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The use of live vegetation in geomorphological experiments: how to create optimal growing conditions

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ABSTRACT: There has been increasing use of live vegetation in laboratory experiments, in particular in fluvial geomorphology. The results from these studies have provided useful insight into the role that vegetation plays in impacting and modifying geomorphic systems. However there has been little published on the seed preparation techniques and vegetation growing conditions required for use in these experiments. This commentary presents results from a series of experiments investigating these factors using *Medicago sativa* and *Avena Sativa*, with the aim of highlighting the optimal growing conditions found to provide a starting point for researchers interested in implementing these techniques. Copyright © 2014 John Wiley & Sons, Ltd.

KEYWORDS: physical modelling; geomorphology; vegetation; vegetation-flow interaction; Medicago sativa; Avena sativa

Introduction

Due to the complex interactions between vegetation and morphology in the natural world, there has been an increase in laboratory experiments using live vegetation to simulate these relationships in recent years in order to investigate ecomorphological processes. Live vegetation has been used in experiments to examine the influence of vegetation on channel morphodynamics (Gran and Paola, 2001; Coulthard, 2005; Tal and Paola, 2007, 2010), as well as patterns of meandering (Brauderick et al., 2009) and braiding (Jang and Shimizu, 2003) and to explore flow induced uprooting (Edmaier et al., 2011; Perona et al., 2012). However, although the results of these experiments are published, details regarding the techniques used for seed preparation and vegetation growing conditions during experiments are limited. These are often restricted to a brief summary of seed preparation and dispersal technique used or focused on root development and root strength (Pollen, 2007; Jiang et al., 2009), meaning that there is no starting point for new researchers wanting to use these techniques. The exception being the work of van de Lagewag et al. (2010). This communication reports on a series of experiments undertaken in controlled conditions to test the optimum conditions for growing vegetation for physical models.

Vegetation Used

Two of the most common vegetation types used in geomorphological experiments are *Medicago sativa* (commonly known as

alfalfa sprouts) and *Avena sativa* (commonly known as catgrass), both are fast growing species and were the focus of the following experiments.

Medicago sativa is a perennial legume which for experimental purposes is used in its sprouting stage. It grows a single stem of approximately 40 mm in height and 1 mm diameter with 2–4 leaves on top after seven to 10 days growth. It has a single main root typically 40 mm in length with branching rootlets when fully developed (Figure 1a). *Medicago sativa* **F1** seeds are dark brown in colour and kidney shaped, approximately 1–2 mm in size. *Avena sativa* is a type of oat cereal; in its early stages of development it grows 3–4 grass blades of approximately 60–80 mm in length with a multiple root system over 50 mm long (Figure 1b) after approximately seven days growth. *Avena sativa* seeds are oval in shape with a husk, typically 5–10 mm in length, covering the inner part of the seed.

Vegetation Growth Experiments

The experiments were conducted in a climate controlled laboratory at the University of Hull, testing a range of different scenarios. Variables considered were seed preparation, water availability and drainage, sand depth of seeding, exposure to light and the seed dispersal method. The seeds for each of the different scenarios were grown in separate containers (160 mm long \times 60 mm wide \times 80 mm deep). The seeding density was one seed per 10 mm² for low density conditions



Figure 1. Photographs of the established vegetation used: (a) *Medicago sativa* sprout and (b) *Avena sativa* (with scale shown).

and two seeds per 15 mm² for high density. The ambient room temperature was maintained by means of an 'Airforce Climate Control' air conditioning unit, with each suite of conditions replicated under different temperatures alternating by a degree between the range of 16 to 22 °C. Water was supplied manually. Sand (0.25–0.7 mm) was used as the growing substrate. Experiments lasted between 7–14 days and the germination rate, stem and root length and development were recorded at daily intervals.

Results

Seed preparation

Gran and Paola (2001) proposed storing seeds in refrigerated conditions prior to use to increase the germination rate of the vegetation, this is supported by horticultural evidence on best practice for seed storage and was thus implemented for these experiments. The influence of pre-soaking the seeds versus planting dry seeds was tested in the first set of experiments. Pre-soaking was found to increase germination rate by approxi-**F2** mately 14% for *Medicago sativa* (Figure 2a) and over 18% for

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Avena sativa (Figure 2b), with stem heights marginally greater for both species following pre-soaking (Figures 2c and 2d).

Pre-soaking resulted in expansion in the size of the seed through water absorption and often the seeds began to germinate, the seeds were therefore ready to sprout when planted. However, it was important to time the pre-sprouting appropriately, so that the seeds were ready to germinate in the substrate immediately but at the same time ensuring that the root system had not developed to such an extent that it would become damaged during seeding. Experiments were therefore also undertaken to determine the optimal number of hours seeds should be soaked for prior to planting. The proportion of seeds germinated for both *Medicago sativa* and *Avena sativa* peaked at 24 hours pre-soaking of the seeds (Figure 3a), although little **F3** variation was found in the stem heights for either species with the different soaking times (Figure 3b).

