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Differential requirements for breaking seed dormancy in biotypes of *Cleome gynandra* and two *Amaranthus* species

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Differential seed dormancy mechanisms arise among plant provenances and biotypes/ecotypes due to adaptations to local environmental cues that synchronise seed germination with optimal periods for seedling survival. This is one of the likely sources of unpredictable and variable germination often noted in semi-domesticated plants such as *Cleome* and *Amaranthus* species. To test this, a study was conducted under laboratory conditions in which two provenances of *Cleome gynandra* L. that differed in pod sizes and two biotypes that differed in leaf markings in each of the species, *Amaranthus hybridus* L. and *Amaranthus retroflexus* L. were subjected to pre-treatments commonly used to break seed dormancy. The study revealed marked differences in the expression of seed dormancy and sensitivity to pre-treatments for breaking seed dormancy between biotypes in each of the three species. The differences were interpreted to reflect either habitat-specific traits in *C. gynandra* biotypes, which came from different environments or genetic differentiation in *A. hybridus* and *A. retroflexus* biotypes, which came from similar environments. Furthermore, the results indicated two types of responses to gibberellic acid (GA₃) in positively photoblastic seeds. In one type, GA₃ replaced light requirements for seed germination. In the second type, light and GA₃ were synergistic in breaking seed dormancy.

Key words: Gibberellic acid (GA₃), germination requirements, light, manure leachate, photoblastic seeds, seed germination, smoke.

INTRODUCTION

Cleome gynandra L. and many of the *Amaranthus* species (for example, *Amaranthus Hybridus* L., *Amaranthus hypochondrioides* L., *Amaranthus dubius* L. and *Amaranthus retroflexus* L.) among many others are indigenised vegetables or pot herbs that form a significant part of the local vegetable diet in South America, Africa, Asia and other tropical and subtropical parts of the world (Kokwaro, 1976; Kaul et al., 1996; Chweya and Eyzaguirre, 1999; Somboon and Pimsamarn, 2006) where they are deeply interwoven with local customs and beliefs. In many parts of sub-Saharan Africa and Asia, they are not formally cultivated, but for years, they have been semi-domesticated volunteer crops in home gardens or on fertile land near homesteads. In most subtropical and tropical regions of the world, the species are generally ruderal plants that

naturally grow on disturbed soil as annual weeds (Burki et al., 1997).

There have been calls for domestication and commercialization of *Cleome* and *Amaranthus* species in sub-Saharan Africa because of their widespread uses (Chweya and Mnzava, 1997; Makokha and Ombwara, 2002). One of the most desirable features for plant domestication is high and uniform seed germination in order to get a good crop stand. Whilst germination from soil seed bank is prolific in disturbed areas, intentionally planted seeds of *C. gynandra* (Ekpong, 2009; Raboteaux and Anderson, 2010) and *Amaranthus* species (Tiryaki, 2009; Chauhan and Johnson, 2009) do not germinate well, and hence, it is difficult to obtain a uniform plant stand. The major underlying cause of poor germination in semi-domesticated plants is usually seed dormancy,

especially in fresh seeds. A number of studies have been conducted on the germination requirements for *Cloeme* and *Amaranthus* species, but the results show considerable variations among studies for both *C. gynandra* (Ochuodho et al., 2004; Ochuodho and Modi, 2005; Ekpong, 2009; Raboteaux and Anderson, 2010) and *Amaranthus* species (Ellis et al., 1985; Aufhammer et al., 1998; Gallagher and Cardina, 1998; Steckel et al., 2004). One possible reason for the conflicting reports on germination requirements of *Cloeme* and *Amaranthus* species could be variations in seed dormancy mechanisms that are either provenance or biotype specific or are specific for a geographic location to which the provenance/biotype is adapted. It is common for wild plants to exhibit location driven selection traits which enhance the successes/fitness of the plant species to a particular environment (Linhart and Grant, 1996). With respect to seed dormancy, location driven selection is expected to result in site-specific adaptation of germination requirements or dormancy traits that favour responses to local environmental cues that synchronise germination with periods that are optimal for seedling survival in that environment (Donohue et al., 2005). Thus, differential germination requirements may exist between provenances or biotypes/ecotypes of a plant species.

The aim of this study was to determine whether provenances/biotypes of each of the species *C. gynandra*, *A. hybridus* and *A. retroflexus* (some of the popular port herbs) differ in seed germination requirements. In this respect, a hypothesis was tested that provenances/biotypes in each of the species differ in their responses to pre-germination treatments commonly used to release seeds from dormancy.

