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Abiotic stresses increase plant regeneration ability

Sara Puijalon, Florence Piola, Gudrun Bornette

Abstract In disturbed habitats, vegetative regeneration is partly ruled by plant reserves and intrinsic growth rates. Under nutrient-limiting conditions, perennial plants tend to exhibit an increased allocation to storage organs. Under mechanically stressful conditions, plants also tend to increase allocation to below-ground biomass and storage organs. We tested whether those stresses acting differently on plants (nutrient level versus mechanical forces) led to similar effect on storage organs and regeneration ability. We measured, for an aquatic plant species, (1) the size and allocation to storage organs (stems) and (2) the regeneration ability of the storage organs. Plant stems were collected in 4 habitats ranked along a nutrient stress gradient, and having encountered null versus significant mechanical stress (flowing water). All stems were placed in similar neutral conditions and left for a period of 6 weeks before measuring their survival and growth. Dry mass allocation to the storage organ (stem) was higher in stressful habitats. Moreover, stress encountered by plants before the experiment significantly affected regeneration: stems of previously stressed plants (i.e. plants that had grown in nutrient-poor or mechanically stressful habitats) survived better than unstressed ones. Stems of plants having encountered mechanical stress before the experiment had increased growth in nutrient-rich habitats but reduced growth in the poorest habitats. These results demonstrate that regeneration could rely on the level of stress previously encountered by plants. Stress could lead to greater regeneration ability following mechanical failure. The possible mechanisms involved in these results are discussed.

Keywords Disturbance · Mechanical stress · Nutrient stress · Regrowth · Storage organ · Survival

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Introduction

Clonal plant species are faced with spatial and temporal variability in their environment, and exhibit several strategies, such as architectural plasticity (Hutchings and de Kroon 1994), physiological integration, and the division of labour (Hutchings and Wijesinghe 1997; Price and Marshall 1999). According to the theory of biomass partitioning, individual plants tend to exhibit a trade-off between traits promoting resource capture and rapid growth, and those promoting resource conservation (Stuefer et al. 1998; Hutchings and John 2004). In this framework, plants that grow under stressful conditions in terms of nutrient level have tissues with a long life-span and low resource loss (Garnier 1992; Ryser and Urbas 2000). They usually allocate a high proportion of vegetative biomass to storage and tend to have a high tissue mass density (Hutchings and John 2004). Other kinds of stresses (e.g. mechanical stress) were demonstrated to induce biomass reallocation within ramets towards an increase in the below-ground biomass (roots and storage organs) compared to that above-ground (Fritz et al. 2004; Puijalon et al. 2005).

Several studies have outlined the benefits of clonal growth (through sprouting or spreading) in habitats characterized by physical damage (e.g. floods, trampling or fire, Barrat-Segretain and Amoros 1996; Bond and Midgley 2003), because this allows an efficient short-distance colonization of open spaces generated by disturbances (disturbances *sensu* Grime 2001, i.e. partial or total destruction of plant biomass, Fahrig et al. 1994; Winkler and Fisher 2002). On this scale, both modelling and experimental evidence suggest that sprouting vigour (survival and regrowth of individuals damaged by disturbance) is partly determined by the plant's levels of reserves (in stolons and other storage organs, as stems or buds), and by the intrinsic growth rate of plants (Iwasa and Kubo 1997; Stuefer and Huber 1999; Gurvich et al. 2005). Concerning the level of reserves, both the size of the storage organ (Piqueras and Klimes 1998) and the quality and quantity of resources stored in their storage organs (Fischer et al. 1995; Landhäuser and Lieffers 2002) rule the regeneration process.

Under natural conditions, plants that allocate a high proportion of vegetative biomass to storage should be favoured in case of damage, as this should allow a rapid regrowth of plant parts involved in resource capture and biomass production (Suzuki and Stuefer 1999; Cornelissen et al. 2003). Surprisingly, despite the relatively extensive literature dealing with plant regeneration (e.g. Cordazzo and Davy 1999; Dietz et al. 1999), only a few papers consider the possible effect of the environmental stress conditions encountered by the plants before the experiments (Clark and Burge 2000; Pysek et al. 2003).

