Scaling of soil water absorption by seeds: an experiment using seed analogues

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Abstract

Scaling of water absorption and water loss by seeds on various soil surfaces was simulated using seed analogues constructed with paper pulp. Three sizes of analogue seeds (large, medium and small) were laid on three types of soil surface (coarse, medium and fine texture). To estimate the amount of water absorbed by a seed during a fixed time interval, the difference in seed weight from the start of the experiment was used. The scaling of water absorption necessary for germination was also studied using actual seeds of 14 species representing a range of seed sizes. Scaling coefficients between the amount of absorbed water by an analogue seed (net water gain) and seed mass were usually lower than 1: small seeds absorbed water more rapidly than large ones. The water loss of analogue seeds was also correlated with seed mass with a scaling coefficient lower than 1, but the amount of water loss itself was far smaller than the absorption. On the other hand, the germination of actual seeds revealed that the amount of water necessary to start germination was proportional to seed mass. Thus, smaller seeds have an advantage over larger seeds in more rapidly attaining the water content necessary for germination. Moreover, small seeds can penetrate through small cracks in the soil surface and thus enjoy a double advantage in a microsite that promotes water absorption and minimizes desiccation.

Keywords: analogue seed, germination, water absorption by seeds, water balance of seeds, water loss from seed

Introduction

Seed germination is a critical phase in the life history of plants, water absorption being the most important prerequisite for seed germination. Water absorption is affected by the size and shape of seeds in relation to soil micro-topography (Harper and Benton, 1966; Foster, 1986). A seed on the soil surface absorbs water from the substrate and loses it to the atmosphere. In order to germinate, the seed must show a net gain of water (Harper and Benton, 1966). For water absorption, contact between seed and substrate is necessary. Size affects the position of the seed on the soil surface, thus affecting the contact. If a seed penetrates into a small crack, it may enjoy twofold advantages, namely good contact with the substrate to absorb water and protection against water loss to the atmosphere (Harper and Benton, 1966). If a seed is sown on the soil across a minute crack, however, the contact will be reduced. Hence, the contact of a seed to a heterogeneous soil surface is determined by the size of the seed in relation to the soil micro-topographic heterogeneity (Pareja and Staniforth, 1985).

Remarkable variations in seed weight among species are known. Seed size within a species, however, is relatively constant (Harper *et al.*, 1970). The constant seed size specific for each species is believed to maximize the parent's reproductive success (Westoby and Rice, 1982), although some have argued that, under some conditions, selection may favour the maintenance of seed size variation within species (Venable, 1992; Geritz, 1995; Sakai and Sakai, 1995). Larger seeds have a greater advantage than smaller ones in competition, germination from deeper soil or litter-cover, and growth as seedlings in harsh environments such as shading and drought (Black, 1956, 1958; Seiwa and Kikuzawa, 1991; Leishman and Westoby, 1994; Leishman *et al.*, 1995). Small-seeded species can produce a great number of seeds and can disperse them widely (Howe and Smallwood, 1982; Augspurger, 1984). Dispersal of seeds is presumed to have fitness advantages for plants (Hughes *et al.*, 1994). Seed size is thought to evolve as a compromise between two counterposed selection pressures, namely more numerous seeds and better chances of establishment (Leishman *et al.*, 1995).

Is there no adaptive advantage of small seeds relative to large seeds in the phase of establishment? Hendrix *et al.* (1991) argued that seedlings germinated from small seeds invest a relatively greater amount of

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resources to root than to shoot and can thus survive under temporary drought conditions. Harper and Benton (1966) sowed seeds of various species on the exposed surfaces of sintered glass plates arranged to supply water under controlled tensions. Based on conclusions derived from the experiments, they suggested that large-seeded species may be at a great disadvantage in open habitats where they are more likely to suffer from desiccation, while small seeds are less liable to desiccation and more likely to be able to germinate in open habitats. Baker (1972), however, showed that large seeded species predominate in drought conditions, although several other researchers criticized his inadequate interpretation of the data (Mazer, 1989; Westoby *et al*., 1992). It is, therefore, still possible that small-seededness is advantageous in germination under temporary drought conditions. Here, we hypothesize that smaller-seeded species can produce greater number of seeds and disperse them widely, thus colonizing an open habitat by germinating quickly. Relationships between water absorption and seed size are more complicated because the shapes of seeds differ among species. If introduced on a completely smooth flat surface of substrate, seeds with a flattened shape will have better contact with the substrate than round ones. In reality, the soil surface is not smooth but irregular; thus, the shape of seeds also affects their orientation on a heterogeneous soil surface (Peart, 1981).

