

ABSTRACTS

*from oral and poster presentations
given at the 2017*

*International Seed Testing Association
the 107th*

*Association of Official Seed Analysts
and the 94th*

*Society of Commercial Seed Technologists
(ISTA/AOSA/SCST)*

annual meeting

held in Denver, Colorado, on

June 14–24, 2017

Potential for Early Counts of Radicle Emergence and Leakage of Electrolytes as Quick Tests to Predict the Percentage of Normal Seedlings

Alison A. Powell*, Linda Kerr, Kazim Mavi, Marie-Hélène Wagner and Stan Matthews†

The potential for early counts of radicle emergence (RE) and electrical conductivity (EC) of seed leachates to predict the production of normal seedlings was investigated in 10 seed lots of radish (78–99% normal seedlings) and 9 seed lots of oilseed rape (> 65% normal seedlings). In radish, germination tests (4 × 50 seeds, top of paper) conducted on three occasions in the experimental period (May and December, 2014; September, 2015) revealed little evidence of deterioration. On each occasion, a 48 h RE count predicted the production of normal seedlings (%) with an $R^2 \geq 0.90$ ($p \leq 0.001$), indicating that over 90% of the variation in normal seedlings was accounted for by RE. Similarly, RE counts on oilseed rape conducted in two laboratories after 3 d (4 × 100 seeds; GEVES, France) or 2 d (2 × 100 seeds; Alexander Harley Seeds, UK) of test initiation gave R^2 values of 0.83 ($p \leq 0.01$) and 0.92 ($p \leq 0.001$), respectively. EC of water-soluble seed leachates for both species (3 replicates of 100 seeds in 40 mL-radish, or 50 mL-oilseed rape) also related to percentage normal seedlings. In radish, EC readings were taken on four occasions during the experimental period. EC readings after 1, 3, 5, 17 and 24 h were highly predictive of normal seedlings ($R^2 \geq 0.83$, $p < 0.001$). An early reading at 3 h gave $R^2 = 0.93$ and a 17 h reading, $R^2 = 0.97$, indicating that early assessment of normal seedlings was possible. A 24 h EC reading also predicted normal seedlings in oilseed rape with R^2 values of 0.88 ($p \leq 0.001$; Harleys) and 0.87 ($p \leq 0.001$; GEVES). EC at 5 h was also significant ($R^2 = 0.45$, $p < 0.05$) and at 17 h, highly significant ($R^2 = 0.75$, $p < 0.01$). Seed lots of oilseed rape that produced over 90% normal seedlings could be identified as having above 75% RE after 2 or 3 d, or an EC at 24 h of $\leq 120 \mu\text{S cm}^{-1} \text{ g}^{-1}$, and in radish, lots with $\geq 80\%$ RE at 48 h or $\text{EC} \leq 160 \mu\text{S cm}^{-1} \text{ g}^{-1}$ at 17 h had 95% normal germination or above. This data reveals the potential for an early RE count or EC reading to predict germination within 24 to 48 h, and will be discussed on the basis of an aging/repair hypothesis. The potential for RE to predict normal germination is being investigated further by ISTA.

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***Avena* Species (Oats): Fatuoid Identification and its Importance to the Seed Industry**

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Fatuoids ('false wild oats') are present in certain varieties of cultivated oats from which they genetically derive, but are not always recognized or properly identified during purity and/or noxious-weed seed analyses. This work aimed to bring awareness to the seed industry that fatuoids exist and should be properly identified, in order to increase accuracy and uniformity during purity and noxious-weed seed analyses of cultivated oat species. Fatuoid seeds have often been mistaken for either other crop or *Avena fatua* L. (wild oat) seeds, a noxious-weed seed. The effects of training, studying the morphology of oat seeds, using seed keys, characteristic charts, and having knowledge of the accurate purity component to properly place these derivatives in, will lead to increased experience and skill levels of analysts, as well as higher accuracy and uniformity in the seed testing industry. Previous studies reporting that fatuoids were frequently placed in the wrong purity component due to misidentification demonstrated that more research, comparing fatuoid characteristics to *A. sativa* L. (cultivated oat) and *A. fatua*, and distinguishing them from other crop and weed seeds, was a need in the seed industry. Acquiring knowledge of various cultivars and derivatives, using references that refer to the details of fatuoid oats, and workshops conducted by experienced analysts, would result in a continual increase of accuracy, uniformity, and compliance in the seed testing industry.

United States Department of Agriculture, Seed Regulatory and Testing Division (Anitra.Walker@ams.usda.gov). Received 27 September 2017.

