University of South Bohemia
Faculty of biological sciences

Master Thesis
(2005)

Comparative study of variously invasive *Oenothera* species: The role of ecophysiological seed characteristics.

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Krčmářová Jana, 2005. Comparative study of variously invasive *Oenothera* species: The role of ecophysiological seed characteristics. Master Thesis, Department of Botany, Faculty of biological sciences, University of South Bohemia, České Budějovice, Czech Republic.

I honestly declare to have worked out this thesis on my own, with the use of cited references.

Krčmářová

Prague, 11th January 2005

Acknowledgements:

I would like to thank all the people who helped me, especially RNDr. Stanislav Mihulka and RNDr. Jana Martínková, Doc. Jaromír Lukavský, RNDr. Jana Kvíderová, RNDr. Elster, Ing. Šantrůček, Mgr. Jiří Kubásek. Special thanks belong to my family, friends and least but not last to Tatra.

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Abstract

Genus *Oenothera* is one of the few alien genera in Central Europe which comprises invasive to rarely occuring species (Mihulka and Pyšek 2001). Comparative studies of related invasive and noninvasive species are thought to offer the most powerful test of what makes a species invasive (Kolar and Lodge 2001).

This study concentrates on germination and early seedlings, because these life stages are in monocarpic non-clonal species like *Oenothera* responsible for the distribution of adults and also for the colonization of new sites (Milberg et al. 2000).

Among attributes which can make species more successful invader scientists count also the pattern of phenotypic plasticity (Weber and D'Antonio 1999, Sexton et al. 2002, Daehler 2003). The studied species produce persistent seed banks of dormant seeds, which respond to environmental signals, which either strengthen or release them from dormancy (Pons 1992). We assume that the invasive species (*O.biennis*) could be able to germinate in broader range of conditions - that it posses a greater array of ecophysiological seed-phenotypes. After confronting the literature the following environmental factors were incorporated: temperature, temperature fluctuations, light intensity, nitrate concentration and presence of allelochemicals (e.g. Pons 1992, Probert 1992, Karssen and Hilhorst 1992, Spalding 2003).

The first experiment concentrated on the complex effect of temperature and light on the germination and early life stages of three *Oenothera* species (*O.biennis*, *O.rubricaulis* and *O. ammophila*) and was performed on a unit for cultivation in a crossed gradient of light and temperature (Kvíderová and Lukavský 2001).

The second experiment tested the complex effect of light and nutrients in the presence and absence of temperature fluctuations on the same set of species.

The third experiment compares the performance of hybridogenous species *O. fallax* and its parental species (*O. biennis* and *O.erythrosepala*).

In some invaders and weedy species allelopathy helps in supression of other species (Ridenour and Callaway 2001, Muir and Majak 1983). A theory stands that the natives do not have tolerance on alien allelochemicals (Hierro and Callaway 2003). *Verbascum thapsus* is a european ecological equivalent of *O. biennis*, which is reported as invasive in North America (Gross and Werner 1978). To examine reciprocal allelopathic effects of these two species, *O. biennis* and *V.thapsus* were subjected to cultivation in the leachate of the germinating seeds of the other species.

Comparative study of variously invasive *Oenothera* species:
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1. Introduction

1.1 Invasions

About fifty years ago people started to notice a strange phenomenon in the nature namely spreading of foreign elements- organisms which were once brought into the area by man himself. During the invading process local species may be supressed, which is especially undesired when rare species are concerned. The composition of vegetation changes as does the whole ecosystem or in the more extreme cases the whole landscape and its functioning (Vitousek et al 1987, Vitousek 1990, Mack et al 2000). The situation considering plants usually ends with a monospecious and/or second-rate growth of undesired species. Today biological invasions are considered as the second largest threat to biodiversity after habitat destruction (Ensering 1999, Mack et al. 2000) and may result in an economical problem as well (Mack 1996). However comparing current changes with the fossil records, one must say, nothing important happens. Still one important think in nature has changed. Man has developed such a dense network of transport, that it helps some species to overcome barriers formally unsourmountable, like big oceans or mountains (Kolar and Lodge 2001).

There were many attempts to explain the phenomenon of invasion. As a result much work has been done concerning this theme and also some theories stood up. The hypothesis of environmental constraints explains the invasions by removal of some former constraint to their spreading. Under changes promoting invasions belong disturbances, break down of barriers (Kolar and Lodge 2001) and nutrient enrichment through agricultural run-offs and acidic depositions (Wedin a Tilman1996, Fenn et al. 1998, Reich et al. 2001).

A common explanation for invasive success of introduced plants is the enemy release hypothesis which posits that natural enemies regulate the species in the native range but are absent from the introduced range (Wolfe 2002, Mitchell and Power 2003, Torchin et al 2003, DeWalt et al. 2004).

Other alternative theory- EICA (Evolutionary increased competitive ability hypothesis) is based on the same preassumption, e. g. absence of herbivores and patogens. It states that the spared costs which would be invested to the defense chemicals in the native range are used to greater growth and allocation in the novel range (Blossey and Nötzgold 1995). Currently is the regulative effect of "enemies" on the population growth seen as overestimated or not operating at all (Van Kleunen and Schmid 2003, Willis et al. 2000).

In the last years scientists started to emphasize the importance of release characteristics, such as frequency of introductions and number of introduced individuals- propagule pressure (Kolar and Lodge 2001).

In studies of individual invasion events authors usually desired to reveal any superior characteristic of the invaders that would distinguish them from non-invasive species, explain the invasiveness and as such be used to predict and prevent other invasions. This hint for generalization revealed that different invaders possess different characteristics which are important in different habitats (Williamson and Fitter 1996). However studies that compare invasive with noninvasive species are still thought to offer the most powerful test of what makes a species invasive (Kolar and Lodge 2001).

1.1.1 Hybrid species

In introduced species there is a strong evidence for hybridization with native relatives or with other introduced taxa (Abbot 1992, Rhymer and Simberloff 1996, Ellstrand and Schierenbeck 2000). Many species of the subsection *Oenothera* are european hybrids of other *Oenothera* species introduced to Europe (Dietrich et al. 1997).

Theoretically genetic mixing can create taxa with extraordinary features and can affect the ecological interactions of such species and lead to greater invasiveness (Ellstrand and Schierenbeck 2000). Hybrids may possess transgressive phenotypes unlike their parents, they can have better fitness, fecundity (Ayres et al. 2003) and moreover their agressive characters can be transmitted back to their parents through backcross (Antilla et al. 2000).

Impact of hybrid species may be sometimes more profound than of their introduced parents and thus the studies of their performance have potential predictive value.

1.1.2 Allelopathy and plant invasion

Invaders often establish virtual monocultures where diverse communities once flourished (Hierro and Calaway 2003). It is thought that introduced species release chemicals on which the native community did not coevolve tolerance and thus is succeptible to them eventhough in the native range no such harmful effects reveal (Hierro and Calaway 2003). Many studies suggest that allelopathy probably contributes to the dominance aquirement of a species (Abdul-Wahab and Rice 1967, El-Ghareeb 1991) and some had found the allelochemicals in invaders (Vaughn and Berhow 1999, Stachon and Zimdahl 1980, Yamamoto 1995).

Many plant species produce allelochemical compounds in all parts of plant (Horsley 1977). The effects on other plants are dependent on concentration, can be highly species or life-stage specific.

1.2 Germination phase and environmental signals

1.2.1 Germination

The study focused on germination stage for several reasons. First, events which occur during the juvenile stage of the plant life history are thought to be the key determinants of the distribution of adults (Templeton and Levin 1979). Second *Oenothera* species -alike most weedy species in temperate climate, form a long-lived seed bank. Some authors suggest that the seed persistence is evolutionary connected with seed size and shape but also with physiological features (Thompson et al. 1993, Thompson et al. 1998). It is primary and secondary seed dormancy which helps seeds to adjust the germination to the appropriate season and to avoid germination during the short periods of the favourable conditions within periods unsuitable for later growth (Donohue 2003). The organism whose sensing mechanisms are well tuned and such ensure the success of seedlings are thought help to species colonize specific system (Martinez-Ghersa et al. 2000) and could play role even in the plant invasions.

Seed phenotypical response is determined by its genotype and by the prevailing environmental conditions during seed development and seed after-ripening (Holdsworth et al. 1999). These factors namely include temperature, photoperiod, light quality,altitude, water and chemical characteristics of the soil and neighbouring vegetation and the same operate in the unfolding of germination. One factor usually influences the effects of other factors and together they form a complex signal.

The transition from a seed to seedling is an irreversible unfolding of a developmental programme and its timing can be plastic (Silwertown and Gordon 1989). When to germinate is an important decision, which commits the seedling to grow in whatever condition elicited the germination. Selection consequently favours environmental cueing mechanisms that decrease the probability of encountering unacceptable growth conditions following germination (Grime 1981). However the ability to withstand the novel habitat conditions or a sudden environmental change and germinate may helps plant to survive at a site and to colonize new sites (Sultan 1987 and 2000).

From comparative studies of native with invasive species that concentrated on fecundity and germination only some had identified any advantage of an invader. They found higher germination rate in *Spartina alternifolia* (Callaway and Josselyn 1992) invading the San Francisco Bay and higher seed production, germination rate and speed in *Banksia ericifolia* (Honig et al 1992). Other studies however did not reveal any superiority.

1.2.2 Factors affecting germination

Light environment has three main qualities that can effect a seed: intensity, spectral composition and periodicity of light (Fenner 1992). The response operates through various light-sensing receptors, from which the phytochrom is best known. There was found a strong interaction of phytochrome mediated processes with other environmental factors (Pons 1992).

Temperature is thought to play a pivotal role in control of germination as it is to a great degree responsible for the synchronization of germination with conditions (Probert 1992). Seasonal fluctuations influence the cycles of dormancy (Baskin and Baskin 1981 and 1993). The diurnal fluctuations serve as well as a signal- they inform about the absence of concurent vegetation and the depth of burial (Probert 1992). Some species are even known to be obligately dependent on the presence of temperature fluctuations (Thompson et al 1977).

Among chemicals normally occuring in the soil, the seed responds especially to to the concentration of nitrate, which reflects the local nutrient status and to the presence and concentration of the allelochemical substances, e.g. the chemicals released by higher plants that play role in inhibition of growth, development or germination. NO_3 as a signal interacts mainly with light and the third factor which cooperates with the letter two are temperature fluctuations (Karssen and Hillhorst 1992).

1.3 Phenotypic plasticity

Plant genotype may produce a broad range of phenotypes in response to variation in abiotic environment (Silwertown and Gordon 1989, Piqliucci 2001), variation in the presence or identity of neighbours or variation in consumer pressure. Phenotypic plasticity is often favoured in habitats with high environmental variability and unpredictability (Van Tierderen 1991). However there must be reliable environmental cues such that individual is able to analyse the situation and express the appropriate phenotype. As such the plastic genotype renders a fitness advantage in environments in which specialized genotypes are less successful (Callaway et al. 2003). Among spatially and temporary heterogenous habitats count also the disturbed habitats where invaders frequently establish (Bradshaw 1965, Schlichtling and Levin 1986, Oble 1995).

Phenotypic plasticity is known to have contributed to invasiveness in several species including *Bromus tectorum*(Rice and Mack 1991), *Avena* spp.(Marshall and Jain 1968), *Pennisetum setaceum* (Williams et al 1995), *Polygonum persicaria*(Sultan and Bazzaz 1993). Most studies involved changes in biomass allocation patterns in response to different environmental conditions, other studied the plasticity in terms of physiological response (Frenot and Gloaguen 1994).

1.4 Study aims

In my study I asked the following questions:

- Do variously invasive species differ in their germinating power, In their seedlings traits or in the range of conditions they are able to germinate in?
- Is the germinating power of chosen *Oenothera* species affected by:
  light climate, temperature, nutrient addition and
  presence/absence of temperature fluctuations?
- Do the chosen experimental treatments affect the morphology and weight of young *Oenothera* seedlings?
- What is the germination power of hybridogenous *O. fallax* compared with the parental species (*O. biennis* and *O. erythrosepala*)?
- Is the germination and early seedling performance of *Oenothera biennis* influenced by the presence of germinating seeds of *Verbascum thapsus* and vice versa?

2. Materials and methods

2.1 Studied species

2.1.1 Genus *Oenothera*

Genus *Oenothera* (Onagraceae) is native to Central, North and South America. These so called evening primroses are however today widely distributed worldwide (Dietrich et al. 1997). Species of the subsection *Oenothera* originate from North America and the centre of their secondary range is Europe. First were brought to Europe about 150 years ago for ornamental purposes and their current invasive status is most probably a result of a garden escape. From the 10 Oenotheras which were introduced only a proportion became widespread, whereas the rest have not changed the area of their distribution from their arrival (Mihulka et al. 2003). Many of the species are results of relatively recent hybridization events and thus of european origin (Dietrich et al 1997).

Species of the subsection *Oenothera* as well as other species of the genus posses a special genom structure. Their chromosomes are connected with each other through reciprocal translocations and they arrange to circles (Renners circles) during meiosis. As a result crossing over is restricted (Cleland 1972) and parental genomes are inherited as unmodified units. Therefore these organisms are permanent translocation heterozygots.

Most of the species are autogamous, which limits the occurence of hybridization, however when such event occurs it becomes fixed immediately (Riesenberg 1997) and hybridization is known to play an important role in the evolution of this subsection.

Majority of species of are biennials and flower the second or the third year of their life. They are typical representatives of the ephemeral community of the early successional sere. They can be found everywhere where there is enough bare ground: in abandoned agricultural fields, railway asides, on river banks and roadside verges (Dietrich et al. 1997). Their habitat preferences are similar both in their native and secondary range (Mihulka et al. 2003).

Studied species are monocarpic thus the persistance of a population at a site depends purely on the ability to continually recolonize the area with the diaspores. Their seeds are relatively small and remain viable for about 80 years (Dietrich et al. 1997) and as such form a persistent seed bank. Small-seeded species are usually thought to require light as a signal of unburial because they can not penetrate from greater depth in the soil or have resources too small fro establishment under vegetation cover. Baskin an Baskin (1993) pointed out that *Oenothera* seeds can quite successfully germinate also in darkness.

The stimulation of germination in such small-seeded species that form persistent seed banks is most probably performed by complex signal of light and nitrate ions (Hilton 1984).

Chosen species come from the same section *Oenothera*, they have similar morphology, phenology and ecology.

Used species (number of localities in six central european countries, Mihulka and Pyšek 2001):

Oenothera biennis (794 localities)
Oenothera rubricaulis (68)
Oenothera ammophila (27)
Oenothera erythrosepala (339)
Oenothera fallax (47)

2.1.2 *Verbascum thapsus* (common mullein)

One more species is embodied in the study- *Verbascum thapsus* (Scrophulariaceae). It is thought to be an european ecological equivalent of *O. biennis* because characteristics and requirements of these two species are very similar. Both species are biennials found on open grounds of abandoned agricultural fields, along rails, on road side verges. Their body posses similar morphology, they both flower in yellow and both are thought to be curative. *Verbascum thapsus* produce greater number of smaller seeds compared to *O. biennis*.

These two species differ in the continent of their origin. *O. biennis* is from North America and currently spreads in Europe. *V. thapsus* is from Europe and is declared to be invasive in USA. As the species colonize similar habitats they can be found growing together.

In my experiment I examined the situation in which they germinate together.

2.2 Used seed sets

species	source population	collection year
Crossed-gradients and phytotron experiments		
Oenothera biennis	Praha- Bubny	2000
O.rubricaulis	Kladno	2000
O.ammophila	Liberec	2000
Klimabox: allelopathy experiment		
O.biennis	Zliv	1999
Verbascum thapsus	Č. Budějovice-Sádka	1999
Klimabox: hybrid performance		
O.biennis	Zliv	1999
O.erythrosepala	Č. Budějovice-Stromovka	1999
O.fallax	Horažďovice	1999

Table 1 brings the place and year of the seed collection.

2.3 Experiment arrangement

Seeds were sown in Petri dishes on five layers of filter paper and watered regularly and sufficiently.

The light spectral change (green shade) was performed by a green gardening folie.

The reduction of light intensity (half-light) was performed by the placement in greater distance from the light source. Further reduction (dark) was carried out by wrapping of the Petri dishes into a three-layered aluminium folie at both sides.

As a fertiliser for experiment with seeds was used a Fertiliser for hydroponia with 45,2 g/l of the total nitrogen (31,6 g/l nitrate N and 13,6 g/l ammonia N), 22,6 g/l of the water-soluable phosphates (P_2O_5) and 56,5 g/l of the water-soluable potassium (K_2O). The fertiliser solution was prepared by diluting 8 ml of the concentrated fertiliser in 1,5 l of water.

The leachates for the allelopathy experiment were obtained by soaking of seeds of *O. biennis* and *V.thapsus* respectively in distilled water two days before the experiment was founded. They germinated well after three days. Half of prepared Petri dishes of each species was than from the beginning watered by this leachate of germinating seeds.

2.3.1 Cross gradients experiment:

Part of the experiments was performed in Botanical institute in Třeboň. A device developed for cultivation of algae on crossed gradients of light and temperature (Kvíderová and Lukavský 2001) was used to find the optimal temperature for germination and the temperature range in which the species in question are able to germinate in.

The unit consists of an aluminium desk, which is cooled on one side and heated on the other side. Both temperatures can be regulated and as a result a temperature gradient stands out. The plate is illuminated by three pieces of sodium lamps NAV TS 400 (OSRAM, D) and two fluorescent tubes. The irradiance can be changed by placing strip filters between the desk and the light sources.

Experimental design

50 seeds per Petri dish, two replicates per treatment;
photoperiod: 15:9 (light:dark)
light climates: unfiltered light (1650 lx), green folio cover (650 lx),
three layered aluminium folio cover (dark);
temperature gradient: 9-29°C, temperature difference between
neighbouring Petri dishes = 4°C.

2.3.2 Phytotron experiment

Fitotron S6CD 97.PPX.F is a closed klimabox, where temperature, light and saturation of some important gases can be modeled in time.

