

Effects of Seed Size on Seedling Size in *Virola surinamensis*; a Within and Between Tree Analysis

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Summary. We conducted a greenhouse study of the effects of initial seed mass on seedling characteristics in a Panamanian population of *Virola surinamensis*, a canopy tree in which mean seed mass of different individuals ranges from 1.34 to 4.04 g. The system is of particular interest because birds preferentially eat fruits of small-seeded plants, leaving seedlings of large-seeded individuals under conditions of potentially severe sibling competition (Howe and Vande Kerckhove 1980).

Effects of differences of mean seed mass between trees are explored using an analysis of variance, while effects of seed-mass variation within crops are demonstrated with a regression analysis. A two-way analysis of variance decisively shows effects of parental source and light condition on seedling height, leaf length, and dry shoot mass (all $P < 0.0001$). *A posteriori* tests show that differences in seedling characteristics reflect differences in initial seed mass, with especially strong differences apparent in shoot mass. Regression of seedling characteristics on initial seed mass shows that variation of seed size within a crop is sufficient to influence shoot mass at 15 weeks ($P < 0.0001$).

Effects of size differences of seeds that land adjacent to each other, either under the parent or in monkey droppings, are documented with growth of pairs of seedlings in pots. Differences in shoot height and mass at 15 weeks are evident when seeds of average size differ by only 0.2 g, and dramatic differences are evident when paired seeds differ by an average of 1.5 g. Seedlings grow more when isolated than when planted with conspecifics.

These experimental results offer indirect support for the hypothesis that small-seeded *Virola* parents secure an advantage in reproduction through differential dispersal, while large-seeded plants produce more competitive seedlings under their own crowns – an advantage most likely to be of importance when frugivores are scarce.

Introduction

A seed contains an embryo and energy reserves; the differences between high and low reserves may mean the difference between vigorous and weak seedlings. Such differences may well spell the outcome of initial establishment of animal-dispersed species in tropical forests; large seeds produce seedlings competitive in the forest understory where light is limiting, while small seeds with rapid germination

colonize openings (Snow 1971). Seed-size advantage also applies to lesser variation within species, where differences in seed mass may be due to environmental influences (Harper 1977), genetic differences between strains of cultivars (Harper and Obeid 1967), or to rare polymorphisms of a discrete nature (Harper et al. 1970). Here we experimentally demonstrate effects of variations in seed mass on seedling characteristics of the neotropical nutmeg, *Virola surinamensis* (Rol.) Warb. (Myristicaceae), which shows wide continuous variation in the seed size of different individuals.

Virola surinamensis is a canopy or emergent tree of Central and South American rainforests (Croat 1978). The natural history of the plant has been discussed at length by Howe and Vande Kerckhove (1980, 1981). Pertinent here is the fact that the tree produces an annual average of 3,000 golfball-sized capsules, each of which dehisces to expose a substantial seed surrounded by a fatty red aril. Several highly frugivorous birds, including toucans (*Ramphastos*), motmots (*Baryphthengus*), trogons (*Trogon*), and a guan (*Penelope*), swallow the arillate seeds and regurgitate or defecate viable seeds singly in the surrounding forest. One monkey (*Ateles*) eats the fruits and defecates viable seeds in pairs or trios. In earlier studies, Howe and Vande Kerckhove (1980, 1981) showed that mean seed size ranged from 1.70 to 2.67 grams dry mass for 17 plants; a far greater variation than that observed in the smaller aril. From 13 to 91% of the available fruits were taken from these trees by fruit-eating animals; 59% of the variance in dispersal success could be attributed to mean seed mass of parent trees. Birds favored plants with small seeds and avoided those with large seeds. The authors suggested that alternating selection on dispersability and competitive seedling establishment favored small-seeded trees during years of high frugivore abundance, and large-seeded plants during years when critical members of the assemblage were absent (see Foster, in press). The implication is that small-seeded individuals colonize distant sites; large-seeded plants occupy the parental stand with vigorous seedlings competitive with other conspecifics.

