USDA APHIS Guidelines for Weed Seed Devitalization Research

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These guidelines are provided by APHIS to aid researchers in designing weed seed devitalization studies. APHIS requests that researchers submit final detailed research workplans or protocols to APHIS for review prior to initiation of studies, especially where any deviations from these guidelines are planned or anticipated. The researchers should continue to communicate with APHIS throughout the study to ensure concurrence on any deviations to the agreed research protocol. APHIS review of research results will consider all uncertainties introduced by deviation from the agreed research protocol, scalability, accuracy or precision limitations of the monitoring devices, variability and inconsistencies in the data, and the level of detail provided about the experimental design. APHIS will also evaluate the strength of the proposed treatment relative to the parameters that were required for seed devitalization during the dose-response testing. APHIS will recommend a "buffer" (i.e., increased treatment dose), where appropriate, to the proposed treatment to reduce operational or other uncertainties in commercial treatments.

Experimental Seeds

- Submit information on the following items: geographic origin of all weed seeds used in the study, weed seed age (if known), seed storage conditions, previous exposure of the seeds to chemicals (e.g., herbicides, fungicides), weed seed condition (i.e., color, size).
- Provide information regarding the possibility of pathogenic spores being associated with the weed seed species to be tested. The potential presence of pathogenic spores may dictate the necessity for additional treatment research.
- Provide a thorough description of all weed seed preconditioning procedures used (e.g., germination stimulators, scarification, stratification) and the conditions under which the seeds are germinated (e.g., temperature, humidity, germination medium).
- Conduct all research trials (i.e., small-scale dose-response research and confirmatory trials) using the species of weed seed for which the treatment is being developed. The use of surrogate species will not be accepted unless the surrogate species can be shown to be equally or more tolerant of the proposed devitalization treatment than the target species. If the treatment is being developed for more than one species of weed seed, perform small-scale dose-response testing to determine the most treatment-tolerant weed seed species. All subsequent testing should be performed using this species.
- APHIS will not extend approval of a weed seed devitalization treatment to species not tested during research. Interception of these species will result in commodity rejection at U.S. ports of entry.
- Retain and specify where voucher specimens have been deposited.

Experimental Design

- Include an untreated control in all experimental replicates. The sample size of the untreated control should be equal to the number of treated, experimental seeds.

- Use germination as the index for seed viability. Grow out all germinated seedlings. Provide complete information on radical differentiation and viability of all germinated seedlings. Use TZ testing in lieu of germination testing, if necessary and as agreed to by APHIS. Provide pictures, as appropriate.
- Conduct small-scale dose-response testing to determine the most treatment-tolerant species of weed seed. Perform this testing individually for each treatment schedule requested for APHIS approval.
  - Include a minimum of four treatment doses that give <100% mortality, one treatment dose expected to cause 100% mortality, and an untreated control in each replicate. Sufficient intervals should be included to characterize exactly when seed viability tapers to zero.
  - Test a minimum of 50 individual weed seeds of each species at each treatment dose in each replicate.
  - Perform a minimum of four replicates of this testing.

  Alternately, submit detailed references documenting the most tolerant weed seed species if small-scale testing will not be conducted to verify that claim.
- Conduct large-scale confirmatory trials for each treatment schedule requested for APHIS approval, using the most tolerant weed seed species.
  - The treatment regimen selected by the researchers for confirmatory testing and proposed to APHIS for publication should be a treatment with an efficacy level of probit-9, or that point on the treatment axis which is predicted to kill 99.9968% of the seeds treated based on the dose-response testing. APHIS may accept a lower level of mortality in certain cases, if it can be justified. APHIS typically requires that all proposed weed seed treatments be confirmed by treating a minimum of 1200 weed seeds total, with no seeds germinating post-treatment.
  - Perform a minimum of four replicates of this work. The replicates should be conducted over a period of time to ensure that the maximum natural variation in response is included in the experimental units.
  - Consider the type of packaging that will be used under operational conditions. This may be an important factor during the research.
  - Hold all weed seeds, following treatment, under the same conditions as control seeds are held. These conditions should maximize seed germination/viability.
- Consider the use of "comparison testing" in lieu of confirmatory testing if the desired schedule involves certain modifications to an existing PPQ Treatment Manual schedule. These comparisons can be accomplished via small-scale dose-response testing.
  - Addition of a new species of weed seed to an existing treatment schedule. Compare the tolerance of the weed seed species which is being proposed for inclusion in the schedule with the tolerance of a species already included in the schedule.
  - Perform testing using the parameters outlined in the existing schedule. Refer to previous bullets for guidelines on conducting dose-response testing. Follow all other recommendations outlined in this document.

- The new weed seed species can be added to an existing schedule if it is equally or less tolerant than that with which it is being compared. If it is more tolerant, it will be necessary to develop a new treatment. This would require both small-scale dose-response testing and large-scale confirmatory trials.

Facilities, Equipment and Monitoring

- Conduct all chemical treatments according to label requirements.
- Provide pictures and/or diagrams, as well as complete information, for the equipment used. This may include the following items: specifications for the treatment unit, information on the monitoring equipment (e.g., type, number, placement, specifications, accuracy), chamber dimensions and construction information (if applicable). Additional information and/or guidelines may be relevant depending on treatment type. Please obtain APHIS approval on all equipment prior to initiating research. Adhere to all standards outlined in the PPQ Treatment Manual.
  http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf

Statistics and Data Submission

- Use appropriate correction factors to account for control mortality, e.g., Abbott's (Abbott 1925). Data where control mortality is ≤5% need not be corrected. Control mortality ≥10% must be explained. Data will not be considered to support treatments where control mortality is ≥20%.
- Analyze dose-response data from all trials using appropriate statistics, such as probit analyses at the LD50, LD95, LD99, and probit-9 levels. APHIS will confirm the most tolerant weed seed species by examining 'raw' mortality/survivorship data.
- Submit the following information: 'raw' mortality/survivorship data from all treatment doses utilized, 'raw' data from the monitoring equipment used, and all other data and information outlined in this document.
- Discuss any potential differences in treatment efficacy that may arise from the 'scaling up' of research-scale to commercial-scale treatments.
- Submit pictures where they will clarify experimental equipment and procedures.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

USDA. PPQ Treatment Manual.
http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf