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GUIDELINE 1: Guidance for temperature treatment efficacy studies

The following guidance is provided to assist researchers in the design of temperature treatment efficacy studies for controlling pests in international trade (Heather & Hallman, 2008). Before designing such studies, ISPM 28 (*Phytosanitary treatments for regulated pests*) should be consulted for details on requirements for submitting data for the evaluation of phytosanitary treatments. If the research is done as a response to a request for market access, the research protocol should be discussed with the importing country before initiating the research. To develop a temperature treatment, the mortality level to be achieved should be specified, at a stated confidence level.

1. Experimental Pest Populations

Pests used in efficacy studies should be no less tolerant to the treatment than would occur under natural conditions. If pest colonies are established for the purposes of supplying pest populations for experimental use, natural infestation should be used whenever possible and colonies should be replenished regularly by wild (naturally occurring) pests.

The environmental conditions, most notably the temperature, in which pests are stored or reared in colonies before experimentation should be suitable to maintain a healthy colony, this including, where appropriate, a constant temperature. Pest mortality, morbidity, fecundity, sex ratio, and growth or development under storage or colony conditions should also be in accordance with international or national standards for that particular species to ensure a healthy, vigorous colony.

The identity of all individuals used in an experiment should be confirmed as being taxonomically equivalent to the stated target pest. Voucher specimens of the target pest should be held in a suitable facility for later taxonomic validation should it be required.

The life stages of the pest treated should correspond to the life stages associated with trade that are most tolerant to the treatment.

If the treatment is being developed for more than one taxonomically related pest, small-scale temperature–time response testing may be undertaken to determine the pest that is most tolerant to the treatment. All subsequent testing may then be performed using this pest.

2. Host Commodity and Infestation

Developmental studies, small-scale temperature–time response research and large-scale confirmatory tests should all be conducted using the commodity for which the treatment is being developed. If the treatment is being developed for more than one commodity, small-scale temperature–time response testing may be undertaken to determine the commodity in which the pest is most tolerant. All subsequent testing may then be performed using this commodity.

The condition of the commodity used in the research should reflect the variability expected in trade commodities. The host commodity should be export market quality and should not have been treated previously with insecticides, fungicides or other chemicals, including soaps, dyes and waxes. If the commodity has been exposed to any of these chemicals, data should be supplied that demonstrate that there are no additive effects to the treatment of the exposed pests.

The host commodity should be infested with the pest in a manner consistent with that which occurs naturally when subjected to treatment application during trade. Natural infestation methods should be used where possible, but artificial infestation may be used where it has been demonstrated that such a population is no less tolerant to the treatment than a naturally infested population. The rate of infestation of the commodity used in testing should not result in a reduction in pest tolerance to the treatment or significant modification of the commodity from that found in trade.

The condition of the treated infested commodity, including packaging or other storage conditions, should be consistent with that found in commodities subjected to treatment during trade.

3. Experimental Design

Treatment efficacy studies may include developmental studies, small-scale temperature–time response research or large-scale confirmatory tests, as required.

Small-scale experiments may be used to determine the following:

- the most treatment-tolerant life stage or condition of the pest
- the likely temperature–time combination that will achieve the desired end-point at the required level of mortality with a specified confidence level
- the likely temperature–time combination that will maintain suitable commodity condition
- the relative level of tolerance of the target pest to the treatment compared with another pest for which sufficient efficacy has already been demonstrated under the same condition (if the target pest is less tolerant to the treatment than the other pest, no further work need be undertaken).

Large-scale confirmatory tests or small-scale temperature–time response tests (for later statistical regression analysis) should then be completed on the temperature most likely to achieve the desired efficacy without causing economically significant levels of damage to the commodity (e.g. without compromising quality standards).

Replicates of treated populations are necessary to allow for adequate statistical analysis. The minimum is three replicates per temperature–time combination in all cases and each replicate treatment should be conducted separately.

Untreated controls are also necessary, with one control per replicate being optimal. The size of untreated controls should be decided depending on the size of the treated population, and they should be held in conditions that do not affect pest survival. Countries may have specific requirements regarding the proportion of the pests that may die in the control for the control to be deemed valid, because high mortality in the control would mean that control mortality could not be separated from the effects of the treatment.

