Effects of an unspecialized soil pathogen on congeneric plant species with different geographic distributions

Vliv nespecializovaného půdního patogenu na rostlinné druhy téhož rodu s odlišným geografickým rozšířením

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This study investigated the potential of a root rot causing fungal pathogen to affect the geographical distribution of plant species. A glasshouse experiment was conducted that compared the responses of congeneric plant species of contrasting range types to the presence or absence of a soil borne fungal pathogen at three different moisture levels. Five congeneric plant species pairs were selected, each with one species present and one absent in humid western Europe: *Centaurea scabiosa–C. stoebe*, *Dianthus deltoides–D. carthusianorum*, *Inula conyzae–I. hirta*, *Potentilla neumanniana–P. cinerea* and *Scabiosa columbaria–S. ochroleuca*. The oomycete *Pythium ultimum* was selected as the root rot causing soil borne pathogen because of its wide geographical distribution, wide host range and reported preference for high soil moisture. The response variables measured included relative growth rates of above- and belowground biomass, leaf number and the shoot-root ratio. Two plant species pairs (*Dianthus* and *Scabiosa*) showed a significant interaction of distribution type with presence of the pathogen. The species with a continental distribution suffered a greater reduction in growth due to the pathogen than the species with an oceanic distribution. However, across all species and genera the pathogen's effect was not dependent on range type or soil moisture and affected only RGR of leaf number. The study revealed that unspecialized fungal pathogens might have an effect on the performance of some plant species and might affect continentally distributed species more than oceanic ones, which might put them at disadvantage when combined with, for example, competition. Nevertheless, this effect was not recorded for all genera tested. Given the little knowledge on the effects of unspecialized pathogens on wild plant species, further studies with different soil borne fungal pathogens causing root rot and an enlarged set of plant species are recommended.

K e y w o r d s: biogeography, biotic interaction, congeneric plant species, glasshouse experiment, *Phytium ultimum*, root rot

Introduction

At continental and global scales, climate is thought to be the most important factor limiting plant ranges (Grace 1987, Woodward 1987, Jäger 1995, Parmesan 1996, Gaston 2003, Araujo & Pearson 2005). Climate affects plant species not only directly, but also indirectly by influencing other environmental variables and biotic interactions (Gutierrez et al. 2005, Sutherst et al. 2007, Costa et al. 2008). Among the latter, competition and herbivory in particular have a large effect, particularly on local plant distribution (Bullock et al. 2000, Pulliam 2000, Bruelheide 2003, Maron & Crone 2006, Morris et al. 2007, Sutherst et al. 2007, Tylianakis et al. 2008). In contrast, information on the role of soil pathogens in influencing plant ranges is scarce and restricted to studies on range expansion of alien

plant species. In this regard, certain successful plant invasions are discussed in the context of reduced soil feedback in the introduced range and the Enemy Release Hypothesis (e.g. Agrawal et al. 2005, Reinhart & Callaway 2006, van Grunsven et al. 2007). Additionally, a number of studies emphasize the important role of specialized phytopathogens in the structuring and maintaining the species composition of plant communities (Connell 1971, Mills & Bever 1998, Van der Putten et al. 2001, Wardle et al. 2004). Unspecialized soil pathogens should have similarly crucial effects as they have the opportunity to switch between different host species (Jarosz & Davelos 1995), although it is likely that some plant species are more susceptible than others. So far, no study has yet explored the role of unspecialized soil pathogens in determining plant distribution. The present study aims to determine whether unspecialized pathogens can affect the growth and performance of wild plant species.

A large number of important phytopathogens are included in the oomycota (*Peronosporomycetes*). Within this group, several species of *Pythium* have wide host ranges (Van der Plaats-Niterink 1981) and are therefore suitable for screening for a general pathogen effect on different plant species. These pathogens are the main cause of damping-off and root rot diseases (Raaijmakers et al. 2009) and can severely reduce plant survival. Many *Pythium* species appear to be highly aggressive, because they induce necrotrophic infections (Jarosz & Davelos 1995, Raaijmakers et al. 2009) and are mainly reported from humid regions (Hendrix & Campbell 1973, Van der Plaats-Niterink 1981, Agrios 1997). However, compared to the many agricultural studies, there are few on the effects of such soil pathogens, with a broad host spectrum, on wild plant species.

