Forage seed quality: dormancy, standards and quarantine

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ABSTRACT

There are many dimensions to the concept of seed quality. Viable seed which will not germinate when provided with all the requirements for germination is dormant. Forage grasses mostly exhibit non-deep physiological dormancy (PD), while physical dormancy (PY) is common in forage legumes where imbibition is prevented by the seed coat's impermeability to water (hard seed). Methods for breaking PD and PY to allow germination testing and/or crop establishment are discussed.

In the seed industry seed quality standards are an important feature of quality assurance and may apply for seed production contracts, seed certification, seed sale and seed importing. Standards for these uses are discussed, with a comment on legislated minimum germination standards which may do little to offer protection to the buyer. Exported seed lots must meet the seed quality standards and phytosanitory/biosecurity requirements of the importing country, but while there is an obvious need to protect against the spread of economically important pests, they should not be used as unnecessary barriers to the seed trade.

Key words: Biosecurity, Germination Testing, Hard Seed, Physiological Dormancy, Scarification, Seed Quality Standards

Introduction

There are many dimensions to the concept of seed quality which may have varying degrees of practical importance for agriculture (Hampton, 2002). Traditionally analytical (or physical) purity and germination capacity have tended to be the only properties of seed considered when assessing forage seed quality, but other components such as cultivar (genetic) purity, seed weight, noxious weed contamination, seed health, moisture content and seed vigour are also important. Rolston (2015) has discussed genetic purity, analytical purity and germination of forage seeds. This companion review examines three additional aspects related to forage seed quality; dormancy and methods for breaking dormancy, seed quality standards and seed requirements for quarantine and biosecurity.

Dormancy

Dormancy is a property of seed that prevents germination of viable seed under external conditions that are adequate to support the germination process itself (Cohn, 2006). Its ecological purpose is primarily to prevent germination in an environment unfavourable for subsequent plant growth. In seeds of most economically important crops the seed dormancy characteristics have been largely removed through selection for rapid and uniform germination. This is not the case for forage species, particularly tropical and subtropical species, where seed dormancy characteristics are still often very similar to wild forms (Atkins *et al.*, 2002).

Baskin and Baskin (2014) described five classes of dormancy: physiological (PD), morphological (MD), morphophysiological, physical (PY) and combinational. In forage

species PD and PY are most common. PD means there is a "physiological inhibiting mechanism" that prevents the germination process, while in seeds with PY, germination is prevented because the seed coat is impermeable to water (the so called "hard seed" condition). PD can be grouped into deep (embryo dormancy), and intermediate and non-deep (coat-imposed; the embryo lacks the resources to overcome mechanical constrains of the covering layers such as the endosperm, palea and lemma and seed coat; Duclos *et al.*, 2013). Forage grasses usually have non-deep PD (Baskin and Baskin, 2015). PY is characteristic of forage legumes (Argel and Paton, 1999). However MD (embryos immature when seed dispersed) has been reported for some tropical grasses including *Brachiaria*, *Digiteria* and *Pennisetum* (Atkins *et al.*, 2002). A recent review of dormancy in forage species has been provided by Baskin and Baskin (2015).

Dormancy breaking methods:

***(i) For germination testing*:** For temperate grasses non-deep PD can be broken by placing imbibing seeds inm cool (5-10°C) conditions for 4-7 days (prechilling – ISTA 2015). The other recommended method is the use of KNO_3 (sometimes used in conjunction with prechilling).

For temperate forage legumes there is often no attempt made to break the hard seed coat to allow germination. Instead the percentage of hard seeds in the seed lot is reported as a separate category on the seed analysis certificate (Scott and Hampton, 1985), the assumption being that any hard seeds are viable and once sown will eventually germinate once the impermeable seed coat is gradually broken down. Alternatively, mechanical scarification (careful piercing, chipping, filing or sandpapering of the seed coat) may be used (ISTA, 2015).

