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Germination of seeds of sicklepod as affected by pre-germination treatments, fruit maturity and depth of sowing: Implication in sown fallow management

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Abstract

Sicklepod (*Senna obtusifolia*) is a multipurpose plant with potentials in improved bush fallow management. Studies were carried out in 1997 and 1998 in the laboratory, nursery and on the field in Ibadan, Nigeria to understand its germination ecology. Studies included germination as affected by: a) pre-germination treatments – sulphuric acid, boiling water and physical scarification, b) fruit maturity, and c) depth of seed burial. Seed coat dormancy was evident in the seeds of sicklepod, especially at dark brown pod maturity stage. Acid-scarification for 2-15 minutes, steeping in boiling water for 10-60 seconds and physical wounding of seed coat resulted in about 100% germination within four days compared to 20% germination obtained in non-scarified seeds at seven days after sowing. Seeds were not physiologically mature enough until the light brown pod stage. At seven days after sowing, seed germination was 100%, 50% and 0% at 0-4 cm, 8 cm and 16 cm soil depth respectively. Results showed that pre-germination treatments will induce early and uniform germination in sicklepod. The latest stage to remove sicklepod for mulching or green manuring to prevent multiplication of seeds in the soil seed bank is yellow pod stage when the embryo is still immature.

Key words: Dormancy, germination ecology, scarification, seed coat, *Senna obtusifolia*.

Introduction

The use of fast growing, deep rooting and nitrogen fixing shrubs in improved fallow management to promote crop growth, reduce weeding frequency, reduce demand for nitrogen fertilizer and control nematodes has been reported (Akobundu, 1993; Holt, 1995; Miiro *et al.*, 2002). Sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby] has been suggested as an improved bush fallow plant (Dupriez and De Leener, 1989; Awodoyin, 2000).

The knowledge of seed germination is necessary for field establishment of a plant and in preventive control of weeds. While in some plants the seeds are pre-conditioned to germinate immediately after dispersal, in many others, though the seeds may be mature and viable, their ready germination is prevented under favourable environmental conditions due to dormancy. Dormancy enables most tropical plants to survive dry seasons and provides insurance against total eradication of populations from the ecosystem (Copeland, 1976; Anderson, 1983; Mortimer, 1990). Of the various dormancy mechanisms, seed coat impermeability to water and gases is peculiar to legumes (Copeland, 1976). Enforced dormancy as a result of depth of seed burial also has implication on establishment of plants and control of weeds.

The seeds of most weed species are pre-conditioned for germination only at fruit maturity, whereas some other seeds either require an after ripening period as in *Striga* sp. (Lagoke *et al.*, 1988) or are pre-conditioned for germination before fruit maturity as in Asteraceae (Ayeni *et al.*, 1984). Understanding the fruit maturity stage at which seeds of a weed species are pre-conditioned for germination is necessary to control its multiplication in the soil seed bank.

In this report we discuss the effects of pre-germination treatments, soil depth and fruit maturity on seed germination in sicklepod with the view of understanding the dormancy mechanism and the stage of physiological maturation of the seeds. The latter seeks to ensure early and uniform establishment of the seedlings in the event of utilizing the plant in sown bush fallow and green manuring.

Materials and methods

The studies were conducted in the laboratory, nursery and crop garden at the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. Ibadan (7° 24'N; 3° 54'E; altitude 234m above sea level), is located in the rainforest-savanna transition zone with a rainfall:evaporation ratio of about 1.0. The germination studies conducted were (a) dormancy removal, (b) effect of fruit maturity on germination and (c) effect of depth of sowing on seed germination.

Dormancy removal

A series of studies were conducted to test germinability of the seeds and investigate methods of inducing early and uniform germination. The seeds used were collected in the wild, in Ibadan, from fully matured and dry fruits whose sickle pods were dark brown in colour, quadrangular in cross section and already dehiscing. Mature seeds are quadrangular in shape. The mean length, breadth and thickness of the seed were 4.63±0.12 mm, 2.32±0.16 mm and 2.91±0.08 mm respectively (n=50). The mean 100-seed weight were 2.16±0.09 g (n=10). The seeds were kept in brown paper envelopes and stored in a laboratory cabinet at prevailing ambient conditions (30°/26°C day/night temperature; 60-90% relative humidity). All germination treatments that were tried aimed at rupturing the seed coat, reducing the seed coat thickness or breaking through the cell layers in the seed coat. These included: acid scarification, physical scarification and boiling water treatment.

