Germination and hardseededness in desmanthus

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Abstract

The mechanisms and control of hardseededness in the 3 Australian cultivars of the genus *Desmanthus* were investigated in a series of experiments in which the effects of various seed-softening treatments, particularly boiling water, were measured. Desmanthus seed is predominantly hard, only defective seeds being normally otherwise. As it has only very brief, early embryo dormancy, hardseededness is the only serious barrier to germination. Seed is most readily softened through rupture of the palisade at the lens (strophiole). The lens is of a typically mimosaceous type which is readily ruptured by immersion in boiling water or less readily by application of pressure to adjacent parts of the testa. Ruptures may consist only of separation of the palisade from underlying tissue, which alone does not confer permeability; mostly they also result in fractures to the palisade that then render seeds irreversibly permeable. The palisade becomes reflective as it separates, which allows the event to be witnessed at the moment of separation if suitable pressure is applied to the testa of an individual seed while it is viewed under magnification.

Brief (4–10 seconds) immersion of high-quality seed in boiling water consistently softened a high proportion of seeds without causing serious damage. Extending the duration of immersion led to a progressive increase in the proportion of seed deaths. Neither previous boiling water treatment nor scarification damage to the testa materially affected results of treatment, but immature and small seeds behaved differently, being more vulnerable to damage than mature seed, and less likely to undergo lens rupture. Adaptation of boiling water treatment to farm-scale seed handling was simple and reliable.

Commercial treatment of seed by an alternative method suitable for greater bulks and consisting of passage through a rice-whitener was checked and found to be successful through a combination of gentle scarification and lens rupture, both attributable to the numerous minor impacts of the process.

Percentage emergence of seedlings from soil in the greenhouse closely followed percentage laboratory germination, except when inferior seed grades were included in the comparison, when emergence was poor. Very little seed softened in soil. Already-permeable seed either germinated rapidly or died, while buried hard seed mostly remained hard and viable even more than a year after sowing.

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Introduction

The genus *Desmanthus* offers promise as a source of pasture legumes for use in northern Australia (Burt 1993), where 3 cultivars, Marc, Bayamo and Uman, were released for pastoral use in 1991. Though all were treated at the time as members of the species *D. virgatus*, they have since come to be regarded as belonging, respectively, to *D. virgatus, D. leptophyllus* and *D. pubescens* (Luckow 1993). For present purposes they are very similar, and the common name ‘desmanthus’ is used here to cover them all.

Desmanthus typically produces seed with high and persistent levels of impermeability to water (hardseededness), which interfere seriously with field establishment. When seed multiplication of the new cultivars began in north Queensland in

1992, there were no suitable methods of seed treatment to reduce hard seed content (soften seed), a position that urgently needed to be remedied.

Desmanthus is a genus of the family *Mimosaceae*, members of which differ from those of the *Fabaceae* in seed form and structure (Corner 1951). The differences extend to the lens or strophiole, an organ of critical importance in the control of hardseededness. The structure, function and manipulation of lenses of fabaceous seeds, particularly of pasture plants, have been widely investigated (Ballard 1973). However, much of the knowledge is not applicable to desmanthus. The form and behaviour of the lens of mimosaceous seeds have been studied only relatively recently, primarily in 2 woody genera, *Albizia* (Dell 1980) and *Acacia* (Tran and Cavanagh 1984). However, lens structure across the *Mimosaceae* seems quite uniform so behaviour of seeds of these genera provides valuable background for desmanthus.

Previous accounts of softening of desmanthus seed were few and conflicting. While the potential of hot water treatment, as well as the (to us) less practical methods of acid and sand scarification, had been demonstrated (Ramamoorthy and Vinya Raj 1990), hot water treatment had also been reported to be unreliable and mechanical scarification ineffective on seed of an unreleased accession, CPI 78382 (Loch and Harvey 1992). Even so, hot water treatment offered advantages if its reliability could be mastered, as it is simple to apply and has been used successfully to soften a wide range of other leguminous seeds. Its application with respect to seed or water temperature and immersion time had been investigated in detail with seeds of a number of fabaceous pasture legumes (McIvor and Gardener 1987) and one mimosaceous shrub, leucaena (Gray 1962).

Desmanthus is the first mimosaceous introduced tropical pasture plant in Australia which would be sown by broadcasting seed. Its use introduces a new and not wholly compatible combination of seed characteristics and requirements, which in turn creates a need for a wider knowledge of the seed than simply what empirical softening treatments to apply. This need, particularly in the context of the establishment of seed crops, led us to a course of experimentation reported here, with the central objective of testing practical methods of overcoming hardseededness while learning as much as possible of the general seed biology of desmanthus.

Methods

Course of investigation

The investigation was conducted step-wise, experiments being planned after consideration of the results of earlier ones. The starting point was to check the potential of hot water treatment for on-farm seed softening, the differing published experiences of such treatment (see Introduction) having introduced uncertainty about its value. Farmers' only means of controlling hot water temperature for treatment is to use boiling water, so boiling water was chosen as the experimental standard, first in a simple exploratory comparison with untreated seed (Experiment 1), then with a range of seed lots, different periods of immersion and different ways of subsequent cooling (Experiments 2 and 3).

While the present study was under way, the licensee of the cultivars, Wrightsons Seeds Australia, was investigating alternatives to hot water for very high volume continuous throughput scarification treatments. On the recommendation of the Satake Milling Company of Sydney, it was considering the passage of seed through a rice-whitener, which scuffs the testa through numerous minor impacts against spinning emery wheels. Seed treated in this way was supplied, tested and compared with seed from the same source treated with boiling water (Experiment 4).

As records accumulated to emphasise the general reliability of boiling water treatment, it became increasingly important to explain the inconsistencies reported by Loch and Harvey (1992), since unpredictable responses form a poor basis for commercial recommendations. Observations made during the earlier experiments suggested a possible cause of the inconsistencies was the behaviour of seeds that had been immature at harvest. Immature seeds were present at very low levels in the samples used in the experiments, which were all of north or central Queensland origin. However, they occurred with much greater frequency in samples received from south Queensland sources similar to those of Loch and Harvey. It, therefore, seemed prudent to check the behaviour under treatment of seed of different visually separable maturity grades, and Experiment 5 was designed for this purpose. To cover all possibilities, the check was later extended to seeds of different size grades (Experiment 7).

As seed crop husbandry evolved, suction harvesting from the soil surface became routine. To disperse soil particles from the otherwise conventionally cleaned seed, washing with water was incorporated. This involved the seed being wet for periods of up to half an hour, with unknown consequences on seed characteristics or response to boiling water. Experiment 6 was conducted to check both the general behaviour of suction-harvested seed and the risks of damage from such treatments.

Finally, using a selection of lines tested in Experiments 5 and 7, the investigation was extended to the measurement of seedling emergence from soil (Experiment 8) to provide some connection between effects detected in laboratory tests and field behaviour.