A further issue to consider with regards to pre-soaking is the ability to disperse seeds on the experimental plot; it can be more difficult to disperse the seeds uniformly when they have been pre-soaked as the seeds tend to clump together. To overcome this the seeds were laid out on trays and air dried for a couple of hours following pre-soaking. However, this method is time consuming, so consideration has to be given to the advantages of pre-soaking on vegetation growth depending on the requirements of the experiment.

Impact of temperature

The temperature that the model vegetation is grown at can be a critical variable in experimental work. It is therefore important to evaluate the impact that temperature will have on vegetation germination and growth rate. Medicago sativa had a higher proportion of seeds germinate over the seven days for all temperatures and appears to be less impacted by temperature variations (with 75% of seeds germinating at 16 °C and 77% at 22 °C and little variation in the intervening temperatures, and root length varying from an average of 27 mm at 16 °C to 31 mm at 22 °C), with the optimal temperature for growth between 18-19°C (Figure 4a). Avena sativa had a generally F4 lower germination rate (only 26% of seeds germinated at 16 °C with a short root length of only 28 mm and only 1-3 roots formed, compared to a germination rate of 42% at 22 °C with an average root length of 44 mm and 3-5 roots), with a sensitivity to the lower temperatures adversely affecting germination (Figure 4b). The larger error bars for each temperature could indicate that other variables may be important in influencing the germination rate of Avena sativa. The stem height for Medicago sativa is similar for all temperatures (Figure 5a), while for Avena sativa F5 the lower temperatures tended to stunt the stem growth, while higher temperatures resulted in stem heights in excess of 100 mm after seven days growth (Figure 5b).

Water temperature can also have a strong impact in maintaining healthy vegetation growth once through the experiment, and variations in water temperature can have an adverse impact on vegetation establishment. An important control on this is whether re-circulated flow is used in the experiment; a recirculating system for the flow can ensure that the water is maintained at a consistent temperature to enable germination and vegetation growth.

Light availability

Lighting for these experiments was controlled by a timercontrolled Thorn-Lopan 250 W, HPS-T sodium lamp. Previous tests by Pedley *et al.* (2009) indicated that when placed 1.5 m



Figure 2. The proportion of seeds for (a) *Medicago sativa* and (b) *Avena sativa* that have germinated through time that were planted with both pre-soaked and dry seeds, and the average stem height through time for (c) *Medicago sativa* and (d) *Avena sativa* under the same conditions.



Figure 3. Time seeds were soaked prior to planting: (a) proportion of seeds sprouted (average value) and (b) average stem lengths for *Medicago* sativa and Avena sativa after seven days growth.



Figure 4. Impact of temperature on seed germination after seven days growth for (a) Medicago sativa and (b) Avena sativa.

above the subject this lighting system delivered the exposure equivalent of full midday northwest European summer sunshine. The light was established at this height and was either constantly supplied to the vegetation or provided on a 50/50 basis, whereby 12 hours of constant light alternated with 12 hours of darkness for a period of 10 growing days. The influence of light availability **F6** seemed to have little impact on the germination rate (Figure 6a) or stem height (Figure 6b) of either species. From observation of

the experiments, the important aspect of light availability was the initial provision of light immediately following seeding. After this, intermittent or constant lighting appeared to be of minimal importance as long as some light was available. Complete withdrawal of light caused growth defects in the vegetation, with weak rooting systems and stunted, yellowing stems and leaves. However, light availability is important in the long-term health of plants and 50/50 lighting (simulating natural day and night



Figure 5. Impact of air temperature on stem height after seven days growth for (a) *Medicago sativa* and (b) *Avena sativa*. Note the difference in scale on the *y*-axis between the two seed types.



Figure 6. The impact of light availability on the (a) proportion of seeds sprouted and (b) stem height for both *Medicago sativa* and *Avena sativa* after seven days growth; light was supplied either constantly for 24 hours or '50/50' in which light was supplied for 12 hours and then withheld for 12 hours.

conditions) are considered optimal during the experiment to maintain healthy vegetation.

Water availability

Substrate saturation and watering frequency were both investigated to determine the impact of water availability. Substrate saturation consisted of saturated and drained conditions: the saturated experiments were conducted in a container from which no water was allowed to drain out of it and so any water that was not taken up by the vegetation or evaporated stayed within the soil profile; under drained conditions the container had drainage holes and so water drained freely out of it. F7 Figure 7a indicates that substrate saturation had little impact in the germination rate of Medicago sativa, but that Avena sativa had 20% higher germination in drained conditions. Both Medicago sativa and Avena sativa respond better to regular watering, with a 20% reduction in seed germination for Medicago sativa watered only once per week and likewise a 10% reduction in Avena sativa (Figure 7b). There was no particular difference noted on the stem heights of the two species under the varying conditions of water availability, although it was observed that saturated substrate resulted in weaker and less developed root systems for both *Medicago sativa* and *Avena sativa*. Completely saturated conditions also resulted in *Avena Sativa* seeds being pushed onto the surface, which could be a disadvantage for certain types of experiment.