Confirmation of this interpretation would require demonstration of genetic differences underlying seed size in this species, as well as demonstration of competitive differences among seedlings derived from seeds of different size. A genetic analysis is outside of the scope of this study, although it should be noted that cultivars of many fruiting plants, including the Old World nutmeg, show dramatic differences

in seed and fruit characteristics (Weil 1965; see Hulme 1971). Here we examine the effects of initial seed mass on seedling characteristics several weeks after germination. The hypotheses tested include: (1) more variation in fresh seed mass exists between than within seed crops, (2) variation in seedling characteristics is consonant with variation in seed mass from different trees, and (3) variation in seedling characteristics is consonant with variation of seed mass within crops. An additional hypothesis, relevant to seeds defecated in pairs or groups by monkeys, is (4) large seeds produce seedlings competitive with those from small seeds growing nearby. Characteristics of interest include height at the apical meristem, length of the longest (of 2–3) leaves, and dry shoot mass 15 weeks after planting. Differences in early juvenile life are presumed to be associated with later differences in survival and vigor (Austenson and Walton 1970; Harper 1977). Unique to this study is documentation of the histories of individual seeds, from fruit drop to measurements 15 weeks after planting, or 12–13 weeks after germination.

Study Site

Viola surinamensis is a canopy tree of the primary rainforest from Costa Rica and Panama to the Guianas and Brazil, with disjunct populations in the Lesser Antilles (Croat 1978). This study was accomplished on Barro Colorado Island, Panama, a seasonal wet forest with an unusually well-known natural history (Croat 1978). Preliminary work established the fact of differential dispersal as a function of fruit characteristics with a sample of 17 fruiting females (Howe and Vande Kerckhove 1980, 1981); a continuing investigation seeks to further document sources of variation in seed dispersal and seed and seedling survival using a larger sample of 28 trees at the same site (Manasse and Howe, unpublished; Howe, unpublished). The four trees that served as seed sources for growth experiments are part of the latter sample of 28.

Methods

The variation in seed mass within and between crops of individual trees was evaluated with collections of 10 randomly selected seeds from 46 *Viola surinamensis* trees. The fresh mass of each seed was measured to the nearest 0.01 g. Such samples are unbiased with respect to size, as indicated by comparisons of seeds shaken down from tree crowns and seeds picked up under the crowns (Howe and Vande Kerckhove 1981).

Effects of seed mass on seedling characteristics in non-competitive situations were determined in a screened enclosure that excluded insects, but admitted natural light and rain. The procedure involved two stages, planting and transplanting.

One hundred randomly selected seeds from each of 4 trees were individually marked, weighed to the nearest 0.001 g, and placed on moist forest soil, in 0.26 l styrofoam cups on 3 July 1980. Perforated cup bottoms facilitated drainage; daily rains or sprinkling with water assured moist soil. Trees were selected with different seed sizes; the mean fresh mass of seeds differed by at least 1 g for each tree (see below). Fifty cups with 1 seed each from each tree were placed on benches exposed to the sun; 50 others from each tree were similarly placed on benches shaded by green

nylon mosquito netting. Conditions on exposed benches approximated those on the forest floor in light gaps; those under netting simulated the shaded forest understory. Final germination was 90%.

The cups in which seeds were planted were too small to allow unhindered growth, so 30 germinating seedlings from each tree (50 from one tree) were transplanted to 3.00 l flower pots on 23 August, before leaves emerged. Limited enclosure space precluded using the entire sample of 400 seedlings; those used for the growth experiments were germinating normally and were at comparable stages of development (radicals 40–60 mm long), with no bias discernible for each tree. Thirty pots from each tree were spaced in alternate fashion on benches in sunlight such that each was surrounded by neighbors from other trees. The 20 extra seedlings from one tree were also placed in the light. Similarly, 30 pots from each of four trees were placed on the shaded benches in alternate fashion, thereby equalizing for position effects. All plants were allowed to grow until 15 October, when height and length of the longest leaf were measured to the nearest 1.0 mm, and above ground shoots were dried to constant mass at 70° C, and weighed to the nearest 0.001 g.

