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Overcoming seed coat dormancy in *Tephrosia bracteolata* Perr. & Guill., a fodder legume of West African Savanna.

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ABSTRACT

Haphazard field establishment due to impervious seed coat dormancy is peculiar to legumes. The effects of physical scarification (sand abrasion, clipping with scissors and pin piercing), exposure to Concentrated sulphuric acid for 0, 10, 20, 30, 40 and 50 minutes, and exposure to boiling water for 0, 5, 30, 60, and 120 seconds on percentage germination and median germination time of *Tephrosia bracteolata* seeds were studied in Ibadan. The results showed that the three methods were effective in overcoming hard seed coat dormancy in *T. bracteolata*. Treatment means in all the pre-germination methods were significantly ($P<0.001$) different. The final percentage germination values were similar and best in 20 and 30 minutes acid exposure time, 5 seconds boiling water exposure time, and in scissors and pin piercing treatments of the physical scarification. The 20 and 30 minutes acid scarification had lowest median germination time of 0.9 days followed closely by 5 seconds boiling water scarification with 1.1 days. Overcoming the impervious seed coat dormancy in *T. bracteolata* will ensure its uniform seedling growth if utilized for fodder bank, grazing reserve and green manure establishment. Boiling water pre-germination treatment for 5 seconds may be adopted for its little skill and low resource demand.

Keywords: Boiling water, dormancy, fodder legume, physical scarification, sulphuric acid, *Tephrosia bracteolata*.

INTRODUCTION

Tephrosia bracteolata Perr. & Guill. is an erect undershrub, 0.6m-2.0m tall in the Papilionoideae subfamily of Fabaceae. It is regarded as weed of roadside and fallow (Akobundu and Agyakwa 1987). It is often encountered on poor soils and in denuded areas. The leaves are imparipinnate with leaflets ranging from 3 to 27. The crude protein content of the leaves remains high throughout the growing season, though higher in the early growing season (17.98%) than in the late growing season (12.98%) (Isichei and Awodoyin 1990). *T. bracteolata* offers many uses as fuel wood and fodder plant. In the derived savanna zone of Nigeria, farmers prepare bales of fresh shoots of the plant for uses in cut-and-carry zero grazing animal husbandry. De-Leeuw (1979) reported that *T. bracteolata* is relished by livestock in Northern Nigeria. Unlike the introduced *Stylosanthes* sp. that cannot withstand competition with perennial grasses (Mohamed-Saleem 1984, Agishi 1985), *T. bracteolata* shares positive association with *Andropogon tectorum*. a tillering fodder grass in the natural habitat, and the two exhibited mutual stimulatory relationship (Isichei and Awodoyin 1990). The plant therefore has high potential in establishing fodder bank and improved grazing reserves.

T. bracteolata is fast growing, and has restricted flowering/fruiting/seed set period that commences in September and lasts till December. The seeds are rectanguloid in shape with the mean length, breath and thickness of 2.92mm, 1.95mm and 0.16mm respectively. 100 seeds weigh 0.88g. The plant readily produces effective nodules on Nigerian soils (Awodoyin 1987). It will therefore be a good green manure plant. In recognition of its soil nutrient restorative value, farmers in the Nigerian derived savanna prefer farmlands under its fallow. A Yoruba proverb that says, "no matter the poor state of the soil, *Tephrosia* (Roroo) will strive on it" lends credence to the recognition of the plant's adaptability to marginal soils.

In spite of its potential as fodder and green manure plant, it has not attracted much attention probably because of inadequate information on its biology including propagation techniques.

T. bracteolata regenerates from seeds and has good seeding ability to perpetuate its population in the natural ecosystem. However, poor and haphazard field establishment from directly planted seeds was observed. This poor establishment is peculiar to many legumes due to seed dormancy resulting from impermeability of seed coat to water

and respiratory gases (Copeland 1976, Egley 1989, Gizachew and Scarisbrick 1999).

In utilizing the plant to establish fodder bank and in green manuring, it is pertinent to understand the appropriate seed treatment method to ensure uniform seed germination and seedling establishment on the field. This study, therefore, had its focus in investigating the response of percent seed germination of *T. bracteolata* to some pre-germination treatments aimed at breaking the impervious seed coat dormancy.

MATERIALS AND METHODS

Seed sources and Treatments

The seeds of *T. bracteolata* were collected from the natural habitat in a denuded area in Ibadan (7°24"N, 3°54"E) with Guineo-Congolian rainforest: dryer type vegetation. The germination study was conducted in March 1997 in the Ecology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan.