Experiments that aim at determining the degree to which seeds of different sizes make contact with the soil surface must control other factors that may influence contact with the soil, such as seed shape. In this paper, we report the result of an experiment using seed analogues designed to exclude other factors and address the following questions:

1. Do small seeds take up water more quickly than large seeds?
2. Is the rate of uptake proportional to seed mass or seed surface area?
3. Is the rate of uptake different on different soil surfaces?
4. Do small seeds lose water more quickly than large seeds, and is the rate of water loss proportional to seed mass or seed surface area?
5. How much water should a real seed imbibe before its germination and is this amount proportional to seed mass?

Materials and methods

Soil preparation

Commercial soil (loess; 'Akadama' brand) for horticultural use was used as a substrate for the experiment. The soil was dried and sieved by four sizes of mesh. Soil particles were divided into three size classes: large (10–18 mm in particle diameter), medium (6–10 mm) and small (3–5 mm). Soils were contained in a polyethylene dish 15 cm in diameter and 3 cm in depth. The dishes were pricked with needles to facilitate drainage. Each dish was set in a larger dish filled with water 5 mm deep. The diameter of the larger dish was 21 cm and its depth 4 cm. In order to saturate the soil in the dish with water, the dish was left in the 5 mm of water in the larger dish for 24 h before the experiment began.

Seed analogues

In order to absorb water freely, we used paper pulp as material for the 'seeds'. We crushed tissue paper made from 100% pulp (COOP Japan, Tokyo) with added water, using a food processor to reduce it to a uniform pulp. We made three sizes of artificial 'seeds' by rolling the pulp into a small ball. To keep the density of artificial seeds constant, a fixed volume of pulp was used. For the large artificial seeds, 10.2 ml of pulp was used to make a sphere 18 mm in diameter; for the medium seeds, 2.6 ml of pulp was used for a sphere 13 mm in diameter; and for small seeds, 0.1 ml of pulp was used for a sphere 4 mm in diameter.

These artificial seeds were dried at 80°C for 24 h. Before the start of the experiment, five artificial seeds from each size class were sampled and weighed. Mean seed mass was 771 mg for the large seeds, 290 mg for the medium seeds, and 9.9 mg for the small seeds. The volumes of the large, medium, and small artificial seeds were 2600, 816, and 31 mm^3, respectively. Material density (seed mass/volume) was not significantly different among size classes (ANOVA; $F_{2,12} = 0.732$, $P > 0.01$).

Water absorption by analogue seeds

To estimate water absorption from substrate to seed, three classes of artificial seeds were sown on three types of soil surfaces. For each trial, five artificial seeds were sown on each soil. Seeds were weighed at several fixed intervals up to 3 h, and then the larger dish was covered to keep the humidity near 100%. After 24 h, we again measured the weight of the artificial seeds and considered them to be saturated with water. Each experiment was replicated three times.

