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Allelopathic effects of *Rumex obtusifolius* leaf extracts against native grassland species

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Summary
In perennial grasslands *R. obtusifolius* (broad-leaved dock) is often surrounded by certain plant species. Thus, it is hypothesized for the current study that *Rumex* can affect their neighbouring plant species by allelopathic interactions. To test this hypothesis, in a series of laboratory and field experiments aqueous extracts of green *R. obtusifolius* leaves were sprayed on seeds of 14 herbaceous plant species (graminoids, non-leguminuous forbs and leguminuous forbs) commonly native to perennial grasslands and its effects on seed germination compared with those with tap water spraying. An allelopathic effect was defined as inhibitory influence of *Rumex* extracts on seed germination relative to tap water. Generally, results of the laboratory experiments showed a species-specific susceptibility of grassland species to *Rumex* extracts; no species was promoted by *Rumex* extracts. All grasses tested were heavily inhibited by *Rumex* extracts, herbs and legumes varied from not affected until heavily inhibited. Spraying of *Rumex* extracts in the field had no effect on germination of these species. The results could be considered in designing seeding mixtures for resowing sward damages to especially contain species not susceptible to *Rumex* allelopathy (e.g. *Trifolium pratense*, *T. repens*, *Plantago lanceolata*) that would thus be assumed to be more competitive against *R. obtusifolius* regrowing in these grasslands.

Keywords: Cultural weed control, pasture, perennial weed, weed ecology

Zusammenfassung
Allelopathische Effekte von Rumex obtusifolius *Blattextrakten gegenüber Grünland-Arten*

Im Dauergrünland ist *R. obtusifolius* (Stumpfblättriger Ampfer) oft von bestimmten Pflanzenarten umgeben. Als Hypothese für die vorliegende Studie gilt, dass *Rumex* benachbarte Pflanzen artenspezifisch durch allelopathische Interaktionen beeinflusst. Um diese Hypothese zu testen, wurden eine Reihe von Experimenten in Labor und Feld durchgeführt, indem wässrige Extrakte von grünen Ampfer-Blättern auf 14 krautige Grünland-Arten (Gräser, Kräuter und Leguminosen) appliziert wurden und deren Wirkung auf die Samen-Keimung mit der Applizierung von Leitungswasser verglichen wurden. Allelopathische Effekte wurden als hemmende Wirkung auf die Samenkeimung relativ zu Leitungswasser definiert. Generell zeigten die Resultate, dass Grünland-Arten spezifisch auf *Rumex*-Extrakte reagieren; keine Art wurde durch *Rumex*-Extrakte gefördert. Bei allen gestesteten Grasarten bewirkten *Rumex*-Extrakte starke Keimhemmung; Kräuter und Leguminosen wurden nicht bis stark gehemmt. Die Applikation der *Rumex*-Extrakte im Feld hatte keinen Einfluss auf die Keimung dieser Arten. Diese Resultate könnten berücksichtigt werden in der Zusammenstellung von Saatmischungen mit Arten die nicht von *Rumex*-Allelopathie beeinflusst werden (z.B. *Trifolium pratense*, *T. repens*, *Plantago lanceolata*) um die Konkurrenzkraft von Neuansaten gegenüber *Rumex* zu erhöhen.

Stichwörter: Kulturelle Unkrautkontrolle, Weideland, ausdauerned Unkraut, Unkrautökologie

Introduction

There is general consensus that in order to develop sustainable methods for non-chemical control of *R. obtusifolius* L., a solid knowledge on the ecology of this weed species is an important prerequisite (ZALLER 2004b). When observing species patterning in grasslands it can be seen that *R. obtusifolius* is often surrounded by certain species growing in the vicinity of this weed species. Generally these patterns can be explained either by competition for space and light or species effects on nutrient dynamics, soil microbial ecology or soil mineralization that may influence growth and distribution of associated plant species (TILMAN 1982; FACELLI and PICKETT 1991, DAKSHINI *et al.* 1999). Another explanation for certain species patterning could be the result of interactions via leaching of allelochemicals from plant tissue and/or through decomposition of plant material containing these substances. The hypothesis that *R. obtusifolius* exerts allelopathic control over meadow species is supported by small-scale distribution of meadow species in the neighborhood of *R. obtusifolius* plants (CARRAL *et al.* 1988). The area affected and the intensity of the allelopathic effect is supposed to increase with the size of the individual *R. obtusifolius* plants (CARRAL *et al.* 1988). Allelopathy is here considered as an interference mechanism in which live or dead plant materials including plant litter release chemicals which exert an effect (usually negative) on associated plants (MULLER 1969).