Conditions immediately before and after the treatment (e.g. during heating up or cooling down) should be equivalent to what would normally be achieved under trade conditions. After treatment, but before and during the analysis of the experimental results, the treated commodity should be held in conditions equivalent to the untreated control.

4. Facilities, Equipment and Monitoring

The facilities and equipment used should ensure adequate control of the ambient conditions during treatment, and be equivalent or similar to those likely to be used in trade.

Treatment monitoring equipment should be able to monitor the temperature of the commodity or the facility with a stated accuracy and frequency over the duration of the treatment. The equipment should be calibrated prior to each trial. The temperatures measured should be that of the commodity close to the pest (where the pest is), and the coolest (for heat treatment) or warmest (for cold treatment) part of the commodity.

Monitoring equipment should be appropriate to accurately determine when the end-point of the treatment has been achieved. Measurements should have appropriate levels of sensitivity and specificity.

5. Data Analysis

It is recommended that, before research is undertaken, statisticians are consulted on the design of treatment efficacy studies and the method of statistical analysis to be used.

Appropriate correction factors should be used to account for control mortality (e.g. Abbott's correction factor (Abbott, 1925)). While results where control mortality is $\leq 5\%$ need not be corrected, control

mortality of $\geq 10\%$ must be explained. Results will not be considered to support treatments where control mortality is $\geq 20\%$ unless this is shown to be normal for the target pest under optimal conditions for survival.

Any potential differences in treatment efficacy that may arise from the scaling up of a treatment from research-scale to trade-scale need to be explained, including those arising from differences in pre-cooling or pre-heating times and the potential impact of these times on pest acclimation or total length of temperature exposure.

Variation in the temperature within and between replicates should be examined in the analysis of the results, and a justification for the required temperature selected should be included in the treatment schedule.

6. Documentation

Accurate and detailed information should be recorded on the pest and host species, the host variety, the origin of the pest, and the host commodity used in the research on temperature treatment efficacy. Information on the condition of the pest and commodity (i.e. stage of maturity, colour, size, physiological condition) at the time of the study should also be documented.

The following should be documented for evaluation in support of treatment efficacy:

- “raw” or unmodified mortality or survivorship data from all temperature–time combinations studied.
- “raw” data from the temperature sensors throughout both the pre-cooling or pre-heating period and the treatment period of each experiment with calibration data for each sensor.
- information showing the location of infested and “filler” commodities (if applicable) as well as sensors to measure air and commodity temperature.
- information on all items outlined in ISPM 28 and in this appendix.

7. References

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

Heather, N.W. & Hallman, G.J. 2008. *Pest management and phytosanitary trade barriers*. Wallingford, UK, CABI. 257 pp.

GUIDELINE 2: Guidelines for research into the efficacy of modified atmosphere phytosanitary treatment

1. Research Materials

It is recommended that specimens of the different life stages of the pest or pests studied are collected and archived as reference specimens of the study organisms, to facilitate decision making in the event of possible disputes on identification. The commodity used in the efficacy studies should be of normal commercial condition.

To perform research on disinfestation treatments for regulated pests using modified atmospheres, it is necessary to know the basic biology of the pests and to define how the pests used in the research will be obtained. Modified atmosphere studies should be carried out on commodities that are either infested naturally or infested with laboratory-reared pests preferably in a natural manner. If artificial infestation or *in vitro* techniques are used, experimental data should be provided to demonstrate that the results are consistent with those obtained with natural infestation. The method of rearing, feeding and refreshing the pest colony should be documented in detail.

2. Measuring and Recording

Instruments used to measure and record the parameters of the modified atmosphere treatment, such as gas concentrations and commodity temperature, should be calibrated and used according to the manufacturer's instructions.

