2000

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Widrlechner, M. P. and Kovach, D. A. (2000), *Seed Sci. & Technol.*, **28**, 11–27

Dormancy-breaking protocols for *Cuphea* seed

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(Accepted August 1999)

Summary

Several species of *Cuphea* exhibit primary seed dormancy. This hampers viability testing and seed regeneration for germplasm managers, plant breeders, and other researchers. An accurate viability test involving embryo excision has been developed. This research explores alternatives to that labor-intensive technique. Seeds of *C. viscosissima* Jacq. lost much of their dormancy after 4 to 6 months of cold, moist storage or after four years of dry storage at room temperature. In either situation, an alternating temperature regimen with light was required during the germination test for optimal nonexcised results. Seeds of *C. viscosissima*, after-ripened at room temperature for seven weeks after harvest, responded well to an alternating temperature regimen with light, if the seeds were first placed under high-humidity conditions in accelerated aging boxes (termed herein as accelerated after-ripening). Response to accelerated after-ripening treatments by older samples of nine other species of *Cuphea*, obtained from the germplasm collections of the North Central Regional Plant Introduction Station, was generally inferior to results obtained by the use of embryo excision. It is possible that the mechanical methods used during the harvesting of the older samples contributed to premature loss of viability and vigor in these seeds. Also, experiments comparing germination tests of freshly-harvested seeds with tests of the same seeds approximately one year later showed that the benefits obtained by using the accelerated after-ripening technique versus controls were less dramatic over time, as increased germinations, probably due to natural after-ripening of the seeds, reduced differences in germination percentages between the two treatments.

Introduction

The monitoring of seed viability is a critical function in conservation of plant genetic resources. Germplasm management decisions are regularly based upon the quantity and quality of each accession held in an active genebank (Sackville Hamilton and Chorlton, 1997). Reliable protocols for seed germination are essential to ensure that genetically representative populations can be produced for multiplication and research. Standards for viability testing have been established (Ellis, Hong and Roberts, 1985) to assist germplasm managers as they assess seed viability, one of the most important determinants of seed quality. For many domesticated, seed-propagated crops, controlled germination tests give reliable measures of seed viability, and established procedures for germination testing for many commonly cultivated crop species are now available (Association of Official Seed Analysts, 1993; International Seed Testing Association, 1993).

Many wild and weedy crop relatives, which may possess extremely valuable genetic diversity, display dormancy characteristics quite unlike their domesticated counterparts.

Relatively little attention has been paid to wild plant species, whose seeds are generally not traded or commercially tested. These taxa pose a special challenge to managers of germplasm collections (Widrlechner, 1997).

In the mid-1980s, the North Central Regional Plant Introduction Station (NCRPIS), in Ames, Iowa, USA, began a long-term project to collect and evaluate germplasm of *Cuphea*, as part of a national breeding and selection effort to develop *Cuphea* as an oilseed crop (Knapp, 1993; Roath, Widrlechner and Kleiman, 1994). *Cuphea* seeds are rich sources of short- to medium-chain fatty acids, with many potential industrial applications. Recent work by S. J. Knapp (1998, written communication) has shown *Cuphea* seed yields sufficient to support the profitable cultivation of this genus as a field crop.

As the NCRPIS assembled collections of *Cuphea* germplasm, it became evident that viability testing would be a limiting factor in managing these collections. Little was known about seed longevity in storage or about methods to induce uniform seed germination. Plant breeding efforts have greatly reduced the dormancy of *C. laminuligera* Koehne and *C. lanceolata* Aiton seeds (Knapp, 1990; Knapp and Tagliani, 1990). Another complicating factor is the indeterminate flowering nature of *Cuphea*. This causes various levels of seed maturity at harvest, hampering efforts to obtain seeds that would store well and germinate easily. One method to induce uniform seed germination, embryo excision (Roath and Widrlechner, 1988), is time consuming, requiring a degree of manual dexterity not needed for typical germination tests. Also, many of the original seed samples that were acquired were too small to allow replicated testing or other destructive experimentation, a common problem for germplasm collections.

This paper reports on a series of experiments conducted to develop alternatives to embryo excision for the viability assessment of *Cuphea* germplasm collections. Tests were conducted both with high-quality seed lots produced specifically for experimentation and with older seed lots of varying quality held by the NCRPIS. A cross-section of taxa was examined to include both biosystematic and chemical (seed oil) diversity.

One experiment consisted of using various alternating temperatures with lighting under high-humidity conditions. This differed from accelerated ageing treatments or from partial-hydration cold stratification in that the temperatures utilized were in a range intermediate between those typical for such treatments. It also differed from priming in that no osmotic solutions or direct contact with the solutions or solid matrix material were required. It differed from all of these in that it used alternating temperatures and lighting, yet was similar to all of these in that the seed was partially hydrated. Its main purpose was to break seed dormancy and the desired effect can occur more rapidly than via cold stratification or natural after-ripening, hence the term 'accelerated after-ripening' is used in this paper.

Materials and methods

A) Seed samples for 1993 and 1994 harvested seeds

Seeds of *C. viscosissima* (PI 534743) were planted and grown in a greenhouse at the NCRPIS during the summers of 1993 and 1994, with harvests on October 12, 1993 and

September 7, 1994. The *Cuphea* plants were grown in a 1:1:1 mixture of Sunshine Agricultural Plug 5 Mix, sand, and a local soil mixture of Nicollet Loam and Clarion Loam. The plants were cultured in pots subirrigated in 1–3 cm of water, supplemented with Peters 21–5–20 fertilizer when the plant leaves began losing their dark green color. Supplemental light (16,000 lumen/m^2, measured at the plant canopy) for 14 hours per day was provided using high-intensity discharge, high-pressure sodium lighting. At the time of harvest, entire plants, still actively growing and flowering (*C. viscosissima* has indeterminate seed production), were bagged in polyester mesh bags. The determining factors for the time of harvest were that many seeds had been produced by the plant, the color of most of the seeds set had turned from green to brown, and that some seeds were beginning to shatter to the ground. The aerial portions from plants of each harvest were dried in a forced-air dryer for 3 to 4 days at 27 °C, followed by one week drying at 27 °C in a room where air was slowly circulated. The seeds were then removed from the plants and allowed to after-ripen at room temperature (23 °C) and approximately 40% RH for an additional $5^1/_2$ weeks, or a total of 7 weeks from harvest to end of the initial after-ripening period. Moisture content (MC) was determined using the International Seed Testing Association (1993) protocol for oil-type seeds. After drying and equilibration, the 1993 seed lot reached a MC of 5.4% wwt, and the 1994 seed lot reached a MC of 4.7% wwt. Seeds used for the *(2) Dry storage treatment of 1993 seeds* study were never stored in the cold, but were kept at room temperature in a manila-type paper envelope. Seeds from the 1993 harvest were used in the *(1) Cold treatments of 1993 and 1994 seeds*, the *(2) Dry storage treatment of 1993 seeds*, and *(3) Alternating-temperature treatments of 1993 seeds* experiments described below. Seeds from the 1994 harvest were used in *(1) Cold treatments of 1993 and 1994 seeds* and *(4) Accelerated after-ripening treatments of 1994 seeds*, which were designed on the basis of results from *(3) Alternating-temperature treatments of 1993 seeds*.

