



## Effect of manure composition on seedling emergence and growth of two common shrub species of Southeast Alaska

Anna Traveset<sup>1,\*</sup>, Teresa Bermejo<sup>2</sup> and Mary Willson<sup>3</sup>

<sup>1</sup>Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Ctra. de Valldemossa Km. 7'5, 07071, Palma de Mallorca, Spain; <sup>2</sup>Universidad de Santiago de Compostela, Facultad de Biología, E-15706, Santiago de Compostela, Spain; <sup>3</sup>Forestry Sciences Laboratory, 2770 Sherwood Lane, 99801, Juneau, Alaska, USA;  
\*Author for correspondence (e-mail: [ieatv@ps.uib.es](mailto:ieatv@ps.uib.es))

Received 19 July 1999; accepted in revised form 20 March 2000

**Key words:** *Rubus spectabilis*, Temperate rainforest, *Ursus arctos*, *Vaccinium ovalifolium/alaskaense*, Vertebrate-seed dispersal

### Abstract

The idea that fecal material accompanying vertebrate-dispersed seeds at deposition sites plays an important role in enhancing seed germination and seedling survival has, surprisingly, little empirical support. The present study attempts to experimentally test this hypothesis. We examined the effect that manure composition from brown bears (*Ursus arctos*), important seed dispersers of *Rubus spectabilis* and *Vaccinium ovalifolium/alaskaense* in the temperate rainforests of Southeast Alaska, has on seedling emergence and growth of these two fleshy-fruited species in their natural habitat. The seeds of *Rubus spectabilis* showed a significantly higher germination rate in manure composed of animal material (mainly deer hair and bones) than in manure consisting of either fruit pulp or vegetation fiber and than in controls (potting soil with no manure added). The final number of germinated *Rubus* seeds was similar between the animal material and the fruit pulp treatments, perhaps due to similar water retention capacities, but was significantly higher than in the vegetation fiber treatment and the control. The germination patterns of *Vaccinium* seeds, in contrast, appeared to be unaffected by the composition of manure in which they were embedded. Seedlings of both species grew faster, and in the case of *Rubus* produced more leaves, when manure consisted of animal material. A principal constituent of bone is calcium phosphate, which may provide important minerals to growing plants. Manure containing vegetation fiber also enhanced seedling growth compared to manure with fruit pulp or the control. For *Rubus*, manure with fruit pulp did not affect seedling length significantly, although the number of leaves per seedling was greater in this treatment than in the control. We conclude that the influence of frugivores on the final fate of seeds of fleshy-fruited plants appears to depend not only upon commonly considered factors such as distance of dispersal, treatment in the digestive tract, and location of deposition, but also on what material the dispersed seeds are embedded in, i.e., on what other food frugivores have consumed along with the fruits.

### Introduction

Passage of the seeds of fleshy-fruited plants through vertebrates' guts has varying results on germination behavior (many studies, reviewed in Traveset (1998)). In addition, when a fruit-eating vertebrate passes seeds through its digestive tract, the seeds are deposited with the residues of whatever the consumer has been eating. The potential fertilizing effects of such

residues in vertebrate manure are often discussed among ecologists concerned with seed dispersal by vertebrates, but little experimental work has been done to test these ideas (Dinerstein and Wemmer 1988; Jones et al. 1991; Malo and Suárez 1995; Miller 1995). Many frugivores, especially in the temperate zone, eat a highly varied diet, with the result that the composition of their faeces varies greatly. Variation in composition is very evident in "carnivo-

rous" mammals that seasonally consume high volumes of fruits (Herrera 1989; Willson 1993). For example, the diet of bears includes vertebrates, vegetative plant parts (stems, leaves, roots), and fruits, and their faeces contain easily distinguishable residues of these dietary components.

