

## Effects of *Aeschynomene histrix* Poir. seed treatment on germination

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**Description of the subject.** Poor germination associated with physical dormancy was experienced in the legume *Aeschynomene histrix* Poir. seeds and can reduce the establishment and growth of this species.

**Objectives.** To evaluate the effects of different pre-planting treatments, including digestion by Lagune cattle or other pre-planting treatments on the germinability of *A. histrix* seeds.

**Method.** The experiment was divided into three phases. Firstly, six Lagune cattle (three young bulls and three heifers) were fed individually with 1,000 seeds and these seeds were subsequently collected from faeces. Secondly, seed germination was compared among seeds defecated by cattle and seeds submitted to seven other pre-planting treatments: control (intact untreated seeds); seeds scarified using sandpaper; and seeds immersed in 80 °C-hot water for 2, 4, 6, 8, and 10 min. Thirdly, we also assessed the effect of crumbling cattle faeces on *A. histrix* germinability.

**Results.** The results show that Lagune cattle can disperse seeds of *A. histrix* with maximum recovery on the second day after ingestion. Of the number of seeds fed 13.42% were recovered. The germination percentage was greatest for sandpaper scarified seeds (96%) and seeds pre-heated during 2 min (86%), but least for digested seeds (4.27%). Breaking-down the dung doubled seedling emergence from digested seeds.

**Conclusions.** As it is desirable to break dormancy of *A. histrix* seeds, the use of mechanical scarification using sandpapering or hot water scarification 80 °C at 2 min may be more beneficial than cattle digestion.

**Keywords.** Scarification, endozoochory, thermal treatment, physical dormancy, Benin.

### Effets des traitements des semences d'*Aeschynomene histrix* Poir. sur la germination

**Description du sujet.** La levée et la croissance d'*Aeschynomene histrix* Poir. peut être réduite à cause d'une dormance tégumentaire.

**Objectifs.** Évaluer les effets du temps de rétention des graines dans le tube digestif des bovins Lagunaires ou d'autres traitements pré-semis sur l'aptitude germinative des graines d'*A. histrix*.

**Méthode.** Premièrement, trois jeunes taureaux et trois génisses ont reçu individuellement 1 000 graines qui ont été recherchées dans les excréments. Deuxièmement, la germination des graines excrétées par les bovins a été comparée à sept autres traitements pré-semis : contrôle (graines intactes non traitées) ; graines scarifiées au papier à scarifier et graines immergées dans de l'eau chaude (80 °C) pendant 2, 4, 6, 8 et 10 min. Troisièmement, nous avons comparé le nombre de plantules qui ont émergé de fèces intactes et émiettées.

**Résultats.** Les bovins Lagunaires peuvent potentiellement favoriser la dispersion des graines d'*A. histrix* avec une récupération maximale le deuxième jour après l'ingestion. Sur le nombre de graines ingérées, 13,42 % ont été récupérées. Le pourcentage de germination était le plus élevé pour les graines scarifiées au papier à scarifier (96 %) et pour les graines préchauffées pendant 2 min (86 %), et le moins élevé pour celles issues du tractus digestif (4,27 %). La décomposition des excréments a doublé la levée des plantules.

**Conclusions.** Pour la levée de dormance des graines d'*A. histrix*, l'utilisation d'une scarification mécanique à l'aide du papier à scarifier ou le trempage des graines dans de l'eau chaude pendant 2 min peuvent être plus bénéfiques que l'ingestion par les bovins.

**Mots-clés.** Scarification, endozoochorie, traitement thermique, dormance physique, Bénin.

## 1. INTRODUCTION

The legume (Fabaceae) *Aeschynomene histrix* (Poir.) is used for pasture improvement in Benin (Tarawali et al., 1988; Peters et al., 1994; Merkel et al., 2000; Adjolahoun, 2008; Houndjo et al., 2019a). However poor germination associated with physical dormancy was experienced in *A. histrix* seeds and can reduce the establishment of this species resulting in uneven establishment (Baskin & Baskin, 1998; Bhatt et al., 2008). Researchers have noted that the hard seeds of many legumes species can be scarified after passing through the digestive tract of cattle, increasing germination capacity and speed (Pakeman et al., 2002; Campos et al., 2008). As it is desirable to break dormancy of legume seeds and contribute to the rehabilitation of degraded grassland in Benin, biological methods such as ingestion by Lagune cattle, mechanical scarification, or hot water treatment, may be of value (Ocumpaugh & Swakon, 1993; Rusdy, 2015; Houndjo et al., 2019b). Our study focused on four main questions:

- are Lagune cattle acting as dispersal vectors for *A. histrix*?
- how does seed retention time in the digestive tract affect seed germination?
- what is the efficiency of treatments with hot water and mechanical scarification on the germination of untreated seeds, compared to cattle digestion?
- how does breaking down the dung affect the seedling emergence of digested seeds?

