J. Range Manage.
46:483-486, November 1993

Effects of cattle ingestion on viability and germination rate of caldén (*Prosopis caldenia*) seeds

RAUL PEINETTI, MARIA PEREYRA, ALICIA KIN, AND ALBERTO SOSA

Authors are assistant professor, Department of Ecology, Facultad de Agronomia-Universidad Nacional de La Pampa, cc 300 Santa Rosa 6300, Argentina.

Abstract

Caldén (*Prosopis caldenia* Burkart) is the dominant tree of the xerophytic open forest in the semiarid pampa of Argentina. Caldén has gradually increased its distribution throughout the region during the past century as a result of livestock grazing in the pampa forest. Caldén has an indehiscent legume fruit that is consumed by livestock during the February–April fruit shedding period (FSP). Both free seeds and pod segments (1 seed inside the endocarp) can be found in cattle dung. Free seeds and pod segments coming from fruits (uningested) and dung (excreted) were collected during the fruit shedding period on 22 February (D1), 7 March (D2) and 27 March (D3) to compare viability and germination rate. Viabilities of uningested free seeds and pod segments averaged 95% and 65%, respectively, and were unaffected ($P \leq 0.01$) by date of collection. Excreted free seed from D1 was 37% viable, which was lower ($P \leq 0.01$) than the 72% viable for D2 and D3. Excreted pod segments averaged only 10% viable seeds. Ingestion increased ($P \leq 0.01$) germination rate of free seed for D1 and D3. However, excreted seed displayed a range of delayed germination response. This diversity would increase the probability of seed germination for a variety of environmental and site conditions.

Key Words: seed dispersal, seed shadow, scarification

Seed dispersal and subsequent plant distribution of trees and shrubs is often expanded via frugivorus animals (Harper et al. 1970, Janzen 1983). Caldén (*Prosopis caldenia* Burkart) is 1 tree species whose distribution has increased in this manner following the introduction of domestic cattle (Koutche and Carmelich 1936, INTA 1980). Caldén is the dominant tree within the xerophytic open forest which covers a large area in the semiarid pampa of Argentina (Cabrera 1976, INTA 1980). Natural population of caldén and other Prosopis species gradually became transformed from savannas to woodland and even impenetrable shrubs. This change is often accompanied by a decrease in production and accessibility of more desirable forage plants (Fisher 1977).

Caldén fruits are valuable livestock forage (Menvielle and Hernandez 1985) during the fruit shedding period (FSP) (February–April) and seeds are frequently encountered in cattle dung. If wildlife or livestock do not eat them, caldén seeds remain below or very near a parental tree. Dispersers often move the seeds from open forest areas into other habitats.

Prosopis species have an indehiscent legume fruit that can split apart in segments (Kingsolver et al. 1977, Scifres and Brock 1972). Each pod segment is normally formed by 1 seed inside a bony endocarp. Both pod segments and free seeds can be propagules. Seeds remain dormant until the endocarp is broken, at least in the case of some *Prosopis* species (Solbrig and Cantino 1975, Hass et al. 1973). Scarification of caldén seeds increases germination (Peinetti et al. 1993), but only limited information exists on the germination requirements of caldén. This study evaluated the effect of light and dark conditions, scarification, and endocarp presence on caldén seed germination. We also analyzed the viability and germination rate of caldén seeds excreted by cattle in comparison with uningested ones at different times during the period of fruit consumption.

Authors thank N. Winzer and R. Camina for their statistical analysis assistance, C. Cabeza and E. Dussart for their critical review of a draft of the manuscript, and S. Suarez Cepeda, E. Cerqueira, and S. Tiranti for their English text correction.
Manuscript accepted 28 Apr. 1993.

Materials and Methods

Study 1: Germination Requirements

Caldén fruits were collected near Colonia Lagos (36°32'S, 64°30'W). The fruits were either split apart into pod segments or separated from the endocarp to obtain free seeds. Six treatments were imposed: free seeds, pod segments, and scarified seeds were each incubated under light or dark conditions. Twenty propagules of each type were placed in a petri dish and each treatment was replicated 10 times. All propagules were incubated for 7 days at 25° C. The optimal temperature for germination is not known, but germination did occur after 48 hours when scarified seeds were incubated at 25° C. Thereafter, the seeds contained within the pod segments were separated from the endocarp, scarified, and incubated for 3 days at 25° C to determine the percentage germination.