3. Establishing and Maintaining Modified Atmosphere Treatment Conditions and Duration of the Treatment

3.1 Preliminary studies

The following steps should be carried out to determine the modified atmosphere treatment conditions required to achieve adequate efficacy:

- (1) The treatment tolerance of the different life stages of the target pest in the commodity should be established to determine the most tolerant stage found in the commodity. The most tolerant stage, even if it is not the most frequent one occurring in the commodity, is the stage that should be used to establish the treatment parameters.
- (2) The relationship between modified atmosphere treatment and response for each life stage should be studied to identify the most tolerant stage at high levels of mortality. The optimum modified atmosphere treatment parameters to cause mortality of the target pest at the most tolerant stage in the commodity need to be determined. The subsequent large-scale or confirmatory studies should be conducted on the most treatment-tolerant life stage in the commodity. Alternatively, if the most tolerant life stage cannot be identified, further research to support treatment efficacy should be conducted on all life stages of the target pest associated with the commodity.
- (3) The influence of variation in commodity properties (e.g. shape, size, maturity, skin thickness) on treatment efficacy should be assessed if such variation may be encountered during the commercial treatment.
- (4) The effect of treatment parameters such as duration, gas concentrations and temperature profile on the efficacy of the treatment should be determined experimentally. If pertinent data do not already exist, it is recommended that treatment response studies are conducted at multiple treatment parameter levels, together with a control. For each treatment level, it is recommended that a minimum of three replicates be conducted, with a minimum total of 120 individuals.
- (5) During the post-treatment observation period of the commodities and associated pests, both treated and control commodities should remain under favourable conditions for survival of the pests. The untreated controls should respond normally for the experiment to be valid, as high control mortality suggests that organisms may have been held under suboptimal conditions. The results from such organisms may be misleading if their treatment mortality is used to predict an

optimum treatment schedule. In general, mortality in the control should be within expected ranges.

3.2 Large-scale or extrapolation (confirmatory) studies

To confirm whether the estimated optimal modified atmosphere conditions and duration at each temperature (or temperature profile) allow an adequate efficacy to be achieved, two methods are recommended: (1) treat a large number of individuals of the most tolerant life stage of the pest while achieving a specified efficacy; or (2) treat the most tolerant stage to give a range of efficacies that may be less than adequate and estimate adequate efficacy using an appropriate regression analysis. The number treated will depend on the required level of confidence.

- Treating a large number of individuals (usually many thousands or tens of thousands), using one set of treatment parameters (commodity, modified atmosphere, duration, temperature) and with no survivors is a direct method of demonstrating treatment efficacy and calculating an associated level of confidence.
- Establishing a modified atmosphere treatment schedule via estimation using regression analysis should be accepted only if the data closely fit the model and the upper 95% confidence interval is used to establish the treatment parameters. This method is useful when it is too difficult or costly to test large numbers of individuals. The resulting modified atmosphere treatment schedule should incorporate an adequate safety margin to ensure that the required treatment efficacy is achieved.

Because the most severe modified atmosphere, duration and temperature measured during confirmatory studies will be used to establish the treatment parameters for the approved treatment schedule, it is recommended that fluctuations in critical atmospheric gas conditions (O₂ and CO₂) and temperature during such studies be minimized.

4. Record Keeping

Records and data from research studies should be retained to validate the data requirements and should upon request be presented to interested parties, for example the NPPO of the importing country, for consideration in establishing an agreed commodity treatment.

GUIDELINE 3: Fumigation research guidelines

1. Research Materials

It is recommended that samples of the different life stages of the pests studied are archived as voucher specimens in order to, among other reasons, resolve possible future disputes on identification. The commodity to be used for efficacy studies should be of normal commercial condition.

To perform research into the control of regulated pests by fumigation, it is necessary to know the basic biology of the pests and to define how the pests used in the research may be obtained. Fumigation experiments should be carried out on commodities infested naturally or infested with laboratory-reared pests preferably in a natural manner. The method of rearing, feeding and refreshing of the pest colony should be documented in detail. If artificial infestation or *in vitro* techniques are used, experimental data should be provided to demonstrate that the results are consistent with those obtained with natural infestation.

