How Fast and Why Do Germinating Guanacaste Seeds (*Enterolobium cyclocarpum*) Die Inside Cows and Horses?[1]

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ABSTRACT

Scarified and unscarified guanacaste seeds were subjected to *in vitro* fermentation using either rumen or caecal inoculum to test their germination response to the fermentation processes of cattle and horses. The fermentation was terminated at various times after inoculation, and the seeds planted under greenhouse conditions to evaluate viability. One day of exposure to either rumen or caecal *in vitro* fermentation killed 90 percent of the scarified seeds, and none survived longer than two days. No unscarified seeds germinated in the flasks. Scarification appears to be a critical process affecting the survivorship of seeds in the guts of large, mammalian herbivores. Because functional differences in the foregut between ruminants and nonruminants impose different chewing patterns and passage rates on these groups, we postulate that these factors are responsible for the lower proportion of scarified seeds in the feces of ruminants. In the large mammalian herbivores, nonruminants function as seed predators while ruminants are better disposal agents for guanacaste seeds.

Guanacaste (*Enterolobium cyclocarpum*: Leguminosae) is a large tree of the Pacific coastal lowlands of the drier portions of Central America (Janzen 1983). Its 10–15 cm diameter flat indehiscent fruits fall to the ground when ripe and are eaten by cattle and horses. These large mammals disperse viable guanacaste seeds in their dung (Janzen 1981a, b, 1982a, b, d, e, f, g), as the Pleistocene (and earlier) herbivorous megafauna of Central America (Janzen and Martin 1982) probably did. Each fruit contains 1–16 seeds (Janzen 1982c), each weighing 300–1100 mg; the seed coat comprises about 60 percent of this weight. When Costa Rican range cattle eat the fruits, most of the seeds survive the 1–8 day trip through the animal in a hard and dormant state (Janzen 1982a). When horses eat the fruits, as many as 70 percent of the seeds are killed by the digestive process, and dormant seeds may remain in the animal as long as 60 days (Janzen 1981b). Another caecal digestor Baird's tapir (*Tapirus bairdii*) likewise kills many of the guanacaste seeds it swallows (Janzen 1981c, 1982c).

The fresh dung of a horse that has been eating guanacaste fruits contains numerous fragments of seed coats and partly digested seeds. It also contains a few seeds that are viable, hard and dormant, and a very few newly germinated seeds. Nearly all of the latter are dead. These observations suggest that if a guanacaste seed can remain hard and dormant in a horse (or other large dispersal agent), it will survive the trip. On the other hand, if it responds to the moist and warm intestinal environment by germinating, it is likely to be killed during some portion of the digestive process. The results of the study reported here suggest that the seed that germinates or is susceptible to germination must leave the animal within a few hours if it is to survive.

As an initial inquiry into such an interaction, we ask the question of how long a scarified (and therefore newly-germinating) guanacaste seed can survive in the gut environment of a large herbivorous dispersal agent. Further, when it dies, what kills it? Replicated *in vitro* cow rumen and horse caecal digestive systems are available and well understood (Van Soest 1982). We used this *in vitro* system rather than put the seeds directly into the animals because of its availability and the greater control possible for the environment of the seed (Goering and Van Soest 1970). The cow rumen and horse caecum systems were used (rather than an intestinal system) because there is circumstantial evidence that large, hard seeds such as guanacaste seeds spend much of their transit time through the dispersal agent in the fermentation sites of the digestive tract (Janzen 1981b).

MATERIALS AND METHODS

Guanacaste seeds were collected from ripe, fallen fruits in Santa Rosa National Park, northwestern Guanacaste

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TABLE 1. *Survival to germination of scarified guanacaste seeds following treatment by simulated cow rumen and horse caecum environments. The number in parentheses is the number of seeds treated.*

Duration of treatment	Air + water	CO_2 + water	Nutrients + CO_2 + water	Inoculum + CO_2 + water + nutrients
Cow				
1 day	90% (40)	33% (40)		11% (200)
2 days	8% (40)	0% (40)		0% (200)
3–6 days	0% (160)	0% (160)		0% (800)
Horse				
1 day	80% (45)	41% (45)	4% (45)	9% (90)
2 days	2% (45)	2% (45)	1% (45)	0% (90)
3–4 days	0% (90)	0% (90)	0% (90)	0% (180)

Province, Costa Rica (May 1979). They were hard, mature, and dormant. In January 1982, several liters of these seeds were soaked in water for a week, and the approximately 18 percent that germinated were removed (it is at least these 18% that never survive the trip through a horse). The remaining seeds were still dormant (and would have remained dormant for months to years in water). Then a large sample of moistened seeds had their coats scarified by soaking them in concentrated sulphuric acid for 45 minutes, rinsing in running water, and drying immediately. Following this treatment, two subsamples of 75 seeds each all imbibed water within 6 hours and germinated between moist toweling within 48 hours at room temperature. Only full-sized and normal-appearing seeds were used in this germination control and in the experiments. Such guanacaste seeds normally display 99-plus percent viability if the seed coat is broken, the seed is moistened, and the environment is well aerated.