MATERIALS AND METHODS

Plant material

The study was conducted in a laboratory at the University of Zululand and involved seed germination of two biotypes each of *C. gynandra*, *A. retroflexus* or *A. hybridus*. The *C. gynandra* biotypes were provenances; one was collected from Harare (17°44' 33" S; 30° 57' 12.66" E; Altitude, 1477 m) in Zimbabwe (biotype 1) and the other was obtained near Nespruit (25°26' 25"S; 30°58' 57" E; altitude of 640 m) in the Mpumalanga province of South Africa (biotype 2). The *C. gynandra* biotypes visually differed in pod sizes; with the pods/capsules of biotype 1 being larger than those of biotype 2. All the *Amaranthus* biotypes were collected from the area surrounding the University of Zululand (28°51' 06" S; 31° 51' 08" E; altitude 102 m) in the KwaZulu Natal province of South Africa. The *Amaranthus* biotypes differed in leaf markings. Leaves of one of the types of *A. retroflexus* (biotype 1) were uniformly green whereas the leaves of the second type (biotype 2) bore leaf markings that were similar to those described by Schaffner (1915). On young leaves, they appeared as dark pink/red V-shapes in the middle of the leaf. On old leaves the markings turned papery whitish, giving a translucent appearance. With regard to the two biotypes of *A. hybridus*, leaves of biotype 1 had no markings, but leaves of biotype 2 had black spots (3 to 4 mm in diameter) along the mid-rib

area of the leaf. The leaf markings, however, tended to fade and disappear with leaf age.

Seed pre-germination treatments

The pre-germination treatments for breaking seed dormancy that were tested on the biotypes included seed aging, chilling (cold stratification), fluctuating temperature, light, gibberellins (GA₃) smoke, nitrogen (N) potassium nitrate (KNO₃), ammonium nitrate (NH₄)₂SO₄. With the exception of manure leachate, these treatments are frequently used to break seed dormancy in the laboratory (Krock et al., 2002; Flematti et al., 2004; Kucera et al., 2005; Finch-Savage and Leubner-Metzger, 2006; Tang et al., 2008; 2010). The molecular and physiological aspects associated with some of the treatments have been reviewed by Kucera et al. (2005), Finch-Savage and Leubner-Metzger, (2006) and Finkelstein et al. (2008). The cattle manure leachate treatment was tested on the assumption that it contains nitrogenous compounds NH₄⁺ and NO₃⁻ formed from the breakdown of manure by micro-organisms, which are known to affect seed germination (Tang et al., 2008; 2010) Also, both *C. gynandra* and the *Amaranthus* species have been observed to germinate prolifically and grow well on peripheries of cattle kraals.

Preparation of manure leachate

An amount of 200 g of cattle manure obtained from the dairy cow unit at the University of Zululand was mixed with 1000 ml of distilled water and left overnight on orbital shaker at low (= 125 rpm) speed. The liquid phase of the mixture was then filtered through Whatman filter paper (No. 42) to obtain a solid-free leachate.

Preparation of smoked water

An amount of 2.0 L of deionised water was placed in a 20 L plastic drum. Smoke was then passed into this drum through an opening at the top of the drum for 2 min via a 90 long x 4 cm diameter flexible tubing from an outlet at the top of a modified 20 L tin drum (Figure 1) in which was burning a mixture of dry and green eucalyptus leaves. At the end of 2 min, the flexible pipe was removed from the inlet of the smoke-receiving drum and the inlet closed tightly, and left overnight to allow the smoke to dissolve into the water.

Seed germination

There were three germination experiments conducted. In Experiment 1, freshly harvested, smoke-treated or non treated seeds of the two biotypes for each of the three species were incubated in 90 cm Petri dishes in darkness or light (main plots) on filter paper soaked/moistened with distilled water, smoked distilled water, cattle manure leachate, 100 µM gibberellic acid (GA₃), 1000 µM K₂SO₄, 1000 µM KNO₂ or 1000 µM NH₄SO₂ solutions (subplots) in five replicates, each of 25 seeds.

In Experiment 2, fresh seeds of the two biotypes of each of the three species were incubated on filter paper moistened with distilled water at constant 27°C or alternately at 27°C for 8 h and 4°C for 16 h in darkness. In Experiment 3, seeds of the *C. gynandra* and *A. retroflexus* biotypes were incubated at ambient temperature on filter paper soaked with distilled water after 3 months of storage at room temperature or at 4°C.

Data recording and analysis

In all three experiments, germination was deemed complete when

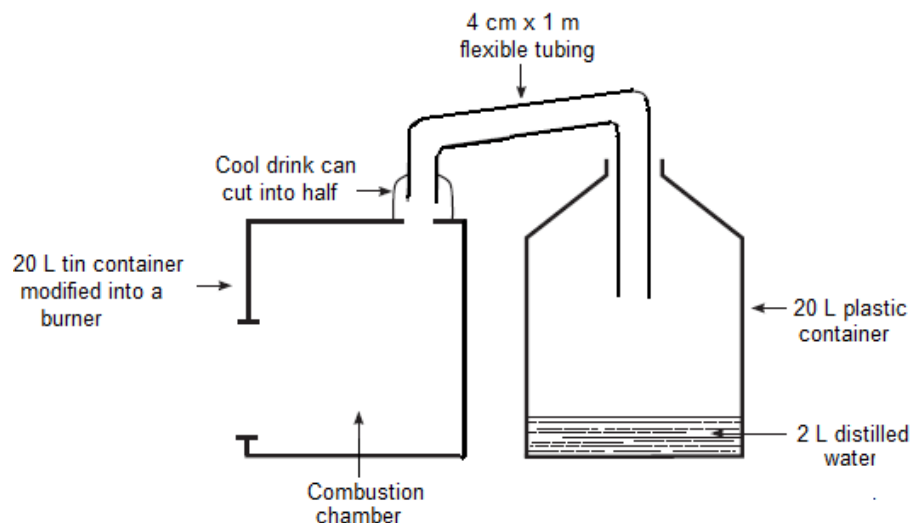


Figure 1. Apparatus used in production of smoke water.