Two experiments in a serie were conducted in order to find whether germination of the species in question is somehow dependent on temperature fluctuations. In the first experiment a regime with a constant temperature was set. The second experimental regime provided seeds with similar energetic income but the temperature fluctuated during the day.

Experimental design

40 seeds per Petri dish, three replicates per treatment;
photoperiod: 15:9 (light:dark)
light climates: full light(50 000 lx) ,
half light (25 000 lx),
three layered aluminium folio cover (dark);
nutrient treatments: half of the dishes was watered with a fertilizer solution;

temperature fluctuations: two experiments were realized

1) steady temperature of 23°C
2) 18° C during the dark period, than 6 hours 25°C, follows a 3 hours long midday peak with 30°C, than another 6 hours of 25°C.

2.3.3 Hybrid experiment:

Common klimabox was used in this experiment with stable temperature of 25°C. The experiment arrangement:

40 seeds per Petri dish, three replicates per treatment;
photoperiod: 15:9 (light:dark) ;
light climates: light (10 000 lx),
three layered aluminium folio cover (dark);
nutrient treatments: half of the dishes was watered with a fertilizer solution.

2.3.4 Experiment on allelopathy

Common klimabox was used in this experiment with stable temperature of 25°C. The experiment arrangement:

40 seeds per Petri dish, three replicates per treatment;
photoperiod: 15:9 (light:dark);
light climates: light, three layered aluminium folio cover (dark);
watering: half of the dishes were watered with a leakage from germinating seeds of the other species.

2.4 Measured traits

Each day or every other day the number of germinated seeds was counted. The seed was declared germinated when its radicule grew more than 1,5 mm. At the end of the experiment total germination percentage was counted and in 10 or more individuals from every Petri dish the following characteristics were measured: the lenght of radicule and hypocotyle and the span of cotyledons. Finally after 14 days the seedlings were weighted.

2.5 Statistical analysis

Data were treated and analysed with program Statistica 5.5 for Windows. PC indexes were computed with the use of Microsoft Excell. Grafical parts were done in the Statistica 5.5.

Morfometrical characteristic were processes as they were, for weight a transformation $\log_{10}$(weight + 1) was used to achieve normal distribution and homoscedasticity.

Data were subjected to the Analysis of variance to obtain the general effects of the used factors. Than each factor was analysed by one-way ANOVA analysis in each combination of other factors. Tukey tests provided the information about the differences, where there were more than two levels of the factor.

3. Results

3.1 Experiment on the crossed gradients of light and temperature

3.1.1. Germination

PERCENTAGE, Summary of all Effects **1-SPECIES, 2-LIGHT, 3-TEMPERATURE**		
	F	**p**
1	202.37	p->0
2	42.37	p->0
3	112.54	p->0
12	2.62	0.045033
13	22.71	p->0
23	3.83	0.000593
123	4.54	0.000005

Table 2. Results (F-values and the achieved levels of probability) from ANOVA with the following factors:
SPECIES (*O.biennis, O. rubricaulis, O.ammophila*),
LIGHT (light, green shade and darkness)
TEMPERATURE (9°C, 13°C, 17°C, 21°C, 25°C, 29°C).

From the studied species *O. biennis* germinates the best, better than *O.rubricaulis* (Tukey, p=0,000121) and *O.ammophila* (Tukey,p=0,000121). The latter two achieved similar final germination percentages.

Figure 1.

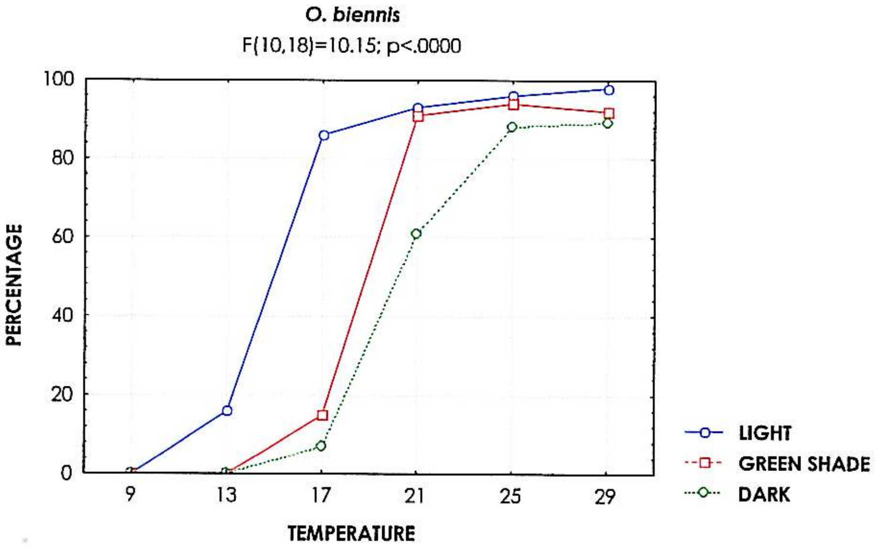
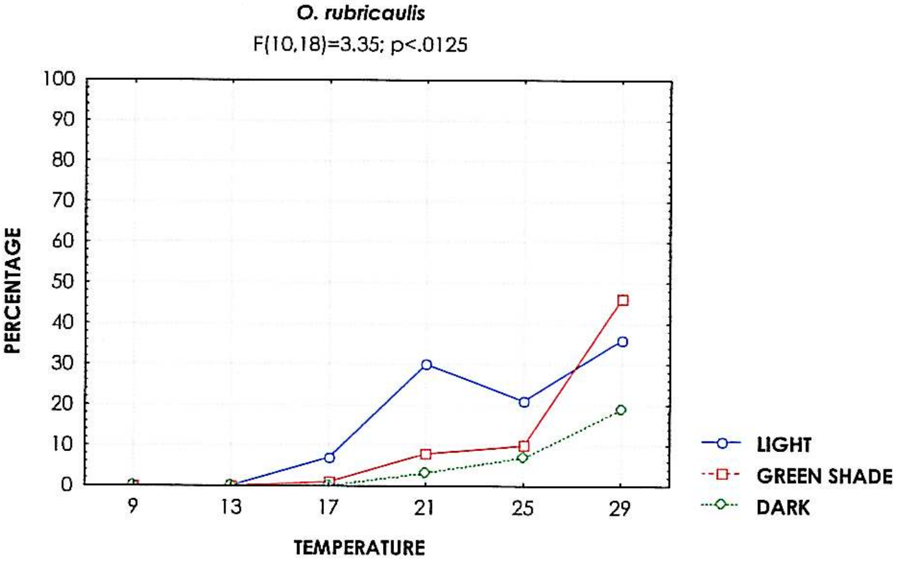


Figure 2.



Description on the next page.

Figure 3.

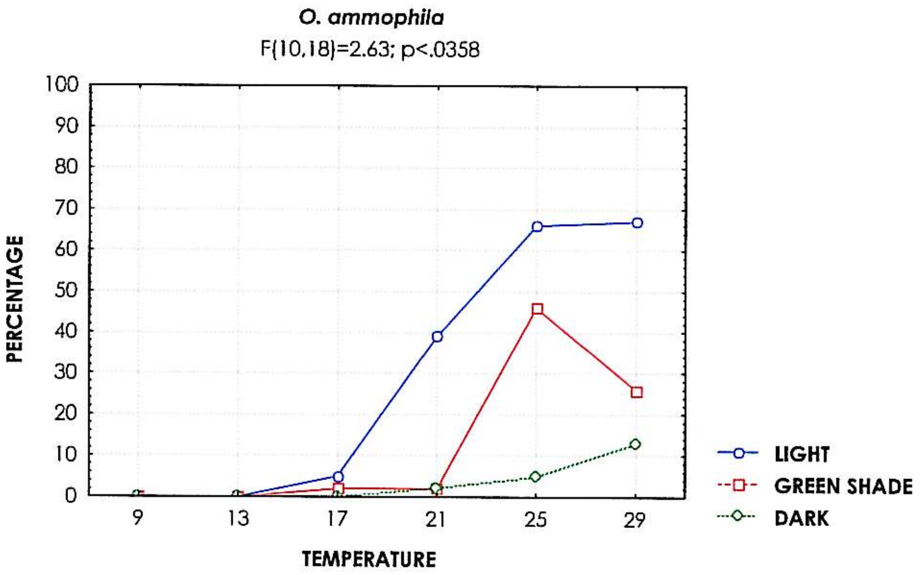


Fig 1,2 and 3 show the final germination percentages in various light treatments and temperatures for each species.

FINAL GERMINATION PERCENTAGE		TEMPERATURE EFFECT	13°C	17°C		21°C		25°C		29°C	
O.biennis	L1	289,1***	16 ± 2,8	86 ± 0	13	93 ± 1,4	13	96 ± 5,7	13	98 ± 0	13
	L2	61,09***	0	15 ± 12,7	21,25,29	91 ± 4,2	17	94 ± 2,8	17	92 ± 2,8	17
	D	15,1*	0	7 ± 9,9	25,29	61 ± 24		88 ± 2,8	17	89 ± 9,9	17
O.rubricaulis	L1	3,09	0	7 ± 4,2		30 ± 19,8		21 ± 1,4		36 ± 0	
	L2	20,16**	0	1 ± 1,4	29	8 ± 0	29	10 ± 5,7	29	46 ± 11,3	17,21,25
	D	10,95*	0	0		3 ± 1,4	29	7 ± 4,2	29	19 ± 4,2	21,25
O.ammophila	L1	10,18*	0	5 ± 1,4	25,29	39 ± 4,2	17	66 ± 14,1	17	67 ± 21,2	17
	L2	1,53	0	2 ± 2,8		2 ± 2,8		46 ± 45,3		26 ± 17	
	D	1,08	0	0		2 ± 0		5 ± 4,2		13 ± 12,7	

Table 3. The final germination percentages ± SD achieved by the species in separate treatments. The numbers bellow the term: TEMPERATURE EFFECT are F-values from the one-way ANOVA analysis of he factor temperature in each combination of other factors. The probability levels are indicated by stars (*p<0,05, ** p<0,01,*** p<0,001).
Light treatments are presented like shortening. L1= full light, L2= green shade and D= darkness.

The effect of temperature is brought by Tab 3. and the effect of light and the differences among species are shown in Tab 4. on the next page.

Summary:

O.biennis showed the greatest tolerance on suboptimal temperature and light. When cultivated in light it was able to germinate in 13°C and in 17°C it achieved nearly 90% germination, with further increase of temperature the germination percentage even increased. *O.rubricaulis* achieved its maximum germination power in 29°C. *O. ammophila* germinated the best in 25°C and 29°C.

O. rubricaulis and *O. ammophila* were not able to germinate in the dark in 17°C and the germination power in 21°C and 25°C did not reach 10%. In green shade *O. rubricaulis* was able to get over 10% germination only in 29°. The germination of *O. ammophila* was the similarly poor in all temperature treatments.

.

Final germination percentage 17°C	LIGHT \ SPECIES	LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
		640,3***		2,13			
O. biennis (B)	43,64**	86 ± 0 RA	L2,D	15 ± 12,7	L1	7 ± 9,9	L1
O. rubricaulis (R)	3,6	7 ± 4,2 B		1 ± 1,4			
O. ammophila (A)	1,8	5 ± 1,4 B		2 ± 2,8			

Final germination percentage 21°C	LIGHT \ SPECIES	LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
		16,91*		571***		11,8*	
O. biennis (B)	3,22	93 ± 1,4 RA		91 ± 4,2 RA		61 ± 24 A	
O. rubricaulis (R)	3,14	30 ± 19,8 B		8 ± 0 B		3 ± 1,4	
O. ammophila (A)	105,31***	39 ± 4,2 B	L2,D	2 ± 2,8 B	L1	2 ± 0 B	L1

Final germination percentage 25°C	LIGHT \ SPECIES	LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
		36,54**		5,1		305,77***	
O. biennis (B)	2,17	96 ± 5,7 R		94 ± 2,8		88 ± 2,8 RA	
O. rubricaulis (R)	6,27	21 ± 1,4 BA		10 ± 5,7		7 ± 4,2 B	
O. ammophila (A)	2,56	66 ± 14,1 R		46 ± 45,3		5 ± 4,2 B	

Final germination percentage 29°C	LIGHT \ SPECIES	LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
		12,81*		16,21*		38,53**	
O. biennis (B)	1,19	98 ± 0 R		92 ± 2,8 A		89 ± 9,9 RA	
O. rubricaulis (R)	7,66	36 ± 0 B		46 ± 11,3		19 ± 4,2 B	
O. ammophila (A)	5,3	67 ± 21,2		26 ± 17 B		13 ± 12,7 B	

Table 4. The final germination percentages ± SD. The tables are divided according to temperture treatments and concentrate on the differences among the light treatments (LIGHT effect) and among species (SPECIES effect). Bellow the term LIGHT and beside the term SPECIES the F-values from one-way ANOVA analysis where the light or species was used as a main factor in the particular combination of the other factors.

L1,L2,D= light treatments significantly differing in the Tukey test in the particular combination of other factors.

B,R,A=species significantly differing in the Tukey test in the particular combination of environmental factors.

The temporal pattern of germination

NUMBER OF GERMINATED SEEDS		
Summary of all Effects;		
1-SPECIES, 2-LIGHT, 3-TEMPERATURE, 4-TIME		
	F	p
1	499.68	p->0
2	50.26	p->0
3	173.93	p->0
4	476.74	p->0
12	4.55	0.003071
13	65.40	p->0
23	5.70	0.000009
14	159.19	p->0
24	28.61	p->0
34	66.24	p->0
123	5.37	p->0
124	2.33	0.000035
134	22.50	p->0
234	3.40	p->0
1234	4.16	p->0

Table 5. Results from the ANOVA analysis with repeated measurements. The characteristics tested was number of germinated seeds, which was counted every other day.

Also the temporal pattern of germination was affected by the used factors (see Figs . *O. biennis* achieved greater germination speed and rate. The course of germination was affected by light climate and temperature. The reduced light availability caused later germination and smaller final germination number. Species germinated later in the cooler treatments.

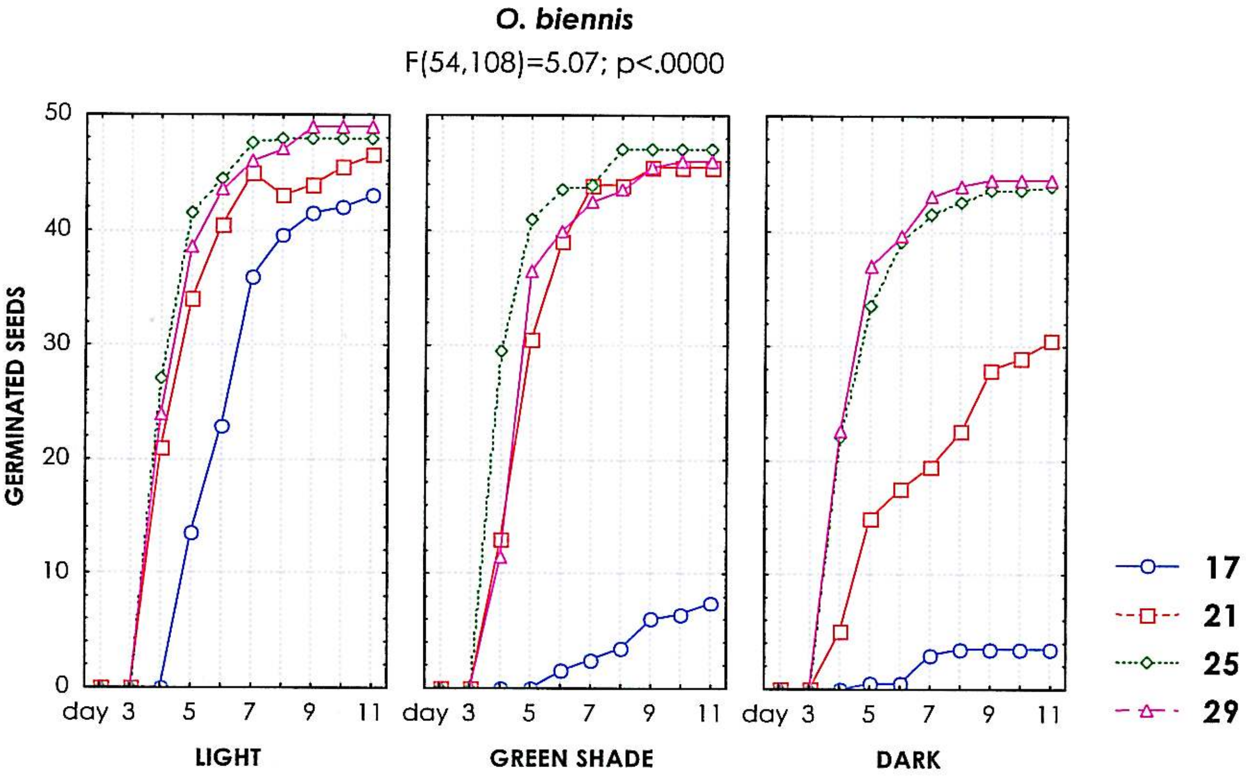


Figure 4. Temporar pattern of germination of *O. biennis* in separate light climates and temperatures.

