Samara Dispersal in Boxelder

An Exercise in Hypothesis Testing

Peter V. Minorsky R. Paul Willing

Pedagogical surveys have shown that traditional approaches to teaching botany, with their emphasis on preserved specimens, prepared slides and slow-to-develop physiological experiments, tend to lead students to one conclusion—plants are boring (Uno 1994). Surveys have also shown that students have a strong preference for hands-on experiments which utilize living material and generate fast results. The incorporation of such experiments into botany or ecology exercises is especially difficult in the "dead" of winter in northern climes. We present here a fun, inexpensive and pedagogically useful laboratory exercise which involves indoor studies of the dispersal properties of the winged fruits (samaras) of boxelder (*Acer negundo* L.) trees. The exercise engages students in the processes of hypothesis testing, experimental design, and data analysis, as well as introducing them to some important concepts relating to functional ecology, succession and plant reproductive biology.

An attractive feature of the exercise presented is its flexibility. If scheduling and the abilities of the students permit, the exercise can easily be re-tailored to incorporate more independent inquiry on the part of the students; alternatively, it could be pared down to be a highly-regimented "cookbook-type" lab. We have adapted sort of a middle ground between these two approaches, so that the exercise does require independent inquiry by the students, but can also be completed in a single, three-hour lab period. In our particular case, a third of this time is devoted to a discussion of statistical analysis (*t*-tests), a step perhaps not necessary at the pre-college level and not further elaborated upon here. For those instructors who can devote more than one lab period to this exercise and wish to use it as the basis for independent projects by their students, we refer readers to an earlier How-To-Do-It publication in this journal (Thomson & Neal 1989) which presents additional pedagogical exercises pertaining to the wind dispersal of fruits and seeds, including the construction of artificial paper samaras, studies of the effects of partial dissections on the dispersal properties of tree-of-heaven (*Ailanthus*) samaras, and the collection in transects of spray-painted samaras dropped from a flagpole. The practical advantage of the present exercise is that it can be completed indoors in a single lab period in a typical classroom (no flagpoles, catwalks or balconies necessary).

Peter V. Minorsky is a Visiting Assistant Professor and **R. Paul Willing** is a Lab Coordinator in the Department of Biological Sciences at Union College, Schenectady, NY 12308-3103; e-mail: willingp@union.edu.

Boxelder Samaras

Samaras are winged fruits morphologically adapted for wind dispersal. The samaras of many species, including ashes (*Fraxinus* sp.), elms (*Ulmus* sp.) and maples (*Acer* sp.) spontaneously develop a spinning motion soon after being dropped. The functional significance of this "autorotation" is to produce lift and drag to counter the forces of gravity, thereby reducing the falling speed of the fruit and increasing the distance it may be transported by horizontal winds (Augspurger 1986). The longer a samara is airborne, the greater are its chances for settling in a gap in the forest canopy far from the shading limbs of the source plant. The selection pressure behind autorotation is thus directed toward improvement of the dispersal potential of the plant.

Boxelder is a dioecious, wind-pollinated, arborescent member of the maple family (Aceraceae), which has a wide distribution in the temperate regions of non-coastal North America. Although other samara-bearing species might conceivably be adapted for use in this exercise, boxelder has four advantages which might not be met by the samaras of other species:

1. Boxelder has a prolonged fruit dispersal season, typically from mid-autumn to early spring, which enables the samaras to be readily located and collected in undamaged form during the "dead" of winter.
2. The samaras are dry at maturity and may descend less quickly than the fleshy samaras of other species. This permits their descent to be measured with a stopwatch in a typical classroom.
3. Unlike most plants, boxelders retain their sterile fruits rather than aborting them, and these sterile samaras develop in parallel with the fertile samaras. Because of insect damage, spontaneous abortion and the relative inefficiency of wind pollination, a large and variable percentage (20 to 60% in our experience) of boxelder samaras are sterile. Thus, the dispersal properties of two naturally occurring samara subpopulations can be studied.
4. Boxelder samaras are of equal area regardless of whether they are sterile or fertile. Sterility in boxelder fruit is cryptic: it is not easy to distinguish between fertile and sterile samaras without a destructive dissection of the seed chamber. Since visible inspection cannot be used reliably to distinguish between fertile and sterile samaras, the dispersal experiments are essentially done "blind."