Table 1. A comparison of seed germinability between biotypes of *A. hybridus*, *A. retroflexus* and *C. gynandra*.

Biotype	Mean seed germination (%)
<i>A. hybridus</i> 1	58.2
<i>A. hybridus</i> 2	50.0
Mean	54.1
LSD _{0.05}	4.07
<i>A. retroflexus</i> 1	80.95
<i>A. retroflexus</i> 2	3.10
Mean	42.02
LSD _{0.05}	1.733
<i>C. gynandra</i> ecoype1	68.95
<i>C. gynandra</i> biotype 2	17.52
Mean	43.24
LSD _{0.05}	3.170

the radical protruded approximately 2 mm. The germination was recorded twice daily for 21 days. Analysis of variance was performed on % germination using Genstart Discovery 3rd Edition. Means of the treatments within each species were separated by the least significance difference at 5% level (LSD_{0.05}).

RESULTS

Experiment 1

Seed germination of the *Cleome* and *Amaranthus* species tested in this study were strongly influenced by the biotype. In *A. retroflexus*, seed germination of biotype 2, with leaf markings was severely suppressed (mean

germination = ca 3%) compared to that for biotype 1 (mean germination = ca. 81%), which had no leaf markings. In *C. gynandra*, the mean percentage germination for biotype 1 (ca 69%) was 3.83-fold that for biotype 2 (ca 18%). With regards to *A. hybridus* biotypes, the mean percentage germination for biotype 2 was significantly lower than that for biotype 1, but the differences between the two biotypes of *A. hybridus* were much smaller than those obtained for biotypes of *A. retroflexus* or *C. gynandra* (Table 1). All biotypes of the two *Amaranthus* species examined were positively photoblastic, whereas those of *C. gynandra* were negatively photoblastic (Table 2). The effect of light on seed germination varied markedly among the biotypes in

Table 2. Effect of light on germination of *A. hybridus*, *A. retroflexus*, and *C. gynandra* biotypes.

Biotype	Percentage germination (%)		Mean
	Light	Dark	
<i>A. hybridus</i> biotype 1	71.0	45.5	58.2
<i>A. hybridus</i> biotype 2	68.9	31.0	50
Mean	69.9	38.3	54.1
LSD _{0.5} for light		4.07	
LSD _{0.5} for biotype x light		5.76	
<i>A. retroflexus</i> 1	84.29	77.62	80.95
<i>A. retroflexus</i> 2	5.71	0.48	3.10
Mean	45.00	39.05	42.2
LSD _{0.5} for light means		1.733	
LSD _{0.5} for biotype x light		2.451	
<i>C. gynandra</i> ecoype1	43.81	94.10	68.95
<i>C. gynandra</i> biotype 2	5.43	29.62	17.52
Mean	24.62	61.86	43.24
LSD _{0.5} for light		3.170	
LSD _{0.5} for biotype x light		4.482	

each of the three species. Light increased seed germination 1.6-fold in *A. Hybridus* biotype 1 with high germinability and 2.22-fold in *A. Hybridus* biotype 2 with low germinability. Similarly, in *A. retroflexus*, although seed germinability was lower in biotype 2 than in biotype 1, light increased germination ca 12-fold in biotype 1, but ca 1.1-fold in biotype 2 compared to germination in dark. In the negatively photoblastic *C. gynandra*, darkness increased seed germination 5.5-fold in biotype 2 which had low germinability compared to 2.15-fold for biotype 1 with higher germinability.

There were marked differences in the effects of imbibition solutions and their interactions with light and biotypes on seed germination of *C. gynandra* and the two *Amaranthus* species tested (Table 3). Between the two *C. gynandra* biotypes, the imbibition solutions did not significantly affect the germination of biotype 2 compared to distilled water under both light and dark conditions. In the case of biotype 1, compared to seed germination in distilled water, that of light-incubated seed was significantly reduced in all imbibition solutions, except smoked water which had similar germination level to that for distilled water (Table 3). By contrast, for dark-incubated seeds of *C. gynandra* biotype 1, there was a tendency for all imbibition solutions to increase seed germination compared to that in water.

The interactions of biotypes with light and imbibition solutions were more marked in the *Amaranthus* species than they were in *C. gynandra*. Exogenously applied GA₃ had no significant influence on seed germination of *A. retroflexus* biotype 2. In the case of *A. retroflexus* biotype 1, all replicates of seeds supplied with GA₃ in the presence of light achieved 100% germination. However,

the germination was not significantly different from that (98%) of seeds incubated in water in the presence of light nor from that (95%) of seeds incubated in GA₃ in darkness. Whilst GA₃ had no effect on germination of dark-incubated seeds of *A. hybridus* biotype 2 compared to water, it was synergistic to light in promoting seed germination of this biotype. The response of *A. hybridus* biotype 1 to GA₃ was different from that of *A. hybridus* biotype 2 in that its seed germination was not affected by GA₃ in the presence of light, but in dark-incubated seeds, GA₃ relieved the severe seed dormancy in *A. hybridus* biotype 1 to the same extent as did light (Table 3).

