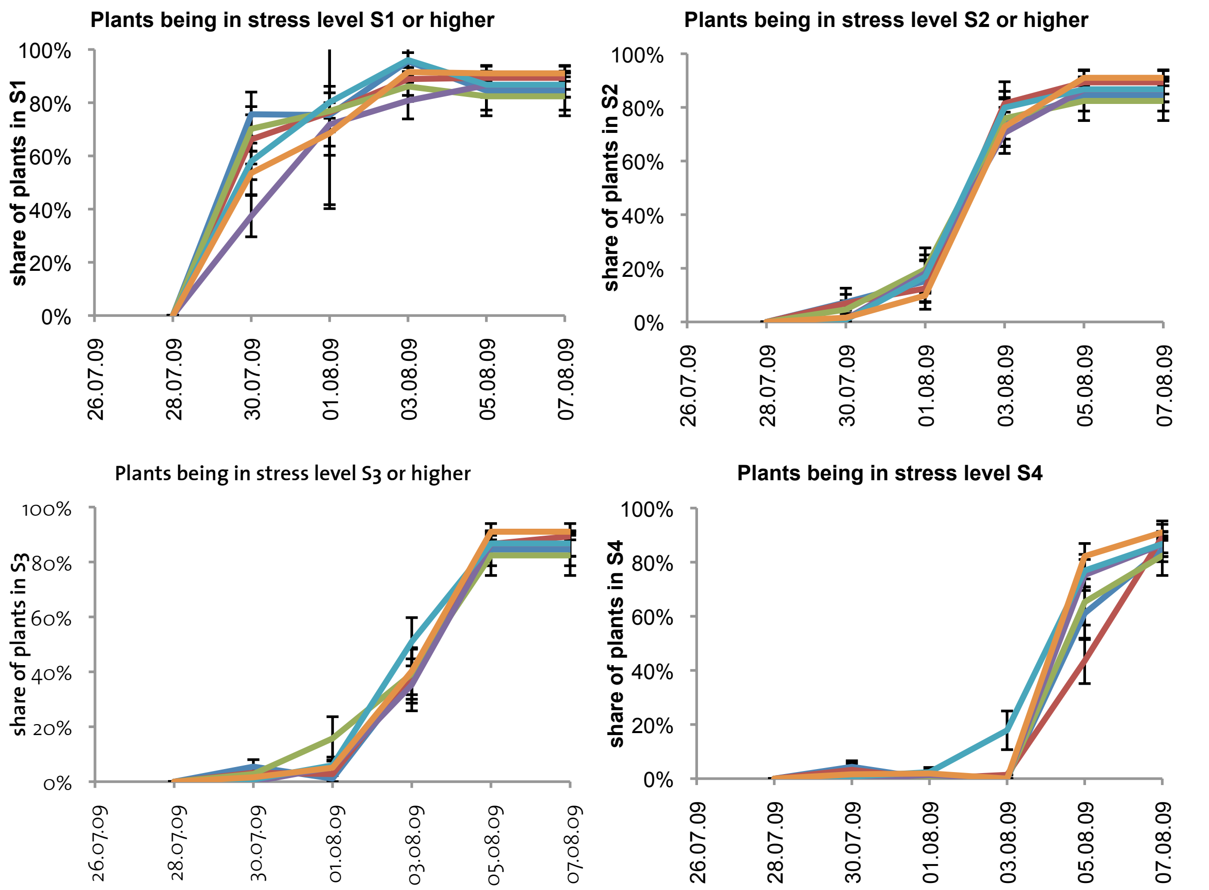

Figure 5.7: The mean value of all stressed plants descended from one pollination-treatment (Inn POP, Inn REG, Inn SELF, Rhone POP, Rhone REG, Rhone SELF) is shown in the above four plots. There is one plot for each stress level S1 to S4. The value axis shows the share of plants already reached the corresponding or a higher stress level, while the category shows the dates of recording.

There is one point in the stress-stage of the experiment where some small but interesting differences did occur. It happened right before all plants dried out, on our second last recording taking place on the 5th of August. Considering figure 5.7 one can see that the curves of S4 on this very date show a higher standard error than we observed so far for other dates. To better investigate this moment we plotted the data for this day in another way (see figure 5.8). As one can recognize there, only 40% of the Inn-REG-plants are in S4, while of the other treatments already 60-80% achieved stress level 4. Then, the Inn individuals did generally seem to have fewer plants in S4 (40-70%) than Rhone ones (70-80%). These differences disappeared on the last measurement, where all plants that originally germinated reached stress level 4. Nevertheless, if water stress would have happened to cease at this moment, the Inn populations might have had a vital advantage

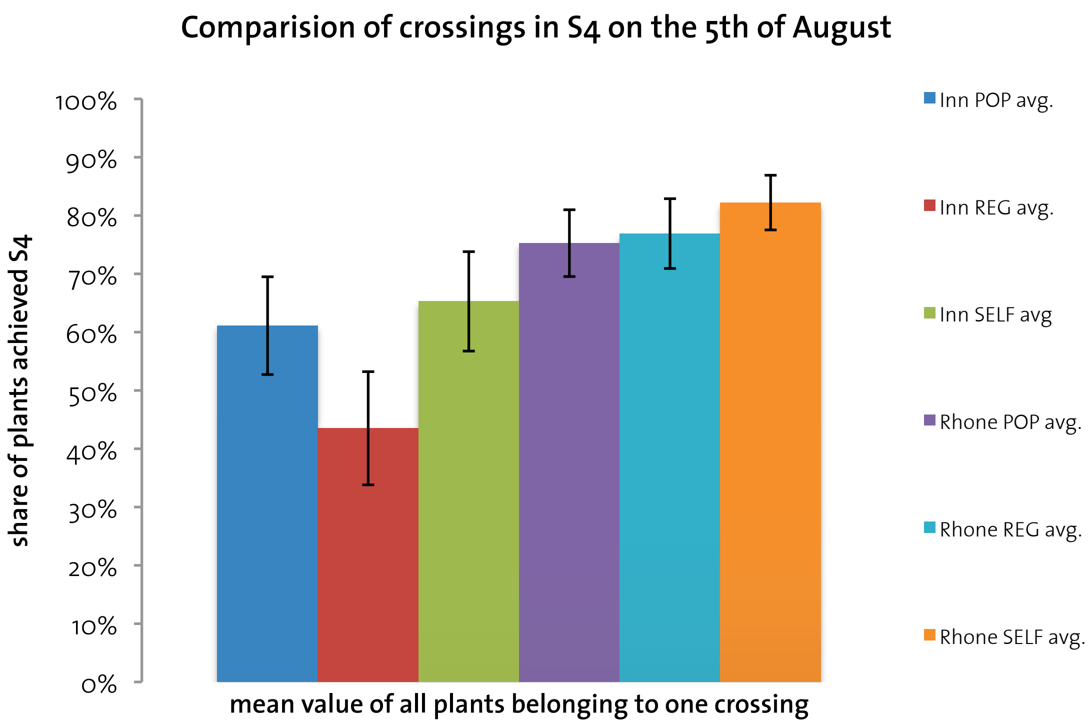

Figure 5.8: Indicated here is the same information as on figure 5.7, but just for stress level 4 and for the 5th of August. On the value axis one can see the share of plants achieved stress level 4. The six different columns represent the six different crossings.

Another particular interesting observation ocurred when we examined the graphs of each single individual. We noticed quite big differences within individuals and within treatments. In figure 5.9 one can see that from the same treatment (POP) and region (Inn), the three individuals (Inn1-01, Inn2-19 and Inn1-12) show a different behavior. The invididual Inn2-19 seems to be the least stressed individual. More or less though, the fluctuations seem to be random. Additional plots comparing individuals of the same region and treatment can be found in appendix 8.9.

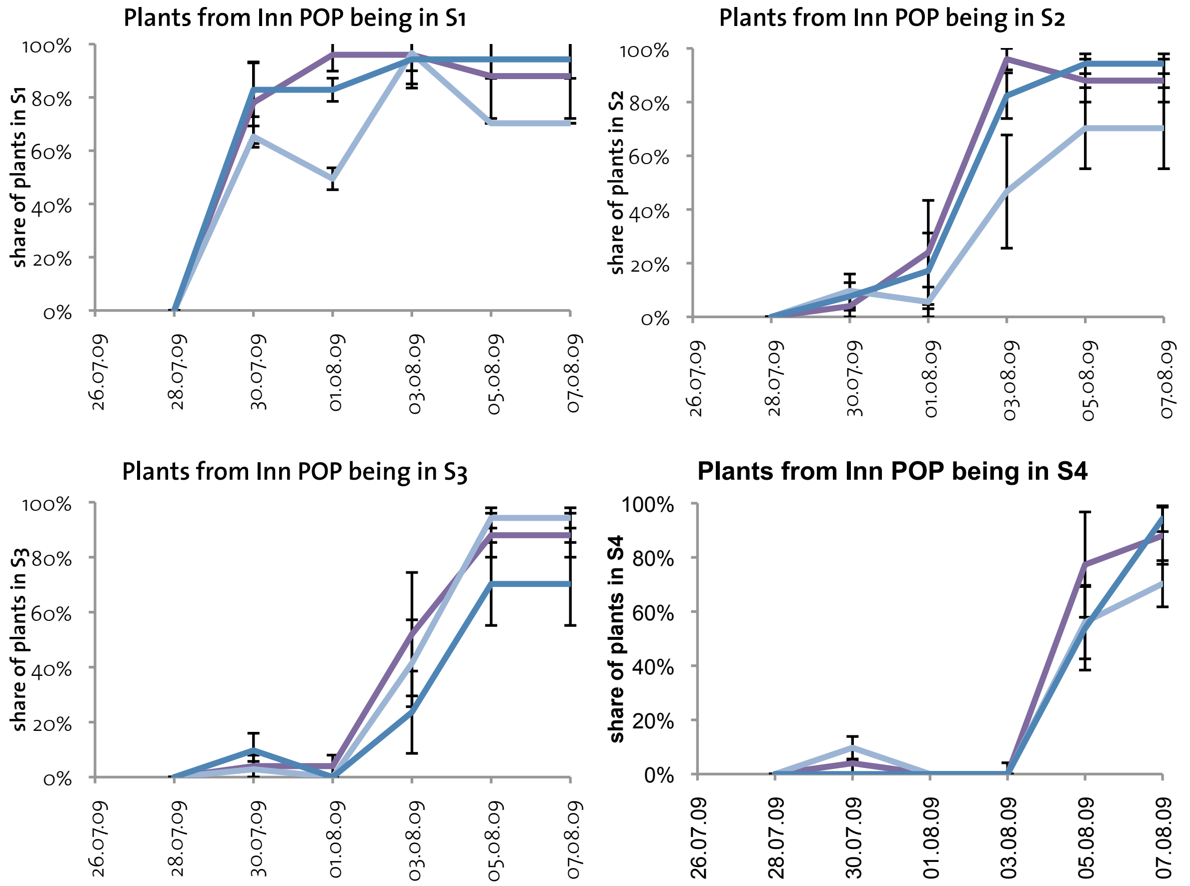

Figure 5.9: In this figure one can see the plots the four stress levels S1 to S4. Only the treatment "POP" and the region "Inn" are taken into account. Compared are the curves of the three Inn/POP individuals (—— Inn1-01, —— Inn2-19, —— Inn1-12).

In figure 5.10 we displayed the comparison between different treatments of the individual Inn1-01 throughout all stress levels S1 to S4. Here the self-pollinated plants descending from the Inn1-01 ancestor clearly showed the least reaction to water stress. The share of stressed plants amounted 60% in the end. The two treatments REG and POP however reached a stress-share of over 80%. Even if one has to point out that the error bars of these individuals are quite large, they do not intersect very much. Interestingly enough, the better answer to water stress of the self-pollinated plants developed towards the end of the experiment. In the beginning, they were stressed as much or even more (see figure 5.10; S3, 1.8.09) as the two other treatments. For more comparisons between the three different treatments within one individual there are more graphs in appendix 8.9.