Study 2: Effects of Cattle Ingestion

Number of Propagules in Dung

The study was conducted at Colonia Lagos in 1990 within an area of 120 ha supporting 1 cattle/ha during the fruit shedding period (FSP) (3 months). The tree population characteristics were described by Peinetti et al. (1991). During the period of caldén fruit shedding, we collected from the soil both fruits and fresh dung (10 samples) via an aleatory design on 3 dates (D1, 22 February; D2, 7 March; D3, 27 March). Fruits were either split apart into pod segments or separated from the endocarp to obtain free seeds. The number of propagules present in dung was expressed on a dry weight basis. At the moment of gathering each fresh dung was weighed, and a subsample (100 g) was extracted, dried, and weighed, to estimate dung dry weight. Samples were kept at 5° C until processing. Free seeds and pod segments were filtered from the excrement through a 1-mm mesh sieve, and then were counted. Differences among sampling dates in the number of free seeds and pod segments present in dung were evaluated by 2-way ANOVA, and free seeds:pod segments ratios were compared separately by 1-way ANOVA.

Seed Viability

Viability of propagules coming from fruits and dung was compared by compositing the collections from each sampling date into the 4 following groups: (1) uningested free seeds, (2) uningested pod segments, (3) excreted free seeds, and (4) excreted pod segments. The percentage of seed viability was determined for each group. Scarified seeds were placed in water-saturated petri dishes for 24 hours. After imbibition the seeds were cut and treated with tetrazolium to determine their viability. Ten petri dishes with 30 propagules each were examined for treatment. Due to a lack of homogeneity of variance, comparisons between excreted and uningested propagules were made for free seed and pod segments separately. In the case of free seeds a 2-way ANOVA was applied. Analyses were executed on arcsine $x^{1/2}$ transformed data and mean comparisons were performed using Tukey's test. The Kruskal-Wallis test (Conover 1980) was used to compare seed viability between excreted and uningested pod segments.

Germination Rate

Germination rate of uningested free seed (UFS) and excreted free seed (EFS) lots for each sampling date were determined (UFS_{D1}, USF_{D2}, USF_{D3}, and EFS_{D1}, EFS_{D2}, EFS_{D3}). Three hundred free seeds were extracted from each lot and placed in 10 petri dishes. All samples were incubated for 4 months at 25° C. Germinated seeds were counted and removed every 24 to 48 hours.

The cumulative Weibull distribution function was fitted to the cumulative germination data for each petri dish. The Weibull function provides a very close description of a wide range of species germination rates, and its parameters allow a direct biological interpretation of different aspects of germination (Brown and Mayer 1988). The 4 parameters of the Weibull function reflect the maximum cumulative germination (M), the rate of germination (k), the lag in the start of germination (l) and the shape of the cumulative distribution (c) (Brown 1987, Brown and Mayer 1988). The lag parameter (l) was not used to compare the lots because the start of incubation in the excreted seeds could not be accurately determined. In these lots, lag was considered to be 3 days, since this approximates the interval between seed ingestion and excretion by cattle (Burton 1948). The maximum germination parameter (M) was not estimated with the Weibull distribution because it could be accurately determined by analyzing germination responses of the scarified ungerminated seeds at the end of the incubation period (4 months). In this way the Weibull distribution was fitted considering only the number of seeds that were able to germinate and not the total number of seeds placed in the petri dish. Thus, the function was:

$$F(t) = 1 - e^{-(t \cdot k)^c}$$

where $F_{(t)}$ is the cumulative germination at time t (days). The rate of germination increases as k increases (the time between the start of germination and when the cumulative germination reaches 63.21% is inversely proportional to k). The shape of the cumulative germination is a good approximation of the normal distribution, when c ranges between 3.25 and 3.61. At lower values of c, cumulative germination is positively skewed while with higher values it is negatively skewed (Brown 1987). Equations were fitted to cumulative germination data with nonlinear least-squares estimation based on the Levenberg-Marquart algorithm (Draper and Smith 1981). The Weibull parameters k and c were classified separately a priori according to 2 factors (range of parameters and seed lot treatments). In each case the Kruskal-Wallis nonparametric test was applied (Conover 1980). The ranges used were: 0–0.01; 0.01–0.06; 0.06–0.25; >0.25 for the k parameter and 0–0.33; 0.33–0.66; >0.66 for the c parameter. Weibull functions showed in Figure 1 correspond to cumulative germination of uningested and excreted free seed at different sample date. Each curve is a representation of the 10 samples of every lot. Every one was constructed considering the midpoint of k and c range that contained the highest number of samples.