The aim of the present work was therefore to compare plants originating from habitats characterized by contrasting stress levels for (1) the size and relative allocation to storage organs and (2) the regeneration ability of storage organs. Two stress factors were considered: nutrient stress and mechanical stress. Under nutrient stress, several studies demonstrated that plants tend to increase their relative allocation to roots and storage organs (Gebauer et al. 1995; Knox and Clarke 2005). Under mechanical stress, we demonstrated that the species under study allocated an increased part of biomass to below-ground organs protected from stress (both roots and storage organs) (Puijalon et al. 2005; Puijalon and Bornette 2006). In the present work, we focused on those two stress factors. We tested whether two stresses acting differently on plants (nutrient stress versus mechanical stress) lead to similar effect on storage organs and regeneration ability. Plants were sampled in habitats ranked along a natural nutrient stress gradient and encountered either null or significant mechanical stress. Dry mass of storage organs was measured to test whether (1)

absolute size of storage organs is affected by stress encountered by plants and (2) allocation to storage organs is greater in stressful habitats. The survival and regrowth rate of plant stems was measured experimentally to test whether the stress previously encountered by plants affected the regeneration ability of their storage organs.

Materials and methods

Species and study sites

The experiment was carried out on an aquatic plant species, *Berula erecta* (Hudson) Coville (Apiaceae, Lesser Water Parsnip) that presents a high ability to regenerate from plant fragments after disturbances (Barrat-Segretain et al. 1999). *B. erecta* is a stoloniferous species, consisting of a rosette of petiolated dissected leaves. Its stem is buried and has a storage function. It measures 30–150 cm high, and colonizes calcareous flowing habitats. The study was carried out in four streams located in the same geological area, and characterized by the same grain-size and water level. Streams differed in their nutrient conditions, and ranged from oligotrophic to meso-eutrophic. Within each stream, plants were sampled in two patches very close to each other (max. 20 m), one standing and one running. Flow velocity was assessed at the patch level. As there were no nutrient sources in the sampled sections of the streams, patches sampled in a given stream were considered similar in terms of water nutrient content. For this reason, the nutrient content of the water was assessed on the stream scale.

Nutrient stress

The functioning of aquatic ecosystems is strongly governed by their nutrient contents (Hansson et al. 1998; Khan and Ansari 2005). In calcareous groundwater-supplied ecosystems, the composition of plant communities has been shown to depend mainly on the phosphate and ammonium concentrations in the water (Carbiener et al. 1990; Elger et al. 2004). The selection of the 4 streams was consequently based on both (1) the occurrence of vegetation groups considered as representative of increasing nutrient levels, and (2) the phosphate and ammonium concentrations in the water. As aquatic plants can also acquire nutrients by roots (Madsen and Cedergreen 2002), and in order to avoid any confounding effect due to the substrate characteristics (through variation in nutrient content or grain-size), we selected patches characterized by coarse substrate (gravels, pebbles). Water samples were collected in each stream on 14 dates spread over 4 months before the sampling date. Ammonium [N-NH₃] and phosphate [PO₄³⁻] concentrations in the water were measured by colorimetric means after standard HACH procedures (HACH Company, Loveland, Colorado, USA). As both nutrients govern plant communities, the 4 streams were equally spaced, between 1 (low nutrient level) and 4 (high nutrient level; Table 1).