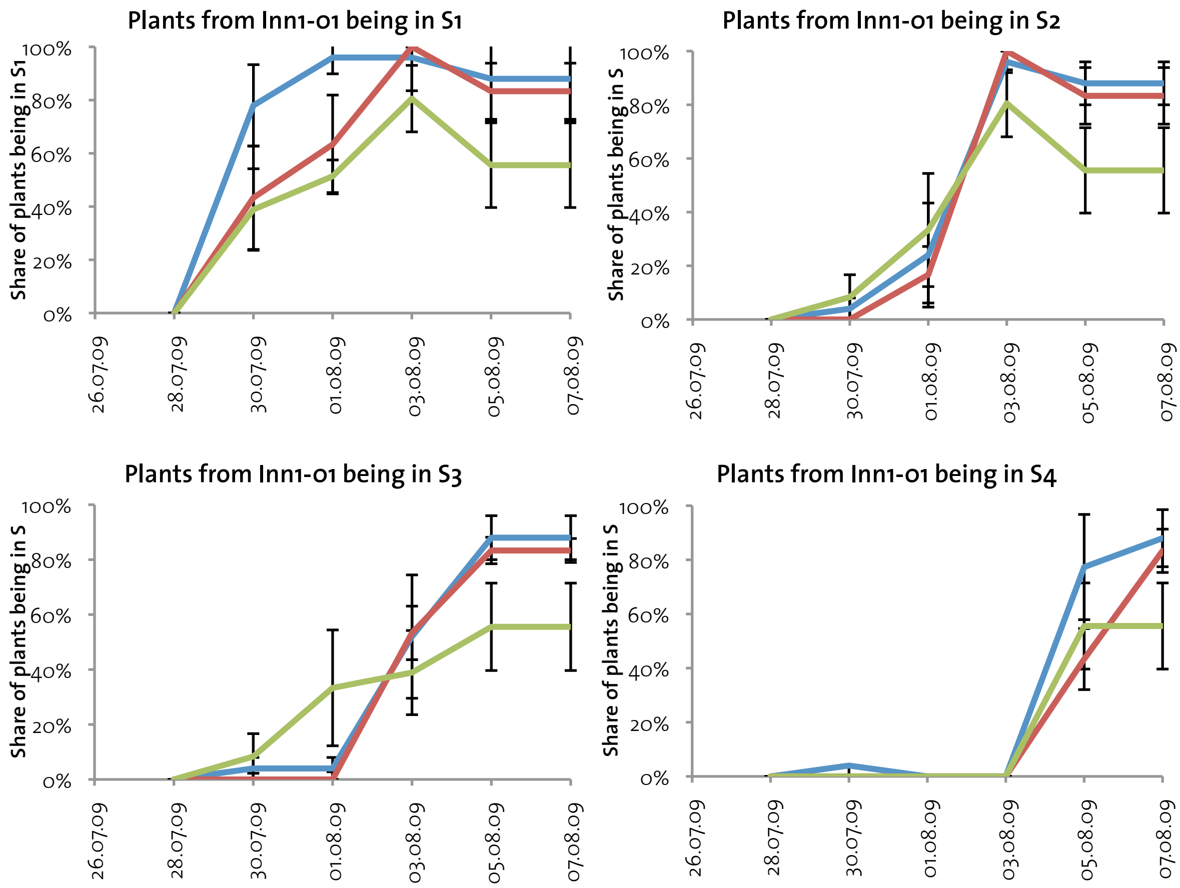

Figure 5.10: Here the four stress levels S1 to S4 are shown. Only the individual Inn1-01 is displayed. Of this individual compared are the three different treatments (POP, REG, SELF)

Both those differences, on the one hand within the same treatment and region, on the other hand within one individual, occurred throughout the experiment. Often it was just one plant or one treatment showing different results.

6. Conclusion and Discussion

The first experiment clearly shows that the preferred habitat of *M. germanica* is very sandy. On the substrate consisting only of gravel, no plant ever germinated. The question remains, if this happened just because the gravel substrate was not wet enough. Maybe the effect is of a smaller amplitude if gravel substrate is flooded, as mentioned in the introduction for *P.nigra* (Guilloy-Froget, 2002). In reality though, gravelly habitats will not be too wet most of the time, either.

Surprisingly, the plants did not well survive on the WSL potting soil. We presumed some microorganisms present on the substrate were the reason for this low survival rate, as they might have infected the plants or have been a competition factor. Interestingly, the few plants that survived on the soil grew very large in the end, so apparently they have been able to profit from the nutrients provided by the soil.

There is a clear answer to our first hypothesis concerning the demands of *M. germanica* on its substrate. It must contain large portions of sand. If small grain sizes are absent in a habitat, so will be seedlings of *M. germanica*. If it is possible to bring back sand to a river-ecosystem through renaturation, *M.germanica* might profit from this additional habitat. However, it is crucial that the newly formed habitats can be colonized from a nearby population.

In the second experiment as well, the substrate had to be very sandy. During this experiment, very few seeds started to germinate on gravel. Concentrating on the ones that developed on sand however, we were able to make some interesting statements.

First of all, it was remarkable how fast the seeds germinated in general and how long they have been able to resist water stress. For two weeks no plant showed any signs of being stressed at all. The question remains if this long absence of stress consequences occurred due to a very high water-storage capacity of the sandy soil. However after several days we observed rather dry pot substrates so we assume these results to be caused by a stress tolerance. This is a clear answer to our second question. Seedlings of *M. germanica* are notably resistant to water stress and are only severely affected if the stress lasts several weeks once the plants have reached secondary leaves.

A fast germinating process followed by a very slow growth combined with a big resistance towards water stress seems to be vital in the habitat of *M. germanica*. The slow growth after the germination process could be due to an enhanced investment into root development. This theory would also accord with our observation of a strong root building, observed during the abortion of the experiment. This would also explain the good resistance towards water stress.

We have been able to note some differences among individuals and pollination treatments concerning germinating and growth capacity. Without going into deep statistical analysis, it seems that individuals from the Rhone population germinate and grow faster than the ones from the Inn population. At the same time, inbred individuals from Rhone performed the best during germinating and growth, while inbred individuals from the Inn were the worst performers during the growth stage of the experiment. During the stress stage of the experiment however, the situation was different. All plants started to dry out 14 days after the start of the water stress and within 10 days all have been dead. No matter where the plants came from, they all reacted very similar to the stress and achieved all four stress levels nearly on the same moment. On the first look, differences only occurred right after the ultimate stress state where all plants have been dead. At this moment the individuals originating from Inn seemed to be less stressed than the ones from Rhone. Hence the ones growing slower have been less susceptible to water stress.

We also noticed that the differences between individuals, within regions and several crossings were high - concerning germination, growth and reaction on stress. Especially it was the case that there have been several individuals behaving completely different than the others. Examples for this were Rh02-20 an Inn1-01. It might be difficult to analyze the data, as those untypical individuals might remarkably lower the significance of the results.

Aside from a profound statistical analysis it would be important to gather ecosystem characteristics and genetic background of the used ancestor plants. With such additional data one can try to explain differences between populations and individuals. For example, the high growth speed of the inbred Rhone individual might be a sign for a high level of self-pollinating rate caused by a rather small population size. But to really make conclusions about the potential consequence of those growing characteristics one has to further investigate this topic.

Our results do match other studies quite well. We found out, like Chen (2007) did for *M.laxiflora*, that saturated, sandy substrate seems to be ideal. We also observed a fast germination as described for several *Salicaceae* by Karrenberg (2002). Also *M. germanica* grew very slow after the fast germination, similar to *Populus nigra* (Guilloy-Froget, 2002). However *M. germanica* demonstrated to be not very susceptible to water stress, unlike *P. nigra*.

Shortly summed up one can say that *M. germanica* does, if on sand, germinate and grow fast, has a good resistance to water stress, but there are big differences within individual plants dependent on what population and ancestor they come from.

7. Literature

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8. Appendix

8.1 “Topfsubstrat” WSL standard

33% (1/3) Wood fibre: "Holzfaser.spezial" (Ricoter)

67% (2/39 Container soil (ökohum gmbh Illigausen):

42% Bark humus

42% Peat

12% Wood fibre

4% Clay

1.25 gr. "Floranid permanent"

1.25 gr. NP 20:20

pH 5.5-6.2

1 gr. "Unikorn 1"

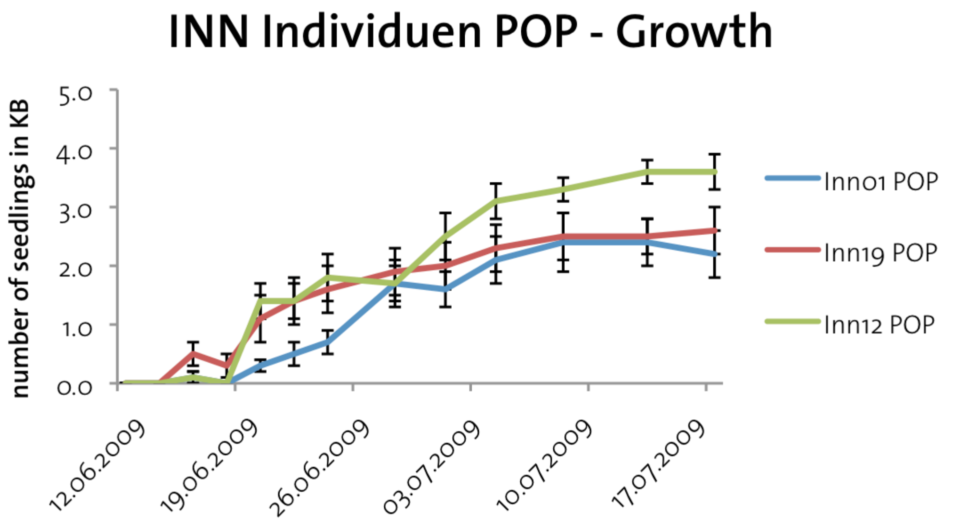
2 gr. Ground horn (pH ~4.5-5)

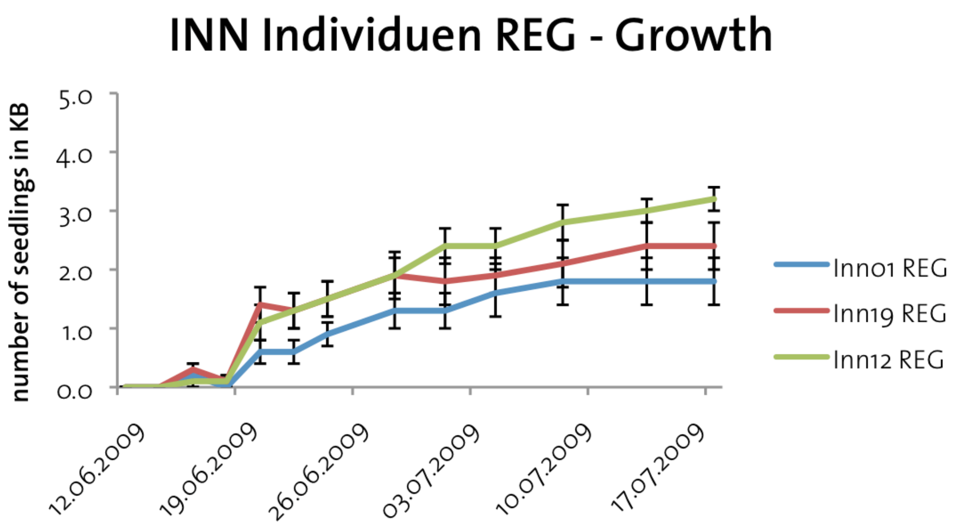
8.2 Notes for the single measurements

Date	Time	T [°C] mom/min/max	Observations
13.6.09	6 pm	28/-/-	some seedlings dried out
15.6.09	10 am	18/-/-	heavy rain – some rain drops into the greenhouse
17.6.09	3-5 pm	34/-/-	-
19.6.09	3-5 pm	18/-/-	during/after heavy rainfall, very wet soil
21.6.09	2-4 pm	20-24/-/-	-
23.6.09	10-11 am	23/-/-	gravel is wet
27.6.09	-	-	-
30.6.09	-	25/-/-	weather really good, gravel wet
3.7.09	-	20/-/-	during cat&dog rainfall
7.7.09	9-11 am	18/-/-	covered, medium rainfall
12.7.09	10-11 am	19/16/39	covered
16.7.09	5-7 pm	37/18/40	sunny, STRESS-START
25.7.09	2-4 pm	25/16/39	cloudy, occasional sunshine, NO STRESS
27.7.09	5 pm	32/16/40	covered, NO STRESS
28.7.09	4 pm	21/16/46	sunny, NO STRESS
30.7.09	-	-	-
1.8.09	-	20/18/39	-
3.8.09	9-10 am	18/18/40	covered, occasional precipitation
5.8.09	8 am	14	sunny

The temperature indicated is the one given by a thermometer situated on the wall of the greenhouse where the experiment took place. "mom" indicates the temperature read off right at the moment of measurement. "max" and "min" stand for the maximum and minimum respectively, that has been measured in the greenhouse since the last reset.