## 2. MATERIALS AND METHODS

### 2.1. Seed material

The investigation involved three complementary activities and morphological characteristics of *A. histrix* seeds used are shown in **table 1**.

### 2.2. Seeds retrieved after cattle digestion

The seed-feeding experiment took place during two periods (from January 2019 to April 2019 and from December 2019 to March 2020). Six cattle (*Bos taurus*),

namely three young bulls ( $114.23 \pm 5.26$  kg) and three heifers ( $95.37 \pm 4.65$  kg), of Lagune cattle breed were offered 1,000 seeds each of *A. histrix* and the percentages of seeds recovered were determined using a method similar to Houndjo et al. (2019b).

All dung produced by each cattle were collected at the end of each 24-hour period in plastic trays for six days, *i.e.*, at 24, 48, 72, 96, 120 and 144 h after consuming the seeds. Total fresh dung of each animal were mixed and three, 200 g aliquots were sampled by collection time. One aliquot was retained for dry matter (DM) determination, one for seed recovery estimates of *A. histrix*, and another for germination assays. Two hundred grams faecal subsample for seed recovery estimates were immediately washed with running water through a series of two stacked sieves with decreasing apertures of 1 mm and 0.25 mm (Jones & Bunch, 1977). The residuals were dried for approximately 24 h at 28 °C in a forced air oven to remove larger particles of digesta and undamaged seeds were separated and counted (Doucette et al., 2001). The average number of seeds retrieved from daily subsamples was extrapolated to the total daily dung amount per animal.

### 2.3. Seed germination

**Germination testing.** Experiments were performed in the Laboratory of Agrostology and Innovation of the University of Abomey-Calavi. Three replicates of a split-plot experiment were used with retention time (24, 48, 72 and 96 h) as the main plots in a randomized complete block design (RCBD), sex (male and female) as the subplots combined to give a total of eight treatments with 24 plots. Soil of the experimental site was collected and sieved and for each treatment and for each replicate, all seeds intact recovered per cattle (40.96 seeds) were placed on the surface of 150 mm diameter pots filled with sieved, sterilized sand and kept moist. The pots were kept in a shade house at ambient temperature (in the range of about 20–29°C) and the number of seeds that germinated was recorded daily until 20 days. The germination of seeds retrieved from cattle dung was compared to the germination of seeds of seven other pre-planting seed treatments: (1) control (intact untreated seeds); (2) sandpaper (intact seeds plus mechanical scarification using sandpaper); and intact seeds were immersed in hot water (80 °C) for 2 (3), 4 (4), 6 (5), 8 (6), and 10 min (7). One hundred seeds were submitted for each pre-planting seed treatment and for each replicate (five replications per treatment), 20 seeds were placed on the surface of 150 mm diameter pots filled with sieved, sterilized sand and kept moist and the germination test was conducted using the standard procedure (ISTA, 1999). Seeds were considered germinated after root emergence (ISTA, 1999). Germinated seeds (germinable seeds) were counted and removed. At the end of the test seeds that

**Table 1.** Pods, seeds, and shells weight of *Aeschynomene histrix* used in the study — *Poids des gousses, graines et enveloppes d'Aeschynomene histrix utilisées dans l'étude.*

Parameter	Average	Range
1,000 pods weight (g)	1.84	1.65 - 1.99
1,000 seeds weight (g)	1.27	1.17 - 1.36
1,000 shells weight (g)	0.56	0.48 - 0.63

Source: Houndjo et al., 2019.

had not germinated were categorized as hard or rotten seeds through examination by touching and piercing with a needle. Hard seeds could not be pierced with the needle (Hassen et al., 2004). Germination percentage (GP), germination speed (GS) and mean germination time (MGT) were also determined using the standard procedure mentioned by Houndjo et al. (2019b).

**Seedling emergence from dung.** In the third part the number of *A. histrix* seedlings emerged from intact (20.48 seeds tested per cattle) and crumbled faeces (20.48 seeds tested per cattle) was assessed using a method similar to Houndjo et al. (2019b). Three replicates of a split-plot experiment were used with retention time (24, 48, 72 and 96 h) as the main plot in a randomized complete block design (RCBD), sex (male and female) as the subplot, dung form (intact and crumbled faeces) as the sub subplot combined to give a total of 16 treatments with 48 plots.