Light and (NH₄)₂SO₄ were synergistic on seed germination of *A. hybridus* biotype 1, but not *A. hybridus* biotype 2. Rather, (NH₄)₂SO₄ was antagonistic to light on germination of the highly positively photoblastic seeds of *A. hybridus* biotype 2 even though (NH₄)₂SO₄ did promote germination of this biotype in darkness as it did in seeds of *A. hybridus* biotype 1 and *A. retroflexus* biotype 1. Light and NH₄SO₄ were also synergistic on seed germination of *A. retroflexus* biotype 2 although the effect was small. In dark-incubated seeds of *A. retroflexus* biotype 2, (NH₄)₂SO₄ had no influence on seed germination.

Smoke treatment severely inhibited seed germination of *A. hybridus* biotypes and *A. retroflexus* biotype 1 in the dark. However, in both *A. hybridus* biotypes, light was able to overcome/relieve the inhibitory effect of smoke water on germination of some of the seeds, but not in *A. retroflexus* biotype 1 whose light-incubated seeds were completely inhibited from germination by smoke water as was the case for its dark-incubated seeds. Seed germination of *A. retroflexus* biotype 2 was slightly

Table 3. Interactions of solution and light on germination of *A. hybridus*, *A. retroflexus* and *C. gynandra* biotypes.

Solution treatment	Percentage germination											
	<i>A. hybridus</i> 1		<i>A. hybridus</i> 2		<i>A. retroflexus</i> 1		<i>A. retroflexus</i> 2		<i>C. gynandra</i> 1		<i>C. gynandra</i> 2	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
100 μ M GA ₃	76.7	74.7	99.3	36.0	100.0	95.3	6.67	0.67	42.00	92.00	6.67	33.33
Deionised water	85.3	44.0	74.0	28.0	98.8	72.7	7.33	0.67	56.00	89.33	5.33	30.00
Smoke water	36.0	0.0	30.7	6.0	0.00	0.0	8.00	1.33	57.33	98.00	5.33	34.67
1000 μ M KNO ₃	85.3	46.0	72.7	40.0	99.3	97.3	2.00	0.67	35.33	90.00	5.33	30.67
1000 μ M K ₂ SO ₄	70.7	48.7	83.3	33.3	98.7	88.7	2.00	0.00	30.67	99.33	4.67	27.33
1000 (NH ₄) ₂ SO ₄	92.0	62.0	54.0	44.0	97.3	91.3	14.00	0.00	41.33	94.00	6.67	30.67
Cattle manure leachate	50.7	43.3	68.0	30.0	96.0	98.0	0.00	0.00	44.00	96.00	4.00	20.67
Mean	71.0	45.5	68.9	31.0	84.3	77.6	5.7	0.5	43.8	94.1	5.4	29.6
LSD _{0.5}			15.24				6.48				11.86	

improved by smoke in the dark.

Compared to water, solutions of KNO₃ and K₂SO₄ significantly increased seed germination of *A. retroflexus* biotype 1 in dark-incubated seeds, but did not significantly affect seed germination in the other biotypes whether administered alone or in combination with light. Cattle manure leachate was antagonistic to light on seed germination of both *A. hybridus* biotypes, and in the case of *A. retroflexus* biotype 2, none of the seeds incubated with manure leachate germinated.

Experiment 2

Alternating temperature (4°C for 16 h/27°C for 8 h) had no effect on the germination of *A. retroflexus* biotype 1, *C. gynandra* biotype 1 and the two *A. hybridus* biotypes (Figure 2). In all these biotypes, seed germination was $\geq 87\%$ in both constant and alternating temperature treatments. By contrast, there were significant differences in germination of *A. retroflexus* biotype 2 and *C. gynandra* biotype 2 between constant and alternating temperature treatments (Figure 2). In the case of *A. retroflexus*

biotype 2, seed germination was 26% at constant temperature. The germination was increased ca. 3.4-fold to 89% when the seeds were incubated at alternating temperatures. In *C. gynandra* biotype 2, seed germination was 55% at constant temperature, and decreased significantly to 44% in seeds incubated at alternating temperatures.

Experiment 3

As in Experiment 1, the germination of biotype 2 was less than that for biotype 1 in both *C. gynandra* and *A. retroflexus* (Table 4). Also, consistent with the results obtained in Experiment 1, light was inhibitory on the germination of both fresh and three-month old seeds of both biotypes of *C. gynandra*. Generally, germination was less in fresh than in the aged seeds of *C. gynandra*, irrespective of the storage temperature (Table 4). In *A. retroflexus*, seed germination of biotype 1 tended to decrease following 3 months of dry storage, but significantly so for seeds stored at ambient temperature. In the case of *A. retroflexus* biotype 2, seed germination markedly improved

from a mean of 2.4% in fresh seeds to 37.16% in 3-months old seeds stored at ambient temperature, and was accompanied by loss in sensitivity to light. By contrast, there was only a slight increase in germination of seeds for the same biotype stored at 4°C (Table 4).