8.3 Further figures concerning individual differences





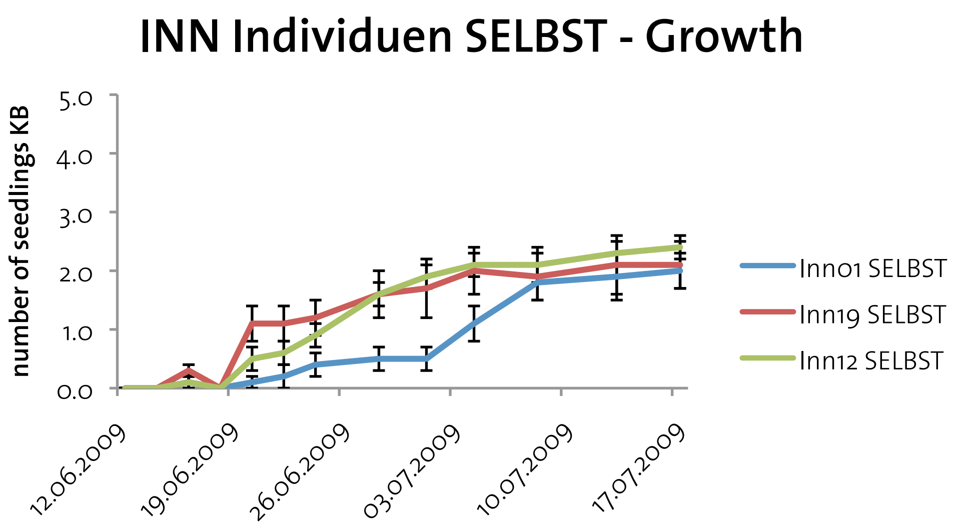
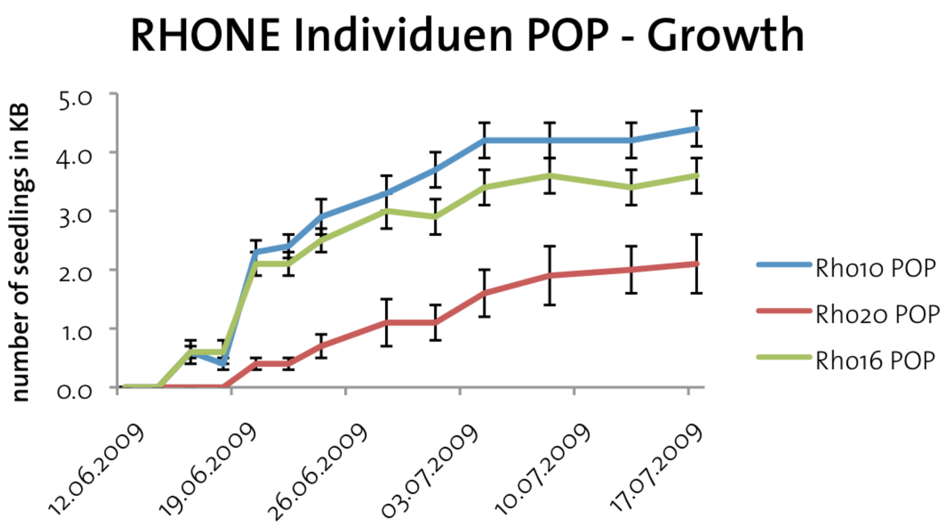
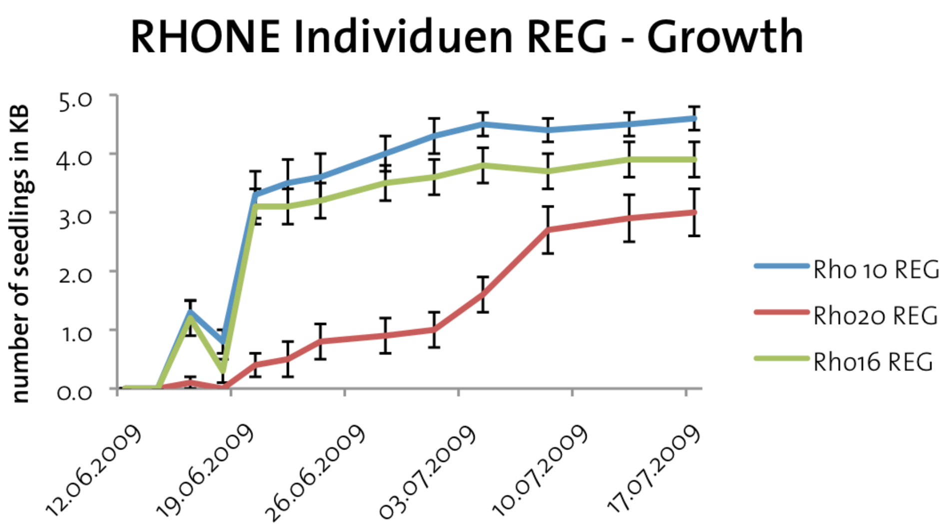


Figure 8.1: Mean number of seedlings in growth state KB for individuals from the river Inn. Separated for the different pollination treatments.





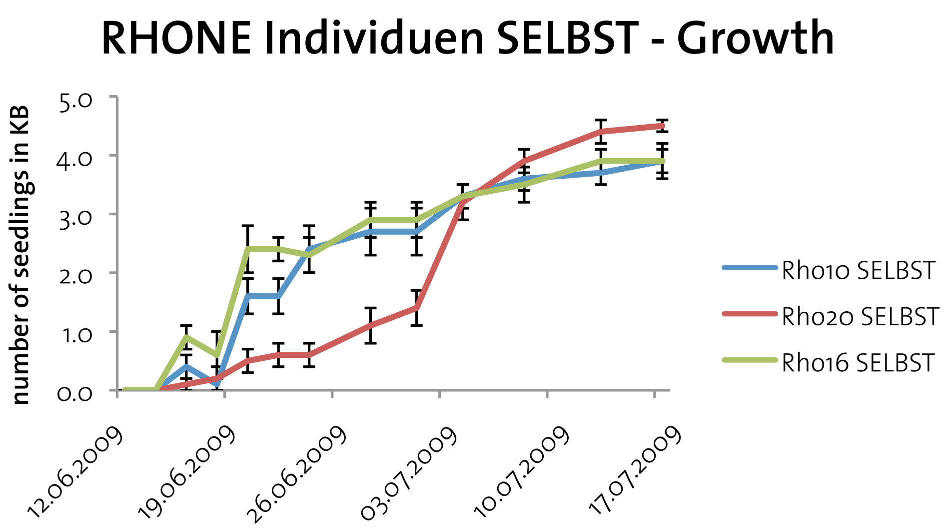
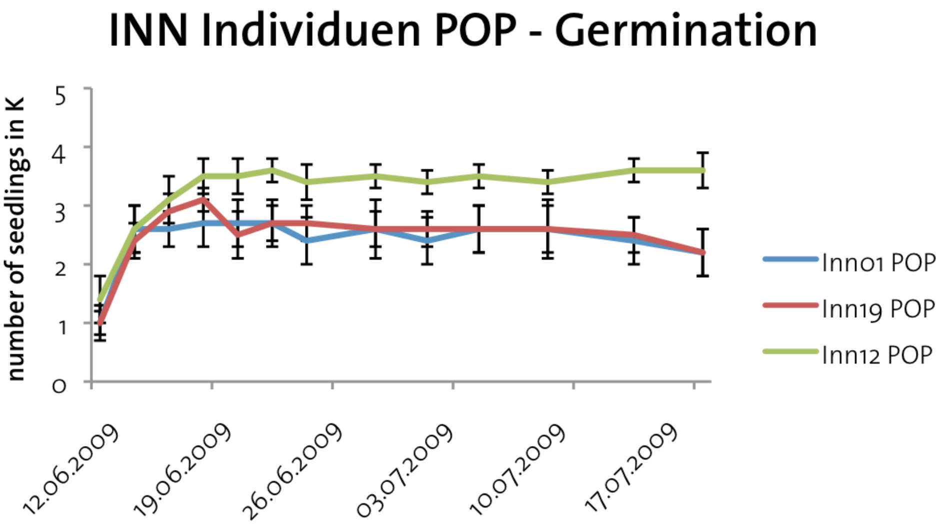
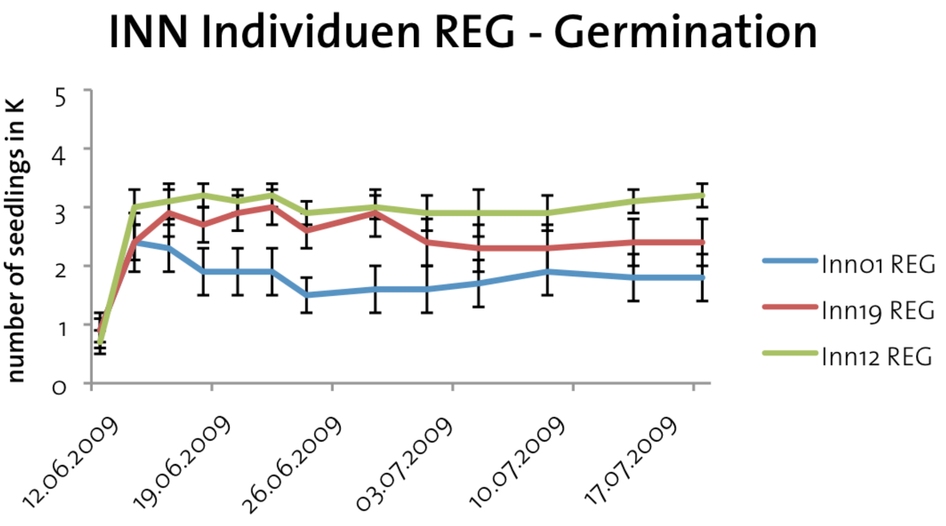


Figure 8.2: Mean number of seedlings in growth state KB for individuals from the river Rhone. Separated for the different pollination treatments.





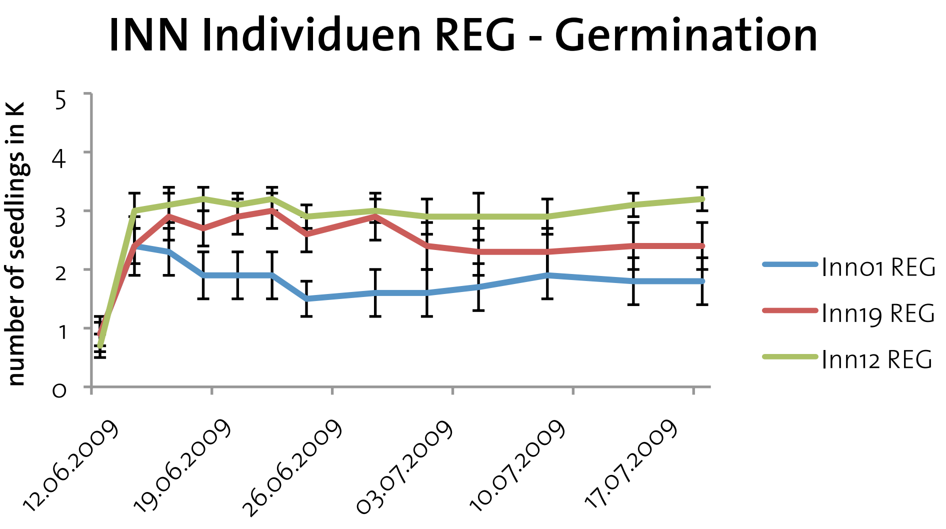


Figure 8.4: Mean number of seedlings in growth state K for individuals from the river Inn. Separated for the different pollination treatments.