Precipitation, and thus humidity, is known to decrease from western to eastern Europe (Crawford 2000). Nevertheless, similar types of vegetation occur in the different regions, such as dry grassland. Additionally, dry grasslands are rich in species, with closely related species being confined to either the western or eastern geographical areas (Jäger 1968, Royer 1991) and replacing each other in the communities there (so called vicariants; Jäger 1972). Such contrastingly distributed species (e.g. oceanic vs. continental) might be due to genetically fixed adaptations to the different climatic and environmental conditions. Consequently, it might be possible to determine whether vicariant species show a different response to unspecialized soil pathogens. More specifically, assuming that pathogens play an important role in humid regions, the expectation is that species from the more humid (oceanic) part of western Europe are less affected by damping-off diseases than those from the drier (more continental) regions.

The objective of this study was to explore these questions, using *Pythium ultimum* as the unspecialized pathogenic model organism. A glasshouse pot experiment was carried out, which involved exposing congeneric species pairs from dry grassland with contrasting distribution patterns in western Europe to the soil pathogen and to three soil moisture levels, in order to test the following hypotheses: (i) The soil pathogen has a negative effect on wild plant species; (ii) Vicariant plant species differ in their susceptibility to this general pathogen. More specifically, whether species with a more oceanic range are less affected than those with a more continental range, and (iii) whether the negative effect of the pathogen on plants is stronger when the soil is moist, were tested.

Materials and methods

Species selection

For the experiment, five genera with two species each were selected (Table 1). The selection criteria were that the two species in a genus should have similar growth forms and habitat requirements and different ranges. In general, the species pairs preferred were those in which one species had a distinctly more west-European and thus oceanic distribution and the other a more continental distribution. As we deliberately allowed the species within a genus to overlap in their geographical range, the species in a genus were chosen in a way that they differed at least in the longitudinal position at the western edge of their ranges (see Table 1). For the sake of simplicity, the species are referred to as oceanic and continental. Using these rigid selection criteria, the species pairs selected represent largely identical plant functional types on a much finer level than the usual classification into grasses, forbs or life forms (Box 1996, Diaz & Cabido 1997). More importantly, the use of species pairs enables one to carry out a phylogenetically independent comparison.

Seeds of all species were collected in the summer of 2005 in central Germany between Göttingen and Halle (for geographical coordinates of the sampling localities see Table 1). In this region, the continental species are close to their western range limit (see Table 1). Taking the seeds of both species from the same region excludes any effects of local geographical adaptation to pathogen presence within species. If local adaptation is involved both species should be expected to be adapted to a similar pathogen load. Seedlings of all species were raised under controlled standardized conditions in a glasshouse.

Pathogen

For the experimental tests *Pythium ultimum* was chosen as the pathogen. This pathogen has a broad host spectrum and a wide geographical distribution. It is one of the most important root-rot causing phytopathogens and is used in many agricultural studies (Hendrix & Campbell 1973, Schüler et al. 1989, Francis & St-Clair 1997, Paulitz & Adams 2003, Levesque & De Cock 2004). In addition, the species is reported to be abundant in humid regions (Hendrix & Campbell 1973, Martin & Loper 1999). The advantage of using *Pythium ultimum* as a pathogen in the screening experiment is that it lacks host-specificity. Although there is no detailed information on which indigenous plant species are susceptible to *Pythium ultimum*, it was assumed that the fungus has the potential to infect all the plant species used in this experiment.

The pathogen strain used was obtained from the Faculty of Organic Agricultural Sciences, Department of Organic Farming and Cropping at the University of Kassel and cultivated on a cornmeal agar (detailed information in Schüler et al. 1989). This strain was isolated from sugar beet and all crop plants tested so far are highly sensitive to this strain (Schüler et al. 1989). The treatment was implemented according to the protocol of Schüler et al. (1989). For the preparation of the inoculation a 5 mm diameter agar disc taken from a seven day old culture of the pathogen was placed on a mixture of autoclaved wheat flour, sand, earth (air dried) and water in the volume ratios 1 : 2 : 2 : 2. This mixture was incubated in the dark at 20 °C in a 500 ml Erlenmeyer flask. After 14 days the pathogen had grown through the whole substrate. This mixture was used as the inoculum (set as 100% *Pythium*) for the experiment. At first it was diluted in several stages with autoclaved fine

Table. – Compilation of species characteristics and description of the collection localities. Continentality value (C), growth form and habitat type from Jäger & Werner (2005); C ranges between 1 and 10; 1 = eu-oceanic, 10 = eu-continental. sg = summer green, eg = evergreen, pl = plurennial.