The seeds were subjected to the following pre-germination treatments:

i. Control. The seeds were soaked in distilled water for 5minutes.

ii. Physical scarification. This is done by, (1) abrasion with coarse sand, (2) piercing with office pin and, (3) chipping with dissecting scissors.

iii. Acid scarification. The seeds were immersed in concentrated sulphuric acid (98% AR H_2SO_4) in a cylinder for 0, 10, 20, 30, 40 and 50 minutes. At each test time some seeds were brought out and washed in perforated plastic cylinder under cold running water for 5 minutes. Final rinsing was done with distilled water.

iv. Boiling Water Treatment. Seeds wrapped up in muslin cloth were steeped in boiling water (100°C) for varying time duration – 0, 5, 30, 60 and 120 seconds.

Germination and Statistical Analysis

In all the treatments, 50 seeds were placed in a petridish (9cm diameter) lined with Whatman No. 1 filter paper that was adequately moistened with distilled water. The treatments were replicated 4 times in a completely randomised design. The petridishes were arranged on tables in the laboratory at room temperature (28°C) where they received diffused light naturally alternated by darkness. The filter papers were moistened every other day with 3ml. Distilled water.

Protrusion of radicle, used as criterion of germination, was recorded as a cumulative number on a daily basis for seven days (Karuiki and Powell 1988). The median germination time, which is the time when 50% of the final number of viable seeds had sprouted (Reghunath *et al.* 1993), was determined from the graphs of time against percentage germination for each pre-germination method.

The percentage germination data were angularly (arcsine) transformed to approximate normality (Little and Hills 1978) and used in the analysis of variance (Sokal and Rholf 1969). Data from each experiment were analyzed separately. Least significant difference at 5% probability level was used to separate means that were significantly different from each other.

RESULTS

The mean cumulative percentage germination of *T. bracteolata* seeds at two, four and seven days after treatment for the various pre-germination treatments are shown in Figure 1. Treatment means in all the pre-germination treatments and in all days of assessment were significantly ($P<0.001$) different (Tables 1 & 2). Also in all the scarification methods, percentage germination in all treatments were significantly better ($P<0.001$) than the control.

Seeds responded quickly to all treatments but best to acid treatments at 20 and 30 minutes exposure time, attaining 95-99% germination within two days (Figure 1). The final percentage germination (at 7 days) were fairly similar in 20 and 30 minutes of acid treatment, in 5 seconds of boiling water treatment and in scissors and pin piercing of physical scarification.

In acid treatment, germination increased as exposure time increased up to 30 minutes but decreased with further exposure. Also in boiling water treatment, germination increased up to five seconds of exposure but decreased afterward. Though exposure in boiling water for 30 seconds was still tolerable, the treatment became lethal beyond. In the physical scarification, seeds subjected to sand abrasion had significantly ($P<0.001$) lower percentage germination when compared to scissors and pin piercing treatments on all days of assessment (Table 1). The final percentage germination in scissors and pin piercing treatments (96.5-99.1%) was almost twice the final percentage germination in sand abrasion treatment (54.1-55.9%).

The median germination times for all treatments were similar and ranged between 0.9-1.3 days for acid scarification, 1.1-1.5 days for boiling water scarification and 1.5-1.6 days for physical scarification (Table 1). The lowest median germination times were recorded at 20 and 30 minutes exposure times (0.9 days) for acid scarification and at 5 seconds exposure time (1.1 days) for boiling water scarification.

The relatively low final percentage germination recorded for 40 and 50 minutes acid exposure time, and 60 and 120 boiling water exposure time were probably due to their lethal effects on the seeds of *T. bracteolata*.

Table 1: Mean cumulative percentage (arcsine) germination of *T. bracteolata* seeds pretreated by Conc. Sulphuric acid, boiling water and physical scarification.