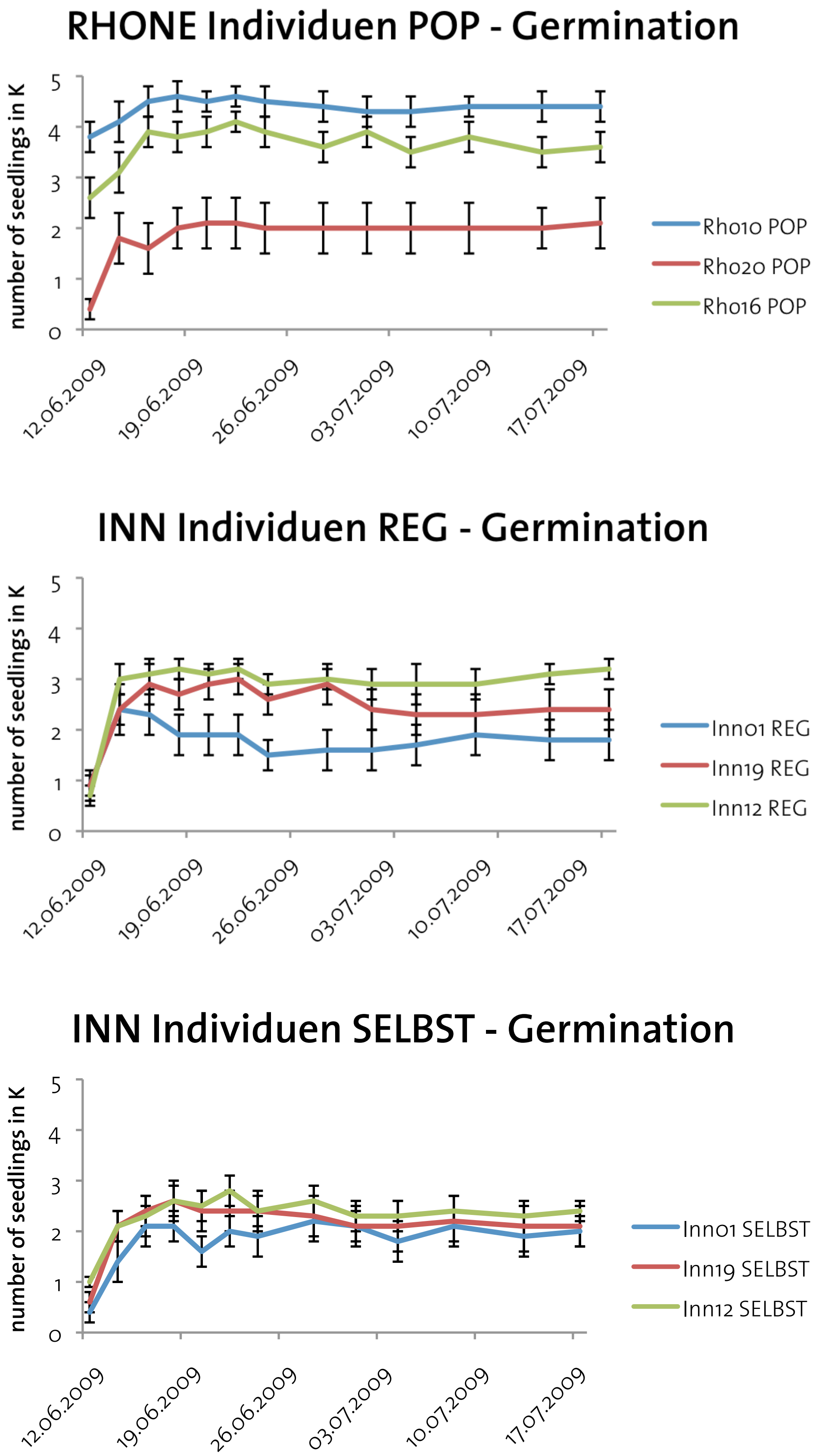


Figure 8.5: Mean number of seedlings in growth state K for individuals from the river Rhone. Separated for the different pollination treatments.

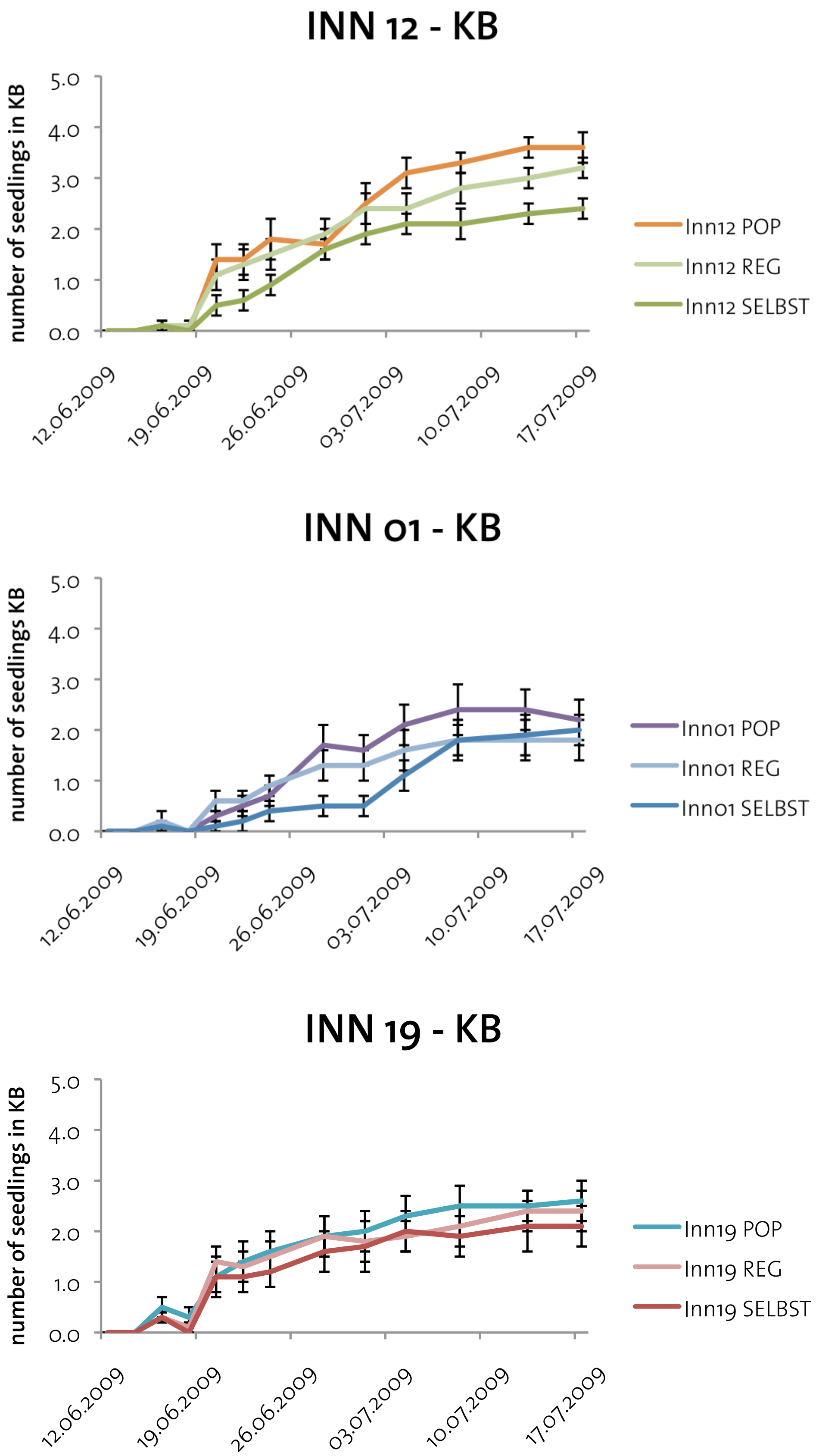


Figure 8.6: Mean number of seedlings in growth state KB for individuals from the river Inn. Pollination treatments are compared for every single individual.

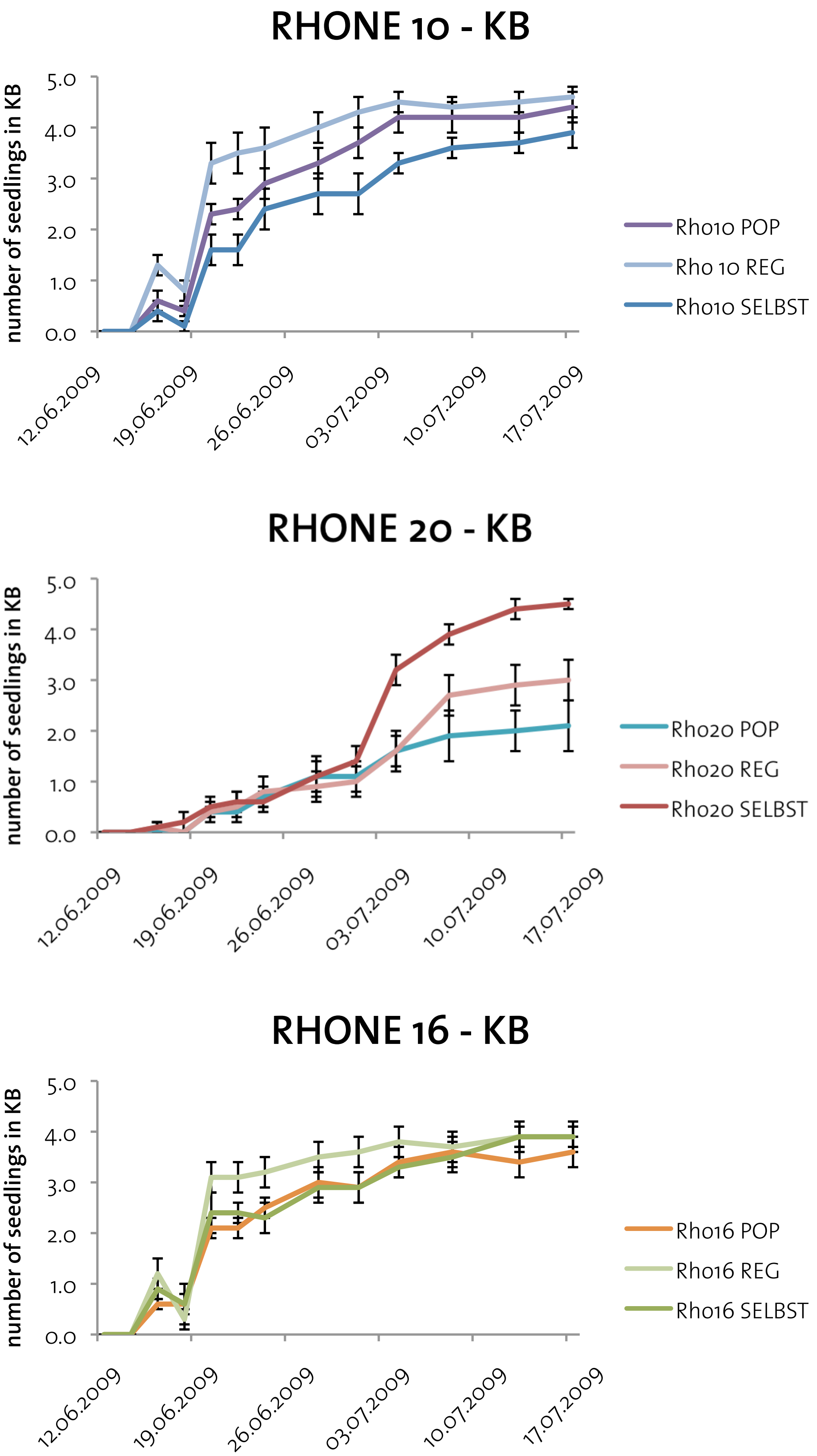


Figure 8.7: Mean number of seedlings in growth state KB for individuals from the river Rhone. Pollination treatments are compared for every single individual.