Plants producing tissues with unfavourable foliage and litter characteristics, like *R. obtusifolius*, are suggested to also contain high levels of secondary metabolites, and could therefore conceivably have a greater allelopathic potential than more palatable plants (WARDLE *et al.* 1998). Indeed, several allelopathic and phytotoxic substances (e.g. oxalic acid, potassium oxalate, malonic acid and potassium malonate) have been isolated from various tissue of *R. obtusifolius* and *R. crispus* (KASAI *et al.* 1982, CHAVES *et al.* 2001). Roots of *R. obtusifolius* have been shown to containing allelochemicals that affect growth of fungi, bacteria and lettuce seedlings (KASAI *et al.* 1982). Decomposing *R. obtusifolius* leaves and their extracts were most toxic for germination and root growth of several grassland species (*Lolium perenne* L., *Trifolium repens* L., *Poa pratensis* L. and *Dactylis glomerata* L.) after seven days decomposition, although the toxicity level was still high after 21 days (Carral et al. 1988). Indications for allelopathic interactions are also effects of the *R. obtusifolius* infestation history showing that germination of *Lolium multiflorum* L. seeds was significantly reduced in previously infested plots compared with previously uninfested plots (LUTTS *et al.* 1987). Laboratory experiments on allelopathic effects of leaf extracts of *R. crispus* also significantly reduced germination of several crop as well as native species (MUMINOVIC 1987; MUMINOVIC 1990; BERES and KAZINCZI 2000). Plants with allelopathic potential against other organisms might not only affect species assemblages but also induce net changes in ecosystem properties, which may in turn impact upon the plant community in the longer term (WARDLE *et al.* 1998).

The objectives of the current study were to determine (1) whether aqueous extracts from fresh leaves of *R. obtusifolius* can affect the germination of native grassland species under controlled conditions, and (2) whether field application of these extracts in a species-rich pasture ecosystem show similar germination rates.

Material and methods

Extracts were prepared of leaves from non-flowering *R. obtusifolius* individuals collected from an organically managed perennial grassland near Bonn, Germany. To prepare these extracts, 1000 g of fresh leaves were blended using a household mixer and soaked in 1 litres of tap water for 24 hours. The extract was filtered through filter paper (Whatman No. 1) and stored at 4°C before applied to seedlings. The pH of the extracts was on average 6.31 (measured with glass electrode).

Allelopathic potential of aqueous *Rumex* extracts was determined by two seedling bioassays (September-November 2002) each using 14 species of native grassland species comprising the three functional groups –grasses (*Alopecurus pratensis* L., *Arrhenatherum elatius* (L.) Beauv. Ex J. & K. Presl, *Dactylis glomerata*, *Poa pratensis*, *Poa trivialis* L.), non-leguminous herbs (*Leontodon autumnalis* L., *Pimpinella major* (L.) Huds., *Plantago lanceolata* L., *R. obtusifolius*, *Sanguisorba officinalis* L., *Taraxacum officinale* G.H. Weber ex Wiggers) and leguminous herbs (*Trifolium pratense* L., *T. repens*