DISCUSSION

Differential germination between biotypes

Germination is one trait that has been found to greatly vary among populations of non-domesticated plant species (Baskin and Baskin, 1998). The variations have been attributed to dormancy traits arising from habitat-specific genetic differentiation that attune seed germination to local environmental cues that synchronise germination with periods that are optimal for seedling survival (Linhart and Grant, 1996). The genotypic differentiation gives rise to biotypes/ecotypes with site-specific, adaptive germination traits (Turesson, 1922; Veasey et al., 2004). In the present study, there were indeed

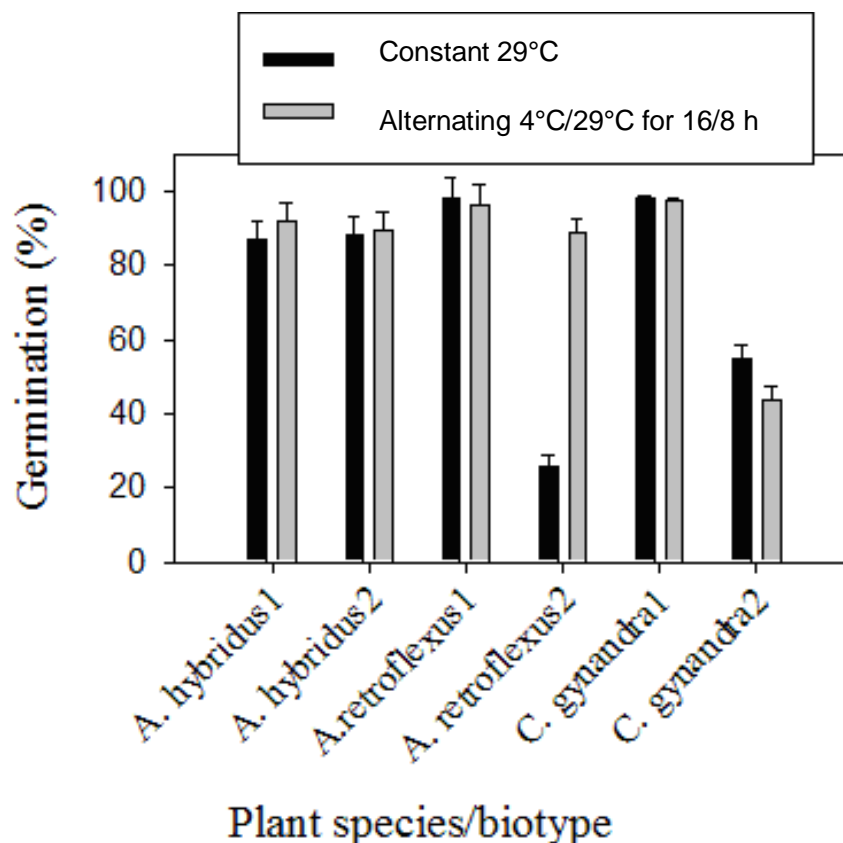


Figure 2. Effect of alternating temperature on germination of *A. hybridus*, *A. retroflexus* and *C. gynandra* biotypes.

marked differences in seed germination between the biotypes of *C. gynandra*, *A. hybridus*, and *A. retroflexus* (Table 1). The mean percentage germination differed between the biotypes by a factor of 0.2 for *A. hybridus*, 3 for *C. gynandra* and 26 for *A. retroflexus*. These differences emanated from differences in seed dormancy expression, and were assumed to reflect either habitat-specific selection in *C. gynandra* biotypes that were collected from different environments or habitat-related genetic drift in *A. hybridus* and *A. retroflexus* biotypes that were collected from similar environments.

In addition to differences in the expression of seed dormancy, the biotypes differed in environmental requirements for breaking seed dormancy (Tables 2, 3 and 4 and Figure 2). In the *Amaranthus* species, although, all biotypes were positively photoblastic, there were differences between the biotypes in the extent to which light promoted seed germination (Table 2). This was particularly marked between biotypes of *A. retroflexus* (Table 2). Similarly, whilst both biotypes of *C. gynandra* were negatively photoblastic, as has been reported previously (Ochuodho and Modi, 2005; Ekpong, 2009; Raboteaux, and Anderson, 2010), there were marked differences in the extent to which darkness promoted germination between the two biotypes (Table 2). These

differences between biotypes were interpreted as reflecting on differential dormancy mechanisms that required different conditions for releasing the seeds from dormancy. In the case of *A. hybridus* and *A. retroflexus*, there were differential interactions of light with GA₃, smoke water and (NH₄)₂SO₄ on seed germination between the biotypes of each of the species (Table 4), which also emphasised the existence of different types of dormancy mechanisms between the biotypes in each of the two *Amaranthus* species tested. In *A. retroflexus*, this was further corroborated by the requirements for alternating temperature to break dormancy in biotype 2, whereas this requirement was absent in biotype 1 (Figure 2) as was also the case with both biotypes of *C. gynandra*. It, thus, seems most likely that the variations in germination requirements for *C. gynandra* (Ochuodho et al., 2004; Ochuodho and Modi, 2005; Ekpong, 2009; Raboteaux and Anderson, 2010) and *Amaranthus* species (Ellis et al., 1985; Aufhammer et al., 1998; Gallagher and Cardina, 1998; Steckel et al., 2004) in the literature may arise from biotype/ecotype or provenance-related differences in dormancy mechanism emanating from local adaptations. Consequently, the presence of between-biotype/provenance differences in dormancy and environmental requirements for breaking seed

Table 4. Interactions of light and seed storage temperature for three months on the germination of *C. gynandra* and *A. retroflexus* biotypes.