Results

Study 1: Germination Requirements

Scarification increased germination percentages and ungerminated seeds did not achieve imbibition. Germination percentage of scarified seeds achieved 100% either in light or dark conditions. In contrast, a very low germination percentage was observed for nonscarified seeds (9% in light and 8% in dark conditions). Though 98% of seeds that had been inside a pod segment were able to germinate, no germination was observed when the pod segments were placed intact in the germination condition (7 days).

Study 2: Effects of Cattle Ingestion

Number of Propagules in Dung

A high number of propagules were found in cattle feces. The mean values and the coefficients of variation for each sampling date were: 211 (85), 654 (54), and 990 (51) free seeds per dung, whereas 322 (100), 262 (60), and 210 (57) pod segments were present per dung sample. The number of propagules expressed on a dry weight basis slightly decreased the coefficients of variation (Table 1). The interaction between number of propagules and date was significant ($p \leq 0.01$). At the end of the sampling period the number of free seeds increased, while the number of pod segments remained unchanged. Therefore the free seeds:pod segments ratio increased (Table 1).

Table 1. Number of free seeds (FS) and pod segments (PS) per dry weight (g) of cattle dung and free seeds:pod segments ratio (FS:PS) on different dates within the fruit shedding period.

Date	Propagules		FS:PS
	FS	PS	
	(no/g)		
22 Feb.	0.408 a[1](77)[2]	0.657 a (46)	0.848 a[3](30)
7 Mar.	1.547 b (68)	0.632 a (44)	2.808 b (36)
27 Mar.	1.986 b (69)	0.429 a (59)	4.844 c (33)

[1]Within propagules and a date, means followed by different letter are different ($P \leq 0.01$).
[2]Coefficient of variation in parentheses.
[3]Means within a column followed by different letter are different ($P \leq 0.01$).

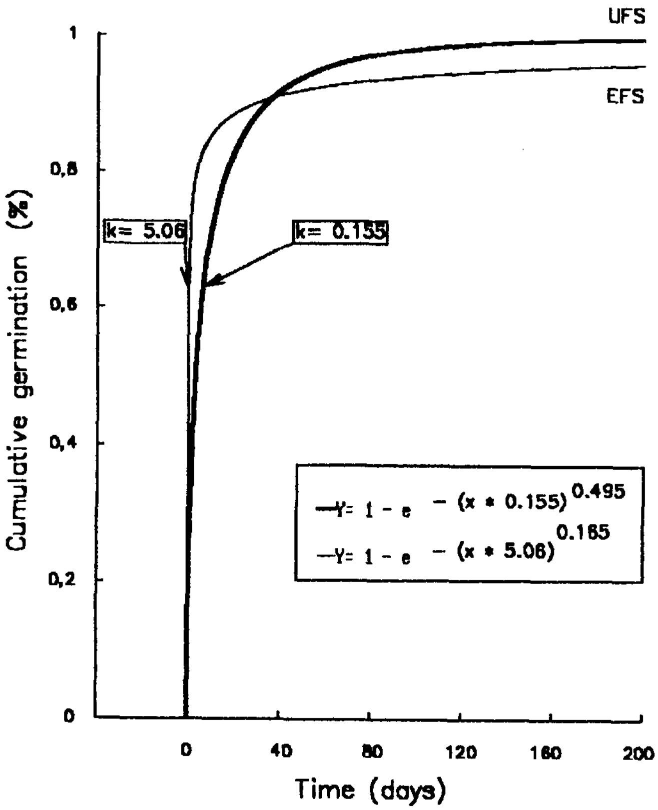
Seed Viability

Passage through the digestive tract of cattle reduced ($p \leq 0.01$) the percentage viability of both free and encapsulated seeds. This effect was more pronounced at the start of the fruit shedding period in the case of free seeds (Table 2). On the first sampling date we observed that some seeds were imbibed when gathered but most of these were not viable. A very low percentage of pod segments in dung contained viable seeds and this percentage did not change as