L., *Vicia cracca* L., *V. sepium* L.). Seed material was obtained from a commercial supplier (Rieger-Hofmann, Blaufelden-Rapoldshausen, Germany). In the current study we preferred aqueous media for preparing extracts to more realistically simulate leaching through rain (INDERJIT and DAKSHINI 1995). Germination rates were determined on 20 seeds of each species that were washed with distilled water and placed in Petri dishes (diameter 12 cm) lined with three circles of filter paper (Whatman No. 1). Five millilitres of the extract were applied after seeds have been placed into Petri dishes. The treatments were replicated 6 times on each date; control treatments received 5 ml of tap water instead of *Rumex* extract. Petri dishes were placed in growth chambers with a 12 hour day/night regime at 22°C. Monitoring of germination started the second day after extract application; a seedling was considered to have germinated when radicle was at least 2 mm long. Test trials with tap water of pH levels ranging from pH 5.5 to pH 7.5 did not show any effect on germination of grassland species (data not shown).

In order to test the allelopathic potential of *Rumex* extracts in the field, 20 ml of the extracts was sprayed on created sward gaps (30 x 30 cm) in an organically managed perennial grassland where 20 seeds of the same species used in the laboratory experiments were sown. Field spraying occurred two times during the first three days after sowing (September 2002). Germination on vegetation gaps was monitored weekly over the course of 6 weeks.

Data on the effect of *Rumex* extract or water spraying were analyzed by repeated measures ANOVAs using a general linear model. Comparisons between germination rates in laboratory and field trials were analysed using a simple correlation after Pearson. All statistical analyses were performed using SPSS for Windows vers. 12.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Overall, germination of the tested grassland species responded highly significantly different to watering with either *Rumex* extract or tap water (repeated measures ANOVA results: treatment $P < 0.001$, species $P < 0.001$, treatment x species $P < 0.001$; data not shown).

All five grass species were highly significantly inhibited in their germination by *Rumex* extracts (Fig. 1). Non-leguminous herbs varied significantly in their response to *Rumex* extract or tap water; with the exception of *P. lanceolata* all other herbs responded with a delayed or inhibited germination to *Rumex* extract spraying (Fig. 2). All four leguminous species tested were also strongly affected in their germination by *Rumex* extracts with both *Vicia* species inhibited and both *Trifolium* species showing a delayed germination (Fig. 3).

Field spraying of *Rumex* extracts on vegetation gaps where the 14 species had been sown had no effect on germination rates of the sown plant species (correlation between germination in laboratory trials vs. field trials: $r = -0.276$, $P = 0.472$; data not shown).

Discussion

Generally, average germination rates across treatments varied greatly between species and were below 10% for *P. major*, *R. obtusifolius* and *V. sepium* and more than 80% for *T. pratense* and *T. repens* with all other species between these rates. Aqueous extracts of *Rumex* leaves selectively caused significant alterations in the germination and growth of seeds of native grassland species. *Rumex* extracts not only caused a delay of germination but for 6 of 14 species completely inhibited germination. This may be due to the presence of growth inhibitory or phytotoxic substances in the extracts that were released during extraction. It also indicates that allelochemicals are partially water-soluble and may be leached from *Rumex* tissue leaching during rain for instance when *Rumex* leaves are no removed after mechanical control through cutting and decomposing on the soil surface where it may affect the growth of newly emerging native seedlings or newly sown species. However, field results of the current study also indicate that this effect may be less pronounced in natural soil since spraying of *Rumex* extracts did not affect species germination. The lack of an effect in field applications can also be explained by the wet conditions during the field experiments that may have diluted allelochemicals while drier conditions might have revealed different results. Interestingly none of the tested species were promoted by *Rumex* extracts, indicating that the usually high contents of nitrogen and other nutrients in *Rumex* tissue (ZALLER 2004a) had no beneficiary effect on seed germination.