The purpose of our study was to determine the consequences for seeds of deposition in bear manure of differing composition. The subject species are common shrubs of the understory of temperate rainforests of southeast Alaska that produce abundant fleshy fruits which are intensively consumed by bears (Traveset and Willson 1997). These species are salmonberry (*Rubus spectabilis*; Rosaceae) and blueberry (*Vaccinium ovalifolium* and *alaskaense*, Ericaceae, which are similar and sometimes synonymized; van der Kloet (1988)). The fruiting period of both *Rubus* and *Vaccinium* is summer, with the peak availability of mature fruits occurring in July. We initially included devil's club (*Oplopanax horridus*; Araliaceae) in the planting experiment, but germination was so poor (< 1%) that no analyzable results were obtained.

## Methods

Feces of brown bears (*Ursus arctos*), of differing composition, were collected on northeastern Chichagof Island (Alexander Archipelago, southeastern Alaska) during July, 1994. Brown bears are abundant on the island (Miller et al. 1997) and are the only species of bear there. A minimum of 10 fresh piles of bear faeces were collected from different sites on the island, placed individually in plastic bags, and taken to the laboratory in Juneau, where they were refrigerated until readied for use. We classified them according to their composition into three groups: (1) those containing mainly animal material, with abundant deer hair and bone, (2) those mainly composed of vegetation fiber, and (3) those in which fruit pulp (mostly of blueberries, as these were the most common fruits in the area at the time) was the dominant component. Manure with the same composition was mixed before using it at the time of seed planting. Seeds of any fleshy fruit present in manure containing animal material or vegetation fiber were removed prior to use with the assistance of a dissecting scope. The faeces with fruit pulp contained thousands of tiny *Vaccinium* seeds, which would have been very difficult to remove. For this reason, we tested the effect

of manure consisting of fruit pulp on seedling emergence and growth only for *Rubus* and *Oplopanax*. All seeds other than *Vaccinium* were extracted. *Vaccinium* seedlings that emerged were removed and not recorded.

All seeds to be planted were gathered from fresh bear feces. Care was taken to choose only viable seeds (intact and filled), discarding those that were apparently aborted or partly digested. For each species, three groups of 10 pots each were used for the different treatments: (1) animal material (ANIMAT, hereafter), (2) vegetation fiber (VEFI), and (3) *Vaccinium* pulp (VAPU). We first filled each pot (9 cm square) with potting (peat) soil, adding a layer, about 1 cm thick, of bear manure on the surface for each treatment. Seeds were placed just below such layer. Another group of 10 pots in which no manure was added served as control. In the first two treatments, 50 seeds of each species to be tested were planted in each pot, whereas in each of the 10 pots with *Vaccinium* pulp, we planted only 50 *Rubus* and 50 *Oplopanax* seeds. Therefore, a total of 2000 *Rubus* and *Oplopanax* seeds and a total of 1500 *Vaccinium* seeds were used for the experiment. The 110 pots were randomly assorted in two 91 × 61 cm trays, which were covered with a 1-cm mesh lid to prevent seed predation by rodents. The trays were in turn surrounded by a fence, to keep out deer and other possible animal disturbances, and placed in a sunny site near the forest edge, where these species usually grow. Seeds were planted on August 30, 1994.

Seedling emergence was first observed in May 2 of the following year, and was recorded weekly until August 3, 1995. Up to five seedlings of each species per pot were marked, by means of colored toothpicks, and the rest were removed. Seedlings were selected as much separated from each other as possible to avoid any possible effect of intraspecific competition within the pot. Growth of these seedlings (expressed in mm/day) was measured at the end of the experiment as the length from soil surface to the tip of the shoot obtained since day of emergence. For *Rubus*, the number of leaves per seedling (easy to count due to their size) was also recorded.

A repeated-measures analysis of variance (RMANOVA) was performed for each species with the general linear model (GLM) procedure in SAS Institute Inc. (1995) to examine differences in seedling emergence patterns among treatments. A one-way ANOVA tested for differences in seedling length among treatments; these data were normalized by us-

ing the logarithmic transformation. The Kruskal-Wallis test was used to examine differences in the number of leaves per seedling; this non-parametric test was used because data could not be normalized with any transformation. All means are accompanied by their standard errors.

