Regeneration of *Phragmites australis* from rhizome and culm fragments: an experimental test of environmental effects, population origin and invasion status

Regenerace *Phragmites australis* z úlomků oddenků a stébel – test vlivu prostředí, geografického původu populace a invazního statutu

Jan Čuda[1], Hana Skálová[1], Laura A. Meyerson[2] & Petr Pyšek[1,3]

[1]*Czech Academy of Sciences, Institute of Botany, Department of Invasion Ecology, CZ-252 43 Průhonice, Czech Republic, e-mail: jan.cuda@ibot.cas.cz, hana.skalova@ibot.cas.cz, pysek@ibot.cas.cz;* [2]*Department of Natural Resources Science, The University of Rhode Island, Kingston, Rhode Island, 02881 USA, e-mail: lameyerson@uri.edu;* [3]*Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ-128 44 Prague, Czech Republic*

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Regeneration from vegetative fragments is common in plants that occur in disturbed and wet habitats but quantitative data comparing regeneration of different plant parts under various environmental conditions are still scarce. *Phragmites australis* is a dominant and a keystone wetland species that is widespread all around the world. It spreads both vegetatively by rhizomes and stolons and generatively by seed. Detached vegetative fragments of culms and rhizomes can support local regeneration after disturbance and spread populations over considerable distances when transported with water, soil and other means. In *P. australis*, there is no information on culm regeneration and how regeneration differs between the clones of different origin and what is the effect of the environment. Here we studied the regeneration of *P. australis* from culm and rhizome fragments over six weeks (mid-June to late July 2017) in a common-garden pot experiment. To simulate various scenarios that can happen in nature, we placed cuttings of culms and rhizomes (representing propagules) in 6-l pots in water, on the sand surface, and buried them 5 cm deep in the sand. We included 19 distinct clones representing populations from three phylogeographic groups (North American invasive, North American native, and European). We tested the effect of phylogeographic group, plant part (culm, rhizome), environment (water, surface, buried), ploidy and genome size on clone regeneration using generalized mixed-effect models. A higher percentage of culms than rhizomes regenerated (69% vs. 37%, respectively). Regeneration was better in fragments placed in water than in those buried and on the sand surface (65%, 50%, and 44%, respectively). Although we found considerable differences in regeneration among particular *Phragmites* populations (ranging from 31% in one of the North American native tetraploids to 90% in a North American invasive octoploid), the effect of the phylogeographic group was not statistically significant. However, phylogeographic group interacted with plant part – culms of the North American invasive populations regenerated better than those of North American natives, while rhizomes did not differ among phylogeographic groups. This difference was most pronounced in the sand-surface treatment. Rhizome fragments produced greater culm- and root biomass than culm fragments and North-American native clones produced the least new biomass of all groups. Lastly, rhizomes regenerated more slowly than culms (16 and 13 days to produce new shoots, respectively), and regeneration was fastest in water. Our results point to a great regeneration ability of culm fragments, which can cope with a wide range of environmental conditions and grow rapidly to produce new plants. We suggest this played an important role in spreading the invasive populations

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in North America, whose culm fragments regenerated better than those of native populations. Culms of invasive populations did not require to be permanently exposed to water for regeneration, which may have also facilitated their spread to drier habitats and their niche expansion beyond wetlands.

Keywords: aboveground biomass, belowground biomass, common garden experiment, common reed, culm, dispersal, disturbance, genotype, invasive plant, rhizome, regeneration, shoot, stem, vegetative reproduction, wetland

Introduction

Modes of reproduction and dispersal greatly influence species distributions; those species that can spread both generatively and vegetatively are competitively favoured because switching between the various modes enables a plastic response to changing environmental conditions (Klimešová & Klimeš 2007). Some aquatic plants can resprout from almost any detached segment, such as *Ceratophyllum demersum* or *Elodea canadensis*, and this kind of spread likely contributed to their cosmopolitan distributions (Cook 1985, GBIF 2020). Others rely on organs like turions, stolons, or rhizomes. Stolons and rhizomes facilitate local spread and establishment of new ramets through connection with the mother plant, which supplies them with nutrients, enhancing their competitiveness and stress tolerance (e.g. Březina et al. 2006). However, these organs also enable long-distance dispersal and stand regeneration following disturbances that not only create propagules by fragmenting plants but also can transport fragments over considerable distances (e.g. Boedeltje et al. 2003, Bhattarai & Cronin 2014). Such disturbances include flash floods (Combroux et al. 2001, Boedeltje et al. 2003, Riis & Sand-Jensen 2006), hurricanes (Bhattarai & Cronin 2014), wave action (Smulders et al. 2017), or earth- and construction works (Bart & Hartman 2003, Brisson et al. 2010). Vegetative dispersal is rather common and diverse in plants that grow in or nearby water (Sádlo et al. 2018) because regenerating fragments are prone to desiccation. For example, Barrat-Segretain et al. (1998) found that plants in sites periodically disturbed by floods produce several types of fragments of which at least one has a high regeneration capacity. The ability of a fragment of the plant body to regenerate depends on its characteristics, such as resistance to desiccation, inundation or sediment burial, or fragment size that interact with the environmental conditions (Bímová et al. 2003, Shen et al. 2005, Weber 2011). Moreover, it has been shown that the ratio between the generative and vegetative reproduction affects the success of alien species introduced beyond the area of their native distribution (Pyšek 1997).

In this study, we assessed regeneration from culm and rhizome fragments in the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. from the *Poaceae* family. *Phragmites australis* is a cosmopolitan species that naturally occurs on all continents except Antarctica (Packer et al. 2017); the populations of European origin, classified as *P. australis* subsp. *australis*, invaded wetlands in North America, leading to alteration of wetland communities and displacement of native American populations, *P. australis* subsp. *americanus* (Chambers et al. 1999, Saltonstall 2002, Meyerson et al. 2009, Packer et al. 2017). *Phragmites australis* is a tall helophytic perennial grass that often dominates both aboveground, where culms grow up to ~4 m, and belowground, where rhizomes form extensive and dense mats (Haslam 1972, Pyšek et al. 2019). It reproduces and spreads both generatively by seed and vegetatively by stolons and rhizomes (Haslam 1972,

Packer et al. 2017). The latter mode of reproduction was thought to prevail (Haslam 1972, Hudon et al. 2005), but the importance of seed dispersal has also been highlighted (Belzile et al. 2010, McCormick et al. 2010). Overall, seed dispersal is important during population establishment following transportation over long distances, while vegetative spread is key in space pre-emption during further phases of population development. However, common reed vegetative spread also occurs over longer distances following disturbance and transport of plant parts elsewhere; this is common along watercourses after floods (Barrat-Segretain et al. 1998, Boedeltje et al. 2003).