Genus	Species	Range type	Continen- taity value (C)	Western range limit (Longitude)	Growth form	Habitat (in central Germany)	Locality of collected seeds	
							Latitude	Longitude
Centaurea	scabiosa	oceanic	1–7	7°W	sg semirosulate, pl polycarpic	calcareous dry and semidry grass-lands, dry meadows and shrub land	51.534445°N Lärchenhügel near Halle	11.884395°E
	stoebe	continental	2–7	7°E	eg semirosulate, pl monocarpic	dry and sandy, partly ruderal or rocky xerothermic grasslands	51.536163°N Franzigmark near Halle	11.886725°E
Dianthus	deltoides	oceanic	2–5	5°W	eg erosulate, pl polycarpic	dry and sandy grasslands, rare in arid environment	51.663636°N Harzgerode	11.138695°E
	carthusianorum	continental	2–4	0°E	semi-eg semirosulate, pl polycarpic	xerothermic grasslands, dry slopes and borders of forests	51.533080°N Seeben near Halle	11.981123°E
Inula	conyzae	oceanic	1–4	8°W	eg semirosulate, pl polycarpic	semidry grasslands, dry shrub land and forests and their edges	51.550842°N Lerchenhügel near Halle	11.869394°E
	hirta	continental	4–6	7°E	eg erosulate, pl polycarpic	continental dry and semidry grass-lands, dry shrub land and their edges	51.536163°N Franzigmark near Halle	11.886725°E
Potentilla	neumanniana	oceanic	2–4	5°W	eg semirosulate, pl polycarpic	rocky, dry and semidry grasslands, dry pine-forests	51.560703°N Faßberg near Göttingen	9.961034°E
	cinerea	continental	3–7	10°E	eg semirosulate, pl polycarpic	continental, rocky, dry and semidry grasslands, dry pine forests	51.503248°N Amselgrund in Halle	11.945086°E
Scabiosa	columbaria	oceanic	2–4	7°W	eg semirosulate, pl polycarpic	calcareous dry and semidry grass-lands, meadows and shrub land	51.556321°N Faßberg near Göttingen	9.965208°E
	ochroleuca	continental	4–6	10°E	eg semirosulate, plpolycarpic	continental, dry and semidry grasslands	51.547988°N Teicha near Halle	11.946029°E

sand to a 10% stem-inoculation-mixture. After that the fivefold volume amount of standard soil (Einheitserde® EE0, Einheitserdewerk Hameln) was added to the stem-inoculation mixture. The resulting substrate mixture was used in the glasshouse experiment. For the pathogen-free treatment standard soil was mixed in the same ratio with autoclaved fine sand as in the 10% stem-inoculation-mixture.

Experimental design

Twelve-week old seedlings were transferred to 500 ml pots containing standardized quantities of uninfected soil (without pathogen: –P) and soil infected with *Pythium ultimum* (+P). These pots were randomly placed in a climate chamber of a glasshouse. Growing conditions were set to 12 hours light at 20 °C and 12 hours dark at 15 °C. There were three soil moisture levels: dry (40% by weight water content = 0.4 g $H_2O \cdot g^{-1}$ dry soil), moist (70%) and wet (100%). Water contents were adjusted daily by weighing the pots. The potted plants developed in the climate chamber for 8 weeks. For each plant species there were eight replicates of each treatment combination, except for *Potentilla neumanniana* with only six replicates per treatment combination. Altogether this makes 46 individual plants per species (36 for *Potentilla neumanniana*) and 468 in the whole experiment.

Measurement of plant performance

At the beginning of the experiment, the leaf number was counted to allow calculations of relative growth rates (RGR). RGR of leaf number is a measure of the relative rates of formation of leaves according to Hunt (1989). Additionally, eight plants of each species were harvested randomly, and roots and shoots were weighed after drying in a laboratory oven at 70 °C for 48 hours. These initial values were used as a biomass reference to calculate RGR of dry biomass. After 8 weeks the experiment ended and all plants were harvested. Leaves were counted and final dry biomass of shoots and roots were measured.