For tropical and subtropical species for which germination test methods are included in the International Rules for Seed Testing (ISTA 2015), methods for breaking dormancy are presented (e.g. Table 1). These are species dependent and include prechilling (seeds imbibed on moist substrate at 5-10°C for 4-7 d); pre-heating (dry seeds heated at 30-35°C with free air circulation for up to 7 days); light (illuminate for 8h in every 24h cycle and during the high temperature period if seeds are germinated at alternating temperature); gibberellic acid (substrate moistened with 0.05% GA_3 solution instead of water); potassium nitrate (substrate moistened with 0.2% KNO_3 solution instead of water), acid scarification (seeds soaked in concentrated H_2SO_4 until seed coat becomes pitted, then washed in running water – time in acid is species dependent); cutting seed (a small cut avoiding the embryo, to allow imbibition).

However, there are many tropical and subtropical forage species for which ISTA does not yet have test methods. Butler (1999) recommended the use of $KN0_3$ for *Axonopus, Bothriochloa, Cynodon, Digitaria, Panicum,*

Table 1. Recommended dormancy breaking methods for germination testing of some tropical/ subtropical forage species (adapted from ISTA, 2015).

Species	Dormancy breaking methods
Andropogon gayanus	Light; KNO_3
Brachiaria decumbens	Light; H_2S0_4
Cenchrus ciliaris	Preheat; prechill
Chloris gayana	Prechill; light
Macroptilium atropurpureum	
Stylosanthes hamata	Cut seed
Urochloa mosambicensis	GA3

Paspalum, Pennisetum and *Setaria* spp, while Atkins *et al.* (2002) reported that a number of grass species including *Bothriochloa, Brachiaria, Chloris, Pennisetum* and *Paspalum* responded to scarification (by mechanical or chemical means). They also reported that several grass species (including *Chloris, Digitaria* and *Panicum*) were stimulated to germinate by smoke, a response now believed to be caused by the presence of the water soluble butenolides which have potent germination promotion action (Flematti *et al.,* 2004).

(ii) For sowing: For temperate forage grasses, a short time in storage post-harvest is sufficient for the non-deep PD to dissipate. For example *Lolium perenne* seed lots may be dormant at harvest, but non-dormant three or four months later and thus able to germinate when autumn sown.

Seed lots of tropical/subtropical grasses such as *Brachiaria decumbens* and *Panicum maximum* however may remain dormant for up to one year post-harvest. For example dormancy in *P. maximum* disappeared between 50 and 300 days of storage (Chin and Hanson, 1999). For these two species, mechanical scarification (Atkins *et al.,* 2002) may be an option for breaking dormancy.

In hand harvested forage legumes, seed is often between 90% to 100% hard. Mechanical harvesting can sometimes provide sufficient scarification to reduce hard seed levels. For example in *Trifolium repens*, hard seed levels of >10% are uncommon, but this is species dependent, with hard seed levels of >20% occurring commonly in *Medicago sativa* for example (Scott and Hampton, 1985). For temperate forage legumes a seed polisher is used to scarify seed lots at or near the end of the seed cleaning process. Seed is fed into the machine and abraded by brushes rotating against a woven wire screen. The intensity of the abrasion can usually be adjusted by alteration of brush speed, distance between the brushes and screen, and throughput rate (Simon *et al.,* 1997). Care must be taken to ensure that this process does not cause damage to the seed thereby reducing germination.

For tropical forage legumes, there is no one technique for the rapid scarification of commercial seed lots (Argel and Paton, 1999). Some success has been reported with the use of a rice polisher which has an abrasive stone wheel which revolves at high speed as seed is passed through the machine (D. Loch, pers. comm. 2010). A reliable mechanical scarification method for tropical forage legumes is urgently required.

Seed quality standards

A standard can be defined as "a measure by which the accuracy or quality of a product is judged", or "a document specifying nationally or internationally agreed properties for a product" (Hampton, 1998). Standards are therefore an important feature of quality assurance. In the seed industry, seed quality standards may apply for seed production contracts, seed certification, seed sale and seed importing.