Fragmentation in *P. australis* is quite common; plants are often broken into pieces by flood, waves, construction works, or management intervention in the reed stands (Hudon et al. 2005, Meyerson et al. 2014). If a disturbance such as a flood occurs, plants can be uprooted or broken and the fragments of shoots, rhizomes, leaves, etc. washed away by running water and dispersed to other areas (Johansson & Nilsson 1993). Importantly, culm fragments are produced more often than those of rhizome because the latter are protected by soil. On the other hand, if rhizome fragments are created, they can be dispersed by water and transported soil, increasing the chance of establishment and likely can survive longer than culm fragments due to stored resources.

Although the species is among the most studied invasive plants in terms of ecology (Chambers et al. 1999, Haslam 2010, Price et al. 2014, Meyerson et al. 2016a, Packer et al. 2017, Pyšek et al. 2020), physiology (Modzder & Zieman 2010, Eller et al. 2017, Pyšek et al. 2019), karyology (Meyerson et al. 2016b, Pyšek et al. 2018) or genetics (Kettenring & Mock 2012, Lambertini 2016), little is known about its vegetative regeneration ability that is crucial for its spread, colonization of new habitats as well as recovery of disturbed populations. There is no information on culm regeneration, and little is known about how regeneration differs between the clones of different origins and what is the effect of the environment. To close this gap, we experimentally tested the regeneration capability (i) of fragments originating from different plant parts, i.e. culms vs. rhizomes, (ii) under different environmental treatments representing situations that can occur in nature, in terms of the mode of burial and water availability, and (iii) compared populations of different origin and invasion status, i.e. native vs. invasive, ploidy levels and genome sizes.

Material and methods

Plant material and experimental setup

We tested the regeneration of *P. australis* culm (i.e. aboveground part of stem) and rhizome fragments in a common-garden pot experiment at the Institute of Botany of the Czech Academy of Sciences in Průhonice, Czech Republic. The experimental garden (49°59'38" N, 14°33'57" E) is located 320 m a.s.l. in the temperate climatic zone, with a mean annual temperature of 8.6 °C and annual precipitation of 610 mm.

On 29 June 2015, plant material from the living collection of *P. australis* populations housed at the Department of Ecology of the Institute of Botany was propagated in 45-l pots filled with sand mixed with 240 g of slow-release fertilizer Osmocote Pro (release time 12–14 months; ICL Specialty Fertilizers). After two years of growing, on 13–14 June 2017, culms and rhizomes were extracted and cut into fragments of standardized

length (20 cm). Before planting, all fragments were weighed on a digital scale with an accuracy of 0.01 g, and nodes were counted as the weight and node number are characteristics known to affect regeneration (Cordazzo & Davy 1999, Weber 2011). The mean fragment weight was 6.7±3.2 g (mean±SD) for rhizomes and 1.4±0.4 g for culms, the mean number of nodes was 6.4±2.3 (range 2–18) for rhizomes and 3.5±1.0 (range 2–7) for culms. Within each clone, fragments of similar thickness were used because individual clones differed in this characteristic (Pyšek et al. 2018). We propagated well-developed rhizomes without terminal buds (Electronic Appendix 1), and cuttings from the basal part of the culm. Culms were cut ~1 cm above the soil surface to clearly distinguish between the regeneration from above- (culm) and belowground (rhizome) plant part. Culm internodes from the basal part were more compressed and lignified compared to those from the middle or apical parts of culms. There were no green leaves at the bottom of culms; we only removed dry leaves when present. For rhizomes, we shortened the roots to ~1 cm so that their biomass did not affect the initial rhizome weight. We included clones of 19 distinct populations representing three phylogenetic groups (North American invasive, North American native, and European, including the whole of Russia; Table 1). This was the same set of clones, except for one that grew too poorly to be included, that were used in our previous experiment focused on the competition among clones (Pyšek et al. 2020).

Table 1. – Characteristics of clones used in the experiment. Note that plants from the whole of Russia are coded as EU native.

Clone ID	Origin and status	Ploidy (x)	Genome size (2C-value in pg)	Country	Latitude	Longitude
D615	EU-native	4	1.89	Russia (Sakhalin)	47°1'48"N	143°18'0"E
FRA3	EU-native	4	1.92	France	44°40'48"N	1°1'12"W
D620	EU-native	4	2.06	Spain	40°43'12"N	0°34'48"E
D643	EU-native	4	2.06	Italy	44°43'12"N	11°31'48"E
D659	EU-native	6	2.96	Russia (Sakhalin)	48°37'48"N	142°47'24"E
D538	EU-native	6	3.02	Romania	45°0'0"N	29°13'12"E
D589	EU-native	8	3.88	Romania	45°0'0"N	29°13'12"E
D553	EU-native	8	3.95	Hungary	47°36'0"N	17°1'48"E
NA134	NA-invasive	4	1.94	USA (MD)	38°35'24"N	76°3'0"W
NA94	NA-invasive	4	1.95	USA (RI)	41°10'48"N	71°34'12"W
NA96	NA-invasive	4	2.17	USA (NH)	43°3'0"N	70°54'0"W
NA159	NA-invasive	4	2.21	USA (RI)	41°21'36"N	71°38'24"W
NA148	NA-invasive	6	3.18	USA (MA)	41°28'12"N	70°45'36"W
USA2	NA-invasive	8	3.90	USA (MA)	42°20'24"N	71°5'24"W
D617	NA-invasive	8	4.12	USA (RI)	41°47'24"N	71°22'12"W
NA124	NA-native	4	2.24	USA (NH)	43°3'0"N	70°54'0"W
NA61	NA-native	4	2.25	Canada (NB)	46°4'12"N	64°43'12"W
NA7	NA-native	4	2.25	USA (NY)	42°56'24"N	76°44'24"W
NA8	NA-native	4	2.30	USA (NY)	42°56'24"N	76°44'24"W