Statistical analysis

To test for a genus-specific treatment effect, the data obtained for each of the five genera were subjected to a three-way generalized linear model (GLM; sum of squares type III) testing for the effects of range type (oceanic/continental), pathogen (presence/absence) and soil moisture (three levels) on each measured response variable (RGR of total biomass, RGR of root and shoot biomass, shoot-root ratio, RGR of leaf number). All factors were considered fixed and the model included all possible interactions. Additionally, Tukey post-hoc tests were carried out for detecting significant differences between the soil moisture treatments. To test for consistency in the responses to the soil pathogen and to soil moisture across all genera, a three-way linear mixed effects model (sum of squares type III) for the same response variables as in the three-way GLMs was performed with genus identity as a random factor and range type (oceanic/continental), pathogen (presence/absence) and soil moisture (three levels) as fixed factors and all possible interactions. These statistical analyses were performed with SAS (GLM with 'proc glm', linear mixed effects model with 'proc mixed', SAS Institute Inc. 2002–2008).

Results

Genus-specific responses

Table 2 lists the GLM results for all calculated relative growth rates (RGR) and the shoot-root ratio separately by species pairs. The species of all congeneric species pairs differed in most of the response variables. Except for one genus (*Dianthus*), there were significant effects of range type on the RGR of total biomass (Table 2). This indicates that, despite having controlled for growth form and habitat requirements, the species within a genus were not completely similar in their growth patterns. However, the responses differed in direction. For example, for total biomass, oceanic species had a higher RGR than the continental species (for *Inula* and *Scabiosa*), whereas the opposite outcome was recorded for continental species (*Centaurea* and *Potentilla*). In contrast, for RGR of leaf number there was either no differences between the range types or higher RGRs for the continental species. However, there was no consistent pattern across all genera and across all response variables.

The pathogen treatment resulted in significant effects only in three species pairs (*Inula*, *Potentilla* and *Scabiosa*, Table 2). In the presence of the pathogen, plants of the genus *Inula* had lower RGRs of root and total biomass than in its absence. Plants of the genus *Potentilla* had higher RGR of leaf number and in the genus *Scabiosa* the shoot-root ratio increased when *Pythium* was present.

In contrast, the moisture treatment was highly effective for all genera and affected most response variables. RGR of biomass and leaf number increased and shoot-root ratio decreased with increasing soil moisture.

In two of the five species pairs (*Dianthus* and *Scabiosa*) the congeners differed in their responses to the pathogen treatment, as seen in a significant range type × pathogen interaction in Table 2. Although no individuals of these two genera died from pathogen infection, the continental species *Dianthus carthusianorum* and *Scabiosa ochroleuca* showed reduced growth in the presence of the pathogen *Pythium ultimum*. In contrast, the oceanic species were not or even positively affected (Fig. 1). For *Dianthus carthusianorum*, the reduction in root growth caused by the pathogen was significant but not in shoot growth, which resulted in a significantly higher shoot-root ratio. In contrast, *Scabiosa ochroleuca* additionally showed a reduced RGR of shoot biomass and of total dry biomass. In consequence, the shoot-root ratio for both *Scabiosa* species was not affected by the pathogen treatment. The other three species pairs showed no significant interactions of range type with pathogen treatment (Table 2, Fig. 1).

Along the moisture gradient, in two of the five species pairs (*Dianthus* and *Inula*) the congeners performed significantly different (Table 2). The oceanic species *D. deltoides* and *I. conyzae* took more advantage of a higher water supply, indicated by higher RGR, than their continental congeners (Fig. 1). The RGR of the continental species *D. carthusianorum* and *I. hirta* was even slightly lower in the wettest treatment (100%) compared to the one with intermediate moisture (70%, see Fig. 1). However, in the species pairs *Centaurea*, *Potentilla* and *Scabiosa* there were no significant differences in the responses of the congeners to the different moisture treatments (Table 2).

Contrary to expectation, there was no interaction between the effects of the moisture and pathogen treatments. Only in the genus *Dianthus* was there a marginally significant effect of the interaction range type × pathogen × moisture. In the high moisture treatment, *Pythium ultimum* reduced the root growth of the continental *D. carthusianorum* and enhanced that of the oceanic *D. deltoides* (F = 3.10, P = 0.0504).