***(i) Seed production contracts*:** The contract between the grower of the seed crop and the owner of or agent for the cultivar will vary within and among countries, but will commonly include standards which must be met or exceeded. For example for:

- germination (e.g. e"90% for *Lolium perenne*; e"75% for *Lablab purpureus*)
- analytical purity (e.g. e"98% pure seed for *Medicago sativa*: e" 90% pure seed for *Stylosanthes guianensis).*

Standards for seed moisture content, seed size and seed health (e.g. free of ergot (*Claviceps*

spp.)) may also be included. Seed production systems for achieving these standards have been presented by Rolston (2015).

***(ii) Seed Certification*:** A seed certification scheme such as the Organisation for Economic Co-operation and Development (OECD) scheme for herbage and oil seeds (OECD, 2015) is designed primarily to maintain genetic integrity and to minimise the risk of physical contamination by seeds of other cultivars (Rolston, 2015). It therefore has standards for cultivar purity only. Individual countries may additionally require standards for other components of seed quality including analytical purity, seed size, and freedom from noxious or undesirable weed seeds, germination and seed health. For example the Indian Minimum Seed Certification Standards for *Trifolium alexandrinum* are: germination (80%), pure seed (98%), inert matter (2%), other crop seeds (10/kg) and weed seeds (10/kg).

A failure to meet any of the quality standards for seed lots entered into certification can result in either the downgrading (e.g. from basic seed to first generation seed) or rejection of the seed lot from certification. For example Rowarth *et al.* (1990) reported that 12% of 537 *Trifolium repens* seed lots produced in one season were either downgraded (for failing to meet the maximum permitted weed seed percentage) or rejected (for the presence of noxious weeds including *Carduus nutans* and *Amsinckia calycina*).

For temperate forage seed species seed certification and standards are long established (Hampton and Scott, 1990), but this is not often the case for tropical species (Loch, 1993), partly because the major tropical forage species tend either to be predominantly or wholly apomictic (e.g. most *Brachiaria* spp., *Cenchrus ciliaris, Panicum maximum*) or are strongly self-pollinating (most legumes) (Hacker and Hanson, 1999). In both cases there is relatively little or no risk of genetic drift through uncontrolled multiplication. End users in the tropics tend not to place a high priority on cultivar uniformity and stability and the need for standards (Loch and Boyce, 2001). While some countries have developed a strong seed certification system for arable crops (e.g. India – Agrawal and Tunwar, 1990; Nepal – NARC, 2014), they have yet to do so for the majority of forage crops (Kumar and Sridhar, 2015; Dinesh Pariyar pers. comm. 2015). Kumar and Sridhar (2015) noted that in India, seed standards for many forage crops have not been formulated, while in Nepal no standards currently exist for forage crops.

(iii) Seed sale: Most countries control by law the production and marketing of seed for sowing, to protect the buyer and seller from uncertain quality and from fraudulent practices, and thereby improve agricultural productivity (Tripp, 1997). Details of seed quality requirements are usually described in regulations rather than the seed law itself (e.g. standards to be applied to germination, analytical and cultivar purity, seed-borne pathogens etc). Systems range from the strict controls over what seed can be bought and sold (i.e. enforcing a set of minimum standards) which apply within the European Community to the "truth-in-labelling" system (a declaration of quality) which applies in the USA. In the former system farmers can only buy seed which has met the minimum standard, whereas in the latter system high quality seed is expected to drive low quality seed off the market (Tripp, 1997). Note that in the "minimum standards" system, many seed companies choose to operate to "higher voluntary standards" (Rolston, 2015).

(iv) ***Seed importing*****:** Most countries have legislation (for Quarantine or Biosecurity) which sets standards relating to the importation of seed. Seed lots may be required to meet both the plant health and seed quality standards of the importing country (see Quarantine/Biosecurity in a later section of this paper).