Mechanical stress

Mechanical stress consisted of exposure to running water. Flow velocity was measured at each patch with a propeller (C2 current meter, OTT Messtechnik GmbH & Co. KG, Kempten, Germany) at a water depth 40% above the substrate, which gives a good

Table 1 Nutrient levels in the 4 streams and mechanical stress levels on the patches sampled

Nutrient level = stream	Patch	Velocity (m s ⁻¹)	[N-NH ₃] (10 ⁻² mg l ⁻¹)	[PO ₄ ³⁻] (10 ⁻² mg l ⁻¹)	Dominant aquatic species
1	Standing	0 ± 0	1.21 ± 1.97	4.82 ± 6.92	<i>Potamogeton coloratus</i> , <i>Berula erecta</i> , <i>Mentha aquatica</i> , <i>Chara major</i>
	Running	0.22 ± 0.06	(a)	(a)	
2	Standing	0 ± 0	3.93 ± 3.77	3.14 ± 3.04	<i>Berula erecta</i> , <i>Mentha aquatica</i>
	Running	0.58 ± 0.06	(ab)	(a)	
3	Standing	0 ± 0	6.43 ± 5.02	3.36 ± 2.88	<i>Sparganium emersum</i> , <i>Berula erecta</i> , <i>Callitriche platycarpa</i> , <i>Elodea nuttallii</i> , <i>Mentha aquatica</i>
	Running	0.31 ± 0.19	(bc)	(a)	
4	Standing	0.04 ± 0.02	8.43 ± 5.10	12.8 ± 10.9	<i>Potamogeton natans</i> , <i>Hippuris vulgaris</i> , <i>Callitriche platycarpa</i> , <i>Groenlandia densa</i> , <i>Sparganium emersum</i> , <i>Berula erecta</i> , <i>Elodea nuttallii</i> , <i>Mentha aquatica</i> , <i>Myriophyllum spicatum</i>
	Running	0.58 ± 0.22	(c)	(b)	

Mechanical stress was assessed using the flow velocity (mean + SD) encountered by plants during the 3-month period preceding the sampling. Nutrient stress was assessed using ammonium and phosphate concentrations (mean + SD) in the water, and the dominant aquatic species of the 4 streams. Streams are ranged from 1 (oligotrophic) to 4 (meso-eutrophic). Different letters indicate significant differences in nutrient concentrations (Tukey-Kramer HSD, $P < 0.05$)

estimation of the average flow velocity in the water column. Flow velocity was measured on 15–17 dates (depending on the stream) with contrasting river discharges, over a 5-month period. Four random flow velocity measurements were made in each patch at each sampling date, avoiding hydraulic shelters (big pebbles, tall plants). These measurements were used to establish a regression between river discharge and flow velocity on the patches. This regression was used to predict the flow velocity encountered by plants during the 3-month period preceding plant sampling.

The average velocity encountered by plants ranged from 0 to 0.04 m s⁻¹, and 0.22 to 0.58 m s⁻¹, for standing and running patches, respectively (Table 1). These latter values have been demonstrated to be highly stressful for plant growth (Puijalón and Bornette 2004; Puijalón et al. 2005).

Plant sampling

Berula erecta is evergreen and totally submerged during winter, whereas it becomes emerged in standing water during summer. At the beginning of spring, plants were totally submerged for several months, and fully encountered the nutrient and velocity conditions of the habitats. The experiment was carried out in April, at the very beginning of the growing season, just before spring regrowth. Two sets of plants were collected in each patch: a set of 8 ramets for measuring dry mass and relative allocation to stem and a set of 50 for measuring the regeneration ability of stems. Individual ramets were collected from

distinct clones in each patch. A ramet of *B. erecta* was defined as a single rooted rosette. The plants were immediately brought to the laboratory for the experiment.

Size and allocation to storage organs

The 64 plants (8 per patch) were collected between 1st and 11th April 2003. Plants were divided into stems (storage organs), roots and leaves. The different parts were weighed after drying for 48 h at 85°C to obtain dry mass (g).

Regeneration ability: experimental design

The 400 plants (50 per patch) were sampled on the 23rd and 24th April 2003. The experiment was carried out in a climate room (air temperature 18°C during the night, rising to 25°C during the day) supplied with natural light, supplemented with additional artificial light (L/D 12–12). In order to measure only the role of the storage organ in plant regeneration, without any confounding effect due to the size of the photosynthetic organs and roots, any horizontal stolon, root and leaf was removed from the stems. The stems were weighed, and placed in 18 × 24 × 10 cm polystyrene boxes on 3 cm wet substrate, composed by 1/3 natural aquatic silt and 2/3 river sand. Five stems of plants originating from the same patch were placed randomly in each box. The boxes were randomly placed in the climate room, and the substrate was maintained wet by adding groundwater during the whole experiment. After 6 weeks, all the plants (stems and newly produced organs) were collected. The survival of each individual was recorded, and the total mass reached by the plants was measured.

