

University of South Bohemia

Faculty of Science



PhD Thesis

**ECOLOGICAL CONSTRAINTS LIMITING THE
ROOT-SPROUTING ABILITY IN WETLAND PLANT
SPECIES**

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ANNOTATION

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Wetland plant species rely largely on vegetative reproduction. Although all types of clonal growth organs are found in wetlands, special adaptations, e.g., turions, fragmentation and budding, are more frequent in true aquatic communities. However, root-sprouting is underrepresented, although it can be beneficial under disturbed conditions. This thesis focuses on ecological constraints potentially hindering root-sprouting in wetlands. This ability was studied in a wetland herb *Rorippa palustris* in relation to life history, injury timing and carbon economy of a plant. In addition, plant regeneration following submergence and severe disturbance was assessed.

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CHAPTER I

Introduction

Introduction

INTRODUCTION

Wetland communities are group of habitats characterized by waterlogged soils and long-term or permanent flooding (Armstrong et al. 1994). These habitats include true aquatic communities with deep water, periodically flooded floodplains, salt marshes with daily inundation and salt stress and also nutrient-poor mires.

The species composition of wetlands is determined by water table fluctuation, anoxia and anaerobic soils with slow decomposition (Sculthorpe 1967). Moreover, there are environmental factors typical of a given type of wetland community. For example, mire communities (bogs, fens) are nutrient limited, whereas species of floodplains and salt marshes have to cope with mechanical stress due to inundation, flooding and grazing (Ellenberg 1988; Esselink et al. 2002; Dormann et al. 2000). In addition, floodplain species often experience alternating flooding and dry periods through the season, whose timing and duration may play an important role in the reproduction and regeneration of species (Klimešová 1994). Elevation is crucial for the distribution of daily inundated salt marsh species and this gradient affects the structure of a salt marsh and is directly related to inundation frequency and hence to sediment deposition, evaporation, aeration, nutrient status, and salinity (Bos et al. 2002).

Ecological and physiological adaptations in wetland species

In comparison to air, the aqueous medium has a greater density and viscosity. It also creates special habitats for plants due to lower diffusion rates, decreased light penetration and various concentrations of soluble gasses. The ancestors of aquatic angiosperms have primary developed in air, but returned to the water environment in the upper Cretaceous and "readapted" to a certain degree (Duarte et al. 1994). However, many aquatic species still posses "terrestrial" traits as a cuticle, functionless stomata, poorly lignified xylem tracheary elements and "terrestrial" reproduction, i.e. flowering and pollination being dependent on air (Sculthorpe 1967).

With increasing water table, sexual reproduction may become problematic, because most submerged genera possess monoecious flowers which are dependent on aerial pollination. However, autogamy and hydrophilic pollination are known in some submerged taxa (e.g. *Ceratophyllum*, *Najas*, *Ruppia*). The risk of sexual reproduction can also be overcome by irregular flowering or vegetative reproduction (Sculthorpe 1967). Seeds of wetland species are often buoyant and their dispersal is facilitated by water current or animals (fishes, waterfowl – Figuerola and Green 2002).

Adaptations to anoxia include development of an internal gas-space system (aerenchyma), air-root formation, reduction in respiration and photorespiration rates, usage of bicarbonate as a carbon source (in alkaline waters), and developing of C₄-like or CAM-like metabolisms (Armstrong et al. 1994; Crawford 1996; Voesenek et al. 2006). At the same time, plants can emerge from the water through increased shoot elongation, which in some species is preceded by an adjustment of leaf angle to a more vertical position (Voesenek et al. 2006).

Species exposed to long-term flooding are known to produce ethylene, which promote stem elongation in order to restore air contact. Submergence, in addition, reduces total biomass and root/shoot ratio of a plant and increases root porosity and the number of adventitious roots formed on stem base (Banga et al. 1995; Chen et al. 2002). However, the duration of inundation which a plant is able to tolerate or survive, differs among species ranging from just one day (intolerant species) to a few months (Chen et al. 2002; Mommer and Visser 2005; Klimešová 1994) with a critical period during the growing season (Crawford 1996).

Contrary to rhizomes, where tolerance of anoxia can last up to several months (i.e., *Acorus calamus* – Weber and Brändle 1996), tolerance of anoxia in roots varies from a few hours to days depending on the season and condition of the organ (Crawford and Brändle 1996, Crawford 2003). The ability of rhizomes to survive long periods of submergence is enabled by high carbohydrate levels, which allow an extended viability for tissues under anoxia. In comparison with rhizomes, roots are also more sensitive to post-anoxic injury (Biemelt et al. 1996).

Clonality in wetlands

Clonality is defined as the production of new, genetically identical offspring with the potential to become independent of the mother organism (Klimeš et al. 1997). In general, clonal reproduction is associated with long-living species in certain conditions, i.e. under high competition, in habitats with restricted seed production, germination and seedling establishment, and under severe abiotic conditions such as anoxia or salt stress (Eckert 2002; Eckert et al. 2003). Clonality is most abundant under cold and wet conditions as well as under dense shade, but is less frequent under disturbed conditions (Klimeš et al. 1997).

Types of clonal growth organs can be classified by their morphological origin: stem, root, leaf; position towards soil surface (above- or below-ground), and spreading ability ranging from a few centimetres per year to freely dispersable propagules. They can also be classified according to their life-span, offspring production and storage capacity (Grace 1993; Klimeš et al. 1997). However, different organs of clonal growth, e.g. rhizomes, stolons, and tubers, provide the species with certain functions. Grace (1993) listed the following functions

(traits): numerical increase, dispersal, resource acquisition, storage, protection, and anchorage (Fig. 1). While stem fragmentation and turion formation result in good dispersal and high numerical increase, rhizomes and tubers serve as protection and storage organs and their spreading ability is poor. By considering these functions associated with clonality, we can see the existence of certain syndromes of characteristics, e.g. storage capacity being negatively correlated with dispersability (Grace 1993, Fig. 1).

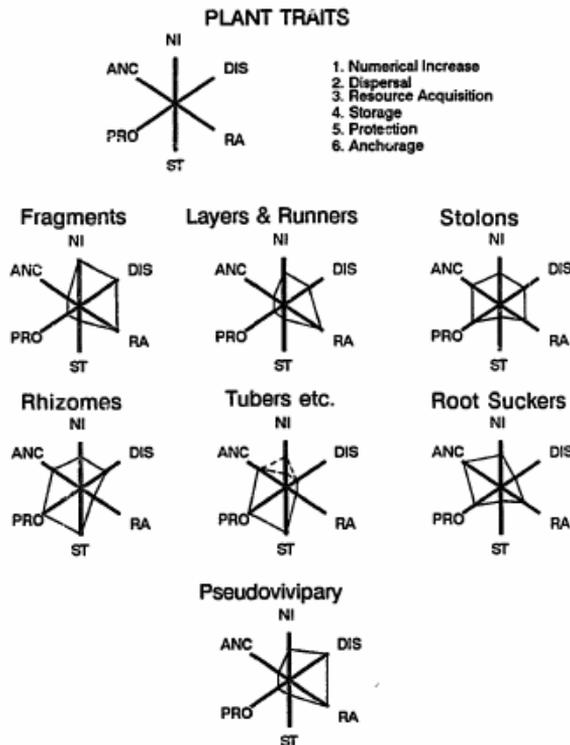


Fig. 1. Illustration of trait syndromes of plants with various modes of clonal reproduction (clonal growth organs). Shown on example plants: fragments (*Myriophyllum*); layers and runners (*Decodon*); stolons (*Justicia*); rhizomes (*Typha*); tubers, bulbs, corms and tap roots (*Sagittaria latifolia* - outer dashed line, i.e. example with high capacity for numerical increase, and *Crinum* - inner dashed line, i.e. example with low capacity for numerical increase); creeping root suckers (*Myrica*); pseudovivipary (*Myriophyllum verticillatum*). Picture adapted from Grace (1993).

The reliance of wetland species on clonal growth is much higher than in terrestrial species, and in most aquatic taxa vegetative reproduction predominates over sexual one (Grace 1993). However, as large areas of wetland communities are dominated by few clones only, fecundity of species may be affected (Charpentier et al. 2000; Eckert 2002). Beside difficulties with sexual reproduction as mentioned above, wetland species grow clonally because lateral growth and adventitious rooting is enhanced in waterlogged soils. In addition, many aquatic species are free floating and do not root in substrate, thus each plant fragment can be considered a plant individual. Vegetative propagules are further dispersed by water currents or accidental floods, which allow downstream colonization (Barrat-Segretain et al. 1998; Boedeltje et al. 2003). However, timing of disturbance is crucial for survival and regeneration of plant fragments (Barrat-Segretain and Bornette 2000). A trade-off between colonization and regeneration ability of fragments was observed: small dispersible propagules show poor establishment, whereas large fragments establish easily.

In general, wetlands and terrestrial habitats host the same types of clonal growth organs, but their proportion can differ between drier and wetter sites, as well as between habitats affected by different stress factors (Klimeš et al. 1997). Compared to clones of plants inhabiting drier habitats, clones of wetland plants are characterised by more extended lateral spread and lower persistence of connections among ramets (van Groenendael et al. 1996). This trend is obvious when comparing clonal growth forms along the whole moisture gradient in various regions (e.g. Central Europe: van Groenendael et al. 1996; Western Himalayas: Klimeš 2008).

Root-sprouting in wetlands

In comparison with clonal growth realized by stem-derived organs, such as stolons or rhizomes, root-sprouting has drawn less attention (Grace 1993; Klimeš et al. 1997). This may be partly attributed to the relatively low percentage of plants spreading by root-derived organs of clonal growth. Moreover, clonal growth by lateral roots is regarded to be typical of opportunistic species growing in disturbed habitats such as arable fields (Klimešová and Martínková 2004), which is probably due to the higher number of buds that can be formed on lateral roots and the higher foraging efficiency of roots compared to rhizomes (Dietz et al. 2002; Klimešová and Klimeš 2003). Although root-sprouting belongs to a rare type of clonal multiplication in all plant communities, it is underrepresented in disturbed wetland and aquatic communities. For example, Grace (1993), dealing with different clonal growth organs in wetlands, listed only three species, i.e. *Myrica heterophylla*, *Rorippa sylvestris* and certain species of *Plantago*.

Root-sprouting is realized by forming adventitious buds on main or lateral roots. In contrast to axillary buds, which are formed from the apical meristems during plant ontogeny, these buds originate “de novo” anywhere on the plant body (Peterson 1975). The formation of adventitious root buds is a typical feature of some plant families (Podostemataceae) or genera (*Rorippa*, *Linaria*, *Euphorbia*), but except of some members of the Orchidaceae family does not occur in monocots (Klimešová and Martínková 2004).

The ability to sprout from root buds can be necessary for overwintering and completing the life cycle of a species, additive to increase plant fitness, or regenerative to insure plant survival after severe injury (Klimešová and Martínková 2004).

Factors triggering root-sprouting in plants are disturbance together with nutrient availability (Peterson 1975). Injury to plant body (disturbance) facilitates or enables the formation of buds on roots, or breaks its dormancy (Martínková et al. 2004b). However, the relationship between nutrient level and root-sprouting is still uncertain (Klimešová and Martínková 2004). In addition, resprouting ability differs during the plant life-cycle depending on age, phenology and life-history mode (Martínková et al. 2004a).

The low abundance of root-sprouting species in wetland communities can be partly explained by phylogenetic constraints: monocots, which are in general unable to form buds on roots, predominate in wetlands and the rate of speciation is low (Duarte et al. 1994; Eckert 2002; Philbrick and Les 1996). However, ecological factors may partake as well: anoxia and/or multiple stress interactions can be lethal to a plant or substantially reduce plant fitness. Moreover, roots compared to rhizomes are less tolerant to anoxia (Crawford and Brändle 1996, Crawford 2003). However, little is known about the role of submergence on root-sprouting ability and the combined effects of submergence and disturbance have not been studied so far (Crawford 1996).

Ecological factors potentially hindering root sprouting ability in wetlands

Anoxia, typical of waterlogged soils, can be overcome by transport of air from upper parts to below-ground, by adventitious roots formation or by metabolically adjustments. However, these adaptations are dependent on species and environmental conditions (Visser et al. 2003).

In wetlands, anoxia is often accompanied by mechanical and other stresses. In extreme cases, flooding can cause uprooting of the whole plant, its fragmentation and transport to downstream areas (Puijalón et al. 2007; Bornette et al. 2008). These interactions, although common, have attracted less attention. For example, anoxia accompanied by mechanical stress is known to determine species richness and the whole community assembly in floodplains (Bornette et al. 1998; Renöfält and Nilsson 2008; van Eck

et al. 2004). Following severe disturbance by flooding the entire community may regenerate through the propagule/seed bank; however, individual species regeneration will depend on its life history and strategy (Barrat-Segretain and Bornette 2000; Boedeltje et al. 2004; Trémolières 2004). Under less severe conditions, anoxia combined with various velocities of the water current can result in morphology adjustment including internode length and position of creeping stems, as observed in *Potamogeton coloratus* and *Mentha aquatica* (Puijalon et al. 2008). The reaction to multiple stresses may also include plastic adjustments of growth form (Puijalon and Bornette 2006; Puijalon et al. 2008) and investment into better anchorage (Barrat-Segretain 2001; Schutten et al. 2005).

Even though plants possess various mechanisms to cope with stress, the timing and duration of a flooding event with regard to plant ontogeny is crucial (Barrat-Segretain and Bornette 2000, Crawford 2003, Johansson and Nilsson 1993). Moreover, the reaction of a single species can change depending on the character of submergence: in *Lotus tenuis* partial submergence evoked shoot lengthening, whereas completely flooded plants were quiescent and their survival depended on stored carbohydrates (Manzur et al. 2009). Submergence combined with injury will prevent a plant to reach the water surface and restore air contact, thus plant survival will be more sensitive to the amount of stored carbohydrates, or, alternatively, to the ability of underwater photosynthesis (Mommer and Visser 2005).

Objectives and content of the thesis

The main objectives of this thesis are: (i) to evaluate the representation of various types of clonal growth organs, especially root-sprouters on the example of wetland communities in the Netherlands, (ii) to determine whether certain clonal traits are selected in different wetland communities, (iii) to assess the role of life history variation, injury timing and starch reserves in the root-sprouting ability of the wetland herb *Rorippa palustris* and (iv) to evaluate the role of submergence and disturbance, i.e. alone and in combination, in the root-sprouting ability of this species.

CHAPTER II focuses on the relation between wetland communities shaped by different stress factors and the representation of clonal growth organs found there. The study also sheds light on relationships between types of clonal growth organs and their functional traits.

CHAPTER III extends the approach used for the evaluation of clonal growth organ preference in various wetland communities (CHAPTER II). In this study we asked whether various wetland communities with different stress factors are preferred by assemblies of plants possessing

specific characteristics (traits) concerning extent of lateral spread, shoot cyclicity, persistence of connections between ramets, and multiplication rate.

CHAPTER IV represents an experimental study with the root-sprouting wetland herb *Rorippa palustris*. In a common garden experiment, its root-sprouting ability was assessed in relation to germination date and injury timing.

CHAPTER V presents an outdoor pot experiment with *Rorippa palustris*. This chapter focuses on the carbon economy of plants differing in life history mode and evaluates the changes in starch allocation following severe injury to the plant body.

In **CHAPTER VI** plants of *Rorippa palustris* originating from different populations and habitats were subjected to flooding and/or severe disturbance. Single and combined effects of these factors on regeneration (root-sprouting), growth, seed production, and winter survival were followed.

Finally, **CHAPTER VII** summarises the main results of this thesis.