A comment on germination standards

As already noted, most countries have legislated minimum germination standards for seed imports (e.g. Table 2) and seed certification schemes (e.g. Table 3), and grower contracts usually contain a minimum germination standard. However, are these minimum germination standards of any value? To explain:

Table 2. Minimum germination standards for imported forage seed lots from a number of countries

Species	Minimum germination	Importing Country
Trifolium repens and *T. pratense*[1]	60%	South Africa
	75%	Argentina
	80%	Canada, EC
	85%	Chile
Lolium perenne	60%	South Africa
	65%	Australia
	80%	Canada, Chile, EC

[1]may also include hard seed

A germination test, when conducted using an internationally agreed method, indicates the percentage of seeds which have produced normal seedlings, abnormal seedlings, and which have failed to produce a seedling (because they are dead or dormant) (ISTA, 2015). While a seed that produces an abnormal seedling has germinated physiologically, the seedling will not be able to emerge from the soil. A germination test result of 90% therefore means 90% normal seedlings. As previously noted, dormancy is a feature for many herbage species, but for grasses the germination test method includes procedures for breaking dormancy and thus allowing germination to proceed, while for forage legumes the percentage of seeds prevented from germinating because the hard seed coat prevents imbibition is reported on the seed analysis certificate (ISTA, 2015).

Seeds can begin to age, or deteriorate physiologically, both pre-and post-harvest (Hampton, 1991), and symptoms of this deterioration include the presence of abnormal seedlings and dead seeds (ISTA, 2015). As the percentage of normal seedlings declines from 100% (excluding the special case of hard seeds in forage legumes), the percentage of abnormal seedlings and/or dead seeds increases (e.g. Table 4). Such physiologically deteriorated (low vigour) seed lots are highly likely to further decline in germination during storage, or transport internationally, and will struggle to perform once sown. Thus the minimum germination standards required for imported seed lots (e.g. Table 1) or for seed certification (e.g. Table 2) signal that it is acceptable to import or sell physiologically deteriorated seed lots, offering little protection to the buyer.

For temperate species, higher voluntary standards are applied in many countries, and therefore this problem can be largely avoided. For tropical species, many of which are relatively new to agriculture, low germination (Table 3) is characteristic of many grasses and legumes (Beavis and Harty, 1999), and the minimum germination standards reflect the production problems which result in low seed quality (Loch and Ferguson, 1999). However, Hare (2015) has recently described tropical forage grass seed production systems in northeast Thailand and northern Laos, which if done correctly, result in high germinating seed lots. It is time perhaps, to reassess minimum germination standards for forage species.

Table 3. Minimum germination percentage standards for some forage species in Australia and India

		Species	Minimum germination (%)
Australia[1]	Grasses	*Brachiaria decumbens*	15
		Chloris gayana	20
		Cynodon dactylon	60
	Legumes[2]	*Desmodium intortum*	70
		Macroptilium atropurpureum	70
		Stylosanthes hamata	60
India[3]	Grasses	*Cenchrus* sp.	30
		Setaria sp.	50
		Sorghum sp.	75
	Legumes	*Medicago sativa*	80
		Stylosanthes sp.	40
		Trifolium alexandrinum	80

[1]from Beavis and Harty (1999); [2]includes hard seed; [3]Indian minimum seed standards for forage crops (undated)

Quarantine and Biosecurity

Any exported seed lots must meet the seed quality standards and phytosanitary/ biosecurity requirements of the importing country. For example in Australia, all seed lots imported into the country must meet Department of Agriculture standards for seed contaminants. These include that seed lots be free of soil (a tolerance of 0.1% is allowed), a tolerance for restricted seed contamination of seed lots (e.g. 60 seeds of *Medicago sativa* per kilogramme in other forage legume seed lots), and seeds of weed species which are prohibited from entry into Australia (for example *Carduus nutans* (nodding thistle), *Pennisetum macrourum* (African feather grass) *Setaria faberi* (giant foxtail)), all of which have a zero tolerance (Department of Agriculture, 2015). Most importing countries have a schedule of regulated (quarantine) weed seeds. Usually an International Seed Analysis Certificate issued by an ISTA accredited seed testing laboratory must accompany the imported seed lot documenting the status of the seed with respect to quarantine impurities. For example in New Zealand, no seed lot will be given biosecurity clearance if it contains unidentified seed, greater than 0.1% by weight of soil particles, or seed of any species listed in the New Zealand Schedule of Regulated (Quarantine) Weed Seeds (MPI, 2014).