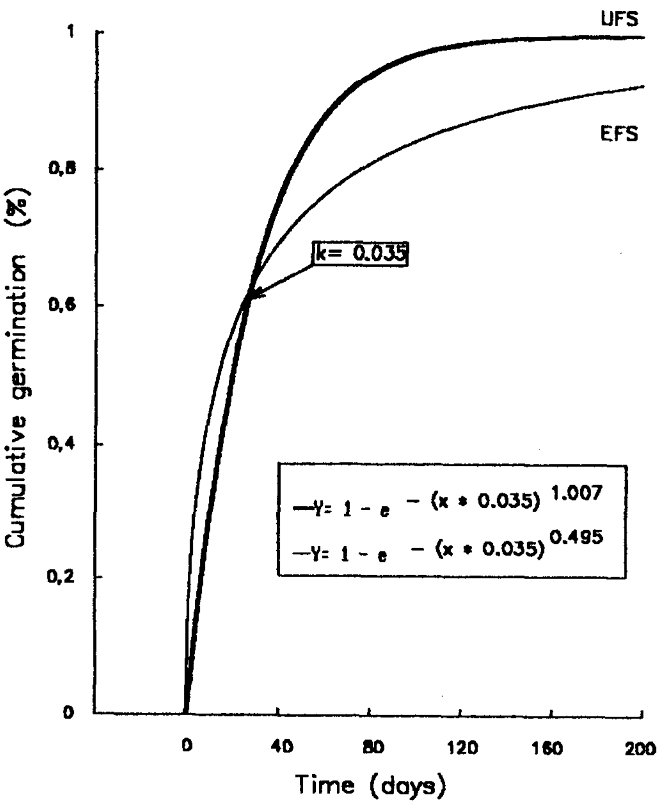
Table 2. Viability (%) of free seeds and pod segments sampled from uningested fruits and cattle dung (excreted) at different dates of Caldén's fruit shedding period.

Date	Free Seeds		Pod Segments	
	Uningested	Excreted	Uningested	Excreted
	(%)		(%)	
22 Feb.	94 a[1](5)[2]	37 c (17)	64 a[3](4)	8 b (40)
7 Mar.	97 a (5)	72 b (13)	66 a (7)	7 b (41)
27 Mar.	94 a (6)	73 b (16)	76 a (5)	16 b (22)

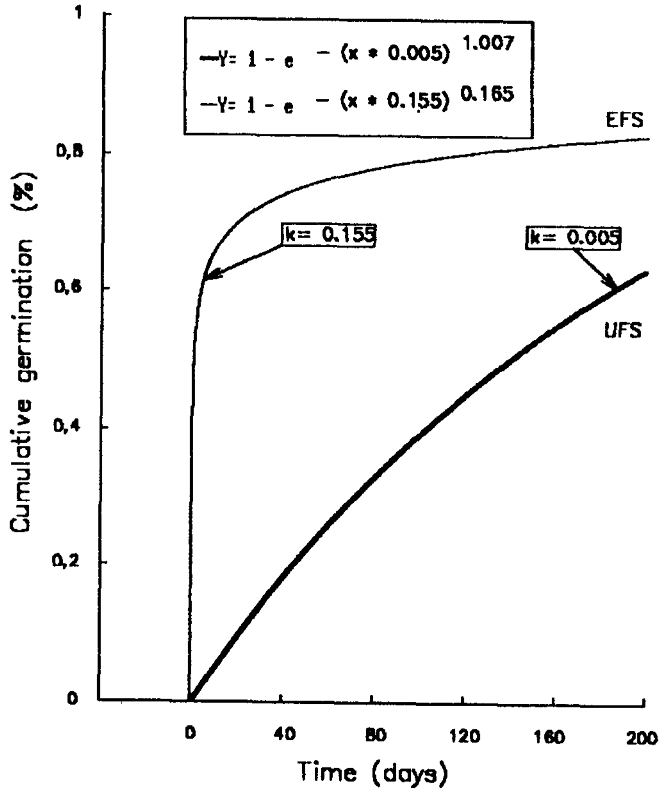
[1]Within free seeds and date, means followed by different letter are different ($P \leq 0.01$).
[2]Coefficient of variation in parentheses.
[3]Within pod segments and date, means followed by different letter are different ($P \leq 0.01$).