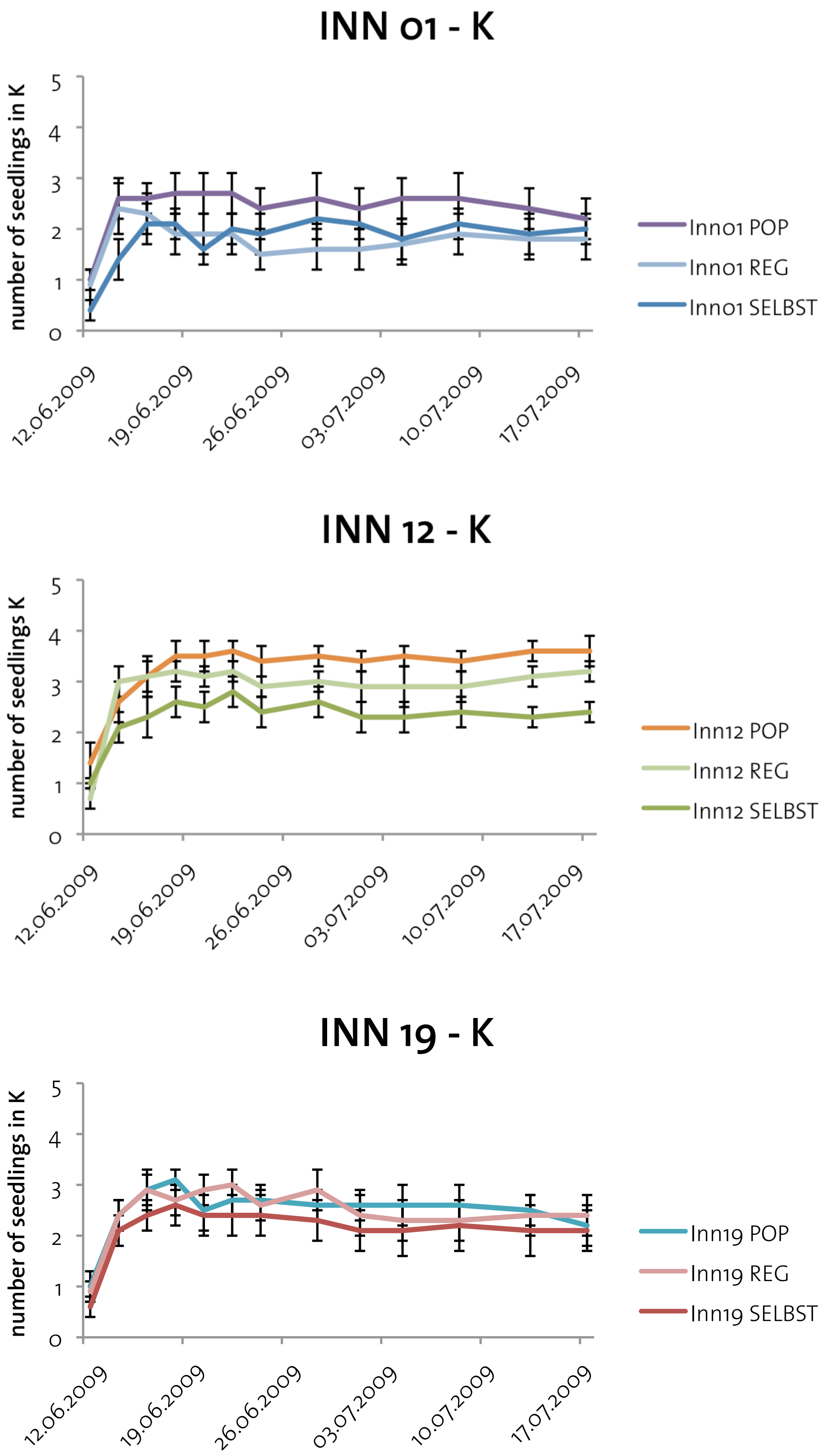


Figure 8.8: Mean number of seedlings in growth state K for individuals from the river Inn. Pollination treatments are compared for every single individual.

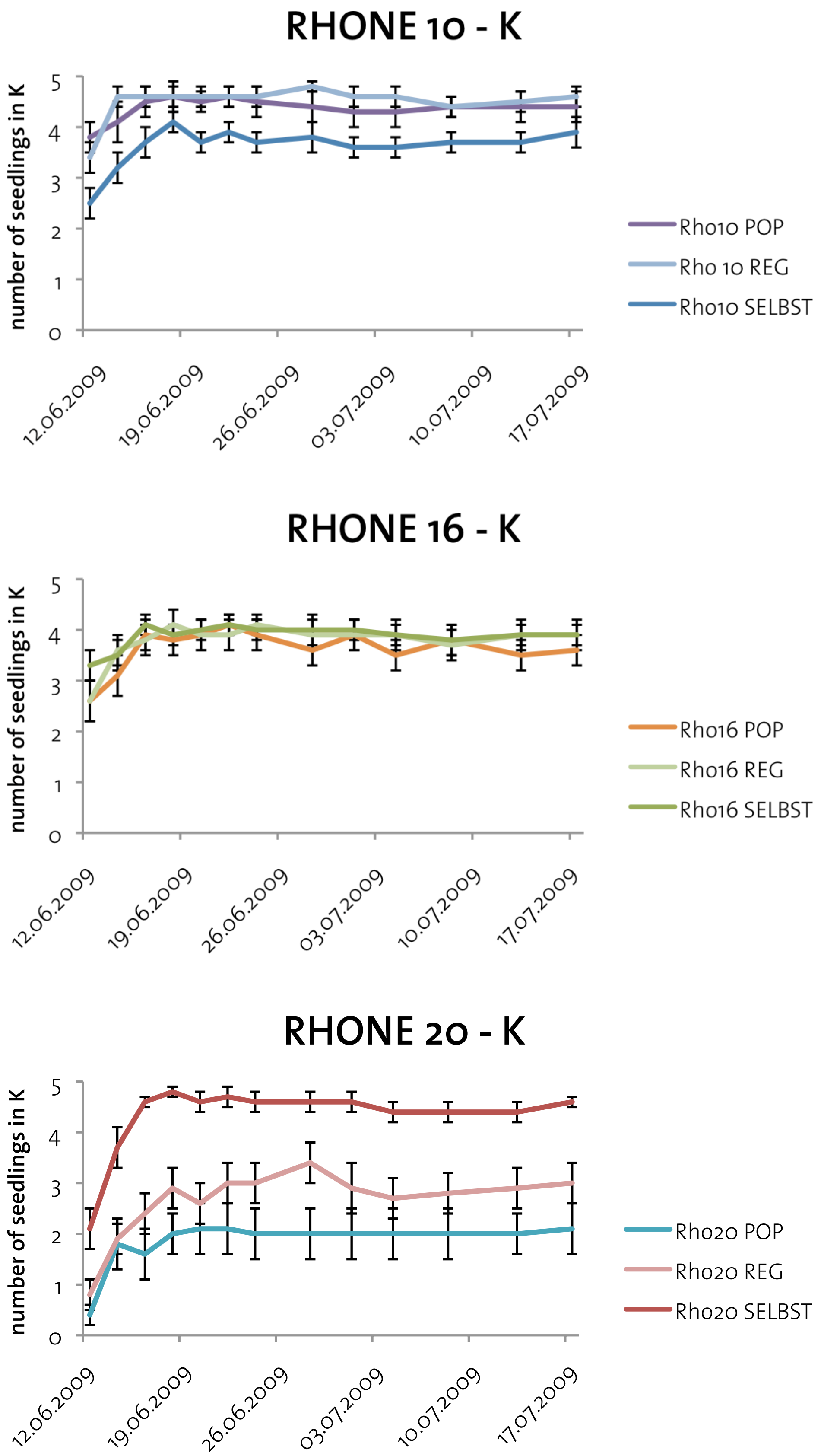


Figure 8.9: Mean number of seedlings in growth state K for individuals from the river Rhone Pollination treatments are compared for every single individual.

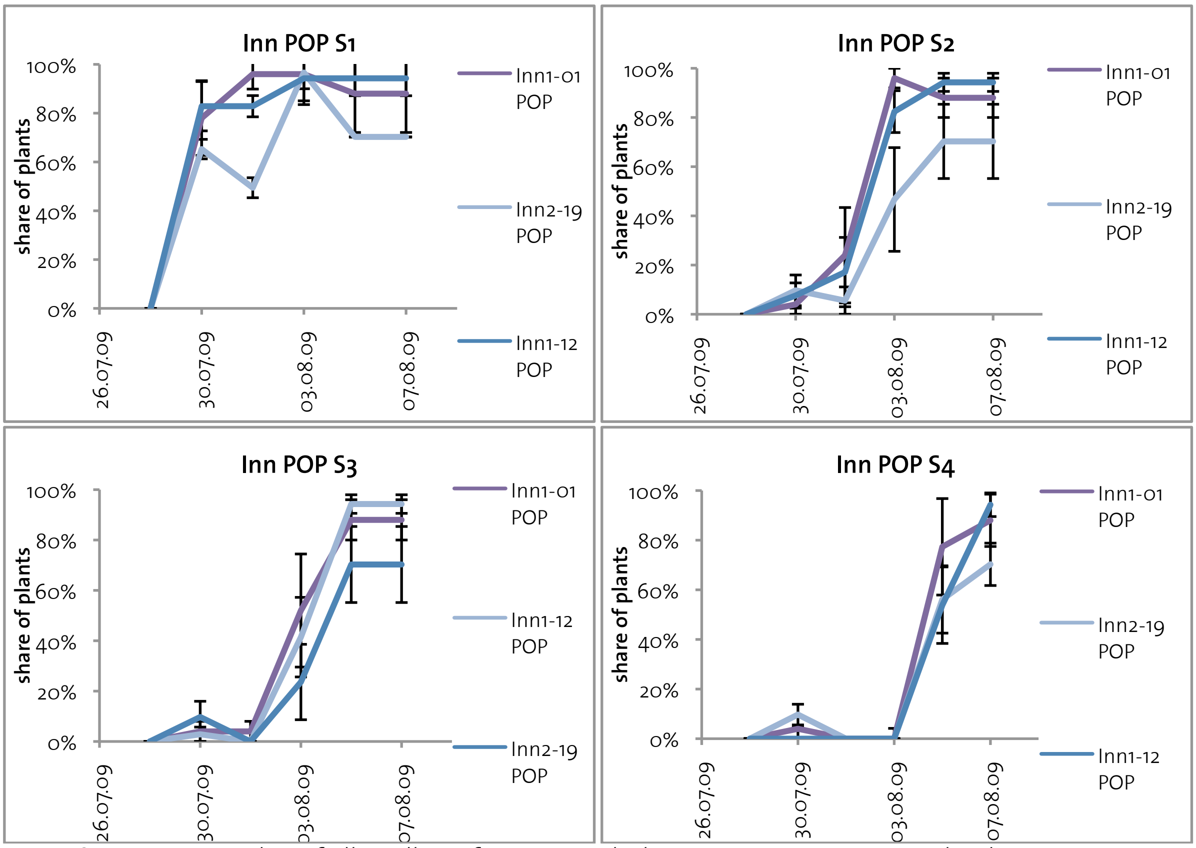


Figure 8.10: Mean number of all seedlings from Inn with the POP treatment in stress level S1 to S4