Water loss by analogue seeds

To evaluate the water loss rate from artificial seeds to the atmosphere, five seeds from each size class, which were saturated with water, were left on an open Petri dish under laboratory conditions. The temperature and moisture conditions (RH) in the laboratory were about 26°C and 40%, respectively. Then, artificial seeds were

Table 1. Mean dry mass of actual seeds of 14 species

Species	Number of germinated seeds	Seed dry mass (mg)
Cucurbita moschata melanaeformis Makino Cucurbitaceae	4	232.50 ± 9.57
Helianthus annus Linn. Compositae	5	122.50 ± 7.59
Maackia amuurense buergeri C.K. Schn. Leguminosae	1	57.4
Xanthium strumarium Linn. Compositae	4	14.00 ± 1.84
Rhus javanica Linn. Anacardiaceae	11	8.64 ± 0.67
Metaplexis japonica Makino Asclepiadaceae	2	4.50 ± 0.40
Ulmus davidiana japonica Nakai Ulmacaea	14	3.52 ± 0.50
Ulmus laciniata Mayr. Ulmaceae	1	3.35
Polygonum sachalinense Fr. Schm. Polygonaceae	7	1.10 ± 0.13
Miscanthus sinensis Anderss. Gramineae	8	0.72 ± 0.15
Taraxacum officinale Weber Compositae	10	0.64 ± 0.07
Alnus hirsuta Turcz. Betulaceae	14	0.59 ± 0.08
Oenothera lamarckiana Ser. Onagraceae	8	0.52 ± 0.07
Verbascum thapsus Linn. Scrophulariaceae	3	0.06 ± 0.01

weighed at several fixed intervals until 22.5 h, when the humidity inside the seeds was at equilibrium with the atmosphere. This was replicated twice.

Water absorption by real seeds

In order to evaluate the scaling of the amount of water necessary for germination, we measured the amount of water absorbed by natural seeds until their germination. The plant species used in this experiment were as follows: *Xanthium strumarium, Cucurbita moschata melanaeformis, Metaplexis japonica, Taraxacum officinale, Helianthus annus, Alnus hirsuta, Ulmus davidiana japonica, Ulmus laciniata, Verbascum thapsus, Polygonum sachalinense, Oenothera lamarckiana, Miscanthus sinensis, Maackia amuurense buergeri* and *Rhus javanica*. These are trees or herbs naturally grown or commonly cultivated in Hokkaido, northern Japan.

Seeds were collected from naturally grown or harvested plants either in the summer or autumn of 1990, and they were immediately rinsed with water and weighed to determine their fresh weight. Then they were put on water-saturated filter paper in a Petri dish. The dish was covered and stored in an incubator regulated to maintain a constant air temperature at 24°C. At 24-h intervals, all seeds were examined to monitor germination and weighed to determine fresh weight. Many seeds were observed to germinate within 5 d of incubation. Seeds which did not germinate within 5 d were discarded. The difference between fresh weight at the time of seed germination when the radicle appeared and initial fresh weight gave the amount of water absorbed until seed germination. After seed germination, the seeds were dried to a constant weight at 70°C, and seed dry mass was obtained. There was approximately 3.8×10^3 of variation between the maximum (232.50 mg in *Cucurbita moschata melanaeformis*) and minimum (0.06 mg in *Verbascum thapsus*) in mean seed dry mass. Twenty seeds per species were used for the experiment, but only data for seeds that actually germinated were used for analysis. The number of germinated seeds varied from one (*Maackia amuurense buergeri* and *Ulmus laciniata*) to 14 (*Ulmus davidiana* and *Alnus hirsuta*). The number of germinated seeds and mean dry mass for each species are shown in Table 1.

Data analysis

The effects of seed size, soil type and time on water absorption by seeds were analysed by a three-way analysis of variance (ANOVA). Water absorption by a seed was expressed as the percentage of water absorbed each time to the total amount of water absorbed in 24 h (at saturation). The percentage absorption data were arcsin-transformed. The effects of seed size and time on water loss were analysed by two-way ANOVA, also using arcsin-transformed data. Statistical analyses were carried out by using a statistical package (SYSTAT) on a Macintosh personal computer. For actual and analogue seeds, the scaling of water absorption was determined for seeds of different sizes. Values of seed dry mass and amount of water absorbed were transformed to log values. The logarithms were then used in linear regression. Thus, these relationships were expressed as $Y = aX^b$, where X is seed mass and Y is absorbed water. If the scaling coefficient b (the slopes of regression lines) was equal to 1.0, the amount of absorbed water was proportional to the seed mass. If net water gain per seed mass was greater in smaller seeds than in larger seeds, the coefficient was less than 1.0.