To simulate different scenarios that may occur for a plant fragment (propagule) in the habitat typically harbouring *P. australis* stands, we placed cuttings of rhizomes and culms in 6-l pots (i) in water, (ii) on the sand surface, and (iii) buried them in the sand

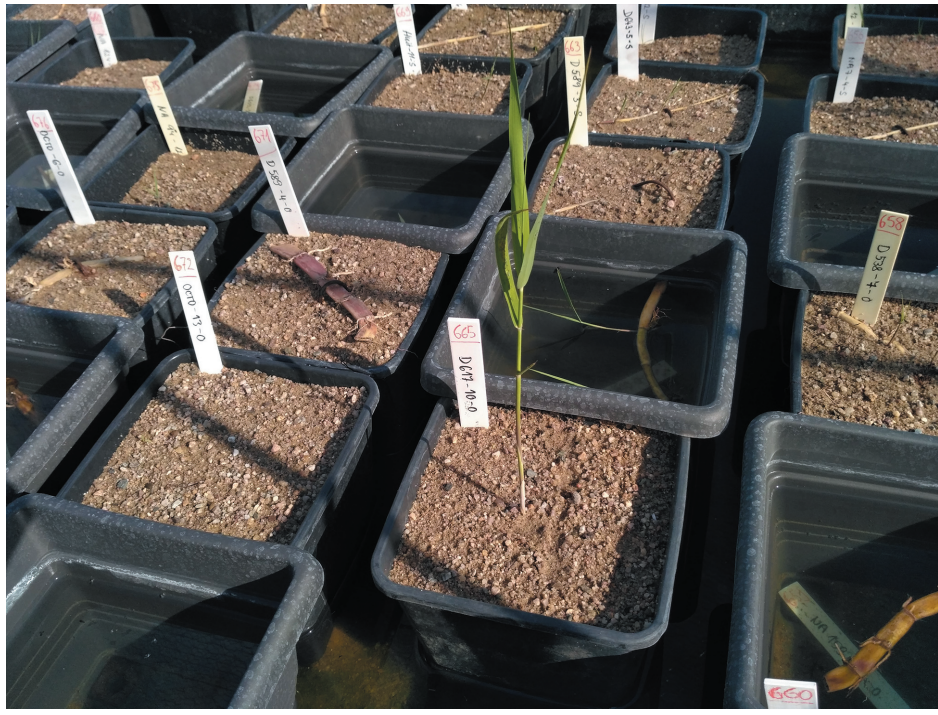


Fig. 1. – Experimental design. Rhizome and culm fragments were placed in water, on the sand surface, and buried 5 cm deep in sand. The plant in the centre is NA-invasive clone D617, regenerating from a buried rhizome fragment. The picture was taken on 10 July 2017, i.e. 27 days after the beginning of the experiment.

(Fig. 1). Pots with sand were 20 × 20 × 23 cm, pots with water 25 × 25 × 20 cm; pot size was chosen to enable placing the fragment diagonally without touching the pot walls. In total, we had 912 pots (19 clones × 3 environments × 2 plant parts × 8 replicates). For the water treatment, we used pots without holes, and water inside the pots was kept at the same level (~5 cm from its upper edge) to ensure similar light conditions for all pots. The fragments were freely floating on the water surface. We used water from a well to fill pots and for watering. We changed the water when algae started to emerge to protect fragments from rotting. The pots with sand had holes in the bottom to ensure permanent uptake of water. The fragments on the sand were attached by a U-shaped piece of wire to fix them to the surface and prevent them from being blown away by the wind. The fragments placed below the sand surface were buried 5 cm deep in a horizontal position.

All pots were randomly placed next to each other in plastic pools, filled with 5 cm of water that kept sand in the pots moist (see Electronic Appendix 2 for details of the experimental setting). A wire mesh protected the beds against birds (magpies steal labels in the garden, and other bird species used stripes of reed leaves as a nest-building material).

All pots were irrigated twice a day, in the morning and evening, for one minute, using common garden sprinklers with subsequent visual control and manual watering of not appropriately watered pots. These arrangements were adopted to prevent the sand-surface desiccation during the day when pots were exposed to direct sun.

Characteristics measured

The experiment was established on 13–14 June 2017, and harvested after 42 days on 25 July 2017. During that period, data were recorded 13 times in an interval of 2 to 4 days (on 20, 23, 26, 28, 30 June, and 4, 7, 10, 12, 14, 17, 20, and 24 July). During each sampling, all pots were checked for fragment new shoot regeneration, i.e. whether new culms were present. At harvest, all fragments were excavated, gently washed in the water, and checked for any sign of regeneration. New roots and culms were clipped, dried in the oven at 60 °C, and weighed on scales with an accuracy of 0.001 g. New rhizomes were recorded only in a few cases, and their biomass was marginal compared to new culms; therefore, it was added to that of the culms.

The regeneration was assessed by using the following measures: (i) regeneration ability (whether new culms or roots were produced at harvest), (ii) regeneration extent (the biomass of the roots and culms produced), (iii) time to regeneration (the number of days since the beginning of experiment till the emergence of the first new culm); and (iv) regeneration ability over time, reflecting changes over time in the formation and die-off of the newly formed culms.

Statistical analysis

The measures of regeneration described above represented the response variables: regeneration ability (new culms or roots produced; yes/no), regeneration extent (weight of new culms and roots; g), time to regeneration (days), and regeneration ability over time (new culms recorded in each of the 13 sampling times; yes/no). We tested the effect of the following predictors: the phylogeographic group with each clone's membership defined based on geographic origin (NA – North America; EU – Europe) and status (NA-invasive, NA-native, EU-native); plant part (culm, rhizome); environment simulated by the treatment (water, sand surface, buried in the sand); ploidy (tetraploid, hexaploid, octoploid); genome size (the amount of nuclear DNA, see Table 1 for data and Pyšek et al. 2018, 2020 for details on measuring karyological characteristics); and time (days, in the analysis of regeneration ability over time) on clone regeneration. Further, we accounted for the effect of initial weight and the initial number of nodes of rhizomes and culms. This was specified as an interaction of plant part × initial weight, and plant part × initial number of nodes in the models. This was done because rhizomes were heavier and had more nodes than culms and including only term initial weight and number of nodes without interaction with plant part would thus mask the effect of the plant part. As the main effects of karyological variables, ploidy and genome size, were not significant for any of the response variables in the preliminary models (tested by dropping each term and comparing the model without it to the full model), we did not include them in further models.