Table 2. – Summary with F values of the three way generalized linear models (GLMs) for the response variables RGR shoot biomass (in $g \cdot g^{-1}$ week^{-1}), RGR root biomass (in $g \cdot g^{-1}$ week^{-1}), RGR total biomass (in $g \cdot g^{-1}$ week^{-1}), shoot-root ratio (in $g \cdot g^{-1}$), and RGR of leaf number (in number number^{-1} $\cdot$ week^{-1}). The three factors are range type (R), pathogen (P) and moisture (M). All possible interactions are included. GLMs were calculated separately for each genus. *** $P < 0.001$, ** $P \le 0.01$, * $P \le 0.05$, (.) $P \le 0.1$.

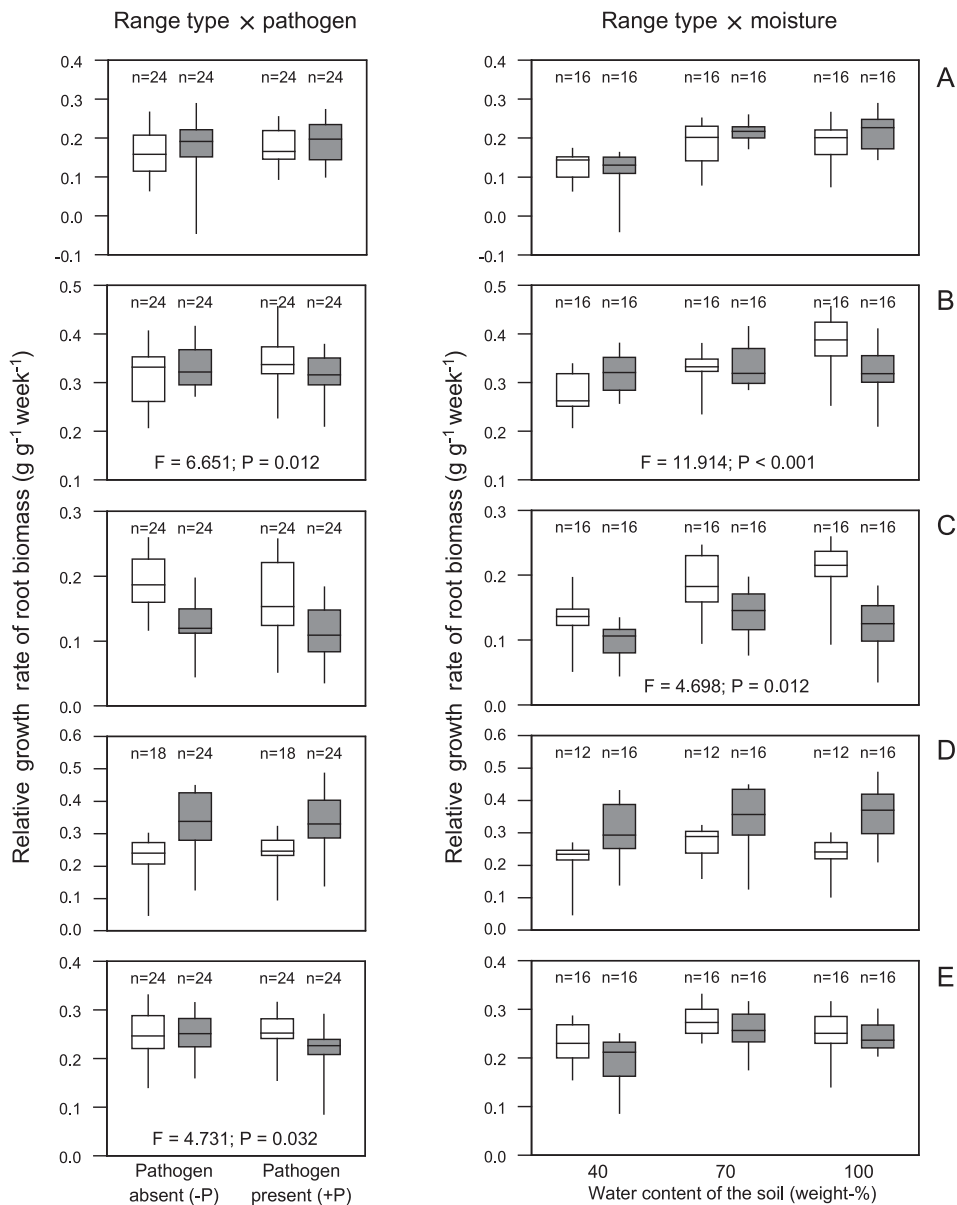| Genus	Source of variation	df	RGR shoot biomass F value P		RGR root biomass F value P		RGR total biomass F value P		Shoot-root ratio F value P		RGR leaf number F value P	
Centaurea	Range type (R)	1	30.00	***	2.88	(.)	10.91	***	11.60	***	161.74	***
	Pathogen (P)	1	0.10		1.66		0.78		2.67		2.61	
	Moisture (M)	2	9.58	***	32.47	***	25.98	***	11.86	***	12.91	***
	R × P	1	0.06		0.02		0.01		0.26		0.10	
	R × M	2	1.25		1.88		0.64		5.93	**	0.42	
	P × M	2	0.55		0.16		0.01		2.17		0.46	
	R × P × M	2	0.92		0.09		0.20		0.18		0.91	
Dianthus	Range type (R)	1	2.84	(.)	0.34		0.48		12.96	***	220.84	***
	Pathogen (P)	1	0.02		0.44		0.15		0.50		0.04	
	Moisture (M)	2	4.33	*	13.12	***	9.23	***	8.24	***	1.62	
	R × P	1	0.35		6.65	*	3.39	(.)	5.07	*	0.14	
	R × M	2	1.18		11.93	***	6.86	***	7.62	***	0.52	
	P × M	2	0.52		0.14		0.04		1.55		0.34	
	R × P × M	2	0.80		3.10	(.)	2.18		2.77	(.)	0.82	
Inula	Range type (R)	1	93.39	***	50.25	***	31.53	***	219.18	***	0.01	
	Pathogen (P)	1	3.14	(.)	4.83	*	4.96	*	0.91		1.05	
