REPORT

Okinawa, Japan
1 - 5 December 2014

Expert consultation on phytosanitary Treatments in *Bactrocera dorsalis* complex

December, 2014

Food and Agriculture Organization of the United Nations

CONTENTS

1. Opening of the Meeting

[1] The Secretariat opened the meeting, noting that it was the second expert consultation that was organized by the IPPC Secretariat, following the Expert Consultation on Cold Treatments (ECCT) held in Argentina in December 2013[1]. The Secretariat thanked the host agency, the Japanese National Plant Protection Organization (NPPO), for the funding provided for the meeting, as well as for hosting the event and making the required logistical and organizational arrangements for its conduct, including appropriate venue selection.

[2] Representing the host agency, Mr. Masato FUKUSHIMA, Director of the Plant Protection Division of the Japanese Ministry of Agriculture, Forestry and Fisheries expressed his gratitude for the experts' interest and participation in the meeting. He also thanked the International Plant Protection Convention (IPPC) Secretariat for organizing the expert consultation and acknowledged the efforts by the facilitators and the steering committee in preparing the meeting. The host also thanked the Okinawa Institute of Science and Technology for providing the meeting venue.

[3] The host mentioned that Okinawa was a befitting venue for this expert consultation in view of its successful eradication of *Bactrocera dorsalis* and *B. cucurbitae* through the sterile insect technique and male annihilation technique, completed in 2003 at the cost of US$250 M. During that period vapor heat treatments were developed for fruits and vegetables to enable trade with other islands in Japan.

[4] The host reminded that *B. dorsalis* was a highly destructive pest of global significance and the Commission on Phytosanitary Measures (CPM) has been working on the production of phytosanitary treatments (PTs) under the International Standard for Phytosanitary Measures (ISPM) 28, *Phytosanitary treatments for regulated pests*. These PTs, based on science would help harmonize treatment standards and avoid trade disputes. He expressed his expectation that experts would deepen their understanding of research concerns regarding the *B. dorsalis* complex in the course of the meeting and identify issues in developing phytosanitary treatments. The host hoped that the meeting would provide an opportunity to establish an enhanced network between experts that would continue to exchange views, information and knowledge in pursuance of IPPC activities.

[5] The opening of the meeting was followed by self-introductions by the participants and observers.

[6] Mr Anthony Robert CLARKE was elected as chairman and Mr Scott MYERS as rapporteur.

[7] The agenda was adopted as presented in Appendix 1 of this report.

[8] The Secretariat provided an overview of the IPPC, explaining the governance structure and the three pillars of work – capacity building, national reporting obligations and standard setting. The Secretariat emphasized that harmonized standards are developed for the benefit of National Plant Protection Organizations (NPPOs), parties to the IPPC, as they can avail themselves of the suite of standards available. This includes diagnostic protocols and phytosanitary treatments, which IPPC members can choose to implement without further justification in order to reduce the number of bilateral agreements needed.

[9] The Secretariat described the four stages of the standard setting process: a list of topics approved by the Commission on Phytosanitary Measures (CPM) (based on IPPC members' submissions to calls for topics and treatments), drafting of standards (including the development of specifications by expert drafting groups, such as the Technical Panel on Phytosanitary Treatments (TPPT) and by way of member consultation), member consultation on the draft standards (including review and revision by technical panels or stewards), and adoption and publication of the standards (following the approval by the Standards Committee of recommendation of a draft ISPM to the CPM, and being subject to the possibility of formal objections by contracting parties).

[1] https://www.ippc.int/core-activities/standards-setting/expert-consultation-on-cold-treatments

[10] A participant queried how formal objections are dealt with if they are of political nature and it was explained that repeated, non-technical objections can be resolved by submitting them to CPM for voting, as is currently the case for several treatments and an ISPM that have received at least one formal objection on non-technical grounds.

[11] Another participant sought clarification whether contracting parties were under obligation to use ISPMs, including treatments, adopted under the IPPC. The Secretariat clarified that ISPMs are not obligatory but can be implemented or used without further justification. By the same token, phytosanitary treatments approved under ISPMs can be applied by contracting parties without the need for providing further demonstration of efficacy, as this had already been established by the experts on the Technical Panel on Phytosanitary Treatments in their review of the submitted data. Conversely, contracting parties are obliged to accept the stated level of efficacy of phytosanitary treatments unless additional justification is provided to support more stringent requirements.