(1) Cold treatments of 1993 and 1994 seeds

Experiments were conducted to measure the effects of the duration of moist, cold treatments on seed germination in *C. viscosissima*. Six replications of 50 seeds each from the 1993 harvest were imbibed with 25 ml of distilled water on two germination papers in plastic boxes (10 cm × 10 cm) at room temperature (about 23 °C) overnight and then held at 4 °C in darkness for defined periods between 1 week and 6 months. Upon removal from the cold treatments, the boxes were transferred to germination chambers programmed for 14 hours at 30 °C with fluorescent light alternating with 10 hours at 20 °C in darkness per 24 hours (typically designated herein as 30C,L,14h : 20C,D,10h). Germination tests lasted 21 days. Lighting, in this and subsequent experiments, consisted of six 15-watt fluorescent light tubes in the germination chamber; illumination was from the sides of the chamber. Three replications were wrapped in aluminum foil during the germination test to exclude light.

Four replications of 50 seeds each from the 1994 harvest were treated as the 1993 seeds, except that the longest cold treatment was 4 months rather than 6 months and that no replications were wrapped to exclude light.

(2) Dry storage treatment of 1993 seeds

Seeds of the 1993 harvest of *C. viscosissima* were stored at room temperature (about 23 °C) and approximately 50% RH (controlled by a room dehumidifier) and were sampled at 6 weeks (to allow for possible after-ripening), and 5, 12, 18, 24, 30, 36, and 48 months after harvest. Four replications of 50 seeds each were germinated as described in *(1) Long-term, moist, cold treatment of 1993 seeds*. Seeds also were germinated at a constant 23 °C temperature with and without illumination. At the close of this experiment, 48 months after harvest, two additional tests were performed to assess viability. The first test was conducted by treating the ungerminated seeds with an aqueous solution containing both 1 mM GA_3 and 12mM KNO_3; any seeds that did not germinate under those conditions also had their seed coats cut. These remained in the germination chambers for an additional two weeks. The second treatment consisted of an accelerated after-ripening protocol, similar to that described in *(4) Accelerated after-ripening treatments of 1994 seeds*, except the two-week treatment was replaced with one-week at 30C,L,14h : 20C,D,10h followed by one week at 30C,L,14h : 5C,D,10h. These seeds were then placed in germination boxes on two blotter papers (10.4 cm × 12 cm, each paper 1 mm in thickness), with 25 ml distilled water, and placed in germination chambers (30C,L,14h : 20C,D,10h) for 21 days.

(3) Alternating-temperature treatments of 1993 seeds

In initial experiments using alternating temperature regimens, the use of KNO_3, GA_3, KNO_3 + GA_3 solutions, as well as the use of accelerated ageing trays also were considered. Three replicates of 50 seeds each of the 1993 harvest of *C. viscosissima* were tested on germination paper with and without KNO_3 [1 mM], GA_3 [1 mM], or KNO_3 [1 mM] + GA_3 [1 mM]. The germination test (30C,L,14h : 20C,D,10h) lasted 21 days. Three replicates of 50 seeds each of the 1993 harvest of *C. viscosissima* were treated for four days on standard germination paper and above water using accelerated-ageing trays as described below in *(4) Accelerated after-ripening treatments of 1994 seeds* in germination chambers (30C,L,14h : 20C,D,10h). This was followed by a germination test under the same temperature-light regimen for 21 days.

(4) Accelerated after-ripening treatments of 1994 seeds

This experiment was conducted to investigate whether an accelerated after-ripening technique could be used to shorten the long after-ripening period noted in *(2) Dry storage treatment of 1993 seeds*. Three replications of 50 seeds each of the 1994 harvest of *C. viscosissima* were held above distilled water using an accelerated ageing box (McDonald and Phaneendranath, 1978). The seeds were placed on a layer of white polyester mesh cloth (Style NC4, wales 16, courses 21; Apex Mills Corp, NY) held on top of the box's brass mesh screen. Boxes were placed in germination chambers for 7 to 28 days under fluorescent lights for 14 hours alternating with 10 hours of darkness and various temperature regimens. Constant temperatures tested were 30, 25, 20, 15, 10, and 5 °C. Alternating temperatures tested were 30/25, 30/20, 30/15, 30/10, and 30/5 °C. The high temperatures were for 14 hours and the low temperatures were for 10 hours per 24-hour

cycle, corresponding to the alternating light and dark cycle, mentioned above. Germination tests lasted 21 days. The seeds were then transferred by removing the polyester cloths from the accelerated ageing boxes and placing them on top of two blotter papers in germination boxes wetted with 25 ml distilled water. The polyester cloth was required because of the development of epidermal hairs on the seed coats (Stubbs and Slabas, 1982). Without the polyester cloth, removal of the seeds from the brass mesh screen of the accelerated ageing boxes was quite difficult as the epidermal hairs were entwined around the mesh screen. Moving the seeds with the polyester mesh cloth was easy as the seeds' epidermal hairs were entwined in the polyester mesh cloth and there was little chance of losing the seeds during the transfer. The germination boxes were placed in germination chambers (30C,L,14h : 20C,D,10h) for 21 days. A MC determination test (based on four replicates) was done on seeds experiencing direct imbibition on blotter paper or seeds experiencing the accelerated after-ripening treatment after three days in germination chambers (30C,L,14h : 20C,D,10h). Measurement after three days was chosen to determine the MC before seeds germinated on paper or developed much contaminate fungi during the accelerated after-ripening treatment. The MC of directly imbibed seed was 61% wwt. The MC of the accelerated after-ripening treated seeds was 33% wwt.