Species/ biotype	Percentage germination							LSD _{0.05}
	Fresh seeds		Three month old seed stored at room temperature		Three month oldseed stored at 4°C temperature		Mean	
	Light	Dark	Light	Dark	light	Dark		
<i>C. gynandra</i> 1	26.00	50.8	44.00	73.60	45.26	74.34	52.33	3.6
<i>C. gynadra</i> 2	6.72	29.28	18.16	46.16	17.52	44.00	26.97	4.1
<i>A. retroflexus</i> 1	90.08	89.36	68.24	77.92	88.56	87.92	83.68	3.6
<i>A. retroflexus</i> 2	2.72	1.76	31.44	42.88	6.96	2.56	14.72	5.1

dormancy such as those observed in this study point towards the need for testing germination requirements for each seed provenance of these less domesticated crops before planting.

Differential germination responses of *Amaranthus* biotypes to GA₃ and light

Gibberellic acid is known to break seed dormancy of several plant species (Peng and Harberd, 2002; Kucera et al., 2005). In this study, the effectiveness of GA₃ in releasing seeds of positively photoblastic seeds from dormancy was variable, and depended on species, biotype and light conditions (Table 4). Noteworthy in the current study is the synergistic interaction of GA₃ and light on seed germination. It is widely accepted that the requirement for light in releasing seeds from germination is partly linked to activation of GA₃ synthesis (Penfield et al., 2005) whereby light activates GA₃ synthesis by elevating transcript levels for GA₃ oxidase genes that encode the final enzyme of the GA₃ biosynthetic pathway (Yamaguchi et al., 1998; Yamauchi 2004; Ogawa et al., 2003). Thus, GA₃ can substitute for light requirements in breaking seed dormancy in plant species whose seeds are positively photoblastic (Hilhorst and Karssen, 1992). There are, however, cases in which GA₃ has failed to replace the

requirement for light. For example, detailed studies with GA₃ (50 mg L⁻¹) by Bell et al. (1999) showed that increased percentage germination of the positively photoblastic species, *Oenothera stricta*, occurred in the light, but blocking endogenous gibberellic synthesis with paclobutrazol, or adding exogenous GA₃ had no effect on the light-induced germination levels. In the current study, exogenously applied GA₃ replaced the requirement for light in *A. hybridus* biotype 1 (Table 4), which is consistent with the activation role of light on GA₃ synthesis (Penfield et al., 2005). However, the hormone failed to substitute for light in dark-incubated seeds of *A. hybridus* biotype 2 as reported for *Oenothera stricta* by Bell et al. (1999) even though the seeds of both species are positively photoblastic (Table 4). Rather, light and GA₃ acted synergistically in releasing the seeds of this biotype from dormancy (Table 4), which cannot be explained by stimulation of GA₃ synthesis. Similarly, there was a tendency for seed germination to respond more to GA₃ in the presence than in the absence of light in *A. retroflexus* biotype 1. The synergism between light and GA₃ suggests that the mechanisms of light effects on seed germination may also involve enhancing the sensitivity/responsiveness of the seeds to GA₃ in some species rather than stimulating synthesis of GA₃ (Hilhorst and Karssen, 1992). The differential interaction

between light and GA₃ among *A. hybridus* biotypes provides indirect evidence that there is more than one physiological process that requires light for breaking seeds dormancy, and the processes differ in sensitivity to GA₃.

It is also noteworthy that it was a fraction of seeds in the seed lots of *A. hybridus* and *A. retroflexus* biotypes 2 whose release from dormancy was promoted by GA₃ in the presence of light. Again this indicates multiple strategies for controlling the release of seeds from dormancy whereby a fraction of the seeds reacts differently from the rest in response to dormancy release factors. The variable dormancy and polymorph germination in other *Amaranthus* species has been attributed to maternal, genetic and environmental factors (Costea et al., 2004). It is not difficult to understand why plants may have variable or multiple dormancy mechanism. In the wild, multiple strategies for breaking seed dormancy ensure a persistent seed bank (Costea et al., 2004) and also act together to fine-tune the release of dormancy to ensure seed germination. Light was not a principal factor in breaking seed dormancy in *A. retroflexus* biotypes (Table 4). In the case of *A. retroflexus* biotype 1, the dark-incubated seeds showed little dormancy, hence, though present, the impact of light was small (Tables 2 and 4). With respect to *A. retroflexus* biotype 2, although, the dark-incubated seeds had

extremely low germination, a very small percentage of the seeds responded to light, and neither were the seeds of this biotype responsive to GA₃ (Table 4). Rather, the severe seed dormancy in *A. retroflexus* biotype 2 was completely alleviated by incubation of imbibed seeds alternately at 27°C for 8 h and 4°C for 16 h in darkness (Figure 2). The later scenario with *A. retroflexus* biotype 2 exemplifies another case of multiple dormancy mechanisms showing a small fraction of seeds requiring light and a larger fraction requiring fluctuating temperature to be released from dormancy.