Throughout the investigation, treatment effects were measured simply by taking dry stored seed, treating a sample of it, then testing both treated and untreated seed and comparing the test results. Treatment effects were judged in all experiments by laboratory germination tests, with additional tests of seedling emergence of selected lines from soil in a greenhouse included in the final experiment.

Observations and digressions into minor treatments meanwhile provided information on background seed behaviour, particularly with respect to the action of the lens. The state of the lens was determined by observation under a dissecting microscope.

Sources of seed

Dry processed seed for the experiments was obtained from the harvest of seed-increase areas and commercial seed crops as the opportunity arose, all 3 cultivars being targeted. The Marc of Experiment 1 was grown at Emerald, central Queensland and the Bayamo near Ayr, north Queensland. All other seed was grown at or near Walkamin on the Atherton Tableland, north Queensland. See Table 1 for details of the seed used in the different experiments.

Treatments and testing methods

Boiling water treatment. Treatment consisted of placing samples of seed in a perforated tea infuser and immersing it in vigorously boiling water in a pan on a hotplate for the specified time, followed immediately by immersion in a high volume of either iced water (0°C) or tap water at ambient temperature (20–25°C).

Germination tests. It was not possible to maintain uniform conditions of light and temperature in germination tests across experiments, but differences in conditions had little effect on outcomes. Tests for Experiments 1, 3 and 4 were done on bench tops in the laboratory in ambient temperatures averaging a little over 25°C, the rest in a germination cabinet at a constant 25°C or 20/35°C (Table 1). All seed was placed on germination pads of paper in trays or petri dishes and kept moist for the duration of the run. Three replicates (100 seeds each) of each line were used. Counts were made daily over the period of each test, and each seed was allocated to a specific category on the following basis:

- *Germinated* seed had an exserted, undamaged radicle and intact healthy cotyledons.

Table 1. Details of seed used and germination tests.

Experiment	Cultivar	Harvest method	Age at test[1]	Test conditions[2]		
				Temp (°C)	Light	Duration (days)
1	Uman	Header	3 m	A	N	7
1	Marc	Hand-swept	3 m	A	N	7
1	Bayamo	Header	3 m	A	N	7
2	Uman	Header	2–14 m	25	8 h	14
3	Uman	Header	3 m	A	N	14
3	Bayamo	Header	3 m	A	N	14
4	Uman	Header	5–8 m	A	N	8
5	Marc	Header	16 d	20/35	8 h	9
6	Marc	Suction	1 m	25	Dark	7
7	Marc	Header	4 d	25	Dark	8
7	Marc	Suction	5 d	25	Dark	8

[1] Age at test in months (m) or days (d) from harvest.
[2] A = ambient temperature; N = natural light; h = hours.

- *Imbibed viable* seed was ungerminated at the end of a run, but had healthy, intact tissues when then dissected out.

These 2 categories were combined to give the SV (soft viable) class of the tables.

- *Hard* seed (H) had not begun to imbibe, and was generally taken to be viable, an assumption checked with the seed used in Experiments 5 and 6. A small proportion of hard seed, averaging 0.6%, was found to be in fact non-viable, but the error was so small as to make correction superfluous.
- *Abnormals* were seeds that had begun to germinate, but were either infected or damaged and judged incapable of producing a healthy seedling.
- *Dead* seed had begun to decompose without starting to germinate.

Abnormal and dead seeds were classed together as non-viable (NV).

Moisture determinations. In the absence of background knowledge of either moisture contents of desmanthus seed or their significance, moisture tests were made on samples of all lines tested, which came from 11 crops grown and dried on 5 different properties and harvested in 3 seasons. For each determination, seed was ground in a mill, weighed, placed in an oven at 120–125°C for 1.5–2 h, cooled over silica gel, and weighed again. Moisture content was calculated by difference on the 'wet-weight' basis.

Data treatment. Most records were expressed as final percentages, determined at the ends of runs, of soft viable (SV), hard (H) and non-viable (NV) seed. Comparable groups of such records were subjected to routine analyses of variance. Differences between untreated and treated seed served to determine effects of treatment in terms of the desired (softening) and undesired (killing) consequences. Each effect was calculated independently of the other, the softening effect as the number of hard seeds rendered permeable as a percentage of the number hard before treatment, and the killing effect as the number killed (or more precisely, rendered non-viable) as a percentage of the number viable before treatment. This method of analysing effects of softening treatments is considered at greater length in an earlier paper (Hopkinson and Paton 1993).

Table 2. Results of germination tests in Experiments 1–3 showing effects of boiling water treatments on survival and softening of seed.

Expt	Cv.[1]	Sec.[2]	Water[3]	Untreated			Treated			Effect[5]	
				SV[4]	H	NV	SV	H	NV	Softening	Killing
				(%)							
1	U	4	Iced	6	89	5	76	4	20	96	16
2	U_1[6]	5	Iced	74	20	6	86	9	5	55	0
2	B_6	5	Iced	9	89	2	91	4	5	95	3
2	M_7	5	Iced	3	96	1	65	32	3	67	2
2	U_2	5	Iced	7	90	2	77	13	10	86	8
2	U_3	5	Iced	7	92	1	73	23	4	75	3
2	U_4	5	Iced	5	93	3	61	35	4	62	2
2	U_5	5	Iced	5	90	5	63	28	9	69	4
2	U_5	60	Iced	5	90	5	51	3	46	96	43
2	U_5	5	Amb	5	90	5	68	24	7	73	2
3	U_5	5	Iced	7	90	3	51	35	13	61	10
3	U_5	10	Iced	7	90	3	60	19	22	79	18
3	U_5	20	Iced	7	90	3	60	11	29	88	26
3	U_5	40	Iced	7	90	3	66	3	32	97	27
3	U_5	60	Iced	7	90	3	46	2	53	98	49
3	U_5	5	Amb	7	90	3	55	28	17	67	15

[1] U = Uman; M = Marc; B = Bayamo. Different subscript numbers indicate seed from different sources.
[2] Duration of immersion in boiling water in seconds.
[3] Condition of cooling water; Amb = ambient temperature.
[4] SV = soft viable, *i.e.* germinated + viable, imbibed, non-germinated; H = hard seed at end of test; NV = non-viable, *i.e.* dead, dying and abnormal seeds.
[5] Softening effect = number of seeds softened as a percentage of hard seed present before treatment; Killing effect = number of seeds killed as a percentage of number of viable seeds before treatment.
[6] Also previously treated with boiling water, hence the high untreated SV value.

Table 3. Results of laboratory tests showing effects of passage of seed through rice-whitener on seed softening and loss of viability, compared with effects of boiling water treatment (Experiment 4).

Cultivar	Treatment[1]	Untreated			Treated			Effect[3]	
		SV[2]	H	NV	SV	H	NV	Softening	Killing
					(%)				
Uman	BW-5	7	91	2	71	15	10	84	8
Uman	RW-20	7	91	2	76	20	4	78	2
Uman	RW-25	7	91	2	86	9	5	90	3
Bayamo	BW-5	6	91	2	90	5	5	95	3
Bayamo	RW-20	6	91	2	86	1	12	99	10
Bayamo	RW-25	6	91	2	86	2	12	98	10

[1] BW-5 = immersion in boiling water for 5 seconds; RW-20 and RW-25 = 20 and 25 seconds, respectively, in a rice-whitener.
[2] SV = soft viable; H = hard; NV = non-viable (dead, dying and abnormal seeds).
[3] Softening effect = number of seeds softened as a percentage of hard seed present before treatment; Killing effect = number of seeds killed as a percentage of number of viable seeds before treatment.