Samara collection may be done by the teacher alone or may be incorporated into an earlier exercise involving a nature walk, tree-keying exercise, or other outdoor activity. Boxelders are commonly found growing along riverbanks, or along the borders of moist fields or railroad embankments. Boxelder often serves as an invading pioneer species during the succession of old fields. During forest succession, boxelders usually succumb prior to the oak-hickory climax stage, probably as a result of too much shading. The samaras of boxelder are paired and about 2.5 to 3.5 cm in length, and occur on drooping racemes 15 to 25 cm long. The natural history of boxelder is well-summarized by Maeglin and Ohmann (1973). It is advisable to collect the samaras a few days before use and let them dry uniformly, especially in the early autumn when the moisture content of the naturally drying samaras is quite variable: fresh, green, fleshy samaras are not suitable. Also, because there can be considerable intraspecific variation in the morphological attributes of *Acer* samaras (Sipe & Linnerooth 1995), it is advisable to collect and pool the samaras from several different specimens.

Three Hypotheses

In this exercise, the students are asked to consider three different hypotheses concerning the respective dispersal of sterile and fertile boxelder samaras.

Hypothesis I is based on the assumption that it would be advantageous to have sterile fruits fall closer to the source plant. Because sterile fruits have zero reproductive potential, they are essentially a lost investment for the source plant. Some of this lost investment might conceivably be recouped if the sterile fruits contributed to the "leaf-litter" immediately beneath the source plants. Indeed, Otto and Nilsson (1981), in attempting to explain the winter-time retention of lower canopy leaves in beeches and oaks, proposed that the delayed shedding of the previous year's leaves may benefit the tree by providing the nearby ground, well-leached by winter's thaw, a fresh supply of soluble nutrients in the early spring. *Hypothesis I, therefore, predicts that the optimal reproductive strategy for species such as boxelder, would be to have fertile samaras land farther from the parental plants and to have sterile samaras fall closer.*

Hypothesis II is based on the notion that ability of boxelder seeds to colonize an old field is limited chiefly by post-dispersal seed predation. Studies of old field colonization have shown that the largest mortality of colonizing tree species is due to predation of seeds by rodents (Gill & Marks 1991). Conceivably, the prolonged dispersal of boxelder fruits over the course of winter may be an evolutionary adaptation for reducing seed predation by winter-foraging mice. The "cryptic sterility" of boxelder samaras may also lower their desirability as a primary food source and may act, therefore, as an effective adaptation for lowering post-dispersal seed predation. If so, then this strategy of "cryptic sterility" would be most effective if the post-dispersal mixing of sterile and fertile samaras were maximal. *Hypothesis II, therefore, predicts that sterile seeds should fall the same distance from the parent as do fertile samaras.*

Hypothesis III is based on the findings of researchers such as Green (1980) who have found that the rate of descent of samaras is highly correlated with the square root of the samara's wing-loading (samara mass divided by wing-surface area). For example, Augspurger and Hogan (1983) found that fruits of *Lonchocarpus* with 0, 1, 2 or 3 seeds had increasingly large values both for (wing-loading)$^{1/2}$ and for rate of descent in still air. Therefore, based on the reasonable assumption that fertile samaras weigh more than sterile ones, *Hypothesis III predicts that fertile samaras, bearing more wing load, should fall closer to the parent than do sterile samaras.*

After having the three hypotheses explained to them, the students are asked to break into groups of three and devise an experiment to decide between them. The students are also encouraged to make preliminary observations concerning the determination of the reproductive status of the samaras, as well as the fruits' general autorotary properties. It should be pointed out to them that samara descent is a two-step process consisting of a rapid, initial "free fall" of about 20 cm, followed by a much slower autorotating descent to the ground.

Experimental Design

Based on their training and inclinations, the students propose a multitude of diverse approaches, some more feasible and some more imaginative than others, to test the predictions of the three hypotheses.

One of the more common suggestions involves collecting the fallen fruits in transects surrounding specimens of boxelders, noting the distances between the site of collection and the presumed parent plants, and then determining the reproductive status of the fruit. Although this research strategy has the advantage of being most natural, questions arise over the uncertainty of assuming that the closest specimen is indeed the source of all the collected fruits and, whether the presumably more nutritious fertile fruits might not be subject to enhanced predation. Although both of these problems are surmountable, their resolution might require many months of outdoor study. It is more feasible to study the question indoors using collected fruits.

Another common proposal involves measuring the differences in the dispersal properties of fertile samaras before and after the seeds have been surgically removed. This approach, too, is discouraged because it is no trivial matter to remove the seed without damaging the samara, and, moreover, such manipulations might well alter the samaras' aerodynamic properties.