(5) Comparison of treatments after one year of storage
Several experiments of the 1994 *C. viscosissima* seed harvest were conducted 12 and 13 months after harvest. The seeds had been kept in a sealed glass jar at 4 °C. Three replications of 50 seeds each were subjected to 30/5, 25/5, 20/5, and 15/5 °C (high temperatures,L,14h : low temperatures,D,10h) on blotter papers or accelerated after-ripening treatments for 14 days, followed by a germination test (30C,L,14h : 20C,D,10h) for 21 days. Untreated controls were also tested.

(6) Embryo excision test of the 1993 seeds
Viability of the 1993 *C. viscosissima* seeds used in *(1) Long-term, moist, cold treatment of 1993 seeds* was determined by using the excised embryo protocol of Roath and Widrlechner (1988). This test was comprised of three replicates with 30 seeds per replicate and placed in a germination chamber (30C,L,14h : 25C,D,10h) for 21 days.

B) Seed samples for 1986 through 1992 cold-stored seeds
Seed samples of *C. angustifolia* Jacq. ex Koehne, *C. carthagenensis* (Jacq.) Macbr, *C. koehneana* Rose, *C. lutea* Rose, *C. painteri* Rose, *C. paucipetala* Graham, *C. procumbens* Cav., *C. tolucana* Peyr., and *C. wrightii* Gray were obtained from the NCRPIS's active collection for use in the five experiments described below. They had been stored at 4 °C, 30% RH for six months after harvest. Additional information about these samples, including the harvest year, accession number, taxonomic section, predominant seed oil, and country of origin, is listed in Table 1.

Table 1. *Cuphea* species from cold storage referenced in *(B) 1986 through 1992 cold-stored seeds.*

Taxon	Section[1]	Accession Number	Year of harvest[2]	Country of Origin	Predominant Seed Oils[1]	Total Oil in Seed[3]
Cuphea painteri	*Diplotychia*	PI 288248	1986	Mexico	Caprylic, 8:0	.
Cuphea angustifolia	*Heterodon*	PI 534664	1986	Mexico	Capric, 10:0	.
Cuphea koehneana	*Heterodon*	PI 534688	1987	Mexico	Capric, 10:0	29%
Cuphea procumbens	*Heterodon*	PI 534714	1990	Mexico	Capric, 10:0	33%
Cuphea tolucana	*Heterodon*	PI 534807	1991	United States	Lauric, 12:0	37%
Cuphea paucipetala	*Heterodon*	PI 561491	1991	Mexico	Capric, 10:0	39%
Cuphea wrightii	*Heterodon*	PI 561512	1991	Mexico	Lauric, 12:0	41%
Cuphea carthagenensis	*Brachyandra*	PI 566706	1992	Brazil	Lauric, 12:0	.
Cuphea lutea	*Heterodon*	PI 578183	1992	Mexico	Lauric, 12:0	.

[1] From Hirsinger and Knowles (1984).
[2] Subsequent storage at 4°C occurred within six months after harvest.
[3] From Germplasm Resources Information Network (GRIN) www.ars-grin.gov

(7) Embryo excision test of cold-stored seeds

In order to ascertain the viability of the nine *Cuphea* species taken from cold storage and used in the experiments described below, nonreplicated samples of 55 seeds each were excised, again following the protocol of Roath and Widrlechner (1988), placed in germination boxes on two blotter papers, and placed in germination chambers (25C,L,14h : 15C,D,10h) for 14 days. This temperature regimen was chosen for these seeds as these were the germination temperatures used in the comparison experiments below.

(8) Accelerated after-ripening treatments of cold-stored seeds

Three replications of 50 seeds each of *C. angustifolia*, *C. carthagenensis*, *C. koehneana*, *C. lutea*, *C. painteri*, *C. paucipetala*, *C. procumbens*, *C. tolucana*, and *C. wrightii* were subjected to an accelerated after-ripening regimen (25C,L,14h : 15C,D,10h) for 14 days. Because of the fungal contamination noted in previous germination tests with *Cuphea* seeds, it was decided that a lower temperature regimen might be more effective than a 30/20°C regimen in providing accurate germination results for a majority of these species. To compare the effect of the accelerated after-ripening treatments, the same temperature and light regimens were given to the same seed lots using two blotter papers instead of the accelerated ageing boxes. Additional tests also were conducted at 15, 25, and 30°C.

(9) Gibberellic acid treatments

Three replications of 50 seeds each of *C. angustifolia*, *C. carthagenensis*, *C. koehneana*, *C. lutea*, *C. painteri*, C. *paucipetala*, *C. procumbens*, *C. tolucana*, and *C. wrightii* were treated with a 1 mM GA_3 solution in various combinations with other treatments. All treatments received 25 ml distilled water or GA_3 solution on two blotter papers placed in plastic germination boxes in germination chambers (25C,L,14h : 15C,D,10h).

The length of germination tests was 21 days after any treatments. Five GA_3 treatments and three distilled water controls were evaluated. One treatment used the GA_3 solution with no additional factors. A distilled water treatment served as a control for GA_3 treatment. Another treatment consisted of a 14-day moist, cold (4 °C) treatment in the presence of the GA_3 solution. Seeds that experienced a cold treatment were imbibed in the GA_3 solution overnight at room temperature (23 °C) before refrigeration. At the close of the moist, cold treatment, some of the GA_3 solution was wicked away and flushed with 20 ml of distilled water in a process resembling the method for applying the GA_3 solution after the cold treatment, which is described next. One treatment consisted of allowing the seeds to experience the 14-day moist, cold treatment in distilled water and then receive the GA_3 solution upon transfer to the 25/15 °C germination regimen. This was accomplished by removing the bottom blotter paper and replacing it with a dry blotter paper, allowing the dry paper to wick away some of the water from the top blotter paper while applying the GA_3 solution slowly to the top blotter paper, which was in contact with the seeds. Another treatment consisted of a 21-day moist, cold treatment in distilled water, followed by application of the GA_3 solution. Still another treatment consisted of first applying the GA_3 solution and allowing the seeds to imbibe the solution for 3 days at 23 °C, then transferring the seeds in their seed germination boxes to cold for 14 days, followed by the 25/15 °C germination regimen. Two final controls for treatments consisted of 14- and 21-day moist, cold treatments with only distilled water.

(10) $CuSO_4$ treatments

Because of occasional, severe fungal growth during the course of other experiments, $CuSO_4$ was tested for effectiveness in reducing fungal growth without reducing overall seed germination. Three replications of 50 seeds each were used in these experiments. After three days imbibition with the 1 mM GA_3 solution, and just before transfer of the seeds to refrigeration, the bottom blotter paper was removed and replaced with a dry blotter paper. Then 15 ml of 0.1, 1.0, or 10.0 mM $CuSO_4$ solution was added slowly to the top blotter paper and seeds. The experimental units were then placed in 4 °C and dark for 14 days, followed by transfer to the standard 25/15 °C germination regimen. Fungal growth was assessed by visual observation.