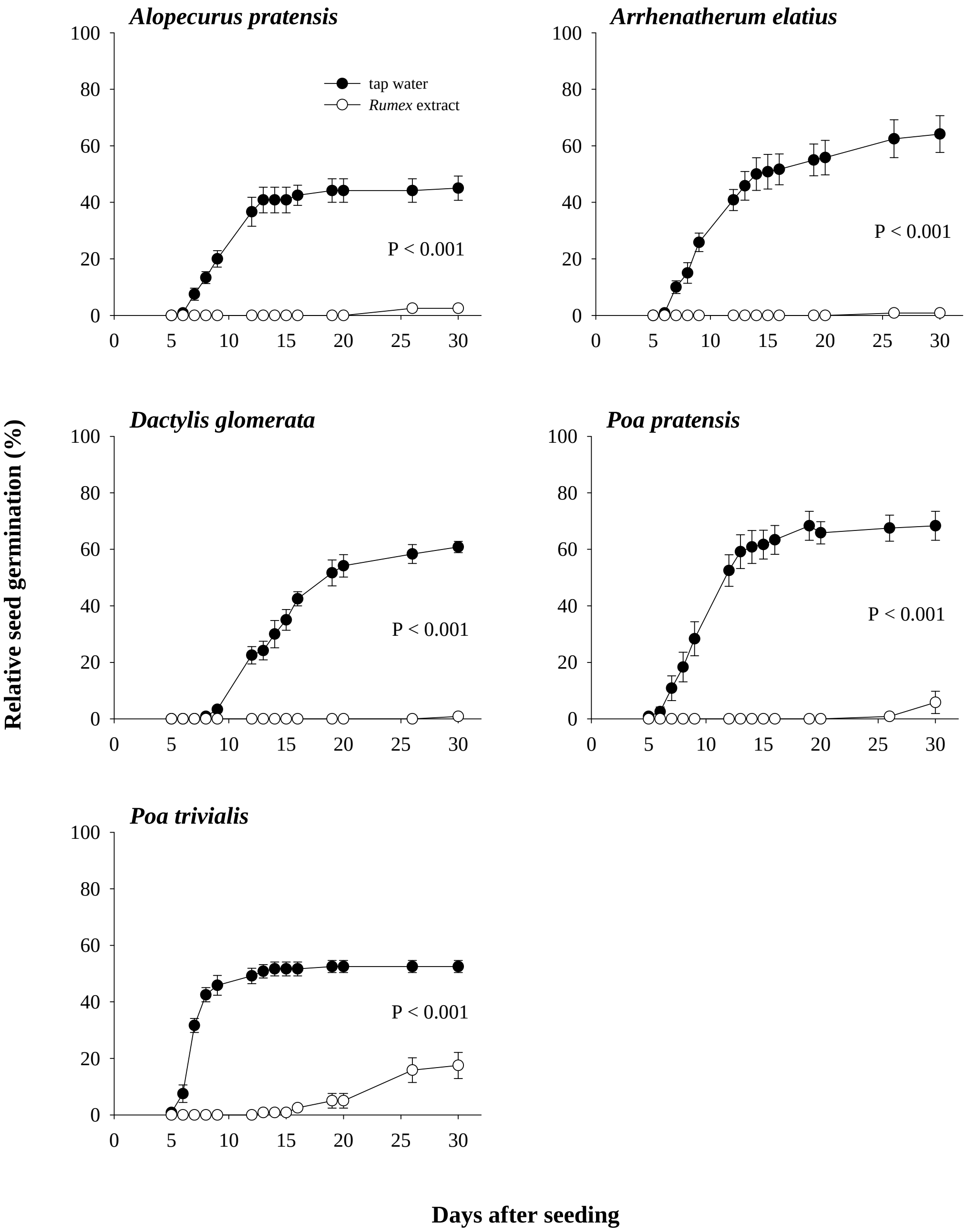

Fig. 1: Seed germination of grass species after watering with either aqueous extracts of *R. obtusifolius* or tap water (means of two dates ± SE, n = 6). P-values denote differences between treatments derived from repeated measures ANOVAs.

Abb. 1: Samenkeimung von Grasarten nach Bewässerung mit wässrigen Extrakten von *R. obtusifolius* oder Leitungswasser (Mittelwerte von zwei Zeitpunkten ± Standardfehler, n = 6). P-Werte bezeichnen Behandlungsunterschiede aus Varianzanalysen für Messwiederholungen.