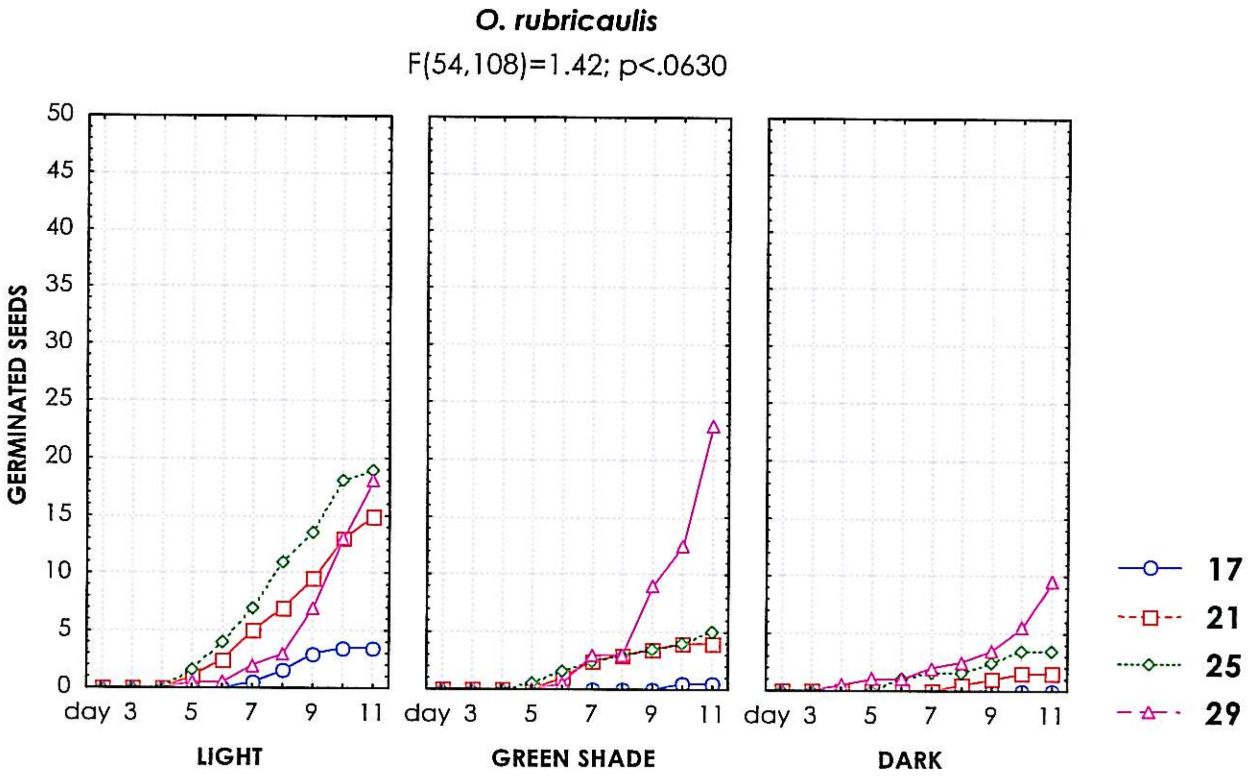


Figure 5. Temporar pattern of germination of *O. rubricaulis* in separate light climates and temperatures.

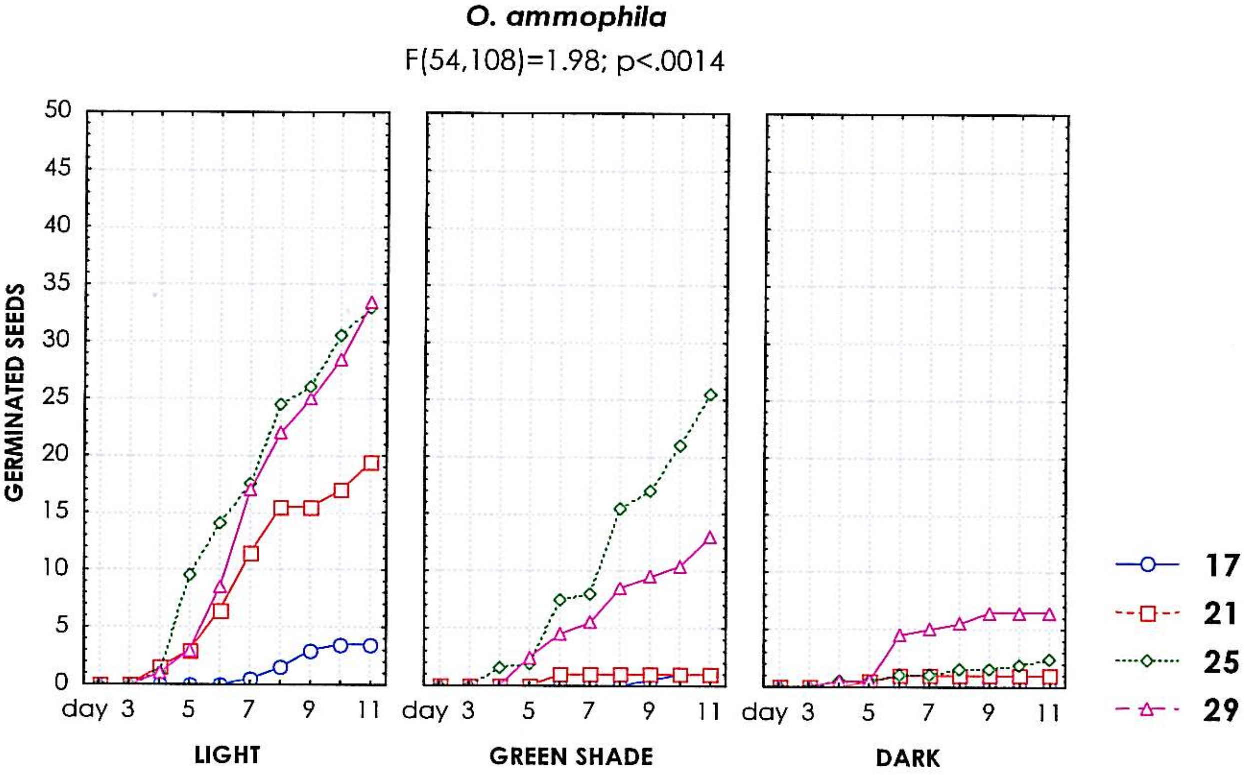


Figure 6. Temporar pattern of germination of *O. ammophila* in separate light climates and temperatures.

3.1.2 Seedling traits

effect	**SPECIES**	**LIGHT**	**TEMPERATURE**	**S × L**	**S × T**	**S × L × T**
Root length	21.93***	3.80*	75.59***	3.02*	11.29***	2.06
Shoot length	56.37***	73.81***	2.07	2.20	11.96***	0.14
Cotyledons	194.94***	20.02***	25.75***	6.09**	6.07**	0.27
Dry weight	255.02**	0.45	1.05	3.18*	7.09**	2.65*

Table 6. Comparison of morphometric traits among species was possible from 25°C, where all three species achieved more than 10% germination in all three light treatments. The table brings Summaries of all effects.
Species effect: the difference among *O. biennis, O. rubricaulis* and *O. ammophila*
Light effect: The difference among light climates (full light, green shade and dark)
Temperature effect: Difference between 25°C and 29°C.

Species differed in all measured characteristics. In general *O. biennis* developed the longest shoot and the greatest cotyledons span, *O.ammophila* developed the longest root and achieved the greatest dry biomass weight. *O.rubricaulis* achieved the smallest values in all measured characteristics.

The significant S×L (species×light) interaction terms indicate species specific responses of the root lenght and span of cotyledons to light. The significant S×T (species×temperature) terms indicate species specific responses of all characters to the change in temperature.

In 21°C *O. biennis* was able to germinate in all light treatments but the latter two species only in light. The differences among species in this treatments are presented in in Tab 7. Tab 8. brings the performace of *O. biennis* in 21°C under separate light treatments. Tab 9, 10,11, 12 bring the light effect and species differences among species in separate traits.

21°C, LIGHT	**SPECIES**	***O. biennis* (B)**	***O. rubricaulis* (R)**	***O. ammophila* (A)**
Root length	6,8**	1,76 (0,52) **A**	1,55 (0,69) **A**	2,3 (0,75) **BR**
Shoot length	1,47	0,48 (0,15)	0,56 (0,41)	0,62 (0,17)
Cotyledons	21,16***	0,53 (0,22) **R**	0,22 (0,12) **BA**	0,32 (0,16) **R**
Dry weight	38.78***	0,000266 (0,000038) **A**	0,000305 (0,000037) **A**	0,000421 (0,000047)**BR**

Table 7. Bellow the term: SPECIES are the F-values from the ANOVA test of the specific (species) effect with the achieved probability levels (* p<0,05,** p<0,01, p<0,001***).
The mean value of the trait in particular species and the SD (in brackets) is brought.
B,R,A=species that in 21°C and light achieved significantly different (Tukey test) trait values.

***O. biennis*, 21°C**	**LIGHT**	**LIGHT (L1)**	**GREEN SHADE (L2)**	**DARK(D)**
Root length	4,4*	1,76 (0,52) **L2 D**	0,89(0,17) **L1 D**	1,69 (0,52) **L1 L2**
Shoot length	72,83***	0,48 (0,15)	1,54 (0,34)	1,36 (0,38) **L1**
Cotyledons	1,41	0,53 (0,2)	0,50 (0,07)	
Dry weight	1,25	0,000266 (0,000038)	0,00257 (0,000029)	0,000246 (0,000028)

Table 8. Bellow the term: LIGHT are the F-values from the ANOVA test of the specific (light) effect with the indications of the achieved probability values (* p<0,05,** p<0,01, p<0,001***).
The mean value of the trait in the particular light treatment and the SD (in brackets) is brought.
L1,L2,D= Light treatments in which *O. biennis* in 21 achieved significantly different trait values.

Table 9.

SHOOT LENGHT		LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
25°C	LIGHT \ SPECIES	2,73		1,33		2,79	
O. biennis	44,48***	1,07 (0,21)	L2 D	1,43 (0,27)	L1 D	1,9 (0,34)	L1 L2
(B)							
O. rubricaulis	5,18*	0,88 (0,34)	D	1,23 (0,45)		1,41 (0,52)	
(R)							
O. ammophila	5,05*	0,88 (0,32)	D	1,25 (0,45)		1,55 (1,01)	L1
(A)							

SHOOT LENGHT		LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
29°C	LIGHT \ SPECIES	48,9***		26,84***		17,82**	
O. biennis	125,18***	1,27 (0,18)	D	1,41 (0,19)	D	2,2 (0,23)	L1 L2
(B)		RA		RA		RA	
O. rubricaulis	6,3**	0,6 (0,28)	D	0,7 (0,38)	D	1,15 (0,71)	L1 L2
(R)		BA		BA		B	
O. ammophila	14,78***	0,89 (0,17)	D	1,15 (0,35)	D	1,62 (0,53)	L1 L2
(A)		BR		BR		B	

Table 10.

ROOT LENGHT		LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
25°C	LIGHT \ SPECIES	15,44***		7,08**		3,83*	
O. biennis	3,03	1,29 (0,37)		1,15 (0,51)	D	1,46 (0,31)	L2
(B)		A		A		R	
O. rubricaulis	1,62	1,19 (0,53)		1,54 (0,78)		1,03 (0,4)	
(R)		A				B	
O. ammophila	1,12	1,95 (0,51)		1,83 (0,53)		1,52 (0,62)	
(A)		BR		B			

ROOT LENGHT		LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
29°C	LIGHT \ SPECIES	9,17***		12,01***		9,47***	
O. biennis	5,07**	1,3 (0,35)	L2	0,97 (0,33)	L1	1,2 (0,35)	
(B)		R		R		R	
O. rubricaulis	13,47***	0,89 (0,24)	L2 D	0,56 (0,21)	L1	0,6 (0,18)	L1
(R)		BA		BA		BA	
O. ammophila	2,12	1,22 (0,4)		0,93 (0,34)		1,13 (0,53)	
(A)		R		R		R	

Tables 9 and 10. Values of root and shoot lenght (SD in the brackets) in separate treatments. Individual characters are distinquished by color. The tables are divided according to temperature treatments and concentrate on the differences among species (species effect) and light climates (light effect).

Light effect: (coloured numbers bellow the term: LIGHT) F-values from the ANOVA test of specific effects (light) with the achieved probability level.

L1,L2,D= light treatments in which the character achieved significantly different value in that particular temperature and species.

Species effect: (bold numbers beside the term: SPECIES) F-values from the ANOVA test of specific effects (species) with the achieved probability level.

B,R,A= species with significantly different value of the character in the given temperature×light treatment.

Table 11.

COTYLEDONS		LIGHT (L1)		GREEN SHADE (L2)	
25°C	SPECIES LIGHT	67,35**		42,14***	
O. biennis	26,32***	0,52 (0,04)	L2	0,45 (0,05)	L1
(B)		RA		RA	
O. rubricaulis	0,45	0,29 (0,05)		0,27 (0,04)	
(R)		BA		BA	
O. ammophila	2,5	0,42 (0,08)		0,39 (0,05)	
(A)		BR		BR	

COTYLEDONS		LIGHT (L1)		GREEN SHADE (L2)	
29°C	SPECIES LIGHT	93,86***		27,03***	
O. biennis	13,22***	0,44 (0,05)	L2	0,38 (0,05) **R**	L1
(B)		RA		R	
O. rubricaulis	0,59	0,25 (0,05)		0,27 (0,06) **BA**	
(R)		BA		BA	
O. ammophila	1,15	0,39 (0,03)		0,37 (0,04) **R**	
(A)		BR		R	

Table 12.

DRY WEIGHT		LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
25°C	SPECIES LIGHT	45,32***		108,04***		20,03***	
O. biennis	0,16	0,000244 (0,000028)		0,000249 (0,000028)		0,000261 (0,00002)	
(B)		RA		RA		A	
O. rubricaulis	0,44	0,000320 (0,000049)		0,000305 (0,000012) **BA**		0,000291 (0,000013)	
(R)		BA		BA		A	
O. ammophila	0,96	0,000381 (0,000046)		0,000407 (0,000026)		0,000407 (0,000098)	
(A)		BR		BR		BR	

DRY WEIGHT		LIGHT (L1)		GREEN SHADE (L2)		DARK (D)	
29°C	SPECIES LIGHT	27,35***		80,31***		48,38***	
O. biennis	5,35***	0,000239 (0,000048)	L2	0,000194 (0,000018)	L1	0,000206 (0,000037)	
(B)		RA		RA		RA	
O. rubricaulis	0,88	0,000308 (0,000047)		0,000328 (0,000057)		0,000347 (0,000024)	
(R)		BA		BA		B	
O. ammophila	2,37	0,000369 (0,000031)		0,000413 (0,000044)		0,000395 (0,000056)	
(A)		BR		BR		B	

Tables 11 and 12. Values of cotyledon span and dry biomass weight (SD in the brackets) in separate treatments. Individual characters are distinquished by color. The tables are divided according to temperature treatments and concentrate on the differences among species (species effect) and light climates (light effect).
Light effect: (coloured numbers bellow the term: LIGHT) F-values from the ANOVA test of specific effects (light) with the achieved probability level.
L1,L2,D= light treatments in which the character achieved significantly different value in that particular temperature and species.
Species effect: (bold numbers beside the term: SPECIES) F-values from the ANOVA test of specific effects (species) with the achieved probability level.
B,R,A= species with significantly different value of the character in the given temperature×light treatment.

The cotyledon span could be compared only between the illuminated treatments, because in the dark these measures were not taken due to the seed coats covering the leaves.

Table 13 show the effect the temperature had in separate species.

O. biennis		TEMP. EFFECT	21°C		25°C		29°C	
SHOOT (cm)	L1	104,87***	0,48 ±0,15	25 29	1,07 ± 0,21	21 29	1,27 ± 0,18	21 25
	L2	41,65***	0,89 ± 0,17	25 29	1,43 ± 0,27	21	1,41 ± 0,19	21
	D	9,1***	1,69 ± 0,52	29	1,9 ± 0,34	29	2,2 ± 0,23	21 25
ROOT (cm)	L1	8,07***	1,76 ± 0,52	25 29	1,29 ± 0,37	21	1,3 ± 0,35	21
	L2	11,32***	1,54 ± 0,34	25 29	1,15 ± 0,51	21	0,97 ± 0,33	21
	D	2,59	1,36 ± 0,38		1,46 ± 0,31		1,2 ± 0,35	
COTYLEDONS (cm)	L1	22,57***	0,53 ± 0,22	29	0,52 ± 0,04	29	0,44 ± 0,05	21 25
	L2	21,34***	0,5 ± 0,07	25 29	0,45 ± 0,05	21 29	0,38 ± 0,05	21 25
DRY WEIGHT (g)	L1	2,15	0,000266 ± 0,000038		0,000244 ± 0,000028		0,000239 ± 0,000048	
	L2	26,34***	0,000257 ± 0,000029	29	0,000249 ± 0,000028	29	0,000194 ± 0,000018	21 25
	D	15***	0,000246 ± 0,000028	29	0,000261 ± 0,00002	29	0,000206 ± 0,000037	21 25

O. rubricaulis		TEMP. EFFECT	21°C		25°C		29°C	
SHOOT (cm)	L1	4,95*	0,56 ± 0,41	25	0,88 ± 0,34	21 29	0,6 ± 0,28	25
	L2	9,99**			1,23 ± 0,45	29	0,7 ± 0,38	25
	D	0,69			1,4 ± 0,52		1,2 ± 0,71	
ROOT (cm)	L1	8,56***	1,55 ± 0,69	29	1,19 ± 0,53		0,89 ± 0,24	21
	L2	28,52***			1,54 ± 0,78	29	0,56 ± 0,21	25
	D	8,93**			1 ± 0,4	29	0,6 ± 0,18	25
COTYLEDONS (cm)	L1	11,66***	0,22 ± 0,12	25 29	0,29 ± 0,05	21	0,25 ± 0,05	21
	L2	0,03			0,27 ± 0,04		0,27 ± 0,06	
DRY WEIGHT (g)	L1	0,12	0,000305 ± 0,000037		0,000320 ± 0,000049		0,000308 ± 0,000047	
	L2	0,43			0,000305 ± 0,000012		0,000328 ± 0,000057	
	D	8,59*			0,000291 ± 0,000013	29	0,000347 ± 0,000024	25

O. ammophila		TEMP. EFFECT	21°C		25°C		29°C	
SHOOT (cm)	L1	8,45***	0,62 ± 0,17	25 29	0,88 ± 0,32	21	0,89 ± 0,17	21
	L2	0,47			1,25 ± 0,45		1,15 ± 0,35	
	D	0,03			1,52 ± 0,62		1,1 ± 0,53	
ROOT (cm)	L1	17,48***	2,3 ± 0,75	29	1,95 ± 0,51	29	1,22 ± 0,4	21 25
	L2	36,64***			1,83 ± 0,53	29	0,93 ± 0,34	25
	D	1,51			1,6 ± 1,01		1,6 ± 0,53	
COTYLEDONS (cm)	L1	4,25*	0,47 ± 0,16	29	0,42 ± 0,08		0,39 ± 0,03	21
	L2	0,69			0,39 ± 0,05		0,37 ± 0,04	
DRY WEIGHT (g)	L1	3,7*	0,000421 ± 0,000047	29	0,000381 ± 0,000046		0,000369 ± 0,000031	21
	L2	0,11			0,000407 ± 0,000026		0,000413 ± 0,000044	
	D	0,04			0,000407 ± 0,000098		0,000395 ± 0,000056	

Table 13. Responses of the measured characterictics to the temperature gradient. They are divided according to species.
L1= full light, **L2**= green shade, **D**= dark
The Temperature effect column brings the F-values from the ANOVA test of the specific effect with the probability level.
(* p<0,05,** p<0,01, ***p<0,001]
21,25,29= temperature treatments differing significantly in the Tukey test in the concrete species light climate combination.