Competitive effects were simulated under light conditions with three sets of seeds of either the same (to the eye) or different sizes. Thirty cups were started with weighed seeds of relatively heavy dimensions (3.7–4.89), light dimensions (2.3–3.29), and mixed dimensions (2.4–5.09). Of these, 25 pairs of each treatment were transplanted, the criterion for selection being an equivalent stage of germination, as discussed above. This eliminated pairs in which one seedling was far ahead of another before leaves emerged. These seeds were planted and ultimately weighed one week later than those in the non-competitive treatments.

Seeds consist of water and dry matter. To determine the relationship between fresh and dry mass, five seeds from each study tree were randomly selected, individually marked, and weighed to the nearest 0.01 g. The seeds were then freeze-dried to constant mass, and re-weighed. To determine the relationship between fresh mass and volume (of interest to bird-dispersal agents), the volume of each fresh seed was estimated using water displacement in a graduated cylinder.

Results

Variation in Seed Size

Samples of 10 seeds from each of 46 trees show enormous variation in seed size. Means for individual trees range from 1.34 to 4.04 g (grand mean = 2.72 g) (Fig. 1). These means are distributed normally; a goodness of fit test does not indicate bimodality suggested by visual presentation ($X^2 = 8.36$, 8 df, $p > 0.1$). A one-way analysis of variance indicates dramatic variation in mean size between trees ($F = 37.30$, $p < 0.0001$), with approximately 78.4% of the variance attributable to differences between trees, and 21.6% attributable to variation within crops. The experimental seeds planted in this study are derived from a non-random selection of trees; not surprisingly the variation between samples of 60 seeds is far greater (92.8%) than the variation within (7.2%). Seeds dropped early in the season are no different in mass than seeds dropped later from the same tree ($t < 2.0$, $p > 0.2$ for three replicates of 10 seeds), confirming a lack

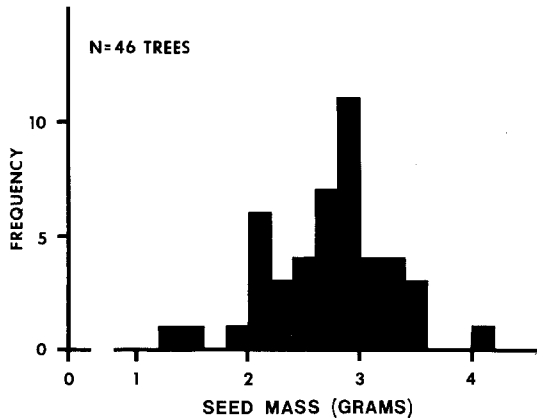


Fig. 1. Distribution of mean seed mass for 10 seeds from each of 46 *Virola surinamensis* trees. The distribution is not significantly different from a normal ($\chi^2=8.36$, 8 df, $P>0.4$)

Table 1. Two-way analysis of variance for *Virola surinamensis* seed and seedling characters, where lighted ($n=30$ per tree) and shaded ($n=30$ per tree) growing conditions are treatment effects for offspring of each of four trees. Shoot characters are measured 15 weeks after planting (12 weeks after germination)

Variable Treatment	F Statistic	Significance
Seed mass (wet)		
Light	0.05	ns
Tree	771.35	$P<0.0001$
Interaction	0.43	ns
Height		
Light	166.29	$P<0.0001$
Tree	90.18	$P<0.0001$
Interaction	1.98	ns
Leaf length		
Light	6.47	$P<0.025$
Tree	79.80	$P<0.0001$
Interaction	3.18	$P<0.05$
Shoot mass (dry)		
Light	50.56	$P<0.0001$
Tree	158.86	$P<0.0001$
Interaction	0.20	ns

of seasonal variation in aril and seed characteristics within crops found in a previous phase of the study (Howe, unpublished).