To test regeneration ability, we used generalized mixed-effect models with the binomial distribution (regenerated yes/no). Clone identity was set as a random factor, as we were not primarily interested in individual clones; however, we a priori expected similar growth patterns within individual clones. For the predictors group, plant part and environment, we tested the main effects and their two-way interactions and we further included the interaction plant part × initial weight, and plant part × initial number of nodes. Models with higher interactions (three- and four-way) were also tested, but did not significantly differ from those with only two-way interactions; therefore, only the latter

are presented. For the weight of new culms and roots, we used linear mixed-effect models and response variables (weight of new culms and new roots) were log-transformed to meet the models' assumptions (normality and homoscedasticity).

In the analyses of regeneration extent, we included only plants that produced new biomass, i.e. zero values were excluded because they were evaluated in the previous step, analyses of regeneration ability. The time to regeneration response variable was square-root transformed to meet the assumptions of the models. The model settings were the same as for regeneration ability, i.e. clone identity was set as a random factor, and only two-way interactions were included.

In analyses of regeneration ability over time, a generalized mixed-effect model with the binomial distribution (the plant was regenerating with new culms at a given time, yes/no) was used. The predictors were the same as for the previous models (group, plant part, environment), but time and its square- and cubic terms were included via "poly" function in R to assess linear and non-linear variations over time. Similarly, clone identity was set as a random factor to account for similar growth characteristics within clones, and pot identity was used as a second random effect nested within the clone to reflect the fact that we repeatedly assessed the same plant fragment in a pot.

The differences in significant terms were tested post hoc by Tukey HSD pairwise comparison of estimated marginal means (Lenth 2018). Apart from R base packages, we used package lme4 for fitting linear and generalized mixed-effect models (Bates et al. 2015), and package emmeans for subsequent multiple comparisons among significant terms (Lenth 2018). Graphs were plotted using the tidyverse package (Wickham et al. 2019). All computations were done in programme R 4.0.2 (R Core Team 2020).

Results

Regeneration ability

The greatest differences were found between plant parts – culms regenerated much better (on average, 69% created new culms and/or roots) than rhizomes (37%, Table 2 and Fig. 2). Regeneration was significantly better in water (65%) than in buried (50%) and sand-surface (44%) treatments (Fig. 2). There were considerable differences in regeneration among individual clones (ranging from 31% in one of the North American native tetraploids to 90% in one North American invasive octoploid); still, the differences among the phylogeographic groups were not significant. There was a significant interaction between the environment and the plant part. Rhizomes performed better in water than on the surface, and those that were buried did not differ from the other two treatments. Culms also regenerated best in water, but they differed significantly from those on the sand surface or buried (Fig. 2). Culms of the NA-native group regenerated less than those of the NA-invasive group, but rhizomes did not significantly differ among groups.

Regeneration ability was also affected by the initial weights of fragments and numbers of nodes, with a positive effect of culm weight and rhizome node number and a negative effect of rhizome weight and culm node number. We did not record any dormant fragments – all fragments either regenerated or were dead (soft and brown-black colour) at harvest time.

Table 2. – Results of the GLME and LME models showing the effect of phylogeographic group (group), environment (env), plant part, initial weight (i weight) and initial number of nodes (i nodes) and their interactions on *Phragmites* regeneration ability (yes/no), new culm and root weight and time to regeneration. Clone identity was included as a random factor in the models. The model included n = 912 pots with 19 clones, regenerating from culms or rhizomes, planted in three environments, each combination in 8 replicates.

	Regeneration ability (GLME)			New culm weight (LME with log)			New root weight (LME with log)			Time to regenerate (LME with sqrt)		
Predictor	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
group	1.1	2	n.s.	9.5	2	0.009	12.5	2	0.002	2.5	2	n.s.
env	23.3	2	<0.001	64.7	2	<0.001	23.7	2	<0.001	229.3	2	<0.001
plant part	83.5	1	<0.001	17.6	1	<0.001	7.2	1	0.007	8.7	1	0.003
group × env	5.1	4	n.s.	3.8	4	n.s.	4.9	4	n.s.	4.2	4	n.s.
group × plant part	6.8	2	0.032	0.5	2	n.s.	2.0	2	n.s.	0.1	2	n.s.
env × plant part	12.8	2	0.002	3.5	2	n.s.	8.5	2	0.014	30.4	2	<0.001
plant part × i weight	10.8	2	0.004	22.9	2	<0.001	35.6	2	<0.001	1.2	2	n.s.
plant part × i nodes	7.3	2	0.026	3.0	2	n.s.	1.9	2	n.s.	0.4	2	n.s.