Acid scarification

The seeds were soaked in concentrated sulphuric acid (98% Laboratory Reagent [LR] H_2SO_4) for 0, 2, 5, 10, 15, 20 and 30 minutes using two cups of different sizes. The outer cup that contained the acid was bigger than the inner perforated cup that contained the seeds. The inner cup with the seeds was lowered into the acid for the duration of the test time. At the end of each test time the inner cup was lifted out and placed under running water for 10 minutes to get the seeds thoroughly washed. Final rinsing was done with distilled water. Fifty (50) scarified seeds were then placed in a petridish (95 mm x 15 mm) lined with Whatman No. 1 (90 mm) filter paper that was adequately moistened with distilled water. The petridishes were arranged in a randomised complete block design with five replicates on a laboratory bench (average ambient tempertature = 28°C; average RH = 75%) where they received diffused light naturally alternated by darkness (13/11 day/night hours). Blocking was necessary to harmonize the effects of uneven light distribution within the laboratory. The filter papers in the petridishes were moistened every other day with 3 ml distilled water. Protrusion of radicle, used as evidence of germination, was recorded as a cumulative number on two, four and seven days after sowing (DAS). All germinated seeds were removed from the petridishes at each assessment. The control seeds were soaked in distilled water for 10 minutes and all other procedures remained the same as treated seeds.

Physical scarification

The matured seeds of sicklepod are quadrangular in shape with the two bases flattened. However, one of the bases draws to a short beak at the region of radicle-plumule protrusion. All the physical treatments were directed at this pointed end, being the most sensitive point. The treatments were;

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- stroking against the cut surface of a nail-file for 10 times to reduce the seed coat thickness,
- Clipping the pointed end with a dissecting scissors,
- Piercing a hole through the seed coat layer with a sharp office pin,
- Untreated seeds served as the control.

Layout, other handling and data collection were as described earlier.

Boiling water treatment

Fifty seeds of sicklepod wrapped in muslin cloth in five packs (replicates) were steeped in boiling water (100°C) on hot plate for 0, 2, 5, 10, 20, 40, 60, 120, 300, 600 and 1200 seconds. The cloth-packs were removed at each test time and placed under running water to cool the seeds. Each pack was then emptied into petridishes lined with Whatman No. 1 filter paper and adequately moistened with distilled water. The handling and monitoring were as described earlier.

Fruit maturity and germination

Four stages of fruit maturity are identifiable in sicklepod (Awodoyin, 2000). These include i) Green (pods are green, fully filled and round in cross section; ii) Yellow (pods are yellow and still round in cross section); iii.) Light Brown (pods are fully brown, quadrangular in cross section, pedicel still green); and iv) Dark Brown (pods have dried up, quadrangular in cross section, dehiscing on convex side, pedicel completely dry). One hundred (100) fruits at each of the four stages of fruit maturity were collected in the wild. The seeds, bulked by maturity and sun-dried for 30 days, were tested for germination either scarified or non-scarified in a 2 (scarification) x 4 (fruit maturity) factorial experiment replicated 15 times. Scarification was done by soaking the seeds in 98% LR H_2SO_4 for 5 minutes. Fifty seeds were placed in each petridish. Seeds were assessed for cumulative number of germinated seeds on the 7th day after setting. Data handling was as described earlier.

Effect of depth of sowing on germination

Mature acid-scarified seeds of sicklepod were used for the study that was conducted in the nursery and on the field in June 1998. Six sowing depths (0, 1, 2, 4, 8 and 16 cm) were investigated.

Nursery experiment

Thirty experimental pots (20cm surface diameter, 22cm depth) were filled with garden topsoil [pH(H_2O)=5.7; total N=0.23%; organic carbon=1.86%]. The texture of the soil was sandy clay loam [62% sand; 17% silt; 21% clay]. The pots were arranged in a randomized complete block design with five replicates on an iron-net table in the open nursery. The six depth treatments were randomly allocated to the six pots in each block. Using a dribbling stick, twenty five holes were made in each pot to the test depths. Two acid-scarified seeds were dropped in each hole. The holes were covered lightly with freshly collected soil. Pots were watered adequately every other day with the excess draining through the perforated base of the pots. Germination was recorded as cumulative number of seeds whose plumule (criterion of germination) had appeared on the soil surface on two, four and seven DAS. All germinated seeds were removed at each assessment. The number of seeds were square-root transformed (Little and Hills, 1978) and subjected to analysis of variance (Gomez and Gomez, 1984). Mean comparison was made using Fisher's Protected least significant difference (LSD) test at 5% level of probability.