[12] In response to a further question as to who could issue formal objections it was answered that these could only be submitted by the official national IPPC contact points from contracting parties.

[13] The Secretariat then provided an overview of the conduct of expert consultations, including the roles and responsibilities of the chair, rapporteur, facilitators, experts, hosts and the Secretariat. It was explained that the aim was to facilitate the exchange of information related to the research and development of *Bactrocera dorsalis* phytosanitary treatments to identify good practices in experimental designs that help address difficulties and to help reduce duplication of research. Through discussion it was hoped that researchers could produce outcomes that would be sufficient to support submissions of Phytosanitary treatments in response to the next IPPC Secretariat's call for treatments.

[14] The session was concluded by the Secretariat presenting the meeting objectives and expected outputs as per the concept note for the expert consultation[2].

2. Administrative matters

[15] The participants list and documents list were presented (see Appendixes 2 and 3) and the local information and logistical arrangements were reviewed by the host.

3. Systematics of *B. dorsalis* complex

[16] The work conducted under a coordinated research project led by the FAO/IAEA Joint Division of Nuclear Techniques in Agriculture on the synonimization of *B. papayae*, *B. philippinensis* and *B. invadens* with *B. dorsalis* based on an integrative taxonomic approach with multiple, independent species delimitation tests was presented (see Appendix 4 for an abstract of this work[3]). It was noted that the large authorship of this recent scientific publication gives great clout to the research findings, and that a number of contracting parties have already accepted the consequent taxonomic revision.

[17] This species synonimization was noted by the experts in the present meeting.

[18] One expert raised a concern that the host lists between *B. dorsalis* and what was formerly considered *B. invadens* were different. Another expert explained that a narrower host list is expected for more recent incursions of a pest species and may widen with increasing time since establishment. It was also brought to attention of the participants that host lists require careful scrutiny by NPPOs with regard to the source and robustness of data presented. By the same token, claims regarding species descriptions and delineations should be carefully analyzed.

[2] https://www.ippc.int/publications/concept-note-0

[3] Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data: http://onlinelibrary.wiley.com/doi/10.1111/syen.12113/abstract

4. History and Development of Phytosanitary Treatments for the *B. dorsalis* complex

[19] The Secretariat provided an overview of the development of phytosanitary treatments under the IPPC framework, including the requirements for treatment submissions specified in ISPM 28 and the working TPPT criteria for treatment evaluation. The challenges encountered in the review of phytosanitary treatment submissions were summarised as follows:

- Models based on dose-response data cannot predict high efficacy required of phytosanitary treatments (as very large sample sizes are needed);
- Excessive mortality in control groups;
- Excessive infestation rates;
- Conflicting statistical methods of publications supporting phytosanitary treatments;
- Inconsistent results owing to variable experimental conditions.

[20] The Secretariat also presented the main points of concern raised by contracting parties during the member consultation period for a series of cold treatments against several tephritid species:

- <u>Clarification of terminology</u>, such as use of “cold treatment” *vs.* “refrigeration”, “consecutive days” *vs.* “24 hrs”, clear description of the plant parts used in experimentation (e.g. fruit), terms requiring clearer definitions (such as replication, repetition, block, endpoint, precooling, validation, confirmatory trials and large scale trials);
- <u>Referencing requirements</u>, such as the sufficiency of one comprehensive, quality study, documentation supporting approval by a country, public access to studies authored by anonymous;
- <u>Applicability of treatments</u>, including the development of generic cold treatments across host varieties or pest species;
- <u>Product quality issues</u>, such as concerns regarding the effect of cold treatments on the quality of treated fruit;
- <u>Experimental conditions</u>, including provision of information on the quality of the commodity, use of correct nomenclature of host varieties, and description of size, weight and shape of the host commodity used.

[21] The need for providing as much detail as possible when describing the experimental conditions used in treatment research was emphasised.

[22] One expert queried whether possible differences in treatment response between target pest populations from different geographical regions are taken into account in the context of harmonization of phytosanitary treatments. In the ensuing discussion it was highlighted that results of seemingly contradictory studies may not be comparable because of differences in experimental design, statistical analysis or methodology for efficacy measurement. Close scrutiny is necessary in verifying and interpreting research results as they may not always prove robust.