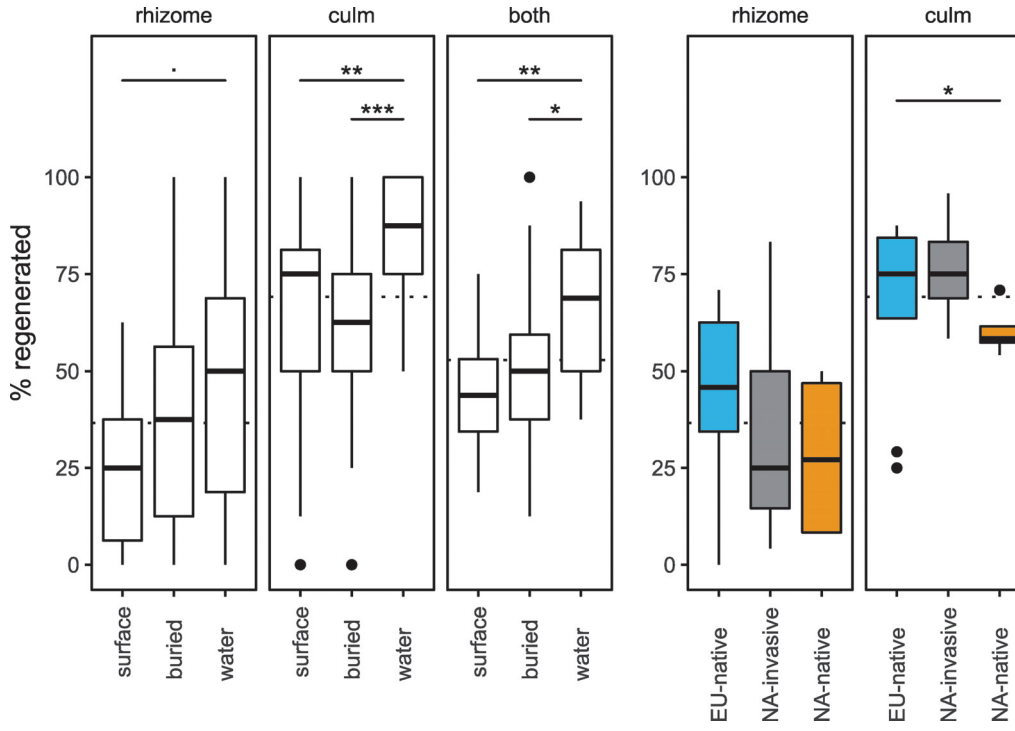


Fig. 2. – Regeneration of *Phragmites* rhizome and culm fragments in three environments over six weeks in relation to plant part and environment (left) and phylogeographic group (right). Dotted lines represent mean values of regeneration within each box. Significance P: . (0.1–0.05), * (0.05–0.01), ** (0.01–0.001), *** (< 0.001), non-significant values not shown. We used mean values of regeneration for each clone, boxplots in first two boxes from the left are based on n = 19, the middle on n = 38, the two on the right on n = 24, 21, 12 for EU-native, NA-invasive and NA-native, respectively.

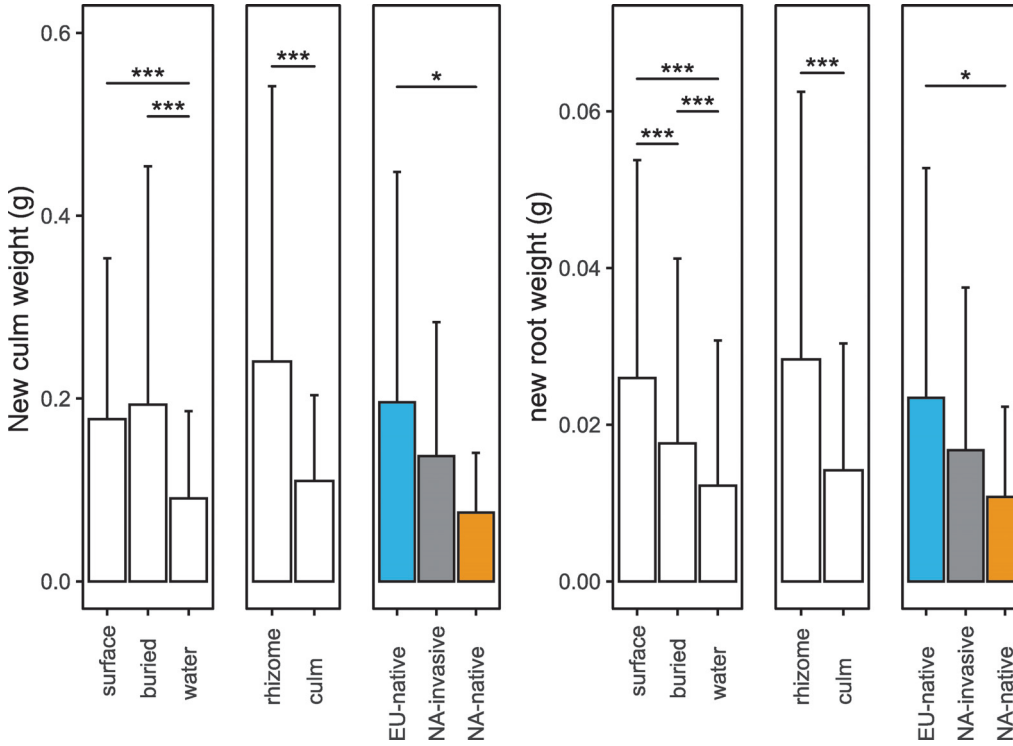


Fig. 3. – The effect of environment (left), plant part (centre) and phylogeographic group (right) on weight of new culms and roots. Bars show mean values, error bars refer to a standard deviation and significance P of the difference is indicated: . (0.1–0.05), * (0.05–0.01), ** (0.01–0.001), *** (< 0.001), non-significant values are not shown.

Regeneration extent

The weight of regenerating culms was most affected by the environment (Table 2); those on the surface and buried produced about twice as much new biomass than those in the water (Fig. 3). The heaviest roots were produced on rhizomes on the surface, those on buried rhizomes had lower weight, and the lightest were those in the water. Culm and root weight also differed among phylogeographic groups – EU-native clones produced significantly more biomass than NA-native clones, while NA-invasive clones did not significantly differ from the other two groups. New culms and roots on rhizomes were about twice as heavy as those on culms, and there was a significant plant part × environment interaction showing that rhizomes built most roots when buried or on the surface. In contrast, the new root biomass of culms was greatest if they were placed on the surface. Lastly, new culm- and root biomass was positively affected by the initial fragment weight, and this effect was more pronounced in rhizomes.

Time to regeneration

Rhizomes regenerated significantly later than culms; on average, it took 16 and 13 days, respectively, to build new culms (Table 2, Fig. 4). The most important factor was the environment – regeneration was faster in water and slower when buried or on the surface.

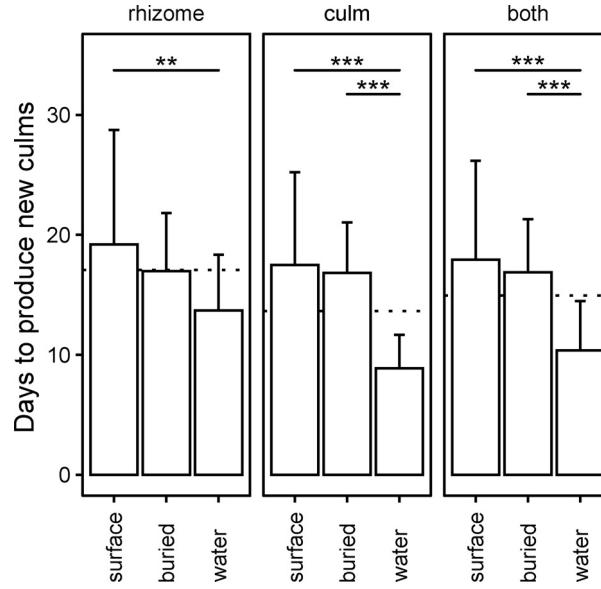


Fig. 4. – Time to emergence of new culms in rhizome and culm fragments under different treatments representing environments. Bars show mean values, error bars refer to a standard deviation and dotted lines mean values within each box. Significance P: . (0.1–0.05), * (0.05–0.01), ** (0.01–0.001), *** (< 0.001), non-significant values are not shown.