(11) KNO_3 treatments

If the treatments described as accelerated after-ripening in *(4) Accelerated after-ripening treatments of 1994 seeds* truly accelerate the after-ripening process, thus reducing primary seed dormancy, we hoped that the addition of KNO_3 after the accelerated after-ripening treatments might help alleviate the remaining levels of seed dormancy and thus increase total germination. Therefore, in a follow-up experiment to *(8) Accelerated after-ripening, constant- and alternating-temperature treatment of cold-stored seeds*, seeds transferred from the 14-day 25/15 °C accelerated after-ripening were placed in germination boxes on two blotter papers imbibed with 25 ml of 20 mM KNO_3 solution. These were placed in germinators at the 25/15 °C regimen and scored for germination for the next 21 days. The germination paper comparative test at the 25/15 °C regimen

received a 20mM KNO_3 imbibition solution at the start of the experiment followed by distilled H_2O as needed during the course of the experiment.

Results

A) 1993 and 1994 harvested seeds

(1) Cold treatments of 1993 and 1994 seeds

For the 1993 seeds, samples that received a dark, long-term, moist, cold treatment germinated at higher levels upon subsequent placement in light than did those samples germinated in darkness (Figure 1). Even after 20 weeks of the cold treatment, germination levels of light-excluded samples were less than those obtained with only 4 weeks of cold treatment and subsequent germination in light. Maximal response to the cold treatment occurred at about 16 to 24 weeks, with germination between 80 and 90%.

The 1994 seeds confirmed the pattern observed in the 1993 seeds that received the cold treatment with subsequent germination in light. After 16 weeks of the long-term, moist, cold treatment, the samples germinated at 95% (Figure 1).

(2) Dry storage treatment of 1993 seeds

The assessment of the effects of dry storage on the reduction of dormancy in *C. visco-*

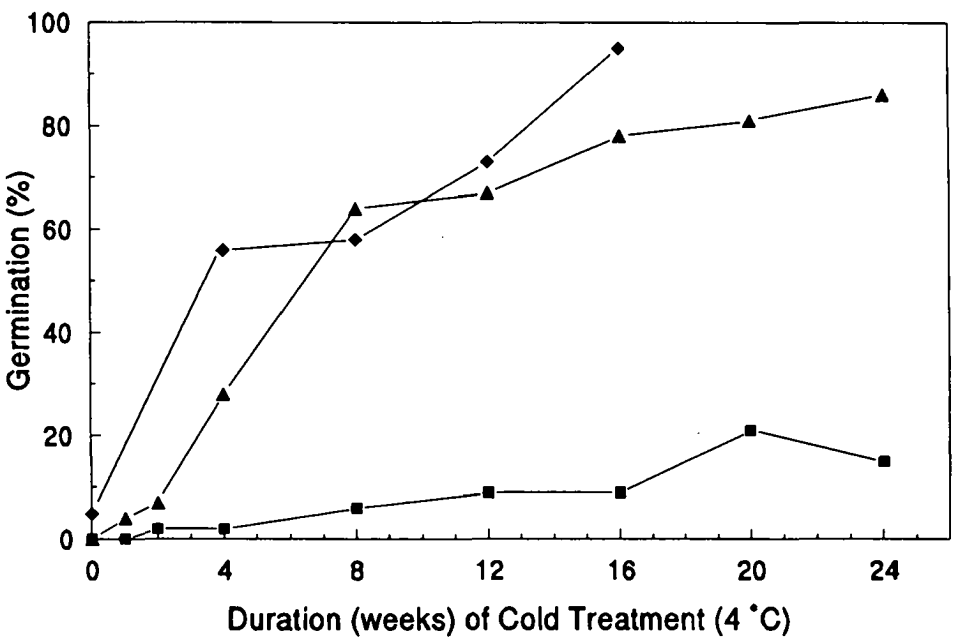

Figure 1. Cold treatments of 1993 and 1994 seeds. Harvest year and germination treatment: ▲–▲, 1993 with light; ■- ■, 1993 in dark; ◆ -◆: 1994 with light.

sissima depended upon the germination protocol (Figure 2). Germination tests conducted at a constant 23 °C and those conducted at 30/20 °C in darkness showed little change throughout dry storage. In contrast, tests conducted at 30/20 °C with illumination showed marked differences, with germination levels increasing with the duration of dry storage. After 4 years of dry storage, germination levels reached 76%.

Tests conducted to determine the viability of seeds that did not germinate following exposure to 30/20 °C in light produced the following results. The first treatment, which included KNO_3 and the cutting of seed coats, was ineffective in inducing additional germination; only 2 of the 38 remaining seeds for all four replications germinated. The second treatment, which followed the protocol established for accelerated after-ripening used in *(4) Accelerated after-ripening treatments of 1994 seeds*, was more effective determining the viability of the seed lot. This treatment was done on 150 seeds (three replicates at 50 seeds per replicate) and yielded an average germination of 92%. Statistical analysis showed that this was significantly greater than the 76% germination achieved by using the 30/20 °C regimen alone. (LSD = 8.6; level of significance (α) = 0.05). Thus, this test showed that even after four years of dry storage, approximately 16% of the seeds were still dormant and unresponsive to the 30/20 °C alternating temperature regimen when tested solely on germination paper.

(3) Alternating-temperature treatments of 1993 seeds

Initial experiments on dormant seeds of *C. viscosissima* had little or no germination when exposed to alternating temperatures and light at 30/20 °C when seeds were placed