	Moisture (M)	2	8.25	***	19.02	***	19.04	***	3.13	*	11.65	***
	R × P	1	0.24		0.41		0.35		0.39		3.37	(.)
	R × M	2	5.38	**	4.70	*	5.97	**	0.37		2.15	
	P × M	2	0.23		0.86		0.97		0.05		1.38	
	R × P × M	2	0.52		0.04		0.10		0.07		1.70	
Potentilla	Range type (R)	1	0.62		33.47	***	4.19	*	18.60	***	2.51	
	Pathogen (P)	1	1.00		0.29		0.62		1.87		4.78	*
	Moisture (M)	2	3.57	*	3.07	(.)	3.42	*	3.30	*	2.47	(.)
	R × P	1	0.12		0.08		0.00		3.14	(.)	1.10	
	R × M	2	0.01		0.78		0.17		7.67	***	0.48	
	P × M	2	0.02		0.06		0.00		1.04		0.68	
	R × P × M	2	0.05		0.27		0.13		0.88		0.83	
Scabiosa	Range type (R)	1	2.81	(.)	6.72	*	13.67	***	60.11	***	37.75	***
	Pathogen (P)	1	0.26		2.29		0.44		7.22	**	0.08	
	Moisture (M)	2	4.91	*	17.37	***	11.72	***	7.71	***	18.49	***
	R × P	1	8.07	**	4.77	*	6.48	*	0.52		0.05	
	R × M	2	1.09		0.90		1.03		0.55		1.33	
	P × M	2	0.01		0.85		0.35		1.26		0.03	
	R × P × M	2	0.40		1.19		0.91		0.25		1.56	

Fig. 1. – Box-and-whisker-plots of the effect of the range type × pathogen interaction (left) and the range type × moisture interaction (right) on the response variable RGR of root biomass for each of the five species pairs investigated: A. *Centaurea*, B. *Dianthus*, C. *Inula*, D. *Potentilla*, E. *Scabiosa*. Species with an oceanic type of distribution are coloured white; species with a continental type of distribution are coloured grey. Significant interaction effects are presented with F and P values in the diagram (calculated in separate three way GLMs for each genus). For species names within genus see Table 1. For the complete statistical results see Table 2.