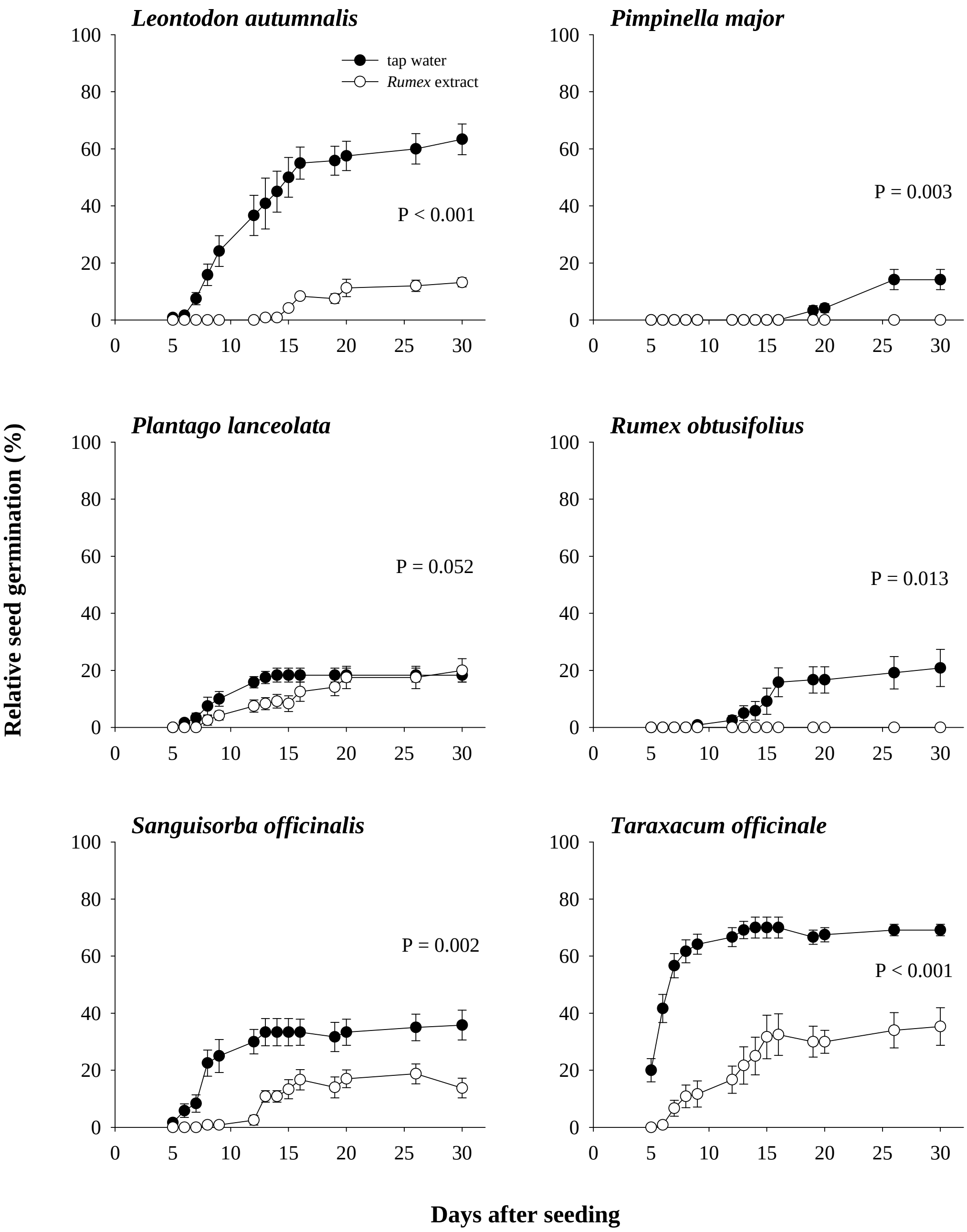

Fig. 2: Seed germination of non-leguminous herbal species after watering with either aqueous extracts of *R. obtusifolius* or tap water (means of two dates ± SE, n = 6). P-values denote differences between treatments derived from repeated measures ANOVAs.