## Results

### *The effect of manure composition on seedling emergence*

The patterns of emergence of *Rubus* seedlings were found to differ significantly among treatments ( $F_{3,36} = 5.18$   $P = 0.004$ ) in the RMANOVA (for the time effect: Wilks'lambda = 0.13,  $F = 15.98$   $P = 0.0001$ , and for the time  $\times$  treatment interaction: Wilks'lambda = 0.10,  $F = 2.70$   $P = 0.0002$ ). Both the ANIMAT and the VAPU treatments resulted in significantly higher emergences than the VEFI treatment and the control group throughout the test period (Tukey's test). Although emergence rate in ANIMAT tended to be greater than in VAPU, the difference was not significant (Figure 1). By the end of the experiment, the final number of emerged *Rubus* seedlings was significantly greater ( $F_{3,36} = 5.38$   $P = 0.004$ ) for the ANIMAT ( $X = 24.6 \pm 2.9$ , i.e. about 50% of the planted seeds) and VAPU treatments ( $X = 22.9 \pm 2.9$ ) than for the VEFI or control treatments ( $X = 14.4 \pm 1.8$  and  $X = 14.4 \pm 1.5$ , respectively) (Figure 1).

For *Vaccinium*, no significant differences among manure treatments ( $F_{2,27} = 0.27$   $P = 0.76$ ) were found in their effect on seedling emergence patterns (Wilk's lambda = 0.11,  $F = 16.86$   $P = 0.001$  for the time effect; time  $\times$  treatment interaction not significant). By August 3, 1995, the average number of emerged seedlings was indistinguishable among treatments, ranging from 16.1 to 20.4 (Figure 1).

### *Effect on seedling growth*

The growth of both *Rubus* and *Vaccinium* seedlings was significantly influenced by the manure treatment ( $F_{3,123} = 6.5$   $P = 0.0004$ , and  $F_{2,142} = 7.48$   $P = 0.0008$ , respectively, considering each seedling as an independent observation). For both species, the ANIMAT and the VEFI treatments enhanced seedling growth significantly more than controls (Figure 2). For *Rubus*, the effect of these two treatments was greater than that of VAPU, which did not differ significantly from con-

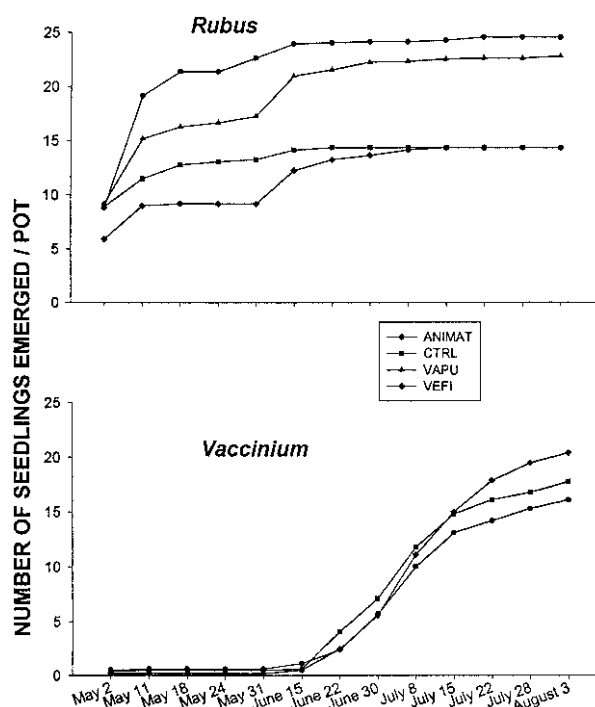


Figure 1. Cumulative seed germination during spring and summer of 1995 for the two species studied in different manure treatments. VEFI: manure consisting of vegetation fiber, VAPU: manure with pulp of *Vaccinium* berries, ANIMAT: manure with animal material, mainly hair and bones, and CTRL: potting soil (no manure added). Fifty seeds of each species had been planted per pot in August 1994.  $N = 10$  pots/treatment.

trols (Tukey's test). Performing the analyses on a per pot basis, the results did not change except for the difference (in this case not significant) between VEFI and control treatments of *Vaccinium*. An effect of density dependence within the pots was discarded, as all treatments (except the *Rubus* controls) had a similar number of seedlings growing per pot. The *Rubus* control pots had the lowest number of seedlings marked (because of the low seed germination) that survived to the end of the experiment, but it was precisely in these pots where seedling growth was lowest (contrary to the expected result if seedling competition within a pot was important). A density dependence effect might have indeed occurred had the experiment lasted for a much longer period.