Palmer Amaranth: Identification from ITS DNA Sequencing

Robert Price*, Toni Bartling, Joshua Kaste, Patrick Woods,
Denise Thiede, Deborah Meyer and Farhad Ghavami

Palmer amaranth (*Amaranthus palmeri* S. Watson), a dioecious annual species native to arid areas of Mexico and the southwestern USA, has become a highly invasive weed of agricultural fields in the southeastern and central USA. It is now listed as a prohibited noxious weed seed in Ohio, Minnesota and Iowa, a state noxious weed in Delaware, and a harmful weed not allowable in exports to China. Seeds of *Amaranthus* species that may be encountered as contaminants in commercial seed lots, e.g., of small grains or of species used in conservation plantings, are often very difficult to distinguish reliably by their morphology. Based on preliminary evidence in the literature, we chose to sequence DNA from the ITS region between the nuclear ribosomal RNA genes, to provide an alternative approach for identifying individual seeds of

Palmer amaranth. To validate this approach, we obtained ITS sequences from known vouchered Palmer amaranth populations from northern and southern California, Arizona, Kansas, Illinois, Indiana and Minnesota, and from ten other *Amaranthus* species including apparent close relatives of Palmer amaranth and several weed species widely established in the USA [*A. albus* L.; *A. arenicola* I. M. Johnst.; *A. blitoides* S. Watson; *A. blitum* L.; *A. californicus* (Moq.) S. Watson; *A. deflexus* L.; *A. powellii* S. Watson; *A. retroflexus* L.; *A. spinosus* L.; *A. tuberculatus* (Moq.) J. D. Sauer]. All of these authenticated samples of Palmer amaranth yielded sequences with a highest identity match of 99% or higher to only Palmer amaranth sequences in BLAST searches of the GenBank database, while those authenticated from morphology as other species gave highest matches of 99% or higher only to species or groups of species other than Palmer amaranth. Thus, the method appears effective in identifying individual seeds as Palmer amaranth or “*Amaranthus* sp., not Palmer amaranth,” and in some cases provides strong evidence as to the other tested species. This approach has been used to identify over 1000 individual amaranth seeds submitted from commercial seed samples. SNPs from the ITS region were found to reliably differentiate between Palmer amaranth and the other *Amaranthus* species in our data and the GenBank database. This has allowed the design of specific primers for PCR tests to assess the presence or absence of Palmer amaranth seeds in bulked samples of up to 100 seeds that may include multiple amaranth species.

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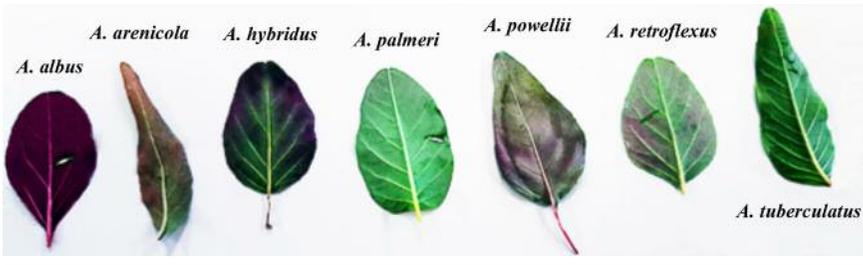
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Sabry G. Elias* and Yeaching Wu

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FIGURE 1. Leaf shape and color patterns of 7 amaranth species after 3 wks of growth in the greenhouse.



petiole and stem color. Seven genetically pure amaranth species were acquired from ARS-USDA Plant Introduction Station, Ames, IA, USA: *A. albus* (tumbleweed), *A. arenicola* (sandhills amaranth), *A. hybridus* (smooth pigweed), *A. palmeri* (Palmer amaranth), *A. powellii* (Powell amaranth), *A. retroflexus* (red-root pigweed), and *A. tuberculatus* (waterhemp). Individual seeds of each species were planted in 50-cell greenhouse trays. Trays were watered as needed and fertilized weekly with 1 tbsp of Miracle-Gro® for every gallon of water. Greenhouse temperature was maintained at $24\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$. Light of approximately $232\text{ mmol m}^{-2}\text{ s}^{-1}$ using high pressure sodium lamps was provided daily from 5:00 pm to 8:00 am. Observations of leaf and stem traits were collected weekly. Palmer amaranth (*A. palmeri*) has been recently listed as a noxious weed in Delaware, Minnesota and Ohio. Leaves of *A. palmeri* were hairless, compared to waterhemp which was pubescent on the stems and leaves. Leaves were also diamond-shaped with a whitish vein on the backside (Fig. 1). Leaves alternated and grew symmetrically around the stem, giving Palmer amaranth a rosette appearance. The leaf petiole of *A. palmeri* is longer than the leaf itself. At three weeks, the stem was light purple at the base and green at the top. According to this study, *A. palmeri* could be differentiated from other amaranth species based on leaf and stem characteristics after a 3-week grow-out in a greenhouse. At different stages of plant development, distinctive features can be used to identify different amaranth species under greenhouse or field conditions. Efforts are underway to develop a DNA test to differentiate *A. palmeri* from other amaranth species.