Abb. 2: Samenkeimung von krautige Arten nach Bewässerung mit wässrigen Extrakten von *R. obtusifolius* oder Leitungswasser (Mittelwerte von zwei Zeitpunkten ± Standardfehler, n = 6). P-Werte bezeichnen Behandlungsunterschiede aus Varianzanalysen für Messwiederholungen.

Allelopathic effects are often due to synergistic activity of several allelochemicals rather than to single compounds (WILLIAMSON 1990) and under field conditions, additive or synergistic effects have been shown to become significant even at low concentrations (EINHELLIG and RASMUSSEN 1978). Substances most likely responsible for allelopathic effects of *R. obtusifolius* are secondary metabolites (CARRAL *et al.* 1988). In the field several other factors simultaneously influence the interference between *Rumex* and neighbouring plants like competition for above- and belowground resources (ZALLER 2004a) or differential use of soil nutrient microsites (J. ZALLER, unpublished data) that may be overriding allelopathic effects. However, despite a large body of experimental evidence that supports the existence of both allelopathy and competition, only very few studies examined their relative roles with the result that both allelopathic leaf leachates and resource competition both contributed to a species' ability to suppress seedling growth of another species (NILSSON 1994, RIDENOUR and CALLAWAY 2001).

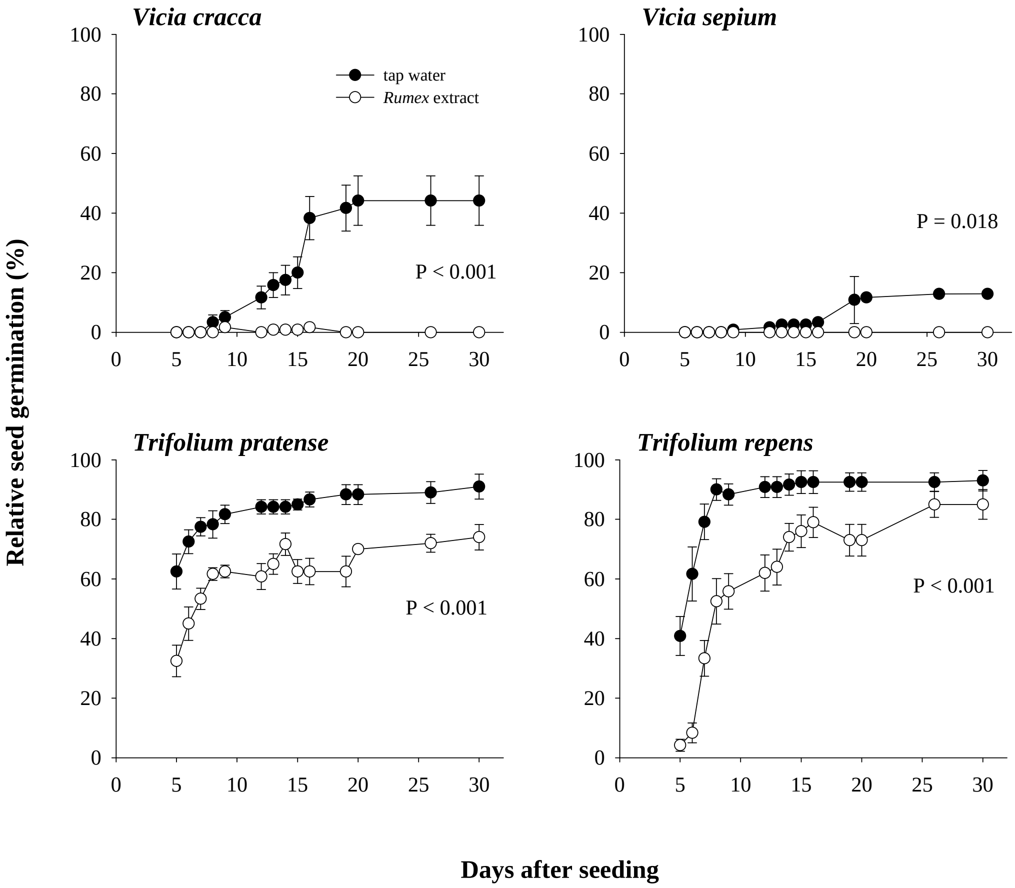

Fig. 3: Seed germination of leguminous species after watering with either aqueous extracts of *R. obtusifolius* or tap water (means of two dates ± SE, n = 6). P-values denote differences between treatments derived from repeated measures ANOVAs.