The same procedures were applied for the relationships between seed dry mass (seed analogues only) and water loss, and a scaling coefficient was also obtained.

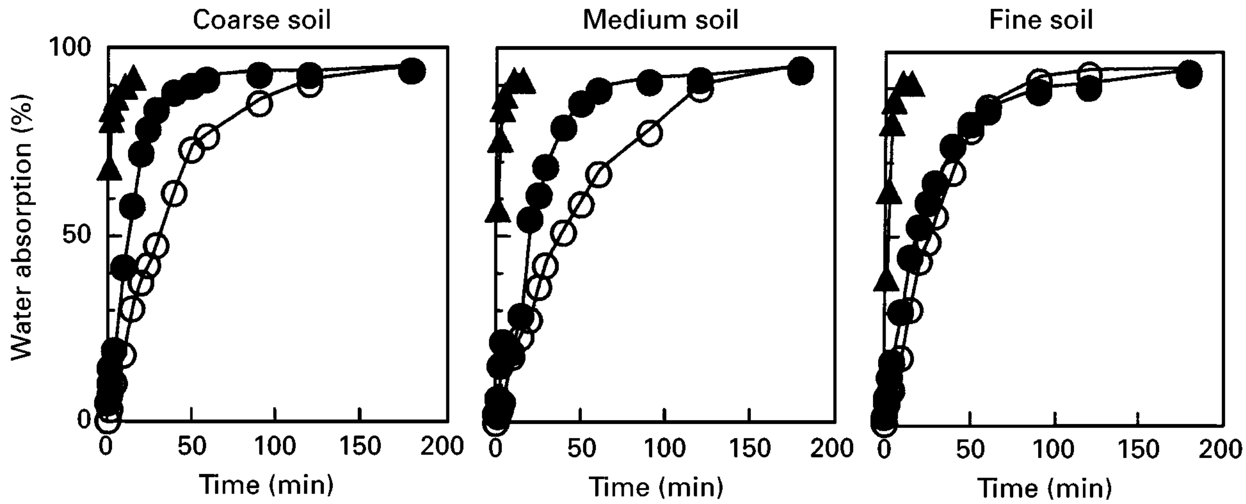

Figure 1. Time course of mean values of net water gain by three sizes of seed analogue on three types of soils. Closed triangles, closed circles, and open circles represent small, medium and large analogue seeds, respectively. Net water gain is expressed as a percentage of the saturated value.

Results

Time course of water absorption by analogue seeds

The amount of water absorbed by a seed until each measuring time was expressed as a percentage of the amount of water absorbed at saturation (until 24 h). The mean percentages of water absorption of three types of analogue seeds on three types of soils are shown in Figure 1. Small seeds always absorbed water rapidly on all soil types. The rate of water absorption by large seeds was the slowest, and the rate of water absorption by medium-sized seeds was intermediate between the two in coarse and medium soils. On fine soil, the mean water absorption of medium and large seeds was quite similar. Three-way ANOVA revealed that there were significant differences ($P < 0.0001$) among seed sizes, soil types, and time and interactions between seed sizes and soil types and seed sizes and time (Table 2).

Time course of water loss by analogue seeds

Mean water loss from three types of seeds in a Petri dish is shown as a percentage of saturated water. Small seeds lost water significantly more rapidly than large seeds, which lost water slowly (Fig. 2).

The rate of water loss was far smaller than that of water gain. Small seeds lost about 15 mg of water in 1 h. On coarse soil, they absorbed twice that amount within only 1 min.