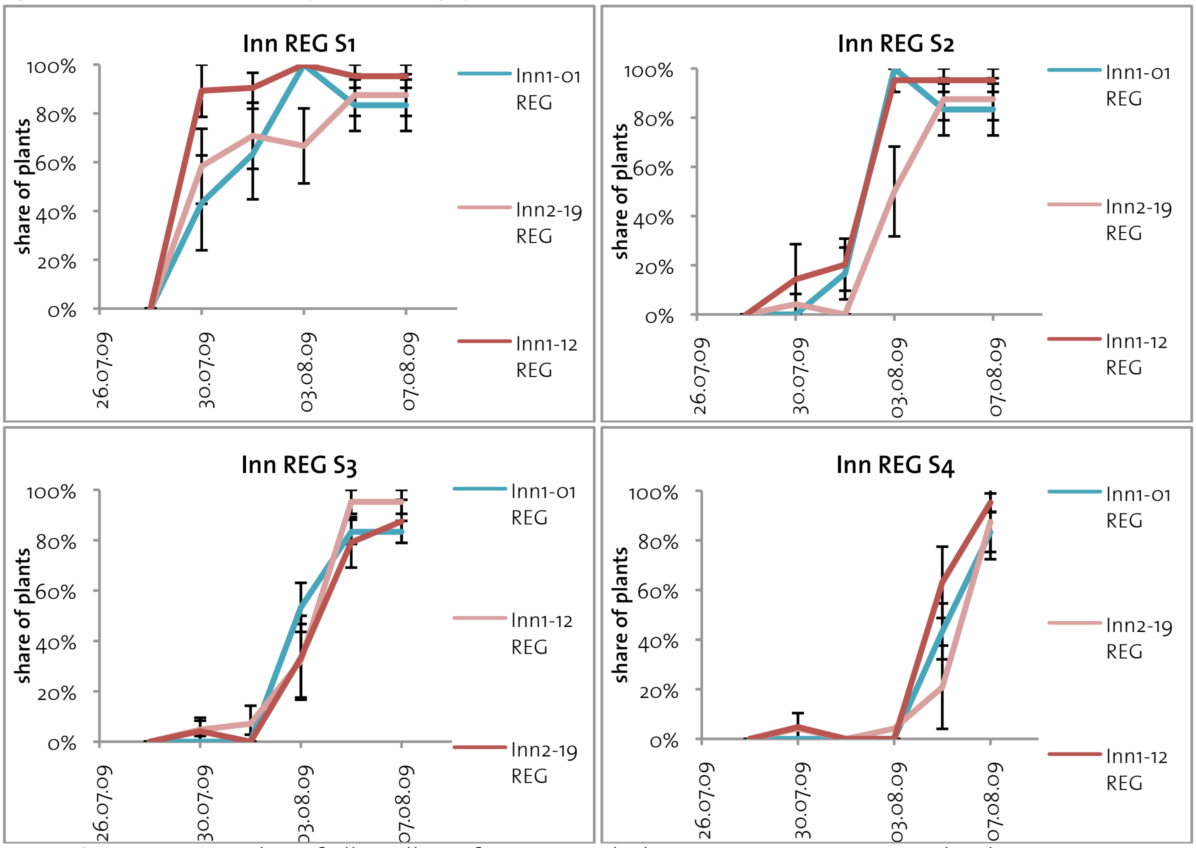


Figure 8.10: Mean number of all seedlings from Inn with the REG treatment in stress level S1 to S4

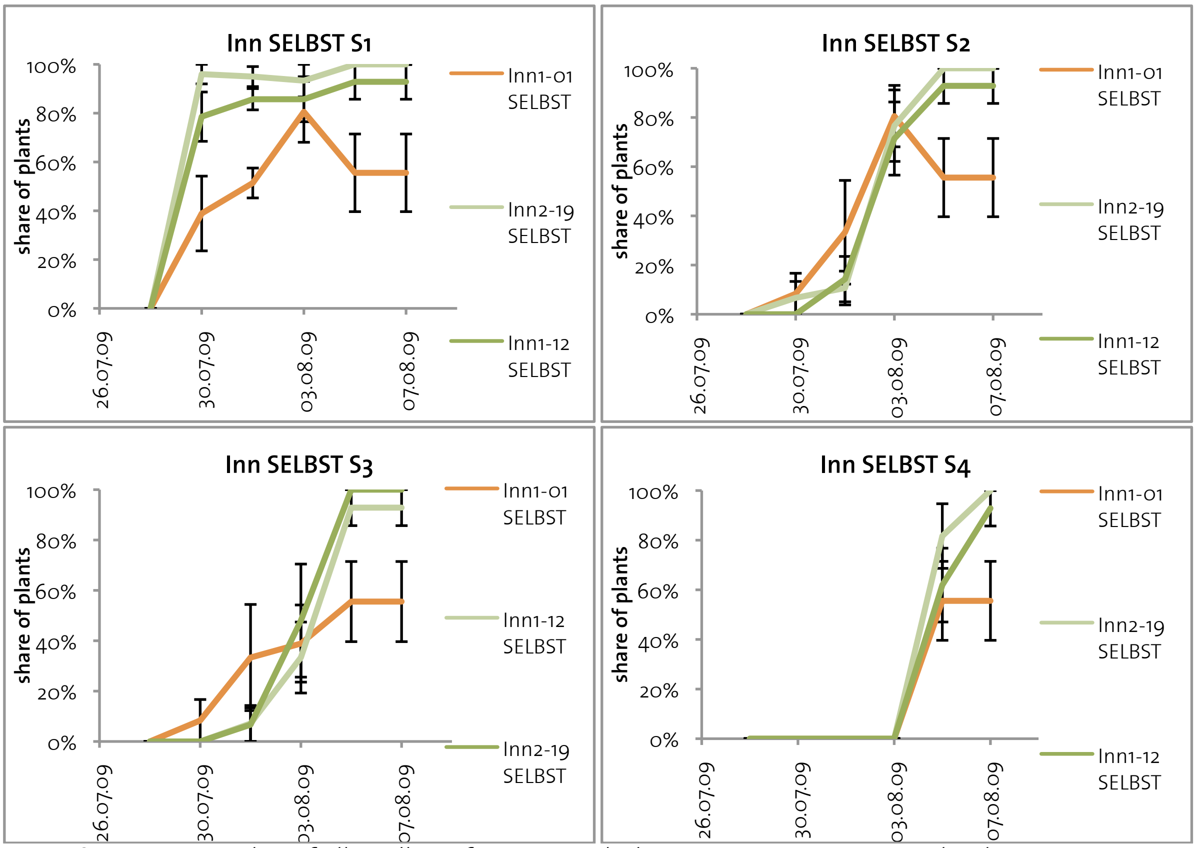


Figure 8.11: Mean number of all seedlings from Inn with the SELF treatment in stress level S1 to S4

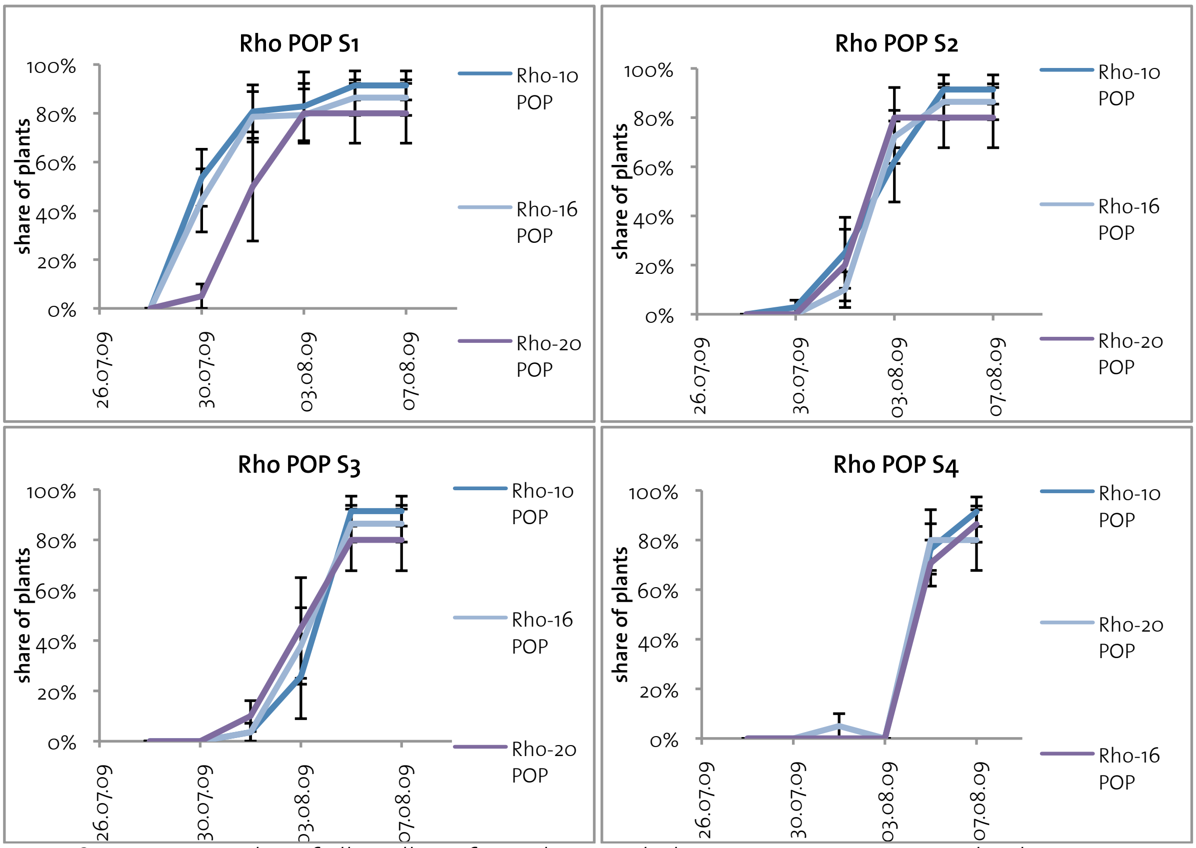


Figure 8.12: Mean number of all seedlings from Rhone with the POP treatment in stress level S1 to S4

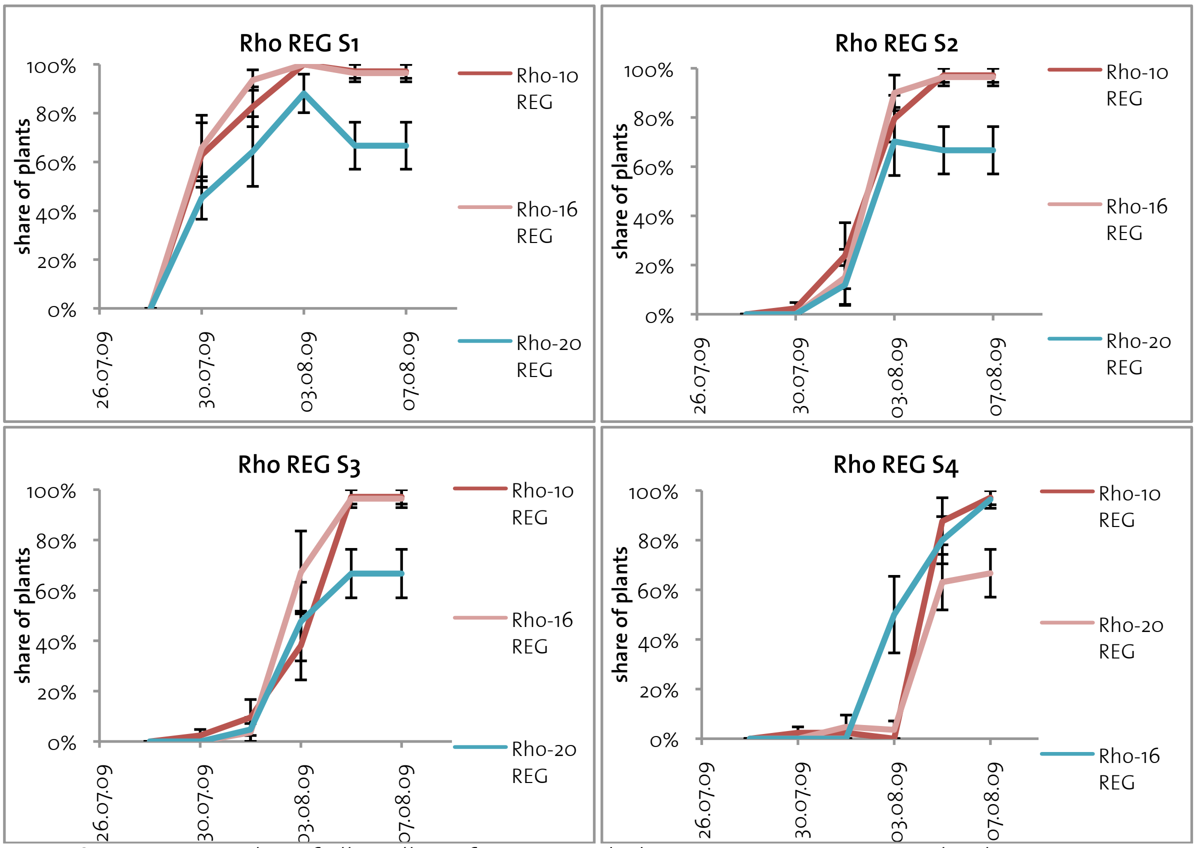


Figure 8.13: Mean number of all seedlings from Inn with the REG treatment in stress level S1 to S4