Experimental details

Table 1 provides details of seed origins and germination test conditions for the first 7 experiments. Table 2 lists the experimental treatments applied in Experiments 1–3 and Table 3 those of Experiment 4, while Table 4 identifies the grades of seed that were separately tested in Experiments 5–7.

Table 4. 100-seed weights of different grades of seed of Marc from first (Experiments 5 and 6) and second (Experiment 7) 1994 crops.

Experiment	Harvest method	Seed grade[1]	100-seed weight
			(mg)
5	Header	Green (4)[2]	374
5	Header	Intermediate (1)	390
5	Header	Mature (93)[3]	377
5	Header	Small(2)[3]	249
6	Suction	Entire	370
7	Header	Big	326
7	Header	Intermediate	227
7	Header	Small[3]	136
7	Header	Entire[3]	238
7	Suction	Entire[3]	272

[1] See Methods for definitions of maturity and size grades.
[2] Numbers in parenthesis are percentages by number of each component.
[3] Indicates seed used in Experiment 8 also.

Experiment 1. This was a purely exploratory experiment restricted to a single treatment (4 seconds in boiling water followed by cooling in iced water) of a single line of Uman seed. The subsequent germination tests, besides determining viability and hard seed content, were used to confirm the connection between visible lens rupture and softening. For this, the state of the lens of each seed was determined by observation under magnification and recorded at the start and (if remaining hard) the end of a test. The identity of each seed was preserved by noting the position on a grid drawn on each germination pad.

Experiment 2. Following promising results from Experiment 1, 7 different seed lines including all 3 cultivars were tested with a single treatment (5 seconds in boiling water followed by cooling in iced water). Two additional treatments were applied to seed of 1 line in a preliminary exploration of effects of varying details of treatment. One extended the duration of immersion in boiling water to 60 seconds, the other involved cooling the seed in water at room temperature. Observations of the states of lenses were conducted and results recorded as in Experiment 1.

Experiment 3. A marked effect of duration of immersion pointed to the need for intermediate treatments to show the shape of the response curve as duration increased. Accordingly, immersion for 5, 10, 20, 40 and 60 seconds was applied to samples of seed of a single line of Uman. Additionally, the comparison between cooling in iced and room temperature water was repeated on one line.

Experiment 4. Samples of commercial seed of 2 cultivars, Uman and Bayamo, each of which had been subjected to 2 different lengths of time in the rice-whitener, was supplied. Previously untreated seed samples of otherwise identical seed were immersed in boiling water for 5 seconds. Samples of untreated seed of both lines and of all treated seed were then tested.

Experiment 5. This was done to determine if a link existed between response to boiling water treatment and seed quality. Different grades of visibly inferior seed were separated by sieving and hand-sorting (Table 4). They consisted of: *green* seed, which was grossly unripe at harvest and remained green of cast after drying; *mature* seed, which appeared ripe and fully developed at harvest; *intermediate* seed, which could not be placed in either of the first 2 categories with confidence and represented only 1% of the whole; and *small* seed, which was dry and apparently ripe at harvest, but was presumed to have suffered some adversity during development. Treated and untreated seed of all grades was then tested, every replicate having been weighed after its seeds had been counted out to provide records of 100-seed weights (Table 4).

Experiment 6. This experiment provided an opportunity to compare header-harvested (already tested in Experiment 5) and suction-harvested seed from the same crop, but its main purpose was to check possible adverse effects on seed quality of the washing of the latter in water after conventional cleaning to disperse remaining soil particles. The procedure involved agitating a slurry of seed and water for several minutes in a cement mixer, draining it over a sieve, and drying it rapidly in a blast of air heated to 35°C. The treated seed remained wet for up to half an hour. Germination tests were conducted on samples of the washed and unwashed bulks (Table 5).

Experiment 7. Here we extended the investigation of possible links between seed quality and response to boiling water treatment (Experiment 5) by testing the behaviour of different size grades. Out of the second 1994 crop of Marc, which contained negligible proportions of green seed, 3 size grades of header-harvested seed were separated by sieving (Table 4) and their behaviour compared with that of 'entire' (that is, ungraded after initial machine-cleaning) samples of both header- and suction-harvested seed from the same crop before and after immersion for 10 seconds in boiling water. As in Experiment 6, 100-seed weights were recorded (Table 4).

Experiment 8. A selection of treated (10 seconds immersion in boiling water) and untreated seed samples already tested in Experiments 5 and 7 (Tables 4 and 5) was taken for tests of seedling emergence from soil in a greenhouse in order to

Table 5. Results of germination tests on different grades of 1994 Marc seed showing effects of boiling water treatments on survival and softening of seed.

	Untreated			Treated[1]			Effect[3]		
	SV[2]	H	NV	SV	H	NV	Softening	Killing	Lenses ruptured
					(%)				
1st crop									
Header-harvested (Experiment 5)									
Green	17	24	59	8	0	92	100	80	89
Intermediate	8	72	21	55	0	45	100	31	98
Mature	1	99	1	98	0	2	100	1	100
Small	6	92	2	92	1	7	99	5	84
Suction-harvested (Experiment 6)									
Unwashed	3	96	0	98	1	1	99	1	
Washed	3	96	1	100	0	0	100	0	
2nd crop									
Header-harvested (Experiment 7)									
Big	9	89	2	80	12	8	87	6	
Intermediate	7	90	3	78	16	6	83	3	
Small	20	62	18	53	19	28	70	13	
Entire	4	94	2	73	21	6	78	4	
Suction-harvested (Experiment 7)									
Entire	7	91	2	71	22	6	75	4	

[1] Seed immersed in boiling water for 10 seconds (Experiments 5 and 7) and 5 seconds (Experiment 6).
[2] SV = soft viable, *i.e.* germinated + viable, imbibed, non-germinated; H = hard seed at end of test; NV = non-viable, *i.e.* dead, dying and abnormal seeds.
[3] Softening effect = number of seeds softened as a percentage of hard seed present before treatment; Killing effect = number of seeds killed as a percentage of number of viable seeds before treatment.

provide comparisons of behaviour in the different environments and to record the fate of hard seed in the longer term. Three replicates of each lot, each of 100 seeds, were sown at a depth of 5 mm in steam-sterilised topsoil from a well structured, basalt-derived krasnozem in seedling tray compartments each of 25 cm^2 surface area. The run began on January 3, 1995 and continued for more than a year. Soil was kept moist over periods when germination was being induced (Days 0–44, 85–100 and 367–384 from sowing), but allowed to dry out at other times to simulate conditions likely to be experienced in the field. Numbers of emerged seedlings were recorded daily during periods of active germination. On Day 390, after the soil had been allowed to dry out, remaining hard seeds were exhumed and separated from soil by sieving and aspiration. They were then examined under a dissecting microscope, scarified individually with a scalpel blade, and subjected to routine germination tests.