The germinable seeds, hard seeds and rotten seeds recovered were submitted, after arcsine transformation, to analysis of variance using Proc GLM of SAS (SAS, 2015) and all data were checked for normality. When Fisher's F values were significant at  $p < 0.05$ , the analysis was continued using Tukey's test at the threshold of  $p < 0.05$  by comparing the means of the germination of seeds retrieved from cattle dung with the germination of seeds of seven other pre-planting seed treatments.

### 3. RESULTS

#### 3.1. Faeces recovery and number of seeds recovered from cattle

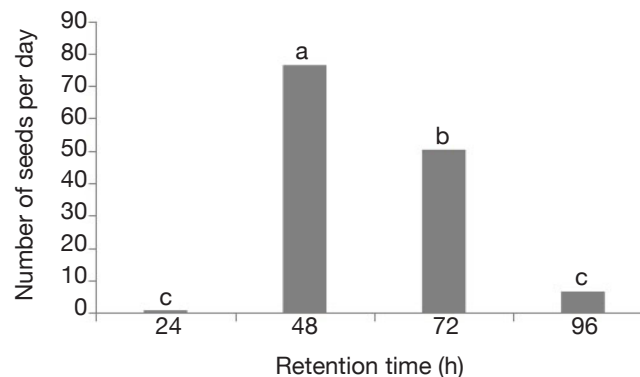
Of the number of *A. histrix* seeds fed,  $13.42 \pm 3.91\%$  were recovered (**Figure 1**), and most seeds were recovered after 2 to 3 days.

#### 3.2. Seed germination after ingestion by cattle

Percentage of germinated seeds of *A. histrix* from the seeds recovered after passage through digestive tract of cattle significantly decreased, as the retention time increased from 48 h (9.16%) to 96 h (1.25%) (Tukey test,  $p < 0.05$ ) (**Table 2**). Seeds recovered at 48 h after ingestion exhibited a significantly low level of germinable seeds (48.51 seeds), and rotten seeds (88.78 seeds) and a high level of hard seeds (336.50 seeds) (Tukey test,  $p < 0.05$ ) (**Table 3**).

#### 3.3. Effects of pre-planting seed treatment on germination parameters

Compared to control seeds ( $26 \pm 4.18\%$ ), the germination percentage was greatest for seeds from mechanical scarification with sandpaper (96%) and



**Figure 1.** Number of seeds per head recovered from cattle faeces — *Nombre de graines par tête récupérées dans les fèces de bovins.*

Different letters indicate significant differences among number of seeds per head recovered from cattle faeces (Tukey test,  $p < 0.05$ ) — *des lettres différentes indiquent des différences significatives dans le nombre de graines par tête récupérées dans les fèces de bovins (test de Tukey,  $p < 0,05$ ).*

**Table 2.** Characteristics of seeds germination after passage through digestive tract of cattle and faeces (dry matter per cattle per day) — *Caractéristiques de la germination des graines après passage dans le tube digestif des bovins et des fèces (matière sèche par bovin par jour).*

Retention time (h)	Percentage of germinated seeds (%)	Mean germination time (day)	Germination speed (seed/day)	Faeces dry matter (g)
24 h	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	227.09 <sup>a</sup>
48 h	9.16 <sup>a</sup>	4.30 <sup>a</sup>	0.48 <sup>a</sup>	172.17 <sup>a</sup>
72 h	6.66 <sup>a</sup>	4.95 <sup>a</sup>	0.36 <sup>a</sup>	224.67 <sup>a</sup>
96 h	1.25 <sup>b</sup>	0.79 <sup>b</sup>	0.05 <sup>b</sup>	256.61 <sup>a</sup>
Overall mean	4.27	2.51	0.226	220.13

For the same column, means followed by the same lower letter are not significantly different (Tukey test,  $p < 0.05$ ) — *Pour la même colonne, les moyennes suivies de la même lettre en minuscule ne sont pas significativement différentes (test de Tukey,  $p < 0,05$ ).*

**Table 3.** Distribution of germinating, hard and rotten seeds after excreted seeds in 1,000 g dry matter faeces per day — *Distribution des graines germantes, dures et pourries après excrétion des graines dans 1 000 g de matières fécales par jour.*