Summary:

Though characters of all studied species respond to temperature, the most profound effect were found in *O. biennis*.

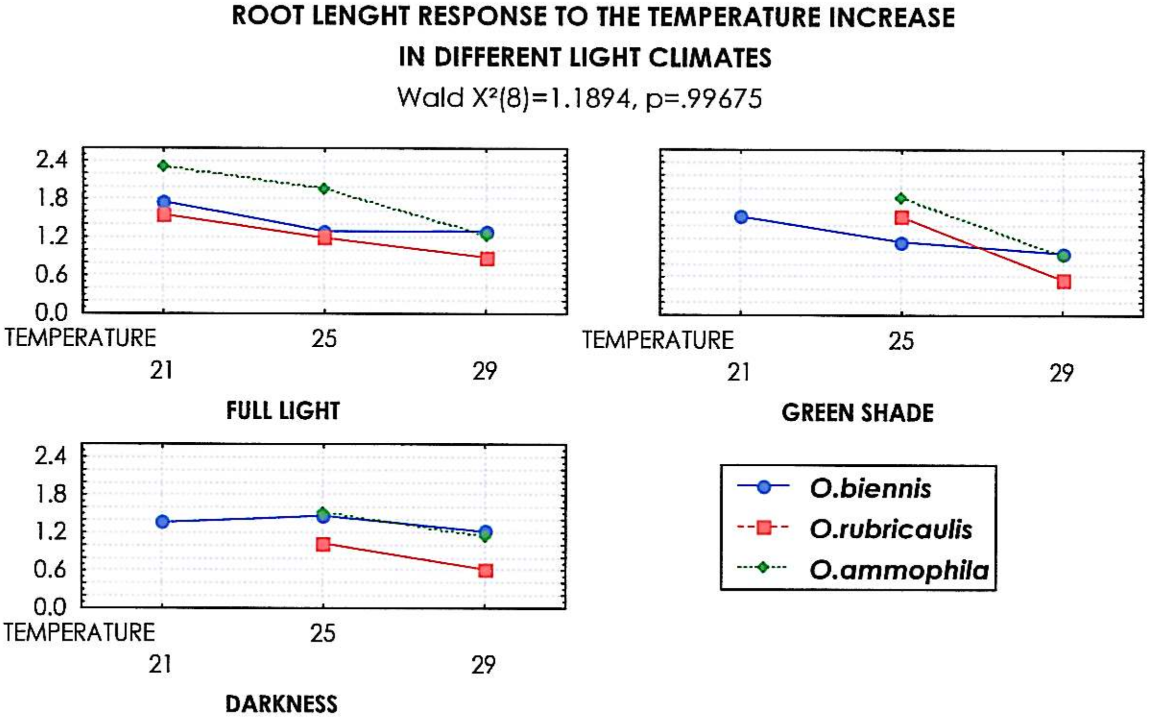
In *O.biennis* shoot gradually increased in all light climates with the temperature increase. The root decreased with the temperature increase.

O.rubricaulis seemed to percieve 29°C as an extreme temperature, majority of measured characteristics achieved the smallest value in this temperature and were greater in 25 (shoot, cotyledons). Root decreased with temperature increase.

In *O. ammophila* the lenght of root and shoot and also cotyledons span decrease gradually with temperature increase in all light climates.

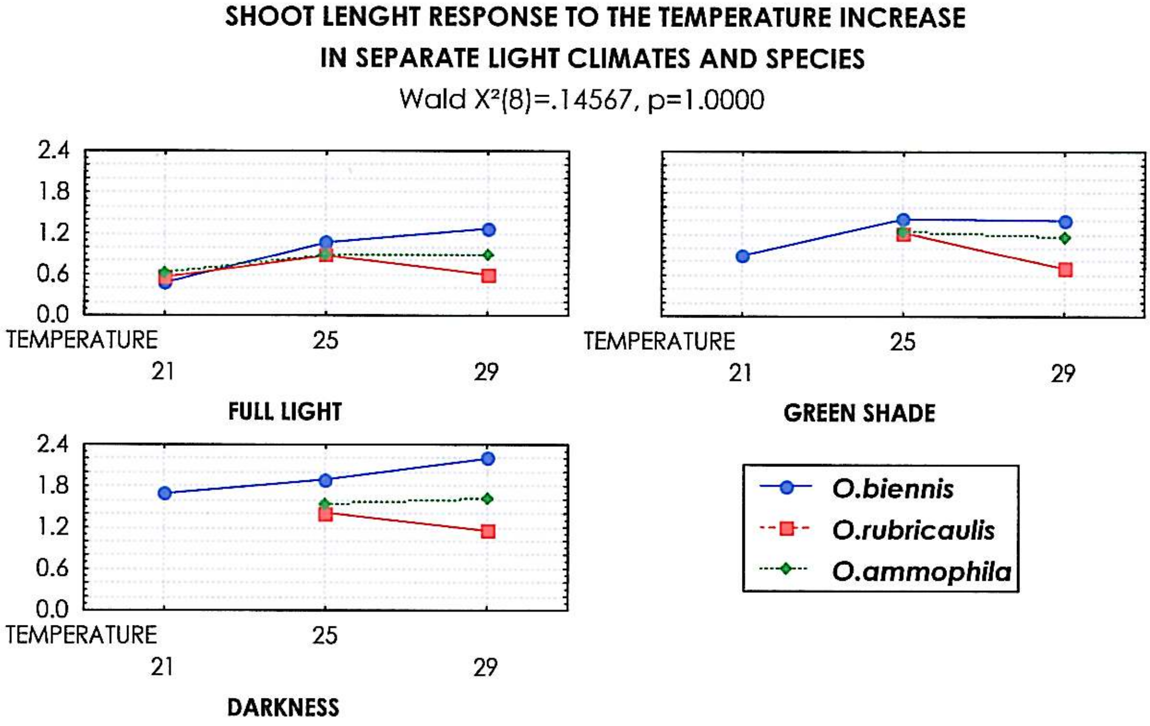
Dry weight showed the smallest change from all characters in all species.

Figure 7.


ROOT LENGHT RESPONSE TO THE TEMPERATURE INCREASE IN DIFFERENT LIGHT CLIMATES

Wald X²(8)=1.1894, p=.99675

Figure 8.


SHOOT LENGHT RESPONSE TO THE TEMPERATURE INCREASE IN SEPARATE LIGHT CLIMATES AND SPECIES

Wald X²(8)=.14567, p=1.0000

Figure 7 and 8 illustrate the effects of light and temperatures on the root and shoot lenght in separate species.

Figure 9.

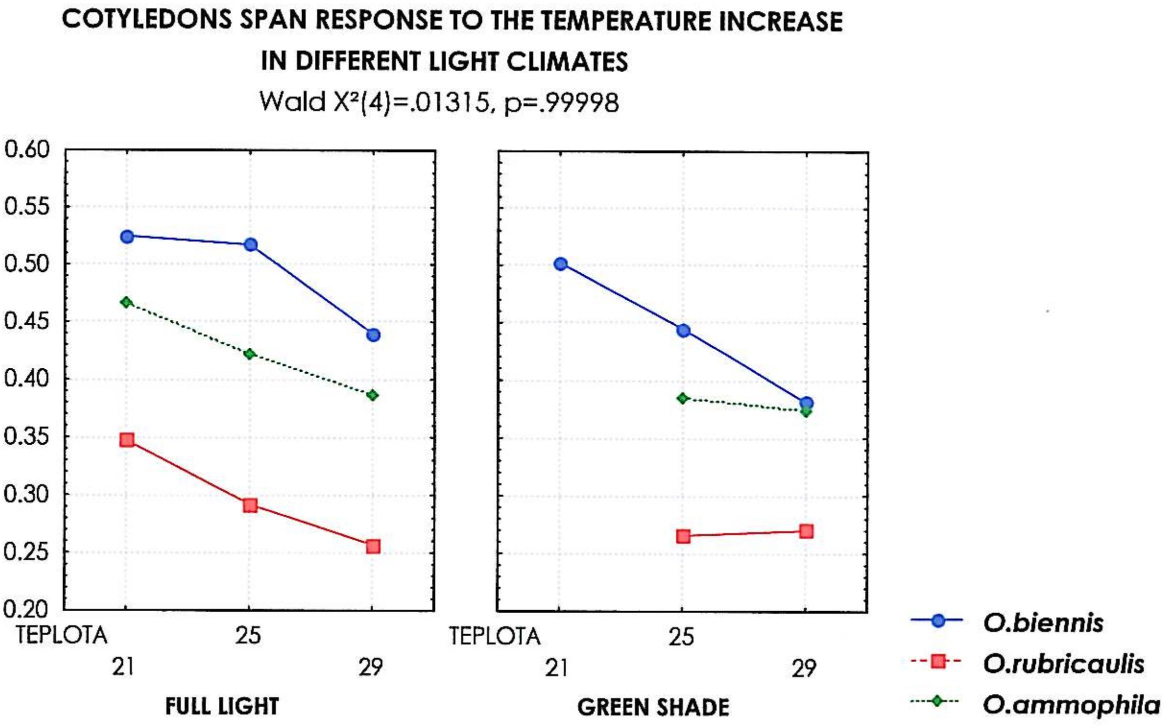


Figure 10.

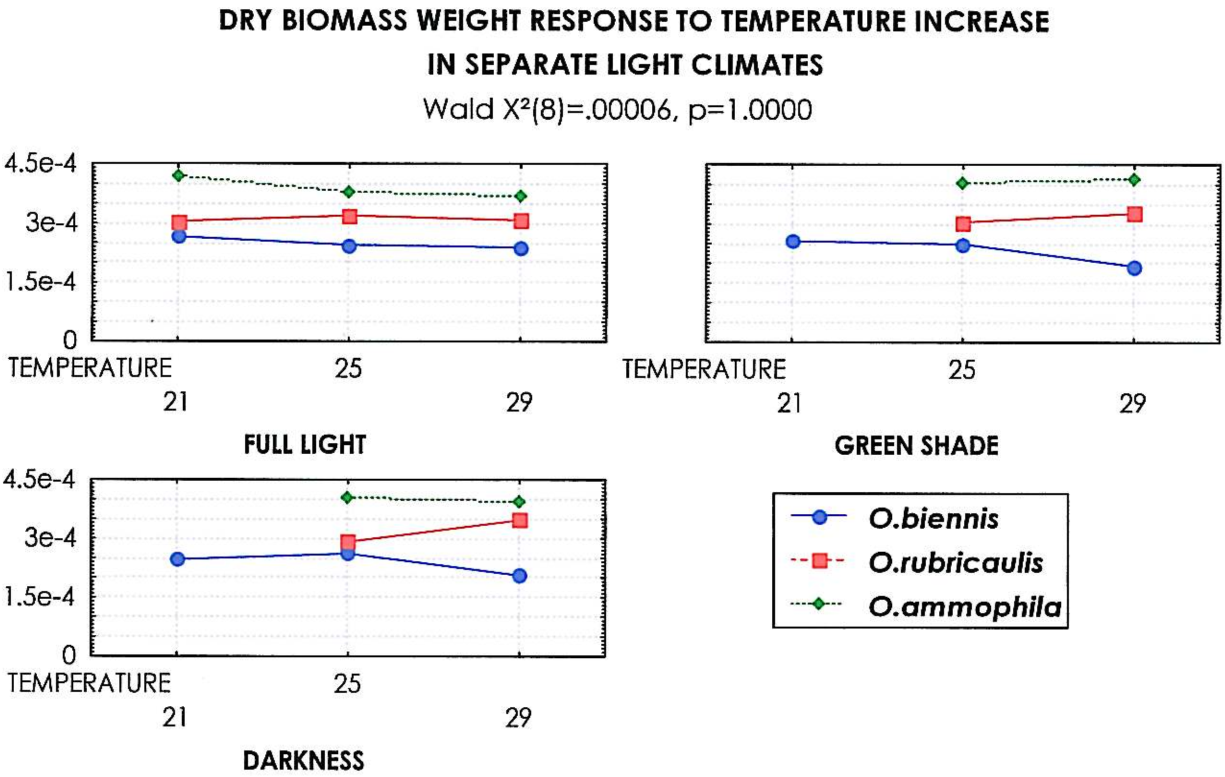


Figure 9 and 10 illustrate the effects of light and temperatures on the cotyledons span and the biomass dry weight in separate species.

3.2 Test on the effect of temperature fluctuations

3.2.1 Germination

All Effects; **GERMINATION PERCENTAGE** **1-SPECIES, 2-FLUCTUATIONS, 3-LIGHT, 4-FERT**	F	p
1	356.53	p->0
2	0.63	0.428554
3	0.98	0.379537
4	1.29	0.260585
12	1.18	0.313545
13	5.11	0.001104
23	86.26	p->0
14	0.04	0.965133
24	21.68	0.000014
34	0.13	0.878474
123	4.83	0.001664
124	1.47	0.236539
134	7.11	0.000070
234	0.91	0.407654
1234	0.98	0.422915

Table 14. Summary of all effects on the final germination percentage. The comparison was performed among
SPECIES: O.*biennis, O.rubricaulis and O.ammophila*
FLUCTUATIONS: experiment with stable temperature and experiment in which temperature fluctuated
LIGHT: full light, half light (achieved by greater distance from the light source) and dark
FERT: unfertilised and fertilised treatments.

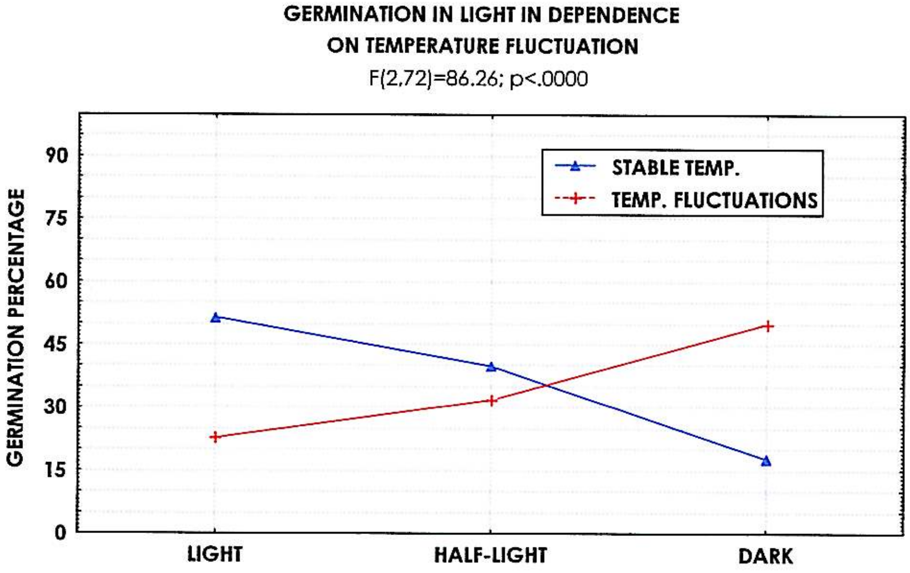
The final germination percentages differed among species. The presence of temperature fluctuations changes the response to other environmental factors (Fig. 11 and 12).

The germination of the there studied species depends on different factors.

PERCENTAGE, Summary of all Effects **1**-FLUCTUATIONS, **2**-LIGHT, **3**-FERTILISER	***O. biennis***		***O. rubricaulis***		***O. ammophila***	
	F	**p**	**F**	**p**	**F**	**p**
1	1.03	0.319446	0.76	0.390983	1.14	0.295485
2	3.22	0.057625	1.41	0.262621	6.16	0.006912
3	0.61	0.441826	0.26	0.617625	0.43	0.516704
12	46.38	p->0	23.06	0.000003	23.16	0.000002
13	14.09	0.000979	3.22	0.085550	5.69	0.025283
23	6.00	0.007699	7.59	0.002795	1.19	0.321685
123	0.69	0.509968	2.33	0.119140	0.15	0.858132

Table 15. The effects of used factors in separate species.

Figure 11 shows the change in response to the light treatments in the presence/absence of fluctuating temperatures.



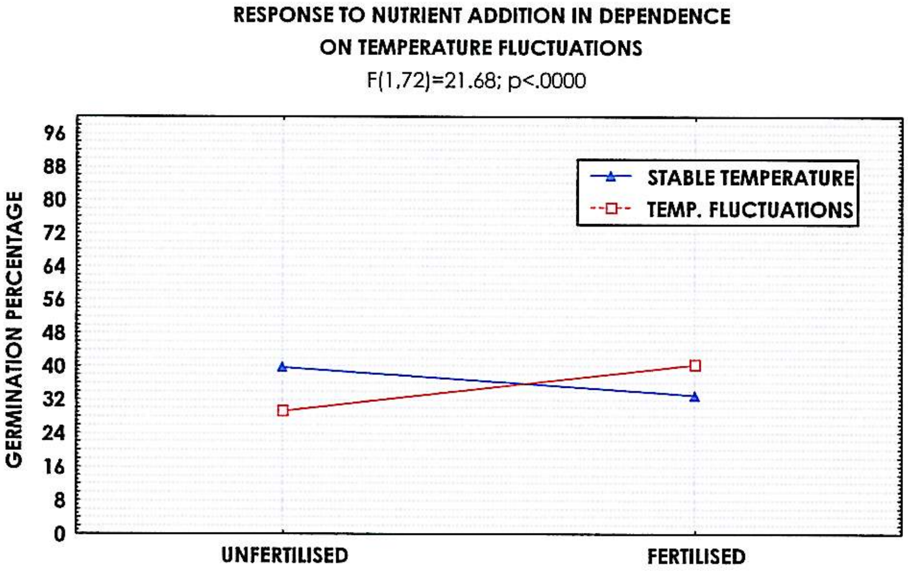


Figure 12 shows the response to nutrient addition in the presence/absence of fluctuating temperatures.