Results and Discussion

Observations of seed characteristics

Desmanthus seeds differ in many ways from those of more familiar pasture legumes, which we learned by observation during the course of these studies. Awareness of these characteristics is necessary for the interpretation of the experimental results, so they are described first.

Seed form. Desmanthus pods shatter readily, allowing naked seeds to be threshed out during header-harvesting or to fall on to the soil surface for suction-harvesting. The free-flowing, individual naked seed is the functional unit in processing, testing and sowing as well as being the ecological dispersal unit.

The seed is roughly lenticular in shape. Its embryonic axis is straight, a characteristic much more common in *Mimosaceae* than *Fabaceae* (Gunn 1981), giving the seed a slightly pointed tip. Gunn noted that a curved axis provided better protection for the radicle, and the conspicuous vulnerability to impact damage of the desmanthus radicle, which protrudes beyond the cotyledons, supports this observation. It was a factor which deterred us from simply increasing the severity of conventional mechanical scarification when normal levels proved ineffective.

The cotyledons are surrounded by endosperm, which is thick in comparison with that of other tropical pasture legume seeds. This tends to pad the seed out even when the embryo is underdeveloped. The dimensions of seeds with immature embryos thus differ less from those of fully developed seeds than is usual. This makes seed that was harvested immature (and would, in other species, shrivel as it dried) unusually difficult to remove during grading. It tends to perpetuate low quality in seed of crops that, through poor synchronisation of ripening or premature curtailment of development, present significant proportions of immature seed to the harvester. Underdeveloped embryos lack structural rigidity and are apt to buckle during drying, causing the testa to stretch over irregular and often angular underlying shapes. This seems further to weaken a testa already defective through having been dried while still incompletely developed. Distortions aid recognition of immature seed during experimentation, but cannot be exploited in bulk grading.

The lens. In other hard leguminous seeds, and evidently desmanthus, the outermost (epidermal) layer of cells of the testa, the palisade, is impermeable to water. It rests on a second (hypodermal) layer of cells which are characteristically thick-walled over most of the area of the testa. These cells possibly serve to absorb impacts (Tran and Cavanagh 1984) and thus reduce the risk of fracture to the palisade. A structure that forms part of the testa is the lens or strophiole, a region of predetermined weakness which becomes the first point of entry of water in all forms of softening other than that of crude physical damage. In desmanthus, it lies on the main ridge of the seed close to the hilum on the side opposite the radicle. The hilar region is usually conspicuous under magnification as it lacks the scurf that commonly covers much of the testa, and consequently often appears darker than surrounding tissue. However, the hilum itself is inconspicuous, its position marked by a small depression. The region of the lens may also form a broader but shallow depression, the position of the lens itself sometimes appearing as a very slight bulge inside it. A vascular strand follows the ridge and loops up to near the surface of the testa in the vicinity of the lens, where it may be visible as a thin line that more clearly marks the position of the lens.

The lens ruptures under tension to allow the initial passage of water through the testa. In fabaceous seeds it is breached by the buckling of elongated columnar palisade cells to form a cleft (Hamly 1932). No cells are fractured, and in some conditions, the cleft may later close (Hagon and Ballard 1970). The cleft seldom penetrates visibly to the surface of the testa. Lenses of mimosaceous seeds so far described are different, both structurally and in the way they are breached. The point of weakness is not the palisade, but the underlying hypodermis, which consists of thin-walled parenchyma in the region of the lens in contrast to the robust hour-glass cells elsewhere (Dell 1980). The testa conforms to thin-shell theory and may experience deformation forces under stress, which cause the palisade to fracture at such weak points (Tran and Cavanagh 1980).

Desmanthus seed is functionally and, so far as can be judged from observation at up to × 75 magnification with a dissecting microscope, structurally of the described mimosaceous type. The entire palisade of its lens separates from the underlying tissue and then, through fractures in the palisade itself, from the rest of the testa, resulting in an irreversible breach. The lens is approximately circular and slightly less than 0.1 mm in diameter. Its condition is readily determined by observation under magnification, at about x 5 to x 40 depending on its state. It may simply separate from the hypodermis, indicated by no more than a change in colour as it becomes reflective and therefore, in most lights, silver ('separated'). It may erupt to produce a dome of reflective tissue ('erupted'). The dome may burst to leave a crater ('exploded'). An erupted or exploded lens is fragile, and may be wiped off simply by contact with other seeds, leaving only the ragged edges of the crater where the cells of the palisade have been torn. Here, we use the word 'ruptured' to cover all these states.

Only intact lenses have so far been observed in untreated seed harvested either manually or mechanically from the standing crop. From this, we infer that the impacts of threshing at harvest do not rupture a detectable proportion of lenses. Erupted and exploded lenses are found only after seed has been subject to some sudden temperature change, such as immersion in hot water. Their appearance suggests a reaction to gas pressure build-up in the air spaces of the mesophyll. Separated lenses occur at low frequencies, usually less than 1%, in weathered seed that has lain for some weeks on the ground.

We can, with practice, cause lens separation by clamping a seed under a dissecting microscope and applying local pressure with a small probe to a part of the testa close to the lens. The lens suddenly turns silver as it separates from the hypodermis. Lenses separated in this way do not erupt. Though usually permeable, they may be slow to allow water to pass, and occasionally remain impermeable indefinitely. In such cases, they show no sign of surface fractures round the edge of the lens. Erupted and exploded lenses are always permeable.

Separation from the hypodermis and fracture of the palisade thus appear to be separate events, and separation alone does not seem to render a desmanthus seed permeable. For this, fracture of the palisade is needed. However, separation clearly weakens the palisade and predisposes it to fracture, and so the two usually occur virtually simultaneously.

Other structures of the testa. A horse-shoe shaped groove, common in related seeds and named by Corner (1951) the pleurogram, lies on either face of the testa. Its function is unknown but, despite appearing to be a point of weakness in the testa, it seems to have no direct role in maintenance or loss of impermeability. In desmanthus, it appears to facilitate stretching of the testa *after* water penetration has begun and during the swelling that accompanies imbibition. The micropyle also appears to allow ready passage of water after penetration has occurred elsewhere, but has not been observed to be the first point of entry. There is thus no reason to attribute to it a role in seed softening.

The testa is often partly or largely covered with a scurf, especially after weathering or hot-water treatment. The scurf is always most conspicuous on mature seeds and may be absent from those dried prematurely. It bears the imprint of the cell tops, and seems to be equivalent to the 'mucilage-stratum' of Corner (1951), that is to say, to include the cuticle and the cell caps of the palisade. It readily gains or loses moisture in response to changes in atmospheric humidity. We have recorded short-term changes in whole-seed weight of up to 4% attributed to this, which represent a potential source of error in measuring seed moisture. However, it clearly has no influence on testa permeability.