Students must also confront the problem of what parameter to measure. Many students propose measuring the horizontal distance a dropped samara travels from the plumbline. However, because of the vagaries and weakness of indoor air currents, this approach, too, is discouraged: the samaras when dropped in still air generally fall close to the plumbline. The simplest and most straightforward suggestion is to measure the time needed for the descent of dropped samaras in still air, and then to determine whether they are sterile or fertile.

A further complication arises because the descent of autorotating samaras has two stages, a rapid free fall and a slower terminal velocity. Because the terminal velocity of the samara is the best descriptor of autorotary descent, and because the very rapid free fall makes little contribution to the total time needed to descend, some authors (e.g. Thomson & Neal 1989) have recommended estimating the rate of descent by dividing height by time. The appropriateness of this simplifying assumption is the topic of one of the questions for discussion. Because there are two velocities involved in descent, however, we prefer to have our students record and present their data as time required to descend a given distance (2.4 m or 8 ft in our case).

Students are also confronted with problems associated with the statistical analysis of their data. One of the more common sources of error in data collection and analysis arises from the non-independence of data values (i.e. pseudoreplication). Students are often tempted to treat repeated measurements taken from the samara as independent values during statistical analysis. This error arises from the misconception that the aim of scientific observation or experiment is to obtain a large number of measurements rather than measurements from a large number of subjects. True replication of measurements, however, is necessary to guard against aberrant single values. We recommend that each group of students measure the descent time of 30 different samaras three times each and determine the average descent time for each of the 30 samaras.

We have found that students can get useful results by standing atop laboratory benches (much to their delight) and dropping samaras from a position parallel and adjacent to the ceiling. (For safety concerns, the samara droppers should wear suitable shoes while standing atop the table). A second student measures the time of descent with a stopwatch (0.01 second resolution), while a third records the data. To avoid mistakes and confusion, it is a good idea to have the students number their samaras with a fine-tipped, indelible marker prior to experimentation. Students should be reminded that each samara should be dropped and timed in a uniform manner to reduce experimental error. The exercise can also be expedited by supplying the students with a standard data sheet. The difference between the descent times of fertile and sterile samaras can be visualized graphically or, in advanced classes, analyzed statistically by means of a two-sample *t*-test (Table 1) as explained in any introductory statistics textbook (see e.g. Sokal & Rohlff 1995).

Another salient property that can be measured is samara mass (Table 1). Although usable measurements can be achieved with a typical laboratory pan balance with a resolution of 0.01 gm, we recommend using an analytical balance. Not too surprisingly, fertile samaras weigh more on average than do sterile samaras (Table 1). Plotting samara mass v. time needed to descend reveals a clear inverse relationship ($r = -0.66$) between the two: fertile samaras fall faster (see Figure 1.) The predictions of Hypotheses I and II are rejected, and the prediction of Hypothesis III is supported.

To calculate wing-loading, the surface area of the samaras must be determined. This parameter can be estimated by photocopying the samaras, and comparing the mass of the sama-