Abb. 3: Samenkeimung von Leguminosen nach Bewässerung mit wässrigen Extrakten von *R. obtusifolius* oder Leitungswasser (Mittelwerte von zwei Zeitpunkten ± Standardfehler, n = 6). P-Werte bezeichnen Behandlungsunterschiede aus Varianzanalysen für Messwiederholungen.

Constrasting results of the allelopathic potential of a species from laboratory and field experiments are frequently reported (STOWE 1979; KEELEY 1988, , HENN *et al.* 1988; MICHELSEN *et al.* 1995, INDERJIT and WESTON 2000) and support the conclusion that bioassays conducted in controlled artificial environments in the absence of soil can greatly overestimate the importance of allelopathy because soil can deactivate secondary metabolites (KROGMEIER and BREMNER 1989). However, in contrast, other studies found significant correlations between allelopathic interactions in laboratory, field or pot experiments (WARDLE *et al.* 1996, BERES and KAZINCZI 2000). Beside the deactivation of allelochemicals in the soil, in the field also other environmental factors such as temperature, length of the photoperiod (LOBON *et al.* 2002) or species diversity (WARDLE *et al.* 1998) are supposed to alter allelopathic activity. Especially the latter factor might also play a role in the current study, since it is suggested that in a species-rich community, allelopathic plant species are less likely to have a dominating effect on plant community structure and that there is less opportunity of plant communities and ecosystem function being impaired by the secondary metabolites of a single plant species (WARDLE *et al.* 1998). In the current study *Rumex* extracts were applied in a diverse organically managed pasture system consisting of more than 50 plant species (TERREN 2002). Therefore it can be assumed that allelopathy is probably less important here than in a more species-poor system. Indeed, successful effects of allelopathic plants in the field are derived mainly from less diverse systems (e.g. WARDLE *et al.* 1998, BOHREN *et al.* 2004).

Not much is known whether allelopathic leachates (extracts) either accumulate at sufficient concentrations or persist long enough under normal growing conditions to inhibit the growth or development of other plants (e.g. CHAVES *et al.* 2003). In the current experiments we only tested extracts from leaves, although roots are also known to have allelopathic potential (NEWMAN and MILLER 1977). Root mediated allelopathy would depend on many factors such as plant densities, root distributions, root densities and microbial activity and would be especially relevant for the very durable rhizomes of *Rumex* species enabling an accumulation of these substances in the soil for a very long time. Generally, allelopathy is thought to be not only unidirectional, thus also *R. obtusifolius* has been shown to be allelopathically affected by other species (CHAVES and ESCUDERO 1997); this might also have implications for non-chemical control of noxious *Rumex* species (CHAVES *et al.* 2003).

In conclusion, results of this study show that the nature of *Rumex* allelopathy seems highly selective where all grass species tested were heavily delayed or inhibited in their germination by *Rumex* extracts, non-leguminous herbs were either inhibited (*L. autumnalis*, *P. major*, *R. obtusifolius*) or not affected (*P. lanceolata*). Leguminous species *V. cracca* and *V. sepium* were inhibited in their germination, while clover species (*T. pratense*, *T. repens*) only showed a delayed germination. Leaching of allelochemicals from live or dead *Rumex* tissue can thus be suspected to also play a role in forming dense monospecific patches in grasslands in which the establishment of other native species is eliminated. Although allelopathy is probably not playing the most important role in creating species patterning in this highly diverse pasture system, results from this experiment could be considered for selecting seeding mixtures for grassland regeneration that contain species less susceptible to *Rumex* allelopathy in order to reduce the tremendous regrowth potential of *R. obtusifolius* in these systems.

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