Field experiment

An area 3.7 m x 3.5 m was cleared, tilled and divided into plots (50 cm x 50 cm) with 10 cm inter-plot spaces. The soil used for nursery experiment above was collected from the site of the field experiment. The six test depths were allocated to plots in a randomized complete block design with each replicated five times. All handling, data collection, transformation and analysis were as in previous section.

Results

Acid scarification

The mean cumulative number of seed germination for sicklepod when soaked in concentrated H_2SO_4 increased with time duration up to 15 minutes from when it decreased with increasing duration (Fig. 1). When the seeds were soaked in acid for 10-30 minutes, maximum germination was obtained within 2 days and there was hardly any additional germination up till the 7th day when the study was finally assessed.

The seeds scarified at short time duration (2 and 5 min.) had increasing number of germination up till the 7th day. In seeds scarified for 5 min. germination increased from about 32% on day two to about 96% on day seven. Scarification for 2 min., however, had minimal effect on germination. It increased germination from 6% on day two to only about 19% germination on day seven. The differences in the

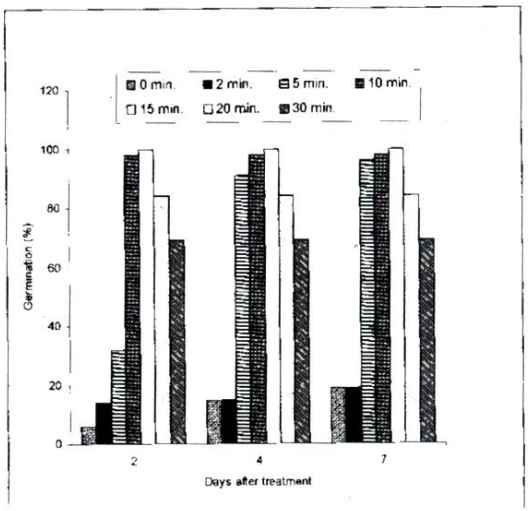

Figure 1. Effect of concentrated sulphuric acid scarification at different time duration on percentage germination of seeds of sicklepod.

mean germination among the time duration were highly significant (P< 0.001) on 2, 4 and 7 days after scarification. The results indicated that the seeds could tolerate acid treatment up till 30 min. (giving about 70% germination). However, observation revealed that while the seedlings resulting from seeds scarified for up to 15 min. were good, the seedlings produced at 20 and 30 min. treatments showed scorching effect of the acid.

Physical scarification

It is evident from the results of physical scarification (Table 1) that breaking open the seed coat favoured uniform germination. In the three physical scarification methods used in this study germination increased from about 80% on day two to 100% on day seven after treatment. The differences among the means of the three treatments were not significant on the three days of observation, but were significantly (P<0.001) higher than the control (Table 1).

Boiling water

Mean cumulative germination increased up to 60 seconds of steeping in boiling water and then abruptly decreased (Table 2). When the seeds were steeped in boiling water for 20, 40 and 60 seconds the mean cumulative germination were substantial (about 80%) from day two after treatment. At final assessment on day seven, steeping for 10, 20, 40 and 60 seconds gave the highest result of about 95% germination. The differences in the mean cumulative germination among the time duration were highly significant (P<0.001) on 2, 4 and 7 days after treatment. At final assessment, differences in the mean germination when the seeds were steeped in boiling water for 10, 20, 40 and 60 seconds were not significant, though the means were significantly (P<0.001) higher than those of other time duration.

Fruit maturity

Seeds from green fruit stage did not germinate and those from yellow stage gave about 4% germination (Table 3). Scarification did not enhance germination at the yellow stage. Germination was high at light brown and dark brown stages of fruit maturity. Though the light brown and dark brown stages were not significantly different in the overall mean cumulative germination, they were significantly (P<0.001) different from green and yellow stages. Effect of maturity on germination in the scarified treatment followed the same trend as above. However, in non-scarified treatment, the light brown

Table 1. Mean cumulative germination (square root transformed) of Senna obtusifolia seeds soaked in Conc.H_2SO_4 at different time duration. (Decode using X^2 - 0.5). Values in parenthesis are percent germination.