Scaling of water absorption by analogue seeds

The net gain of water by a seed per unit time is related to its size. The relationships between the two are expressed as linear regressions on double logarithmic scales (Fig. 3) or,

$$Y = aX^{b},$$

where X is seed size and Y is absorbed water. Small

Table 2. Three-way ANOVA for water absorption

Source	df	Sum of squares	Mean square	F-value	P-value
Seed size	2	45.557	22.779	529.246	0.0001
Soil type	2	1.268	0.634	14.727	0.0001
Time	12	188.257	15.688	364.504	0.0001
Seed size × Soil type	4	2.023	0.506	11.748	0.0001
Soil type × Time	24	0.613	0.026	0.594	0.9398
Time × Seed size	17	3.301	0.194	4.511	0.0001
Seed size × Soil type × Time	33	1.288	0.039	0.907	0.6203
Residual	1344	57.845	0.043		

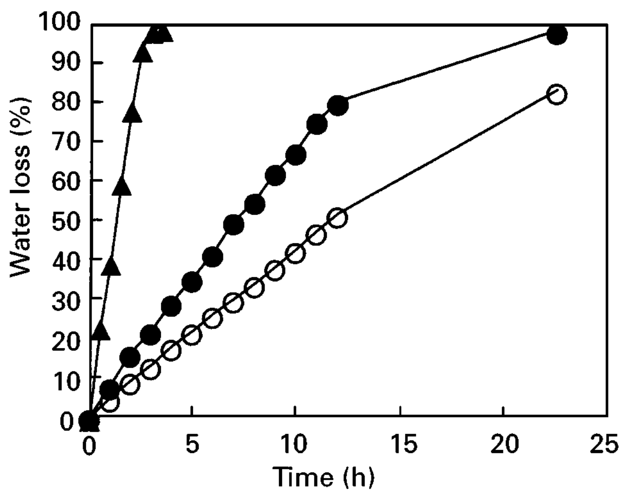

Figure 2. Time course of mean value of water loss from three sizes of seed analogue on a Petri dish. Closed triangles, closed circles and open circles represent small, medium and large analogue seeds, respectively. Water loss is expressed as a percentage of the saturated value.

seeds absorbed water rapidly, while larger seeds did so rather slowly. Therefore, the slopes of the regressions are rather small for short periods of absorption time. In 5 min, the scaling coefficient (b) in the coarse soil was 0.425, increasing to 0.648 in 15 min and reaching 0.897 in the saturated phase. Scaling coefficients on the medium and the fine soils were smaller than the coefficient on the coarse soil (data not shown). Even in the saturated phase, the scaling coefficients were 0.526 and 0.600 on medium and fine soils, respectively.

Scaling of water loss by analogue seeds

The water loss from a seed left on a Petri dish was also plotted on a double logarithmic scale (Fig. 4), and a linear regression against seed dry mass was obtained. The scaling of water loss in 1 h was allometric; that is, coefficient b was 0.563. After sufficient time had elapsed (3.5 h for small seeds and 22.5 h for larger seeds), the scaling coefficient became 0.821, suggesting that the scaling of water loss was still allometric, which means that small seeds were nearly dried up by that time, but larger seeds were still wet.

Scaling of water absorption by real seeds

The relationship between log-transformed values of dry mass of real seeds and amount of water absorbed until germination is shown in Figure 5, and the regression equation was obtained as follows,

$$Y = 0.086X^{1.031},$$

where X is seed dry mass (mg) and Y is the amount of water absorbed by a seed during the period of germination (mg per germination period), representing the amount of water necessary for germination. Since the regression equation was highly significant ($r^2 = 0.98$, $P < 0.001$) and the scaling coefficient ($b = 1.031$) was not significantly different from 1.0 ($P < 0.001$; Student's *t*-test), we concluded that the amount of water necessary for germination is proportional to the seed mass.