(a)



(b)



(c)

Fig. 1. Weibull equation fitted to the cumulative germination proportion at different sampling dates, where UFS = Uningested Free Seed. EFS = Excreted Free Seed, and k = rate of germination. Figures A, B, and C correspond to the 3 sampling dates, 22 Feb., 7 Mar., and 27 Mar., respectively.

the fruit shedding period progressed (Table 2).

Germination Rate

Weibull function allowed a good fitting to the cumulative germination data. In each sample the Marquat parameter was much lower than 120, and r^2 was significant. Germination rate (k) of uningested seeds decreased ($p \leq 0.01$) during the fruit shedding period (Fig. 1). Germination rates increased when the seeds passed through the intestinal tract at the first and third sampling date (Fig. 1a and c). For the second date the k values were not different from the control (kEFSD2 = kUFSD2 (Fig. 1b). The shape of the cumulative distribution (parameter c) shows similar responses across sampling dates (Fig. 1). However, cumulative germination at the start of incubation was higher in the uningested free seed UFSD1 lots than UFSD3 ($p \leq 0.01$). Nevertheless the effect of passage through the intestinal tract was present on all sampling dates $cUFSD_1 > cEFSD_1$; $cUFSD_2 > cEFSD_2$; $cUFSD_3 > cEFSD_3$ $P<0.01$). So the cumulative germination of excreted seeds was positively skewed in relation to unconsumed lots. In this way c shows a scarification effect in the second sampling date in spite of equal k values.

Discussion and Conclusions

Germination Requirements

As in *P. glandulosa* (Scifres and Brock 1972), caldén germination is not regulated by light. The seed coat delays germination in caldén because it interferes with water uptake. This is the overriding factor that cause dormancy and delays germination in most hard seeds of Leguminosae (Bewley and Black 1982). Caldén seed germination was inhibited when the seed was inside the seed pod. Consequently, if environmental factors are favorable, germination will depend on seed pod rupture and seed scarification by natural degradation or dispersal agents.

Effect of Cattle Ingestion

Some fruit characteristics, such as indehiscence, delayed maturation, dry season fruit drop, variable fruit size, variation in fruit seediness and hard seed, are considered adaptations for dispersal by animals (Janzen 1982). Caldén's fruit characteristics and its large number of viable seeds that remain in cattle feces indicate that cattle could be effective seed dispersers. However, though most seeds pass unharmed through the digestive tract, cattle may defecate them in inappropriate sites that restrict germination (Janzen 1981, Janzen 1983).

Caldén's seed coat is a barrier that prevents water flux entry, generally, water flux density equal to an appropriate force (the negative gradient of a suitable potential) divided by some resistance (Nobel 1983). Delayed germination of caldén seeds increases as the fruit shedding period advances. This is probably the result of increased coat resistance to water. Passage through the digestive tract enhances germination rate of the surviving caldén seeds. This scarification effect is generally accepted for the hard legume seeds; however, it has not been reported in other species (Janzen 1981).

Viability and enhanced germination rate effects by cattle consumption apparently depend upon seed coat resistance values. In this way both effects were higher at the start of the fruit shedding period as the seed coat resistance probably was lower than at the end of the fruit shedding period. Yet it is notable that in the middle of the fruit shedding period scarification effect was less pronounced than at the end. This indicates that other factors such as seed:fruit pulp ratio, tenacity to other fruit parts (e.g., fibers), or hard endocarp elasticity (Janzen 1983) likely affect the viability and germination rate.

Seed that is sufficiently scarified by a disperser to allow rapid germination usually either dies in the animal or in the dung. Yet, when seed is not consumed, the high degree of dormancy probably would not be adequate for plant dispersion.

The magnitude of these 2 responses is principally determined by when the fruit is consumed. It is surprising that even if passage through the digestive tract increases germination rate by scarification, the excreted seeds exhibit a wide range of delayed germination levels. This delayed germination diversity could increase the germination probability under varying site and environmental conditions.