Time (minutes)	Days after treatment		
	2	4	7
0	1.83 (6)	2.81 (15)	3.20 (19)
2	2.71 (14)	2.97 (15)	3.14 (19)
5	4.08 (32)	6.78 (91)	6.96 (96)
10	7.05 (98)	7.10 (98)	7.10 (98)
15	7.11 (100)	7.11 (100)	7.11 (100)
20	6.53 (84)	6.53 (84)	6.53 (84)
30	5.90 (69)	5.90 (69)	5.90 (69)
LSD (P≤0.001)	0.33	0.26	0.24

stage had significantly (P<0.001) higher mean germination followed by dark brown, yellow and green stages in the descending order. Scarification significantly (P<0.001) enhanced germination at the light brown and dark brown stages. There was a strong interaction (P<0.001) between scarification and pod maturity.

Depth of sowing

There was early seedling emergence at 1 cm depth in the nursery and on the field. At 4 days after sowing (DAS), seedlings had emerged from all depths except 16 cm. No seedlings emerged at 16 cm depth on 7 DAS (Table 4). There were significant (P<0.001) differences among the test depths on 2, 4 and 7 DAS. Emergence on the surface was significantly (P<0.001) higher than emergence at all other depths on 2 and 4 DAS. On 7 DAS, however, depths 0, 1, 2 and 4 cm were not different from one another but had significantly (P<0.001) higher germination than 8 and 16 cm depth treatments. Substantial emergence (nursery = 50%; field = 63%) was recorded at 8 cm depth.

Discussion

The germination study showed that there is inherent dormancy in sicklepod seeds. Seed coat dormancy is the case of this phenomenon in sicklepod seeds as shown by improved germination after treatment by reducing the thickness (acid scarification), breaking up (physical scarification) and rupturing

Table 2. Mean cumulative germination (square root transformed) of Senna obtusifolia seeds steeped in boiling water for different time durations (Decode using X^2 - 0.5). Values in parenthesis are percent germination.

Time (seconds)	Days after treatment		
	2	4	7
0	1.60 (4)	2.98 (17)	3.27 (20)
2	4.08 (32)	4.37 (37)	4.51 (40)
5	4.57 (41)	4.78 (45)	5.00 (49)
10	4.86 (46)	6.44 (82)	6.82 (92)
20	6.44 (82)	6.83 (92)	7.01 (97)
40	6.36 (80)	6.56 (85)	6.93 (95)
60	6.67 (88)	6.76 (90)	6.93 (95)
120	4.27 (35)	4.61 (42)	4.93 (48)
300	3.13 (19)	3.60 (25)	3.85 (29)
600	2.77 (14)	3.50 (24)	3.59 (25)
1200	1.36 (03)	1.51 (4)	1.58 (04)
LSD (P≤0.005)	0.21	0.013	0.11

Table 3. Mean cumulative germination (square root transformed) of scarified and unscarified seeds of Senna obtusifolia at different stages of pod maturity (Decode using X^2 - 0.5). Values in parenthesis are percent germination.

Scarification	Maturity				
Scarification	Green	Yellow	Light Brown	Dark Brown	Mean
Scarified (S1)	0.71 (0)	1.64 (4)	6.93 (95)	7.04 (98)	4.08
Unscarified(S2)	0.71 (0)	1.46 (3)	3.68 (26)	2.75 (14)	2.15
Maturity mean	0.71	1.55	5.31	4.90	-

LSD P≤0.001 (scarification averaged over maturity treatment) = 0.25
LSD P≤0.001 (maturity averaged over scarification treatment) = 0.31
LSD P≤0.001 (maturity at the same scarification treatment) = 0.41.

(boiling water) of the seed coat overcame the dormancy and improved the germination. The peculiarity of seed coat dormancy to legumes and effectiveness of concentrated H_2SO_4 in overcoming the dormancy had been reported in *Acacia* sp., *Chamaecytisus palmensis* and *Tephrosia bracteolata* (Masamba, 1994; Demel Teketay, 1997; Gizachew and Scarisbrick, 1999; Awodoyin *et al.*, 2000).

Dormancy confers longevity on the seeds and enables them to survive harsh conditions in the dry season. The longevity may be up to 20 years in *Striga* sp. (Lagoke *et al.*, 1988) and *Senna (Cassia) obtusifolia* (Cock and Evans, 1984), and 15 years in *Eichhornia crassipes* (Kim, 1988). The impervious seed coat provides a delaying mechanism that prevents germination under conditions which might prove unsuitable for establishment, allows endozoic dispersal and recolonization after fire (Egley, 1989). Lack of additional germination from day 2 through day 7 at 20 min. acid treatment may indicate that the embryo of the non-germinating seeds were killed by the acid.