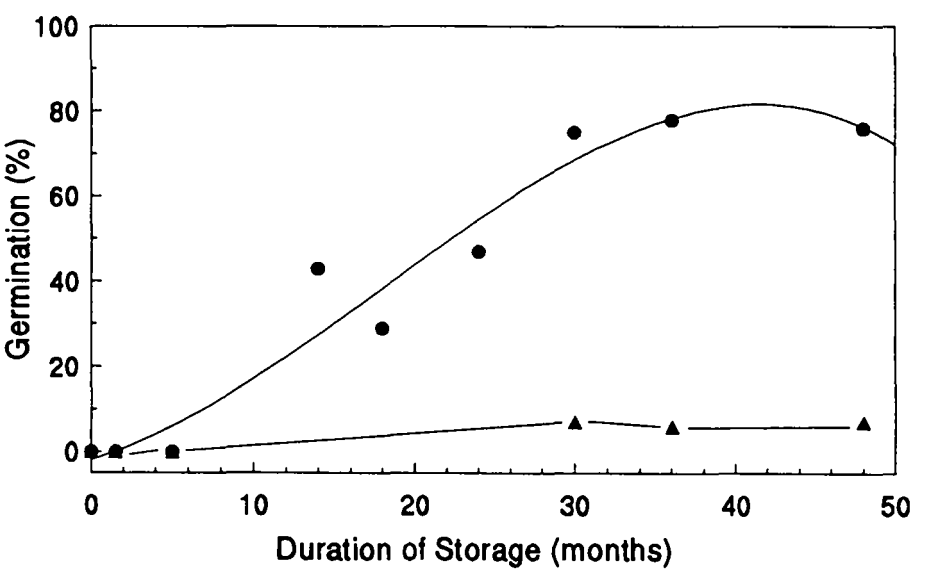

Figure 2. Dry storage treatment of 1993 seed. Germination treatments: ● 30 / 20°C with light; ▲ 30 / 20°C in dark. Equation for 30 / 20°C with light: $y = 0.00165x^3 - 0.0888x^2 + 1.1689x - 1.8199$. $R^2 = 0.9475$.

on germination paper. This was true even with the addition of GA_3 or KNO_3. However, *Cuphea* seeds that have been stored for some time do appear to respond to KNO_3 treatments (A. Logan-Miller, National Seed Storage Laboratory, personal communication). An accelerated after-ripening treatment of four days in duration also failed to promote germination.

(4) Accelerated after-ripening treatments of 1994 seeds

The following experiments utilized accelerated ageing trays for accelerated after-ripening (*aa*) treatments at various temperature regimens with light. Germination response to *aa* treatments at constant temperatures of 20 °C and 25 °C were minor, ranging from 0 to 22% germination (Table 2). A greater response at constant temperature 30 °C occurred for the one-week duration *aa* treatment, indicating a response to the higher temperature. Longer *aa* treatments resulted in less germination, indicating that seed deterioration was occurring in this high-moisture, high-temperature environment. As the *aa* treatment temperatures decreased, the effect of a cold treatment can be seen. Germination percentages ranged from 48 to 55% for the four-week long *aa* treatments at the 5, 10, and 15 °C temperatures.

Alternating temperature regimens with light dramatically increased the germination response of dormant *C. viscosissima* seeds (Table 3) with the trend being that the greater the amplitude of temperature change, the greater the promotion of germination. As in the constant temperature *aa* treatments, the higher overall temperatures with a low to moderate amplitude of change (30/20 °C and 30/25 °C) showed their highest germination response for the one-week *aa* treatment and declining germination percentage for longer durations. This indicated that seed deterioration was occurring at these higher temperatures when the *aa* treatment was given for greater than one week. This was not surprising as the seed MC was approximately 33% during the *aa* treatments. The highest germination results occurred at the 30/10 °C and 30/5 °C temperature regimens,

Table 2. Constant temperature, *aa* treatments of 1994 seeds.

	Duration of Treatment			
Temperature of *aa*	1 week	2 weeks	3 weeks	4 weeks
30 C	40 a	19 ab	2 bc	6 b
25 C	4 d	13 b	0 c	3 b
20 C	4 d	17 ab	15 b	22 b
15 C	11 c	30 ab	34 a	52 a
10 C	8 cd	29 ab	45 a	55 a
5 C	29 b	32 a	42 a	48 a

Results shown are in percentage germination. Percentage germination for controls was 3%. Temperatures indicate the temperature of treatment only. All germination tests were conducted at 30/20 °C. Statistical analysis: grouping of means is indicated by lower case letters. Comparisons are for temperatures for each duration of treatment, i.e., groupings run vertically. Although the germination test lasted 21 days, very few seeds germinated after seven days.

Table 3. Alternating temperature, *aa* treatments of 1994 seeds.

	Duration of Treatment			
Temperature of *aa*	1 week	2 weeks	3 weeks	4 weeks
30/25 C	39 ab	13 c	13 b	3 c
30/20 C	25 b	7 c	3 b	3 c
30/15 C	44 ab	38 b	15 b	20 bc
30/10 C	69 a	97 a	86 a	65 ab*
30/5 C	49 ab	96 a	95 a	95 a

Results shown are in percentage germination. Percentage germination for controls was 3%. Temperatures indicate the temperature of treatment only. All germination tests were conducted at 30/20°C. Statistical analysis: grouping of means is indicated by lower case letters. Comparisons are for temperatures for each duration of treatment, i.e., groupings run vertically. Although the germination test lasted 21 days, very few seeds germinated after seven days.

*Germination occurred in one replicate in the *aa* boxes of this treatment causing zero percentage survival during the germination test which resulted in high variance for the 4 weeks treatment.

though seeds in the 30/10°C regimen, four weeks duration, had seeds germinate during the *aa* treatment in one replicate. Both had germination means above 90% for the two-week duration treatment and were statistically indistinguishable. Controls averaged 3% germination throughout the duration of the experiment.

(5) Comparison of treatments after one year of storage

After one year of dry, cold storage (4°C), *C. viscosissima* seeds still exhibited strong dormancy when using the 30/20°C germination test (Table 4). However, an alternating temperature treatment on blotter paper at the 25/5 and 30/5°C regimens resulted in about 50% germination for both tests. Though the germination results from treatments on blotter paper were always less than the *aa* treatments, the results indicated that older seeds responded better to alternating temperatures. These results have a direct implication on the results seen in the older, cold-stored *Cuphea* seeds in *(8) Accelerated after-ripening treatments of cold-stored seeds.*

(6) Embryo excision test of the 1993 seeds

The results of the replicated excised embryo test on the 1993 harvested seeds of *C. viscosissima* had a mean of 88% viability with a range from 83 to 97%. The results for the 1993 seeds in *(1) Cold treatments of 1993 and 1994 seeds* had a mean of 86% germination with a range from 82 to 90%. The means of the these two experiments were not significantly different.

B) 1986 through 1992 cold-stored seeds

(7) Embryo excision test of cold-stored seeds

Three of the nine species tested, *C. koehneana*, *C. painteri*, and *C. wrightii,* displayed

Table 4. Comparison of treatments after one year of storage. Data reported in percentage germination. Standard errors in parentheses.