Countries will also have a system of official rules (legislation and regulation) to prevent the introduction and/or spread of quarantine pests and pathogens. Many importing countries specify phytosanitary import requirements and require a combination of import permits and phytosanitary certificates for the international movement of a seed lot (Cockerell, 2006). For example the New Zealand Ministry for Primary Industries has an Import Health Standard for Importation of Seed for Sowing, which specifies the phytosanitary requirements that must be met for compliant seed for sowing to be given biosecurity clearance to enter New Zealand (MPI, 2014). In setting these requirements the Ministry has been required to ensure that they are:

(i) technically justified

(ii) do not impose unjustified technical

Table 4. Some possible germination test results for seed lots which meet an 80% minimum germination standard (Hampton, 1998)

Species	Seed lot	Normal seedlings (%)	Abnormal seedlings (%)	Hard seed (%)	Dead (%)
Lolium perenne	1	80	18	-	2
	2	80	3	-	17
	3	80	9	-	11
Trifolium repens	1	80	18	1	1
	2	80	2	1	17
	3	80	10	2	8

barriers to trade

(iii) provide an appropriate level of biosecurity protection (i.e. prevent the entry of unwanted organisms) (Hampton, 2010). For example for seed of *Medicago* spp. imported into New Zealand, the named quarantine pests are pea early browning virus, peanut stunt virus, *Trogoderma granarium* and *Xanthomonas campestris* pv. *alfalfae*. The phytosanitary certificate for the seed lot must contain a declaration that the *Medicago* seeds "have been inspected in accordance with appropriate official procedures and found to be free of *Trogoderma granarium*" (the regulated pest), and "sourced from a pest free area" or "pest-free place of production", and "free from Pea early browning virus, Peanut stunt virus and *Xanthomonas campestris* pv. *alfalfae*". (MPI, 2014). The exporting country is therefore required to conduct the seed health testing or inspections so that the declaration can be made. In other countries, there may also be a requirement for retesting/inspection of the imported seed lot after arrival in the country, as is the case, for example in Iran (M. Dehgan-Shoar, pers. comm. 2015).

A comment on the unjustified use of phytosanitary regulations

As reported by Hampton (1998), a seed-borne pathogen (a *Phoma* sp.) was detected by the importing country on a weed seed (*Viola*) in a New Zealand exported forage grass seed lot. Shipments of further grass seed lots to the importing country were suspended because the pathogen was declared not to occur in the importing country. Subsequent a costly investigation by New Zealand officials proved that the pathogen had first been recorded in the importing country in the 1920s, and while not common, was certainly present. Exports from New Zealand were eventually allowed to resume.

In a second example, after over one hundred years of exporting New Zealand forage grass seed lots to another country, the importing country introduced a new list of regulated seed-borne pathogens. New Zealand seed exporters were then required to assure freedom from a number of these seed-borne pathogens which had been recorded in New Zealand, but are of no economic significance. This would have required seed health testing for these pathogens, an expensive exercise complicated by the fact that internationally agreed seed health testing methods for these pathogens did not exist. Government to government negotiations resulted in a revision of the importing country's regulated pest list to the satisfaction of both parties (Hampton, 2002).

The first example was, in the New Zealand seed industry's view, a non-tariff barrier to international trade, while the second example appeared to result from a lack of access to scientific information on the pathogens in question (Hampton, 2010). As noted by McGee (1997), "The world's phytosanitary system should protect against

the spread of economically important pests without causing unnecessary barriers to the international movement of seeds".

Conclusions

While dormancy is not usually considered a problem for temperate forage seed lots, little is known about dormancy breaking methods for germination testing of tropical and subtropical forage species or of effective methods for reducing dormancy in seed lots for sowing.

Seed quality standards are required by seed companies, for seed certification, for seed sale and for seed imports. However, when the purpose of a standard is to offer protection to the buyer, the level at which the standard is set must be able to serve this purpose. For tropical and subtropical species in particular, the often very low germination standards need to be reviewed. This applies equally to locally produced and imported seed lots. Seed import standards must always be technically justified, provide the required level of biosecurity protection, and not impose unjustified technical barriers to trade.

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