Retention time (h)	Excreted seeds in 1,000 g DM	Germinable seeds	Hard seeds	Rotten seeds
24 h	2.70 <sup>c</sup>	0.00 <sup>b</sup>	0.9 <sup>b</sup>	1.84 <sup>b</sup>
48 h	473.80 <sup>a</sup>	48.51 <sup>a</sup>	336.50 <sup>a</sup>	88.78 <sup>a</sup>
72 h	278.00 <sup>b</sup>	17.48 <sup>b</sup>	240.70 <sup>a</sup>	19.83 <sup>b</sup>
96 h	25.50 <sup>c</sup>	0.76 <sup>b</sup>	23.30 <sup>b</sup>	1.44 <sup>b</sup>
Overall mean	195.01	16.68	150.34	27.97

For the same column, means followed by the same lower letter are not significantly different (Tukey test,  $p < 0.05$ ) — *pour la même colonne, les moyennes suivies de la même lettre en minuscule ne sont pas significativement différentes (test de Tukey,  $p < 0,05$ )*. 0: in these time periods, no seeds were recovered — *dans ces périodes, aucune graine n'a été récupérée.*

2 min heat treatment (86%), and least for seeds from passage through the digestive system of cattle (4.27%) (Tukey test,  $p < 0.05$ ) (Figure 2, Tables 2 and 4). Again germination speed of seeds immersed in hot water for 2 min is the highest 8.32 seeds/day, and lowest for seeds recovered from the faeces (0.23 seeds/day) (Tables 2 and 4). The fastest (3.03 day) mean germination time was recorded in the seeds immersed in hot water for 2 min and the slowest (5.12 day) was recorded in the control (Tables 2 and 4).

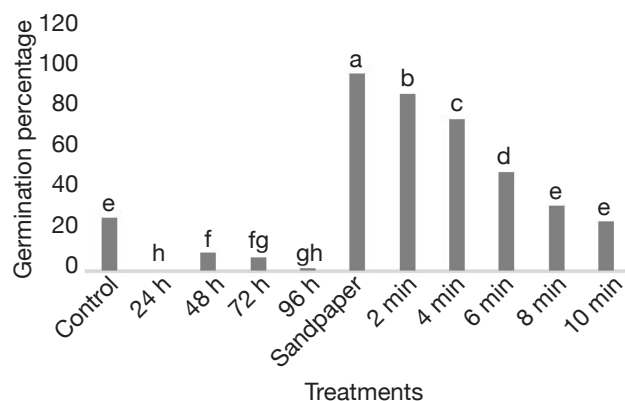
### 3.4. Establishment in trays of *A. histrix* seedlings from faeces

The number of seedlings emerging out of crumbled faeces (3.52 plants per cattle) was significantly higher ( $p < 0.05$ ) compared to seedling emergence out of intact faeces (1.35 plants per cattle) but remained very low.

## 4. DISCUSSION

A small percentage of ingested seeds of *A. histrix* passes through the Lagune cattle gastro-intestinal tract. The percentage of *A. histrix* seeds retrieved (13.42%) from cattle dung is similar to those obtained for other herbaceous and shrub species eaten by cattle (6.2-80%) (Gardener et al., 1993; Houndjo et al., 2019a). Therefore it is suggested that Lagune cattle are potential vectors of *A. histrix*. However cattle digested considerably higher amounts (86.58%) of *A. histrix* seeds. In practice (animal nutrition), legume seeds have long been considered as a source of high quality protein for grazing ruminants. Percentage of germinated seeds of *A. histrix* significantly decreased, as the retention time increased from 48 h (9.16%) to 96 h (1.25%). These results corroborated the findings of Rahimi et al. (2016), who reported that the viability of excreted

seeds declines with the length of time seeds spend in the digestive tract. However consumption of seeds by cattle is not effective for increasing the germinability of *A. histrix* seeds, as we estimate that 4.27% (5.73 seeds) of *A. histrix* seeds would still be potentially able to germinate from the total recovered seeds (134.2 seeds). Seeds recovered at 48 h after ingestion exhibited a



**Figure 2.** Germination percentage of treated *Aeschynomene histrix* seeds — *Pourcentage de germination des graines d'Aeschynomene histrix traitées.*

Control: intact untreated seeds; seeds recovered in the cattle trial with their retention time (24, 48, 72 and 96 h); sandpaper: mechanical scarification using sandpaper; seeds were immersed in hot water (80 °C) for 2, 4, 6, 8, and 10 min — *contrôle : graines intactes non traitées ; les graines récupérées dans l'essai sur les bovins avec leur temps de rétention (24, 48, 72 et 96 h) ; papier à scarifier : scarification mécanique à l'aide du papier à scarifier ; les graines ont été immergées dans de l'eau chaude (80 °C) pendant 2, 4, 6, 8 et 10 min* ; different letters indicate significant differences among germination percentage of treated *A. histrix* seeds (Tukey test,  $p < 0.05$ ) — *des lettres différentes indiquent des différences significatives entre les pourcentages de germination des graines d'A. histrix traitées (test de Tukey,  $p < 0,05$ ).*