The number of leaves per seedling in *Rubus* at the end of the experiment differed significantly among treatments ( $\chi^2 = 29.49$ ,  $P = 0.0001$ ). It was highest in the ANIMAT treatment, intermediate in VEFI and VAPU treatments, and lowest in the control group (Figure 3). The same result was obtained when the analysis was performed on a per pot basis.

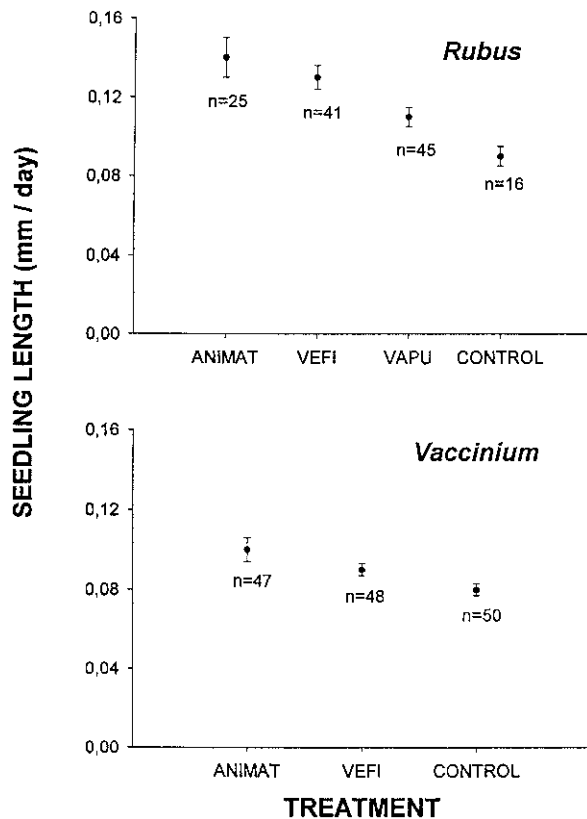


Figure 2. Mean growth rate of *Rubus* and *Vaccinium* seedlings in the different manure treatments. The number of seedlings measured (n) is given for each treatment. Bars represent the standard error of the mean.

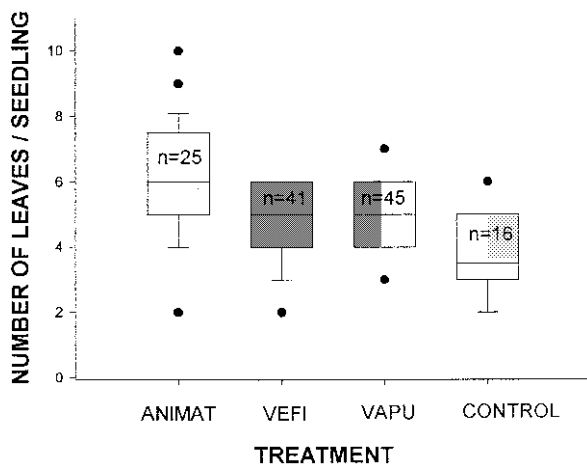


Figure 3. Number of leaves per seedling of *Rubus* at the end of the experiment for each different treatment. The median, 90<sup>th</sup>, 75<sup>th</sup>, 25<sup>th</sup> and 10<sup>th</sup> percentiles, outliers and error bars are shown. The number of seedlings measured (n) is given for each treatment.

## Discussion

Vertebrate frugivores appear to influence the probability of seedling emergence and growth not only by ingesting seeds and defecating them at particular microhabitats and distances from the mother plant, but also by the type of food they have consumed along with the seeds. The results of our experiments show that frugivores that deposit seeds with animal material may convey greater benefit to seeds and/or seedlings than those that have fed mostly on vegetation. Furthermore, even if manure consists only of plant material, the effect on seedling emergence and/or growth may depend upon whether it is composed of mainly fruit pulp or contains mostly vegetation fiber. At least for *Rubus*, fruit-pulp manure appears to enhance emergence more than vegetation fiber manure, while the latter enhances seedling growth more than the former.