[23] A summary of the history of phytosanitary treatments for species in the *B. dorsalis* complex (see Appendix 5) was provided and a list of approved treatments collated of NPPO approved treatments was presented to the group.

[24] The question was posed as to whether comparisons of treatments are being conducted where multiple studies exist for the same pest and host. An expert explained that such an effort is currently underway for cold treatments and that the same will be done for heat treatments (as part of the ongoing work program of the Phytosanitary Temperature Treatments Expert Group (PTTEG)[4], which was formed as an outcome of the ECCT).

[25] In line with the above discussion on possible population differences, it was again brought out that a close look must be paid to the research underpinning the findings, as variation in treatment response or

[4] https://www.ippc.int/partners/international-organizations/phytosanitary-temperature-treatments-expert-group

efficacy may be an artifact of the research methodology (including treatment level, type of confirmatory testing, temperature prescription, etc). If more generic approaches to treatments are to be developed, it needs to be definitively established whether the population differences play a factor or not. It was strongly emphasized that further research is needed specifically on any untested assumptions made in the context of treatment prescriptions.

[26] It was noted that a list of approved phytosanitary treatments for the *B. dorsalis* complex constituted a key output from this expert consultation. All participants were requested to verify the information presented, as pertinent to their regions and/or trading partners, and add any treatments that might have been omitted.

[27] One of the experts pointed out that the approved vapour heat and high-temperature forced air treatments presented have, to his knowledge, never actually been commercially applied because of industry concerns regarding fruit damage. The experts considered that industry involvement in treatment development and evaluation is important as its primary interest is the quality of the traded commodity, thus complementing the NPPOs' main concern of preventing pest spread.

[28] Regarding the challenges encountered with the data review and evaluation process, one of the facilitators highlighted the concerns already summarised at the start of the current session. He stressed in particular the importance of submitters providing the complete set of relevant information, addressing in full the requirements listed in the treatment submission form. In this context, the expert, who is also a TPPT member, advised that the TPPT is currently developing guidelines to assist NPPOs and RPPOs in proper and complete treatment submissions and these guidelines, finalised and endorsed by the SC, would be made available when the next call for phytosanitary treatments is made by the IPPC Secretariat.

[29] The chairman briefly summarised the outcomes of the discussion of the session, highlighting that a common understanding was reached of the importance of tracking down sources of data variability between studies and of following internationally accepted research protocols to eliminate any artificial variations in efficacies.

5. Expert presentations

[30] The experts each gave a presentation on their respective area of research relating to phytosanitary treatments in the *B. dorsalis* complex (abstracts of all presentations can be found in Appendix 4). Particularly noteworthy discussions points following the presentations are summarized in the below paragraphs.

[31] The presentation on development of a cold treatment for *B. dorsalis* in South Africa, which satisfied the phytosanitary requirements of a major importing country, served as an example of the benefit harmonised phytosanitary treatment standards would bring about by allowing access to multiple trading partners. By the same token, the very similar research conducted for vapour heat treatments against *B. dorsalis* in Indonesia, Thailand and Vietnam, respectively, illustrated how international standards would reduce duplication of research efforts.

[32] The experience of hot water immersion treatments for *Anastrepha* species in fruits in Mexico was presented and informed the ongoing and planned work in Mozambique on hot water treatments for phytosanitary disinfestation of *B. dorsalis* with regard to the research design. This information exchange was deemed very useful as the comparatively low-tech treatment options are greatly needed for African fruit commodities to access various markets.

[33] Several expert presentations touched on systems approaches for fruit fly control and market access and these were thought to be interesting developments in view of the importance of increasing integration of pre- and post-harvest treatments and phytosanitary measures. The issue of host status was also mentioned in several presentations as being of great importance in the context of market access.

[34] The chairman concluded the session by noting that common approaches and constraints in developing phytosanitary treatments for *B. dorsalis* complex species were well-articulated and identified in the course of the presentations and ensuing discussions.

6. Development of phytosanitary treatments for the *B. dorsalis* complex (experimental design and other factors)

[35] The facilitator introduced the background document for the session, entitled "Development of phytosanitary treatments for the *Bactrocera dorsalis* complex" (see Appendix 6). It was noted that differences continue to prevail between the commercial environment and the research supporting phytosanitary treatments and that researchers must be mindful of the assumptions made in their work that such variables do not reduce treatment efficacy. Researchers ought to make as few assumptions as possible, whether stated or not, and explain and justify those that are made.