Treatments	Mean Percentage Germination Days After Treatment 2	4	7	Median Germination time (Days)
		Concentrated sulphuric acid		
0 minutes	24.7	35.3	37.2	2.05
10 minutes	54.1	58.8	62.5	1.00
20 minutes	83.0	88.0	90.0	0.90
30 minutes	88.0	90.0	90.0	0.90
40 minutes	50.5	62.9	65.7	1.30
50 minutes	49.3	54.8	56.9	1.15
Mean	58.26	64.95	67.06	-
LSD(0.05)	2.77	5.39	4.19	-
		Boiling Water		
0 seconds	24.7	36.3	38.7	2.10
5 seconds	67.7	82.5	85.1	1.10
30 seconds	50.8	67.9	68.6	1.38
60 seconds	38.7	43.0	50.8	1.40
120 seconds	32.6	32.6	40.9	1.50
Mean	42.86	53.01	56.80	-
LSD(0.05)	4.22	7.76	5.98	-
		Physical Scarification		
Control	25.1	36.8	38.9	2.15
Sand abrasion	33.5	42.7	47.9	1.50
Scissors	50.6	70.6	83.5	1.60
Pin piercing	50.1	74.7	83.9	1.60
Mean	39.81	56.19	63.53	-
LSD(0.05)	5.05	5.61	6.78	-

DISCUSSION

The results showed that impervious seed coat may be the cause of dormancy in *T. bracteolata*. Trials aimed at reducing the thickness (acid scarification), breaking up (physical scarification) and rupturing (boiling water scarification) the seed coat effectively improved the germination. The breaking of seed coat dormancy in legumes using sulphuric acid, boiling water and physical scarification has been demonstrated by other works (Duguma *et al.* 1988, Karuiki and Powell 1988, Todd-Bockarie *et al.* 1993, Gizachew and Scarisbrick 1999).

Copeland (1976) reported that hard seed coat creates barrier to water uptake and entry of gases in most legumes. He further reported that the mechanism of imperviousness in the seeds of legumes may be due to deposition of suberin, lignin and cutin in the seed coat membranes or across the micropylar opening in the seed coat. The presence of continuous layer of tightly packed palisade cells in the seed coat of legumes may also create barrier to water and gases uptake (Egley 1989). The hard seed coat dormancy maintains a low constant moisture content in legume seeds, confers long longevity on them and enables them to survive harsh conditions in the dry season. It also allows endozoic dispersal of seeds and recolonization after forest fire (Egley 1989).

Overcoming the seed coat dormancy drastically by acid scarification in the laboratory explains the gradual action of permanently present weak acids in the soil in breaking the seed coat barrier to the uptake of water and gases to induce germination. The increase in percentage germination with increase in acid exposure time may explain the importance of storage length in the soil seed bank to precondition seeds of *T. bracteolata* for germination. Since seeds are deposited in the soil seed bank at different times, this may explain the stratification of seed germination exhibited by *T. bracteolata* in natural habitat. The stratification of germination provides good insurance against total eradication of a particular plant species, most especially weeds, from the natural ecosystem. Duguma *et al.* (1988) reported a direct relationship between length of storage time and percentage germination in seeds of *Leucaena leucocephala* stored in laboratory and in screen house.