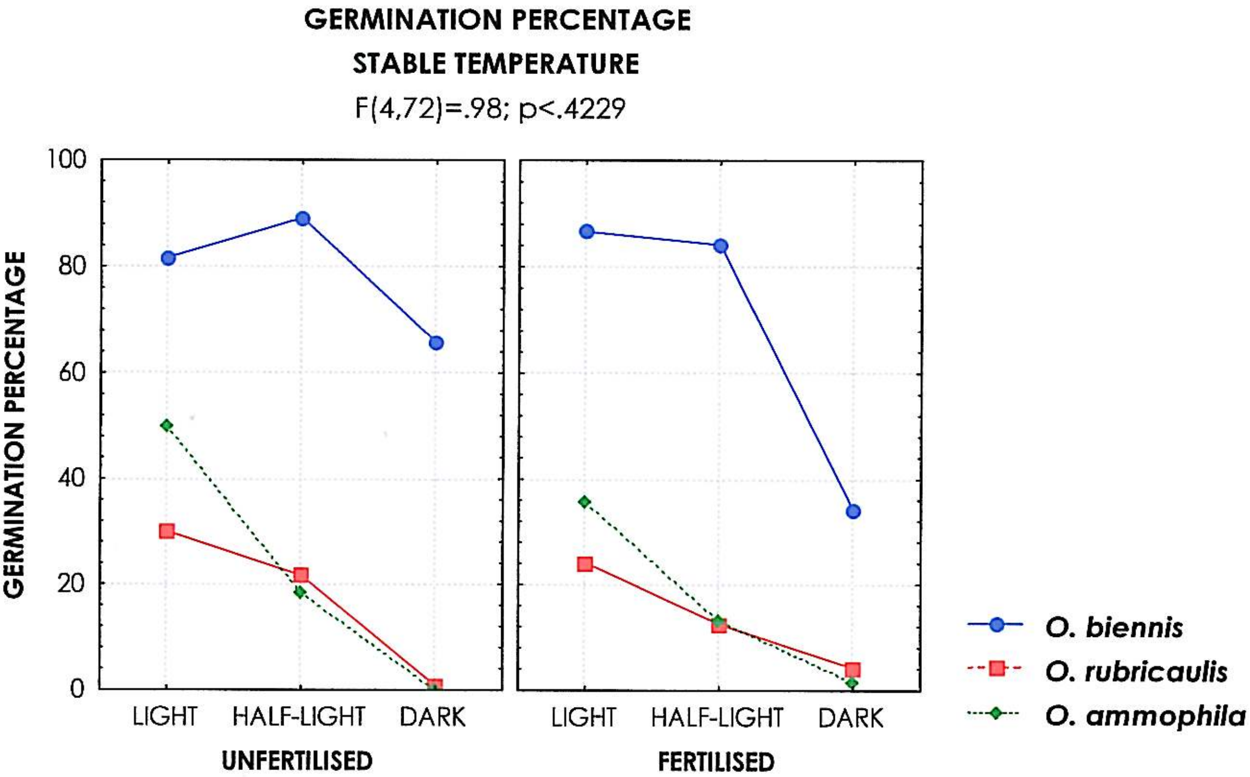


Figure 13 shows the final germination percentage achieved by diferent species in the separate light/nutrients treatments in stable temperature.

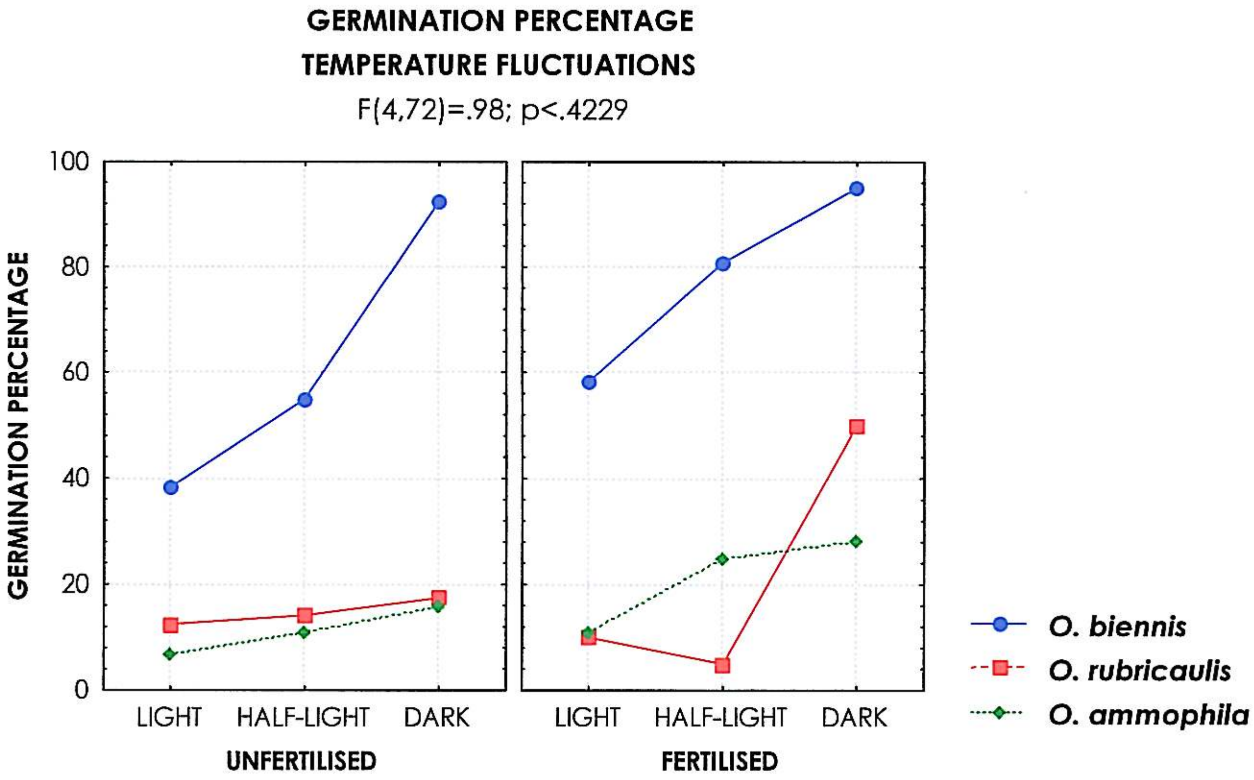


Figure 14 brings the achieved final germination percentages achieved by the studied species in separate light/nutrients treatments, when the temperatures fluctuate.

SPECIES	LIGHT	FERTILISER	FLUCTUATIONS	STABLE TEMPERATURE	FERT EFFECT		FLUCTUATION EFFECT
					∼∼∼	——	
O. biennis	light	no	38,3 ± 21,28	81,7 ± 7,64	2,05	0,77	11,04*
		yes	58,3 ± 11,55	86,7 ± 6,29			13,93*
	half light	no	55 ± 10,9	89,2 ± 1,44	16,56*	1,38	28,98**
		yes	80,8 ± 1,44	84,2 ± 7,22			0,62
	darkness	no	92,5 ± 5	65,8 ± 11,22	0,38	6,62	14,03*
		yes	95 ± 5	34,2 ± 18,09			31,53**
O. rubricaulis	light	no	12,5 ± 5	30 ± 5	0,6	0,67	18,38*
		yes	10 ± 2,5	24,2 ± 11,27			4,52
	half light	no	14,2 ± 10,41	21,7 ± 16,07	1,89	0,83	0,46
		yes	5 ± 5	12,5 ± 6,61			2.45
	darkness	no	17,5 ± 2,5	0,8 ± 1,44	10,14*	0,61	100***
		yes	50 ± 17,5	4,2 ± 7,22			17,59*
O. ammophila	light	no	6,7 ± 5,2	50 ± 17,32	1,25	1,65	17,22*
		yes	10,8 ± 3,82	35,8 ± 8,04			23,68**
	half light	no	10,8 ± 5,77	18,3 ± 5,77	0,89	0,68	2,53
		yes	25 ± 25,37	13,3 ± 8,78			0,57
	darkness	no	15,8 ± 1,44	0	16,07*	1	361***
		yes	28,3 ± 5,2	1,7 ± 2,89			60,24**

Table 16 summarize the effect of temperature fluctuations on each combination of species×light×nutrients. It also brings the fertilization effect on each combination of fluct×species×light.

Summary:

In all three species germination was influenced by presence/absence of temperature fluctuations. When light was available, the species germinated better in the absence of temperature fluctuations. However, in the dark, they germinated better in presence of temperature fluctuations.

The germination was not profoundly affected by the nutrient addition, it caused an increase in germination number in *O. biennis* in the half light, and in *O.rubricaulis* and *O. ammophila* in the dark.

Table 16 shows the effect of light and the differences among species in separate treatments.

FLUCTUATIONS			**LIGHT (L1)**		**HALF-LIGHT (L2)**		**DARK (D)**	
		LIGHT \ SPECIES	**5,07**		**$20,9^{**}$**		**$517, 75^{***}$**	
UNFERTILISED	***O. biennis* (B)**	$11,63^{**}$	38,3 ± 21,28 D		55 ± 10,9 D	RA	92,5 ± 5 L1 L2	RA
	***O. rubricaulis* (R)**	0,42	12,5 ± 5		14,2 ± 10,41	B	17,5 ± 2,5	B
	***O. ammophila* (A)**	3,03	6,7 ± 5,2		10,8 ± 5,77	B	15,8 ± 1,44	B
		LIGHT \ SPECIES	**$44,69^{***}$**		**$20,72^{**}$**		**$29,05^{***}$**	
FERTILISED	***O. biennis* (B)**	$19,18^{**}$	58,3 ± 11,55 L2 D	RA	80,8 ± 1,44 L1	RA	95 ± 5 L1	RA
	***O. rubricaulis* (R)**	$16,22^{**}$	10 ± 2,5 D	B	5 ± 5 D	B	50 ± 17,5 L1 L2	B
	***O. ammophila* (A)**	1,13	10,8 ± 3,82	B	25 ± 25,37	B	28,3 ± 5,2	B
STABLE TEMPERATURE			**LIGHT (L1)**		**HALF-LIGHT (L2)**		**DARK (D)**	
		LIGHT \ SPECIES	**$15,93^{**}$**		**48.94^{***}**		**$99,4^{***}$**	
UNFERTILISED	***O. biennis* (B)**	$6,81^{*}$	81,7 ± 7,64	RA	89,2 ± 1,44 D	RA	65,8 ± 11,22 L2	RA
	***O. rubricaulis* (R)**	$7,12^{*}$	30 ± 5 D	B	21,7 ± 16,07	B	0,8 ± 1,44 L1	B
	***O. ammophila* (A)**	$17,28^{**}$	50 ± 17,32 L2 D	B	18,3 ± 5,77 L1	B	0 L1	B
		LIGHT \ SPECIES	**$42,98^{***}$**		**$88,08^{***}$**		**$7,6^{*}$**	
FERTILISED	***O. biennis* (B)**	$18,85^{**}$	86,7 ± 6,29 D	RA	84,2 ± 7,22 D	RA	34,2 ± 18,09 L1 L2	RA
	***O. rubricaulis* (R)**	4,07	24,2 ± 11,27	B	12,5 ± 6,61	B	4,2 ± 7,22	B
	***O. ammophila* (A)**	$18,1^{**}$	35,8 ± 8,04 L2 D	B	13,3 ± 8,78 L1	B	1,7 ± 2,89 L1	B

Table 16 reveals the differences among the three studied species in each experimental environment and the effect of light.

123 = F- value for light effect in the particular species×fluctuation×nutrient situation,

L1 l2 D = significantly differing light treatments in the particular species×fluctuation×nutrient situation

123 = F- value for species effect in the particular fluctuation× nutrient ×light situation.

B R A = significantly differing species in that particular fluctuation× nutrient ×light situation

Summary:

O. biennis shows greatest sensitivity to light signal. It responds to light changes in presence and in absence of temperature fluctuations.

O. biennis achieved superior final germination percentages in the great majority of used treatments.

O. ammophila and *O. rubricaulis* germinate poorly when temperature fluctuates and nutrient status is low. When the nutrient status is higher, *O.rubricaulis* reaches its maximum germination percentage in this test and is significantly stimulated by dark.

In constant temperature *O. ammophila* shows greater reactivity to light. In the light treatments irrespectively of their nutrient status, it achieves the maximum germination percentage in this test.

3.2.2 Seedling traits

SEEDLING TRAITS, Summary of all effects								
1-SPECIES, 2-FLUCTUAT, 3-LIGHT, 4-FERTILIS								
	root lenght		**shoot lenght**		**cotyledons**		**dry weight**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**
1	50.33	p->0	83.08	p->0	75.88	p->0	13.91	0.000002
2	0.27	0.604567	30.15	p->0	41.76	p->0	9.20	0.002705
3	75.29	p->0	243.39	p->0	6.59	0.010560	64.58	p->0
4	18.39	0.000021	5.64	0.017852	18.60	0.000020	1.70	0.193347
12	7.03	0.000955	9.28	0.000106	0.52	0.596022	13.65	0.000003
13	3.27	0.011337	50.46	p->0	0.69	0.499619	2.75	0.029198
23	10.66	0.000028	26.13	p->0	0.06	0.812098	8.60	0.000252
14	1.04	0.355082	0.39	0.680458	5.28	0.005418	5.64	0.004060
24	5.10	0.024271	6.05	0.014153	0.03	0.854677	1.11	0.292449
34	0.48	0.622023	4.41	0.012543	2.16	0.142088	2.36	0.096612
123	0.31	0.874213	8.44	0.000001	0.26	0.774964	10.99	p->0
124	6.18	0.002190	1.93	0.145610	2.20	0.112452	0.79	0.453918
134	1.38	0.240270	0.44	0.782109	1.55	0.212668	0.62	0.650940
234	0.24	0.783985	4.29	0.014058	2.18	0.140163	1.18	0.308975
1234	1.54	0.188203	2.72	0.028894	1.82	0.163998	1.87	0.117243

Table 17 brings the F-values and the achieved levels of probability from ANOVA analysis with the following factors (levels):
SPECIES (*O. biennis, O.rubricaulis, O.ammophila*)
FLUCTUAT= fluctuations (constant temperature, temperature fluctuations)
LIGHT (light, half-light, darkness)
FERTILIS= fertiliser (unfertilised treatments, fertilised treatments)

Following tables show the effects of fertilization, temperature fluctuations and light on the studied characters and the differences in achieved characters among species in different treatments. The detailed description of the tables is bellow the Tab 21. Units of individual characters: root lenght [cm], shoot lenght [cm] and cotyledon span [cm]; dry biomass weight [g].

Table 18.

ROOT LENGTH	LIGHT	FERTILISER	FLUCTUATIONS	STABLE TEMPERATURE	FERTILISER EFFECT		FLUCTUATION EFFECT
					~~	——	
O. biennis	light	no	1.32 ± 0,4	2.22 ± 0,58	30,71***	1,86	43,86***
		yes	2.33 ± 0,83	2.41 ± 0,6			0,27
	half light	no	1.25 ± 0,41	2.± 0,51	8,52**	3,14	21,4***
		yes	1.75 ± 0,41	2.25 ± 0,68			5,39*
	darkness	no	0.62 ± 0,19	0.77 ± 0,36	54,32***	8,42**	4,17*
		yes	1.12 ± 0,35	0.55 ± 0,19			61,38***
O. rubricaulis	light	no	1.26 ± 0,6	1.09 ± 0,51	2,33	5,99*	0,54
		yes	0.88 ± 0,36	1.49 ± 0,58			7,68*
	half light	no	0.74 ± 0,32	0.87 ± 0,33		2,53	1,32
		yes		1.14 ± 0,67			
	darkness	no	0.40 ± 0,17		23.09***		
		yes	0.75 ± 0,3				
O. ammophila	light	no		1.8 ± 0,73		0,71	
		yes	2.46 ± 1,35	1.66 ± 0,65			5,81*
	half light	no	0.79 ± 0,54	1.57 ± 0,77	5,14*	1,21	7,05*
		yes	1.59 ± 1,01	1.27 ± 0,5			0,96
	darkness	no	0.86 ± 0,39		4,77*		
		yes	1.2 ± 0,56				

FLUCTUATIONS			LIGHT (L1)		HALF-LIGHT (L2)		DARK (D)	
	ROOT LENGTH	SPECIES LIGHT	0,11		5,41**		15,61***	
UNFERTILISED	**O.biennis (B)**	39,46***	1.32 ± 0,4 D		1.25 ± 0,41 D	RA	0.62 ± 0,19 L1 L2	BA
	O.rubricaulis (R)	18,04***	1.26 ± 0,6 L2 D		0.74 ± 0,32 L1 D	B	0.40 ± 0,17 L1 L2	BA
	O.ammophila (A)	0,17			0.79 ± 0,54	B	0.86 ± 0,39	BR
		SPECIES LIGHT	9,78***		0,25		13,15***	
FERTILISED	**O.biennis (B)**	35,89***	2.33 ± 0,83 L2 D	R	1.75 ± 0,41 L1 D		1.12 ± 0,35 L1 L2	R
	O.rubricaulis (R)	1,1	0.88 ± 0,36	BA			0.75 ± 0,3	BA
	O.ammophila (A)	5,93**	2.46 ± 1,35 D	R	1.59 ± 1,01		1.2 ± 0,56 L1	R

STABLE TEMPERATURE			LIGHT (L1)		HALF-LIGHT (L2)		DARK (D)	
	ROOT LENGTH	SPECIES LIGHT	25,58***		31,47***			
UNFERTILISED	**O.biennis (B)**	83,64***	2.22 ± 0,58 D	RA	2.± 0,51 D	RA	0.77 ± 0,36 L1 L2	
	O.rubricaulis (R)	2,97	1.09 ± 0,51	BA	0.87 ± 0,33	BA		
	O.ammophila (A)	0,8	1.8 ± 0,73	BR	1.57 ± 0,77	BR		
		SPECIES LIGHT	19,73***		20,61***			
FERTILISED	**O.biennis (B)**	107,02***	2.41 ± 0,6 D	RA	2.25 ± 0,68 D	RA	0.55 ± 0,19 L1 L2	
	O.rubricaulis (R)	2,51	1.49 ± 0,58	B	1.14 ± 0,67	B		
	O.ammophila (A)	3,26	1.66 ± 0,65	B	1.27 ± 0,5	B		

Table 19.