Responses across genera

The species with contrasting distributions differed consistently in RGR of leaf number, but not in RGR of biomass (Table 3). Surprisingly, across all genera and moisture treatments RGR of leaf number was higher in the presence of the pathogen compared to the control (–P: 0.055, +P: 0.062 number number^{-1} · week^{-1}). However, the other response variables were not affected by the pathogen. As in the genus-wise analyses, the moisture treatments had a strong effect on all response variables (Table 3).

Contrary to expectation, there were no significant interactions of the pathogen treatment with the range type of the plant species or with moisture, indicating that the pathogen effect differed inconsistently between the continental and oceanic species or along the moisture gradient (Table 3).

Table 3. – Summary with F values of the linear mixed effects model for the response variables RGR shoot biomass (in g · g^{-1} · week^{-1}), RGR root biomass (in g · g^{-1} · week^{-1}), RGR total biomass (in g · g^{-1} · week^{-1}), shoot-root ratio (in g · g^{-1}), and RGR of leaf number (in number number^{-1} · week^{-1}). The three fixed factors are range type (R), pathogen (P) and moisture (M) with genus identity as random factor. All possible interactions are included. *** $P < 0.001$, * $P \leq 0.05$.

Source of variation	df	RGR shoot biomass		RGR root biomass		RGR total biomass		Shoot-root ratio		RGR leaf number	
		F value	P	F value	P	F value	P	F value	P	F value	P
Range type (R)	1	2.65		0.74		0.28		22.12	***	170.03	***
Pathogen (P)	1	0.20		0.01		0.03		0.46		3.93	*
Moisture (M)	2	21.04	***	45.24	***	43.21	***	7.31	***	22.31	***
R × P	1	0.40		2.02		1.42		1.01		1.12	
R × M	2	0.94		0.87		1.20		0.08		0.76	
P × M	2	0.03		0.31		0.24		0.26		0.06	
R × P × M	2	1.09		1.27		1.30		0.64		1.21	

Discussion

The first hypothesis that *Pythium ultimum* has a negative effect on wild plant species is only partly supported by the data. There was only one genus (*Inula*), in which both species were negatively affected by *Pythium ultimum*, in two genera (*Centaurea* and *Potentilla*) both species were unaffected and in another two genera (*Dianthus* and *Scabiosa*) the two congeneric species differed in their responses to the pathogen. Most species either did not respond to the pathogen or showed no consistent effects across all studied response variables. It might be criticized that the low pathogen effect might have a methodological background (e.g. caused by the inoculation procedure), which is highly improbable. The substrate variants used were all mixed simultaneously and very thoroughly to avoid differences between different lots. For this the well-established procedure described in Schüler et al. (1989) and the same *Pythium ultimum* strain was used. This strain is known to have a high infection potential and to reduce the fresh biomass of pea seedlings (*Pisum sativum*) by up to 76.8% (Schüler et al. 1989). However, in contrast to almost all other experiments carried out so far, seeds from wild plants rather than from crop species were used. In his review, Morris et al. (2007) points out that crop plants have undergone intensive breeding

with the aim of maximizing production, which might have resulted in poorer defence mechanisms. Thus, crops might be more susceptible to herbivores and pathogens than non-crop plants. This would also imply that pathogen concentrations in experiments with wild plants have to be higher than in experiments with crops. The concentration of *Pythium ultimum* used in our study was indeed higher than the one used by Schüler et al. (1989), but still might not have been high enough to cause severe effects. It is also known that *Pythium ultimum* can infect seeds prior to germination and that with increasing seedling age there is a decreasing susceptibility to *Pythium ultimum* (Mellano et al. 1970, Martin & Loper 1999). In this study 12 week-old seedlings were used, which might have been more robust than earlier stages. However, although it was initially planned to establish germination experiments in the presence of pathogens, this was impossible because of the great variation in germination rates of the collected seeds.

Nevertheless, genus-specific differences in infection potential were identified, showing that despite the pathogen's wide host range, not all wild plant species are suitable hosts. It is known that *Centaurea maculosa* Lam., a species closely related to *C. stoebe* that is well studied because of its invasiveness in North America, produces root exudates as defence against herbivores, pathogens and competitors (Callaway et al. 1999, Prithiviraj et al. 2007). Possibly, these mechanisms are also effective against *Pythium*. However, there is no published information on this for other species.