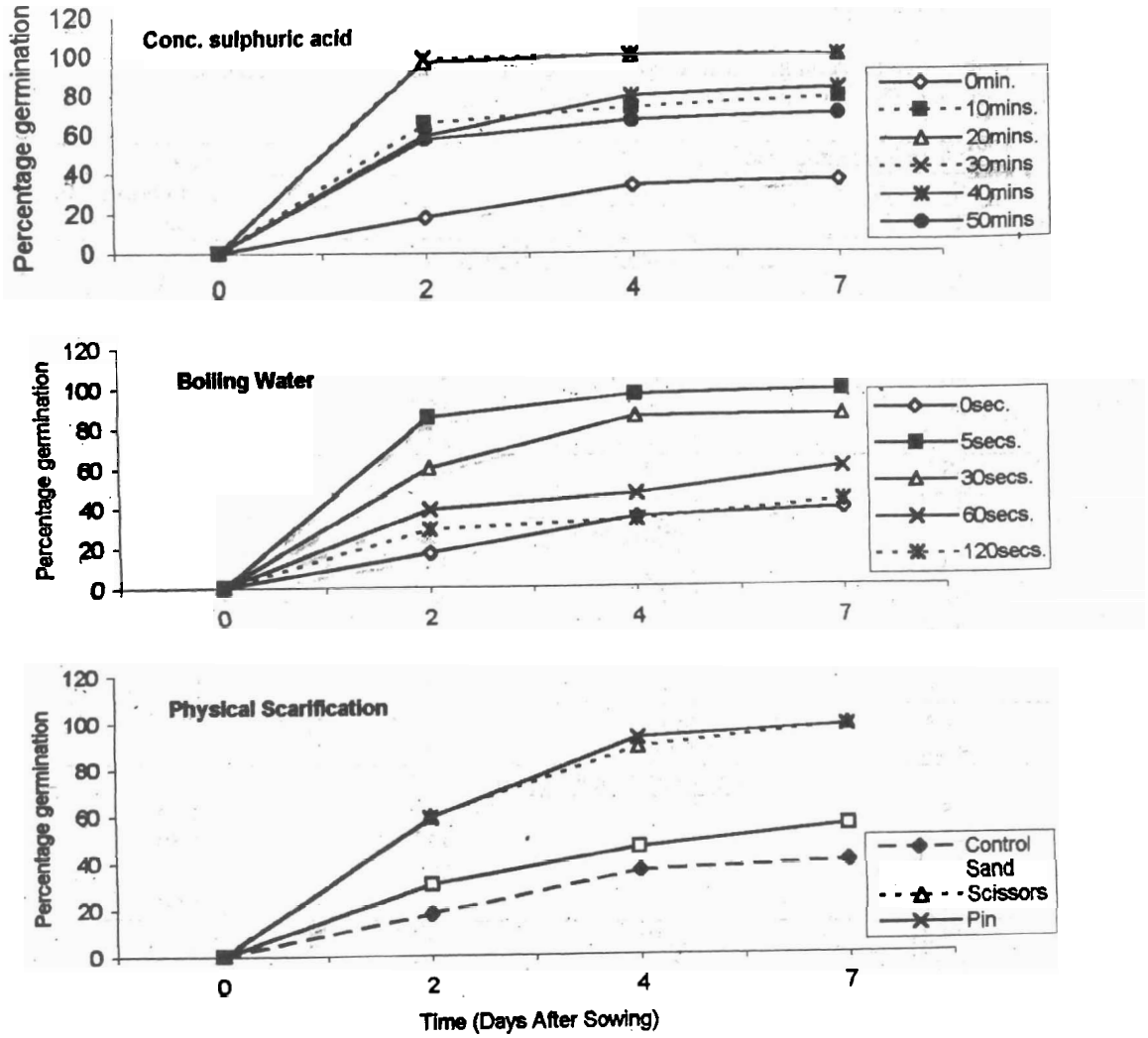

Figure 1. Mean cumulative percentage germination of seeds of *Tephrosia bracteolata* pretreated by conc. sulphuric acid, boiling water and physical scarification.

The breaking of dormancy by boiling water may explain how moist heating resulting from burning of thrash that precedes cropping overcomes seed coat dormancy in the soil. Also the physical scarification may explain how abrasion of the seed coat caused by ploughing and harrowing, and charring of seed coat by field burning overcomes hard seed coat dormancy. Mohamed-Saleem (1984) reported that boiling water pre-germination treatment is generally good to overcome hard seed coat dormancy in legumes.

The relatively low final percentage germination recorded for 40 and 50 minutes acid exposure time, and 60 and 120 seconds boiling water exposure time were due to their lethal effects on the embryo. Lethal effects of boiling water treatment has been reported in various works (Duguma *et al.* 1988, Todd-Bockarie *et al.* 1993). Mohammed-Saleem (1984) explained that legume seed stock pretreated by boiling water has 'false start' whereby the first flush does not survive. To compensate for this false start, he recommended that one-third of the seed stock to be used for field establishment should not be treated. The effect of seed:hot water and seed:acid (W/V) ratios was not considered in this study, though Duguma *et al.* (1988) reported that the ratios influenced germination results in *L. leucocephala*.

Effectiveness of boiling water treatments in overcoming hard seed coat dormancy in *T. bracteolata* is an advantage considering the little skill involved and low resource demand.

Table 2. ANOVA showing the effects of acid scarification, boiling water and physical scarification pre-treatments on germination of seeds of *T. bracteolata* (n=50).

Sources of Variation	df	Mean Square Day 2	Day 4	Day 7
Concentrated sulphuric acid				
Exposure	5	2224.40	1745.95	1656.53
Time		***	***	***
Error	18	18.64	13.69	8.27
CV(%)	-	7.41	5.70	4.29
Boiling Water				
Exposure	4	1131.91	1781.31	1556.69
Time		***	***	***
Error	15	8.16	27.57	16.39
CV(%)	-	6.66	4.22	7.13
Physical Scarification				
Treatment	3	636.58	1473.19	2217.35
Method		***	***	***
Error	12	11.26	13.85	20.25
CV(%)	-	8.43	6.62	7.08

*** - significant at 0.1% probability level.

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