One reason why seeds in manure with either animal material or fruit pulp show the highest emergence rates may be the water retention capacity, probably greater in those two treatments than in vegetation fiber or in potting soil. *Rubus* seeds, but not *Vaccinium* seeds, have been found to be greatly affected by passage through vertebrate frugivores' guts, either bears or birds, ingested seeds showing a greater emergence rate than control (un-ingested) seeds (Traveset and Willson 1997). One trait that may contribute to such difference in response to gut passage between the two species is seed coat thickness (Traveset and Willson 1997). The seed coat of *Rubus* is presumably more permeable to water after passing through bears and thus responds better to humidity than that of *Vaccinium* seeds.

Manure consisting of animal material had the greatest effect on seedling growth for both study species. While protein digestion represents a supply of nitrogen, bones provide a source of calcium phosphate which may be limiting growth in these rainforest soils (Willson et al. 1998); indeed bone meal is a commonly used garden fertilizer. All other manure treatments may supply some other nutrients or improve the moisture regime of the seedling, thus explaining the higher seedling growth in them compared to the control group (potting soil). These results are consistent with those of other studies. Dinerstein and Wemmer (1988) reported a significant increase in seedling growth of *Trewia nudiflora* when seeds were embedded in rhinoceros dung. Miller (1995) found that seedling growth of several *Acacia* species in

dung varied depending upon the species of herbivore that had deposited the dung, attributing this result also to differences in the nutrient content and in the water-holding capacity of such dung.

It is possible that toxic compounds present in the frugivores' manure affect seed and/or seedling survival, as it has been found in some studies (Welch 1985; Marambe et al. 1993; Malo and Suárez 1995). Some of our marked seedlings actually died after several days or weeks of growing normally, and this might well be due to such toxic compounds. Seeds of some Mediterranean pasture species have been found to be unable to colonize dung pats, and seedling growth is inhibited in cattle dung (Malo and Suárez 1995). Animal wastes are known to contain phenolic compounds and fatty acids which act as germination inhibitors for some plants, affecting, for instance, the activity of enzymes that regulate the rate of germination (Marambe et al. (1993) and references therein). Working with African acacias, Miller (1995) also found that seed germination in dung was significantly lower than in soil, although seedling growth did not differ between the two treatments.

The shrub species we studied are also dispersed by birds, mainly thrushes, in this region (Willson 1994; Traveset et al. 1995; Traveset and Willson 1997, 1998). Birds excrete relatively high amounts of white urates when eating invertebrates, but watery material often colored with fruit pigments when eating fruits. The variation in fecal composition in these dispersal agents may also affect seed and seedling ecology, and it would certainly be worthwhile to examine this in future studies.

The effect of gut passage on seed germination behavior of *Rubus* and *Vaccinium* is similar between bears and birds (Traveset and Willson 1997), but the effect on establishment success is probably quite different, for several reasons. The number and density of seeds in the faeces is much higher for bears, which is likely to affect seed and seedling survival (e.g. Barnea et al. (1992); Willson (1993) and unpublished data). The large number of seeds in bear dung attracts seed predators, mainly rodents (Bermejo et al. 1998; Willson 1993), and after germination, seedlings probably encounter intense competition. On the other hand, the rich and copious excrement of a carnivore such as a bear may provide more nutrients to a seedling, over a longer period of time, than the small scats of birds. Even if the period between bear defecation and seed germination can be several months, and the dung pile may have partly disaggregated when seeds

begin germinating (due to rain, rodent disturbance, natural decay, etc.) it is likely that nutrients leached from fecal material have enriched the ground beneath the initial deposition spot. It is not unusual, however, to see seedlings of different species, including the two studied here, emerging from bear dung piles that look intact. Whether the consistency of these piles differs significantly among habitats (within forest vs. open pastures, for instance), and the consequences for the seeds, is unknown, and it is a question that certainly deserves more attention.