Time to regeneration did not differ among phylogeographic groups and was not affected by the initial weight or number of nodes.

Regeneration ability over time

Regeneration ability (presence of new stems at a given time) of fragments significantly changed over time, and time interacted with the other factors (Table 3). Regeneration was fastest in water, where almost half of the fragments regenerated after one week. Still, the regeneration rate somewhat dropped again after 24 days, often due to the death of some newly formed shoots (Fig. 5). Regeneration was initially faster on the sand surface than for buried fragments, but after 10 days, the buried fragments sped up and finally regenerated more quickly (Fig. 5). Phylogeographic groups significantly differed in their regeneration dynamics (Table 3). North American native clones were much slower than NA-invasive and EU-native clones (Fig. 5). Culms of all phylogeographic groups regenerated faster than rhizomes, and their regeneration rate did not slow down towards the end of the experiment (Fig. 5). Regeneration ability over time varied with plant part and environment. Culms in water regenerated fastest, while rhizomes on surface slowest, and there was a marked decline in regenerated rhizomes in water and culms in the burial treatment. In contrast, the percentage of regenerated culms on the surface was more or less increasing until the end of the experiment (Fig. 5). This pattern also differed marginally among phylogeographic groups: NA-invasive culms performed best on the surface, while NA-native culms and rhizomes regenerated worst in this treatment (Fig. 5).

Table 3. – Results of the GLME model, showing the effect of phylogeographic group (group), environment (env), plant part, time since the beginning of the experiment (days) and their interactions on regeneration ability of *Phragmites* fragments over time (presence of new stems at a given time). Clone and pot identity were included as random factors in the model. The model included n = 912 pots with 19 clones measured 14 times, regenerating from culms or rhizomes, planted in three environments, each combination in 8 replicates.

	Regeneration ability over time (GLME)		
Predictor	χ^2	df	P
group	7.9	2	0.019
env	165.1	2	<0.001
plant part	147.0	1	<0.001
time	503.3	3	<0.001
group × env	8.9	4	0.064
group ×plant part	6.2	2	0.044
env × plant part	6.6	2	0.037
group × time	18.2	6	0.006
env × time	241.4	6	<0.001
plant part × time	23.4	3	<0.001
group ×env × plant part	2.3	4	n.s.
group × env × time	15.5	12	n.s.
group × plant part × time	7.1	6	n.s.
env × plant part × time	120.0	6	<0.001
group × env × plant part × time	19.1	12	0.085

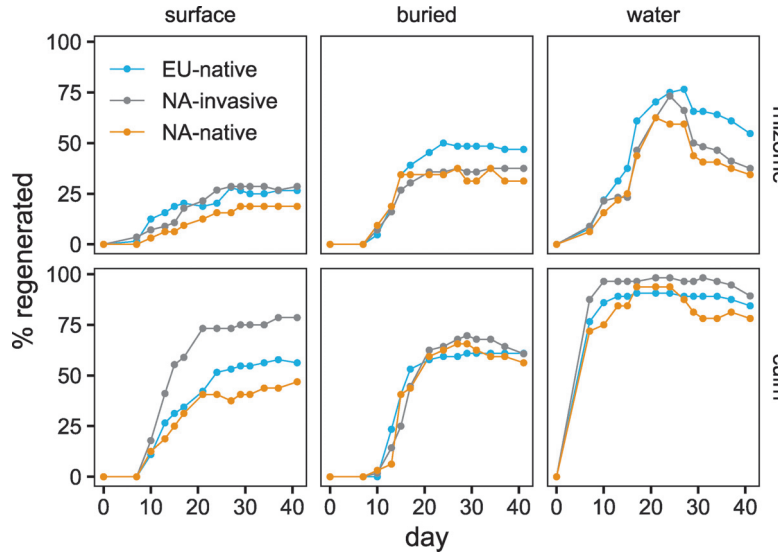


Fig. 5. – Regeneration ability over time (presence of new stems at a given time) in plant fragments during the 41 days of the experiment. Lines consist of 14 time measurements; each datapoint in EU-native is based on n = 64 (8 clones × 8 replicates), in NA- invasive on n = 56 (7 × 8) and in NA-native on n = 32 measurements (4 × 8).

Discussion

Importance of culm fragments in Phragmites regeneration and dispersal

Our results show that fragments from culms of *Phragmites australis* regenerated better than those from rhizomes. In this species, dispersal by vegetative fragments is considered as an important vector of spread (Haslam 1972). However, rhizomes are often reported in the literature as the only option of *P. australis* fragmental propagation, without mentioning the culms (League et al. 2006, Juneau & Tarasoff 2013, but see Véber 1978). On the contrary, our results strongly suggest that culm fragments play an important role in this species' spread. The dispersal of *Phragmites* fragments occurs during various events that disturb reed stands, such as floods (Combroux et al. 2001, Fér & Hroudová 2003), roadside maintenance (Brisson et al. 2010, Juneau & Tarasoff 2013) or hurricanes (Bhattarai & Cronin 2014). Unlike rhizomes, culms are not protected by a soil layer and are more exposed to damage; they are also more likely to be transported due to the disturbance. For example, a flash flood first breaks off the above-ground parts of *Phragmites*, whereas rhizomes remain in the ground. Only floods of great intensity can erode the soil around plants, uproot, and transport rhizomes or whole plants to another place. Out of riverbanks, typically on floodplains, where the damaging force of water is not so strong, underground plant structures are rarely damaged, but culms can break off. Analogously, this holds for other disturbances mentioned earlier, e.g. culm fragments are more often produced during roadside maintenance, like mowing of road verges and ditches. In contrast, rhizome fragments are only produced during earthworks, such as construction works and ditch dredging (Bart & Hartman 2003). The dredging of drainage ditches is performed once in 5–50 years (Dollinger et al. 2015) while mowing usually occurs once or twice a year.