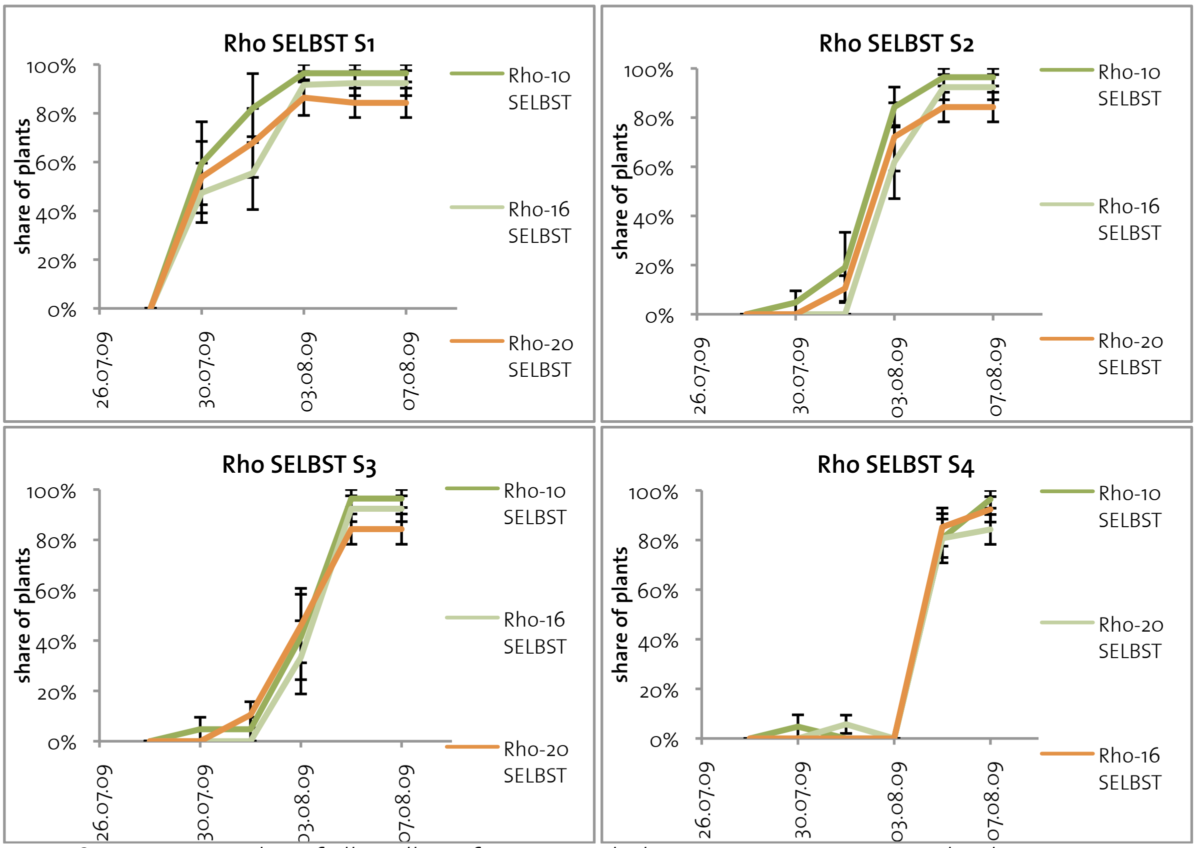


Figure 8.10: Mean number of all seedlings from Inn with the SELF treatment in stress level S1 to S4

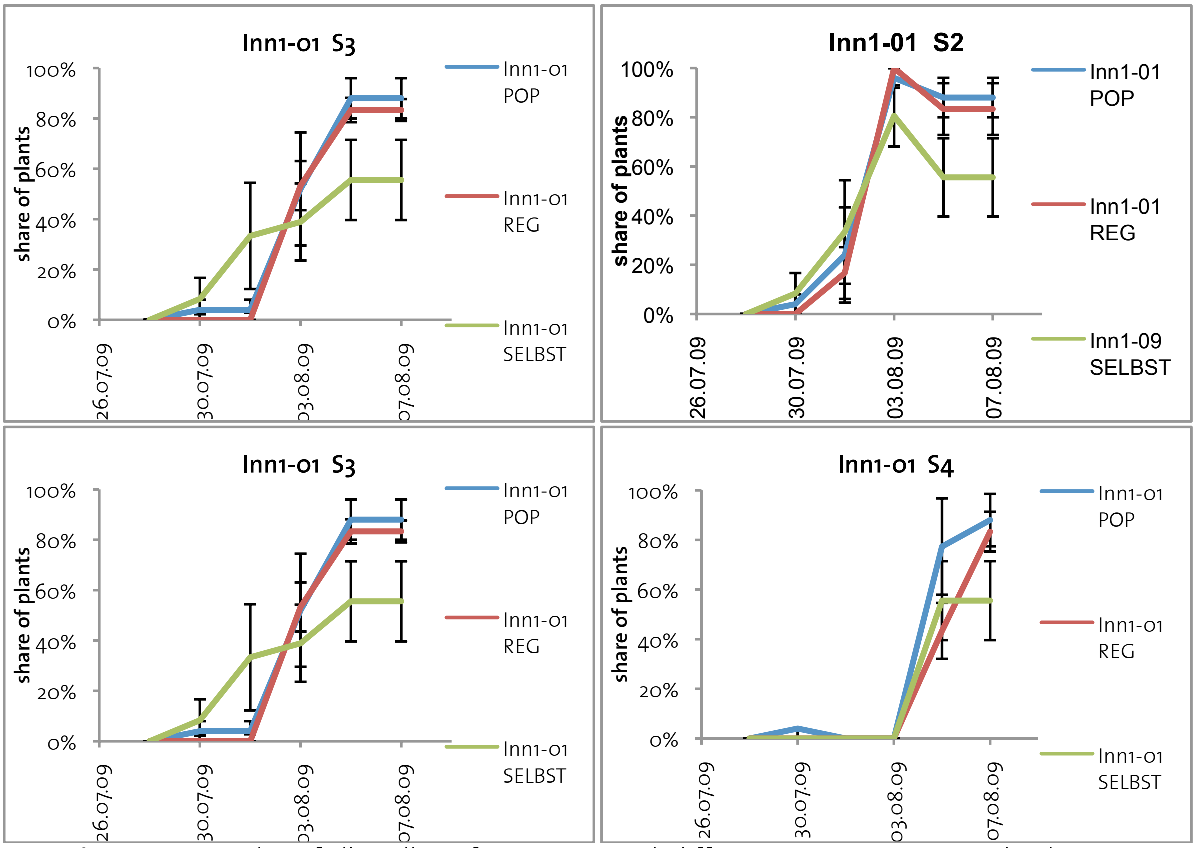


Figure 8.14: Mean number of all seedlings from Inn1-01 with different treatments in stress level S1 to S4

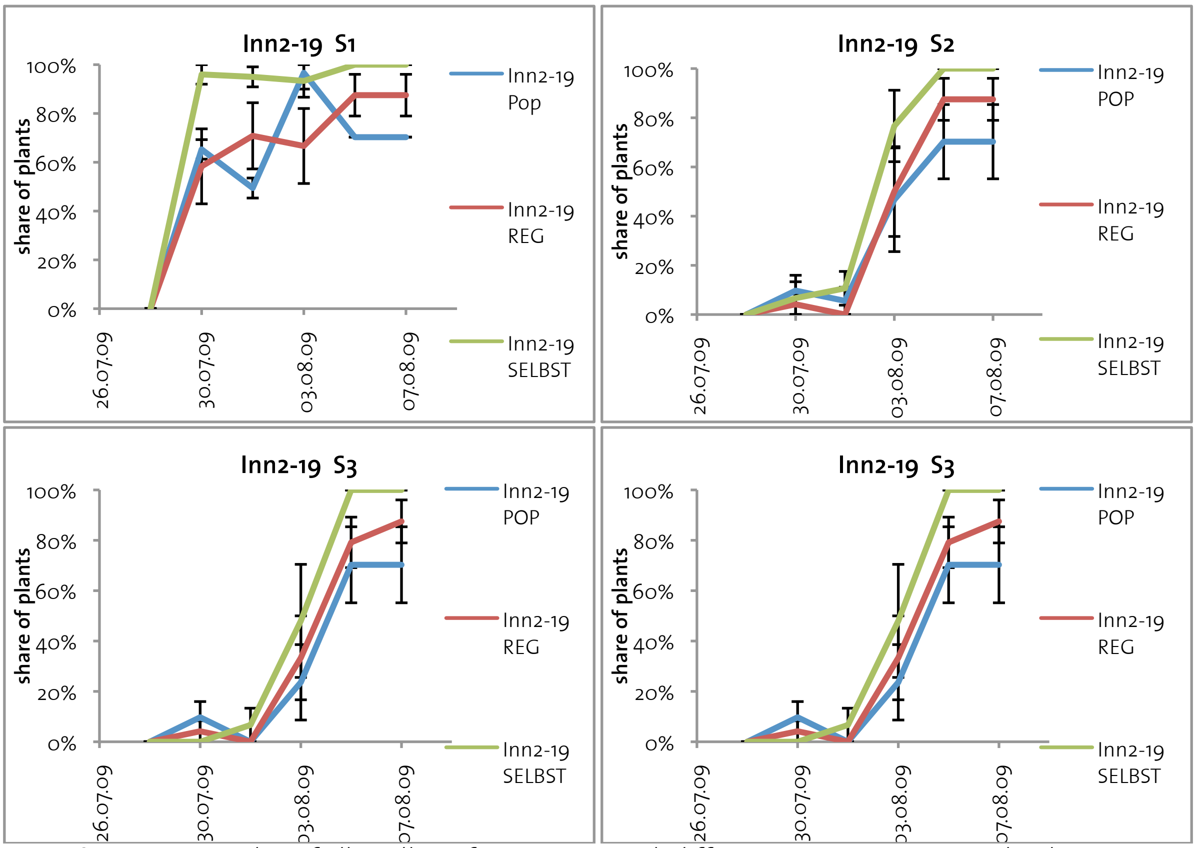


Figure 8.15: Mean number of all seedlings from Inn2-19 with different treatments in stress level S1 to S4