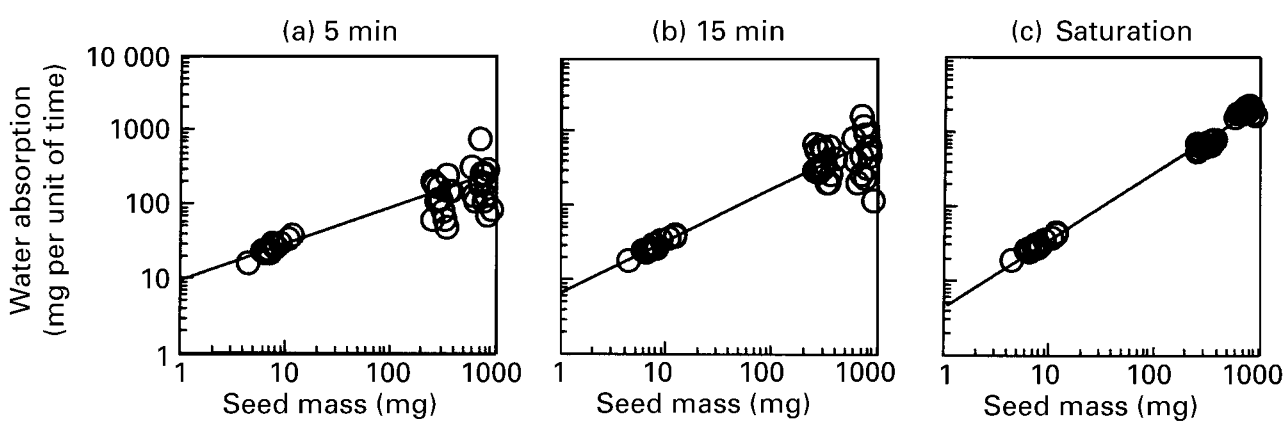

Figure 3. Relationships between net water gain and dry mass of analogue seeds on coarse soil at different intervals from the start of experiment. Water absorption (*Y* mg per unit of time) is expressed against seed mass (*X* mg) as $Y = aX^b$.
(a) $a = 12.9$; $b = 0.425$; $r^2 = 0.777$; $P < 0.0001$. (b) $a = 9.03$; $b = 0.648$; $r^2 = 0.87$; $P < 0.0001$. (c) $a = 5.41$; $b = 0.897$; $r^2 = 0.994$; $P < 0.0001$.

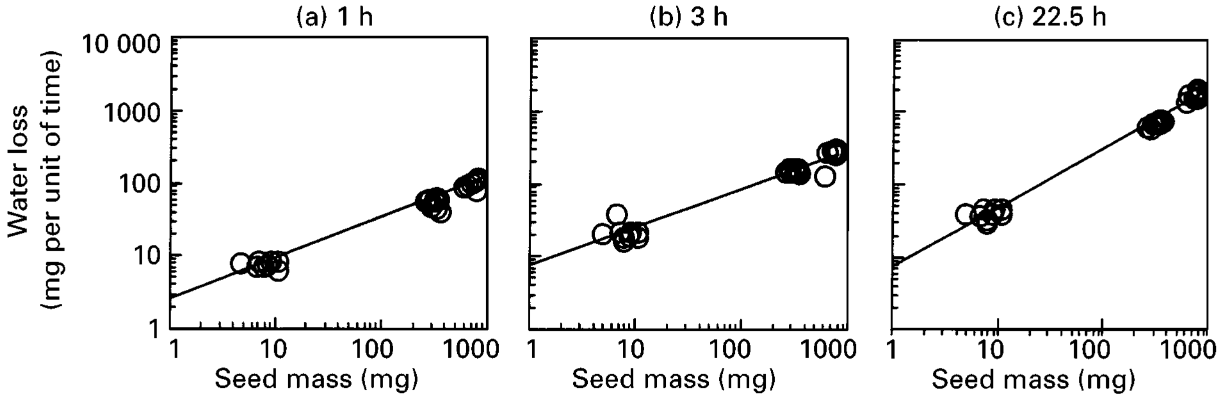

Figure 4. Relationships between water loss and dry mass of analogue seeds on a Petri dish at different intervals from the start of experiment. Water loss (*Y* mg per unit of time) is expressed against seed mass (*X* mg) as $Y=aX^b$;
(a) a = 2.62; b = 0.563; $r^2 = 0.965$; $P < 0.0001$. (b) a = 7.65; b = 0.554; $r^2 = 0.965$; $P < 0.0001$. (c) a = 8.05; b = 0.821; $r^2 = 0.990$; $P < 0.0001$.