Treatments:	1994 Seed		
	Months dry, cold-stored since harvest	Treated & germinated on germ paper (GP)	*aa* treated, then germinated on germ paper
Control (no treatments)	12	1 (1)	–
aa: 14 days @ 30/5 °C	12	46 (10)	80 (14)
aa: 14 days @ 25/5 °C	12	51 (4)	63 (18)
Control (no treatments)	13	2 (1)	–
aa: 14 days @ 20/5 °C	13	37 (9)	72 (10)
aa: 14 days @ 15/5 °C	13	19 (6)	34 (9)

Treatments indicate the temperature of treatment only. All germination tests were conducted at 30/20 °C. Statistical analysis: (1) Alternating temperature treatments, using GP or *aa*, were significantly different than controls. (2) *aa* treatments were significantly different than GP across temperatures. (3) 30/5, 25/5, and 20/5 °C were significantly different than 15/5 °C, across treatments (GP or *aa*).

germination levels below 20% (Table 5). The other six samples ranged between 69 and 95%. Because this experiment was conducted without replication, it is not possible to make statistically based conclusions about observed differences. However, we are confident in the validity of these results as a measure of the overall viability of our samples because we were unable to induce germination levels in excess of those observed via embryo excision.

(8) Accelerated after-ripening treatments of cold-stored seeds

The promotive effects of the accelerated after-ripening treatments of dormant *C. viscosissima*, reported in *(4) Accelerated after-ripening treatments of 1994 seeds*, were not seen in all nine *Cuphea* species taken from cold storage. Species that showed improvement over controls had only moderate increases. In this experiment, the highest germination levels occurred when using alternating temperatures with or without an *aa* treatment (Table 5). In light of the results of *(5) Comparison treatments after one year of storage*, these results are not surprising. Even though stored at cold temperatures, older seeds responded to alternating temperatures without need of *aa* treatments.

(9) Gibberellic acid treatments

In comparison with the control, the addition of GA_3 produced statistically significant increases in the germination of only one species (*C. procumbens*) (32 versus 13%, control), but the actual magnitude of this increase was small in relation to the germination levels measured by embryo excision (Table 5). The GA_3 with two-week cold treatments, *(b) 2-wk cold, w/ GA_3* and *(c) 2-wk cold then GA_3*, and the GA_3 with three-week cold treatment, *(d) 3-wk cold, then GA_3*, for *C. procumbens* had germination percentages significantly greater than the control whether GA_3 was added before or after the

Table 5. Dormancy breaking methods tested on nine species of *Cuphea* seed. Germinated on germination paper at 25/15°C regimen. Data reported in percentage germination. Standard errors in parentheses. Least significant differences at bottom of replicated data.

	PI 288248 *C. painteri*	**PI 534664** *C. angustifolia*	**PI 534688** *C. koehneana*	**PI 534714** *C. procumbens*	**PI 534807** *C. tolucana*	**PI 56149** *C. paucipetala*	**PI 56151** *C. wrightii*	**PI 566706** *C. carthagenensis*	**PI 578183** *C. lutea*
Controls:									
Control	0 (0)	33 (1)	5 (2)	13 (4)	77 (1)	35 (4)	0 (0)	70 (3)	22 (1)
Accelerated After-ripening Treatments:									
AA, 15/5	0 (0)	25 (4)	7 (3)	10 (2)	77 (6)	46 (7)	1 (1)	83 (5)	33 (7)
AA, 25/15	1 (1)	47 (6)	9 (3)	23 (6)	80 (1)	55 (5)	1 (1)	86 (4)	29 (10)
AA, 25/15, KNO_3	0 (0)	41 (3)	11 (2)	47 (8)	84 (2)	61 (5)	0 (0)	89 (4)	38 (3)
AA, 30/5	0 (0)	39 (2)	9 (3)	51 (4)	77 (1)	56 (7)	1 (1)	87 (2)	20 (8)
Germ Paper Treatments:									
GP, 15/5	0 (0)	29 (4)	9 (1)	6 (1)	78 (1)	46 (5)	1 (1)	79 (3)	45 (2)
GP, 25/15	1 (1)	29 (4)	7 (2)	10 (5)	63 (4)	44 (9)	1 (1)	77 (4)	17 (1)
GP, 25/15, KNO_3	0 (0)	25 (4)	10 (3)	62 (3)	89 (4)	23 (3)	1 (1)	85 (3)	31 (4)
GP, 30/5	0 (0)	37 (4)	5 (1)	18 (2)	57 (3)	35 (6)	1 (1)	64 (21)	6 (2)
GP, 15	0 (0)	0 (0)	2 (1)	1 (1)	38 (2)	14 (3)	3 (1)	35 (9)	17 (2)
GP, 23	0 (0)	0 (0)	3 (1)	9 (2)	59 (3)	60 (2)	1 (1)	35 (12)	20 (1)
GP, 30	0 (0)	0 (0)	3 (2)	6 (0)	34 (2)	0 (0)	0 (0)	15 (11)	0 (0)
Cold Treatments:									
2 weeks cold	0 (0)	26 (4)	7 (2)	19 (4)	49 (2)	29 (4)	1 (1)	72 (8)	25 (2)
3 weeks cold	0 (0)	39 (2)	8 (2)	39 (7)	43 (6)	41 (5)	0 (0)	83 (6)	28 (2)
Gibberellic Acid Treatments:									
a) GA_3	0 (0)	38 (2)	13 (4)	32 (3)	73 (4)	42 (5)	1 (1)	81 (4)	30 (4)
b) 2-wk cold w/ GA_3	0 (0)	40 (5)	7 (1)	42 (2)	65 (4)	41 (5)	1 (1)	65 (12)	27 (5)
c) 2-wk cold, then GA_3	0 (0)	42 (5)	12 (2)	36 (5)	71 (7)	45 (4)	1 (1)	59 (2)	39 (4)
d) 3-wk cold, then GA_3	0 (0)	39 (2)	7 (2)	42 (3)	57 (1)	42 (3)	1 (1)	81 (2)	35 (7)
e) 3-da GA_3, 2-wk cold	1 (1)	41 (3)	3 (2)	33 (5)	63 (5)	31 (4)	1 (1)	60 (4)	41 (3)
f) 3-da GA_3, 2-wk cold, 0.1 mM $CuSO_4$	1 (1)	37 (2)	4 (3)	20 (7)	64 (2)	27 (1)	1 (1)	82 (1)	23 (5)
g) 3-da GA_3, 2-wk cold, 1 mM $CuSO_4$	0 (0)	35 (2)	7 (2)	33 (3)	62 (4)	33 (6)	1 (1)	84 (4)	30 (2)
h) 3-da GA_3, 2-wk cold, 10mM $CuSO_4$	1 (1)	25 (1)	11 (4)	30 (3)	67 (5)	31 (3)	0 (0)	88 (2)	31 (1)
LSD's for above:	1	10	7	12	10	14	2	20	12
Excised Embryos (non-replicated):									
GP, 25/15	2	69	13	78	89	84	15	95	84