[36] A table from the document was reviewed by the experts which list factors differing between commerce and research and for which a lack of data may exist. It was agreed that the variables needed scrutiny to determine whether they require further research to assess their potential impact on treatment efficacy. It was also noted that while research should be conducted as quickly and easily as possible, it also has to be applicable to commercial conditions.

[37] The expert group undertook to prioritise the variables listed in terms of the perceived need for further investigation as to their effect on the efficacy of phytosanitary treatments. The factors mentioned most by the experts present were the source of the target pest used in the experiment (feral *vs*. colony), the mode of infestation (natural *vs*. artificial), the treatment dose (such as cooling or heating rates), determination of treatment start times, determining treatment temperature from experimental conditions, commodity variety and pest populations from different geographical regions.

[38] The participants agreed that collaboration was needed among them and other phytosanitary treatment researchers in addressing these research concerns in an effort to determine the effects of these factors on treatment efficacy.

[39] Several treatment factors were discussed in greater detail and are summarised in the below paragraphs:

Artificial vs. natural infestation:

[40] Participants noted that many studies will continue to use artificial infestation due to practical limitations of naturally infesting some host fruit commodities. Several participants noted that among these limitations in using natural infestation were (i) the difficulty to obtain sufficiently high infestation rates to demonstrate treatment efficacy; (ii) concerns regarding commodity quality as fruit may not be optimal for infestation at commercially suitable ripening stages; (iii) the lack of precision in determining survival rates when the number of eggs deposited in naturally infested fruit is unknown.

[41] Upon participants' queries, the group was reminded that the treatment submissions should demonstrate that artificial infestation results are more tolerant to the treatment than with natural infestation in order to meet the requirements applied by the TPPT. Researchers were encouraged to include treatment data obtained with natural infestation alongside the experiments with artificially infested fruit so as to add to the scant body of information on the effect of method of infestation on phytosanitary treatment efficacy. Different studies to date show differing results with regard to tolerance of insects obtained from artificial *versus* natural infestation, yet in many cases, treatments derived from studies using artificial infestation are more conservative compared to those from studies using natural infestation. The Secretariat noted that it should be considered that phytosanitary treatments should not be more restrictive than can be technically justified.

Host

[42] Ease of infestation of host fruit on treatment efficacy was discussed and it was noted that more data is needed to shed light on this issue. If harder-to-infest hosts require less severe treatments, as the few

studies in the literature suggest, then the possibility should be investigated of deriving generic treatments for fruit fly species across a host range based on data obtained for the easiest-to-infest host (requiring the toughest treatment). It was concluded that more work is needed to obtain this type of fundamental information.

Ramp up/down time

[43] The expert group considered that ramp-up or ramp-down times can influence treatment efficacy. While cold treatments may be more effective if the cool down period occurs over a long perid, essentially extending the treatment time. The group discussed establishing standards for defining cooling periods and how treatment time is initiated (e.g. when all temperature probes reach the target temperature, vs when 50% of probes reach target temperature. Similarly, it is becoming increasingly apparent from reviewing the various heat treatment protocols that a single temperature applied for a certain amount of time is not directly transferrable if the ramp-up/down times are different. If such an effect is conclusive, the ramp-up/down periods must be specified in heat treatment schedules.

[44] With regard to data review for proposed phytosanitary treatments going through the IPPC standard setting process, one participant noted that the research underpinning treatment submissions should be made available for the scrutiny of contracting parties during member consultation. The importance was noted of accessing and understanding the technical justification for treatment schedules in an effort to convince contracting parties and industry that the IPPC treatments are scientifically justified.

7. Operational conditions commercial practicability

[45] The background to the session included a summary by the Secretariat of the issues that had been identified in the ECCT report with regard to confirmatory trials and commercial practicability of phytosanitary cold treatments for fruit flies. These operational aspects in relation to cold treatments thus complemented the earlier presentations which focused mostly on high temperature treatments.

[46] In the context of the most suitable implementation technology used in approved phytosanitary treatments, it was noted that in some exporting countries, they rely entirely on the importing countries' prescriptions, including pre-treatment, treatment and post-treatment procedures.