Both culms and rhizomes need contact with soil to root; therefore, an additional disturbance is needed for successful establishment unless the plant fragments arrive at a newly formed surface. Rhizome fragments typically recruit from plants growing in heavily disturbed sites, are usually transported with soil (if not spread by water which washes the soil off), and are buried under sediment, which protects them from desiccation. Culm fragments that land on vegetation or litter do not survive, but shallow puddles can provide them with optimal conditions, where a plant can grow before roots reach the substrate. In water, culm fragments can float for several weeks (the majority was still floating at the end of the experiment due to hollow culms and rhizomes; each fragment contained 1 to 17 cavities) and potentially travel tens to hundreds of kilometers. Such distances can be inferred from flow velocity ranging between ~ 0.9 km/h during normal runoff conditions to 10 km/h during floods, as reported for three rivers in Poland (Wyżga 1999). Lastly, it needs to be noted that rhizomes, unlike culms, are less dependent on fragmentation timing. Rhizome fragments can be produced, dispersed and regenerate all year round, although their regeneration capability varies with time (Juneau & Tarasoff 2013). In contrast, culms regenerate only when young shoots rich in carbohydrates are present, usually not before May in central Europe (Véber 1978).

Heavier fragments produce more biomass

Although regeneration ability in rhizomes was lower than in culms, rhizomes produced new culms and roots that were twice as heavy which probably increases their survival and

competitiveness following transport to new sites. The higher regeneration extent of rhizomes was probably related to their greater initial weight compared to culms (mean±SD: rhizomes 6.8±3.2 g, while culms 1.4±0.5 g). Rhizomes are storage of reserves such as carbohydrates, nitrogen and phosphorus (e.g. Granéli et al. 1992, Klimeš et al. 1999), while culms are primarily a supportive and partly assimilative structure with low energy reserves. The advantage of rhizomes, as storage organs, is their ability to induce more vigorous resprouting from their reserves. Regenerating culms are more dependent on resources available on site. The regeneration capability increases with fragment size; bigger fragments regenerate better and produce more biomass, as previously reported for *Phragmites* (Bart & Hartman 2003, Juneau & Tarasoff 2013), *Arundo donax* (Wijte et al. 2005), *Solidago canadensis* and *S. gigantea* (Weber 2011), *Reynoutria* taxa (Bímová et al. 2003), and *Salvinia natans* (Zhang et al. 2019). Fragment size can further interact with the environment; large fragments with smaller specific surface area are less prone to desiccation and can induce faster growth in the beginning regardless of available nutrients at a site (Bart & Hartman 2003). However, small fragments disadvantaged by a limited nutrient reserves may profit from eutrophication and become more competitive. This needs to be kept in mind because spread of invasive genotypes of *P. australis* was found to be linked with a high nutrient availability (Silliman & Bertness 2004).

The effect of environmental conditions on regeneration

Our results show that both culm and rhizome fragments can cope with a range of conditions that occur in nature, like being covered with a substrate or exposed on the soil or water surfaces. The regeneration ability of fragments was best in water, which suggests that they must deal with water loss on the soil surface, and probably also with soil pathogens and suboptimal light conditions when buried. However, the fragments in water produced the least biomass, as the lack of nutrients probably limited them. The overall poor regeneration ability on the sand surface was especially pronounced in rhizomes. This was likely caused by poor rhizome protection against water loss due to the highly porous rhizome surface, similar to other wetland plants (Armstrong et al. 2006). The culms performed better, probably due to more efficient barriers against water loss, such as a thick cuticle on the epidermis and abundant cuticular wax (Lau et al. 1978), which may also protect the culms pathogens. The regeneration ability in culms buried under the sand was lower than in those placed on the surface; the most plausible explanation is that growing through the layer of sand is too demanding for shoots poorly supported by a small amount of reserves in the absence of photosynthesis.

On the other hand, rhizomes performed best when buried – in this case, the 5 cm layer of sand is unlikely to be limiting for growth because horizontal rhizomes can regenerate from a depth of more than 1 m, depending on soil and hydrological conditions (Haslam 1970). In water, culms did not show as strong a dieback as rhizomes after approximately three weeks of planting. A possible explanation could be that soil pathogens that are abundant on rhizomes decayed some newly formed culms. Véber (1978) reported a 20–25% mortality of young *P. australis* plants as a result of even slight and inconspicuous damage to rhizomes.

It needs to be noted that we did not test regeneration in a brackish environment, which encompasses a large portion of habitats invaded by *P. australis* in North America (e.g. McCormick et al. 2010). Salinity increases the osmotic stress; Bart & Hartman (2003)

found that rhizome fragments regenerated worse and produced less and lower culms in brackish environment. Wave action also damages buds and newly resprouting roots and shoots, which decreases the chance of their establishment.

Methodological issues

The regeneration capability of fragments varies greatly over the season; Juneau & Tarasoff (2013) report 71.1% regeneration in *Phragmites* rhizomes collected in November, but only 15.6% in those in June. This variation is caused by the changing amount of resources in rhizomes, that are transported from culms to rhizomes during the growing period, and then in spring allocated to the growth of above-ground tissues (Granéli et al. 1992). This may explain the relatively good performance of both rhizome and culm fragments in our experiment because both of them possessed some resources (carbohydrates, nitrogen and phosphorus) when the experiment was running. For logistic reasons, namely the need to propagate plants sufficiently to establish the experiment, we collected the rhizomes in late spring. It is likely that rhizomes collected in autumn, winter, or early spring when they still contain the resources accumulated from the previous season would regenerate better.

We observed worse regeneration and decay of soft herbaceous culms that were not lignified yet, during propagation of clones that we received to our collection from abroad (J. Čuda, personal observation). Véber (1978) compared different types of *Phragmites* propagation (clone division, layering, rhizome and culm cuttings, seed) for commercial use. He reported that cuttings from the apical part of the culm kept in fishpond water with mud achieved the highest root-taking capacity of 9%, which is much less than for our cuttings from the culm base in water (84.9%). The better regeneration from basal internodes in our experiment can be explained by the fact that these fragments have more buds and shorter internodes than is the case for the upper parts. Buds are aggregated close to the ground, where they are better protected from damage (Klimešová & Klimeš 2007).