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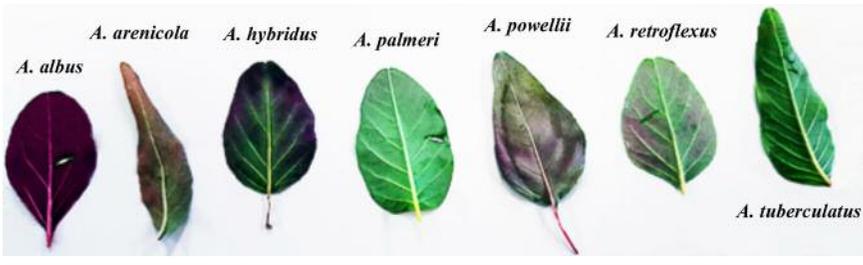
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Identification of Secondary Noxious Brome Species in the Canadian *Weed Seeds Order* (2016)

Jennifer Neudorf, Angela Salzl and Ruoqing Wang*

Four brome species, field brome, Japanese brome, cheat and downy brome, are classified as 'secondary noxious' under the Canadian *Weed Seeds Order* (2016). These species have been introduced into Canada from temperate regions of Europe and Asia and from northern Africa. Japanese, brome, cheat and downy brome are widespread in Canada; field brome is found in Ontario. These species may be found as weeds in either domestic or imported seeds. The florets of these secondary noxious brome species could be morphologically similar to brome species not on the *Weed Seeds Order*. Seed morphological features of these brome species (Figs. 1–4) are described below.

Field brome (*Bromus arvensis* L.): Floret is long and narrow with a size of 7.0–9.0 mm (L) × 1.0–1.5 mm (W), oval-shaped and tends to have straight sides. Floret base is weakly ridged and ends abruptly in the side view. Lemma is thin and papery with translucent edges, with smooth or short hairs on the upper half. Palea tends to be of similar length as the lemma, with long and thin teeth. Caryopses can be reddish-brown or purplish, and are weakly to strongly curled. Awn is long, arises below the split in the lemma, and is about 6–11 mm.

Cheat (*Bromus secalinus* L.): Floret is long and wide with a size of 6.5–8.5 mm (L) × 1.8–2.5 mm (W), oval-shaped with curved sides. Floret base is weakly ridged, wide and ends abruptly in the side view. Lemma is thick and smooth with short hairs along the curled edges. Palea is similar in length to both the lemma and caryopsis, with short and thin teeth. Caryopses are reddish-brown and strongly curled. Awn is short, arises from the top of the lemma, and is about 3–6 mm.

Japanese brome (*Bromus japonicus* Houtt.): Floret is long and wide with a size of 7.0–9.0 mm (L) × 1.0–2.0 mm (W), oval-shaped with a flared top. Floret base is strongly ridged and humped in the side view. Lemma is papery with translucent edges, with short or long hairs on the upper half. Palea tends to be

FIGURE 1.
Field Brome (*Bromus arvensis*)

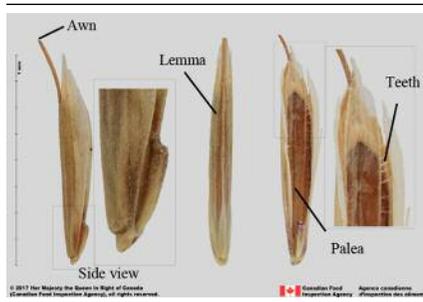


FIGURE 2.
Cheat (*Bromus secalinus*)

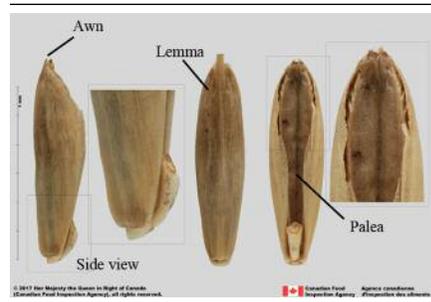


FIGURE 3.
Japanese brome (*Bromus japonicus*)