The *in vitro* system consisted of 168 Erlenmeyer flasks (125 ml) maintained at 40°C in a water bath and subjected to occasional shaking. Each flask received 10 scarified seeds and two unscarified seeds, 40 ml of water (48 flasks) or an equal volume of buffered nutrients, and rumen fluid (120 flasks; *cf.* Goering and Van Soest 1970). Half of the water-containing flasks and all the rumen-inoculated flasks were incubated under a positive CO_2 pressure to ensure anaerobic conditions similar to the rumen. After 1–6 days, four of the aerobic controls, four of the anaerobic controls, and 20 of the experimental flasks were removed. The guanacaste seeds were rinsed and immediately planted in moist soil at normal greenhouse temperatures.

The design of the caecal experiment was the same, except that 15 scarified seeds were placed in each flask, six instead of eight controls were removed each day, and the experiment ran for only 4 days. Further, the solutions in the experimental flasks were of two types, a solution of nutrients and water, or a buffered nutrient-water mix inoculated with horse caecal fluid. Each day three inoculum-free experimental flasks and six inoculated experimental flasks were removed, and their seeds planted.

The unscarified seeds were planted at the same time as the scarified ones. The greenhouse germination trials were terminated when the scarified seedling plants had pushed out of their seed coats, or the seed contents were obviously dead and rotting.

Rumen fluid was obtained from a fistulated cow and caecal fluid was mixed from two fistulated ponies. An anaerobic environment was maintained during transport and preparation of the inoculum (Goering and Van Soest 1970).

All experiments were conducted between 8 and 26 February 1982, using the facilities and animals of the Animal Science Department, Cornell University.

RESULTS

Only one day of warm anaerobic rumen-like conditions was needed to kill all but 11 percent of the scarified guanacaste seeds (Table 1). No scarified seeds survived in this environment for 2 or more days. Only scarified seeds in a wet and anaerobic environment had a significantly higher survivorship than in rumen fluid ($P < .001$, Fisher exact probability test) but a significantly lower survivorship than the 90 percent survival in a wet, aerobic environment ($P < .001$, Fisher exact probability test). However, only 8 percent of the scarified seeds survived the wet, aerobic environment for 2 days (none survived this long in any other cow environment), and none survived longer. No unscarified seed was caused to germinate by the various treatments.

The results obtained from the horse caecum-like conditions duplicated those from the cow rumen conditions, with respect to all treatments. However, the addition of nutrient solution to the anaerobic flasks, uninoculated with caecal fluid, proved to be as lethal to the seeds as the inoculation treatment. Again, no unscarified seed germinated by the various treatments.

DISCUSSION

A guanacaste seed that is sufficiently scarified to begin germination in a cow or horse has less than 24 hours to get out or it will be killed. A seed that is nicked by the teeth, has a thin or otherwise penetrable seed coat (such as those that germinate without scarification immediately after being removed from the fruit), or remains in the digestive tract long enough for chemical etching (scarification) of the seed coat is doomed. Such seeds are the source of the seed coats and partly digested seeds found in cow and horse dung when these animals have been feeding on guanacaste fruits. Being a "good" dispersal agent requires a balance between swallowing the seed in the first place (*e.g.*, Janzen 1981a) and not abrading or degrading the seed coat during transit to the point where the seed is susceptible to moisture as a germination cue. This balance should also be affected by the rate of seed passage; since horses pass seeds much more slowly than cows, the internal environment of horses should be proportionately more dangerous to seeds whose seed coats are susceptible to digestive processes.

Does a guanacaste seed have an equal chance of being scarified, and thereby likely killed through germination during its trip through a horse and cow? Ruminants and nonruminants digestive systems differ in ways that affect the communition and passage of food particles (Demment and Van Soest 1985). Ingested food is chewed and swallowed by both groups but for the ruminants food particles are regurgitated and rechewed (ruminated) to reduce particle size. Upon ingestion horses chew food only once, but more thoroughly initially, than do ruminants (Grenet *et al.* 1984). Ingested particles of high specific gravity and smaller size are more rapidly passed through ruminants (Welch 1982) and have a low probability of remaining in the dorsal rumen from which particles are regurgitated for rumination. Furthermore, horses chew the fruits thoroughly, spitting out the seeds (Janzen 1981a) while cattle do not spit out the seeds while ruminating (Janzen 1982a). Although the remainder of the trip through the gut is physically gentle, the greater retention time of seeds in the horse (Janzen 1981b) increases the probability of chemical scarification.