Statistical analysis

Size and allocation to storage organs

The effect of both stress factors on size of storage organ were studied using a two-way ANOVA, with flow velocity and nutrient level considered as fixed factors.

The effects of both stress factors on dry mass allocation to storage organ were studied using allometric relationships, which allow comparisons at a common plant size and which is a more accurate and less biased method than calculating ratios (Jasienski and Bazzaz 1999). We used an analysis of covariance (ANCOVA) to test how stress factors and their interaction affect stem allocation, i.e. relationships between stem dry mass and total dry mass. The ANCOVA was carried out on the stem dry mass against the total dry mass (covariate), nutrient level, flow velocity, and all interactions involving both factors and the covariate. Both nutrient level and flow velocity were treated as fixed categorical predictors.

Stem survival

Stem survival is a binary variable. Consequently, it was studied with a logistic regression (generalized linear model with a binomial distributed variable and a logit link function, McCullagh and Nelder 1989). As stem survival was expected to depend on their initial mass, the initial fresh mass of the stems was used as a covariate in the model. The effects of both

stress factors and their interaction on stem survival were analysed using a logistic model, treating both stress factors as fixed categorical predictors, and initial fresh mass as a covariate. A generalized linear model carried out on survival data grouped by box, with both stress factors and their interaction as fixed effect did not reveal significant over-dispersion of data (i.e. response variance did not significantly exceed the binomial variance, residual deviance = 78.4, df = 72, $P = 0.28$). Consequently the box effect was not considered in the final analysis. Significance of the fixed effects was tested using Wald statistic.

Plant regrowth

The effects of both stress factors (nutrient level and flow velocity) on plant regrowth was tested by examining the relationships between initial fresh mass of stems and final fresh mass of plants. As the final fresh mass of individuals was significantly linearly correlated with the initial fresh mass of the stems (for the whole set of plants, linear regression, $P < 10^{-3}$, $N = 295$ and in each group), the effect of both stress factors on plant regrowth was tested using an analysis of covariance (ANCOVA) with the initial fresh mass as a covariate. This method allowed us to investigate how both stress factors encountered by plants affected plant regrowth, i.e. the relationship between initial mass of stems and final fresh mass of plants. A mixed model analysis of covariance, examining the effects of both stress factors and their interaction on plant regrowth, was carried out with the initial fresh mass as covariate, flow velocity, nutrient level, and all interactions involving these terms as fixed effects, and one random box effect nested within nutrient level*flow velocity. This first model did not reveal any significant effect of box ($P = 0.62$), and consequently this factor was not considered in the final model. Both nutrient level and flow velocity were treated as fixed categorical predictors. As the covariate (initial fresh mass of stems) was significantly correlated with the categorical factors (nutrient level and flow velocity), the ANCOVA was performed on a subset of stems to meet the assumption of the absence of collinearity between the covariate and the categorical predictors (Quinn and Keough 2002). This subset was composed of the living individuals having the largest range of initial fresh mass (covariate) values common to all groups in order to maximize the overlap of fresh mass values between groups. This subset consisted of 29 and 41 stems for nutrient level 1, 22 and 27 stems for nutrient level 2, 11 and 28 stems for nutrient level 3, and 8 and 14 stems for nutrient level 4, for both standing and running patches, respectively.

All masses were \log_e -transformed prior to analysis to improve the normality of residuals and the homogeneity of variance. For all analyses, interaction terms between the two stress factors and the covariate (if present) were first introduced in the model. Non-significant higher order interaction terms were then stepwise removed to obtain the final model; i.e. backward selection (Quinn and Keough 2002).