REFERENCES

- Armstrong W, Brändle R, Jackson MB (1994) Mechanisms of flood tolerance in plants. *Acta Bot. Neerl.* 43: 307-358.
- Banga M, Blom CWPM, Voeselek LACJ (1995) Flood-induced leaf elongation in *Rumex* species: effects of water depth and water movements. *New Phytol.* 131: 191-198.
- Barrat-Segretain M-H (2001) Biomass allocation in three macrophyte species in relation to the disturbance level of their habitat. *Freshwater Biol.* 46: 935-945.
- Barrat-Segretain M-H, Bornette G (2000) Regeneration and colonization abilities of aquatic plant fragments: effect of disturbance seasonality. *Hydrobiologia* 421: 31-39.
- Barrat-Segretain M-H, Bornette G, Hering-Vilas-Bôas A (1998) Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquat. Bot.* 60: 201-211.
- Biemelt S, Albrecht G, Wiedenroth EM (1996) The effect of post-hypoxia on roots in *Senecio* and *Myosotis* species related to the glutathione system. *Folia Geobot. Phytotax.* 31: 65-72.
- Boedeltje G, Bakker JP, ten Brinke A, van Groenendael JM, Soesbergen M. (2004) Dispersal phenology of hydrochorous plants in relation to discharge, seed release time and buoyancy of seeds: the flood pulse concept supported. *J. Ecol.* 92: 786-796.
- Boedeltje G, Bakker JP, Bekker RM, van Groenendael JM, Soesbergen M (2003) Plant dispersal in a lowland stream in relation to occurrence and three specific life-history traits of the species in the species pool. *J. Ecol.* 91: 855-866.
- Bornette G, Amoros C, Lamouroux N (1998) Aquatic plant diversity in riverine wetlands: the role of connectivity. *Freshwater Biol.* 39: 267-283.
- Bornette G, Tabacchi E, Hupp C, Puijalon S, Rostan C (2008) A model of plant strategies in fluvial hydrosystems. *Freshwater Biol.* 53: 1692-1705.
- Bos D, Bakker JP, de Vries Y, van Lieshout S (2002) Long-term vegetation changes in experimentally grazed and ungrazed back-barrier marshes in the Wadden Sea. *Appl. Veg. Sci.* 5: 45-54.
- Charpentier A, Grillas P, Thompson JD (2000) The effect of population size limitation on fecundity in mosaic populations of the clonal macrophyte *Scirpus maritimus* (Cyperaceae). *Am. J. Bot.* 87: 502-507.
- Chen H, Qualls RG, Hiller GC (2002) Adaptive responses of *Lepidium latifolium* to soil flooding: biomass allocation, adventitious rooting, aerenchyma formation and ethylene production. *Environ. Exp. Bot.* 48: 119-128.
- Crawford RMM (1996) Whole plant adaptations to fluctuating water tables. *Folia Geobot. Phytotax.* 31:7-24.
- Crawford RMM (2003) Seasonal differences in plant responses to flooding and anoxia. *Can. J. Bot.* 81: 1224-1246.
- Crawford RMM, Brändle R (1996) Oxygen deprivation stress in a changing environment. *J. Exp. Bot.* 47: 145-159.
- Dietz H, Kohler A, Ullmann I (2002) Regeneration growth of the invasive clonal forb *Rorippa austriaca* (Brassicaceae) in relation to fertilization and interspecific competition. *Plant Ecol.* 158: 171-182.
- Dormann CF, van der Wal R, Bakker JP (2000) Competition and herbivory during salt marsh succession: the importance of forb growth strategy. *J. Ecol.* 88: 571-583.
- Duarte CM, Planas D, Peñuelas J (1994) Macrophytes, taking control of an ancestral home. In: Margalef R (ed.): *Limnology now: a paradigm of planetary problems*. Elsevier. 553p.
- Eckert CG (2002) The lost of sex in clonal plants. *Evol. Ecol.* 15: 501-520.

- Eckert CG, Lui K, Bronson K, Corradini P, Bruneau A (2003) Population genetic consequences of extreme variation in sexual and clonal reproduction in an aquatic plant. *Mol. Ecol.* 12: 331-344.
- Ellenberg H (1988) *Vegetation ecology of central Europe*. 4th edition. Cambridge University Press. Cambridge. 731p.
- Esselink P, Fresco LFM, Dijkema KS (2002) Vegetation change in a man-made salt marsh affected by a reduction in both grazing and drainage. *Appl. Veg. Sci.* 5: 17-32.
- Figuerola J, Green AJ (2002) Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. *Freshwater Biol.* 47: 483-494.
- Grace JB (1993) The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. *Aquat. Bot.* 44: 159-180.
- Johansson ME, Nilsson C (1993) Hydrochory, population dynamics and distribution of the clonal aquatic plant *Ranunculus lingua*. *J. Ecol.* 81: 81-91.
- Klimeš L, Klimešová J, Hendriks R, van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H, van Groenendael J (Eds.), *The Ecology and Evolution of Clonal Plants*. Backhuys Publishers, Leiden, The Netherlands, pp. 1-29.
- Klimešová J, Klimeš L. (2003) Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham-Sparrow's model? *Oikos* 103: 225-229.
- Klimešová J, Klimeš L (2006) CLO-PLA 3: a database of clonal growth in plants, <http://clopla.butbn.cas.cz/>
- Klimešová J (1994) The effects of timing and duration of floods on growth of young plants of *Phalaris arundinacea* and *Urtica dioica*: an experimental study. *Aquat. Bot.* 48: 21-29.
- Klimešová J, Martínková J (2004) Intermediate growth forms as a model for study of plant clonality functioning: an example with root sprouters. *Evol. Ecol.* 18: 669-681.
- Manzur ME, Grimoldi AA, Insausti P, Striker GG (2009) Escape from water or remain quiescent? *Lotus tenuis* changes its strategy depending on depth of submergence. *Ann. Bot.* 104: 1163-1169.
- Martínková J, Kočvarová M, Klimešová J (2004a) Resprouting after disturbance in the short-lived herb *Rorippa palustris* (Brassicaceae): an experiment with juveniles. *Acta Oecol.* 25: 143-150.
- Martínková J, Klimešová J, Mihaluk S (2004b) Resprouting after disturbance: an experimental study with short-lived monocarpic herbs. *Folia Geobot.* 39: 1-12.
- Mommer L, Visser EJW (2005) Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. *Ann. Bot.* 96: 581-589.
- Peterson RL (1975) The initiation and development of root buds. In: Torrey JG, Clarkson DT (Eds.), *The development and function of roots*. Acad. Press, London. pp 125-161.
- Philbrick CT, Les DH (1996) Evolution of aquatic angiosperm reproductive systems. *Bioscience* 46: 813-826.
- Puijalon S, Bornette G (2006) Phenotypic plasticity and mechanical stress: biomass partitioning and clonal growth of an aquatic plant species. *Am. J. Bot.* 93: 1090-1099.
- Puijalon S, Lena JP, Bornette G (2007) Interactive effects of nutrient and mechanical stresses on plant morphology. *Ann. Bot.* 100: 1297-1305.
- Puijalon S, Léna J-P, Rivière N, Champagne J-Y, Rostan J-C, Bornette G (2008) Phenotypic plasticity in response to mechanical stress: hydrodynamic performance and fitness of four aquatic plant species. *New Phytol.* 177: 907-917.

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- Renöfält BM, Nilsson C (2008) Landscape scale effects of disturbance on riparian vegetation. *Freshwater Biol.* 53: 2244-2255.
- Schutten J, Dainty J, Davy AJ (2005) Root anchorage and its significance for submerged plants in shallow lakes. *J. Ecol.* 93: 556-571.
- Sculthorpe CD (1967) *The biology of aquatic vascular plants.* Arnold. London. 610p.
- Trémolières M (2004) Plant response strategies to stress and disturbance: the case of aquatic plants. *J. Biosci.* 29: 461-470.
- van Eck WHJM, van de Steeg HM, Blom CWPM, de Kroon H (2004) Is tolerance to summer flooding correlated with distribution patterns in river floodplains? A comparative study of 20 terrestrial grassland species. *Oikos* 107: 393-405.
- Visser EJW, Voesenek LACJ, Vartapetian BB, Jackson MB (2003) Flooding and plant growth. *Ann. Bot.* 91: 107-109.
- Voesenek LACJ, Colmer TD, Pierik R, Millenaar FF, Peeters AJM (2006) How plants cope with complete submergence. *New Phytol.* 170: 213-226.
- Weber M, Brändle R (1996) Some aspects of the extreme anoxia tolerance of the sweet flag, *Acorus calamus* L. *Folia Geobot. Phytotax.* 31: 37-46.

CHAPTER II

Distribution of clonal growth forms in wetlands

Sosnová M, van Diggelen R & Klimešová J (2010)

Aquat. Bot. 92: 33-39

Clonal growth forms in wetlands

DISTRIBUTION OF CLONAL GROWTH FORMS IN WETLANDS

ABSTRACT

Clonal multiplication is a predominant type of reproduction in wetland species. However, both wetlands and plant organs of clonal growth are diverse, thus due to different stress factors operating in various wetlands preponderance of plants with specific clonal growth organs (CGOs) can be expected. To test this hypothesis the CGO spectra of wetland communities of the Netherlands were analysed, including a bog, a fen, heathland, a floodplain, river beds, fresh water pools, open salt water and a salt marsh. Moreover, it was evaluated whether different CGOs are characterised by different functional traits (shoot cyclicality, persistence of connections between ramets, number of offspring produced per year and lateral spread per year) in wetland species. Data on types of CGO, i.e., epigeogenous and hypogeogenous rhizomes, fragments & budding plants, stolons, tubers & bulbs, root-splitters, root-sprouters and special adaptations (turions) as well as their functional traits, were taken from the CLO-PLA 3 database. CGO spectra of wetland communities were analysed using two methods: comparison of observed vs. expected CGO spectra based on presence/absence data and multivariate analysis (CCA) for inter-community differences considering species frequency. Moreover, relationships between CGOs and their functional traits were tested using multidimensional contingency tables. Apart from 26 % of non clonal species, the majority of wetland species was rhizomatous (51 %). Other types of CGO were represented in less than 10 % of species and root-derived CGOs were underrepresented (< 2 %) in comparison with terrestrial habitats. Among communities, fresh water pools and open salt water hosted higher proportion of species with fragments (~ 10 %) and turions (~ 30 %). Multivariate analysis divided wetland communities along the disturbance and hydric (water) gradients. Highly disturbed communities (salt marshes) were characterised by non-clonal species and species with root-derived CGOs. Aquatic communities (fresh water pools and open salt water) hosted species with ability to spread by fragmentation and turions, contrary to permanently wet communities (bog and wet heathland) with prevalence of species with epigeogenous rhizomes. It was also confirmed that the CGOs of wetland species differed in their traits. The most important functional trait characterising individual CGOs in the wetland flora was the degree of lateral spread (explained variability: 53 %) followed by duration of persistence of connections between ramets (explained variability: 74 %), which is in accord with earlier distinguished strategies of clonal growth: integrator/splitter and spreading/non-spreading clones.

Clonal growth forms in wetlands

Keywords: clonal growth organ, the Netherlands, wetland plant community, functional trait

Nomenclature sources: Kubát et al. (2002), phytosociological alliances according to Schaminée et al. (1995, 1996, 1998).

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ABSTRAKT

Klonalita je převažujícím typem rozmnožování rostlin v mokřadech. Ovšem typy orgánů klonálního růstu podobně jako druhy mokřadů se liší. Díky působení rozdílných stresových faktorů v různých mokřadech lze také očekávat jiné zastoupení typů klonálních orgánů v nich. Tato hypotéza byla testována na příkladu mokřadních společenstev Nizozemí. Mokřadní společenstva zahrnovala: rašeliniště, slatiniště, vlhké vřesoviště, nivy řek, říční břehy, sladkovodní a slanomilná společenstva s otevřenou vodní hladinou a slanomilné trávníky. Navíc bylo zkoumáno, jestli určité typy klonálního růstu mohou být charakterizovány určitými funkčními charakteristikami (znaky: cyklicitou prýtlů, vytrvalostí spojení mezi rametami, počtem vyprodukovaných potomků a laterálním šířením). Data o typech klonálních orgánů (at. epigogenních a hypogeogenních oddencích, fragmentech a pučících rostlinách, stolonech, hlízách a cibulích, rozpadu kořene a odnožování z kořenů a speciálních adaptací) spolu s jejich funkčními charakteristikami byla získána z databáze CLO-PLA 3. Spektra typů klonálních orgánů byla analyzována za užití dvou metod: porovnáním očekávaných a zjištěných spekter typů klonálních orgánů (binomická data) a mnohonásobnou analýzou, která brala v potaz druhovou frekvenci. K testování vztahu mezi typem klonálních orgánů a funkčními vlastnostmi byly použity mnohorozměrné kontingenční tabulky. Ze všech analyzovaných druhů bylo 26 % druhů neklonálních a 51 % druhů tvořila oddenky. Ostatní typy klonálního růstu byly nalezeny u méně než 10 % druhů a klonální typy odvozené z kořenů byly vzácné (< 2 % druhů). Společenstva s otevřenou vodní hladinou (ať už sladkovodní či slaná) měla typické zastoupení orgánů klonálního růstu: vyšší zastoupení druhů s fragmenty (~ 10 %) a turiony (~ 13 %). Mnohorozměrná analýza rozdělila mokřadní společenstva podle gradientu vlhkosti a disturbance. Narušovaná společenstva (slanomilné trávníky) byla typická neklonálními typy a druhy s orgány klonálního růstu odvozenými od kořenů, zatímco vodní společenstva (sladká a slaná voda) byla charakterizována druhy fragmentujícími se a tvořícími turiony. Epigeogenní oddenky byly naopak typické pro permanentně vlhká společenstva typu rašeliniště a vřesoviště. Tato práce také prokázala, že se jednotlivé klonální orgány liší ve svých znacích. Nejdůležitějším funkčním znakem charakterizujících jednotlivé typy klonálních orgánů bylo laterální šíření následované vytrvalostí spojení mezi rametami (vysvětlená variabilita . 53, resp. 74 %), což odpovídá již dříve rozlišeným strategiím typu integrator/splitter a šířící/nešířící (spreading/non-spreading) se klon.

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Clonal growth forms in wetlands

CHAPTER III

Clonal growth in wetlands: Is there a relationship between clonal growth traits and habitat conditions?

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[submitted]

Clonal traits

CLONAL GROWTH IN WETLANDS: IS THERE A RELATIONSHIP BETWEEN CLONAL GROWTH TRAITS AND HABITAT CONDITIONS?

ABSTRACT

Question: Which sets of clonal growth traits are typical of plants inhabiting wetland communities shaped by different stressors?

Location: Wetland communities of the Netherlands.

Methods: We analysed clonal traits of plants occurring in the wetlands of the Netherlands. Wetlands included bogs, fens, wet heathlands, floodplains, river beds, fresh water pools, salt marshes, and open salt waters. Clonal growth traits (including number of offspring, lateral spread, persistence of connections between ramets, shoot cyclicity, and bud bank characteristics) were analysed with multivariate techniques using species frequency data and with permutation tests using presence/absence data.

Results: Based on species frequencies, clonal plants in true aquatic communities (those with permanent water table, anoxia and low disturbance, i.e., fresh water pools, open salt water, and river beds) were characterized by the abundant production of easily spreading offspring, monocyclic shoots, and annual connections between ramets (splitting clones). In contrast, clonal plants in low-productive bog communities were characterized by polycyclic shoots and low offspring production. Clonal plants in salt marsh communities had intermediate traits. The specificity of true aquatic communities and salt marshes was confirmed by permutation tests that gave equal weight to rare and abundant species. However, other wetland communities were characterized by the entire range of clonal traits.

Conclusions: Particular wetland communities characterized by distinct stress factors showed shifted sets of clonal traits, i.e., they had specific, specialized traits. Other wetland communities, however, contained species that were characterised by the entire range of clonal traits.

Keywords: functional traits; the Netherlands; wetland plant communities; bud bank

Nomenclature source: Kubát et al. 2002, phytosociological alliances according to Schaminée et al. 1995, 1996, 1998.