Light inhibition of *Cleome* seed germination

It is generally assumed that germination of small-seeded species is positively photoblastic (Milberg et al., 2000; Jankowska-Blaszczuk and Daws, 2007), which enable the species to germinate on top of the ground or where the resultant seedling can immediately take advantage of light to synthesize food before the storage food reserves of the small seed run out. In spite that *C. gynandra* is small-seeded, reports on its germination in the literature consistently show that its seeds are negatively photoblastic (Raboteaux and Anderson, 2010; Ochuodho and Modi, 2007) as was the case in the present study (Table 4). Ochuodho and Modi (2007) attributed the negative photoblastic reaction of *C. gynandra* seeds to biosynthesis inhibition of gibberellins by light taking into cognisance that photo-inhibited seeds recovered germination capacity after treatment with GA₃ (Ochuodho, 2005). Different to the results of Ochuodho (2005), in the present study, 100 µM GA₃ did not relieve photo-inhibition on seed germination of *C. gynandra* (Table 4). In this respect, the results of the current study were in agreement with those obtained by Bell et al. (1999) who did not get a positive response to GA₃ treatment in the germination of the negatively photoblastic species *Trachyandra divaricata*.

Effects of potassium and nitrogenous compounds on seed germination of *Cleome* and *Amaranthus* biotypes

Solutions of K salts and nitrogenous compound have all been previously implicated in relieving seeds from dormancy (Mayer and Polkjaoff-Mayber, 1963; Hendricks and Taylorson, 1972; Bell et al., 1999; Pérez-Fernández and Rodríguez-Echeverría 2003; Alboresi et al., 2005; Vandellook et al., 2008). In the present study, 1000 µM solutions of both KNO₃ and K₂SO₄ were generally ineffective in breaking seed dormancy in fresh seeds of *C. gynandra* and the two *Amaranthus* species tested (Table 4). Many seeds that respond positively to light have also been observed to respond positively to NO₃⁻, and studies that show a positive interactions (synergism)

between NO₃⁻ and light in positively photoblastic species are also common (Bungard et al., 1997; Bell et al., 1999; Tang et al., 2008; 2010). In contradiction to these studies, in the current study, there was no positive interaction between KNO₃ (1000 µM) and light on seed germination of the two *Amaranthus* species tested despite that their seeds were positively photoblastic. In contrast to KNO₃, 1000 µM (NH₄)₂SO₃ interacted positively with light on seed germination of *A. hybridus* biotype 1 and *A. retroflexus* biotype 1 (Table 4). However, in *A. hybridus* biotype 2, (NH₄)₂SO₄, interacted negatively with light on seed germination, which once again emphasized genetic differences between the biotypes in the control of seed dormancy in both *Amaranthus* species.

Effects of manure leachate on seed germination of *Cleome* and *Amaranthus* biotypes

C. gynandra and many of the *Amaranthus* species characteristically emerge prolifically and grow vigorously in places peripheral to cattle kraals and in deposit areas for refuse and ash at homesteads in Africa. It is therefore reasonable to assume that nitrogenous compounds among others in the leachate from cattle manure and refuse and the “smoke effect” in the ash may be responsible for high plant emergence in these areas in addition to supporting good plant growth of *Cleome* and *Amaranthus*. The cattle manure leachate was thus expected to positively affect seed germination via enhancement by NH₄⁺ and NO₃⁻ forms of N from organic matter decomposition. The effects of cattle manure leachate on seed germination did not however mirror the effects of any of the N forms (NH₄⁺ or NO₃⁻). In contrast to (NH₄)₂SO₄ which had synergism with light on seed germination of *A. hybridus* biotype 1, cattle manure leachate was antagonistic to light on the germination of this biotype. It also had a tendency to antagonise light on the germination of *A. hybridus* biotype 2. The basis of the antagonistic effects of cattle manure leachate on germination is unknown.

Effects of smoke on seed germination of *Cleome* and *Amaranthus* biotypes

Smoke has in recent years received considerable attention in relation to its promotion on seed germination (Van Staden et al., 2000; Pérez-Fernández and Rodríguez-Echeverría, 2003), its promotive effect being attributed to butenolide, 3-methyl-2Hfuro[2,3-c]pyran-2-one, a compound found in plant material derived smoke (Van Staden et al., 2004; Flematti et al., 2004; 2005). It is, however, emerging that smoke may also have negative effects on seed germination. Roche et al. (1997) observed that smoke from plant derived material inhibited the germination of *Bursaria spinosa*, *Drosera gigantea*,

Ptilotus polystachys and *Schoenia cassiniana*. Also, Gomez-Gonzalez et al. (2008) noted that smoke reduced germination in 8 of the 18 plant species from the Chilean matorral vegetation they tested. In the present study, smoke from burning leaves of a *Eucalyptus* spp. suppressed seed germination of the two *Amaranthus* species tested, but not that of *C. gynandra*. Roche et al. (1997) suggested that the negative effects of smoke in sensitive species may emanate from supra-optimal concentration of smoke derivatives responsible for enhancing germination. Recently, Light et al. (2010) discovered a butenolide (3,4,5-trimethylfuran-2 (5H)-one) from plant-derived smoke, which has antagonistic actions on seed germination. Therefore, as suggested by Light et al. (2010), there is a possibility that the effect of smoke on seed germination could depend on the balance between the germination promoting butenolide (3-methyl-2H-furo[2,3-c]pyran-2-one) and the germination inhibiting butenolide (3,4,5-trimethylfuran-2(5H)-one), and the differential responses of plant species to smoke may be related to their sensitivity to the ratio of the two butenolides.

