Update on the Identification of Palmer Amaranth (*Amaranthus palmeri*)

**Purpose:** Palmer amaranth has been classified as prohibited noxious weed by Ohio and Minnesota. Seed testing labs conducting a 25,000 seed noxious exam are now classifying *Amaranthus* species that can't be differentiated from *Amaranthus palmeri* as noxious weed seeds (see SCST Executive Board's Recommendations below). The Minnesota Department of Agriculture supported the validation of a DNA test that will allow single seeds of *Amaranthus* spp. to be tested using DNA sequencing to determine if the seed is *Amaranthus palmeri* or another Amaranthus species. This test will help seed producers qualify their seed as free of *Amaranthus palmeri*. This method should be used in conjunction with field inspections and standard good practices for seed production.

**Test Method:** Internal transcribed spacer (ITS) sequencing is a common method used in species identification in plants (see reference below). Standard PCR is used to amplify the ITS region followed by direct sanger sequencing to obtain the DNA sequence. The sequence information is compared to the library of sequences in GenBank. A 20 seed validation of 10 *Amaranthus palmeri* and 10 other *Amarathus* species has been conducted in two labs, California Dept. of Ag and Eurofins BioDiagnostics, Inc. The results were 100% accurate and allowed each lab to validate that all *Amaranthus palmeri* seeds were correctly identified. Research Scientists, Robert Price and Toni Bartling are planning to publish these methods in a peer-reviewed journal in 2017.

This test can be conducted on any seed and takes about 2 weeks to complete. Alternative DNA methods are being evaluated including the development of pooled seed methods.

**Validated Labs and Contact Information:**

California Department of Food & Agriculture
Robert Price, Ph.D., Senior Seed Botanist/Plant Taxonomist
Seed Laboratory and Herbarium
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Eurofins BioDiagnostics, Inc.
Denise Thiede, Ph.D., Vice President
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River Falls, WI 54022
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715-426-0246

**Test Protocol**

Sample submission instructions:

Amaranthus seed that is found in the 25,000 seed Noxious Weed exam should be submitted for DNA testing to obtain a statistically accurate report of the Amaranthus germplasm that is in the lot.

Individual seeds may be submitted for testing. The most economical approach is to ask your seed testing provider to submit seeds from multiple seed lots at the same time. Seeds from each seed lot can be submitted in coin envelopes or Ziploc baggies with sample information and seed number listed on each bag. Each seed will be entered as a single sample and results will be issued on a single seed basis.

BioDiagnostics can pass on efficiencies in testing batches of 24-48 seeds or 49-94 seeds to seed companies by processing multiple samples at the same time. All samples must arrive at the same time to be entered as a batch. Labs testing native seeds can assist with batching.

**Alternative Methods:**
Seed producers may also use a growout to evaluate whether seeds are *Amaranthus palmerii*. Illinois Crop Improvement is currently offering this service as a greenhouse growout. Pure amaranth seeds for planting can be submitted directly to the greenhouse. Purity and separation requests on samples where amaranth contamination is suspected will be handled by the seed laboratory and then shared with the greenhouse. The growout process adds an additional level of uncertainty beyond the normal sampling variance by relying on the weed seeds to germinate for identification. Seeds that do not germinate cannot be evaluated. The greenhouse growout also takes more time to complete. Illinois Crop will offer to outsource samples to the above named labs first and will continue to offer the growout service for Amaranthus species, as well as any other species, indefinitely.

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References:

White, T.J., Bruns, T., Lee, S. and Taylor, J., (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Chapter 38. Pages 315-322. In: PCR Protocols: a Guide to Methods and Applications (M. Innis, D. Gelfand, J. Sninsky and T. White, eds.). Academic Press, Orlando, Florida.

**SCST Board's Recommendation-Dec 19 2016**

Palmer amaranth, *Amaranthus palmeri*, has recently been found as an incidental seed in Revegetation and Rangeland kinds in Iowa and Minnesota. This is an annual plant native to the southern United States. It is undesirable and considered a noxious weed in Ohio and Minnesota.

The seed is visually indistinguishable from many other *Amaranthus* species, which causes concern for identification. Due to this we advise extreme caution when reporting *Amaranthus* species. Seed analysts must take into consideration if possible the location of production, any available field inspection information, state where seed was produced, type of crop, etcetera when making species determination for *Amaranthus* contaminants. If the laboratory has no access to this type of information and it is impossible for the laboratory to definitively determine if the *Amaranthus* in question is or is not *Amaranthus palmeri*, it is imperative that any *Amaranthus* be listed as *Amaranthus sp*. and classified as noxious on a Report of Analysis. A disclaimer stating that the species cannot be determined and may be *Amaranthus palmeri* should be included with this. Inaccurate identification of *Amaranthus sp*. on a Report of Analysis may be cause stop sales and further action by regulatory officials.

Efforts are being made to find a marker for PCR testing to identify *Amaranthus palmeri* when found as a contaminant. However, this will take time. We direct you to report identification as described above to further prevent the spread of this undesirable weed.

*SCST Executive Board*