INTRODUCTION

Most wetland and aquatic plants propagate predominantly by vegetative means (Grace 1993; Barrat-Segretain et al. 1998; Boedeltje et al. 2003, 2004; Combroux & Bornette 2004). Various reasons have been proposed to explain this reliance on vegetative reproduction, and these include species phylogeny (Eckert 2002), life history constraints (Charpentier et al. 2000), or species ecology (Santamaría 2002). Compared to clones of plants inhabiting drier habitats, clones of wetland plants are characterised by larger lateral spread and lower persistence of connections among ramets (van Groenendael et al. 1996). Although this trend is obvious when comparing clonal growth forms along the whole moisture gradient in various regions (e.g., Central Europe: van Groenendael et al. 1996; Western Himalayas: Klimeš 2008), it is lost after phylogenetic correction. This does not necessarily mean that clonal traits typical for wetland plants are merely by-products of selection for other traits in a common ancestor. For example, clonality itself is thought to be important for successful radiation of monocots in wetlands (Duarte et al. 1994; Philbrick & Les 1996).

On a finer scale, wetland communities ranging from fresh or salt water bodies to sites affected by a daily or seasonally fluctuating water table show a distinct pattern of different characters of clonal growth types in relation to habitat conditions (Sosnová et al. 2010). Also on small spatial and temporal scales within one habitat, various and even contradictory selection pressures affect the success of growth strategies (Bornette et al. 1998, 2008; Puijalón et al. 2005; Dunn et al. 2006). For example, aquatic communities are rich in plants with turions or easily fragmented stems, communities in waterlogged soils contain a high portion of rhizomatous species, while communities that are flooded daily typically contain plants with root-derived clonal organs and annual species (Sosnová et al. 2010). Nevertheless, growth form or clonal organ types are only surrogates for function (Grace 1993; Sosnová et al. 2010), and as hypothesised by Grace (1993), various wetland communities with different stress factors may be preferred by assemblies of plants possessing specific characteristics concerning extent of lateral spread, shoot cyclicity, persistence of connections between ramets, and multiplication rate. To the best of our knowledge, however, a direct analysis is lacking as to whether plants with certain clonal growth traits prefer particular wetland communities.

In this study, therefore, we test the conceptual model developed by Grace (1993), i.e., we test the hypothesis that the particular traits of a plant assemblage depend on the given community type. Moreover, we ask whether these traits can be predicted with the knowledge from experimental studies. Based on the Grace's model, the following five predictions were examined: *i*) A high degree of environmental heterogeneity caused by both biotic and/or abiotic factors will select for a high rate of dispersal. Species possessing greater

lateral spread will capitalize on their ability to quickly explore suitable patches (cf. Roughgarden 1979; Macek et al. 2010). *ii*) Low nutrient availability, which is likely to cause slow plant growth, will select for a high local persistence, i.e., polycyclic shoots (Jónsdóttir & Watson 1997). *iii*) Low light availability, caused by large neighbours or shading in deep water, will select for a high carbon-storage capacity enabled by long-lived connections between ramets and production of larger offspring. Further, the formation of emergent foliage will be associated with the storage of the large amounts of photosynthate that are required to produce large clonal offspring (Grace & Wetzel 1982). *iv*) High disturbance intensity will select for numerical increase via clonal multiplication, i.e., plants will tolerate disturbance via clonal multiplication (Grime 2001). As an alternative to the last prediction, *v*) high disturbance will also select for efficient bud protection, i.e., plants will avoid disturbance (Klimešová & Klimeš 2007).

In contrast to the above predictions, it is also possible that species in different wetland types will not possess common traits related to clonality but will diverge either to increase the opportunities for coexistence in a community (Grime 2006) or because of the importance of plant traits not associated with clonality (Bornette et al. 1994; Boedeltje et al. 2008). Grace (1993) also stated that natural selection for traits other than those associated with clonality might constrain the validity of the proposed model. However, the present study is among the first to test predictions based on individual species with real data on clonal growth traits of an assembly in a specific wetland type against its general background. To test the predictions, we used two analyses. First, we considered only the presence of species, and both rare and abundant species had the same effect or weight in the analysis. Second, we considered species frequencies. Obtaining the same results with both analyses would support Grace's conceptual model of how clonality and habitat interact to affect wetland plant communities.

METHODS

To evaluate the spectra of traits of different wetland communities, we used two methods: (i) : comparison by contingency tables of traits of one particular alliance (observed spectra) with random trait spectra made up from permutations of all species from all wetland alliances (expected spectra), and (ii) comparison by multivariate analysis (canonical correspondence analysis: CCA) taking into account species frequencies. The vegetation of the Netherlands, which encompasses all major wetland types in Europe, is used as an example in this study.

Studied plant communities

We selected all major types of wetland communities occurring in the Netherlands: bogs, wet heathlands, fens, floodplains, river beds, fresh water pools, salt marshes, and open salt waters. Environmental characteristics of each community are given in Table 1. According to the treatise concerning vegetation of the Netherlands (Schaminée et al. 1995, 1996, 1998), we chose phytosociological alliances typical of a given community (Table 1) and included all listed species in the analysis. In the treatise, all species occurring at a frequency of more than 10% in at least one of the alliances of an order were listed, whereas species rare in all alliances were omitted. The amount of relevés per alliance differed, ranging from several hundreds to several thousands.

Table 1. Wetland communities of the Netherlands with descriptions of their characteristic environmental factors and relevant phytosociological alliances (according to Schaminée et al. 1995, 1996, 1998).

Community	Environmental characteristic	Alliances
Bogs	Mire community. Water table at the soil surface, acidic (pH < 5.5), nutrient-poor, precipitation-fed, low vegetation. Rather homogenous. Rarely disturbed.	Rhynchosporion albae, Caricion lasiocarpae, Ericion tetralicis, Oxycocco-Ericion
Wet heathlands	Nutrient-poor habitat. Dwarf-shrub vegetation. High spatial variation. Fluctuating water table during wet/dry season. Medium disturbances by herbivory or fire.	Littorellion uniflorae, Hydrocotylo-Baldellion, Eleocharition acicularis
Fens	Mire community with low vegetation. Base-rich, slightly acidic to neutral (pH > 5.5), fed by ground/surface water.	Caricion nigrae, Caricion davallianae
Floodplains	Dynamic systems with tall vegetation, intensive flooding (winter/spring) and dry summer periods. High nutrient availability due to flooding deposits and internal (re)mobilisation of nutrients.	Phragmition australis, Caricion gracilis, Caricion elatae, Lolio-Potentillion anserinae, Calthion palustris, Alopecurion pratensis
River beds	Eutrophic littoral zones. High spatial variation, muddy, fluctuating water table. High nutrient availability.	Sparganio-Glycerion, Cicution virosae, Oenanthion aquaticae
Fresh water pools	Spatially homogenous, rather deep water, oxygen stress. In this study fresh water pools include emergent communities and truly open-water communities. High nutrient availability.	Lemnion minoris, Lemnion trisulcae, Nymphaeion, Hydrocharition morsus-ranae, Parvopotamion, Ranunculion peltati, Nitellion flexilis, Charion fragilis, Charion vulgaris
Salt marshes	Habitat between mainland and sea. High nutrient availability. Low vegetation. Regularly flooded by the sea; mechanical and salt stress.	Zosterion, Spartinion, Thero-Salicornion, Puccinellion maritimae
Open salt waters	Spatially homogenous, rather deep water, salt and oxygen stress, high nutrient availability.	Charion canescentis, Zannichellion pedicellatae, Ruppion maritimae

Clonal growth traits

We obtained the data on traits related to clonality from the database of clonal growth in plants (CLO-PLA 3, Klimešová & Klimeš 2006). We used the following traits: lateral spread, shoot cyclicity, persistence of connections between ramets, number of offspring produced per year, and bud bank characteristics (occurrence and seasonality; Table 2). In the case of multiple records per species in the database, the record with the most-filled cells was used. Because most clonally growing species use more than one type of clonal growth organ (e.g., *Potamogeton* spp. can spread via turions, fragmentation, rhizomes, and stem tubers; Klimeš & Klimešová 1999), the traits related to clonality may also obtain different categories. However, we used only one category for each species, i.e., the one providing the highest offspring production and lateral spread (but see multivariate analysis section below). Non-clonal species, representing 26% of the Netherlands wetland flora, were not included in the analyses.

Apart from clonal growth traits, we also used data on seasonality and vertical distribution of buds in the bud bank (Klimešová & Klimeš 2007). The bud bank characteristics in the CLO-PLA 3 database are arranged according to the position of buds on shoots in relation to the soil surface: more than 10 cm above soil surface, from 0 to 10 cm above the soil surface, at the soil surface, from 0 to -10 cm below the soil surface, and greater than 10 cm below the soil surface. The bud bank per layer is further characterised by the number of buds (0, 0–10, >10 buds per shoot) and their seasonality (seasonal and perennial).

Table 2. Selected clonal traits and their descriptions and categories (separated by semicolons) used in the study. Data were extracted from the CLO-PLA3 database (Klimešová & Klimeš 2006).

Trait	Description	Categories
Lateral spread	Distance between parent and offspring ramet (m/year).	<0.01; 0.01–0.25; >0.25; dispersable
Shoot cyclicity	Life-span of a shoot in years, i.e., until shoot flowering.	1; 2; >2
Persistence of connections	Persistence of connections between parent and offspring ramets (in years).	1; 2; >2
Number of offspring	Offspring ramets produced per year and per mother ramet.	<1; 1; 2–10; >10
Occurrence of buds	All buds on plant potentially used for vegetative regeneration. Numbers of buds per shoot are assessed from the number of nodes or leaves.	0; 1–10; >10
Seasonality of buds	Types of bud bank derived from the mode of perennation of bud-bearing organs.	seasonal; perennial

Wetland species pool

For illustration of typical trait spectra found in wetland plants of the region, we made a wetland species pool. This pool consisted of all higher plants of the Netherlands with Ellenberg indicator values for moisture ≥ 6 (Botanisch Basisregister, CBS, 1993). Ellenberg indicator values are empirically determined preferences based on occurrence of the species in plant communities along environmental gradients, with preferences generally assessed as ranging from 1 (lowest preference) to 12 (highest preference) on an ordinal scale.

Data analysis

(i) Contingency tables

Occurrence of traits within all wetland species was permuted (500 permutations), and the average of a random sample (expected value; n always being identical to species number in corresponding alliance) was compared with the trait occurrence within an individual alliance (observed value) by contingency tables (Pearson χ^2 test). The same procedure was followed for all traits. Rare species in this analysis had the same weight as common ones.

(ii) Multivariate analysis

Traits found in different alliances were also compared using the direct method of ordination analysis (CCA). This approach accounted for species frequencies and included the fact that many plant species possess several types of clonal growth organs (see above) and thus fall into more than one category of a particular clonal trait. We used the frequency of species per alliance as the species data set. The data on clonal traits were coded as dummy variables, standardised by samples before entering the analysis and used as explanatory variables. Bud bank characteristics were excluded from this analysis because of their complex structure. The CCA was performed with CANOCO (ver. 4.5, ter Braak & Šmilauer 2002), and differences between alliances were tested with Monte Carlo permutation tests (499 permutations under a reduced model).

RESULTS

Clonal traits of wetland species

We found slow lateral spread ($< 25 \text{ cm year}^{-1}$) in 84% of wetland species, i.e., species with Ellenberg values for moisture > 6 , and lateral spread exceeding 25 cm year^{-1} in 8% of wetland species. Only 7% of the species were able to produce freely dispersable propagules (Fig. 1a). Most plants produced monocyclic or dicyclic shoots (59 and 30% of species, respectively; Fig. 1b). Perennial connections between ramets prevailed over one- or two-

seasonal connections (54% vs. approximately 22% in one- vs. two-seasonal connections; Fig. 1c). Most wetland species produced at least one clonal offspring per year (44% of the species produced just one offspring, 41% produced 2–10 offspring per year); few species produced more than 10 or fewer than 1 clonal offspring per year (Fig. 1d).

Most of the reserve buds were located on the shoot base or up to 10 cm belowground (Fig. 2). A seasonal bud bank prevailed aboveground, whereas belowground buds were mostly perennial (Fig. 2).

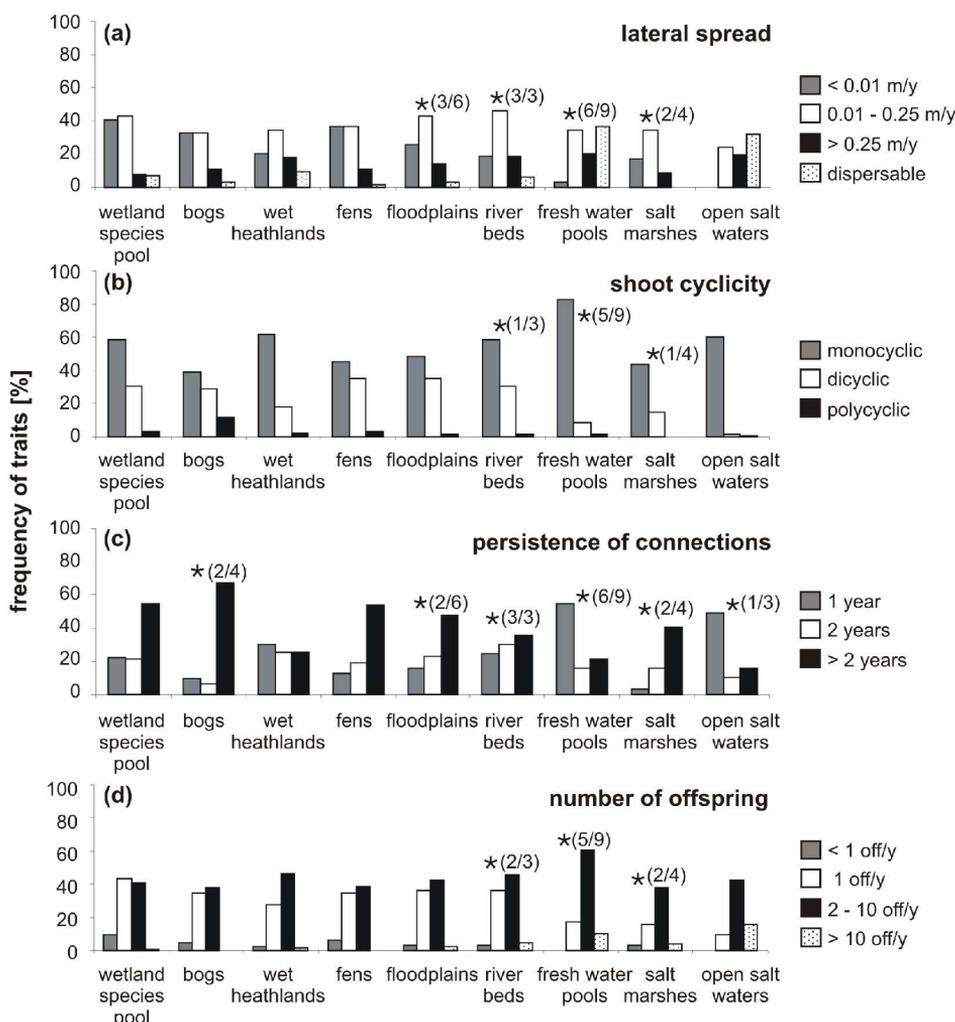


Fig. 1. Frequency of clonal traits, i.e., (a) lateral spread, (b) shoot cyclicity, (c) persistence of connections between ramets, and (d) number of offspring, in wetland communities of the Netherlands. The wetland species pool was defined as all species from the Dutch flora with Ellenberg's indicator values for moisture > 6 ($n = 428$). Columns represent the mean percentage of a particular trait in a given community type. Asterisks denote significantly different results ($p < 0.05$) between observed and expected trait spectra; the proportion of significant results is indicated in parentheses (number of significant alliances/all alliances belonging to a particular community; for results of tests, see Table 4).