In the two genera *Dianthus* and *Scabiosa* the species with a continental distribution responded negatively to the pathogen, as seen in a lower RGR of biomass compared to the oceanic species. This supports the second hypothesis of a reduced growth performance of plant species that do not occur in humid western Europe with a presumed higher pathogen presence. Although the effect of the pathogen was not lethal, growth reductions have the potential to reduce a species' ability to withstand competition, infestations of other pathogens and biomass loss due to herbivory (Van der Putten et al. 2001). Individual population sizes may decrease and long-term changes in community structure may occur (Burdon 1991), leading to a disadvantage in regions where the pathogen occurs. Consequently, at least for some continentally distributed species, root-rot causing pathogens such as *Pythium ultimum* can actually be considered as detrimental biotic agents, and thus, a potential factor determining the western limits of the distribution ranges in Europe.

However, the third hypothesis that the effects of pathogen increase with increase in soil moisture has to be rejected. Such an effect was only observed in the genus *Dianthus*, while in all other genera the pathogen effect was not associated with high soil moisture. It might be that *Pythium ultimum* is either unable to affect most of the chosen species or is not as dependent on high soil moisture as generally assumed. This view is also supported by a study of Paulitz & Adams (2003), who found *Pythium ultimum* predominantly in coarse-textured dry soils. However, this study was carried out in Washington State, where the samples were collected in a dry summer, which might have had an influence on the outcome of the investigation. In contrast to Paulitz & Adams (2003), Larkin et al. (1995) found the *Pythium ultimum* infection of alfalfa seedlings to be positively correlated with rainfall. Similarly, an experimental study of Lifshitz & Hancock (1984) revealed that *Pythium ultimum* sporangia survived better in moist soil at moderate temperatures of between 15–21 °C. However, in all these studies the pathogen was cultivated or tested on crops. Almost nothing is known about the infection of wild plant species by unspecialized *Pythium* species and its relationship with environmental variables.

Conclusions

The results partly support the hypothesis that unspecialized pathogens have the potential to influence the distribution of wild plant species, especially those that naturally occur in less humid climates. This is the first test of the effect of an unspecialized pathogen on several wild plant species from different environments, which highlights the need for further studies with different unspecialized pathogen species, a larger set of wild plant host species and more intensive field surveys to evaluate the in situ effect of this biotic constraint.

Acknowledgements

Souhrn

Práce se zabývá vlivem houbového patogenu, působícího hnilobu kořenů, na geografické rozšíření rostlinných druhů. Ve skleníkovém pokusu byla sledována reakce dvojic druhů téhož rodu s rozdílným rozšířením na přítomnost patogenu při třech různých hladinách vlhkosti. Bylo testováno pět dvojic, z nichž vždy jeden druh se vyskytuje v humidní západní Evropě a druhý nikoli: *Centaurea scabiosa–C. stoebe*, *Dianthus deltoides–D. carthusianorum*, *Inula conyzae–I. hirta*, *Potentilla neumanniana–P. cinerea* and *Scabiosa columbaria–S. ochroleuca*. Jako patogen byla vybrána houba *Pythium ultimum* ze skupiny *Oomycota*, která má velké geografické rozšíření, široký okruh hostitelů a preferuje vlhké půdy. U rostlin byla měřena relativní růstová rychlost nadzemní a podzemní biomasy. Dvě dvojice druhů (*Dianthus* and *Scabiosa*) vykazovaly průkaznou interakci mezi typem rozšíření a přítomností patogenu. U druhů s kontinentálním rozšířením způsobil patogen větší redukci růstu než u druhů oceanických. Při testování celého druhového souboru však vliv patogenu nezávisel na typu rozšíření nebo půdní vlhkosti a projevoval se pouze v relativní růstové rychlosti listů. Výsledky ukazují, že hostitelsky nespecializované houbové patogeny mohou alespoň u některých rostlinných druhů ovlivňovat rozšíření; tento vliv může být větší u druhů s kontinentálním rozšířením a pokud se k němu přidá např. vliv kompetice, může to pro ně znamenat nevýhodu. O vlivu nespecializovaných houbových patogenů na divoce rostoucí rostliny je známo velmi málo, proto jsou žádoucí další studie, které by testovaly vliv i jiných druhů houbových patogenů na širší spektrum rostlinných druhů.

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