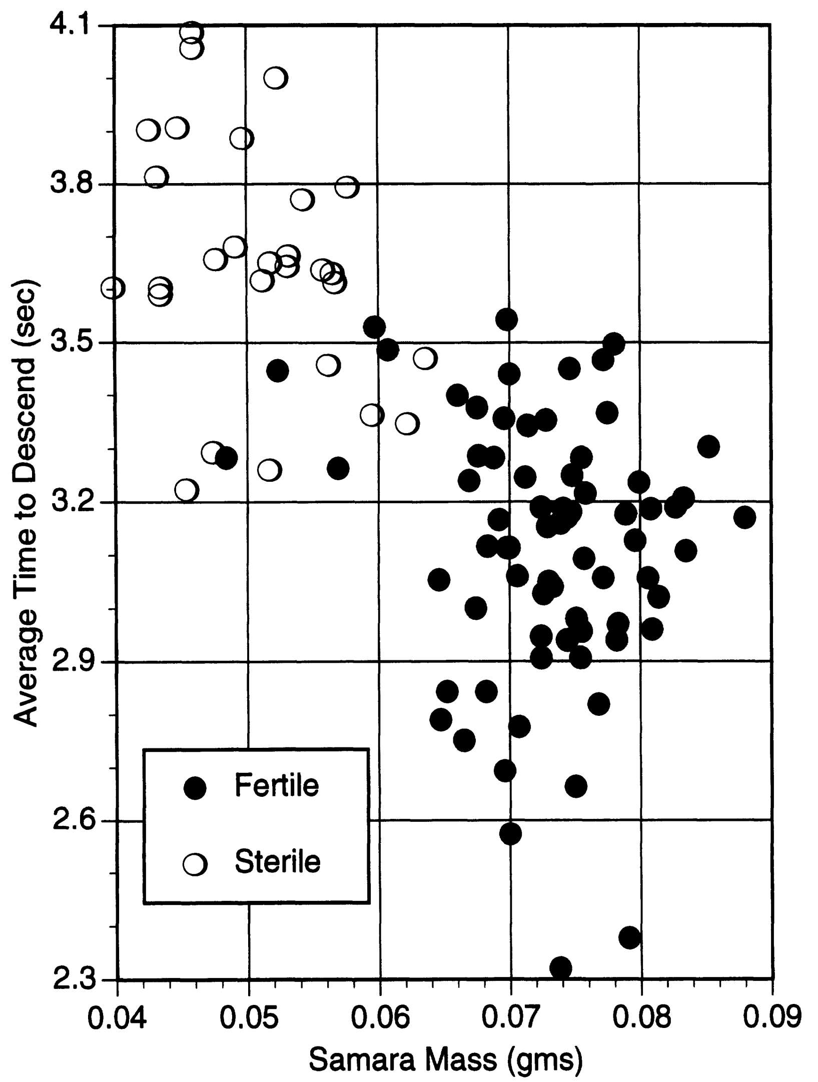

Figure 1. Relationship between boxelder samara mass and time needed to descend 2.4 m (n = 100). Note the clear separation between the sub-populations of fertile and sterile samaras.

Table 1. Summary of experimental results demonstrating that fertile boxelder samaras on average weighed more and descended a distance of 2.4 m more quickly than did sterile samaras. No significant difference was found between the average surface areas of fertile and sterile samaras.

	Number (n)	*Average Mass (gm) ± Standard Deviation*	*Average Surface Area (cm²) ± Standard Deviation*	*Average Descent Time (sec) ± Standard Deviation*
FERTILE	72	0.0727 ± 0.0070	3.27 ± 0.35	3.11 ± 0.25
STERILE	28	0.0508 ± 0.0062	3.24 ± 0.42	3.65 ± 0.23
TWO SAMPLE *T*-TEST		$t = 15.28$ significant; $P < 0.001$	$t = 0.89$ not significant; $P > 0.05$	$t = 19.29$ significant; $P < 0.001$

ras' individual photocopied images to the mass and area of the photocopied sheet. An analytical balance (0.0001 g resolution) must be used to measure the weight of the photocopied samaras. Cutting out the samara images and weighing them is tedious and also doubles the length of the exercise. Because of time constraints, it may be preferable to inform the students that previous researchers (this study) have determined no significant difference between the surface area of sterile and fertile boxelder samaras (see Table 1). When the $(\text{wing-loading})^{1/2}$ parameters for the data points presented in Figure 1 were plotted vs. time needed to descend, an even stronger correlation ($r = -0.75$) was determined (data not shown).

The experiment's support of Hypothesis III offers two important lessons. The first is that hypotheses, no matter how intuitively attractive they are, need to be objectively tested. The second is that there are limits to evolution. Even though boxelders might benefit if the dispersal strategies suggested by Hypotheses I or II were correct, this does not mean that boxelders have evolved or will evolve these strategies. There are morphological, developmental and physical constraints to evolution. Because of such constraints, birds have not evolved helium-filled sacs and cats have not evolved stainless steel claws. Similarly, because of aerodynamic constraints, boxelders have not evolved sterile fruits that fall closer to the source plant.

Questions for Discussion

1. In our experimental design, we ignored the fact that there are two components to the descent of samaras: a very rapid free fall and a slower autorotation (the terminal velocity). Propose an experiment to determine whether the differences between the descent of sterile and fertile samaras are due to differences in the length of the free fall or in the terminal velocity.
2. Guries and Nordheim (1984) have suggested that blade length is an important parameter in determining the length of a samara's free fall. Samaras with long blades require a longer initial free fall than do samaras with short blades. In what ways would you expect the samaras of a small maple species such as *Acer pennsylvanicum* (striped maple or moosewood) to differ from those of a tall species such as sugar maple (*Acer saccharum*) or red maple (*Acer rubrum*)?
3. Like many wind-pollinated trees in the temperate zone, boxelder forms flowers in the early spring before the leaves expand. What is a possible advantage of releasing pollen before the leaves expand? With this in mind, how might boxelders benefit aerodynamically by releasing their samaras during winter?
4. Can you think of any other common plants that use wind to disperse their seed or fruit? Do these seeds or fruits also autorotate?
5. If lighter seed masses cause slower rates of samara descent and greater seed dispersal, would individual boxelders that produce on average smaller seeds than normal be at a selective advantage? Why or why not?

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