Clonal traits

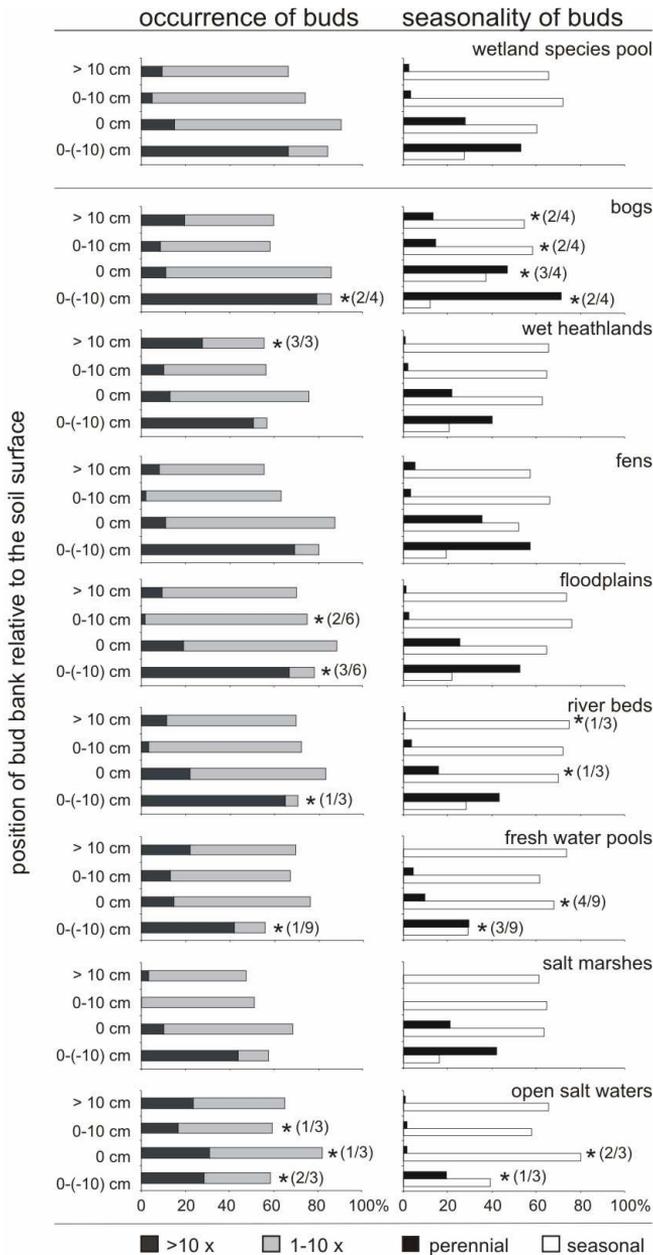


Fig. 2. Occurrence and seasonality of bud bank in different soil layers within studied wetland habitats. The wetland species pool includes all species from the Dutch flora with Ellenberg's indicator values for moisture > 6 ($n = 428$). Columns represent the mean percentage of species with particular bud bank characteristics in a given wetland type. Asterisks denote significantly different results ($p < 0.05$) between observed and expected trait spectra; the proportion of significant results is indicated in parentheses (number of significant alliances/all alliances belonging to a particular community; for results of tests, see Electronic appendix).

Trait types based on presence/absence data

Particular wetland communities differed in observed trait spectra, but significant results were seldom found for all of its alliances, i.e., trait spectra of some (but usually not all) alliances belonging to a particular community were different from the expected trait spectra (Table 3). Traits in which observed and expected spectra frequently differed were lateral spread and persistence of connections between ramets. Wet heathlands and fens did not differ in any trait from the expected trait spectrum, whereas river beds and fresh water pools had distinct trait spectra in most cases (Table 3).

The dispersal of ramets was rather intermediate with prevalence of species spreading less than 25 cm per year in floodplains, river beds, and salt marshes. Spatially stable fresh water pools were occupied by species producing freely dispersable propagules (Fig. 1a, Table 3).

We found a short local persistence, i.e., a prevalence of monocyclic shoots, in one alliance belonging to river beds and salt marshes. In addition, nutrient richness of fresh water pools in the Netherlands corresponded well with the prevalence of monocyclic shoots (Fig. 1b, Table 3).

Long-lived persistence of connections among ramets was found in salt marshes, bogs, and floodplains, whereas fresh water pools and open salt waters were typical of fast-splitting clones, i.e., short persistence. River beds were characterised by an equal proportion of splitting and persistent clones (Fig. 1c, Table 3).

High clonal multiplication was found in fresh water pools, and intermediate offspring production was typical of river beds and salt marshes (Fig. 1d, Table 3).

Bog species possessed a perennial bud bank situated mostly belowground, whereas species of open salt waters formed seasonal bud banks aboveground (Fig. 2; for detailed results, see Appendix).

Trait spectra considering species frequency

The whole multivariate model (CCA) was significant at $P = 0.002$ ($F = 2.2$). The first canonical axis explained 18.2% of the variability (499 permutations; eigenvalue: 0.785, $F = 2.25$, $P = 0.002$), and the second axis explained an additional 15.2% of the variance in the data. The first axis was positively correlated with production of freely dispersable propagules, monocyclic shoots, and annual connections between ramets, which were typically found in fresh water pools and open salt waters. The second axis assigned polycyclic shoots to bogs and medium offspring production together with biennial connections of ramets to floodplains and salt marshes (Fig. 3).

Clonal traits

Table 3. Comparison between observed (species of a particular alliance) and expected trait spectra (based on 500 permutations of all species, n always identical to species number in correspondent alliance). Tested by contingency tables (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; if not indicated, the compared spectra were not significantly different).

Wetland type	Alliances	Lateral spread	Shoot cyclicity	Persistence of connections	Number of offspring
Bog	Rhynchosporion albae	1.42	0.45	1.36	2.18
	Caricion lasiocarpae	1.01	0.46	0.81	1.13
	Ericion tetralicis	2.68	4.91	10.95**	1.22
	Oxycocco-Ericion	2.69	3.65	10.68**	1.80
Wet heathland	Littorellion uniflorae	4.88	0.43	1.05	2.65
	Hydrocotylo-Baldellion	3.27	1.37	1.48	2.03
	Eleocharition acicularis	4.40	0.67	1.63	0.49
Fen	Caricion nigrae	4.51	3.57	1.15	1.91
	Caricion davallianae	4.08	1.90	0.80	3.23
Floodplain	Phragmition australis	6.21	0.81	3.33	2.17
	Caricion gracilis	13.33**	1.50	6.06*	3.94
	Caricion elatae	8.77*	1.18	6.28*	2.39
	Lolio-Potentillion anserinae	4.61	0.98	4.15	0.64
	Calthion palustris	8.65*	1.33	5.57	1.78
	Alopecurion pratensis	5.60	1.16	1.74	1.91
River beds	Sparganio-Glycerion	24.79***	4.50	13.64**	11.88**
	Oenanthion aquaticae	18.69***	3.04	10.53**	5.84
	Cicution virosae	31.51***	13.85***	22.88***	14.49**
Fresh water pools	Lemnion minoris	19.66***	8.68*	11.28**	9.20*
	Lemnion trisulcae	20.43***	10.45**	12.23**	11.22*
	Nymphaeion	21.97***	11.71**	19.92***	11.13*
	Hydrocharition morsus-ranae	9.10*	3.72	3.81	4.10
	Parvopotamion	21.37***	9.50**	9.09*	11.03*
	Ranunculion peltati	19.01***	11.21**	11.38**	10.80*
	Nitellion flexilis	1.14	0.57	1.68	1.99
	Charion fragilis	2.27	0.78	2.14	1.28
	Charion vulgaris	2.63	4.25	7.14*	0.97
Salt marsh	Zosterion	0.81	0.05	1.93	0.71
	Spartinion	3.53	2.40	1.71	1.40
	Thero-Salicornion	17.38***	6.11*	6.94*	8.80*
	Puccinellion maritimae	10.43*	5.59	9.61**	15.32**
Open salt water	Charion canescens	2.86	1.29	1.96	1.60
	Zannichellion pedicellatae	2.26	1.09	2.34	2.67
	Ruppion maritimae	7.68	2.42	9.03*	5.54
		DF=3	DF=2	DF=2	DF=3

DISCUSSION

Based on frequency of species, the data and analyses in this study indicate that plants of wetland communities affected by different stress factors possess diverse types of clonal traits. Based on the presence rather than on the frequency of species, however, there was an association between clonal traits and species presence in aquatic communities (fresh water pools and riverbeds), i.e., one generally can use clonal traits to predict which species are present in aquatic habitats. The plant communities in these latter two open-water wetland communities have species with a distinct spectrum of traits including high offspring production, good dispersability, fast-splitting clones, and monocyclic shoots. These results

indicate strong environmental filtering on recruitment from the local species pool in open-water communities, which causes convergence in plant traits. In other community types, the whole diversity of clonal and bud bank traits was found, even when some traits were typical for frequent species and others were found in rare species only. This finding well supports the idea of Grime (2006) that competition and disturbance operate simultaneously with diversifying and converging effects on community assembly. A similar trend was observed in terrestrial communities by Thompson et al. (2010).

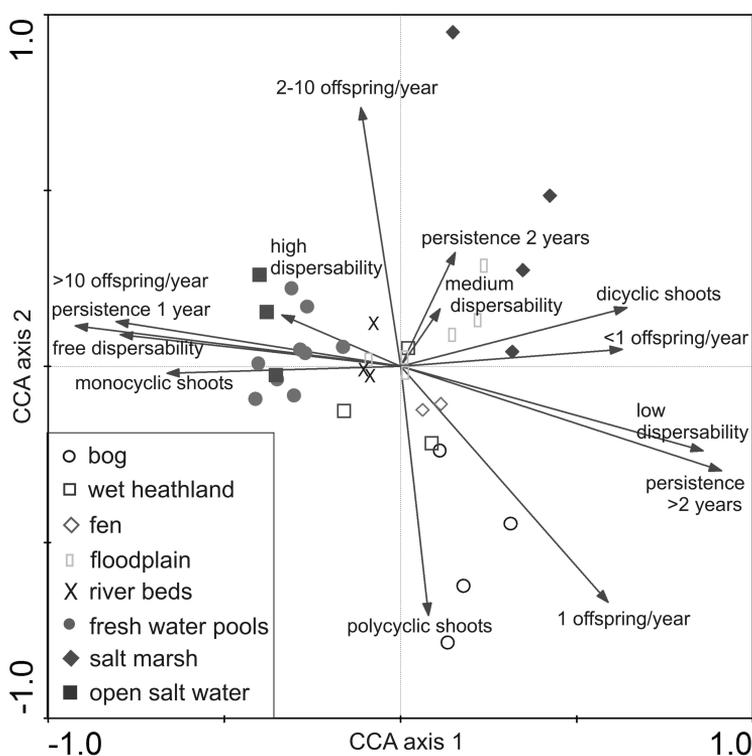


Fig. 3. Canonical correspondence analysis (CCA) ordination diagram of clonal traits used as explanatory variables for species composition in wetland communities. Arrows show clonal trait correlations. For explanation of clonal traits, see Table 2. Wetland communities are represented by different symbols. CCA axis 1 explained 18% of the variability (Eigenvalue: 0.785, $F = 2.25$, $P = 0.002$), CCA axis 2 additionally explained 15.2% of the variability.

That open-water communities have specific clonal traits is related to their colonization histories and to the qualities of water relative to air as a growing medium. Relative to air, water has a higher density and greater viscosity, a lower diffusion rate, and decreasing light

availability with increasing water table (Duarte et al. 1994). In addition, the aquatic environment provides optimal conditions for the production of low-cost vegetative propagules (Grace, 1993). These propagules are easily fragmented and can be transported over considerable distances within lakes and slowly flowing waters (Barrat-Segretain & Bornette 2000, Boedeltje et al. 2003). The ability of clones to rapidly divide may also prevent losses associated with traumatic breakages due to occasional disturbance in open-water communities (Hay & Kelly 2008). Although the colonization ability of vegetative propagules may be great, vegetative clones are vulnerable to desiccation and have short life spans. Consequently, seeds play the most important role in colonization by aquatic vegetation (Nielsen et al. 2006; Pollux et al. 2007).

In addition to the open-water communities, two other communities harboured specific sets of clonal and bud bank traits when species frequency was taken into account: nutrient poor mires (bog and fen) and salt marshes together with floodplains.

Bogs and fens formed a separate group characterised by low multiplication rates and polycyclic shoots. This confirms the classical idea that the production of perennial connections among ramets (de Kroon & Schieving 1990; Jónsdóttir & Watson 1997) is an advantageous clonal growth strategy in these resource-poor environments. The hypothesis that integrators prevail over splitters in dry and resource-poor habitats is based on experimental work and has been supported in an analysis of the flora of central Europe (van Groenendael et al. 1996) and of the western Himalayas (Klimeš 2008). Salt marshes and floodplains, on the other hand, were characterized by clones with medium lateral spread, high persistence of connections, and medium offspring production. Both communities are affected by daily or seasonally fluctuations in the water table, and salt marshes species must also cope with salt stress.

Our analysis based on presence/absence data failed to support predictions modified from Grace (1993) (see Introduction) for most of the communities. Species assemblies in these communities seem not to result from the filtering caused by one strong environmental constraint. Instead, the assemblies have diverse traits that enable co-existence of species (Kahlert et al. 2005; Grime 2006; Moles et al. 2009). Our analysis was inconsistent with the five predictions stated in the Introduction, as discussed below:

i) Freely dispersable offspring were not favoured in heterogeneous habitats but rather prevailed in homogeneous aquatic communities. In addition, the spreading ability of salt marsh species was slow, indicating the importance of additional selection forces, e.g., salt stress and other biotic interactions. Because the salt marsh vegetation consists of a high proportion of annual species, colonization and spreading by seeds can be important (Alvarez et al. 2005; Nielsen et al. 2006; Sosnová et al. 2010). Also, the need for osmotic protection

reduces the growth of plants and favours species forming integrated clones in order to supply ramets with water or nutrients (Pennings & Callaway 2000).

ii) High shoot turnover was typical of plants in nutrient-rich fresh water pools as predicted in Introduction, but the results were not significant for other wetland communities. Although nutrient availability in the sediment and water column affect the composition of aquatic plant communities (Santamaría 2002), high turnover rate of shoots in open-water communities should only be a consequence of different nature of aqueous medium. Especially submerged and free-floating species produce mobile offspring, which can be easily detached from the plant body and spread over long distances. These propagules can easily obtain nutrients directly from the water column. On the other hand, species with tightly connected systems predominate in 'periodically drier' wetland communities (floodplains and river beds), where the opportunity for fragment dispersal is reduced (Santamaría 2002).

iii) Reduced light availability typical for aquatic communities did not select for persistent clones but rather for fast disintegrating (splitting) ramets. Similarly, bogs and salt marshes, which have low vegetative biomass, contain species with long-lived connections between ramets, traits that are probably a by-product of the slow growth and decomposition rates in bogs. Extensive storage is clearly not associated with habitats limited by light but rather with nutrient-limited habitats, where photoassimilates cannot be used for growth and are stored in belowground organs (Čížková et al. 1996; Macek & Rejmánková 2006, 2007).

iv) Contrary to the prediction, relatively stable open-water communities had the highest offspring production, whereas river beds and daily inundated salt marshes produced fewer offspring.

v) Species growing in open-water communities had a seasonal bud bank situated in the water column, which could be an adaptation to herbivory or desiccation. Also, the diversity of bud-bearing organs in aquatic plants (turions, plant fragments, rhizomes) is regarded as an adaptation to disturbance, which may also include mechanical stress or extreme water level fluctuations (Combroux & Bornette 2004). In bogs, on the other hand, where a perennial belowground bud bank dominated, disturbances are rare, and this trait is probably related to the benefit of high persistence in a habitat with low nutrients (Nordbakken 2000).

In conclusion, Grace's predictions based on the clonal traits of individual species were supported in communities with distinct habitat characteristics. Most habitats, however, hosted the whole spectra of clonal traits, and most of the predictions based on the clonal traits of individual species knowledge were not supported. The relationships between these traits and environmental factors seem to be complex and cannot be easily explained by the results of experimental studies with individual species.