For the ANOVA and the two ANCOVAs, tests for trends and planned comparisons were performed on least squares means (LS Means or adjusted means) to test hypotheses on the response variable. Tests for trends were performed to test for linear trend in the stem allocation and regrowth across nutrient levels, i.e. to examine the effect of increasing nutrient level on stem allocation and regrowth. Planned contrasts were used to test for specific differences in stem allocation and regrowth between flow velocities within each nutrient level.

The significance level was 0.05 for all analyses. All statistical analyses were performed using JMP 5.1.2 (2004, SAS Institute Inc.) and R (R-2.3.1, R Development Core Team-2006) statistical software.

Results

Size and allocation to storage organs

Stem dry mass was significantly affected by nutrient level, but not by flow velocity, nor by interaction between flow velocity and nutrient level (Table 2, Fig. 1a). Stem dry mass significantly increased for increasing nutrient level (Fig. 1a, $F^1_{56} = 44.6$, $P < 10^{-3}$, test for linear trends in LS Means across nutrient levels, for both velocities).

Stem dry mass was significantly related to total dry mass, as well as to both stress factors and their interaction (Table 2). Allocation to stem decreased with increasing nutrient level for both flow velocities (Fig. 1b, $F^1_{55} = 9.0$, $P = 0.004$ and $F^1_{55} = 17.3$, $P < 10^{-3}$, tests of linear trends for standing and running velocity, respectively). The effect of flow velocity on stem allocation differed depending on the nutrient level (Fig. 1b): stem allocation was significantly higher in running water for the two sites with the lowest nutrient level ($F^1_{55} = 5.8$, $P = 0.02$ and $F^1_{55} = 17.5$, $P < 10^{-3}$, contrast test on LS Means on standing versus running patches of nutrient levels 1 and 2, respectively), whereas it was not affected by flow velocity in the two other sites ($F^1_{55} = 2.0$, $P = 0.16$ and $F^1_{55} = 0.2$, $P = 0.63$, contrast test on LS Means on standing versus running patches of nutrient levels 3 and 4, respectively).

Stem regeneration after disturbance

Stem survival

The survival of stems was not significantly affected by their initial fresh mass, when tested for the whole set of individuals (logistic model, $P = 0.25$, $N = 400$), and it was consequently not considered in the final model. Stem survival was significantly

Table 2 Factors affecting the absolute stem dry mass and stem dry mass allocation relative to total dry mass

Factor	Absolute stem dry mass			Stem allocation relative to total dry mass		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Total dry mass ^a				1	69.27	<10 ⁻³
Nutrient level ^b	3	15.28	<10 ⁻³	3	5.11	0.003
Flow velocity ^b	1	0.92	0.34	1	14.18	<10 ⁻³
Nutrient level ^b × flow velocity ^b	3	<i>1.29</i>	<i>0.29</i>	3	2.94	0.04
Total dry mass ^a × flow velocity ^b				1	<i>0.02</i>	<i>0.89</i>
Total dry mass ^a × nutrient level ^b				3	<i>0.58</i>	<i>0.63</i>
Total dry mass ^a × flow velocity ^b × nutrient level ^b				3	<i>1.88</i>	<i>0.15</i>
Error	59			55		

A two-way ANOVA ($N = 64$, $R^2 = 0.44$, error mean square = 0.48 for the final model) and a two-way ANCOVA ($N = 64$, $R^2 = 0.77$, error mean square = 0.21 for the final model) were used for absolute stem dry mass and relative allocation, respectively. Non-significant interaction terms that were stepwise dropped are indicated in italics (*F* and *P*-values correspond to the model with higher order interaction where the term was present). The degree of freedom of error corresponds to the final model. a and b indicate the covariate and categorical predictors, respectively

Fig. 1 Absolute dry mass of stems (a) and stem dry mass allocation relative to total dry mass (b) according to stress levels (nutrient level and flow velocity) encountered by plants. Points are adjusted means (LS Means $\log_e(\text{stem dry mass}) \pm \text{SE}$) predicted from the two-way ANOVA for stem dry mass and from the two-way ANCOVA for stem dry mass allocation. Nutrient levels 1–4 represent increasing nutrient level. For the significance of the differences observed, see Table 2 and text