SHOOT LENGTH	LIGHT	FERTILISER	FLUCTUATIONS	STABLE TEMPERATURE	FERTILISER EFFECT		FLUCTUATION EFFECT
					~~	——	
O. biennis	light	no	0.27 ± 0,37	0.2 ± 0,04	1,03	$15,17^{***}$	1,39
		yes	0.21 ± 0,03	0.24 ± 0,05			$8,25^{**}$
	half light	no	0.19 ± 0,02	0.22 ± 0,03	0,08	3,52	$6,21^{*}$
		yes	0.2 ± 0,04	0.23 ± 0,05			$5,85^{*}$
	darkness	no	1.88 ± 0,34	1.81 ± 0,32	$4,64^{*}$	1,63	0,74
		yes	2.06 ± 0,35	1.95 ± 0,5			1,03
O. rubricaulis	light	no	0.12 ± 0,04	0.15 ± 0,04	0,29	0,65	2,04
		yes	0.11 ± 0,02	0.14 ± 0,04			2,26
	half light	no	0.11 ± 0,03	0.16 ± 0,05		2,99	$13,12^{***}$
		yes		0.13 ± 0,07			
	darkness	no	1.11 ± 0,55		0,24		
		yes	1.18 ± 0,58				
O. ammophila	light	no		0.19 ± 0,05		0,17	
		yes	0.24 ± 0,09	0.19 ± 0,06			$4,79^{*}$
	half light	no	0.19 ± 0,03	0.26 ± 0,06	0,16	0,39	$15,12^{***}$
		yes	0.19 ± 0,05	0.25 ± 0,08			4,26
	darkness	no	1.57 ± 0,67		1,6		
		yes	1.31 ± 0,63				

			LIGHT (L1)		HALF-LIGHT (L2)		DARK (D)	
FLUCTUATIONS	***SHOOT LENGTH***	SPECIES LIGHT	**1,08**		**$34,02^{***}$**		**$14,82^{***}$**	
UNFERTILISED	***O.biennis* (B)**	$229,95^{***}$	0.27 ± 0,37	D	0.19 ± 0,02 **R**	D	1.88 ± 0,34 **R**	L1,L2
	***O.rubricaulis* (R)**	$32,77^{***}$	0.12 ± 0,04	D	0.11 ± 0,03 **BA**	D	1.11 ± 0,55 **BA**	L1,L2
	***O.ammophila* (A)**	$42,27^{***}$			0.19 ± 0,03 **R**	D	1.57 ± 0,67 **R**	L2
		SPECIES LIGHT	**$21,5^{***}$**		**0,03**		**$30,16^{***}$**	
FERTILISED	***O.biennis* (B)**	$690,26^{***}$	0.21 ± 0,03 **R**	D	0.2 ± 0,04	D	2.06 ± 0,35	L1,L2
	***O.rubricaulis* (R)**	$26,46^{***}$	0.11 ± 0,02 **BA**	D			1.18 ± 0,58	L1
	***O.ammophila* (A)**	$31,5^{***}$	0.24 ± 0,09 **R**	D	0.19 ± 0,05	D	1.31 ± 0,63	L1,L2

			LIGHT (L1)		HALF-LIGHT (L2)		DARK (D)	
STABLE TEMPERATURE	***SHOOT LENGTH***	SPECIES LIGHT	**$11,2^{***}$**		**$23,46^{***}$**			
UNFERTILISED	***O.biennis* (B)**	$841,52^{***}$	0.2 ± 0,04 **R**	D	0.22 ± 0,03 **RA**	D	1.81 ± 0,32	L1,L2
	***O.rubricaulis* (R)**	1,48	0.15 ± 0,04 **BA**		0.16 ± 0,05 **BA**			
	***O.ammophila* (A)**	$15,09^{***}$	0.19 ± 0,05 **R**	L2	0.26 ± 0,06 **BR**	L1		
		SPECIES LIGHT	$24,08^{***}$		$18,73^{***}$			
FERTILISED	***O.biennis* (B)**	$458,7^{***}$	0.24 ± 0,05 **RA**	D	0.23 ± 0,05 **R**	D	1.95 ± 0,5	L1,L2
	***O.rubricaulis* (R)**	0,15	0.14 ± 0,04 **BA**		0.13 ± 0,07 **BA**			
	***O.ammophila* (A)**	$7,06^{*}$	0.19 ± 0,06 **BR**	L2	0.25 ± 0,08 **R**	L1		

Table 20.

COTYLEDONS	LIGHT	FERTILISER	FLUCTUATIONS	STABLE TEMPERATURE	FERTILISER EFFECT		FLUCTUATION EFFECT
					~~	—	
O. biennis	light	no	0.55 ± 0,11	0.69 ± 0,09	13,41***	7,05**	28,49***
		yes	0.71 ± 0,2	0.76 ± 0,15			1,44
	half light	no	0.56 ± 0,08	0.61 ± 0,1	6,66*	42,53***	3,34
		yes	0.63 ± 0,04	0.83 ± 0,17			15,03***
O. rubricaulis	light	no	0.39 ± 0,08	0.49 ± 0,12	0,2	5,26*	4,22*
		yes	0.38 ± 0,07	0.58 ± 0,14			14,16***
	half light	no	0.36 ± 0,06	0.48 ± 0,11		0,71	15,09***
		yes		0.44 ± 0,16			
O. ammophila	light	no		0.56 ± 0,13		1,76	
		yes	0.63 ± 0,24	0.62 ± 0,2			0,0088
	half light	no	0.41 ± 0,12	0.52 ± 0,13	0,75	0,5	4,15
		yes	0.48 ± 0,24	0.56 ± 0,14			0,96

FLUCTUATIONS			LIGHT (L1)	HALF-LIGHT (L2)
	COTYLEDONS	**LIGHT \ SPECIES**	**12,84****	**18,59*****
UNFERTILISED	***O.biennis* (B)**	0,06	0.55 ± 0,11 **R**	0.56 ± 0,08 **RA**
	***O.rubricaulis* (R)**	1,24	0.39 ± 0,08 **B**	0.36 ± 0,06 **B**
	***O.ammophila* (A)**			0.41 ± 0,12 **B**
		LIGHT \ SPECIES	**10,01*****	**4,11**
FERTILISED	***O.biennis* (B)**	1,99	0.71 ± 0,2 **R**	0.63 ± 0,04
	***O.rubricaulis* (R)**		0.38 ± 0,07 **BA**	
	***O.ammophila* (A)**	1,84	0.63 ± 0,24 **R**	0.48 ± 0,24

STABLE TEMPERATURE			LIGHT (L1)		HALF-LIGHT (L2)	
	COTYLEDONS	**LIGHT \ SPECIES**	**24,94*****		**11,44*****	
UNFERTILISED	***O.biennis* (B)**	10,35**	0.69 ± 0,09 **RA**	l2	0.61 ± 0,1 **RA**	l1
	***O.rubricaulis* (R)**	0,06	0.49 ± 0,12 **BA**		0.48 ± 0,11 **B**	
	***O.ammophila* (A)**	1,06	0.56 ± 0,13 **BR**		0.52 ± 0,13 **B**	
		LIGHT \ SPECIES	**10,45*****		**33,7*****	
FERTILISED	***O.biennis* (B)**	3,66	0.76 ± 0,15 **RA**		0.83 ± 0,17 **RA**	
	***O.rubricaulis* (R)**	6,27*	0.58 ± 0,14 **B**	l2	0.44 ± 0,16 **B**	l1
	***O.ammophila* (A)**	0,86	0.62 ± 0,2 **B**		0.56 ± 0,14 **B**	

Table 21.

DRY WEIGHT	LIGHT	FERTILISER	FLUCTUATIONS	STABLE TEMPERATURE	FERTILISER EFFECT		FLUCTUATION EFFECT
					~~	——	
O. biennis	light	no	0.000524 ± 0,00011	0.000508 ± 0,000059	0,85	1,73	0,22
		yes	0.000580 ± 0,000145	0.000532 ± 0,000053			1,7
	half light	no	0.000497± 0,00011	0.000407 ± 0,000088	0,07	15,2***	3,82
		yes	0.000509 ± 0,000058	0.000495 ± 0,000046			0,43
	darkness	no	0.000202 ± 0,000017	0.000322 ± 0,00003	3,29	1,94	191,17***
		yes	0.000215 ± 0,000024	0.000300 ± 0,000044			38,32***
O. rubricaulis	light	no	0.000425 ± 0,000049	0.000419 ± 0,000098	2,43	0,08	0,01
		yes	0.000271 ± 0,000128	0.000435 ± 0,000114			3,59
	half light	no	0.000275 ± 0,000051	0.000422 ± 0,000044		0,88	22,07**
		yes		0.000456 ± 0,000059			
	darkness	no	0.000303 ± 0,000042		2,01		
		yes	0.000340 ± 0,000043				
O. ammophila	light	no		0.000492 ± 0,000072		2,21	
		yes	0.000808 ± 0,000395	0.00058 ± 0,000161			2,18
	half light	no	0.000441 ± 0,000071	0.000416 ± 0,000054	0,25	2,1	0,27
		yes	0.000609 ± 0,000445	0.000506 ± 0,00014			0,2
	darkness	no	0.000433 ± 0,000005		0,69		
		yes	0.00045 ± 0,000067				

FLUCTUATIONS	DRY WEIGHT	SPECIES LIGHT	LIGHT (L1)	HALF-LIGHT (L2)	DARK (D)
			1,39	**7,39***	**154,66***
UNFERTILISED	**O.biennis (B)**	65,28***	0.000524 ± 0,00011 D	0.000497± 0,00011 R D	0.000202 ± 0,000017 RA L1 L2
	O.rubricaulis (R)	7,13*	0.000425 ± 0,000049 L2 D	0.000275 ± 0,000051 B L1	0.000303 ± 0,000042 BA L1
	O.ammophila (A)	0,66		0.000441 ± 0,000071	0.000433 ± 0,000005 BR
		SPECIES LIGHT	5,01*	0,36	80,59***
FERTILISED	**O.biennis (B)**	63,78***	0.000580 ± 0,000145 D	0.000509 ± 0,000058 D	0.000215 ± 0,000024 RA L1 L2
	O.rubricaulis (R)	2,23	0.000271 ± 0,000128 A		0.000340 ± 0,000043 BA
	O.ammophila (A)	1,58	0.000808 ± 0,000395 R	0.000609 ± 0,000445	0.00045 ± 0,000067 BR

STABLE TEMPERATURE	DRY WEIGHT	SPECIES LIGHT	LIGHT (L1)	HALF-LIGHT (L2)	DARK (D)
			4,05*	**0,1**	
UNFERTILISED	**O.biennis (B)**	31,36***	0.000508 ± 0,000059 R L2 D	0.000407 ± 0,000088 L1 D	0.000322 ± 0,00003 L1 L2
	O.rubricaulis (R)	0,01	0.000419 ± 0,000098 B	0.000422 ± 0,000044	
	O.ammophila (A)	4,82*	0.000492 ± 0,000072 L2	0.000416 ± 0,000054 L1	
		SPECIES LIGHT	**3,31**	**0,52**	
FERTILISED	**O.biennis (B)**	65,88***	0.000532 ± 0,000053 D	0.000495 ± 0,000046 D	0.000300 ± 0,000044 L1 L2
	O.rubricaulis (R)	0,08	0.000435 ± 0,000114	0.000456 ± 0,000059	
	O.ammophila (A)	0,62	0.00058 ± 0,000161	0.000506 ± 0,00014	

Tables 18, 19, 20 and 21 bring trait values ± SD achieved by the studied species in separate treatments.
The first concentrates on the FERTILIZATION and FLUCTUATION effect the second on the SPECIES and LIGHT effect.
The values with stars are F-values from the one-way ANOVA tests with the FERTILIZATION, FLUCTUATION, SPECIES and LIGHT respectively as the main factors in the concrete combination of other factors. The stars indicate the achieved probability value (* p<0,05, **p<0,01, *** p<0,001)
L1,L2,D= light treatments in which the character achieved significantly different value
B,R,A= species that developed significantly different trait value.

3.3 Performance of hybridogenous *O. fallax* and its parental species (*O. biennis* and *O. erythrosepala*)

3.3.1 Germination

PERCENTAGE, Summary of all Effects		
1-SPECIES, 2-LIGHT, 3-FERTILISER		
	F	**p**
1	166.28418	p->0
2	167.827332	p->0
3	5.19424438	0.031847
12	143.23381	p->0
13	1.98920858	0.158729
23	46.7482033	p->0
123	27.6151085	0.000001

Table 22. Summary of effects the used factors had on the final germination percentage.
Species effect: differences among *O.biennis, O.erythrosepala, O.fallax*
Light effect: differences between light and dark treatment
Fertiliser effect: differences between unfertilised and fertilised treatment

Final germination percentage was different in the studied species. *O.erythrosepala* did not germinate at all (except three individuals), *O. biennis* germinated the best and *O. fallax* less (Tukey, p=0.000173), it achieved about 70% of the germination power of *O. biennis*.

In the dark the germination power generally significantly diminished (Tukey, p=0.000152). The species differed in their response to light treatments. While *O. biennis* nearly ceased to germinate in the dark and reached nearly 100% in the light, *O. fallax* germinated similarly in both light treatments.

With the adition of nutrients germination power slightly rised (Tukey, p=0.031973), but further analysis has shown, but its impact depended on light.

Due to virtually no individuals of *O. erythrosepala*, this species has been omitted from the following analysis.

Table 23 shows germination differences of the remaining species- *O. biennis* and *O. fallax*.

	O. biennis	***O. fallax***	**p**
light	92,5 ± 2,5	53,3 ± 6,29	0,000763
light, fertilised	95,5 ± 6,61	21,7 ± 20,21	0,000174
dark	0,83 ± 1,44	10 ± 2,5	0,86985
dark, fertilised	15,8 ± 3,82	58,3 ± 6,29	0,000403

Table 23. Achieved final germination percentages of studied species in separate treatments. P-value from the Tukey test is given.

O. biennis germinates better than *O. fallax* in the light, independently of the nutrient status. In the dark situation is different. When the nutrient status is low, both species germinate minimally. When the nutrients are optimal *O. fallax* responses by greater increase of the germination power and achieves better results than *O.biennis*.

species	fertiliser	light	dark	p(L)
O. biennis	**no**	92,5 ± 2,5	0,83 ± 1,44	**0,000174**
	yes	95,5 ± 6,61	15,8 ± 3,82	**0,000174**
	p(F)	**0,999938**	**0,402984**	
O. fallax	**no**	53,3 ± 6,29	10 ± 2,5	**0,000356**
	yes	21,7 ± 20,21	58,3 ± 6,29	**0,001384**
	p(F)	**0,005302**	**0,000213**	

Table 24. Final germination percentages and their differences revealed by Tukey tests.
p(F)= the probability that the fertiliser does not have effect in the concrete species-light combination.
p(L)= probability that light climate does not affect the germination in the concrete species-fertiliser combination.

In *O. biennis* the nutrient addition caused unsignificant increase in the germinated seeds in both light treatments. In *O.fallax* the nutrient addition significantly increased the germination number in the dark, but decreased the germination number in the light.

The course of germination

NUMBER OF GERMINATED SEEDS		
Summary of all Effects		
1-SPECIES, 2-LIGHT, 3-FERTILISER, 4-TIME		
	F	**p**
1	74.67	p->0
2	62.70	p->0
3	5.63	0.025969
4	165.83	p->0
12	78.19	p->0
13	1.52	0.239055
23	10.45	0.003546
14	41.89	p->0
24	43.41	p->0
34	0.86	0.511245
123	8.40	0.001713
124	27.35	p->0
134	0.37	0.955916
234	19.03	p->0
1234	8.65	p->0

Table 25. Summary of all effects from the ANOVA with repeated measurements. The character tested was the number of germinated seeds, which were counted every second day.

The course of germination differed between *O. fallax* and *O. biennis*, between light and dark and also between the fertilised and unfertilised treatments.

Species differed in the adjustment of the course of germination to the light conditions.