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REFERENCES

- Alvarez, M.G., Tron, F. & Mauchamp, A. 2005. Sexual versus asexual colonization by *Phragmites australis*: 25-year reed dynamics in a mediterranean marsh, Southern France. *Wetlands* 25: 639-647.
- Barrat-Segretain, M.-H. & Bornette, G. 2000. Regeneration and colonization abilities of aquatic plant fragments: effect of disturbance seasonality. *Hydrobiologia* 421: 31-29.
- Barrat-Segretain, M.-H., Bornette, G. & Hering-Vilas-Bôas, A. 1998. Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquatic Botany* 60: 201-211.
- Boedeltje, G., Bakker, J.P., Bekker, R.M., van Groenendael, J.M. & Soesbergen, M. 2003. Plant dispersal in a lowland stream in relation to occurrence and three specific life-history traits of the species in the species pool. *Journal of Ecology* 91: 855-866.
- Boedeltje, G., Bakker, J.P., ten Brinke, A., van Groenendael, J.M. & Soesbergen, M. 2004. Dispersal phenology of hydrochorous plants in relation to discharge, seed release time and buoyancy of seeds: the flood pulse concept supported. *Journal of Ecology* 92: 786-796.
- Boedeltje, G., Ozinga, W.A. & Prinzing, A. 2008. The trade-off between vegetative and generative reproduction among angiosperms influences regional hydrochorous propagule pressure. *Global Ecology and Biogeography* 17: 50-58.
- Bornette, G., Amoros, C. & Lamouroux, N.L. 1998. Aquatic plant diversity in riverine wetlands: The role of connectivity. *Freshwater Biology* 39: 267-283.
- Bornette, G., Henry, C., Barrat, M.H. & Amoros, C. 1994. Theoretical habitat templates, species traits, and species richness – aquatic macrophytes in the upper Rhone river and its floodplain. *Freshwater Biology* 31: 487-505.
- Bornette, G., Tabacchi, E., Hupp, C., Pujalon, S. & Rostan, J.C. 2008. A model of plant strategies in fluvial hydrosystems. *Freshwater Biology* 53: 1692-1705.
- CBS 1993. *Botanisch Basisregister*. CBS, Heerlen.
- Charpentier, A., Grillas, P. & Thompson, J.D. 2000. The effects of population size limitation on fecundity in mosaic populations of the clonal macrophyte *Scirpus maritimus* (Cyperaceae). *American Journal of Botany* 87: 502-507.
- Čížková, H., Lukavská, J., Přibáň, K., Kopecký, J. & Brabcová, H. 1996. Carbohydrate levels in rhizomes of *Phragmites australis* at an oligotrophic and a eutrophic site: A preliminary study. *Folia Geobotanica & Phytotaxonomica* 31: 111-118.
- Crombroux, I.C.S. & Bornette, G. 2004. Propagule banks and regenerative strategies of aquatic plants. *Journal of Vegetation Science* 15: 13-20.

- de Kroon, H. & Schieving, F. 1990. Resource partitioning in relation to clonal growth strategy. In: van Groenendael, J. & de Kroon, H. (eds.) *Clonal growth in plants: regulation and function*, pp. 113-130. SPB Academic Publishing, The Hague, The Netherlands.
- Duarte, C.M., Planas, D., Peñuelas, J. 1994. Macrophytes, taking control of an ancestral home. In: Margelef, R. (ed.), *Limnology Now: a Paradigm of Planetary Problems*. pp. 59–79. Elsevier, Amsterdam, The Netherlands.
- Dunn, R.R., Colwell, R.K. & Nilsson, C. 2006. The river domain: why are there more species halfway up the river? *Ecography* 29: 251-259.
- Eckert, C.G. 2002. The lost of sex in clonal plants. *Evolutionary Ecology* 15: 501-520.
- Grace, J.B. & Wetzel, R.G. 1982. Niche differentiation between two rhizomatous plant species: *Typha latifolia* and *Typha angustifolia*. *Canadian Journal of Botany* 60: 46-57.
- Grace, J.B. 1993. The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. *Aquatic Botany* 44: 159-180.
- Grime, J.P. 2001. *Plant strategies, vegetation processes and ecosystem properties*. Wiley, Chichester, UK.
- Grime, J.P. 2006. Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. *Journal of Vegetation Science* 17: 255-260.
- Hay, M.J.C. & Kelly, C.K. 2008. Have clonal plant biologists got it wrong? The case for changing the emphasis to disintegration. *Evolutionary Ecology* 22: 461-465.
- Jónsdóttir, I.S., & Watson, M.A. 1997. Extensive physiological integration: an adaptive trait in resource-poor environments? In: de Kroon, H. & van Groenendael, J. (eds.) *The ecology and evolution of clonal plants*, pp. 109-136. Backhuys Publishers, Leiden, The Netherlands.
- Kahlert, B.R., Ryser, P. & Edwards, P.J. 2005. Leaf phenology of three dominant limestone grassland plants matching the disturbance regime. *Journal of Vegetation Science* 16: 433-442.
- Klimeš, L. & Klimešová, J. 1999. CLO-PLA 2 – a database of clonal plants in central Europe. *Plant Ecology* 141: 9-19.
- Klimeš, L. 2008. Clonal splitters and integrators in harsh environments of the Trans-Himalaya. *Evolutionary Ecology* 22: 351-367.
- Klimešová, J. & Klimeš, L. 2006. CLO-PLA 3: a database of clonal growth in plants. URL: <http://clopla.butbn.cas.cz/>
- Klimešová, J. & Klimeš, L. 2007. Bud banks and their role in vegetative regeneration – a literature review and proposal for simple classification and assessment. *Perspectives in Plant Ecology, Evolution and Systematics* 8: 115-129.
- Kubát, K., Hrouda, L., Chrtěk, J., Kaplan, Z., Kirschner, J. & Štěpánek, J. 2002. *Klíč ke květeně České republiky*. Academia, Praha. [Key to the Flora of the Czech Republic].
- Macek, P. & Rejmánková, E. 2006. The effect of long-term submergence on functional properties of *Eleocharis cellulosa* Torr. *Aquatic Botany* 84: 251–258.
- Macek, P. & Rejmánková, E. 2007. Response of emergent macrophytes to experimental nutrient and salinity additions. *Functional Ecology* 21: 478–488.

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- Macek, P., Rejmánková, E. & Lepš, J. 2010. Dynamics of *Typha domingensis* spread in *Eleocharis* dominated oligotrophic tropical wetlands following nutrient enrichment. *Evolutionary Ecology* (under review).
- Moles, A.T., Warton, D.I., Warman, L., Swenson, N.G., Laffan, S.W., Zanne, A.E., Pitman, A., Hemmings, F.A. & Leishman, M.R. 2009. Global patterns in plant height. *Journal of Ecology* 97: 923-932.
- Nielsen, U.N., Riis, T. & Brix, H. 2006. The importance of vegetative and sexual dispersal of *Luronium natans*. *Aquatic Botany* 84: 165-170.
- Nordbakken, J.-F. 2000. Fine-scale persistence of boreal bog plants. *Journal of Vegetation Science* 11: 269-276.
- Pennings, S.C. & Callaway, R.M. 2000. The advantages of clonal integration under different ecological conditions: A community-wide test. *Ecology* 81: 709-716.
- Philbrick, C.T. & Les, D.H. 1996. Evolution of aquatic angiosperm reproductive systems. *BioScience* 46: 813-826.
- Pollux, B.J.A., Jong, M.D.E., Steegh, A., Verbruggen, E., van Groenendael, J.M. & Ouborg, N.J. 2007. Reproductive strategy, clonal structure and genetic diversity in populations of the aquatic macrophyte *Sparganium emersum* in river systems. *Molecular Ecology* 16: 313-325.
- Puijalón, S., Bornette, G. & Sagnes, P. 2005. Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species. *Journal of Experimental Botany* 56: 777-786.
- Roughgarden, J. 1979. *Theory of population genetics and evolutionary ecology: an introduction*. MacMillan, New York, US.
- Santamaría, L. 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologica* 23: 137-154.
- Schaminée, J.H.J., Stortelder, A.H.F. & Weeda, E.J. 1996. *De vegetatie van Nederland (v. 3) – graslanden, zomen, droge heiden*. Opulus Press, Uppsala Leiden.
- Schaminée, J.H.J., Weeda, E.J. & Westhoff, V. 1995. *De vegetatie van Nederland (v. 2) – wateren, moerassen, natte heiden*. Opulus Press, Uppsala Leiden.
- Schaminée, J.H.J., Weeda, E.J. & Westhoff, V. 1998. *De vegetatie van Nederland (v. 4) – kust, binnenlandse pionier milieus*. Opulus Press, Uppsala Leiden.
- Sosnová, M., van Diggelen, R. & Klimešová, J. 2010. Distribution of clonal growth forms in wetlands. *Aquatic Botany* 92: 33-39.
- ter Braak, C.J.F. & Šmilauer, P. 2002. *CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5)*. Microcomputer Power, Ithaca.
- Thompson, K., Petchey, O.L., Askew, A.P., Dunnett, N.P., Beckerman, A.P. & Willis, A.J. 2010. Little evidence for limiting similarity in a long-term study of a roadside plant community. *Journal of Ecology* 98: 480-487.
- van Groenendael, J.M., Klimeš, L., Klimešová, J. & Hendriks, R.J.J. 1996. Comparative ecology of clonal plants. *Philosophical Transactions of the Royal Society of London. Series B- Biological Sciences* 351: 1331-1339.

Electronic appendix. Comparison between observed (species of a particular alliance) and expected bud bank characteristics (based on 500 permutations of all species). Tested by contingency tables ($P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; na not available; if not indicated, the compared spectra were not significantly different). DF (occurrence of buds) = 2; DF (seasonality of buds) = 1.

Wetland type	Alliances	Bud bank > 10 cm		Bud bank 0-10 cm		Bud bank 0 cm		Bud bank 0-(10) cm		Bud bank < -10 cm	
		Occurrence	Seasonality	Occurrence	Seasonality	Occurrence	Seasonality	Occurrence	Seasonality	Occurrence	Seasonality
Bog	Rhynchosporion albae	2.01	0.23	3.09	0.24	0.17	0.34	2.17	0.11	0.00	na
	Caricion lasiocarpae	1.25	3.19	1.89	2.00	0.00	5.56*	5.17	1.44	0.35	na
	Ericion tetralicis	3.57	7.18**	3.20	4.10*	0.20	5.45*	8.43*	6.72**	0.35	na
	Oxyocco-Ericion	2.65	6.12*	2.17	4.13*	0.16	5.85*	7.24*	7.85**	0.35	na
Wet heath land	Litorellion uniflorae	6.55*	1.02	3.37	1.02	0.21	0.07	1.25	0.00	0.35	na
	Hydrocotylo-Baldellion	6.67*	0.35	2.56	0.00	1.20	0.21	3.68	0.07	0.00	na
	Eleocharion acicularis	9.10*	1.02	3.00	0.00	0.66	1.98	3.74	0.40	0.35	na
Fen	Caricion nigrae	1.23	2.81	2.61	1.03	0.97	2.61	5.63	1.21	1.05	na
	Caricion davallianae	1.74	1.36	1.51	0.21	0.61	1.57	4.87	1.10	0.87	na
Flood plain	Phragmition australis	0.71	1.03	0.17	0.00	4.15	2.56	6.50*	0.30	1.70	na
	Caricion gracilis	0.99	1.03	1.88	0.21	3.54	0.91	4.68	0.15	1.71	na
	Caricion elatae	0.20	0.34	0.92	0.21	3.11	0.58	6.94*	0.00	1.71	na
	Lolio-Potentillon anserinae	0.15	2.05	3.12	3.09	0.58	0.34	0.06	0.92	2.14	na
	Callithion palustris	2.77	0.12	6.48*	0.12	3.56	0.75	8.99*	2.01	0.08	na
	Albopetron pratensis	4.07	2.76	7.32*	1.34	2.46	0.09	5.32	1.93	0.86	na
River beds	Sparganio-Glycerion	0.32	1.04	0.31	0.00	2.74	8.45*	3.00	1.45	1.06	na
	Oenanthon aquaticae	1.13	4.13*	0.15	0.00	4.42	3.57	4.92	0.91	1.06	na
	Cicution virosae	0.31	1.03	0.40	0.21	4.37	0.84	9.33**	0.32	1.71	na
Fresh water pools	Lemnon minoris	2.93	1.02	1.42	1.04	1.32	2.57	1.99	0.62	1.11	na
	Lemnon fluitulae	2.92	1.02	1.13	1.03	1.10	2.57	2.98	0.53	1.10	na
	Nymphaeion	3.19	2.05	3.86	0.00	0.93	8.07**	3.93	4.98*	1.38	na
	Hydrocharition morsus-ranae	2.64	0.35	2.49	0.00	1.85	5.14*	2.14	3.44	0.72	na
	Pavoptamion	1.71	2.05	2.17	0.00	0.95	7.03**	5.28	4.17*	0.71	na
	Ranunculion peltati	1.76	2.06	2.19	0.00	0.94	9.04**	6.62*	4.80*	0.71	na
	Nitellion flexilis	5.66	1.03	4.38	0.00	2.83	0.98	1.90	0.47	1.08	na
	Charion fragilis	4.90	0.36	4.64	0.36	1.78	1.78	1.51	0.12	0.35	na
	Charion vulgaris	2.99	1.02	1.94	0.36	1.59	3.39	2.44	0.96	1.06	na
	Zosterion	0.67	na	0.67	na	0.83	1.14	0.88	0.53	na	na
Salt marsh	Spartinion	0.44	na	0.42	na	0.00	0.00	1.11	1.14	na	na
	Thero-Salicornion	3.07	1.03	1.29	1.03	2.44	0.44	0.60	1.25	0.00	na
	Puccinellion maritimae	0.39	1.02	2.04	1.02	0.50	0.26	0.54	1.09	0.35	na
Open salt water	Charion canescens	1.48	na	2.44	na	1.33	2.33	0.48	0.40	1.08	na
	Zannichellion pedicellatae	4.20	0.35	2.37	0.35	0.20	7.88**	6.13*	1.12	1.06	na
	Ruppion maritimae	2.79	1.05	10.95**	na	11.34**	5.86*	10.89**	8.89**	1.03	na

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CHAPTER IV

Life-history variation in the short-lived herb *Rorippa palustris*: effect of germination date and injury timing

Klimešová J, Sosnová M & Martínková J (2007)

Plant Ecol. 189: 237-246

LIFE-HISTORY VARIATION IN THE SHORT-LIVED HERB *RORIPPA PALUSTRIS*: EFFECT OF GERMINATION DATE AND INJURY TIMING

ABSTRACT

Life-history variation in annuals is known to be caused by size requirements for photoinduction of flowering, but the importance of germination date and injury was overlooked so far even though they may play an important role in disturbed habitats. To test the effect of germination date and timing of injury on life-history variation of an annual plant, we performed a two-year pot experiment with the root-sprouting herb *Rorippa palustris*. Plants belonging to six different cohorts, and sown at monthly intervals from April to September, were injured (all stem parts removed) in three ontogenetic stages: vegetative rosettes, flowering plants and fruiting plants. Plants from the April, and partly from the May, cohort behaved as summer annuals: they started to bolt at the same time, resprouted and overwintered poorly. Plants from the June cohort flowered in the first season as well, but they entered the bolting stage a month later than the preceding cohorts, produced the least fruits, but overwintered successfully and flowered again the second year (polycarpic perennials). Cohorts germinating after the summer solstice did not flower during the first year and, with the exception of the September cohort, overwintered successfully and flowered the second year (winter annuals). After injury, the pattern of life-histories was the same as in control plants, although generative reproduction was interrupted by injury in the first year of the experiment. About one quarter of plants injured in the vegetative stage regenerated after injury irrespective of cohort, however, regeneration was enhanced in larger plants. Regeneration of plants in the flowering and fruiting stages depended on date of injury in relation to day length, being the most successful after the summer solstice. Life-history variation, together with the ability to resprout after severe injury in the pioneer wetland herb *Rorippa palustris* is caused by its ability to germinate throughout the season. Even the second tested factor, the effect of injury, is modified by germination time. The experiment points to a complex nature of factors affecting life-history variation as well as resprouting after severe injury in short lived plants.