cold treatment. In comparing the GA_3 with two-week cold treatments (42 and 36% germination, (b) and (c) treatments) with the two- and three-week cold treatments (no GA_3) (19 and 39%, respectively) for *C. procumbens*, the addition of GA_3 replaced the need for a third week of cold treatment. However, when GA_3 was added after a three-week cold treatment, *(d) 3-wk cold, then* GA_3, no further increase in germination occurred. It may be that a 'low-vigor factor' is involved in *Cuphea* seed germinations that can only be alleviated quickly in the laboratory by using the embryo excision method (Roath and Widrlechner, 1988). The seed coat of *Cuphea* seeds may present its own barrier to germination until external stimuli such as long-term, cold moist conditions over winter or microbial activity in the soil help reduce the structural integrity of the seed coat. Differing views on this point have been expressed by Bewley and Black (1982) and Copeland and McDonald (1995). This low-vigor factor may be a natural phenomenon of *Cuphea* seeds, the result of poor quality seeds due to poor growing or harvesting conditions, or may be an indicator that the seeds are nearing the loss of viability (Harrington, 1972).

(10) $CuSO_4$ *treatments*

Few significant differences in germination levels were observed among the three $CuSO_4$ levels, and those few were inconsistent across species (Table 5). In *C. procumbens* and *C. carthagenensis*, significant increases in germination percentages were detected, while *C. tolucana* had a significant decrease in germination. Additionally, the effectiveness in controlling fungal contaminants was not noticeable in these species because they had little to no fungi present in the control tests. In the other six species, the degree of fungal control was directly related to the concentration of the $CuSO_4$ treatment. But at the highest concentration, $CuSO_4$ retarded the growth of the seeds' radicles. Only after the $CuSO_4$ solution was diluted by the addition of distilled water (as needed during the course of the germination test) was the root system no longer prevented from growing. In about a third of the seeds that germinated at the highest concentration of $CuSO_4$, most of the root growth was due to the development of secondary roots. But again, no real benefit in germination response was shown by using $CuSO_4$ treatment. Our results lead to the conclusion that the use of $CuSO_4$ as an antifungal treatment has no benefit for *Cuphea* germination tests.

(11) KNO_3 *treatments*

The addition of 20 mM KNO_3 to the germination solution in some of the treatments of *(8) Accelerated after-ripening treatments of cold-stored seeds* tests did not show any increases in germination means for eight of the species tested, whether the seeds received the *aa* treatment or not (Table 5). One species, *C. procumbens*, showed a significant increase in germination for the 25/15 °C regimen for both the *aa* and germination paper treatments (Table 5).

Discussion

Figures 1 and 2 illustrate the nature of dormancy in *C. viscosissima* seeds. Both 1993

and 1994 harvests required at least 4 months of cold, moist treatment to germinate in light at levels above 80%. Results of the tests of long-term, moist, cold treatments in complete darkness revealed the light-requiring nature of *C. viscosissima* seeds (Figure 1).

Results shown in Figure 2 revealed four important facts about seed dormancy and viability of *C. viscosissima*. First, the seeds remained at high levels of germinability after four years of storage at room temperature. As noted earlier, the moisture contents of the seeds used in this experiment were about 5%. Results of Dickie, Ellis, Kraak, Ryder, and Tompsett (1990) and Robertson, Chapman, Wilson and Russell (1984) suggest that seed quality of stored seeds is more dependent on seed moisture content than on the temperature of storage. Second, levels of seed dormancy decreased with time. This result agrees with observations reported by Grabe, Garbocik and Kaliangile (1985) for *C. wrightii* and is typical of many species. Third, light was required for seed germination, at least while some level of seed dormancy was still present. Fourth, seed germination percentages were increased if alternating temperatures were part of the germination regimen. From the results shown in Figures 1 and 2 at least four factors influence the release of seed dormancy in *C. viscosissima*: 1) time of storage – four-year dry stored seeds germinated better than newly harvested seeds; 2) temperature of storage – long-term, moist, cold-treated seeds released dormancy faster than dry, room temperature storage; 3) light – percentage germination occurring in the presence of fluorescent light was greater than seeds germinated in darkness; and 4) alternating temperatures – seeds tested under alternating temperatures germinated at much higher percentages than did seeds tested under constant temperatures.

Table 3 shows that *C. viscosissima* seeds harvested from plants grown under optimal conditions in the greenhouse had high germination percentages when subjected to an *aa* treatment of high amplitude temperature changes followed by an alternating temperature germination regimen with light. This regimen may mimic that found naturally in relatively arid regions with wide diurnal temperature fluctuations. This method could prove a valuable test for seed viability of newly obtained *Cuphea* seeds, possibly eliminating the need for tedious embryo excisions for viability testing and grow-outs for seed increases. The advantage of the *aa* method appears to decrease the longer the seeds are stored, as seen in Table 4. The caveat comes with seeds produced from plants grown under suboptimal conditions, as suggested from the low-viability species results shown in Table 5.

Research done by Edwards (1981, 1986) found for three species of *Abies* that drying pre-chilled seeds to approximately 37% MC helped maintain the release of dormancy when stored at 2 to 5 °C between 3 and 12 months. In some cases, the storage of partially hydrated seeds even aided in the release of seed dormancy. The *aa* treatments of this present research can also be considered a type of partial hydration. However, a major difference between the two methods is the length of time required. It took several months of constant, cold-temperature storage of partially hydrated seeds after a pre-chill treatment to help release dormancy. The *aa* treatments using alternating temperatures helped release seed dormancy after only two weeks of treatment.