Effects of seed storage period and temperature on germination of *Cleome* and *Amaranthus* biotypes

The post harvest duration of dry seed storage (after ripening) is an important factor in the dormancy release equation of seeds, and is a common requirement for loss of primary dormancy and for promoting seed germination (Bewley, 1997; Leubner-Metzger, 2003; Kucera et al., 2005; Finch-Savage and Leubner-Metzger, 2006). Yepes (1978) determined that *C. gynandra* seeds have a rest period (latency) that extends to the 5th month after collection, and active germination starts 6 months after harvest. In contrast Ekpong (2009) determined that *C. gynandra* seeds broke dormancy in three months after harvest whether stored at room temperature or at 15°C. The current study tested the effect of a three-month seed storage period on seed germination of *C. gynandra* and *A. retroflexus* biotypes stored at room temperature (20 to 32°C) or at cold temperature (4°C), and the results show differential effects of the three-month seed storage period and of storage temperature on seed germination of the biotypes tested in both species. Seed germination in dark of *C. gynandra* biotype 1 increased 1.45-fold from ca 51% in freshly harvested seeds to ca.74% after three month storage, irrespective of the storage temperature. In *C. gynandra* biotype 2, storage temperature did not affect germination as in biotype 1, which was consistent with results obtained by Ekpong (2009). However, although, seed germination of *C. gynandra* biotype 2 in the dark increased after three month storage, it was below 50% (≤46.16%) compared with that (74%) of biotype 2. Thus, it does seem from the current results that the duration of after-ripening requirement for *C. gynandra* depends on

provenance or biotype, hence the discrepancies between reports for this species. Both biotypes of *C. gynandra* were still strongly and negatively photoblastic at three months of storage, which appears to indicate that this feature may not be subject to change by an after-ripening period. With respect to *A. retroflexus*, germination of seeds stored at room temperature decreased after three months of storage in biotype 1, which had very little dormancy in freshly harvested seeds. The cause for the decrease in seed germination of *A. retroflexus* biotype 1 was not determined, but induction of secondary dormancy by the storage conditions cannot be ruled out. In biotype 2, which had strong dormancy in freshly harvested seed (germination<3%), there was improvement in germination of three-month old seeds stored at room temperature (ca. 31 and 43% germination in light and dark, respectively), but dormancy in seeds stored at 4°C, was maintained as in the fresh seeds. In addition to improvement in germination of three-month old seeds in *A. retroflexus* biotype 2, the tendency for requirement for light was absent in seeds stored at room temperature.

Conclusion

The study revealed large differences in the expression of seeds dormancy and associated environmental requirements for breaking seed dormancy between biotypes in each of the three species (*C. gynandra*, *A. hybridus*, and *A. retroflexus*) tested. The differences were interpreted as reflecting on either habitat-specific driven selection in *C. gynandra* biotypes that were collected from different environments or habitat-related genetic drift in *A. hybridus* and *A. retroflexus* biotypes that were collected from similar environments.

The biotypes of the two *Amaranthus* species tested were positively photoblastic, whereas those of *C. gynandra* were negatively photoblastic, and in both cases the response to light varied with the biotype. Interactions of light and GA₃ on the germination of positively photoblastic seed of *Amaranthus* biotypes suggested that there were two different light-related physiological processes/mechanisms involved in the release of positively photoblastic seeds from dormancy, and the two mechanisms are affected by GA₃ differently. In one mechanism, exogenously applied GA₃ can replace the requirement for light as it did in *A. hybridus* biotype 1. In the second mechanism, light requirement is not replaced by GA₃, but rather, GA₃ is synergistic to light in releasing seed germination as was the case in *A. hybridus* biotype 2. The former mechanism in which GA₃ replaces light is consistent with the reports that light releases dormancy by stimulating GA₃ synthesis, whereas in the later mechanism, light appears to be required to enhance the sensitivity of the seeds to GA₃. The two mechanisms may act independent of each other as exemplified by the two *A. hybridus* biotypes.

With respect to other imbibition solutions tested, 1000 μM solutions of both KNO_3 and K_2SO_4 were generally ineffective in breaking seed dormancy in fresh seeds of *C. gynandra* and the two *Amaranthus* species tested, whereas 1000 μM $(\text{NH}_4)_2\text{SO}_4$ interacted positively with light on seed germination of at least one biotype each of *A. hybridus* and *A. retroflexus*. Smoke water was antagonistic to seed germination of all *Amaranthus* biotypes to different degrees, but did not affect the germination of *C. gynandra* biotypes. Also, cattle manure leachate was generally antagonistic to germination of *Amaranthus* biotypes.

A three-month storage period improved the germination of the two *C. gynandra* biotypes tested to different extents, but the storage temperature did not affect the final germination %. Also, the three-month storage period did not affect the negative photoblastic nature of *C. gynandra* seeds. In *A. retroflexus* there was differential sensitivity of seed germination to storage and to storage temperature between its biotypes, which reflected differences in expression of seed dormancy.

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