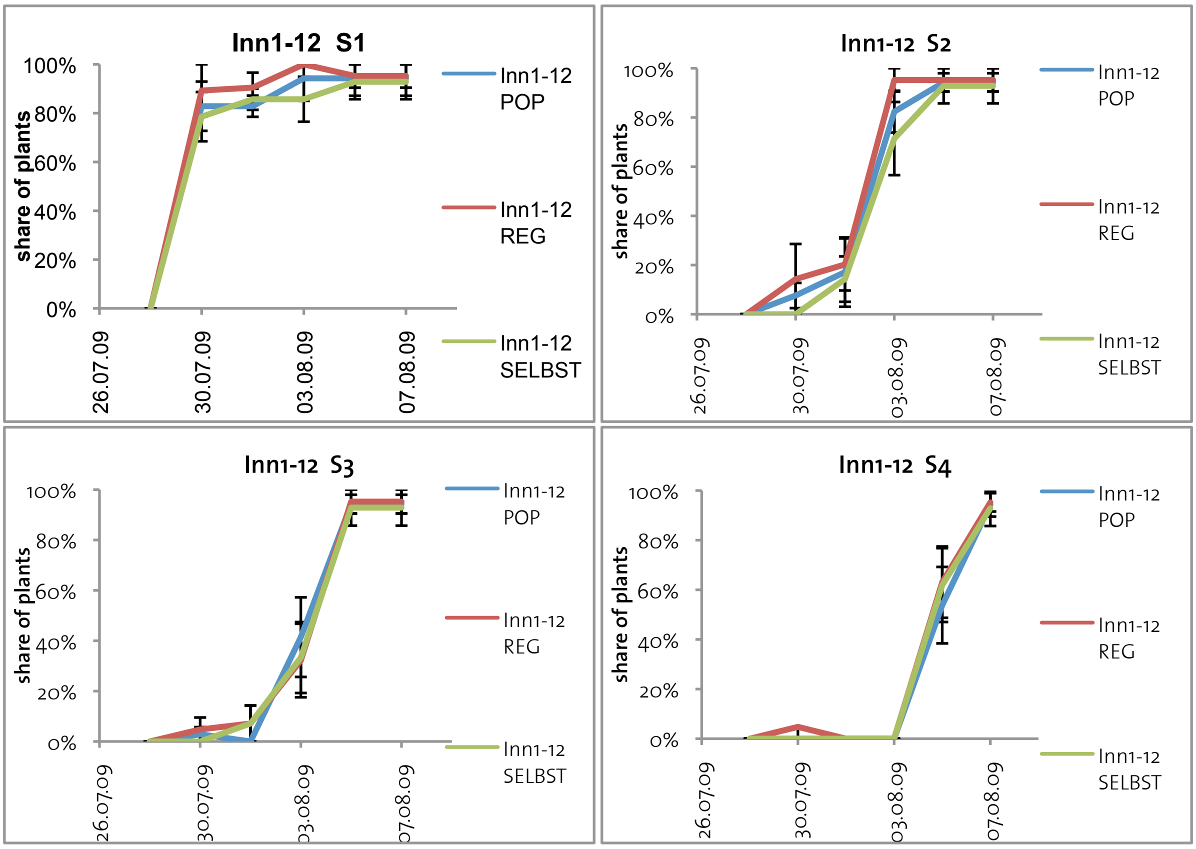


Figure 8.16: Mean number of all seedlings from Inn1-12 with different treatments in stress level S1 to S4

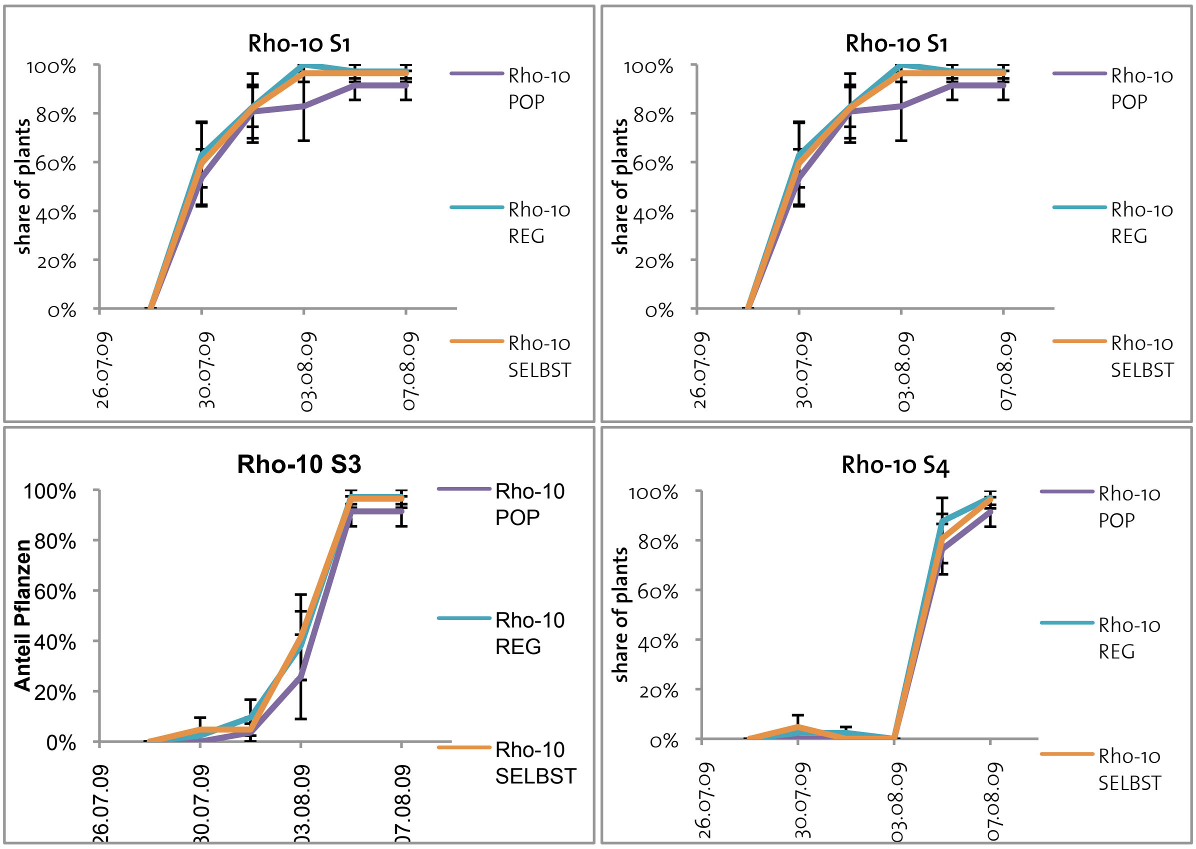


Figure 8.17: Mean number of all seedlings from Rho-10 with different treatments in stress level S1 to S4

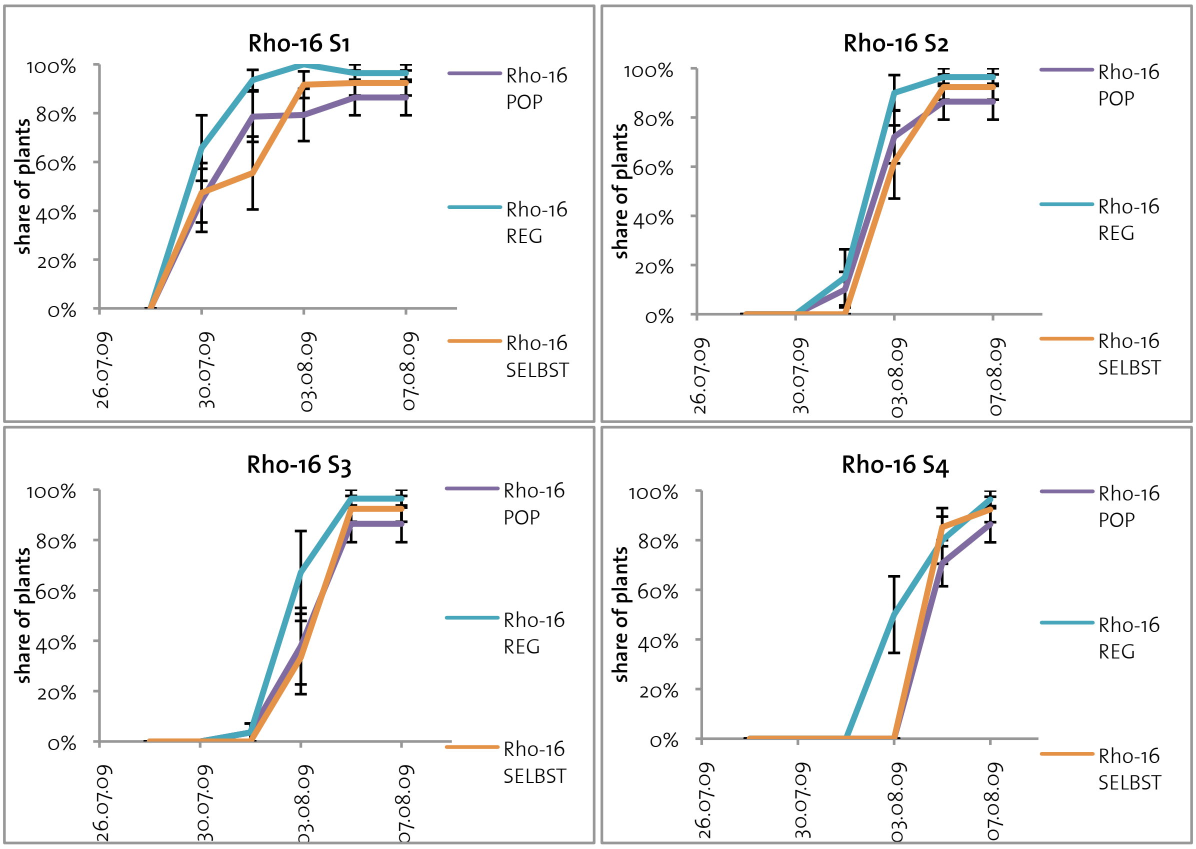


Figure 8.18: Mean number of all seedlings from Inn1-01 with different treatments in stress level S1 to S4

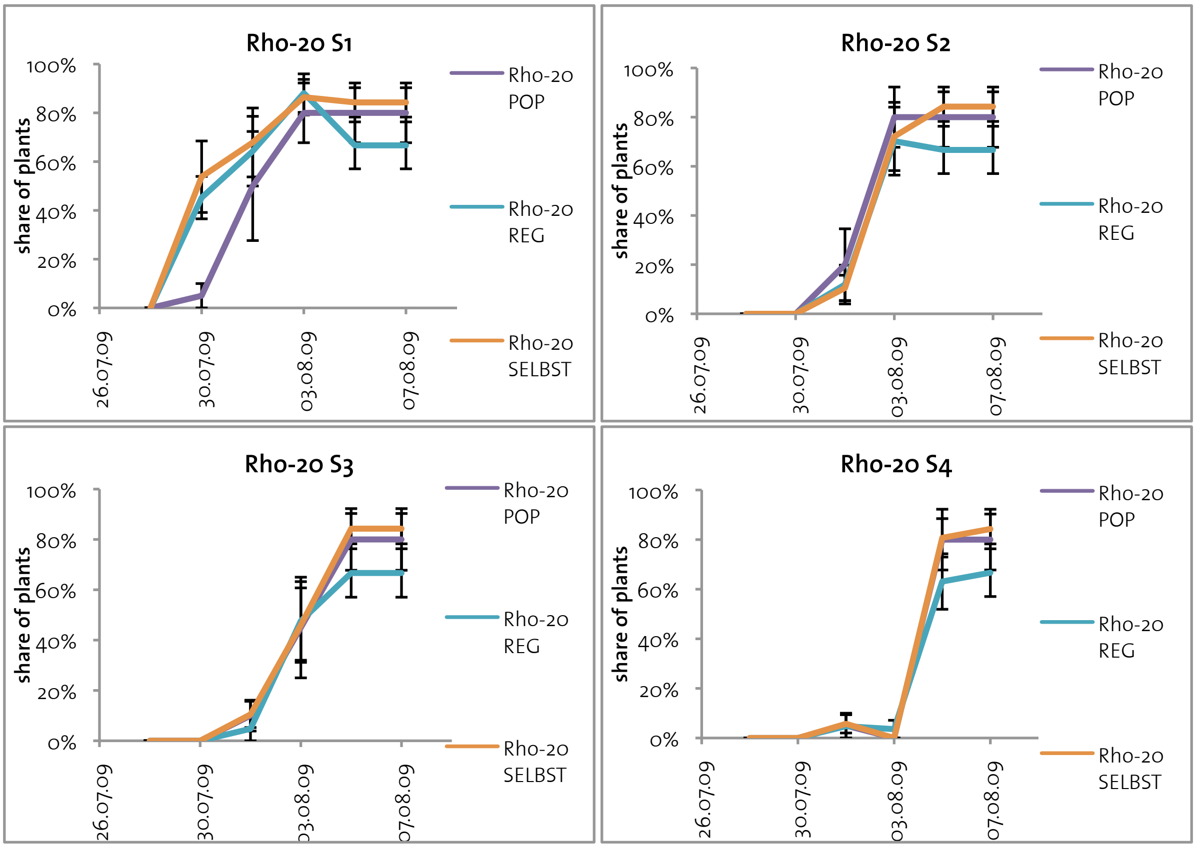


Figure 8.19: Mean number of all seedlings from Rho-20 with different treatments in stress level S1 to S4