The 3 cultivars of desmanthus differ slightly in the shape and size of the seed, the shape of the pleurogram, and the outward appearance of the lens and hilar region; but all appear to be alike with respect to the essential structure and function of the critical organs.

Confirmation of lens function

In Experiment 1, 99.6% of seeds with visibly ruptured lenses germinated within 7 days. The lenses of those that did not germinate, in this experiment and wherever examined later, did not rupture but merely separated, and showed no surface fractures of the palisade. Some seeds observed to be in this state germinated late.

Solutions of iron salts coming into contact with imbibing palisade cells of some types of leguminous seeds rapidly stain them black (Ballard 1973). Palisade cells of desmanthus seeds react conspicuously to 3-mM ferrous ammonium sulphate solution, as used on other types of seed (Ballard *et al.* 1976). Steeping of desmanthus seeds in such solutions allowed the point of first entry of water to be identified. Occasionally tears occurred in the testas of immature seeds, apparently caused by the stresses associated with buckling of the embryo during drying, and these became points of first entry. These seeds were readily detected and eliminated from consideration because imbibition was more rapid than is possible through the lens, and staining occurred within half an hour of immersion. The point of first water entry into remaining lens-ruptured seeds was then invariably the lens.

These observations together confirm the connection between lens rupture and water penetration.

Experimental variation in results of tests

Results of germination tests are mostly expressed as means of percentages by number of seeds in particular classes. Their calculated standard deviations should correspond closely with the theoretical values inferred from the binomial expansion, provided the experimental method ensures purely random sampling and uniformity of conditions. After each series of tests, we found this to be the case. The average theoretical standard deviation of germination values over 9 runs exceeded the equivalent calculated value by a mere 0.26%, a difference of no statistical consequence.

As an index of reliability of estimates of means of such values as germination percentages, an accurate enough rule-of-thumb is to rely on theoretical standard errors. These vary from about ±2.9% at a mean of 50% to ±1.3% at a mean of 5%. Similarly, differences between means of 9–4%, depending on their position over the same range, may be assumed to be statistically significant at $P < 0.05$.

In soil emergence tests, the variance of percentage emergence was, as is to be expected, greater. Even so, calculated standard deviations averaged only 1.4 times the theoretical values. Standard deviations of means of percentages of viable exhumed hard seeds were 1.5 times the theoretical value.

General patterns of germination and emergence

In the absence of dormancy or impaired vitality, the course of seed germination tended to follow a simple and consistent pattern (Figure 1). It was set primarily by the pattern of uptake of water, and variation in the interval between visible imbibition and radicle emergence was slight. The mean duration of the imbibed ungerminated state was about a day.

Exceptions to this pattern were recorded to a significant extent only in one series of tests, begun only 5 days after harvest (Experiment 7). They are shown in Figure 2 in comparison with seed harvested earlier from the same area, which behaved normally. In the absence of other impediments to germination like low vigour due to age, delays in the germination of imbibed seed must be attributed to the presence of embryo dormancy. When an index of the effect is juxtaposed to seed age combining all records of all 3 cultivars for which seed age was known (Table 6), such dormancy is seen to be short-lived and restricted to fresh seed.

Table 6. Relationship between age of seed at testing and early dormancy.

Age of seed (months)	Viable permeable seed imbibed but ungerminated at Day 7 of routine test
	(%)
0.15	52.9
0.5	13.4
3	4.0
3	0.8
14	1.9

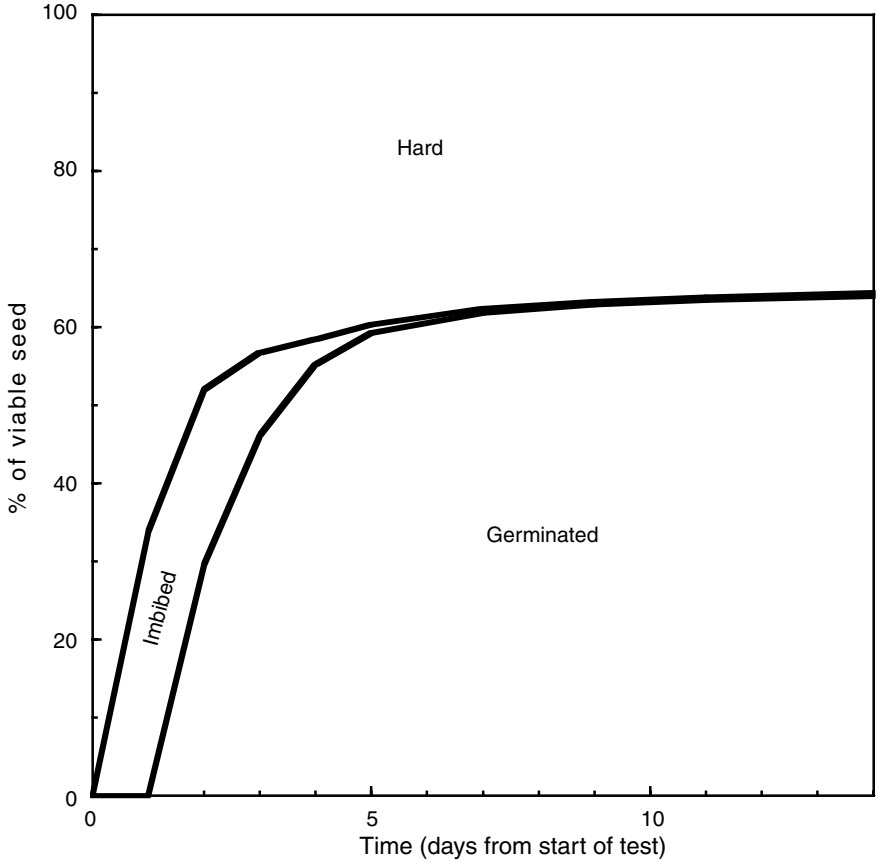

Figure 1. Typical pattern of germination of non-dormant seed under laboratory conditions. The figure is a composite derived from the behaviour of 1346 viable seeds of 1993 Uman after treatment with boiling water.

Germination in laboratory tests was normally virtually complete within a week of the start of the test, and few remaining seeds germinated without specific treatment to break impermeability. Germination of buried seed in soil (Experiment 8) followed much the same pattern. The measured property, seedling emergence, proceeded more slowly than laboratory germination, partly because it is a later stage of the germination process. Mean time for seedlings to emerge from soil in the greenhouse was 7.2 days, compared with a mean germination time for the same lines of seed in laboratory tests of 4.0 days. Very few seedlings emerged after the first flush, which arose from seeds that were permeable at sowing. Averaged across all lines of seed used, 23.9% had produced seedlings by Day 14, and 27.7% when the pots were dried off on Day 44. A further period of watering between Days 85 and 100 produced only another 0.7%, and another between Days 367 and 384 only 0.03%, despite the survival of 32.6% as a reservoir of hard seed in the soil.