Measures of wet mass at seed drop are closely allied with measures of volume and dry mass. The relationship between dry seed mass and wet seed mass for 20 seeds is described by the linear regression:

$$\text{dry mass (g)} = 0.70 \text{ wet mass (g)} + 0.06$$

with a strong correlation ($r=0.91$). Similarly, the association between seed volume at fruit drop and wet mass is described by the relation:

$$\text{volume (ml)} = 1.08 \text{ wet mass (g)} - 0.19$$

with an even stronger correlation ($r=0.97$). Clearly, there is substantive variation in solid mass exclusive of water (see Howe and Vande Kerckhove 1981), as well as a strong

Table 2. Seed and shoot characteristics for 30 seeds at planting and 30 seedlings at 15 weeks after planting under light and shaded conditions for each of 4 *Virola surinamensis* trees. Pairs of means not significantly different within each light treatment are indicated by "ns".^a Means are accompanied by standard errors

N	Seed wet mass (g)	Shoot height (mm)	Longest leaf length (mm)	Shoot dry mass (g)
Light Treatment				
30	1.25±0.02	10.0±0.2	11.4±0.2	0.41±0.02
30	2.19±0.04	11.2±0.3	14.6±0.3	0.73±0.03
30	2.74±0.04	13.8±0.3	14.3±0.4	0.89±0.04
30	3.53±0.07	14.2±0.3	16.3±0.3	1.10±0.05
Dark Treatment				
30	1.29±0.02	12.9±0.3	11.5±0.8	0.28±0.01
30	2.22±0.05	14.0±0.3	14.5±0.3	0.54±0.02
30	2.68±0.05	15.7±0.3	16.2±0.3	0.72±0.02
30	3.54±0.06	17.5±0.4	16.9±0.6	0.94±0.04

^a Duncan Multiple Range Test, with $P<0.05$

Table 3. Seed influence on seedling characteristics within a *Virola surinamensis* crop. Regression equations for the relationship between height, leaf length, and shoot mass (dry) and initial seed mass (wet). Shoot characteristics were measured 15 weeks after planting; 12 weeks after germination

Equation	N	r^2	$P \leq$
height (mm) = 1.92 seed (g) + 7.11	50	0.07	0.06
leaf length (mm) = 2.97 seed (g) + 8.01	50	0.14	0.01
shoot mass (g) = 0.34 seed (g) - 0.02	50	0.27	0.0001

relationship between the weight of seeds carried by birds and the volume of space taken in the crop.

Effects Between Trees

A two-way analysis of variance on shoot characteristics at 15 weeks after planting shows unequivocal influences of seed source and light conditions on early seedling growth (Table 1). Interaction effects are negligible, excepting the interaction of light conditions on tree for the variable leaf length. *A posteriori* Duncan Multiple Range Tests indicate that most means for each variable are significantly different for different trees within the light and shade treatments; few cases of indistinguishable shoot characteristics emerge (Table 2). All measures within the lighted or shaded treatments increase in magnitude in conformance with seed mass, thereby implicating seed reserve as an important determinant of shoot characteristics.

Effects Within Crops

A regression of seedling variables on initial seed mass, using a sample from one tree of 50 seedlings grown under lighted conditions, indicates that variation in seed size is great enough within some tree crops to promote differences in seedling vigor (Table 3). Shoot mass, the most important correlate of general vigor, is plotted against initial seed mass (Fig. 2). Regressions of shoot mass against initial seed mass are less telling with smaller samples of 30, but still indicate some influence of seed reserves on seedling vigor. Under