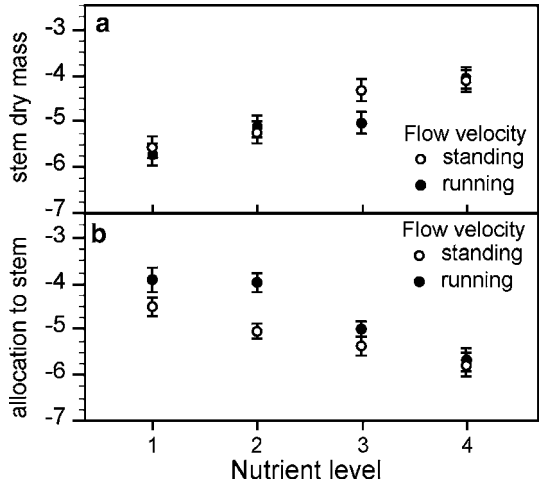


Table 3 Factors affecting stem survival (logistic model, $R^2 = 0.41$, $\chi^2 = 189.4$, $P < 10^{-3}$, $df = 4$, $N = 400$)

Factor	df	Wald statistic (χ^2)	<i>P</i>
Nutrient level	3	77.17	$<10^{-3}$
Flow velocity	1	50.94	$<10^{-3}$
Flow velocity \times nutrient level	3	6.28	0.10

Initial fresh mass was not included in the final model as it did not significantly affect stem survival, when tested for the whole set of individuals. The Wald statistic used to test the significance of the parameters, degrees of freedom (df) and *P*-values (*P*) are indicated. Non-significant interaction terms that were stepwise dropped are indicated in italics (χ^2 and *P*-values correspond to the model with higher order interaction where the term was present)

affected by nutrient level and flow velocity, but not by the interaction between flow velocity and nutrient level (Table 3). An increased stress level (i.e. a decreasing nutrient level or high flow velocity) corresponded to a significant increase in stem survival (Fig. 2).

Plant regrowth

The regrowth rate of stems was not significantly affected by their initial fresh mass. Indeed, the slope of the regression between $\log(\text{final fresh mass})$ and $\log(\text{initial stem fresh mass})$ for the whole set of individuals ($R^2 = 0.18$, $P < 10^{-3}$) did not significantly differ from 1 ($t_{178} = -1.74$, $P = 0.08$). The final fresh mass of plants was significantly related to the initial fresh mass of stems (positive effect), both stress factors and their interaction (Table 4). In standing water, the increase in nutrient level was significantly related to a decrease in plant regrowth (Fig. 3, $F^1_{171} = 70.4$, $P < 10^{-3}$, test for linear trend in LS Means across nutrient level, for standing patches). This pattern was also consistent in running water (Fig. 3, $F^1_{171} = 7.4$, $P = 0.007$, test for linear trend in LS Means across nutrient level, for running patches). The effect of flow velocity on stem regrowth differed depending on the nutrient level (Table 4, Fig. 3). Running water resulted in (1) a significantly reduced

Fig. 2 Stem survival (%; $n = 50$ individuals per patch) observed in relation to stress level (nutrient level and flow velocity) encountered by plants under natural habitat conditions before sampling. Nutrient levels 1–4 represent increasing nutrient level. For the significance of differences observed, see Table 3

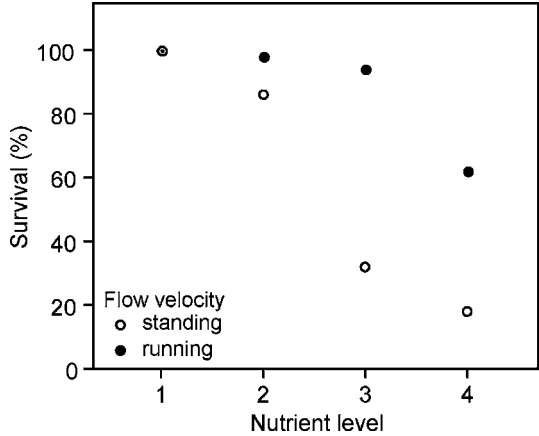


Table 4 Factors affecting the regrowth of stems (assessed through the relationship between the final fresh mass of individuals and the initial stem fresh mass) tested with a two-way ANCOVA ($N = 180$, $R^2 = 0.49$, error mean square = 0.29, for the final model)