**Table 4.** Characteristics of seeds germination for the various other pre-planting seed treatments — *Caractéristiques de la germination des semences pour les différents autres traitements pré-semis.*

Treatments	Germinable seeds (%)	Hard seeds (%)	Rotten seeds (%)	Mean germination time (day)	Germination speed (seed/day)
Control	26.00 <sup>e</sup>	54.00 <sup>a</sup>	20.00 <sup>d</sup>	5.12 <sup>a</sup>	1.19 <sup>d</sup>
Sandpaper	96.00 <sup>a</sup>	0.00 <sup>c</sup>	4.00 <sup>e</sup>	3.24 <sup>b</sup>	6.26 <sup>ab</sup>
2 min	86.00 <sup>b</sup>	8.60 <sup>b</sup>	5.40 <sup>e</sup>	3.03 <sup>b</sup>	8.32 <sup>a</sup>
4 min	74.00 <sup>c</sup>	9.80 <sup>b</sup>	16.20 <sup>de</sup>	3.80 <sup>ab</sup>	4.90 <sup>bc</sup>
6 min	48.00 <sup>d</sup>	10.00 <sup>b</sup>	42.00 <sup>c</sup>	4.14 <sup>ab</sup>	3.07 <sup>cd</sup>
8 min	32.00 <sup>e</sup>	12.00 <sup>b</sup>	56.00 <sup>b</sup>	3.99 <sup>ab</sup>	2.03 <sup>d</sup>
10 min	24.00 <sup>e</sup>	8.00 <sup>b</sup>	68.00 <sup>a</sup>	3.97 <sup>ab</sup>	1.54 <sup>d</sup>
Overall mean	55.14	14.62	30.22	3.90	3.90

For the same column, means followed by the same lower letter are not significantly different (Tukey test,  $p < 0.05$ ) — *pour la même colonne, les moyennes suivies de la même lettre en minuscule ne sont pas significativement différentes (test de Tukey,  $p < 0,05$ ).*

significantly high degree of hard seeds (336.50 seeds) and a low level of germinable seeds (48.51 seeds), and rotten seeds (88.78 seeds) (Tukey test,  $p < 0.05$ ). In the Guinean Soudanian agroecological zone of Benin, *A. histrix* hard seeds present in the soil seed bank could be softened by temperature fluctuation during the dry season between August to mid-September and December to March (Jaganathan et al., 2018). Overall germination percentage of the intact untreated seeds (26.00%) was significantly higher (Tukey test,  $p < 0.05$ ) than that of seeds recovered from faeces (4.27%). This is in agreement with previous studies on herbaceous seeds dispersal *via* endozoochory (Shayo & Uden, 1998; Razanamandranto et al., 2004; Viero et al., 2018). The germination percentage was greatest for seeds from mechanical scarification with sandpaper (96%), intermediate for seeds from hot water scarification (52.80%), and least for seeds from passage through cattle tracts (4.27%) (Tukey test,  $p < 0.05$ ). Mechanical scarification has a positive effect on breaking physical dormancy because sandpapering may have cracked the lignified palisade cells and consequently the seed could absorb water and oxygen, swell and germinate (Yildiztugay & Kucukoduk, 2012). Hot water scarification at 2 min soaking significantly increased germination percentage of *A. histrix* from 26% (intact untreated seeds) to 86% but with the longer time of exposure, the values of the germination speed decreased. As it is desirable, to sow pastures with *A. histrix* seeds, the use of mechanical scarification using sandpapering or hot water scarification at 80 °C during 2 min may be more beneficial, giving high germination percentage and uniform germination. The result demonstrates that breaking down the dung doubled seedling emergence (Mancilla-Leytón et al., 2012; Eichberg et al., 2016). However it would be necessary to evaluate the palatability of these

seeds under field conditions and the germination and establishment of these species in faeces in the field.

## 5. CONCLUSIONS

The passage of *A. histrix* seeds through the digestive system of Lagune cattle reduces its germination, and they presented inferior germination compared to seeds from mechanical scarification with sandpaper and seeds from hot water scarification. As it is desirable, to break dormancy of *A. histrix* seeds, the use of mechanical scarification using sandpapering or hot water scarification at 80 °C during 2 min may be more beneficial giving high germination percentage and uniform germination.

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