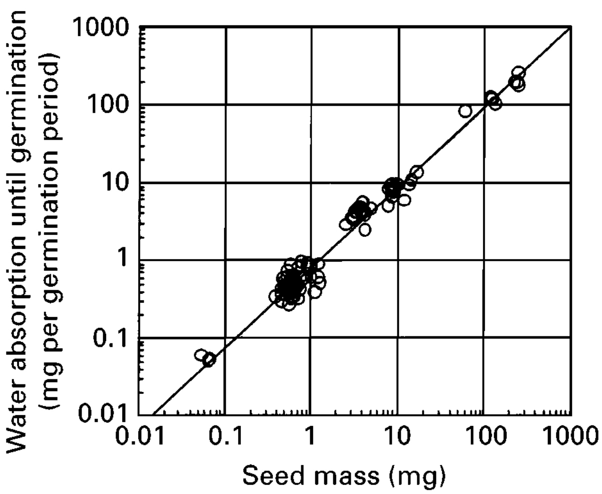

Figure 5. Net water gain necessary for germination against dry mass of actual plant seeds. Seeds of 14 species naturally grown or cultivated in Hokkaido, northern Japan, were used.

Discussion

The amount of water absorbed by actual seeds until germination was proportional to seed mass (Fig. 5). Each seed must absorb more than 90% of its mass in water by the time of germination. Although this relation was obtained from the comparison of the 14 species, it may indicate a universal trend with some exceptions, such as oily seeds. Hence, smaller seeds require a lesser total amount of water to germinate than large seeds.

Efficiency of water gain from the substrate is important for seeds to germinate. In order to analyse the advantages and disadvantages of different sizes of seeds concerning water absorption and water loss, we used seed analogues made up of paper pulp to avoid problems arising from inter-specific differences in shape, and in surface morphology and materials of seeds. Even on the same substrate, small seeds always gained water more rapidly than larger seeds (Fig. 1). The surface area of a seed is proportional to two thirds the power of its mass, as in the case of a sphere. Thus, smaller seeds have relatively greater surface area against seed mass than larger seeds. If the water absorption is proportional to the surface area of the seeds, it must scale to approximately two thirds the power of seed mass. The scaling coefficients in net water absorption to seed mass observed in this study changed with time intervals of water absorption. However, they were always less than 1.0, and some of them were quite similar to two thirds. It was concluded that small seeds are usually more rapid in net water gain than larger seeds.

Even if smaller seeds are quicker in water absorption than larger ones, this advantage will be cancelled out when the water loss rate is also proportional to the surface area of the seeds. The scaling coefficients of water loss rates obtained in this study were also less than 1.0. However, the amount of water loss itself was far smaller than that of water absorption. Therefore, in this study, net water gain was determined by the water absorption rate alone, which may also be true for seeds in many cases outside the laboratory, since actual seeds have mechanisms to imbibe water effectively and to hold water against desiccation.

However, when water loss rate is comparable to the rate of water absorption, the scaling of water loss must be taken into account. Harper and Benton (1966) found a lower germination rate in larger seeds than in smaller seeds on a sintered glass plate. They argued that the effect of desiccation is not as acute for small seeds as it is for large ones because small seeds have a larger contact : surface area ratio. This contact : surface area ratio may change depending on the nature of soil surface micro-topography. Harper *et al.* (1965) observed that the water uptake and germination of seeds in several species were higher when the surface of the soil was irregular than when it was smooth. Harper and Benton (1966) suggested that the cracks present in irregular soil provided sites with relative humidity where the seeds were protected from excessive desiccation. When the seeds are larger than the crack, however, they are bound to lie on the soil surface across the crack, and thus the area of seed contact with the soil surface will be reduced. The contact area between seed and substrate will change with the relative size of seeds to the size of soil surface cracks. Therefore, whether a seed can benefit from soil surface irregularity or not will depend on its relative size. Hegde *et al.* (1991) noted that the frequency distribution of number of species of different seed sizes is positively skewed: there are numerous species with small-sized seeds and relatively rare species of large-sized seeds. They pointed out that this distributional pattern resembles the fractal nature of soil surface micro-topography: small cracks are numerous and large cracks are relatively rare.