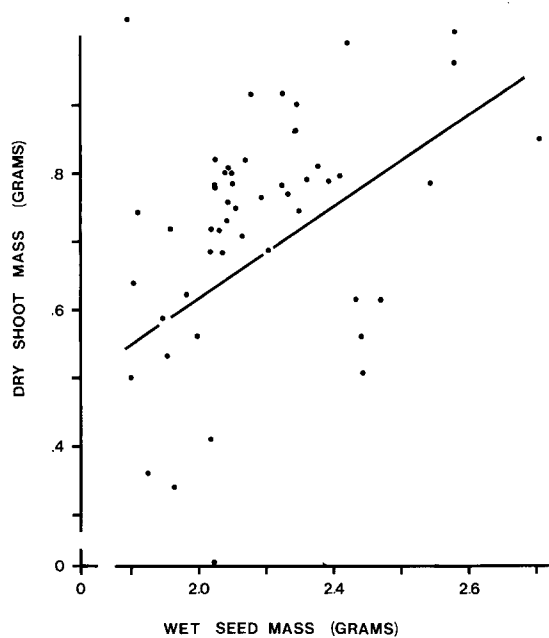


Fig. 2. Regression of shoot mass at 15 weeks against fresh mass of the seed, for seeds from one *Virola surinamensis* crop. The plot shows that minor differences in initial seed mass (1.8–2.8 g) may significantly influence seedling growth ($r^2=0.27$, $P<0.0001$). See Table 3

shaded conditions, significant relationships occurred in samples of 30 from three trees ($r^2=0.21$, 0.14, and 0.18, $P<0.01$, <0.05 , and <0.025 , respectively). Under lighted conditions, significant relationships occurred in samples of 30 from two trees ($r^2=0.16$ and 0.31, $P<0.05$ and <0.005 , respectively). Neither regression was significant at one tree ($r^2=0.02$, 0.01). The fourth sample under lighted conditions is that of 50, discussed above (Table 3). While most variation in seed size is between rather than within tree crops, even minor differences recorded within a sample of seeds from an individual tree are often sufficient to influence seedling size.

Effects of Seedling Proximity

Seedlings that grow under the parental crown or that germinate in monkey droppings are in close contact with each

other, and might be expected to compete for nutrients. The influence of proximity on seedling growth depends on initial seed size. Seeds differing by a gram or more produce dramatically different seedling sizes, as might be expected from seedlings that grow alone (Table 4). Most important, seeds of approximately average mass produce differential seedling growth when they differ by as little as 0.21 g, while differences of similar magnitude in the mass of relatively large seeds produce no effect at 15 weeks (Table 4).

It would be informative to know if seedlings grown in competition are smaller than those grown in isolation. The ideal analysis would be one of covariance of slopes of regressions of shoot characteristics on initial seed mass for samples from the same trees grown in pairs and in isolation. The scatter of the regressions precludes such an analysis here, but one comparison is instructive. One sample of seeds germinated alone averaged 2.74 ± 0.04 g ($n=30$), while the smaller seeds in the “mixed” treatment averaged 2.64 ± 0.06 g ($n=25$). These seed masses are similar ($t=1.25$), but the resulting shoot masses are not (0.89 ± 0.04 vs. 0.79 ± 0.03 g, respectively; one tail $t=1.82$, $P<0.05$). A similar comparison is possible for the heaviest seeds in the “large” and “mixed” treatments (Table 4). The heaviest seeds from the “mixed” treatment (4.16 ± 0.10 g) are smaller than the heaviest seeds from the “large” treatment (4.20 ± 0.06 g; $t=3.33$, $P<0.01$), but produce seedlings heavier than those from the “large” treatment (1.21 ± 0.05 vs. 1.07 ± 0.05 g; $t=2.00$, $P<0.05$). The first comparison suggests that seedlings do better growing alone than in close proximity with a conspecific, while the second comparison indicates that seedlings do better with a small than large competitor.

Discussion

Variation in seed mass within and between *Virola surinamensis* crops clearly influences seedling characteristics. Large seeds confer an early advantage to isolated seedlings under either lighted and shaded conditions, and some evidence indicates an advantage to shoots from large seeds that grow in close proximity with those from small seeds. These results are especially significant because dispersal agents differentially remove fruits from trees producing small seeds with high aril to seed ratios (Howe and Vande

Table 4. Seedling properties of seeds germinated in close proximity, as occurs in monkey droppings. Means are accompanied by standard errors. Comparisons of means are indicated by F-statistics