Literature Cited

Bewley, J.D., and M. Black. 1982. Physiology and biochemistry of seeds in relation to germination. Tome I. Springer Verlag. Berlin Heidenberg, N.Y.

Brown, R.F. 1987. Germination of *Aristida armata* under constant and alternating temperatures and its analysis with the cumulative Weibull distribution as a model. Australian J. Bot. 35:581-591.

Brown, R.F., and D.G. Mayer. 1988. Representing cumulative germination. 2. The use of the Weibull function and other empirically derived curves. Ann. Bot. 61:127-138.

Burton, G.W. 1948. Recovery and viability of seeds of certain southern grasses and lespedeza passed through the bovine digestive tract. J. Agr. Res. 76:95-103.

Cabrera, A.L. 1976. Regiones fitogeográficas Argentinas. Tome II. Enciclopedia Argentina de Agricultura y Jardinería. ACME. Buenos Aires.

Conover, W.L. 1980. Practical nonparametric statistics. John Wiley and Sons, N.Y.

Draper, N., and H. Smith. 1981. Applied regression analysis. John Wiley and Sons, N.Y.

Fisher, C.E. 1977. Mesquite and modern man in southwestern North America. p. 177-188. *In:* B.B. Simpson (ed). Mesquite: its biology in two desert scrub ecosystems. Dowden, Hutchinson & Ross. Strousburg.

Harper, J.L., P.H. Lovell, and K.G. Moore. 1970. The shapes and sizes of seeds. Annu. Rev. Ecol. Sys. 1:327-356.

Hass, R.H., R.E. Meyer, C.J. Scifres, and J.H. Brock. 1973. Growth and development of Mesquite. Texas Agr. Exp. Sta. Res. Monogr. 1:10-19.

INTA. 1980. Inventario Inegrado de los Recursos Naturales de la provincia de La Pampa. ISAG. Buenos Aires.

Janzen, D.H. 1981. *Enterolobium cyclocarpum* seed passage rate and survival in horses, Costa Rica Pleistocene seed dispersal agents. Ecology 62:593-601.

Janzen, D.H. 1982. Variation in average seed size and fruit seediness in a fruit crop of a guanacaste tree (Leguminnosae *Enterolobium cyclocarpum*). Amer. J. Bot. 69:1169-1178.

Janzen, D.H. 1983. Dispersal of seeds by vertebrate guts. p. 233-262. *In:* D.J. Futuyma and M. Slatkin (eds). Coevolution. Sinaver Assoc. Sunderland, Mass.

Kingsolver, J.M., C.D. Johnson, R.S. Swier, and A.L. Teran. 1977. Prosopis fruits as a resource for invertebrates. p. 108-122. *In:* B. Simpson (ed). Mesquite: its biology in two desert scrub ecosystems. Dowden. Hutchinson & Ross. Strousburg.

Koutche, V., and J.N.F. Carmelich. 1936. Contribución al conocimiento de los bosques de la República Argentina: Estudio forestal del caldén. Tome 37. Boletín del Ministerio de Agricultura de la Nación.

Menvielle, E.E., and O.A. Hernandez. 1985. El valor nutritivo de las vainas de caldén (*Prosopis caldenia* Burkart). Revista Argentina de Producción Animal. 35:548-550.

Nobel, S.P. 1983. Biophysical plant physiology and ecology. Foreman Co., San Francisco.

Peinetti, R., O. Martinez, and O. Balboa. 1991. Intraspecific variability in vegetative and reproductive growth of a *Prosopis caldenia* population in Argentina. J. Arid Environ. 21:37-44.

Peinetti, R., C. Cabeza, M. Pereyra, and O. Martinez. 1993. Observaciones preliminares sobre la diseminación del calden *(Prosopis caldenia)* en la región semiárida de Argentina. Turrialba. In press.

Scifres, C.J., and J.H. Brock. 1972. Emergence of honey mesquite seedlings relative to planting depth and soil temperatures. J. Range Manage. 25:217-219.

Solbrig, O.T., and P.D. Cantino. 1975. Reproductive adaptations in Prosopis (Leguminosae Mimosoideae). J. Arnold Arboretum. 56:398-412.