Key words: adventitious buds, bud bank, disturbance, flowering, long-day, wetland

Life-history variation in *R. palustris*

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ABSTRAKT

Změny v životním cyklu jednoletek mohou být způsobeny nutností dosáhnout určité velikosti pro indukci kvetení, ale důležitost data vyklíčení a narušení byla dosud opomíjena, ačkoliv může ovlivnit životní cyklus zejména u rostlin na narušovaných stanovištích. K objasnění této problematiky jsme založili 2-letý nádobový experiment s kořenoodnožujícím druhem rukví bažinou. Rostliny z 6 následujících kohort (vysévané každý měsíc od dubna do září) byly narušeny ve třech ontogenetických stádiích (vegetativní růžice, kvetoucí a plodící rostliny). Rostliny z dubnové (a částečně i květnové) kohorty se chovaly jako jednoletky (summer annuals): začaly kvést synchronně, schopnost odnožování a přežívání zimy byla malá. Rostliny z červnové kohorty také kvetly první sezónu, ovšem o měsíc později nežli předchozí kohorty a vytvořily méně semen. Tyto rostliny přezimovaly úspěšně a opakovaně kvetly další sezónu (polykarpické trvalky). Rostliny z kohort, které vyklíčily až po letním slunovratu, již první sezónu nekvetly a s výjimkou zářijové kohorty úspěšně přezimovaly a kvetly další sezónu (ozimé jednoletky – winter annuals). Narušené rostliny se po regeneraci chovaly podobně jako rostliny kontrolní, ačkoliv narušení přerušilo investici do reprodukce. Zhruba jedna čtvrtina rostlin narušených jako vegetativní růžice regenerovala, a to nezávisle na kohortě, i když větší rostliny byly úspěšnější. Regenerace rostlin narušených jako kvetoucí nebo plodící závisela na datu narušení vzhledem k délce dne, přičemž nejlépe regenerovaly rostliny narušené po letním slunovratu. Variabilní životní cyklus a schopnost regenerovat z kořenů po narušení u mokřadního druhu rukve bažinné je dáný jeho schopností klíčit v průběhu celé sezóny. Datum vyklíčení má vliv i na druhý testovaný faktor – efekt narušení. Tento experiment přibližuje komplexní vliv několika faktorů na životní cyklus a schopnost odnožování z kořenů u krátkověkých narušovaných druhů.

Zbytek této kapitoly byl uveřejněn v *Plant Ecology*, Volume: 189, Issue: 2, Pages: 237-246, Published: APR 2007.

CHAPTER V

Life-history variation in the short-lived herb *Rorippa palustris*: The role of carbon storage

Sosnová M & Klimešová J (2009)

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LIFE-HISTORY VARIATION IN THE SHORT-LIVED HERB *RORIPPA PALUSTRIS*: THE ROLE OF CARBON STORAGE

ABSTRACT

Carbon storage is commonly found among perennials, but only rarely in annuals. However, many short-lived species may behave as annuals or short-lived perennials depending on the date of germination, photoperiod or disturbance. Due to the trade-off between investments into current reproduction versus survival, these life history modes presumably differ in carbon allocation. In this study, we aimed to evaluate how carbon storage is affected by germination date and disturbance in an outdoor pot experiment with the short-lived *Rorippa palustris*. Plants from autumn and summer cohorts were injured in different ontogenetic stages (vegetative, flowering and fruiting) and the starch content in roots was assessed. Plants from the autumn cohort invested more carbon into growth and reproduction, whereas plants from the summer cohort invested preferentially into reserves. However, injury changed the allocation pattern: in plants from the autumn cohort, injury prevented allocation to reproduction and thus injured plants had a larger carbon storage at the end of the season than control plants; injury at the flowering and fruiting stage caused depletion of reserves for regrowth in plants from the summer cohort, resulting in lower starch reserves compared to control plants. We suggest that life history variation in *Rorippa palustris* can be caused by changes in its carbon economy: when all resources could not be used for flowering due to weak photoinduction or loss of flowering organs due to injury, part of the resources is stored for over wintering and reproduction in the next year.

Keywords: starch, life-history mode, recovery from injury, wetland plants, carbon allocation

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ABSTRAKT

Vytrvalé druhy na rozdíl od jednoletém ukládají uhlík běžně. Nicméně, mnoho krátkověkých druhů se může chovat jako jednoletka nebo krátkověká trvalka v závislosti na datu vyklíčení, fotoperiodě nebo disturbance. Toto chování se ale zákonitě liší v alokaci uhlíku, protože rostlina musí upřednostnit buď investici do současné reprodukce nebo do přežívání. Tato studie se zabývala změnami v alokaci uhlíku u krátkověkého druhu *Rorippa palustris* v závislosti na datu vyklíčení a disturbance. Rostliny z podzimní a letní kohorty byly narušovány v různých ontogenetických stádiích (vegetativní, kvetoucí a plodící) a byl sledován obsah škrobu v kořenech. Zatímco rostliny z podzimní kohorty investovaly do růstu a reprodukce, rostliny z letní kohorty spíše tvořily zásoby. Po narušení ovšem došlo ke změně v alokaci uhlíku: u podzimní kohorty došlo k přerušení investice uhlíku do reprodukce a narušené rostliny vykazovaly větší zásoby uhlíku na konci sezóny, zatímco u letní kohorty narušení vedlo ke snížení obsahu zásobního škrobu v kořenech vlivem nutnosti obnovení nadzemní biomasy a to zejména u rostlin narušených jako kvetoucí nebo plodící. Získané výsledky naznačují, že různé chování (life history variation) u tohoto druhu může být způsobeno změnami v hospodaření s uhlíkem: pakliže rostlina nemůže využít všechny uhlík ke kvetení, ať už díky slabé indukci kvetení nebo ztráty kvetoucích prýtů v důsledku poranění, uschová si uhlík pro přezimování a reprodukci v příští sezóně.

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CHAPTER VI

The consequence of flooding and injury on performance of
wetland root-sprouting herb *Rorippa palustris*

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[manuscript]

THE CONSEQUENCE OF FLOODING AND INJURY ON PERFORMANCE OF WETLAND ROOT-SPROUTING HERB *RORIPPA PALUSTRIS*

ABSTRACT

Root-sprouting belongs among the rarest types of clonal growth organs found in wetland communities. This underrepresentation can be explained by predominance of monocots and low speciation rate common in wetlands, however, various functional constraints may participate, e.g., higher energy costs of root formation and sprouting under hypoxic conditions. In this study we asked, whether the root-sprouting ability in a wetland plant is hindered by flooding stress. The impact of oxygen shortage was evaluated in a greenhouse pot experiment with six populations of *Rorippa palustris* originated from two contrasting habitats: wetland populations, which often experience flooding and, ruderal populations from mechanically disturbed sites. Plants were subjected to following treatments: flooding for a period of 7 days, injury by aboveground biomass removal, injury followed by flooding 7 days, and no manipulation (control). Wetland populations were delayed in phenology compared to ruderal ones, but this difference disappeared in time. No other differences in stress response between wetland and ruderal populations were found. Flooded plants showed fast vertical growth, but neither their survival nor seed production was affected. Injured plants regenerated within few days following injury, were smaller and produced less seeds. Combination of injury and flooding was detrimental for plants from both habitats causing mortality in 80 % of plants. Regeneration in survival plants took several weeks and seed production was reduced. The survival of populations of this species experiencing flooding combined with disturbance is difficult and depends on seed bank. The results indicates possible limitations of the root-sprouting ability in wetlands, however, to reveal the true reasons, more experimental work is needed.

Keywords: root buds, stress survival, tolerance to submergence, injury, contrasting habitats

INTRODUCTION

The combination of stress together with frequent and severe disturbance represents harsh conditions, for which plants could hardly be adapted (Grime 2001). Following severe disturbance event, plant regeneration is additionally reduced by nutrient deficiency and other stress factors. In ecosystems, however, only few restricted areas are not inhabited by plants, which can be caused by temporal and spatial instability of such extreme conditions and by the potential of species to recolonise the place from adjacent areas. The ecosystems typical of combination of stress and severe disturbance are tidal zones, riverine littoral zones with temporal inundation combined with disturbance, and fire prone habitats, where aridity is combined with recurrent fires and herbivory (Bornette et al. 2008, Noble and Slatyer 1980).

In riverine littoral zones, plants have to cope with disturbance and various stress factors including water table fluctuations, movement of water currents, silt deposition, and erosion. These processes form gradients in river floodplain with the highest levels of stress and disturbance found near the stream (Bornette et al. 2008, Pujalon et al. 2007). On the community level, the existence of gradients in river floodplain results in vegetation zonation (Bornette et al. 2008, Renöfält and Nilsson 2008, van Eck et al. 2004); on the population level it leads to adaptation of life histories (Trémolières 2004), and on the individual level it ends in morphological and physiological adaptations (Crawford 2003, Visser et al. 2000, Voesenek et al. 2006). Only few species, however, are able to cope with multiple stresses acting near rivers, and they can employ rather contrasting strategies for survival. For example, persistent species resist water current by good anchorage and flexible stems, some species can regenerate through bud bank after being covered by sediment of fragmented by erosion, and other species can recolonise suitable areas by freely floating plant parts or by seeds (Hanley and Lamont 2002, Kallioda et al. 1991, Lowe et al. 2010, Trémolières 2004, Willby et al. 2000).

In plants from riverine habitats, the strategy of regeneration through bud bank is usually realized by buds situated on belowground organs of stem origin, i.e., rhizomes. However, plant regeneration following severe disturbance can be realised by adventitious sprouting from roots, which is a strategy commonly found in species growing in “terrestrial” disturbed habitats such as arable fields (Klimešová and Klimeš 2007). Regrowth and spread through lateral roots is regarded to be typical of opportunistic species due to the high number of buds which can be produced on lateral roots and the higher foraging efficiency of roots compared to rhizomes or stolons (Dietz et al. 2002, Klimeš and Klimešová 1999). Although root-sprouting is common under dry “terrestrial” conditions, it is rarely found in wetland and aquatic habitats (Grace 1993, Sosnová et al. 2010). This could be attributed to the high proportion of monocotyledonous plants in wetlands, i.e., monocots are rarely root-sprouters.

However, apart from this phylogenetic reason, root-sprouting ability in wetlands can be also hindered by detrimental effects of anoxia and disturbance, because *de novo* formation of buds on roots is more oxygen demanding compared to sprouting from preformed axillary buds on rhizomes (Peterson 1975, Philbrick and Les 1996). Contrary to rhizomes, where tolerance of anoxia can last up to several months (i.e., *Acorus calamus* - Weber and Brändle 1996), in roots it varies from few hours to days depending on the season and condition of the organ (Crawford and Brändle 1996, Crawford 2003). The ability of rhizomes to survive long periods of submergence is enabled by high carbohydrate levels, which allow an extended viability of tissues under anoxia. Roots are also more sensitive to post anoxic injury (Biemelt et al. 1996).

To test the ecological hypothesis presuming damaging effects of disturbance and anoxia on the root-sprouting ability in wetland species, we established a pot experiment with a short-lived perennial herb *Rorippa palustris*. It occurs on a broad scale of wet habitats with distinct disturbance and flooding regimes, thus we could expect that local adaptations could play a role in regeneration ability of this species. Although population differentiations could be found inside one region (Kawecki and Ebert 2004) and may increase with distance among sites (Becker et al. 2006, Galloway and Fenster 2000, Leiss and Müller-Schärer 2001), local differentiation between neighbouring populations of contrasting habitats can be even stronger than differentiation at a European scale (Bischoff et al. 2006) and small scale gradients of flooding duration may select for special genotypes as well (Lenssen et al. 2004). To test the possible role of local adaptations in *R. palustris*, we used plants originating either from ruderal or wetland habitats and expected higher tolerance to flooding stress in wetland populations and better regeneration ability in ruderal populations. Single and combined effects of disturbance and short-term inundation were tested.

METHODS

Study species

Rorippa palustris (Brassicaceae) is short-lived root-sprouting species. It is able to germinate throughout the growing season and depending on germination date it behaves as an annual, winter annual or short-lived perennial. In this species, the root-sprouting ability is triggered by disturbance and depends on plant size and age (Klimešová et al. 2004, Martínková et al. 2004). *R. palustris* occurs on river banks and fishpond bottoms with spring or summer floods or, on the other hand, it is found on wet ruderal places like path margins or wet depressions in arable fields, where it can experience severe disturbance.

Pot experiment

Seeds of *R. palustris* originated from wetland populations (Zliv brook, Zliv meadow, Zdice) or wet ruderal sites (Nový Bydžov, Jindřichův Hradec, České Budějovice) were obtained in the 2006 season. Seeds were collected from several plants of a given population and mixed. Wetland populations originated from wet meadows near dikes and warps of small rivers, whereas ruderal populations were collected in mowed road ditches and disturbed wet depressions near construction sites. In March 2007 seeds were germinated on Petri dishes in fluctuating temperatures (approx. 24/10 °C day/night). Two weeks after germination (13th April 2007) a total of 480 seedlings (80 seedlings per population) were planted into pots (one seedling per pot, 13 x 13 x 13 cm) filled with a mixture of pure washed sand and standard garden soil (AGRO CS a. s., Česká Skalice) at a volume ratio 2 : 1. A fine-textured textile was placed on the bottom of each pot to avoid root penetration. Pots were placed into containers (24 pots per container, 82 x 97 x 40 cm, 20 containers) located in open greenhouse to prevent water level fluctuations, but enable free air circulation. Plants were watered regularly; water level was kept 7 cm below the pots' soil surface; following stress application the water level was lowered to 9 cm below the soil surface.

Plants were regularly treated with insecticides Mospilan (Mospilan 20SP, 20% Acetamiprid, AgroBio Opava) and Fast K (0.003% Deltamethrin, Prost a. s., Náměšť nad Oslavou) to avoid aphid and other pests attacks.

Treatments

In the age of two months (9th June 2007), twenty randomly chosen plants from each population were subjected to one of the following treatments: (i) control, (ii) injury by removal of all aboveground biomass, (iii) flooding for 7 days and (iv) injury combined with flooding. Removal of all aboveground biomass resulted in loss of all axillary buds in plant, thus plant was forced to regenerate from adventitious buds originated on roots. For the flooding treatment the water level was elevated up to 10 cm above the soil surface, i.e. plant rosette was submerged, but the plant was allowed to reach water surface by stem elongation. After 7 days, the water table was lowered and kept at 9 cm below the soil surface. This decrease in water table should simulate natural groundwater decline during summer months. During the vegetation season 2007 mortality, growth and seed production of plants was followed. Maturing seeds were trapped into fine-textured fabric tied round the stem; the fabric allowed free air circulation and light penetration. When all seeds were ripen and/or shoot dead, the shoots wrapped in fabric were cut off and all seeds were carefully poured out. Seeds were subsequently weighted and its total number was estimated from the weight of exactly 50 seeds from each plant.

All plants were moved outdoors for overwintering without any frost protection. The following spring (April 2008) all living plants were counted and moved back into open greenhouse. In the season 2008, plants were measured and seed production was followed. In a monthly step, starting in May 2008, plants were fertilized with tablet fertilizer (1 tablet fertilizer per pot, dates on 6th May, 3rd June, 10th July, 12th September 2008, Universal garden fertilizer, ASB Grínland, nutrient content: N 0.1g/pot, P 0.06 g/pot, K 0.7 g/pot). The same procedure was followed in the 2009 season (plants were fertilized on 22nd April, 26th May, 22nd June, 21st July, 19th August, and 21st September 2009).

Statistics

The effects of individual population and habitat (ruderal, wetland) on growth of control plants, i.e., leaf length, height of plant, were tested by repeated measurement analysis of variance. In control plants, nested design analysis of variance was used to test the effect of population and habitat (population nested in habitat) on height of plant, cumulative height of plant, seed weight, weight of 50 seeds, and seed number.

The effect of treatment application on plant characteristics was tested by nested design analysis of variance and post hoc Tukey (HSD) tests (population nested in habitat – Table 1).

The data collected in the season 2008 (i.e., 26th May, 3rd June, 11th June, 18th June, 23rd June, 30th June, 7th July) were tested by factorial analysis of variance (factors: habitat and treatment), and the effect of season was tested by repeated measurement analysis of variance. The relation between cumulative shoot height and the seed number in various treatments was assessed by simple linear regression.

Generalized linear models (GLM) with binomial distribution (link function = logit, test = χ^2) were used to test the relationship between population, habitat and initial aboveground biomass on the regeneration ability. Similarly, the relation between plant height and seed number on the overwintering probability was tested.