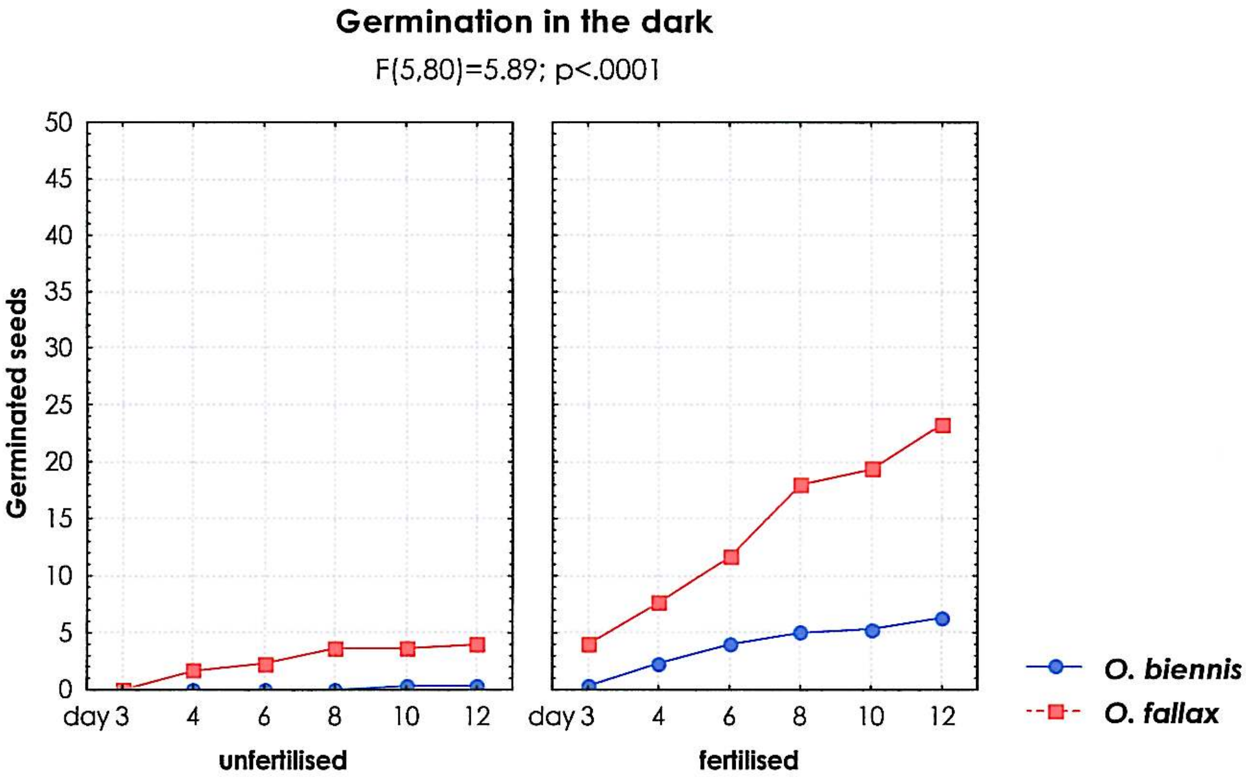


Figure 15 show the course of germination in the dark.

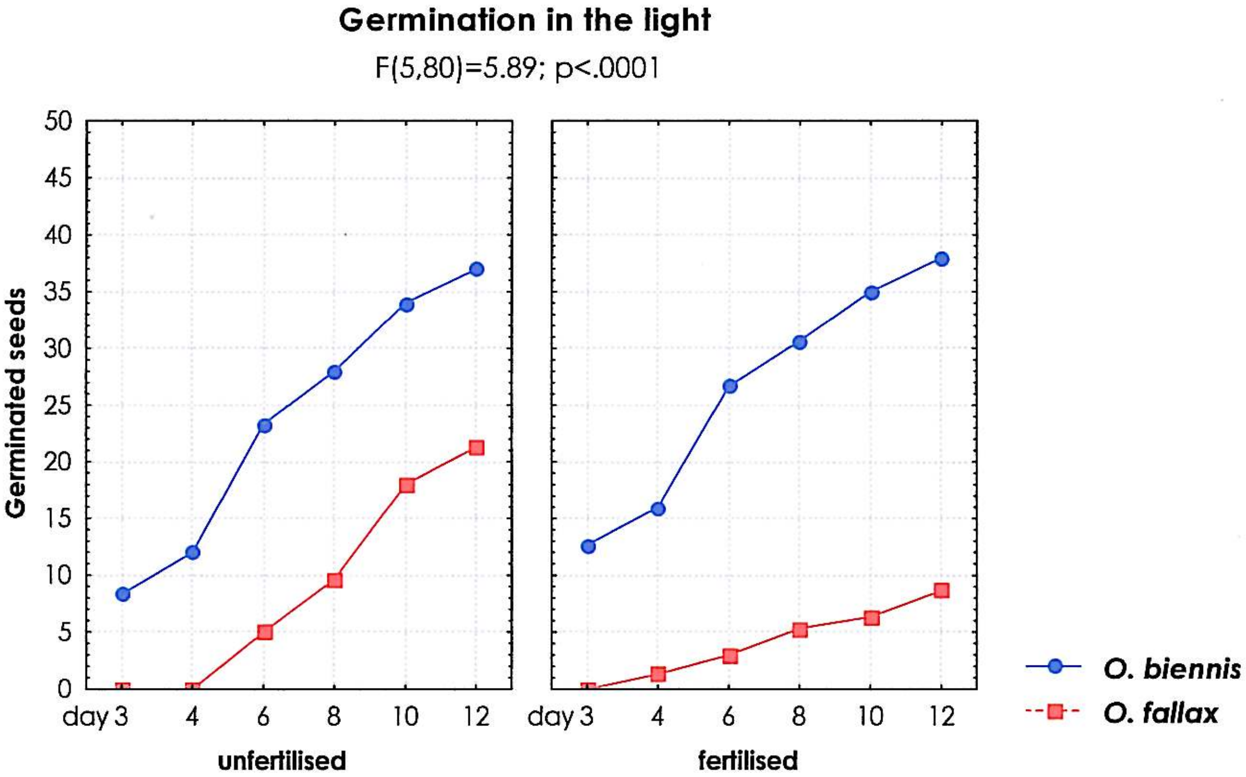


Figure 16 shows the course of germination in the light.

3.3.2 Seedling traits

1-SPECIES, 2-LIGHT, 3-FERTILISER				
	F-value			
	root	**shoot**	**cotyledons**	**dry weight**
1 *O. biennis* × *O.fallax*	0.01	59.68***	0.15	5.83*
2 light×dark	17.17***	160.43***		48.34***
3 fertiliser yes×no	0.85	8.14**	25.77***	12.91***
12	10.77**	64.32***		14.53***
13	0.08	26.13***	2.73	4.17*
23	12.18***	3.94***		5.8 *
123	0.76	25.83***		22.87***

Table 26. F-values from the ANOVA analysis for all measured morphometric characteristics and dry biomass weight.
The analysis of cotyledons span was performed only between the light treatments, where the leaves were already out of the seed coat.

Species differed in shoot lenght and dry biomass weight. The light had effect on all measured characteristics.

ROOT	**fertiliser**	**light**	**dark**	**light effect**
O. biennis	**no**	2,89 ± 1,07		
	yes	1,66 ± 0,99	0,91 ± 0,45	9,32**
	fert.effect	25,07***		
O. fallax	**no**	1,96 ± 0,81	1,11 ± 0,64	9,84**
	yes	1,02 ± 0,88	1,46 ± 0,3	360,43***
	fert.effect	13,26***	4,22*	

Table 27. Root lenghts ± SD in various treatments.
Numbers bellow the term **light effect** and next to the term **fert.effect** (fertilization effect) bring the F-values from the one-way ANOVA analysis in the particular combination of other factors.

In the light both species reacted to the addition of nutrients by shortening of the root. In the dark *O. fallax* when fertilised developed significantly longer root. The light affected the root lenght in both species in both nutrient treatments (Tab 27).

ROOT LENGTH	***O. biennis***	***O. fallax***	**species effect**
light	2,89 ± 1,07	1,96 ± 0,81	15,29***
light, fertilised	1,66 ± 0,99	1,02 ± 0,88	4,89*
dark, fertilised	0,91 ± 0,45	1,46 ± 0,3	19,83***

Table 28. Root lenghts achieved by the two species in the separate treatments.
Numbers bellow the term: species effect bring the F-value from the ANOVA analysis of the specific effects.

Species differed from each other in the root lenght in all treatments (Tab 28).

SHOOT	**fertiliser**	**light**	**dark**	**light effect**
O. biennis	**no**	0,23 ± 0,09		
	yes	0,32 ± 0,09	1,43 ± 0,67	98,63***
	fert.effect	16,49***		
O. fallax	**no**	0,2 ± 0,03	2,46 ± 0,68	360,43***
	yes	0,28 ± 0,08	1,96 ± 0,48	189,84***
	fert.effect	23,96***	5,97*	

Table 29. Shoot lenghts ± SD in various treatments.
Numbers bellow the term light effect and next to the term fert.effect(fertilization effect) bring the F.values from the one-way ANOVA analysis with light or fertilization as the main effect in the particular combination of other factors.

In the light both species reacted on the addition of nutrients by the increase of the shoot lenght. In the dark *O. fallax* reacted on the nutrient enhancement by shortening of the shoot. In all fertilization treatments the dark cultivation caused the prolonging of the shoot (Tab 29). Differences between species brings Table 30.

SHOOT LENGTH	***O. biennis***	***O. fallax***	**species effect**
light	0,23 ±0,09	0,2 ± 0,03	2,23
light, fertilised	0,32 ± 0,09	0,28 ± 0,08	1,44
dark, fertilised	1,43 ± 0,67	1,96 ± 0,48	7,96**

Table 30. Shoot lenghts achieved by the two species in the separate treatments. Numbers bellow the term: species effect bring the F-value from one-way ANOVA with the main effect **species.**

COTYLEDONS	**unfertilised**	**fertilised**	**fert.effect**
O. biennis	0,62 ± 0,14	0,76 ± 0,27	7,03**
O. fallax	0,54 ± 0,1	0,81 ± 0,29	22,06***
species effect	7,35**	0,37	

Table 31. Cotyledons span ± SD in the light treatments with different nutrient status. The numbers bellow the fert.effect (fertilization effect) and beside the term:species effect are F-values from one-way ANOVA analysis with species or fertilization as the main effect in the particular combination of other factors.

Both species cotyledons enlarge in nutrient rich treatments (Tab 31).

DRY WEIGHT	**fertiliser**	**light**	**dark**	**light effect**
O. biennis	**no**	0,001075 ± 0,0001904		
	yes	0,0009804 ± 0,0002356	0,000767 ± 0,000209	5,89*
	fert.effect	12,1**		
O. fallax	**no**	0,0008259 ± 0,0001089	0,00078 ± 0,000103	0,58
	yes	0,0010607 ± 0,0001424	0,0007308 ± 0,000933	37,63***
	fert.effect	0,8	19,33***	

Table 32. Dry biomass weight ± SD in different treatments. The numbers bellow the term: light effect and beside the term: fert. effect (fertilization effect) are F-values from the one-way ANOVA analysis with light or fertilization as the main effect in the particular combination of other factors. The stars indicate the achieved probability level. The data in tables are real dry biomass weights, but the F-values were obtained by analysis of log-transformed data.

O. biennis reacted on the nutrient addition by smaller accumulation of the biomass. *O. fallax* responded to nutrient enhancement differently in the light, where the biomass grew and in the dark, where the biomass diminished. In both species the dry biomass weight in the dark diminished when the treatment was fertilised (Tab 32).

DRY WEIGHT	***O. biennis***	***O. fallax***	**species effect**
light	0,001075 ± 0,0001904	0,0008259 ± 0,0001089	23,57***
light, fertilised	0,0009804 ± 0,0002356	0,0010607 ± 0,0001424	0,74
dark, fertilised	0,000767 ± 0,000209	0,0007308 ± 0,000933	0,28

Table 33. Differences between species in the dry biomass weight in separate treatments. Bellow the term: species effect the F-values are presented from the one-way ANOVA with the species as main effect in the particular combination on other factors.

Species seedlings dry biomass weight did not differ in the fertilised treatments, but in the unfertilised light treatment *O. biennis* developed significantly more biomass (Tab 33).

3.4 Test on allelopathic potential of *O. biennis* and *V. thapsus*.

3.4.1 Germination

PERCENTAGE, Summary of all Effects **1-SPECIES, 2-LIGHT, 3-PRESENCE**		
	F	**p**
1	9.52	0.00710
2	355.46	0.00000
3	8.35	0.01068
12	10.13	0.00578
13	12.78	0.00253
23	10.13	0.00578
123	7.25	0.01599

Table 34. Summary of effects on final germination percentage. Factors tested: species (*O.biennis*, *V.thapsus*), light (light, dark cultivation), presence (watering by water or by the leachate from germinating seeds of the other tested species).

V. thapsus differed from *O. biennis* in final percentage of germinated seeds. *V. thapsus* achieved greater (Tukey, p=0.000009) numbers, with a general mean final germination percentage of 61% to 48% of *O.biennis*. Used light climates and allelopathic climates had effect on germination of the studied species. The response on light is affected by the presence of other species and the response on the complex signal presence-of-other-species-germinationg-seeds and light affects both species differently.

Table 35.

				light effect		allelopathy effect
		water	**leachate**	**water**	**leachate**	
O. biennis	**light**	92,5 ± 2,5	97,5 ± 2,5	3025***	3306,25***	6
	dark	0,8 ± 1,44	1,7 ± 1,44			0,5
V.thapsus	**light**	95,8 ± 3,82	93,3 ± 7,64	6,58	417,24***	0,26
	dark	54,2 ± 27,88	1,7 ± 1,44			10,61*

Figure 17.

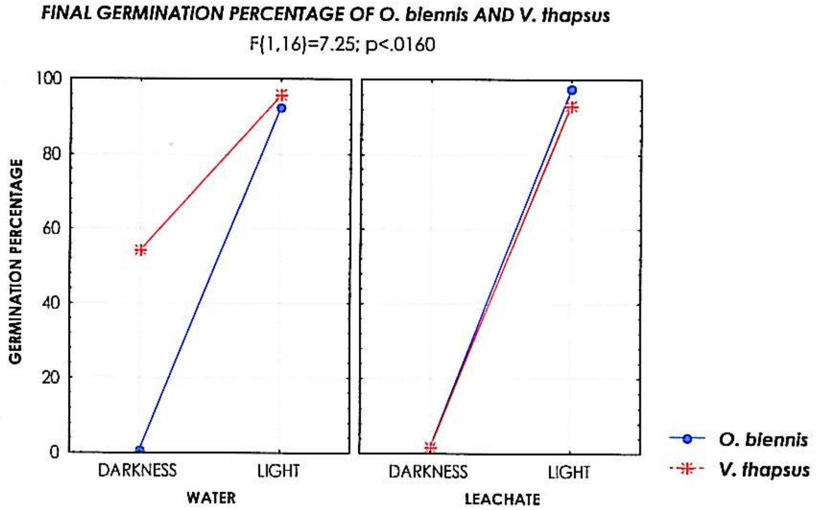


Table 35. Final germination percentages ± SD in used treatments and the effects of the factors light and presence of the other species (leachate).
The values given bellow the terms: light effect and allelopathy effect are the F-values from one-way ANOVA with the main effect light or allelopathy in the concrete combination of other factors.

Figure 17. Final germination percentages of the studied species when they germinated alone (left) and in the leachate from the other species germinating seeds (right).

In the light species achieved similar germination percentages. *O. biennis* germinated in the dark only minimally independently on the nature of watering. *V. thapsus* achieved when watered by water, similar germination percentage both in the light and in the dark. The situation changed, when the *V.thapsus* was watered with the leachate. Particularly in dark its germination dropped significantly.

The course of germination

NUMBER OF GERMINATED SEEDS		
Summary of all Effects		
1-SPECIES, 2-LIGHT, 3-PRESENCE, 4-TIME		
	F	p
1	26.26	0.000102
2	562.23	0.000000
3	3.45	0.081559
4	124.02	0.000000
12	0.43	0.522986
13	24.73	0.000138
23	13.45	0.002082
14	4.88	0.000599
24	41.12	0.000000
34	7.38	0.000010
123	0.49	0.491974
124	11.96	0.000000
134	0.42	0.830915
234	3.90	0.003247
1234	17.10	0.000000

Table 36. Summary of all effects from ANOVA analysis with repeated measurements. To the tested effects:
SPECIES (*O. biennis* and *V.thapsus)*,
LIGHT (light and dark)
PRESENCE (water or seed-leachate)
TIME (numbers of germinated seeds counted every other day).
The F-values and achieved probability levels are presented.

If we concentrate in the table of results only on the time phenomenon, we can see, that the number of germinated seeds grows in time, the germination procedes differently in the light (the numbers are greater) and in the dark and the nature of watering also affects the course of germination. The germination numbers of *V. thapsus* are in the majority of days superior to that of *O.biennis*. However the final number of germinated seeds in the light was the same in both species.

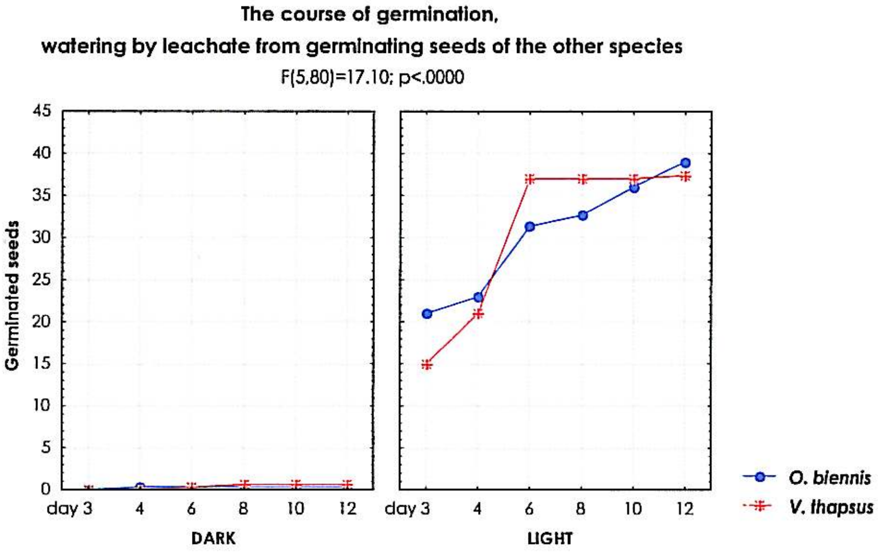


Figure 18. Temporal pattern of germination in the two light treatments when O. *biennis* was watered by the seed-leachate of *V.thapsus* and *V.thapsus* by the seed-leachate of *O. biennis*. The y-axis represents the number of germinated seeds

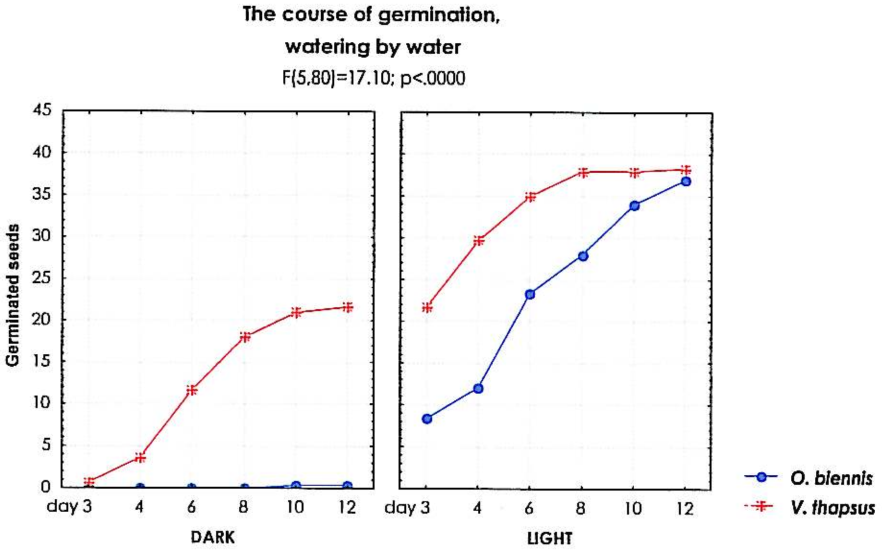


Figure 19. Temporal pattern of germination in the two light treatments when O. *biennis* and *V.thapsus* were watered by water. The y-axis represents the number of germinated seeds

3.4.2 Seedling traits

The comparisons of developed morphometric characteristics was not performed between the dark treatments, because *O. biennis* did not reach even 10% germination here and such analysis would not bring any reasonable results. Table 37 shows the results of Analysis of variance in studied characteristics.