In this experimental system, small artificial seeds also had an advantage in relation to soil micro-topography. They usually penetrated into cracks on the coarse soil and frequently into cracks on the medium soil (K. Kikuzawa and H. Koyama, unpublished observations). They never did so on the fine soil. In a crack, the contact area of seed surface to substrate increases and results in the increase in water absorption rate. On the other hand, larger seeds sometimes penetrated into cracks on the coarse soil, but sometimes they lay across a crack. In the latter case, the contact area between seed surface and substrate was reduced. On the medium-soil surface, large seeds frequently lay across a crack (K. Kikuzawa and H. Koyama, unpublished observations). Although large seeds usually lay across cracks on the fine soil, the contact : surface area ratio was assumed to be reduced less on the fine soil than on the other soils, since here the cracks were smaller than those on the medium and coarse soils. The changes in the water absorption rate of each size of seeds on each soil may reflect the changes in the relative area ratio of contact surface to the whole surface of a seed.

The above arguments indicate a double advantage of small seeds relative to large seeds in water absorption on soil surfaces. They have the advantage of quicker water absorption because of their large surface area to mass ratio, and they also have the advantage of being able to penetrate into large- as well as small-sized cracks, while larger seeds can only utilize large cracks. Rapid water absorption is advantageous on the bare soil, where the water content of the soil surface rapidly declines after rainfall (H. Koyama and K. Kikuzawa, unpublished results). Outside of the laboratory, small-seeded species imbibe water rapidly and elongate their radicle to penetrate deeper soils, where water content is rather high and stable, before the surface soil becomes desiccated again. In the same situation, large seeds could not do so, since their rate of water absorption is low, and hence they could not take up enough water to germinate before the surface soil dried. Moreover, once they germinate, seedlings of small seeds can avoid desiccation by developing relatively larger root to shoot ratios (Hendrix *et al*., 1991).

In conclusion, the difference in scaling coefficients between the amount of water necessary for germination ($b = 1$) and that actually gained ($b < 1$) suggests an advantage of small seeds over large seeds with respect to water balance. Moreover, the ratio of contact area to the substrate and that to the atmosphere will determine the water balance between absorption and loss, as was suggested by Harper and Benton (1966).

It is known that small-seeded species are often found in open habitats (Salisbury, 1942; Foster and Janson, 1985; Foster, 1986), and many authors attribute this trend to seed dispersibility, which is advantageous in reaching and colonizing open sites (Howe and Smallwood, 1982; Augspurger, 1984). This study suggests that small seeds are also at an advantage during the phase of germination on bare soil in open habitats, where the soil surface tends to be desiccated and available water supply is highly fluctuating. On the other hand, in shaded habitats where leaf litter is usually accumulated, the soil water content is relatively high and stable (H. Koyama and K. Kikuzawa, unpublished results). Therefore, water availability is not critical for seeds to germinate. In these habitats, larger seeds are at an advantage because greater maternal investment helps ensure that the seedling can penetrate leaf litter and deep soil by developing a large shoot and radicle (Gross, 1984), by out-competing other plants through more rapid shoot growth (Stanton, 1984; Winn, 1985), by establishing from deeper soil horizons when buried (Black, 1956), and by tolerating shady conditions (Grime and Jeffery, 1965; Foster, 1986).

Several extensions of the present study may be possible. The analogue seeds may be used in a field as an indicator of soil micro-topography. If analogue seeds were laid on the soil surface within a fixed time and water absorption was measured, the results would indicate the water condition and micro-topographic heterogeneity of the soil. Several suggestions may be drawn from the results of the present study on the practice of site preparation for natural and artificial forest regeneration. In order to promote natural regeneration, soil surfaces should be scarified to facilitate surface micro-topographical heterogeneity. If seeds are sown artificially, several sizes of seeds should be mixed to utilize the variety of surface cracks fully.

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