RESULTS

Growth of control plants in the 2007 season

In the 2007 season, control plants from wetland habitat were delayed in phenology compared to ruderal ones (repeated measurement ANOVA, leaves: $p > 0.05$; plant height: $F = 10.4$, $p < 0.01$), i.e. wetland plants started bolting later. However, this difference disappeared in time (Fig. 1) and plants from both habitats did not differ in stem height at the time of seed maturity ($p > 0.05$). Similarly, plants from various populations differed in growth of shoot

(repeated measurement ANOVA, plant height: $F=10.0$, $p<0.001$) but not in the length of leaves ($p>0.05$). At the time of seed maturity certain plant populations keep on lower plant height (nested design ANOVA, $F=2.833$, $p<0.05$).

Total seed weight and seed number produced by plants originated in different habitats and populations did not differ ($p>0.05$). Wetland populations produced heavier seeds compared to ruderal ones (nested design ANOVA, $F=10.43$, $p<0.01$) and seed number was positively related to cumulative plant height (simple linear regression, $R=0.678$, $p<0.001$).

Table 1. Growth parameters, regeneration and overwintering of plants subjected to various treatments in three consequent seasons.

		controls	flooded	injured	injured + flooded
season 2007	height of plant [cm]	45.92 ± 0.91a	49.82 ± 0.94b	18.37 ± 0.77c	6.88 ± 1.33d
	$F=345.2^{***}$				
	cumulative height of plant [cm]	61.54 ± 1.37a	65.02 ± 1.37a	39.90 ± 2.30b	11.42 ± 2.80c
	$F=96.15^{***}$				
	total seed weight [g]	0.72 ± 0.03a	0.65 ± 0.03a	0.16 ± 0.01b	0.07 ± 0.02b
	$F=99.29^{***}$				
weight of 50 seeds [mg]	1.94 ± 0.03a	2.05 ± 0.03a	2.00 ± 0.04a	2.17 ± 0.14b	
	$F=3.58^*$				
seed number	18946 ± 839a	16979 ± 814a	4257 ± 329b	2247 ± 648b	
	$F=94.08^{***}$				
mortality [No]		1	4	21	96
season 2008	over wintering [No]	23	15	49	14
	height of plant [cm]	40.70 ± 2.24	42.47 ± 2.49	41.38 ± 1.69	47.29 ± 3.37
	$F=0.675$ n.s.				
	cumulative height of plant [cm]	99.50 ± 11.05	91.13 ± 12.07	111.59 ± 7.75	102.29 ± 14.58
	$F=0.1512$ n.s.				
	total seed weight [g]	2.37 ± 0.34	1.73 ± 0.27	2.31 ± 0.22	2.20 ± 0.41
	$F=0.558$ n.s.				
weight of 50 seeds [mg]	2.22 ± 0.08	1.91 ± 0.11	2.20 ± 0.05	2.18 ± 0.12	
	$F=1.786$ n.s.				
seed number	53368 ± 8021	46449 ± 7522	51704 ± 4921	49820 ± 8630	
	$F=0.126$ n.s.				
season 2009	over wintering [No]	4	4	8	1

Means ± standard errors are shown, $n=120$. The values were calculated for living plants only. Data of the 2007 season were tested by nested design ANOVA (population nested in habitat), and post hoc Tukey (HSD) test. Results of F -tests for the effect of the treatment are given. Data of the 2008 season were tested by factorial ANOVA. (* $P<0.05$; *** $P<0.001$; n.s.: non-significant).

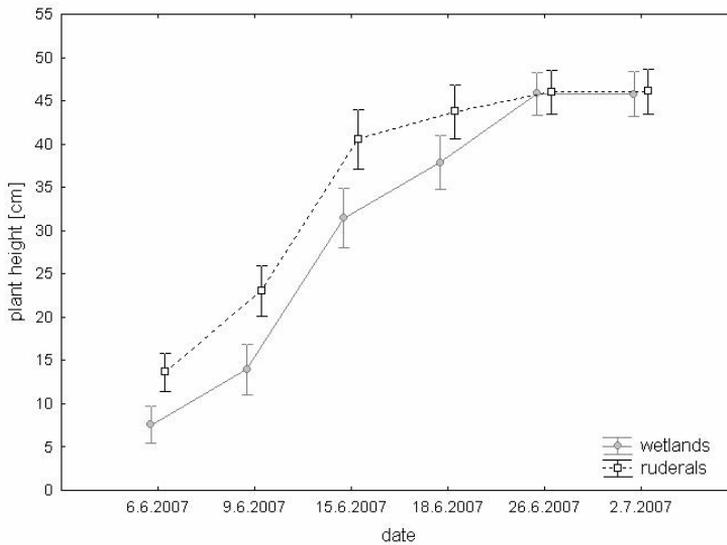


Fig. 1. Growth of control plants from June to July 2007. Plants started bolting in the beginning of June and seeds were mature in August 2007. Height of longest shoot is shown. The stem height after the mid July remained stable. Means and confidence intervals for wetland (in grey) and ruderal (in black) populations are shown.

Treatment application

Flooding had no effect on mortality of plants, whereas injury caused some mortality (12 %), irrespective of population, habitat or initial biomass production (GLM, $p > 0.05$). The combination of injury and flooding was detrimental for most of plants (80 % of mortality), irrespective of habitat or population (GLM, $p > 0.05$) and only plants with high initial biomass regenerated (GLM, $\chi^2 = 24.05$, $p < 0.001$) (Table 1, Fig. 2).

Treatment application affected all measured characteristics of plants (Table 1). Immediate response of plant to flooding included fast lateral growth and changes in leaves orientation. This effect remained significant till seed ripening (height of plant – Table 1), although cumulative plant height was not affected. There was a trend towards lower seed production in flooded plants (Table 1).

Injured plants lost all of their aboveground biomass and were forced to regenerate from root buds, which resulted in smaller plants (decrease plant height and cumulative plant height) at the end of season. Total seed weight and seed number were also lowered by the treatment application (Table 1). The majority of injured plants were able to regenerate within 7 days and the regeneration success was not affected by the amount of plant biomass

removed by injury, although smaller plants failed to regenerate more frequently. Regenerated plants started bolting in the beginning of July, i.e. four weeks after injury.

Combination of injury and flooding resulted in high mortality. Regeneration was low and took 3-6 weeks. Moreover, the regenerated plants were small and produced hardly any seeds (Table 1). The weight of seeds (weight of 50 seeds) was high compared to plants from other treatments.

In plants subjected to single treatment, the number of seeds was positively correlated to cumulative shoot height (simple linear regression, flooded plants: $R=0.696$, $p<0.001$; injured plants: $R=0.453$, $p<0.001$), but this effect was not significant in plants subjected to the combination of injury and flooding (Fig. 3).

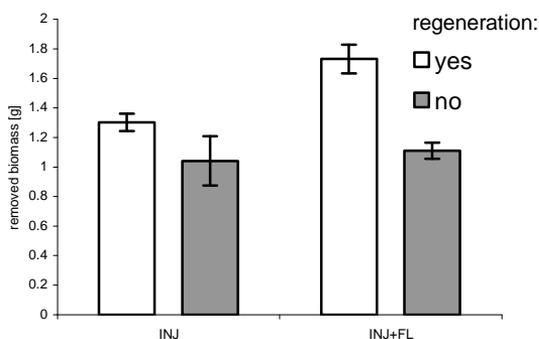


Fig. 2. Regeneration ability related to the biomass removed by injury. Means and standard errors are shown. Illustrated for plants subjected either to injury or to the combination of injury and flooding.

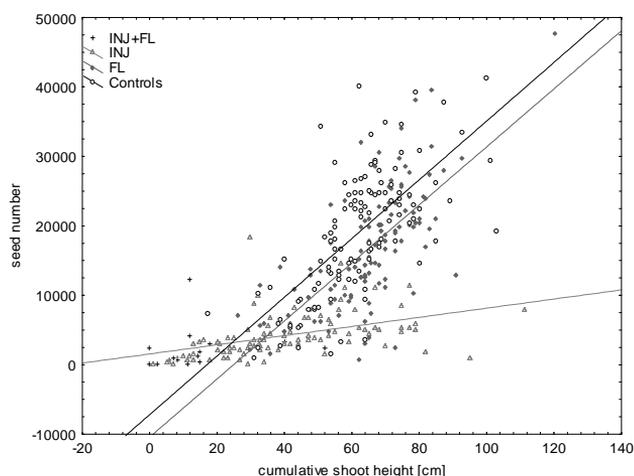


Fig. 3. Seed number (estimated from total seed weight and weight of 50 seeds) related to cumulative plant height. Data for the season 2007.

Overwintering and polycarpy

Overwintering of control and flooded plants was low (19% of control plants, 12.5% of flooded plants – Table 1). By contrast, winter survival in injured plants was successful in 41% of cases. In plants subjected to the combination of injury and flooding, 14 out of 24 regenerated plants overwintered. Except of two individuals, all overwintered plants also flowered and produced seeds in the 2008 season. Smaller plants and plants with lower seed production overwintered more likely (GLM, cumulative shoot height: $\chi^2 = 32.56$, $p < 0.001$; seed number: $\chi^2 = 31.26$, $p < 0.001$), however this is due to higher overwintering success of injured plants.

The next season (season 2008), plants started bolting in May, produced higher shoots (repeated measurement ANOVA, $F=95.48$, $p < 0.001$) and had higher seed production (seed number: $F=103.2$, $p < 0.001$). Weight of 50 seeds did not differ among seasons. Population origin and treatment application in previous year were not important for plant performance. Few individuals (17 plants) were still alive in the 2009 season and reproduced repeatedly. All plants were dead in the 2010 season.

Mean number of seeds produced by all plants in seasons 2007 & 2008 was lowered in flooded plants and plants subjected to the combination of injury and flooding. However, higher overwintering success in injured plants resulted in compensation in seed production in the 2007 season (Fig. 4).

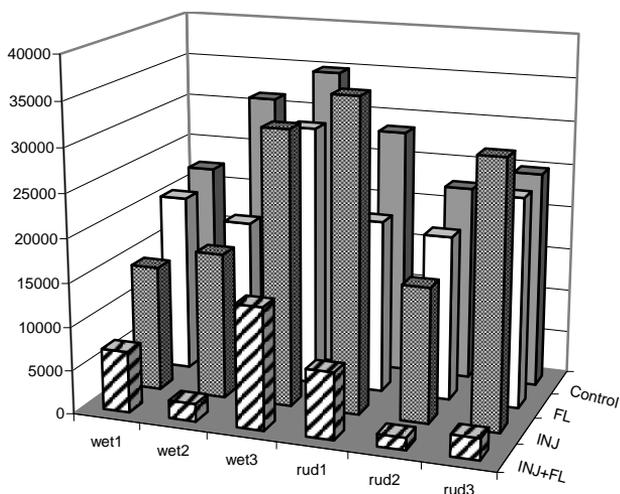


Fig. 4. Mean number of seeds produced by all individuals in seasons 2007 & 2008. Counted for all populations and treatments. Populations: wet1 (Zliv brook), wet2 (Zliv meadow), wet3 (Zdice), rud1 (Nový Bydžov), rud2 (Jindřichův Hradec), rud3 (České Budějovice). Treatments: Control (control plants), FL (flooded plants), INJ (injured plants), INJ+FL (injured and flooded plants).

DISCUSSION

Our results support the idea that regenerative root-sprouting is constrained by submergence. Whereas *Rorippa palustris* was able to cope either with submergence or with severe disturbance if acting separately, the combination of both treatments resulted in large mortality and reduced plant fitness. Plants originating from populations occurring in different habitats, i.e., ruderal and wetland, did not show different reaction to single or combined treatments.

Effect of habitat of origin

It seems that *Rorippa palustris* is a plastic species with high ability to cope with harmful conditions as simulated in our experiment. Although plants from ruderal habitat started bolting few days earlier, this advantage lasted shortly and disappeared with flowering and setting seeds. Further on, no difference in shoot height and seed production was detectable, through ruderal populations produced lighter seeds. Despite the high mortality, the regeneration ability of this species was similar in plants originating from both habitats, i.e., individuals regenerated without respect to disturbance and flooding regimes operating in habitats of their origin. Although local adaptations were observed in many plant species, i.e., flooded plants originating from wetland populations of *Ranunculus repens* were better adapted to submergence than ruderal ones (Lynn and Waldren 2003) and *Ranunculus reptans* showed local adaptation along small scale gradient of flooding duration (Lenssen et al. 2004), other studies fail to detect any local adaptation in a species. For example, in *Rumex palustris*, phenotypic plasticity in growth of submerged plants could not be linked to habitat type (Chen et al. 2009). As *Rorippa palustris* and *Rumex palustris* are both selfing short-lived species with versatile life history, we can speculate, that the lack of local adaptation might be due to life history variation and extensive seed production: populations of those species are less dependent on *in situ* survival, but rather rely on recolonisation from seeds.

Effect of treatments

Stress and disturbance application, contrary to habitat origin, had a significant adverse effect on plant performance, namely on seed production and survival. Seed production in flooded plants was lowered only marginally, but it was four times reduced in injured plants. However, injured plants survived winter period more successfully and were able to compensate their seed production in the following season. This implies good ability of the species to recolonize the areas disturbed by flooding.

Flooding in *Rorippa palustris* resulted in fast vertical growth and changes in leaves orientation, which is a common strategy enabling plant to escape hypoxia by reaching water surface (Crawford 2003, van der Sman et al. 1993). This avoidance reaction, however, was not possible in plants subjected to both treatments and thus experiencing severe anoxia. Following the disturbance event – the loss of all aboveground biomass of a plant, new sprouts were visible within few days and plant started flowering a month later. Following the disturbance event combined with flooding, new sprouts in surviving plants appeared in 3-6 weeks.

Seed production of both injured and injured and flooded plants was low compared to flooded and control plants. This was caused by changes in plant architecture, as adventitious shoots are known to have reduced number of branches in comparison with intact plants (Bartušková and Klimešová, *in press*), and reduced branching and shoot size result in reduced seed set (Piippo et al. 2005).

Effect of internal factors

Only plants with high biomass production at the time of injury were able to regenerate (Fig. 2) indicating high demands on stored reserves for resprouting. As we already reported for *Rorippa palustris* (Sosnová and Klimešová 2009), plants with bigger aboveground biomass also possess larger starch reserves, which are known to be a key factor for plant recovery following defoliation under flooding conditions (Peña-Fronteras et al. 2009, Striker et al. 2008). The change from aerobic to energetically demanding anaerobic metabolism because of flooding (Crawford and Brändle 1996) was probably the reason for the high mortality in completely submerged injured plants. Similarly, under excessive moisture or in the periods of heavy rains, fragments of root-sprouting *Barbarea vulgaris* showed low regeneration success (Tachibana et al. 2010).

Contrary to adverse effects of disturbance on plant survival and fitness in the first year, this treatment increased winter survival probability of the plants. Although investments of carbon to regrowth immediately after disturbance were needed, the carbohydrates were not fully used for generative reproduction and this probably lead to higher probability of overwintering (see also Sosnová and Klimešová 2009). By extending their life span, the plants were able to compensate for seed production in the following season. This strategy was observed in other monocarpic plants (Klimešová et al. 2007, Martínková et al. 2008, Piippo et al 2009) and can be regarded as a reason for their success in disturbed habitats.

CONCLUSIONS

To conclude, plant responses to injury and flooding treatments did not differ between ruderal and wetland populations. Flooding stress promoted stem elongation in plants, but decreased seed production. Injury alone caused low mortality of plants and reduced seed production, but the combination of injury and flooding was fatal for the majority of plants and strongly reduced seed set. While injured plants were able to resprout from root buds in a few days, it took several weeks to plants subjected to combination of injury and flooding. In order to regenerate, the root-sprouting plant needs to form buds on roots and this process is energetically costly even in well aerated soil, thus the *in situ* persistence of plant populations of this species will require regeneration from seed bank in case of concurrence of flooding and severe disturbance.

The presented study is based on single species, thus to obtain more representative results, we need to examine more root-sprouting species, especially perennials which resprout from roots spontaneously (*Rorippa amphibia*, *Sium latifolium*, *Limonium vulgare*, etc.). Additionally, direct measurements of oxygen demands of root fragments or whole plants and their comparison with rhizomes could contribute to proper testing of ecological constraints limiting the root-sprouting ability in wetland species.