Treatment	<i>n</i>	Fresh seed mass (g)	Shoot height (mm)	Leaf length (mm)	Dry shoot mass (g)
Lightest	25	3.89 ± 0.07	13.69 ± 0.30	16.00 ± 0.54	0.96 ± 0.04
Large		F = 11.68 **	F = 1.06	F = 1.08	F = 3.03
Heaviest	25	4.20 ± 0.06	14.17 ± 0.29	16.70 ± 0.41	1.07 ± 0.05
Lightest	25	2.67 ± 0.03	13.27 ± 0.28	15.70 ± 0.23	0.84 ± 0.03
Small		F = 21.10 **	F = 9.86 **	F = 3.69	F = 13.90 **
Heaviest	25	2.88 ± 0.03	14.54 ± 0.29	16.24 ± 0.16	0.99 ± 0.02
Lightest	25	2.64 ± 0.06	13.14 ± 0.34	15.14 ± 0.34	0.79 ± 0.03
Mixed		F = 162.56 ***	F = 11.56 **	F = 9.23 *	F = 45.96 ***
Heaviest	25	4.16 ± 0.10	14.82 ± 0.36	17.00 ± 0.30	1.21 ± 0.05

* $P<0.01$ ** $P<0.001$ *** $P<0.0001$ (one-way analysis of variance)

Kerckhove 1980, 1981). High dispersability might offset an advantage to large seeds that produce particularly vigorous seedlings. The first issue is whether seed size is subject to natural selection, the second issue is the interpretation of possible adaptive significance of seed-size variation reported here.

Plant reproductive output may be influenced by either environmental or genetic factors. Crop size is generally susceptible to soil, water, and light conditions in the immediate vicinity of the plant (Salisbury 1942; Harper 1977). Aside from density effects on seed mass in some grasses (Rabinowitz 1979), seed size appears more stable in the face of environmental variation than other reproductive properties of plants (Harper et al. 1970; Harper 1977). Fruit characteristics in *Virola surinamensis* are not obviously associated with vine cover, leaf size, drainage, crop size, or trunk dimensions. Further, seed and aril weights are stable from year to year; most seed-size variation remains between rather than within plants. Environment probably has some effect on fruit characteristics, but genetic variation is at least as likely to underlie variation in fruit characteristics as in herbs and grasses (Harper 1977) or tropical trees of agricultural importance (Hulme 1971; Burley and Styles 1976), including the Old World Nutmeg (Weil 1965). It is reasonable to suggest that natural selection acts upon *Virola* seed variation.

Current understanding of the natural history of *Virola surinamensis* offers some insight into the ecological significance of seed-size variation (Howe and Vande Kerckhove 1981). Birds remove approximately two thirds of the crop of this tree, allowing 1,000 or more seeds to drop directly under the crown. Only 1–7 juveniles greater than a year in age occur under the crowns; as many occur within 5 meters of the crown edge, where seed fall is much lighter. This suggests at least a three to five fold advantage to very local dispersal beyond the crown edge. Other things being equal, large seed size should confer an advantage to seedlings growing in competition under the crown, in clumps left by monkey droppings, or singly throughout the forest. But all things are not equal, because frugivores consistently deplete small-seeded trees rather than large-seeded ones. Large-seeded plants have an advantage when seedlings must compete, but not when fruit-eating animals are common. The overall implication is that large-seeded individuals have a reproductive advantage in unusual years when frugivores are rare or absent (Foster in press), but that small-seeded plants have an advantage in dispersability when fruit-eating animals are present.

What selective interpretation can be put on the distribution of seed sizes in *Virola surinamensis*? The abundance of fruit-eating animals sometimes varies dramatically from year to year (Foster in press) and visitor loyalty varies from one part of the forest to another (Howe 1981), but some dispersal agents are present in most years at most places.

Extreme alternating selection is unlikely. Furthermore, the distribution of mean seed sizes is normal, not bimodal, indicating that strong disruptive selection is not in operation. The best explanation is that we have identified sources of selection on extremes of a seed-size distribution that is maintained by mild alternating selection on quantitative traits. What we see as intermediate seed sizes probably constitutes an evolutionary trade-off between dispersal and establishment capabilities that is adaptive for most plants in most years.

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