Most experimental plots, especially those replicating fluvial environments, will have a bed slope and so obtaining uniform water availability across the bed can be difficult. Both seed species require a constant water supply and the best way to achieve this is to continuously run a low flow to ensure uniform water distribution and that areas of the bed do not dry out (see Tal and Paola, 2007, for an example of how this was achieved in a series of river experiments).

Seed dispersal

An important consideration in the growth of experimental vegetation is the method of seed dispersal. Firstly, the depth of seed coverage was considered, Figure 8 shows the proportion **F8** of seeds sprouted and stem height for seeds left uncovered on



Figure 7. The impact of water availability on the proportion of *Medicago sativa* and *Avena sativa* seeds sprouted: (a) the influence of having free draining or saturated conditions on germination and (b) the impact of watering frequency.



Figure 8. The proportion of seeds that sprouted (upper graph) and maximum stem growth (lower graph) through time for both *Medicago* sativa and Avena sativa under different depths of sand covering the seeds: (a) 10 mm sand depth, (b) 5 mm sand depth and (c) seeds left on surface with no sand coverage.



Figure 9. Proportion of seeds sprouted dependent on seed dispersal technique: (1) 'surface' – seeds spread on the surface and then covered by substrate, (2) 'mixed' into the substrate or (3) using a combination of 'both'.

the surface and buried by 5 mm and 10 mm substrate respectively (the sediment was sprinkled over the top of the seeds, it was not patted down or compacted to replicate sediment burial in a natural environment). These results clearly show a dramatic reduction in the vegetation growth when the seeds are left bare on the surface, this is due to the failure of the seeds to establish root systems before they dry out from exposure. This was observed in the experiments even when the seeds were regularly watered, but increasing the water availability and/or using different sediment sizes could improve the germination rate of uncovered seeds.

It is clear that the seeds of both species need to be covered to enable growth, and if individual plants are being considered in an experiment it is recommended that seeds are each pushed an equal distance into the sediment to enable roots to germinate underneath the sediment. However burial is not always possible in a dynamic experimental environment when minimal landform disturbance is required. Therefore three different seed dispersal methods were trialled to find the most effective method of achieving seed dispersal: (1) seeds were distributed evenly on the surface and then covered with a thin layer of substrate (approximately 5 mm); (2) seeds were mixed into the substrate (the specified number of seeds was mixed into the sand prior to filling the container); and (3) a combination of the previous two methods was adopted. The surface dispersal method produced the highest germination rate for F9 both Medicago sativa and Avena sativa (Figure 9) and allowed for an even distribution of seeds across the surface. Whereas mixing the seeds with the substrate prior to seeding cause the most observable damage to the delicate

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roots that had sprouted during pre-soaking and thus reduced the vegetation growth.

Conclusion

Medicago sativa has a wide range of growing conditions and has demonstrated resilience to changes in temperature, water and light availability, with consistently higher germination rates than Avena sativa. The optimal temperature for growth was found to be 18-19 °C but it can prosper over a wider range, with best growth recorded under constant light and in a drained substrate. Avena sativa is more sensitive to temperature and performs better at higher temperatures, with 20-22 °C having the highest germination rate and stem growth, it also needs a drained substrate and frequent watering to thrive. Storing the seeds in a refrigerator prior to use was found to prolong the seed life and accelerate germination rate on exposure to room temperature. In these experiments pre-soaking of both species of seeds for up to 24 hours and an early supply of light following seeding was found to be important to ensure rapid germination and establishment of the vegetation.

Both *Medicago sativa* and *Avena sativa* can grow in a range of temperatures, with preparation of seeds prior to use helping to increase germination success and therefore vegetation establishment. The final choice of species will be dependent on the scaling and vegetation requirements of the specific experiments. For example, *Avena sativa* has the advantage that the larger sized seeds are easy to handle,

however this can create obstacles in the flow, potentially creating turbulence. In comparison, the smaller seed size of Medicago sativa is often comparable to the substrate material being used and so is less obtrusive, and the more resilient seed structure means that it is more durable than Avena sativa. The size of model vegetation and root length that is appropriate will also be important when considering the scaling of the experiment in question. This commentary was limited to growing conditions in sand using two common model vegetation types, but aims to start off a discussion of how to optimize the preparation and use of live vegetation in geomorphological experiments. With the hope that this will provide guidance for those that are new to this method and to begin a practice of sharing the knowledge on how vegetation is used in experimental work, as well as the results of these, with the wider academic community.

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