Factor	df	<i>F</i>	<i>P</i>
Initial fresh mass of stem ^a	1	71.76	$<10^{-3}$
Nutrient level ^b	3	31.39	$<10^{-3}$
Flow velocity ^b	1	6.69	0.01
Nutrient level ^b × flow velocity ^b	3	9.08	$<10^{-3}$
Initial fresh mass of stem ^a × flow velocity ^b	1	<i>1.87</i>	<i>0.17</i>
Initial fresh mass of stem ^a × nutrient level ^b	3	<i>0.87</i>	<i>0.46</i>
Initial fresh mass of stem ^a × flow velocity ^b × nutrient level ^b	3	<i>1.60</i>	<i>0.19</i>
Error	171		

Non-significant interaction terms involving the covariate that were stepwise dropped are indicated in italics (*F* and *P*-values correspond to the model with higher order interaction where the term was present). The degree of freedom of error corresponds to the final model. a and b indicate the covariate and categorical predictors, respectively

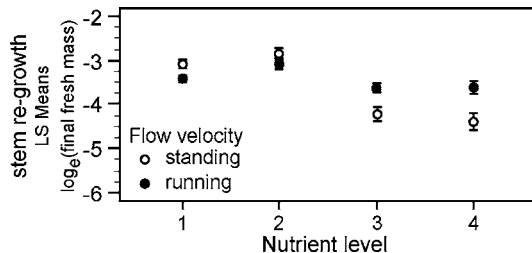


Fig. 3 Stem regrowth in relation to stress level (nutrient level and flow velocity) encountered by plants under natural habitat conditions before sampling. Points are adjusted means (LS Means \log_e (final fresh mass) \pm SE) predicted from the two-way ANCOVA. Nutrient levels 1–4 represent increasing nutrient level. For the significance of differences observed, see Table 4 and text

regrowth at the lowest nutrient level ($F^1_{171} = 4.6$, $P = 0.03$, contrast test on LS Means for running versus standing patch); (2) a non-significant effect on stem regrowth at nutrient level 2 ($F^1_{171} = 1.4$, $P = 0.24$, contrast test on LS Means for running versus standing patch) and (3) a significantly increased regrowth at the highest nutrient levels 3 and 4 ($F^1_{171} = 10.0$, $P = 0.002$ and $F^1_{171} = 11.4$, $P < 10^{-3}$, contrast test on LS Means for running versus standing patch for nutrient levels 3 and 4, respectively).

Discussion

Plant allocation to storage organs

Variation in allocation to storage organs along the nutrient gradient, in the absence of mechanical stress, were consistent with previous studies. Indeed, our results demonstrated that plants originating from nutrient-poor habitats had a higher allocation to storage organs, even if the absolute dry mass of storage organs followed the opposite pattern (i.e. increased size for increasing nutrient level). Mechanical stress did not affect absolute dry mass of storage organs, but did affect, in accordance with our hypothesis, the relative allocation to storage organs. Indeed, when significant (i.e. for two of the nutrient levels), the effect of mechanical stress was consistent with our hypothesis, as it resulted in an increased allocation to storage organs.

The patterns observed in the present study could result either from plastic response to the local habitat conditions, or from the local selection of genotypes by stress factors (Van Der Meijden et al. 2000). Whatever the origin of the variation (either genetic or phenotypic), the study demonstrated that the stresses encountered by plants can affect the size and relative allocation to storage organs.