Boiling water treatment

Boiling water treatment typically softened a high proportion of seeds while (provided the duration of immersion was brief) killing very few, substantially increasing the percentage of soft viable seed (Table 3). The only exception was the very low green seed fraction of one crop where the soft viable percentage was reduced (Table 5). As this was a minor constituent of the whole sample (Table 4), the overall effect was insignificant. Since the tests were done on samples representing virtually all commercial lots from 1993 and most pre-commercial seed-increase lots over 3 seasons, the benefit may be taken as general.

Cooling with iced water proved to be unnecessary: in 2 separate comparisons with cooling with water at ambient temperature, it made no detectable difference (Table 2). Nevertheless, its use was retained in order to fix the duration of exposure to high temperature as exactly as possible.

Treatment of seed which had been treated a year before caused a further, albeit lesser, increase in germination without further death (Table 2). Previously scarified seed responded to treatment in a similar way to intact seeds. Tetrazolium staining of cotyledon tissue which had been exposed directly to boiling water through fractures showed only a shallow outer layer to be dead (minor undescribed tests). We concluded that a history of prior softening treatments need not disqualify seed from treatment with boiling water.

Effects of increasing duration of immersion in boiling water (Figure 3; Table 2) showed that most of the benefit in terms of softening was achieved after only brief immersion, whereas the numbers of seeds killed increased progressively as time of exposure increased. In other words, brief immersion produced the most satisfactory result, with no significant net gain beyond 10 seconds. The divergence of the killing and softening curves was wide (Figure 4), indicating that accurate timing of immersion (so long as it is brief) is not critical — an important practical attribute.

Experiment 8 confirmed that the benefits of immersion extended to seedling emergence from soil (Table 7). It also illustrated that a further consequence of treatment was a massive reduction in the amount of long-term seed survival in soil.

The physiological lesson emphasised by these results was that the softening effect (the action of boiling water on the lens) was almost instantaneous, while the killing effect (due presumably to

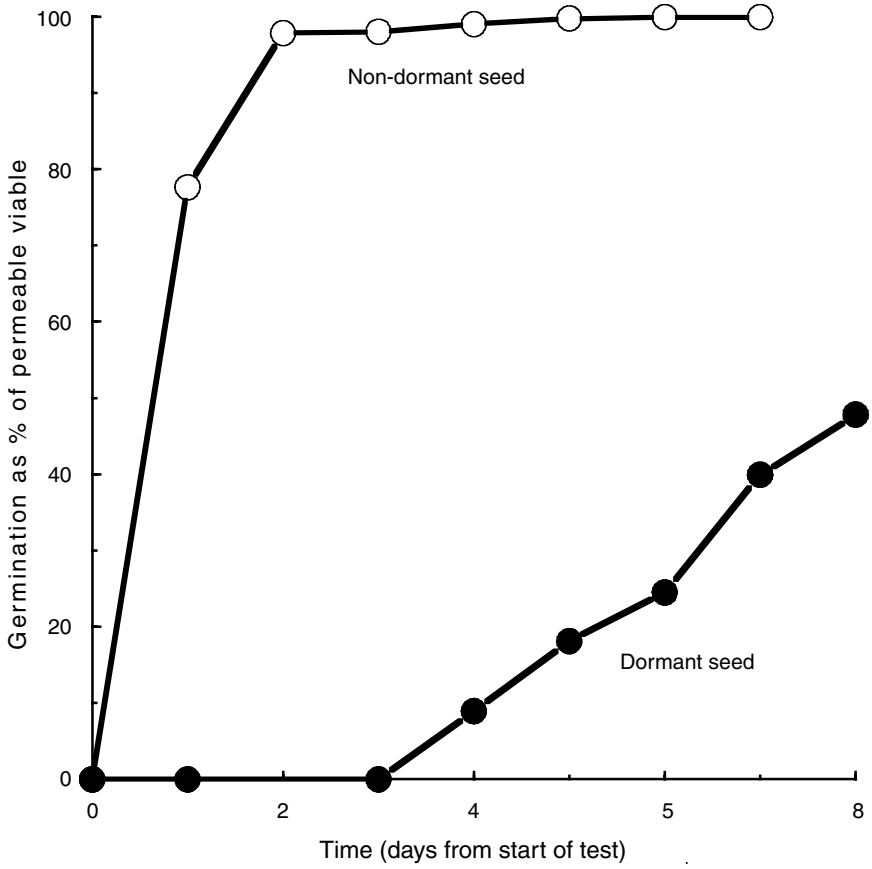

Figure 2. Illustration of the effect of early dormancy on the course of germination under laboratory conditions, comparing the behaviour of freshly harvested dormant and older non-dormant seed of 1994 Marc.

the conduction of heat across the testa and into the embryo) was progressive.

Table 7. Comparisons of response to boiling water treatment of normal and small seed tested in laboratory conditions and in soil in the greenhouse, records being expressed as percentages of total seed used in each test (Experiment 8).

	Normal seed[1]		Small seed[2]	
	Untreated	Treated[3]	Untreated	Treated[3]
Laboratory tests[4]				
Soft viable	4	81	13	73
Hard	95	15	77	10
Non-viable	2	5	10	18
Soil tests[5]				
Emergents	6	70	10	18
Surviving hard	76	4	43	1
Expiring in soil	16	22	38	63

[1] Combines mature header-harvested seed of Experiment 5 and entire header- and suction-harvested seed of Experiment 7 (overall average weight 294 mg/100 seeds) (see Tables 4 and 5).
[2] Combines small header-harvested seed of Experiments 5 and 7 (average weight 193 mg/100 seeds) (See Tables 4 and 5).
[3] Immersed in boiling water for 10 seconds.
[4] Soft viable = germinated + viable, imbibed, non-germinated; Hard = hard seed at end of test; Non-viable = dead, dying and abnormal seeds.
[5] Numbers of seeds expiring in soil are not directly measurable and are inferred from the differences between numbers of viable seeds sown (estimated from the lab tests) and numbers accounted for as emergents (emerging seedlings) and exhumed hard seeds.

Atypical behaviour of defective seeds

Preliminary observations. In Experiments 1, 2 and 3, a greater number of seeds was softened by boiling water treatment than could be accounted for by the observed increase in the number of ruptured lenses. An analysis of the behaviour of 20 groups of seed showed that treatments which increased the number of ruptured lenses by an average of 56% raised the number of softened seeds by 68%. This difference of 12% was highly significant ($P < 0.001$) judged by a paired t test. Obviously, either not all ruptures were detected or there were points other than the lens at which seed could be softened. The most common reason for the difference seemed to be an increase in the incidence of torn testas following treatment. The association of torn testas with the distorted shape of immature seeds (see *Confirmation of lens function*) suggested that immature seeds might contribute disproportionately to the difference. In addition, in Experiment 3, seeds with ruptured lenses were consistently heavier than those that remained intact, the difference averaging 13%, as if the lenses of light seeds responded less readily to treatment. These observations all raised the suspicion that small or immature seed behaved anomalously, so Experiments 5, 7 and 8 were devised to check this hypothesis.