The dehiscent nature of *Cuphea* fruits makes seed harvesting difficult. *Cuphea* fruits develop a few mature seeds at a time, often shattered by wind or rain over a long period. No detailed information was available as to the growing or drying conditions of the nine *Cuphea* species tested that were harvested from 1986 to 1992 and then stored in 4 °C, 25 to 40% RH. In some cases, the seeds were harvested while many of them were still green in color. This was due to late flowering and the indeterminate inflorescences. Additionally, samples often were dried in forced-air dryers from two weeks to two months, compared to the three to four days of forced-air drying received by the *C. viscosissima* seeds grown and harvested for experiments shown in Figures 1 and 2, and in Tables 2 and 3. Comparing the favorable results shown in Table 3 with the less than favorable results shown in Table 5 as well as the higher germination percentages of the embryo excision tests, our results indicate that some of the seeds tested in *(8) Accelerated after-ripening treatments of cold-stored seeds* (Table 5) were of low vigor and others were of very low viability. This could be due to the suboptimal growing and harvesting conditions, or due to differences in harvesting methods. Roath (1992) reported that damage to *Cuphea* seeds from vacuum harvesting or combine harvesting was significantly greater than with hand-harvested seeds. Also, germination percentages of hand-harvested seeds were significantly greater than combine-harvested or vacuum-harvested seeds. Unfortunately, hand harvesting of *Cuphea* seeds is tedious (Roath, 1992). But hand harvesting might result in longer-storing, higher-vigor, higher-germinating seeds. In addition, less time would be consumed by using alternating temperature and light regimens for *Cuphea* germination tests than spent on tedious embryo excisions.

Whereas the use of KNO_3 or GA_3 alone did not increase percentage germination with the older, cold-stored *Cuphea* seeds, the short, moist, cold treatments combined with the use of GA_3 promoted germination in some accessions (Table 5). The results of these experiments indicate that to avoid labor-intensive embryo excisions, two alternative approaches to assess viability of *Cuphea* species are the use of a two-week *aa* treatment (30C,L,14h : 20C,D,10h or 30C,L,14h : 5C,D,10h) or a two-week moist, cold treatment combined with GA_3, both methods followed by a germination test at 30C,L,14h : 20C,D,10h for 21 days. It is further recommended that harvests of *Cuphea* seeds for germplasm centers should avoid harvesting by vacuum or combine methods, unless systems can be devised to remove low-quality seeds from machine-harvested samples.

Acknowledgements

We wish to thank Dr. Allen Knapp, Dr. Steven Knapp, and Dr. Loren Wiesner for their useful critiques of this manuscript and Dr. Loren Wiesner, National Seed Storage Laboratory, for suggesting the term 'accelerated after-ripening' to describe the protocol described in experiments 4 and 5. Journal Paper No. J-18313 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1018 and supported by Hatch Act and State of Iowa funds.

The use of brand names is not to be construed as endorsement of the product by the USDA-Agricultural Research Service.

References

Association of Official Seed Analysts. (1993). Rules for testing seeds. *Journal of Seed Technology*, **16**, 1–113.

Bewley, J. D. and Black, M. (1982). *Physiology and Biochemistry of Seeds in Relation to Germination.* Springer-Verlag, New York.

Copeland, L. O. and McDonald, M. B. (1995). *Principles of Seed Science and Technology. Third Edition.* Chapman & Hall, New York.

Dickie, J. B., Ellis, R. H., Kraak, H. L., Ryder, K. and Tompsett, P. B. (1990). Temperature and seed storage longevity. *Annals of Botany*, **65**, 197–204.

Edwards, D. G. W. (1981). Improving seed germination in *Abies*. *Combined Proceedings International Plant Propagators' Society*, **31**, 69–78.

Edwards, D. G. W. (1986). Special prechilling techniques for tree seeds. *Journal of Seed Technology*, **10**, 151–171.

Ellis, R. H., Hong, T. D. and Roberts, E. H. (1985). *Handbook of Seed Technology for Genebanks. Volume II. Compendium of Specific Germination Information and Test Recommendations.* International Board for Plant Genetic Resources, Rome.

Grabe, D. F., Garbocik, C. and Kaliangile, I. (1985). Seed technology in *Cuphea*. In *Cuphea* Domestication and Agronomic Development in Oregon, pp. 4–39. *Annual Report to SDA and USDA-ARS.* Oregon State University, Corvallis, OR.

Harrington, J. F. (1972). Seed storage and longevity. In *Seed Biology* (ed. T. T. Kozlowski), vol. 3, pp. 145–245. Academic Press, New York.

Hirsinger, F. and Knowles, P. F. (1984). Morphological and agronomic description of selected *Cuphea* germplasm. *Economic Botany*, **38**, 439–451.

International Seed Testing Association. (1993). International rules for seed testing. 1993. *Seed Science and Technology*, **21**, (suppl, rules), 1993.

Knapp, S. J. (1990). Recurrent mass selection for reduced seed-dormancy in *Cuphea lanceolata* and *Cuphea laminuligera*. *Plant Breeding*, **104**, 46–52.

Knapp, S. J. (1993). Breakthroughs towards the domestication of *Cuphea*. In *New Crops* (eds. J. Janick and J.E. Smith), pp. 372–379. J. Wiley and Sons, New York.

Knapp, S. J. and Tagliani, L. A. (1990). Genetic variation for seed dormancy in *Cuphea laminuligera* and *Cuphea lanceolata*. *Euphytica*, **47**, 65–70.

McDonald, M. B., Jr. and Phaneendranath, B. R. (1978). A modified accelerated aging seed vigor test for soybeans. *Journal of Seed Technology*, **3**, 27–37.

Roath, W. W. and Widrlechner, M. P. (1988). Inducing germination of dormant *Cuphea* seed and the effects of various methods on seedling survival. *Seed Science and Technology*, **16**, 699–703.

Roath, W. W. (1992). Effects of harvest methods and storage conditions on seed germination of two *Cuphea* species. In *Proceedings: the First International Conference on New Industrial Crops and Products, October 8–12, 1990.* Riverside, California (eds. H. H. Naqvi, A. Estilai, and I. P. Ting), pp. 183–187. Association for the Advancement of Industrial Crops, Riverside, CA., USA.

Roath, W. W., Widrlechner, M. P. and Kleiman, K. (1994). Variability in *Cuphea viscosissima* Jacq. collected in east-central United States. *Industrial Crops and Products*, **3**, 217–223.

Robertson, J. A., Chapman, G. W., Wilson, R. L. and Russell, R. B. (1984). Effect of moisture content of oil type sunflower seed on fungal growth and seed quality during storage. *Journal of American Oil Chemists Society*, **61**, 768–771.

Sackville Hamilton, N. R. and Chorlton, K. H. (1997). Regeneration of accessions in seed collections: a decision guide. *IPGRI Handbook For Genebanks #5.* International Plant Genetics Resources Institute, Rome.

Stubbs, J. M. and Slabas, A. R. (1982). Ultrastructural and biochemical characterization of the epidermal hairs of the seeds of *Cuphea procumbens*. *Planta*, **155**, 392–399.

Widrlechner, M. P. (1997). Managerial tools for seed regeneration. *Plant Varieties and Seeds*, **10**, 185–193.