Summary of all Effects **1-SPECIES, 2-LIGHT, 3-PRESENCE**	F-value			
	ROOT LENGHT	**SHOOT LENGHT**	**COTYLEDONS**	**DRY WEIGHT**
1 *O. biennis* × *V.thapsus*	1.14	203.06***	281.25***	28.16***
2 light × dark	33.84***	134.72***		50.64 ***
3 water × leachate	0.49	0.41	12.25***	0.27
12	8.03**	220.25***		53.36***
13	0.85	0.16	5.2*	0.07
23	0.47	0.81		0.41
123	0.83	1.29		0.02

Table 37. F-values from the ANOVA analysis of the SPECIES, LIGHT and PRESENCE factors. The stars indicate the achieved level of significance (*p<0,05;**p<0,01; ***p<0,001)

Comparison of the species performance was possible only in the light and is brought by Table 38.

	light			
	ROOT	**SHOOT**	**COTYLEDONS**	**DRY WEIGHT**
F-value	*O.biennis* × *V.thapsus*	*O.biennis* × *V.thapsus*	*O.biennis* × *V.thapsus*	*O.biennis* × *V.thapsus*
water	10,1**	0,41	102,54***	379,55***
leachate	55,67***	39,47***	196,33***	149,49***

Table 38. F-values from the one-way ANOVA analyses from the each environmental treatment with the SPECIES as the main factor.

O. biennis seedlings were generally robuster than those of *V. thapsus*.

Table 39.

Root lenght (cm)		light	dark	allelopathy effect in light	light effect
O. biennis	**water**	2.89 ± 1,07		0,3	
	leachate	3.04 ± 1,26			
V.thapsus	**water**	2.18 ± 0,61	0.61 ± 0,23	64,48***	175,24***
	leachate	1.08 ± 0,33			

Tables 39, 40, 41 and 42 bring the values of the measured characters: root lenght
shoot lenght
dry weight
that the studied species achieved in separate treatments.
On the right the F-values from tests of the specific effects are given:
Allelopathy effect= the difference in the character between watering by the leachate and water;
Light effect= the difference in the character between light and dark treatments.

Table 40.

Shoot lenght (cm)		light	dark	allelopathy effect in light	light effect
O. biennis	**water**	0.23 ± 0,09		3,56	
	leachate	0.27 ± 0,07			
V.thapsus	**water**	0.24 ± 0,04	2.11 ± 0,4	40,6***	721,66***
	leachate	0.16 ± 0,05			

Table 41.

Dry weight (g)		light	dark	allelopathy effect in light	light effect
O. biennis	**water**	0.000466 ± 0,0000826		3,29	
	leachate	0.000418 ± 0,0001052			
V.thapsus	**water**	0.0000673 ± 0,0000191	0.0000574 ± 0,0000161	15,08***	4,56
	leachate	0.0000437 ± 0,0000101			

Table 42.

Cotyledons (cm)		light	allelopathy effect
O. biennis	**water**	0.62 ± 0,14	0,52
	leachate	0.6 ± 0,11	
V.thapsus	**water**	0.37 ± 0,04	75,17***
	leachate	0.27 ± 0,05	

The span of cotyledons was not measured in the dark treatment, because at the end of the experiment majority of plants had their leaves still hidden in the seed coat. Thus the search for differences was performed only in the light treatments.

4. Discussion

4.1 Experiment on the crossed gradients of light and temperature

Oenothera biennis had the greatest temperature range tolerated from the three species. That correspons our primary hypothesis that the phenotypical plasticity of ecophysiological seed responses will be greater in the more successful invader. This could help *O.biennis* to at least germinate at new sites and as the germination percentages are usually very high, the chance that some will become established is all the more greater.

The absolute failure or poor germination in the darkness observed in *O.rubricaulis* and *O. ammophila* in majority of temperatures, could be ascribed to light-requirement expected in small seeded species with persistent seed banks.

The overall poor germination *O. ammophila* and *O. rubricaulis* in the dark and shaded treatments could be ascribed to the requirement of the temperature fluctuation as it has been recorded especially in species of disturbed habitats (Thompson 1974, Thompson and Grime 1983) The next experiment showed that these species germination in the dark really is stimulated by the temperature fluctuations.

The range of temperatures the seed is able to germinate in is influenced by other environmental factors. Usually other factors e.g. light move the treshold of the required temperature. This phenomenon was seen also in our experiment. Seeds in treatments with better light availability were able to germinate in cooler temperature and vice versa- seeds in warmer treatments were able to germinate even when shaded. The lower germination percentage achieved in cooler treatments is probably due to the fact that smaller proportion of seeds was satisfied with the temperature and able to respond (Probert 1992).

The retarded beginning of germination in the cooler treatments can be explained by the longer time needed in lower temperatures to acomplish the germination process (Probert 1992). As well the quicker germination observed in greater light availability can be ascribed to the promoting effect of light in given temperature (Pons 1992).

O.biennis usually develops the greatest shoot lenght and cotyledon span, which I assume reflects the better potential for light competition in *O.biennis* than in other species.

The fact that dry weight was affected the least from all characters in all species may be due to the resource limitation, which limits the total allocation of resources and results in necessary trade-off in allocation in different structures.

I think that the most profound differences among the studied species lie in their germination behaviour.

4.2 Test on effect of temperature fluctuations

Oenothera species belong under species, which sense and respond to alternating temperatures. The fact that they form persistent seed banks and are not good competitors makes their establishment dependent on the presence of gaps. The identification of gaps is crucial and it is thought to operate through two mechanisms: light exposure triggers the germination or the buried seed senses large diurnal temperature fluctuations, which indicate, that the formerly isolating layer has been removed (Thompson and Grime 1977). Such requirement for alternating temperatures was seen in many species (Steinbauer and Grigsby 1957, Thompson et al. 1977).

In *Oenothera* species the light is required in constant temperatures whereas in darkness there is need for temperature fluctuations. The effect of fluctuating temperatures is known to interact with the levels of phytochrome in plants and thus the light is known form a complex signal with the diurnal fluctuations (Probert et al. 1987). I think my observations indicate that there are at least two germination programs which are unfolded by different complex light-fluctuations signals and the responses are probably exclusive. That would explain, why the effects of light are so antagonistic in the presence and absence of temperature fluctuations. In concordance with this theory is also the observation that the *O.rubricaulis* and *O.ammophila* germination was less reactive on light in temperature fluctuations than in the absence of them.

Nutrient addition had an impact on the germination percentage only in the presence of temperature fluctuations, usually in dark or shaded treatments. We can assume that *Oenothera* species react on the complex signal of fluctuations and nitrate concentration. The higher nutrient status can serve as an assurance of resources needed for penetrating from the depth of burial or they can indicate absence of concurental vegetation (Karssen and Hilhorst 1992).

O. biennis seedling morphology was from the three species again the most affected by the course of temperature, nutrient enhancement and light.

In all cases the span of first leaves was greater in the fertilised treatment. Theoretically are the species that are able to allocate soil resources in fertile conditions to leaf production thought to be highly competitive (Grime 1979, Ryser and Notz 1996). The question is whether this observation in cotyledons could be extrapolated to normal leaves.

In great majority of light climates the root of *O. biennis* was greater under constant temperature. Considering the high germinability of *O.biennis* seeds in all light treatments, we could expect it would germinate also under vegetation canopy, where the temperature fluctuations are less obvious (Thompson et al 1977, Van Assche and Vanlerberghe 1989). In these cases perhaps the investment in the root could help compensate water or nutrient stress resulting from the neighbouring vegetation. *O. rubricaulis* developed in constant temperature greater leaves. Such investment to leaves could be also enhance the chance to survive under canopy when the seed already germinated.

4.3 Performance of hybridogenous *O.fallax*

In this experiment *O. biennis* was not able to germinate in the dark. The dark-dormancy is a common feature of seeds of the species with persistent seed bank. By this mean the germination in soil is prevented. In small seeded species like *O.biennis* the reserves in the seed may not be sufficient for latter seedling development. The light-requirement is also thought to be significant for gap detection (Pons 1992), because the disturbance usually destructs the concurental vegetation and offers the seed an opportunity to succeed.

The fact that *O. biennis* was able to germinate in the dark successfuly in previous experiments and not here is mainly due to the choice of seeds. During the exepriments of orientation and the test experiments the *O. biennis* seed set had become depleted and another seed set had to be used. The population differentiation is known in many species of introduced colonizers (Brown and Burdon 1983, Novak and Mack 1995, Kaufman and Smouse 2001), thus the observed differences could be ascribed to this phenomenon.

O.fallax was able to germinate in light and in dark. The effect of nutrients depended on the presence of light. It is suggested that the effect of NO_3 works nearly allways in complex with other environmental factors especially light (Hilton 1984). They both supply the seed with information about local environment and vegetation (Frankland and Taylorson 1983, Pons 1989) . The effectiveness of nitrate to change the dormancy level is thought to depend on the level of active phytochrom level P_{fr} . The levels of these chemicals form complex signal and may replace each other to some extent (Pons 1992). The persisting seeds can contain preexisting P_{fr} for long periods (Cone and Kendrick 1985). We can conclude, that the Pfr level in *O.fallax* seeds was in the dark unsufficient to break dormancy in majority of seeds and when the concentration of nitrates rose, the combination of the two factors was sufficient to break dormancy in greater percentage of seeds.

The reduction of germination percentage seen in the light may work on the same basis. However the Pfr level is expected to rise in the light and together with the higher nitrate level, the complex signal may get over the higher germination treshold especially when these plants prefere usually water and resource poor bare terrain.

The total failure of *O. erythrosepala* to germinate could be caused by total dependence of the germination on other factors: e.g. on temperature fluctuations.

In the used experimental conditions *O. fallax* was able to germinate better than *O. erythrosepala* and also than *O. biennis*, when the light was not available. In the light however *O. biennis* germinated better. The requirements for germination of the hybridogenous species thus differed from both parental species. The fact that the germination requirements of the hybrid were different was expected as the process of hybridization is usually followed by niche differentiation, otherwise the hybrid usually does not establish in the concurence with the parental species (Riesenberg 1997). As a result the hybrid can be more successful in particular conditions (Ayres et al. 2003).

Both species showed similar sensitivity to the used factors. Root of both species irrespectively of the light climate was shorter in the fertilised treatment. This observation is consisten with other studies, that have reported the relative increase of the root allocation in nutrient-poor environments (Aerts 1999).

4.4 Test on allelopathy potential in *O. biennis*

The germination of *V. thapsus* in the dark was retarded by the leachate from *O.biennis* seeds. In the light no supression of germination was found in both species. I asssume that the fact that the allelopathic effect showed only in the dark treatment may be due to the connection between the environmental stress and greater production of secondary metabolites (Gershenzon 1984).

However in light the characteristics of *V.thapsus* seedlings: lenght of radicule, hypocotyl, the span of cotyledons and the dry biomass weight- all were smaller- supressed by the *O. biennis* seed-leachate.

Alongside with the allelopathic supression of germination which is a well known process namely in the succession (Rice 1983) these substances can also reduce seedling elongation (Kheradnam and Bassiri 1980, Rice 1988, Eskelsen and Crabtree 1995).

Eventhough the allelopathic ablities were found in *O. biennis*, we can't tell to what extent are the results extendable. Petri dishes bioassays probably overestimate the effects of allelochemicals because they do not count with other factors which operate in the field conditions such as seed densities and soil characteristics, microclimate, and microbial activity(Keeley 1988, Stowe 1979,Wardle et al. 1998). Furthermore, the mobility of these compounds in natural soil might be less due to buffering or immobilization (Callaway and Hierro 1993).

O. biennis germination was not affected by the seed-leachate of *V. thapsus*. Each plant stage can have different allelochemical potential on different species. The effect of allelochemicals furthermore depends on the environmental conditions. Number of studies have found germination and early seedlings supressing effect of the leachates from shoots in *Tribulus terrestris* (El-Ghareb 1991), from leaves in *Centaurea maculosa* (Muir and Majak 1983) and *Bunias orientalis* (Steinlein et al. 1996). Sometimes the allelochemicals are released from the decaying plant material, while the fresh plant does not show allelopathic potential like in *Carduus nutans* (Wardle et al. 1995).

V. thapsus may thus supress *O. biennis* in other situation or life stage.

5. Conclusions

from ?

O.biennis was able to germinate in greater range of environmental conditions. It usually showed the greatest sensitivity to all used factors. Its germination percentages greatly exceed those of *O.rubricaulis* and *O. ammophila*.

Morphometric characteristics of *O.biennis* are not exclusively superior to those of the other species, but are again affected by the used factors to the greatest extent.

It revealed that the all chosen environmental characteristics: temperature, light, temperature fluctuations, nutrients and allelochemicals affect at least in some cases the germination of *Oenothera* species.

The investigation of the performance of hybridogenous *O.fallax* found out, that this species requirements for germination differ from both parental species and thus it established its own germination niche.

There was found possible allelochemical potential in *O. biennis*. The leachate from its germinating seeds supressed in darkness the germination of *V.thapsus*. No impact in opositte direction was observed.

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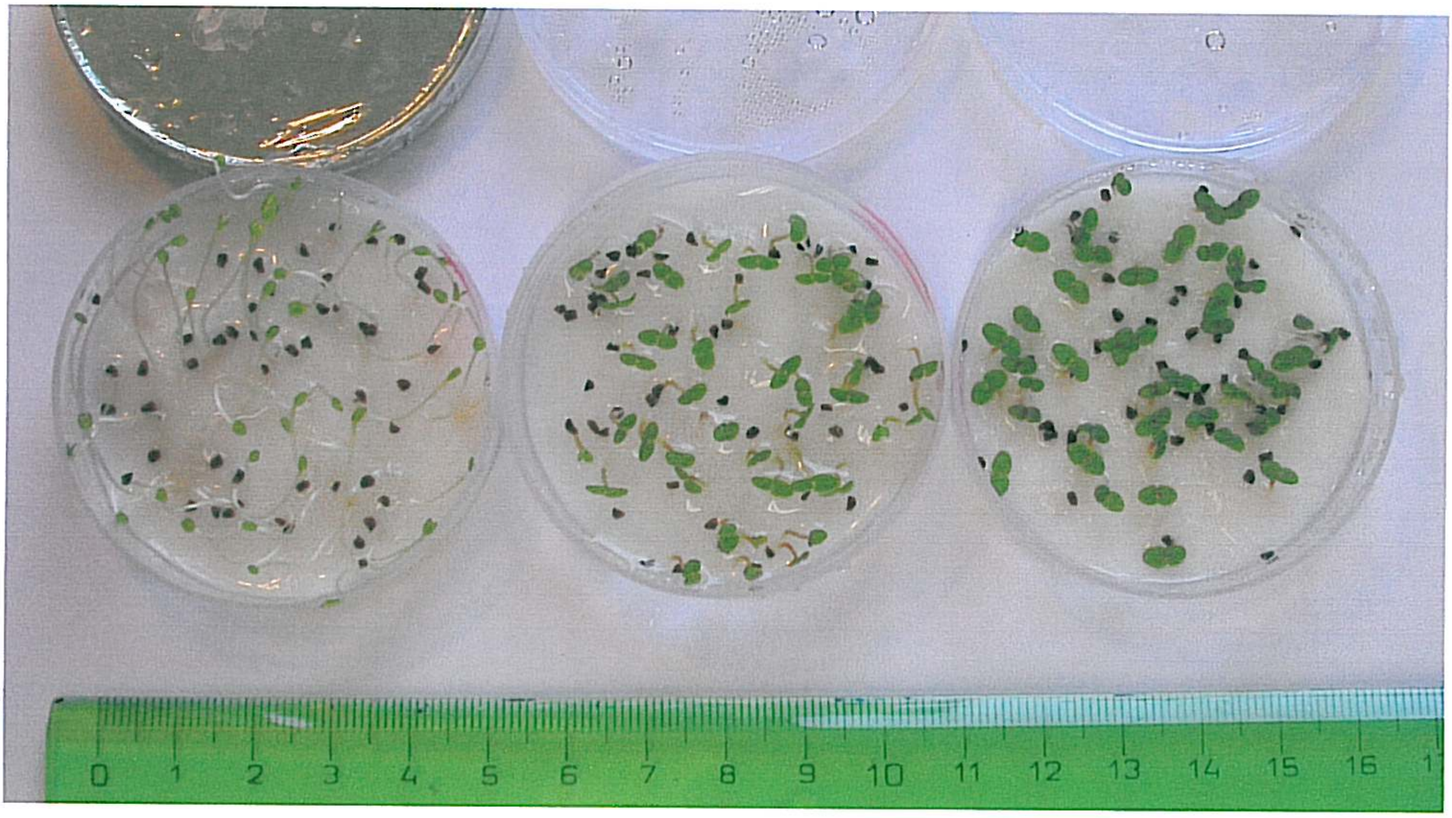
Appendix

Seedlings of *O. biennis.*

A) 25°C, light increases from left to right.



B) full light, temperature increases from left to right