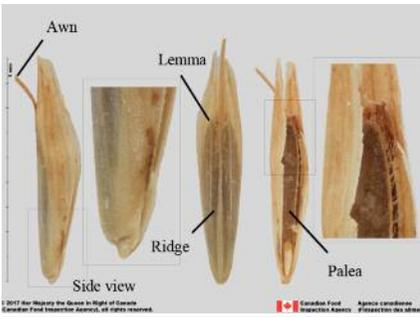
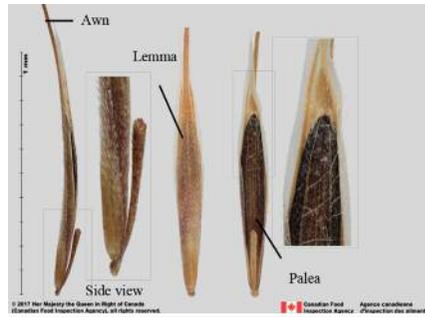


FIGURE 4.
Downy brome (*Bromus tectorum*)



a similar length as the caryopsis, with long and thin teeth. Caryopses can be reddish-brown or purplish, and are flat or weakly curled. Awn is long, arises below the split in the lemma, and is about 8–13 mm.

Downy brome (*Bromus tectorum* L.): Floret is very long and narrow with a size of 9.0–12.0 mm (L) × 1.0–2.0 mm (W), oval-shaped and flattened, and appears arched in the side view. Floret base is weakly ridged, narrow and appears notched in the side view. Lemma is papery and hairy with wide translucent edges and top; hairs can be short or long. Palea tends to be as long as, or shorter than, the caryopsis, with very long and thin teeth. Caryopses tend to be purple and lie flat. Awn is very long, appears as part of the lemma, and is about 10–18 mm.

Acknowledgment: Thanks to Jo Jones for taking the images used in this abstract.

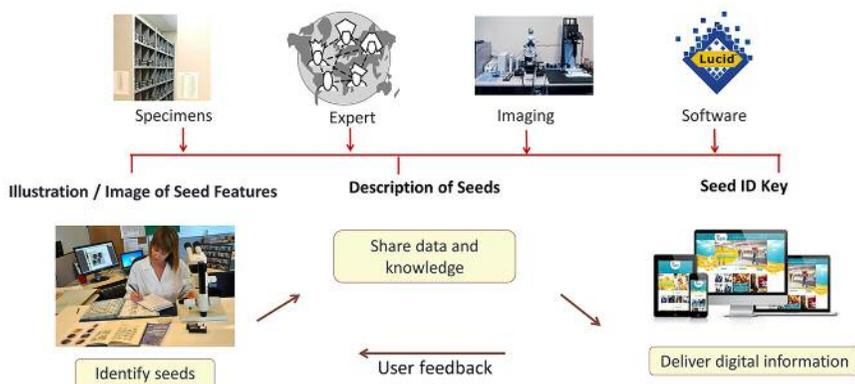
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Development of a Digital Tool for Seed Identification

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Seed identification. Identifying unknown seeds, especially those of noxious weeds, is a routine and important diagnostic test in seed certification. Seed identification is primarily based on seed morphological features according to taxonomic classification. Digital identification tools allow seed identification features to become accessible virtually, making them an important work tool and reference in a seed laboratory.

Digital tool development. The following is a digital tool development flow-chart and required resources:



Digital tool main components: a) *Web navigation.* A web-based identification tool provides a complete tool kit that satisfies the end user's needs. In addition to the main components of ID fact sheets, image gallery, interactive key matrix (e.g., Lucid Key), it should also include a home page, user instructions and other resources such as a glossary, literature references, contact and feedback information. b) *Fact sheets.* Fact sheets provide complete identification descriptions and associated morphological features in multimedia: text description of seed features, images or illustrations of seeds such as feature close ups, population variation of seeds, complete shape or color profiles, and species surface or specific feature descriptions. A comparison of similar species is also desirable in various formats, e.g., a comparison chart, descriptions of morphological differences or identification tips, and images or illustrations. c) *Identification key.* The interactive taxonomic key matrix is assisted by computer programs or software (e.g., Lucid Key) to develop identification keys for plant families, genera or species, allowing variable stages of identification. Identification keys can have media assistance, and feature guides and advice for end users. User-friendly seed ID keys should not require special training on the software. A good computer program should also have tolerance for feature selection errors and uncertainties. d) *Image gallery.* The image gallery hosts seed feature illustrations or image collections designed to assist in identification, such as sorting by taxonomic classification according to family and genus; seed feature categories such as seed size, seed color, or seed shape; or other search needs such as key words, scientific or common names. This is a reference tool that can be used for initial identification screening, training, or to familiarize users with seed morphologies.

Acknowledgments: Thanks to Taran Meyer, Janessa Emerson and Yimeng Wang for images and illustrations used in this abstract.

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