This experiment was primarily focused on fragment regeneration (resprouting only with using reserves), but we also recorded the first growth phase of newly formed plants (growth with using nutrients from the substrate). Fragments in water were disadvantaged as they could not establish by means of rooting in the substrate. Therefore, a proper assessment of the growth phase would be to establish fragments in soil because substrate properties substantially influence plant growth.

Lastly, it needs to be noted that the emergence of the buried culm fragments was delayed by having to grow through the sand layer. However, recording when they started to regenerate would have required sand layer removal and would not have been possible without disturbing the fragment's growth. Therefore, we assumed that the time needed to grow through the sand is probably just the difference between regeneration in water and under the sand, which was 3.3 and 8.0 days for rhizomes and culms, respectively. Some of the regenerating fragments may have died before reaching surface, while regeneration in water was immediately recorded.

The role of regeneration in Phragmites invasion

The phylogeographic groups differed in regeneration ability of culm fragments. North-American native populations performed worse than populations that are invasive on this continent to which they were introduced from Europe (Meyerson et al. 2009, 2010,

Meyerson & Cronin 2013, Packer et al. 2017, Pyšek et al. 2019). The superior regeneration performance of culm fragments of North-American invasive populations was most pronounced if the fragments were placed on the sand surface, where they regenerated by 40% and 68% better than both European native and North-American native populations, respectively. This pattern might have been reflected in the spread of the alien genotypes in North America and their habitat niche. The invasive alien populations perform better than natives in drier, disturbed and nutrient-rich habitats like ditches along highways (Lelong et al. 2007, Jodoin et al. 2008, Brisson et al. 2010) and agricultural drainage ditches (Maheu-Giroux & de Blois 2007). These well-drained habitats are suitable for *Phragmites* growth and served as source areas for colonization of more hostile habitats (Bart & Hartman 2003). Importantly, the establishment of culm fragments in nutrient-rich habitats may help them to overcome their handicap with initial low reserves stored.

Further, long linear landscape structures, such as ditches, interconnect disturbed and natural habitats, which enables the spread of *Phragmites* on a regional scale (Maheu-Giroux & de Blois 2007, Brisson et al. 2010). The initial spread was mostly due to seed dispersal (Maheu-Giroux & de Blois 2007), later on, the role of vegetative spread, both in terms of site colonization in connection with the maternal plant and long-distance dispersal by fragments, increased with time since introduction. Fragments are more competitive and flexible concerning the environment than seedlings because they can use stored nutrients. Fragments are also more resistant than seedlings to damage during the establishment phase because they can regenerate from multiple points. Clonality facilitates invasiveness in wet and cold climatic areas and is advantageous in less disturbed and more natural habitats (Pyšek 1997). The invasion of *Phragmites* in Canada was slower than in the United States due to colder climate and shorter growing season, restricting both vegetative and generative reproduction (Marie-Victorin 1995).

Our results show that resprouting from vegetative fragments is an important factor influencing the establishment and spread of *Phragmites*. Therefore, the management of invasive stands should monitor the number of fragments produced during various disturbance events and their chance to establish and/or spread. Important is their ability to survive and form roots, which readily happens in water, making the establishment of a new clone possible once the fragments get in contact with soil. Even small fragments are able to regenerate, but the damage of buds and sleeping buds by crushing, which are located at nodes, minimizes the chance of establishment.

See www.preslia.cz for Electronic Appendices 1–2

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Souhrn

Rákos obecný (*Phragmites australis*) je dominantní a klíčový druh mokřadů, vyskytující se po celém světě. Rostliny se rozmnožují a šíří semeny i vegetativně. Části stébel a oddenků oddělené od mateřské rostliny napomáhají po disturbanci regeneraci populace na stanovišti, kde roste, ale mohou přispět k jejímu šíření na značnou

vzdálenost, pokud jsou přeneseny jinam. Zkoumali jsme regeneraci částí stébel a oddenků po dobu šesti týdnů (červen až červenec) v pokusné zahradě. Fragmenty z těchto částí rostlin jsme umístili do šestilitrových květináčů ve vodě, na povrch písku a zahrabané pod 5 cm vrstvu písku, čímž jsme simulovali situace, ke kterým dochází v přírodě. Pracovali jsme celkem s 19 populacemi, které pocházely ze tří fylogeografických skupin (severoamerické původní, severoamerické invazní a evropské populace). Testovali jsme vliv fylogeografické skupiny, části rostliny (stéblo, oddenek), prostředí (voda, povrch, zahrabáno) na regeneraci klonu pomocí zobecněných lineárních modelů se smíšenými efekty. Schopnost regenerace byla vyšší u stébel (regenerovalo jich 69 %) než u oddenků (37 %); fragmenty celkově lépe regenerovaly ve vodě (65 %) než zahrabané (50 %) či na povrchu písku (44 %). Fylogeografické skupiny se celkově nelišily ve schopnosti regenerace, ačkoliv jednotlivé populace se značně lišily, v rozmezí od 31 % u severoamerického původního tetraploida až po 90 % u jednoho ze severoamerických invazních oktoploidů). Fylogeografická skupina nicméně interagovala s částí rostliny: stébla severoamerických invazních populací regenerovala lépe, než stébla původních, a to především na povrchu písku. Oddenky vytvořily více nové biomasy (stébel a kořenů) než stébla a severoamerické původní populace vytvořily ze všech skupin nejméně nové biomasy. Oddenky regenerovaly pomaleji než stébla (nová stébla se vytvořila za 16, respektive 13 dnů od začátku pokusu) a regenerace byla nejrychlejší ve vodě. Naše výsledky ukazují velkou regenerační schopnost i rychlou regeneraci rákosu ze stébel, a to za různých podmínek prostředí. To mohlo přispět k šíření severoamerických invazních populací, které regenerovaly lépe než původní populace. Regenerace ze stébel severoamerických invazních populací byla navíc mnohem úspěšnější v sušších podmínkách na povrchu písku, což může mít vliv při jejich šíření do sušších habitatů.

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