In short, bears seem to play a double role (one direct and one indirect) in the plant-frugivore system. They transport seeds away from the parent plant, often to suitable sites for germination and growth, and they can also enhance emergence of seedlings and facilitate their establishment. Our experiments demonstrate that the degree of influence depends upon what other food they have consumed along with the fruits. Enhanced emergence and growth in these northern temperate forests may be especially advantageous, as seedlings that have attained a greater size before the cold winter comes presumably have greater chances of survival.

### Acknowledgements

We thank B. Cannon, M. Cannon, T. Comet, A. Rodríguez, and M. Stubbs for valuable assistance both in the field and the lab. Two anonymous reviewers made valuable comments on the manuscript. This study is framed within projects PB96-0860 and PB97-1174 financed by DGICYT.

### References

- Barnea A., Yom-Tov Y. and Friedman J. 1992. Effect of frugivorous birds on seed dispersal and germination of multi-seeded fruits. *Acta Oecologica*. 13: 209–219.
- Bermejo T., Traveset A. and Willson M.F. 1998. Post-dispersal seed predation in the temperate rainforest of Southeast Alaska. *Canadian Field Naturalist*. 112: 510–512.
- Dinerstein E. and Wemmer C.M. 1988. Fruits *Rhinoceros* eat: dispersal of *Trewia nudiflora* (Euphorbiaceae) in lowland Nepal. *Ecology*. 69: 1768–1774.
- Herrera C.M. 1989. Frugivory and seed dispersal by carnivorous mammals, and associated fruit characteristics, in undisturbed Mediterranean habitats. *Oikos*. 55: 250–262.

- Jones R.M., Noguchi M. and Bunch G.A. 1991. Levels of germinable seed in topsoil and cattle faeces in legume-grass and nitrogen fertilized pastures in South-East Queensland. *Australian Journal of Agricultural Research*. 42: 953–968.
- Malo J.E. and Suárez F. 1995. Establishment of pasture species on cattle dung: the role of endozoochorous seeds. *Journal of Vegetation Science*. 6: 169–174.
- Marambe B., Nagaoka T. and Ando T. 1993. Identification and biological activity of germination-inhibiting long-chain fatty acids in animal-waste composts. *Plant Cell Physiology*. 34: 605–612.
- Miller M.F. 1995. *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing by rodents. *African Journal of Ecology*. 33: 194–210.
- Miller S.D., White G.C., Sellers R.A., Reynolds H.V., Schoen J.W., Titus K. et al. 1997. Brown and black bear density estimation in Alaska using radiotelemetry and replicated mark-resight techniques. *Wildlife Monographs*. 133: 1–55.
1995. SAS/STAT Guide for Personal Computers. Version 6 Edition. SAS, Cary, NC.
- Traveset A. 1998. Effect of seed passage through vertebrate frugivores guts on germination: a review. *Perspectives in Plant Ecology and Evolution*. 1: 151–190.
- Traveset A., Willson M.F. and Gaither J.C. 1995. Avoidance by birds of insect-infested fruits of *Vaccinium ovalifolium*. *Oikos*. 73: 381–386.
- Traveset A. and Willson M.F. 1997. Effect of birds and bears on seed germination of fleshy-fruited plants in temperate rainforests of southeast Alaska. *Oikos*. 80: 89–95.
- Traveset A. and Willson M. 1998. Ecology of the fruit-color polymorphism in *Rubus spectabilis*. *Evolutionary Ecology*. 12: 331–345.
- van der Kloet S.P. 1988.: 1–201 The genus *Vaccinium* in North America. Research Branch, Agriculture Canada, Publication 1828.
- Welch D. 1985. Studies in the grazing on heather moorland in North-East Scotland. IV. Seed dispersal and plant establishment in dung. *Journal of Applied Ecology*. 22: 461–472.
- Willson M.F. 1993. Mammals as seed-dispersal mutualists in North America. *Oikos*. 67: 159–176.
- Willson M.F. 1994. Fruit choices by captive American robins. *Condor*. 96: 494–502.
- Willson M.F., Gende S.M. and Marston B.H. 1998. Fishes and the forest: expanding perspectives on fish/wildlife interactions. *Bioscience*. 48: 455–462.