Stem survival and regeneration after disturbance

Several studies demonstrated that the sprouting ability of plants is positively related to the quantity of carbohydrates stored (de Groot and Wein 2004; Schwilk and Ackerly, 2005). As nutrient and mechanical stresses previously encountered by plants affected the size of storage organs, they were expected to alter plant survival and regeneration rate after breakage and loss of biomass. Our results demonstrated that plants that had previously grown in stressful habitats (either nutrient-limiting or mechanically stressful) survived better than those that had previously grown in unstressful environments. Furthermore, we demonstrated that, in nutrient-rich habitats, plants previously submitted to mechanical stress regrew better than unstressed ones. Even if both stresses resulted in similar positive effects regarding plant survival and regrowth, these effects were not directly linked to absolute size of storage organ. Consequently, in the present situation, differences in survival and regrowth of stems could involve differences in the nature of the resources stored. Indeed, the nature of resources stored is partly ruled by the nutrient level of the habitat where the plant has grown (Fischer et al. 1995; Améziane et al. 1997; Cuzzuol et al. 2005; Kavanova and Gloser 2005). For instance, in nutrient-rich habitats, Steinbáchová-Vojtísková et al. (2006) demonstrated that *Typha* individuals tend to store more nitrogen and non-structural carbohydrates, whereas in oligotrophic conditions, individuals of *Phragmites* accumulate preferably starch (Kubin and Melzer 1996).

Even if less documented, mechanical stresses may also modify the quality of stored resources through alteration of the density of tissues of storage organs (e.g. Biddington and Dearman 1985; Fritz et al. 2004; Puijalon and Bornette 2004). The effects of stresses on the quality of resources stored could lead to an absence of correlation (as in the present study), or even to a negative correlation between the size of the storage organ and resprouting ability (Cruz et al. 2003).

In the present study, stresses previously encountered by plants seem to have a delayed effect on future plant survival and growth. Such delayed effects of environmental conditions after cessation of the factor (i.e. carry-over effect) can be observed for very contrasting traits: e.g. survival (Huber et al. 2004), growth (Thellier et al. 2000), reproduction (Van Zandt and Mopper 2002) or traits of offspring ramets (Schwaegerle et al. 2000; Agrawal 2001; Puijalon and Bornette 2006). Such effects could be mediated by plant size (Huber et al. 2004), but previous studies also demonstrated that some delayed effects can be independent of plant size. In the present study, differences between survival and regrowth of storage organs were not directly linked to their size. In such situations, the quality of resources stored, or variations in hormonal concentrations or alteration of genetic expression could be involved in the response observed (Schwaegerle et al. 2000; Agrawal 2001; Van Zandt and Mopper 2002).

Concluding remarks

The present experiment is an attempt to understand how mechanical stress and nutrient limitation rule the size of the storage organ, and its ability to survive and regrow after loss of biomass. Aquatic plants can be easily fragmented (e.g. because of water motion or animal displacements). Our results indicate that plant regeneration after mechanical damage depends on the conditions they previously encountered: regeneration from storage organs would contribute efficiently to individual maintenance in stressful habitats (either due to nutrient limitation or mechanical stress), whereas it would be less efficient in unstressful ones.

The survival of plant species after biomass loss relies either on the regeneration of individuals from their preserved parts (sprouting), or on the recruitment of individuals from the seed-bank (Lavorel et al. 1997; Deiller et al. 2002; Garcia and Zamora 2003). As suggested by previous studies (Bellingham and Sparrow 2000; Mundhara and Rashid 2001; Bond and Midgley 2003), a trade-off occurs between regeneration and recruitment in plant populations. In stressful situations (e.g. nutrient limitation or mechanical stress), the dominance of sprouting could lead to a lower genetic diversification of plant populations. On the other hand, eutrophication is a process that has affected many ecosystems, and particularly aquatic ones, for at least a century (Brinson and Malvarez 2002; Khan and Ansari 2005). The present results suggest that eutrophication should negatively affect the tolerance of clonal species to biomass loss, either through a lower regeneration efficiency, or through a lower survival rate for dispersing fragments, because many aquatic species are frequently dispersed through fragment drift (Kubin and Melzer 1996, Boedeltje et al. 2004).

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