Scarification in concentrated H_2SO_4 for 2 min. may not be adequate to overcome the seed coat dormancy in sicklepod. Also the results may be indications of tolerance of acid soils by sicklepod. Breaking of dormancy by acid scarification underscores the effectiveness of soil acidity in gradually overcoming impervious seed coat dormancy to induce seed germination. The short drastic action of concentrated H_2SO_4 in the laboratory may explain the way the permanently present weak humic acid in the soil gradually softens the hard coat.

Treatment in boiling water for between 10 and 60 sec. favoured good germination. Treatment in boiling water may be likened to moist heating that is obtained in the natural situations, especially during burning of thrash that precedes cropping or heating of the bare soil by the intense solar radiation at the commencement of rainy season.

Physical scarification favours germination of seeds of sicklepod. This may be likened to abrasion of seed coat caused by ploughing and harrowing on the field, chewing and ruminating by animals, and charring of the seed coat by field burning.

These results may suggest that seed coat wounding, as well as thickness reduction and seed coat rupturing may enhance imbibition of water and subsequent seed germination. Also, once the dormancy is adequately broken maximum germination was obtained within two days due to rapid uptake of water. This may explain the rapid nature of weed seeds at water uptake following short or irregular showers.

The results of effect of maturity stages on germination suggests that the embryos were not fully mature at yellow fruit stage. Also, the results of the trial may indicate that if the seeds of sicklepod must be prevented from building up in the soil seed bank, the latest stage to cut back the plants or remove the fruit is at the yellow stage of fruit maturity.

Acid-scarification did not improve germination at the yellow fruit stage. This suggests that the poor

Table 4. Mean cumulative germination (square root transformed) of seeds of Senna obtusifolia as affected by depth of sowing in the nursery and field (Decode using X^2-0.5). Values in parenthesis are percent germination.

Depth of sowing	Nursery			Field		
	Days after sowing			Days after sowing		
	2	4	7	2	4	7
0	4.34(37)	5.92(69)	6.96(96)	3.20(19)	4.37(37)	7.05(98)
1	3.22(20)	5.09(51)	6.85(93)	2.05(7)	3.77(27)	7.01(97)
2	0.71(0)	4.96(48)	6.92(95)	0.71(0)	2.54(12)	6.94(95)
4	0.71(0)	4.96(48)	6.93(95)	0.71(0)	2.19(9)	7.02(98)
8	0.71(0)	2.87(15)	5.05(50)	0.71(0)	1.91(6)	5.65(63)
16	0.71(0)	0.71(0)	0.71(0)	0.71(0)	0.71(0)	0.71(0)
LSD($P \leq 0.001$)	0.13	0.27	0.27	0.19	0.29	0.22

coat at the light brown and dark brown stages were responsible for the enhancement of germination by acid-scarification. In non-scarified seeds, the highest germination recorded at the light brown stage may be due to less lignifications of the seed coat hence less hard seed coat dormancy. The strong interaction between scarification and seed maturity may further demonstrate the increasing seed coat dormancy as fruits matured and dried up. Copeland (1976) reported that as fruits and seeds of most legumes mature there is deposition of lignin and suberin in the seed coat and across the micropylar opening to create a water-proofing effect, which prevents ready imbibition of water by the seed coat.

Deep burial of the seeds up to 8 cm did not prevent germination of the seeds. However, burial up to 16 cm depth prevented seed germination and establishment of seedlings. This may indicate that ploughing up to 16 cm depth will prevent infestation of the field by sicklepod. Nonetheless, due to the presence of seed coat dormancy, subsequent ploughing may bring up the deep-buried seeds for reinfestation. Lack of germination at lower soil depth might be due to low O_2/CO_2 ratio (Akobundu, 1987) and poor light condition (Demel Teketay, 1997).

Conclusion

The seeds of sicklepod are not physiologically mature at yellow fruit stage but have matured adequately at light brown stage. Acid, boiling water and physical scarification treatments induced early and uniform germination of mature seeds. Considering the cost of concentrated H_2SO_4 and the skill required in handling, it may not be readily adaptable by peasant farmers. However, the boiling water treatment for 10-60 sec. gives a good alternative. Seeds should be sown within 0-4cm soil depth to realize the uniform germination. The use of sicklepod as fallow plant and in green manuring may be readily acceptable by West African farmers because it is locally utilised as laxative and pot-herb, especially in Cameroon, Ethiopia, Ghana, Nigeria, Senegal and Sudan (Gbile, 1986; Dupriez and De-Leener, 1989; Schippers, 2000).

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