While the digestive process itself may be sufficiently intense to directly kill (digest) the germinating seed, it appears that the anaerobic environment alone (as mimicked by the CO_2 environment) is only marginally safer than the inoculated solution. Even aerobic water was a safe environment for only one day. Numerous planting experiments not reported here have made it clear that guanacaste seeds generally die if they germinate and remain under water or in very water-logged soil. It is likely that the rapidly metabolizing seedling has high oxygen requirements that cannot be met unless the seed is well-aerated. The few scarified guanacaste seeds that survived 1 or even 2 days in the anaerobic environment might well have been individuals that were slow to initiate germination and therefore were actually subject to the negative effects of the experimental environments for less time than the protocol suggests.

While none of the unscarified seeds germinated in the 6 days (cow) or 4 days (horse) of the experiment, there is indirect evidence that eventually the seed coats of some unscarified guanacaste seeds will be degraded by the digestive process if they remain in the animal long enough (as in the case with their tenure in the soil). For example, when a horse's dung is monitored for guanacaste seeds for many weeks after the horse has been fed guanacaste seeds, both hard and dormant seeds, and partly digested, newly germinated seeds appear in the dung (Janzen 1981b). The latter seeds must have been hard and dormant in the horse for weeks before finally germinating and being killed. Since a germinating seed changes its specificity gravity, it is even possible that it was the act of germination that caused such seed to leave its lodging and move on.

Seed contents are generally of very high nutrient value (Short and Epps 1976, Grodzinski and Sawicka-Kapusta 1970), even though some may also be quite toxic to specific animals. Newly germinated guanacaste seeds are highly edible to some rodents (Janzen 1982d), and it is likely that guanacaste seed contents would represent a net positive addition to the usual diets of large herbivores. Guanacaste seed contents were found to have a 91 percent true digestibility (*in vitro* determinations at 48 hours) and a 20.5 percent crude protein content. It is possible that the very slow passage rate of dense, hard seeds in large herbivore fodder such as guanacaste fruits is more than serendipitous. If the seed passage can be slowed to where marginally germinable seeds do germinate, then the animal may obtain a relatively high-quality diet in addition to the dispersal reward offered by the fruit. Such an argument also applies to small legume seeds consumed with foliage rather than through directed fruit consumption (Janzen 1984).

The grazing ruminants have evolved as consumers of forages and the retention of food particles is a function of their size and shape (Demment and Van Soest 1985). Forages enter the rumen as long, thin particles with low specific gravity and are reduced through time to small sizes of high specific gravity. The rumen system links physical characteristics to probability of passage (Smith 1968, Welch 1982) and therefore ensures that all forage particles spend long periods in the rumen before escape to the lower tract. When ingested, the short, dense seeds behave like forage particles which have been retained for a long period. They have a high probability of escape and show short transit times in cows (Janzen 1982a). However, transit times are very long in the horse. This difference occurs because no such well developed mechanism for sorting particles exists in the hind gut of equids (Demment and Van Soest 1985). In fact, seeds of high specific gravity appear to collect *in cecum* and are retained for long periods. Therefore, both in terms of scarification and retention times, ruminants appear to have gut functions less likely to kill seeds than do nonruminants.

The results of this study with artificial rumen and caecal conditions are consistent with the conclusion that the adaptive significance of large mammals to the guanacaste tree is in dispersing the seeds rather than in scarifying them. In nature, as in the flasks, seeds that are sufficiently scarified to germinate in the mammal do so, and are killed. Those that remain unscarified remain dormant and survive the trip. However, newly germinated guanacaste seeds may also be found in horse and cattle dung. Such seeds have usually exited in a hard and dormant state, but shortly after their seed coats are penetrated by moisture. While their seedlings are picturesque and seem to underscore the importance of the large mammal as a dispersal agent and scarifying agent of guanacaste seeds, other studies (*e.g.*, Janzen 1982d) show that such newly germinated seeds are likely to be killed by seed predators or to be poorly rooted. The seed with a reasonable chance of surviving is the seed that not only does not germinate inside the dispersal agent but is also not so scarified during passage that it germinates immediately in the dung. Rather, the surviving seeds are those that become incorporated into the litter as the dung is decomposed and at some later time have their dormancy broken by seed coat degradation in the soil.

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