2. Instrument Recording

Instruments used to record fumigation parameters, such as gas concentration and enclosure and commodity temperature, should be calibrated, certified and used according to the manufacturer's instructions. Routine calibration of all measuring instruments should be conducted periodically.

3. Estimating and Confirming Optimal Gas Concentration and its Duration for the Treatment

3.1 Preliminary studies

The following steps should be carried out to estimate the dose required to achieve an adequate efficacy:

- (1) The treatment tolerance of the different life stages of the target pest that may be present in the commodity should be established to determine the most tolerant stage. The most tolerant stage, even if it is not the most frequent one occurring in the commodity, is the stage that should be used to establish the treatment parameters.
- (2) The treatment tolerance of different shapes, size and varieties of the commodities should be established to determine if they may influence the treatment outcome.
- (3) The optimal fumigant concentration and treatment duration at each temperature should be determined experimentally. If pertinent data do not already exist, it is recommended that at least five dose levels and a control are used for each pest life stage, temperature, and shape or size of commodity, with a minimum of 120 individuals where possible for each of the doses and a minimum of three replicates. The relationship between optimal fumigant concentration and its duration and response for each life stage and temperature should be determined to identify the most tolerant stage. The optimum dose to kill the pest at the most tolerant stage in the variety or commodity type where the target pest shows the highest tolerance needs to be determined. The remainder of the research should be conducted on the most fumigant-tolerant life stage in the variety or commodity type where the target pest shows the highest tolerance at each temperature.
- (4) During the period of post-treatment observation of the commodities and associated pests, both treated and control commodities must remain under favourable conditions for survival of the pests. The untreated controls must respond normally for the experiment to be valid. Any study where the control mortalities are high indicates that the organisms were held and handled under suboptimal conditions. The results from such organisms may be misleading if their treatment mortality is used to predict an optimum treatment dose. In general, mortality in the control should not exceed 10%.

3.2 Large-scale or extrapolation (confirmatory) studies

To confirm whether the estimated optimal fumigant concentration and its duration at each temperature allows an adequate efficacy to be achieved, two methods are recommended: (1) treat a large number of

individuals of the most tolerant life stage of the pest while achieving a specified efficacy; or (2) treat the most tolerant stage to give a range of efficacies that may be less than adequate and estimate the adequate efficacy using a regression analysis. The number treated depends on the required level of confidence.

- Treating a large number of individuals (usually many thousands or tens of thousands), using one set of treatment parameters (commodity, concentration, duration, temperature) and with no (or nearly no) survivors is a direct method of demonstrating treatment efficacy and calculating an associated level of confidence.
- Establishing a treatment schedule via estimation using regression analysis should be accepted only if the data closely fit the model and the upper 95% confidence interval is used to establish the treatment parameters. This method is especially useful when it is too difficult or costly to test large numbers of individuals. The resulting treatment schedule should incorporate an adequate safety margin to ensure that the required treatment efficacy is achieved.

Because the most severe fumigant concentration and duration at each temperature measured during the confirmatory studies are used to establish the required parameters for the approved treatment, it is recommended that fluctuations in fumigant concentration and temperature during such studies be minimized.

4. Record Keeping

Records and data from research studies should be retained to validate the data requirements and should upon request be presented to interested parties, for example the NPPO of the importing country, for consideration in establishing an agreed commodity treatment.

Publication history

- 2017-11 The Standards Committee (SC) requested the Secretariat to remove Appendix 1 (Guidance for temperature treatment efficacy studies) from the draft ISPM Requirements for the use of temperature treatments as phytosanitary measures and to incorporate the text into the IPPC Procedure Manual for Standard Setting as a Technical Penal on Phytosanitary Treatments procedure.
- 2018-05 For consistency with the other standards on requirements for treatments, in their May 2018 meeting, the SC removed Appendix 1 from draft ISPM on Requirements for the use of fumigation treatments as a phytosanitary measure and requested the Secretariat to include this in the procedural manual for standard setting.
- 2018-11 As the research protocols from the draft ISPMs are research guidance materials and not part of the standard setting procedure, the SC decided to combine the research guidance material for the different treatment types and make them publically available on the International Phytosanitary Portal.

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