Experiments 5, 7 and 8. Green seed of Marc in Experiment 5 was of very low quality initially, and was largely killed by treatment that had little adverse effect on mature seed (Table 5). A high proportion of seeds was already permeable before treatment. Some perhaps had been harvested before their testas had completed their development, and in some the testa tore during drying. In such seeds, while the response of the lens was irrelevant to the outcome, it often failed to rupture, as if a pressure difference across the testa was needed to produce the required eruption, but failed to develop if the testa was breached. Seed that was intermediate in condition was also intermediate in response, while mature seed reacted consistently and favourably to boiling water.

The second crop from the same area, used in Experiment 7, produced smaller seed overall, and the small grade was much lighter than that of Experiment 5 (Table 4). The softening effect on all seed was less complete than in Experiment 5. A relatively high proportion of small seed failed to soften, and a relatively high proportion was killed, with treatment (Table 5).

When the comparisons were extended to soil tests, the differences between small and normal seed became more conspicuous (Table 7). The records are bulked for presentation, seed lots within each grade behaving essentially alike. Small seed, while producing more emergent seedlings from untreated seed than normal seed as a result of having more initially soft seeds, suffered far more mortality overall than normal seed. It fared especially badly after boiling water treatment, more so than germination tests indicated, producing far fewer seedlings than normal seed and leaving almost no surviving hard seed in the soil.

Thus, when fractions of low grade seed were separated out and compared with good seed, the results vindicated earlier suspicions derived from observations on unsorted samples. Defective seed, whether immature through premature harvest or small through probable adversity during development, was less satisfactorily responsive to

boiling water treatment than fully developed seed. Embryos were more vulnerable to damage, and lenses less likely to be ruptured.

When small seed was excluded, the success of emergence from soil was more or less consistent with laboratory germination. The relationship between numbers of soft viable seeds and of emergents over the 6 lines of seed was close to linear (r = 0.985) and almost proportional, numbers of emergents averaging about 80% of the soft viable fraction.

Treatment with rice-whitener

Treatment in a rice-whitener, whether for 20 or 25 seconds, was highly effective in softening seed without causing unacceptable numbers of deaths, producing a very similar overall result to that of 5 seconds in boiling water (Table 3). Responses in the 2 seed lots differed a little, the Bayamo being softened more completely than Uman and after a shorter period of treatment, but suffering rather more death.

Observation of seed before and during imbibition showed effects of both lens rupture and scarification. The scarification was unusual in consisting of multiple gentle scuffings rather than the relatively few large fractures that sanding disc scarifiers cause. In addition, the scuffing was effective primarily on that part of the testa covering the radicle protrusion. The combination of this effect and lens rupture caused conspicuous imbibition and swelling of the radicle before moisture had penetrated the testa elsewhere.

The average percentage of separated lenses was much higher in Bayamo (91%) than in Uman (54%), possibly a reflection of the more exposed position of the lens of the former on the raphe. Our observation of lens separation after multiple small impacts in the rice-whitener led us to use pressure to nearby parts of the testa (described earlier) to cause observable separation under the microscope.

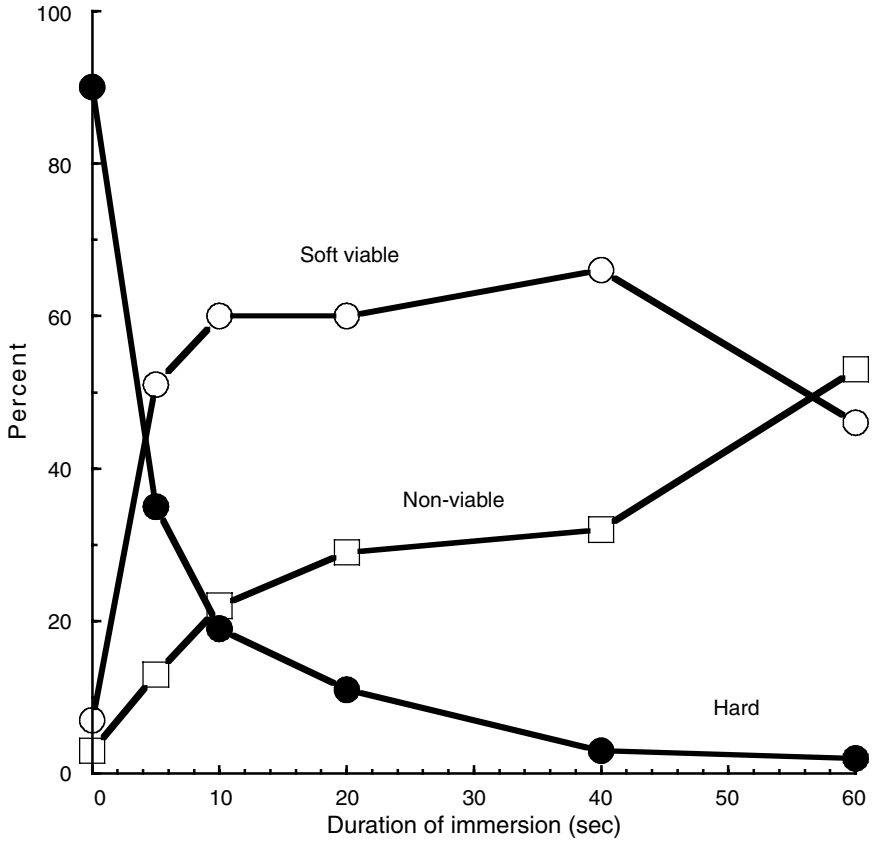

Figure 3. Effects of duration of immersion in boiling water on behaviour of 1993 Uman seed in Experiment 3, recorded after 14 days in laboratory germination tests.

Behaviour of suction-harvested seed

The adoption of suction-harvesting raised 2 questions: did suction-harvested seed differ from header-harvested seed in either its characteristics or its reaction to softening treatments?; and were there any adverse consequences of the wetting which occurred when suction-harvested seed was washed with water to disperse soil particles?

Comparison of the records of the most closely matched pairs of samples of Marc seed [first crop, header-harvested mature and suction-harvested unwashed; second crop, header-harvested entire and suction-harvested entire (Table 5)] shows strong similarity between members of each pair. Clearly neither harvest method caused much softening, despite the violence of the impacts sustained during both. Harvest method seems to have little influence on the vital characteristics of mature desmanthus seed.

Results of Experiment 6 alleviated concerns about an adverse effect of washing on suction-harvested seed (Table 5), with no detectable effect of washing on test performance before or after boiling water treatment, and no visible sign that imbibition had begun in the small number (3%) of permeable seeds present at washing. We conclude that, if the described procedure is followed, seed can be washed and dried with impunity.

Moisture content

Combine-harvested seed dried with forced draught at elevated temperature (commercial and experimental lots considered together) averaged 8.4% moisture content (range 7.4–9.5%). The two suction-harvested lots had moisture contents of 7.6 and 5.2% as harvested. The latter, ground

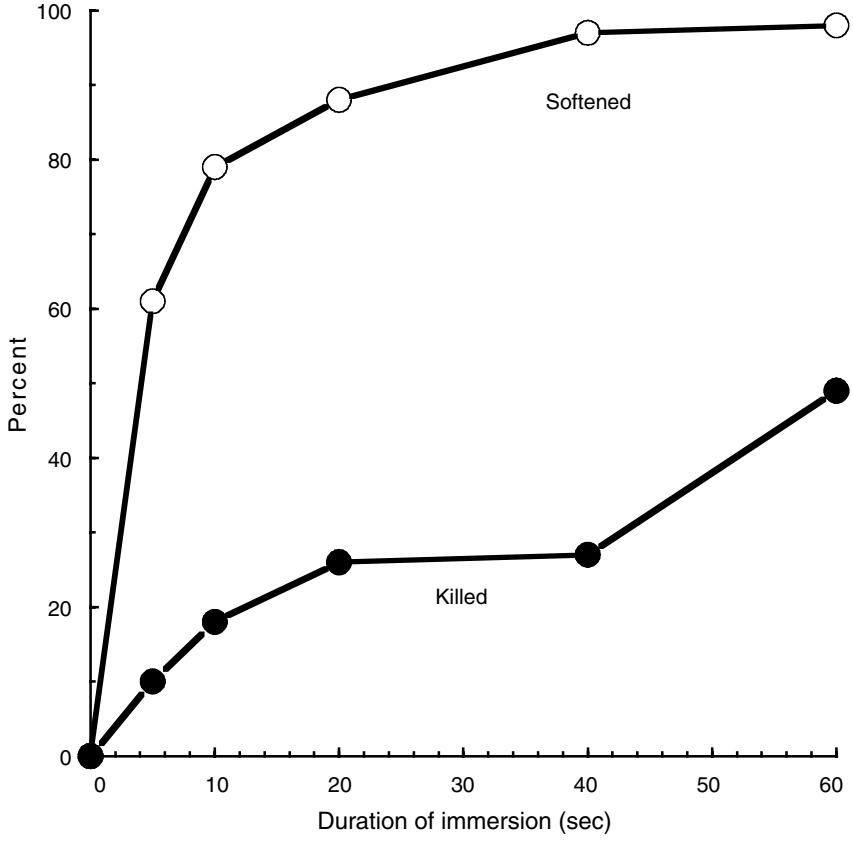

Figure 4. Effects of duration of immersion in boiling water on softening and killing effects on 1993 Uman seed in Experiment 3. Effects are calculated as the number of seeds affected (*i.e.* softened or killed) as a percentage of the number available to be affected (*i.e.* initially hard or initially alive).

and placed in a confined atmosphere, drew the equilibrium relative humidity of surrounding air down to 23%. No evidence of internal moisture regain in storage was recorded, despite long periods of high ambient air humidity (>70%). In Experiment 8, the average moisture content of hard seed in soil was 1% lower when exhumed than it had been when sown a year earlier.

Two inferences may be made from such records. Firstly, although thermal damage to the embryo from immersion in boiling water will theoretically increase as seed moisture content over the period of immersion increases [an effect clearly detectable, for example, when dry heat is applied to leguminous seeds (Hopkinson and Paton 1993)], there is no indication of it having done so. Presumably, the heat dose received was insufficient, at least with brief immersion, to produce such an effect, which means that the risk of unwitting damage to seed through treatment at high moisture content is low. Secondly, dry hard desmanthus seed continues to lose moisture at low ambient humidity while not regaining it at high humidity. It shares this ability with at least some other mimosaceous seeds (Tran and Cavanagh 1984), in spite of the absence of the structures of the hilum that control it in fabaceous seeds (Hyde 1954). Tran and Cavanagh had some evidence that the lens might provide a route for at least some diffusive loss of moisture in mimosaceous seeds, while Gunn (1981) reported an unpublished suggestion by Hyde that the pleurogram of *Albizia* might facilitate irreversible drying. The route remains unknown in desmanthus seeds.

Commercial implications

Our concern, if use of boiling water is to be promoted for commercial treatment, is about the reliability of the effects across seed lots, particularly avoiding serious damage to seed. Our records indicate low risk of damage. Indeed, investigation of potential causes of variation in effect — provenance, cultivar, moisture content, duration of treatment, cooling method, prior treatment — has emphasised the robustness of the method. The only serious inconsistency seems likely to arise from the anomalous behaviour of inferior (small or immature) seed. This is no cause for concern with crops grown in north Queensland where the incidence of defective seed has invariably been very low. However, there are indications that it introduces a risk for seed produced under the cooler winter conditions of south-east Queensland. Cox (1998) recorded small seed, poor germination and very low levels of hardseededness in Bayamo near Gympie, which he attributed to frost damage; but better all-round quality in Marc, which escaped frosting through earlier seeding. We have noted high levels of immature seed in samples from multiplication plots near Gayndah. In the light of such occurrences, inconsistencies in treatment effect like those reported by Loch and Harvey (1992) might be expected in other seed produced in southern Queensland. However, they do not translate into a commercial problem as commercial seed has not been grown there. We are therefore confident that boiling water treatment of commercially produced seed will be both effective and reliable.

Given this confidence, we devised a method for on-farm treatment of seed to be used for sowing individual seed crops (our most urgent priority). Quantities of several kg of seed were placed in a freely draining cage made for the purpose with fly-screen and wire, which was then immersed for 5 seconds in a large container of boiling water, removed, promptly immersed in an overflowing 200 L drum of tap water, removed when cool, drained, and then dried immediately with a strong flow of air heated to 35°C. We successfully treated several hundreds of kg of seed with this method. Meanwhile, the commercial initiative of using a rice-whitener proved equally satisfactory for bigger-scale treatment of bulk seed for sale for extensive pasture sowing. Thus, the challenge of raising the immediate germination capability of desmanthus seed has been overcome.

There remains, however, one deficiency in desmanthus seed that is not overcome by seed treatment. Seed that is hard at sowing has very little capacity to soften afterwards. The buried seed of Experiment 8 provides a rather artificial example, but the same characteristic is clearly evident from earlier records (Burrows and Porter 1993) showing the average rate of softening of seed placed in various positions in a pasture to be only 9% per year of the hard seed originally sown. A more recent comparison of hard seed breakdown in dry seeds of numerous leguminous species stored at high temperature has emphasised the particular intransigence of desmanthus in its resistance to softening (McDonald 2000).

Protracted retention of hardseededness in sown seed places a lot of dependence on success of initial establishment, which in many environments is a risky policy. Dependence might be reduced if treatments were available that predisposed seeds to later softening. Taylor (1981) demonstrated such a predisposition in subterranean clover. Since then, several other mediterranean pasture legumes have been shown to share this ability. All, however, are fabaceous species with structurally different lenses from those of desmanthus, and different mechanisms of lens rupture. Optimism based on the existence of a precedent would thus be misplaced. Relevant precedents might be better sought in the behaviour of other mimosaceous seeds, though we ourselves have failed to find any.

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