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REFERENCES

- Bartušková A., Klimešová J. Reiteration in the short lived root-sprouting herb *Rorippa palustris*: does the origin of buds matter? Botany [in press].
- Becker U., Colling G., Dostál P., Jakobsson A., Matthies D. (2006) Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. *Oecologia* 150: 506-518.
- Biemelt S., Albrecht G., Wiedenroth EM. (1996) The effect of post-hypoxia on roots in *Senecio* and *Myosotis* species related to the glutathione system. *Folia Geobot. Phytotax.* 31: 65-72.
- Bischoff A., Cremieux L., Šmilauerová M., Lawson CS., Mortimer SROV., Doležal J., Lanta V., Edwards AR., Brook AJ., Macel M., Lepš J., Steinger T., Müller-Schärer, H. (2006) Detecting local adaptation in widespread grassland species - the importance of scale and local plant community. *J. Ecol.* 94: 1130-1142.
- Bornette G., Tabacchi E., Hupp C., Pujalon S., Rostan C. (2008) A model of plant strategies in fluvial hydrosystems. *Freshwater Biol.* 53: 1692–1705.

The consequence of flooding and injury

- Chen X., Huber H., de Kroon H., Peeters AJM., Poorter H., Voeselek LACJ., Visser EJW. (2009) Intraspecific variation in the magnitude and pattern of flooding induced shoot elongation in *Rumex palustris*. *Ann. Bot.* 104: 1057-1067.
- Crawford RMM., Brändle R (1996) Oxygen deprivation stress in a changing environment. *J. Exp. Bot.* 47: 145-159.
- Crawford, RMM. (2003) Seasonal differences in plant responses to flooding and anoxia. *Can. J. Bot.* 81: 1224-1246.
- Dietz H., Kohler A., Ullmann I. (2002) Regeneration growth of the invasive clonal forb *Rorippa austriaca* (Brassicaceae) in relation to fertilization and interspecific competition. *Plant Ecol.* 158: 171-182.
- Galloway LF., Fenster CB. (2000) Population differentiation in an annual legume: Local adaptation. *Evolution* 54: 1173-1181.
- Grace JB. (1993) The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. *Aquat. Bot.* 44: 159-180.
- Grime JP. (2001) *Plant strategies, vegetation processes, and ecosystem properties*, Wiley, Chichester, 417 p.
- Hanley ME., Lamont BB. (2002) Relationships between physical and chemical attributes of congeneric seedlings: how important in seedling defence? *Funct. Ecol.* 16: 216-222.
- Kalliola R., Salo J., Puhakka M., Rajasilta M. (1991) New site formation and colonizing vegetation in primary succession on the western Amazon floodplains. *J. Ecol.* 79: 877-901.
- Kawecki TJ., Ebert D. (2004) Conceptual issues in local adaptation. *Ecology Letters* 7: 1225-1241.
- Klimeš L., Klimešová J. (1999) Root sprouting in *Rumex acetosella* under different nutrient levels. *Plant Ecol.* 141: 33-39.
- Klimešová J., Klimeš L. (2007) Bud banks and their role in vegetative regeneration - A literature review and proposal for simple classification and assessment *Perspect. Plant. Ecol.* 8: 115-129.
- Klimešová J., Martínková J., Kočvarová M. (2004) Biological flora of Central Europe: *Rorippa palustris* (L.) Besse. *Flora* 199: 453-463.
- Klimešová J., Sosnová M., Martínková J. (2007) Life-history variation in the short-lived herb *Rorippa palustris*: effect of germination date and injury timing. *Plant Ecol.* 189: 237-246.
- Leiss KA., Müller-Schärer H. (2001) Performance of reciprocally sown populations of *Senecio vulgaris* from ruderal and agricultural habitats. *Oecologia* 128: 210-216.
- Lenssen JPM., van Kleunen M., Fischer M., de Kroon H. (2004) Local adaptation of the clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. *J. Ecol.* 92: 696-706.
- Lowe BJ., Watts RJ., Roberts J., Robertson A. (2010) The effect of experimental inundation and sediment deposition on the survival and growth of two herbaceous riverbank plant species. *Plant Ecol.* 209: 57-69.
- Lynn DE., Waldren S. (2003) Survival of *Ranunculus repens* L. (creeping buttercup) in an amphibious habitat. *Ann. Bot.* 91: 75-84.
- Martínková J., Klimešová J., Mihulka S. (2008) Compensation of seed production after severe injury in the short-lived herb *Barbarea vulgarit*. *Basic Appl. Ecol.* 9: 44-54.
- Martínková J., Kočvarová M., Klimešová J. (2004) Resprouting after disturbance in the short-lived herb *Rorippa palustris* (Brassicaceae): an experiment with juveniles. *Acta Oecol.* 25: 143-150.
- Noble IR., Slatyer RO. (1980) The use of vital attributes to predict successional changes in plant-communities subject to recurrent disturbance. *Vegetatio* 43: 5-21.

- Peña-Fronteras, JT., Villalobos MC., Baltazar AM., Merca FE., Ismail AM., Johnson DE. (2009) Adaptation to flooding in upland and lowland ecotypes of *Cyperus rotundus*, a troublesome sedge weed of rice: tuber morphology and carbohydrate metabolism. *Ann. Bot.* 103: 295-302.
- Peterson RL. (1975) The initiation and development of root buds. In: Torrey JG., Clarkson DT. (Eds.) *The development and function of roots*. Academic Press, London, pp. 125-161.
- Philbrick CT., Les DH. (1996). Evolution of aquatic angiosperm reproductive systems. *BioScience* 46: 813-826.
- Piippo S., Hellstrom K., Huhta A-P., Rautio P., Tuomi J. (2009) Delayed flowering as a potential benefit-decreasing cost of compensatory regrowth. *Botany* 87: 837-844.
- Piippo S., Huhta A-P., Rautio P., Tuomi J. (2005) Resource availability at the rosette stage and apical dominance in the strictly biennial *Erysimum strictum* (Brassicaceae). *Can. J. Bot.* 83: 405-412.
- Puijalon S, Lena JP, Bornette G (2007) Interactive effects of nutrient and mechanical stresses on plant morphology. *Ann. Bot.* 100: 1297-1305.
- Renöfält BM., Nilsson C. (2008) Landscape scale effects of disturbance on riparian vegetation. *Freshwater Biol.* 53: 2244-2255.
- Sosnová M., Klimešová J. (2009) Life-history variation in the short-lived herb *Rorippa palustris*: The role of carbon storage. *Acta Oecol.* 5: 691-697.
- Sosnová M., van Diggelen R., Klimešová J. (2010) Distribution of clonal growth forms in wetlands. *Aquat. Bot.* 92: 33-39.
- Striker, GG., Insausti, P., Grimoldi, AA. (2008) Flooding effects on plants recovering from defoliation in *Paspalum dilatatum* and *Lotus tenuis*. *Ann. Bot.* 102: 247-254.
- Tachibana M., Itoh K., Watanabe H., Nakayama S. (2010) Mode of reproduction of *Barbarea vulgaris* in two different habitats in Tohoku, Japan. *Weed Biol. Management.* 10: 9-15.
- Trémolières M. (2004) Plant response strategies to stress and disturbance: the case of aquatic plants. *J. Biosci.* 29: 461-470.
- van der Sman AJM., Joosten NN., Blom CWPM. (1993) Flooding regimes and life-history characteristics of short-lived species in river forelands. *J. Ecol.* 81: 121-130.
- van Eck WHJM., van de Steeg HM., Blom CWPM., de Kroon H. (2004) Is tolerance to summer flooding correlated with distribution patterns in river floodplains? A comparative study of 20 terrestrial grassland species. *Oikos* 107: 393-405.
- Visser EJW., Bogemann GM., van de Steeg HM., Pierik R., Blom CWPM. (2000) Flooding tolerance of *Carex* species in relation to field distribution and aerenchyma formation. *New Phytol.* 148: 93-103.
- Voesenek LACJ., Colmer TD., Pierik R., Millenaar FF., Peeters AJM. (2006) How plants cope with complete submergence. *New Phytol.* 170: 213-226.
- Weber M., Brändle R. (1996) Some aspects of the extreme anoxia tolerance of the sweet flag, *Acorus calamus* L. *Folia Geobot. Phytotax.* 31: 37-46.
- Wilby NJ., Abernethy VJ., Demars BOL. (2000) Attribute-based classification of European hydrophytes and its relationship to habitat utilization. *Freshwater Biol.* 43: 43-74.

CHAPTER VII

Summary of results

SUMMARY OF RESULTS

The main objectives of this thesis were: (i) to evaluate the representation of various types of clonal growth organs, especially root-sprouters on the example of wetland communities of the Netherlands, (ii) to determine whether certain clonal traits are selected in different wetland communities, (iii) to assess the role of life history variation, injury timing and starch reserves in the root-sprouting ability of the wetland herb *Rorippa palustris*, and (iv) to evaluate the role of submergence and disturbance, i.e. alone and in combination, in the root-sprouting ability of this species.

THE MAIN RESULTS OF THIS THESIS ARE SUMMARISED AS FOLLOWS:

The representation of various clonal growth organs and traits in wetland communities

Wetland communities are considered a heterogeneous group of communities affected by different biotic and abiotic factors. These factors may select for various species possessing different clonal growth characteristics (organs and traits). Therefore, on the example of the main wetland communities found in the Netherlands, we examined the spectra of clonal growth organs and traits connected to clonality. The spectra were analysed with multivariate techniques using species frequency and with permutation tests using presence/absence data.

We found that most wetland species use rhizomes, whereas root-derived organs of clonal growth are rare in all wetland communities except of salt marshes, where root-sprouting species (e.g. *Limonium vulgare*, *Artemisia maritima*) together with annual non-clonal species were relatively abundant (CHAPTER II).

Among communities, true aquatic communities, i.e. fresh water pools and riverbeds were specific in their representation of clonal growth organs. This was true both in case we used species frequencies data and when using presence/absence data. These communities were inhabited by species able to spread by fragmentation, budding and turion formation, which can be regarded a special aquatic adaptation (CHAPTER II).

We also confirmed that the clonal growth organs of wetland species differ in their traits. The most important functional trait characterising individual clonal growth organs was the degree of lateral spread followed by duration of persistence of connections between

ramets. In general, we were able to find certain trade-offs between functional traits realized by various clonal growth organs (Fig. 1)

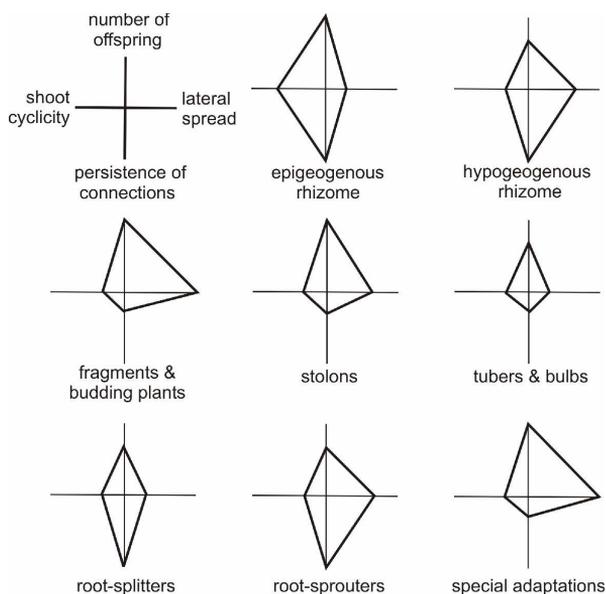


Fig. 1. Types of clonal growth organs and the most common combination of categories for each trait. Trait description and characters are adapted from Klimešová and Klimeš (2006) and given in Methods part of the Chapter II. Example plants for various types of clonal growth organ include: *Carex pseudocyperus* (epigeogenous rhizome), *Glyceria fluitans* (hypogeogenous rhizome), *Elodea canadensis* (fragments & budding plants), *Callitriche palustris* (rooting stem), *Dactylorhiza maculata* (tubers & bulbs), *Campanula trachelium* (root-splitters), *Rorippa austriaca* (root-sprouters), *Potamogeton gramineus* (special adaptations).

True aquatic communities were also specific in their representation of clonal traits (CHAPTER III). Species growing in fresh water pools and river beds were characterized by high offspring production, good dispersability, fast splitting clones and monocyclic shoots. These results indicate strong environmental filtering on recruitment from the local species pool in open water communities causing convergence in plant traits. However, in other communities, a high diversity of clonal and bud bank traits was found, even when some traits were typical of frequent species and others were found in rare species only.

The role of life history variation, injury timing and starch reserves on the root-sprouting ability

After showing the underrepresentation of root-sprouting in wetlands (CHAPTERS II & III), we focused on ecological factors potentially affecting the resprouting ability in wetland plant species. We explored both intrinsic factors, e.g. life history mode and starch reserves, and external factors represented by different stress types, e.g. submergence and injury.

Time of germination was a factor principally affecting life history variation in the short-lived root-sprouting herb *Rorippa palustris*. Plants germinated before summer solstice behaved as summer annuals, whereas individuals germinated later in the season postponed flowering to the next season (winter annuals). Moreover, plants germinating more closely to summer solstice acted as polycarpic perennials (CHAPTER IV). Severe injury had little effect on the life histories of this species and merely caused the postponement of reproduction. However, probably because of the changes in starch allocation injured plants overwintered more successfully, i.e. injury prevented allocation of starch to seed production (CHAPTER V).

Time of injury in relation to plant ontogeny and phylogeny was important for sprouting ability. Young plants (<6 weeks) and plants with low biomass production were less successful in resprouting (CHAPTER IV). Starch concentration was positively correlated with above-ground biomass (CHAPTER V): smaller plants regenerated less frequently, especially when disturbance was combined with submergence stress (CHAPTER VI). Thus, the amount of starch in roots may be a key factor limiting the regeneration ability of this species.

The role of submergence and disturbance

We evaluated the effects of injury and/or submergence on the root-sprouting ability of the wetland herb *Rorippa palustris* (CHAPTER VI). Submergence had only little effect on plant growth, fitness and winter survival, while injury caused some mortality, largely reduced seed production and increased overwintering probability in plants. However, the combination of injury and submergence was detrimental and caused mortality in 80% of plants. Root-sprouting success following injury was high and quick. New sprouts were visible within 7 days following disturbance, but several weeks were necessary for regeneration of plants subjected to the combination of submergence and disturbance (CHAPTER VI).

Although we expected that root-sprouting ability can also be affected by a particular population or population origin, this was not confirmed in our study. Wetland populations (wet meadows, river banks) were not better adapted to submergence stress, and ruderal populations (edges of arable fields, urban habitats) were not able to withstand disturbance and severe injury better. The only characteristic which was found to differ in plants originated from contrasting habitats was seed weight, as wetland populations produced heavier seeds

compared to ruderal populations. This might be interpreted as supply of offspring by the mother (maternal effect); however, it needs further study.

General conclusion and future perspectives

This thesis evaluates the role of root-sprouting in various wetland communities differing in abiotic as well as biotic conditions. With the exception of salt marshes, root-sprouting is underrepresented in all communities and known for few wetland species only. Further, we evaluated the role of ecological factors in resprouting. In manipulative experiments we showed that this ability is affected by plant size and age and corresponds well to the starch content in roots. We also showed that stress has a great impact on resprouting, seed production and winter survival. Combination of injury and submergence was detrimental for most plants causing high mortality. We also concluded that carbon economy plays an important role in the life cycle and regeneration ability of a plant. It seems that root-sprouting is energetically costly and inefficient in waterlogged conditions.

The studies presented raise some questions for future research. Firstly, there is a need of more experimental studies and observations into other root-sprouting wetland species, especially perennials (*Rorippa amphibia*, *Sium latifolium*, *Limonium vulgare*, etc.). Secondly, the role of other ecological factors potentially important for root-sprouting ability, e.g. leaf and root herbivory, should be addressed. And thirdly, direct measurements of oxygen demands of root fragments or whole plants are needed. Although not proved for the model species, also the possible effects of genetic variation should be kept in mind, as well as a maternal effect of plants.