[47] A long discussion ensued on the possibility of devising generic treatments and it became apparent that the participants had various conceptions of what constituted a generic treatment. It was concluded that if this terminology is used, it is important to define in clear terms what "generic treatments" mean. The general understanding seemed to be that of a consolidation of several similar treatments covering more than one crop/species combination (e.g. a generic phytosanitary treatment for a group of pest insects on a particular host, or a generic treatment for one pest insect on a range of host species). It was clarified that, on the other hand, that harmonization of phytosanitary treatments is a different matter and not to be confused with generic treatments.

[48] With regard to a discussion point on differences between treatments approved within bilateral agreements, the question was posed whether generic treatments would work for importers or whether bilateral fine-tuning would always be required. It was noted that agreement was required on important points in measuring treatment variables (such as the number and placement of probes *inter alia*). A participant from an importing country emphasized in this context that prescription of treatment protocols and verification of their proper application were different matters, with the latter always being required to satisfy import country requirements. Another participant showcased that the NPPO in his country uses generic export protocols and merely fine-tunes them to meet specific import requirements.

[49] When discussing the practical extension of treatment schedules from large scale trials into commercial application, the main points made by participants were that (i) simple protocols are required for use by industry; (ii) commercial aspects must be duly considered (e.g., temperature and time requirements for cold treatments); (iii) operational conditions should not be built into the prescription unless essential to

the treatment, i.e., when affecting treatment efficacy (such as, for instance, cooling time following heat treatment).

[50] The issues of treatment starting point, temperature ranges and thresholds were explored by the participants. Participants were reminded that every treatment has a degree of error, such as upper and lower temperature limits between which a heat treatment is effective. There was a consensus among participants that in this regard, the specification of a single treatment starting point is required for industry, such as a commercial starting temperature based on a mean of all probes. In this context, a researcher from an importing country explained that his NPPO has recently decided to establish the upper temperature limit for cold treatments by using the mean effective dose, rather than severest extreme of the tested range, thus effectively lowering the severity of the treatment. This development was received with interest and welcomed by the participants in the expert consultation, noting they would follow suit and consider such changes to their methods for setting treatment doses, effectively resulting in treatments that are less stringent than currently prescribed.

[51] The session was concluded by recalling that there was a lot of data generated to obtain market access and that an effort should be made by NPPOs to submit these data in response to the IPPC Secretariat's call for phytosanitary treatments.

8. Overview and Conclusions

[52] The chairman noted that the meeting objectives had been reached in the course of the expert meeting and the discussions held, in that:

(1) the phytosanitary treatment researchers from various countries around the world discussed and shared the scientific and practical issues related to the development of fruit fly treatments to control pest species within the *B. dorsalis* complex and discussed acceptable common approaches;

(2) phytosanitary treatments for pest species within the *B. dorsalis* complex used in different countries around the world were identified and compiled (refinement of the information will continue);

(3) the treatment experts explored the various constraints in developing fruit fly treatments and identified common methods to address these constraints.

[53] The chairman concluded that two of the expected outputs had been delivered by the expert consultation during the earlier meeting sessions: (1) a list of NPPO or RPPO approved phytosanitary treatments used to control pest species within the *B. dorsalis* complex was compiled (see Appendix 6); (2) with regard to agreement on a common approach to the development of phytosanitary treatments for fruit flies – including methodologies, statistical analyses, carrying out confirmatory trials, evaluating and submitting – it was concluded that the guidance material available to assist with submissions of treatment proposals through the IPPC standard setting process would be increasingly utilized by phytosanitary treatment experts in developing their treatment research.

[54] With regard to the output of a plan for future collaboration among the experts involved in the meeting, lengthy discussions were held on the feasibility of continuing the discourse on phytosanitary research in *B. dorsalis* complex as a stand-alone expert group vis-à-vis the integration of this group with the larger PTTEG group.

[55] After exploration of the various avenues for future collaboration, a clear consensus was reached that there should not be a multiplicity of groups – rather, the *B. dorsalis* expert group will work under the umbrella of the larger PTTEG group and will consider the formation of specific focus groups of interested researchers, such as on *Bactrocera* treatment research in a particular region or on cold treatment issues, *inter alia*. Since the PTTEG already has an established mechanism of communication, also, with the Technical Panel on Fruit Flies, a rapport on systems approaches to controlling *B. dorsalis* could be maintained with the latter through this avenue.

[56] As part of the plans for collaboration among the experts it was decided, after discussion, to produce a joint-review paper, authored by the meeting participants, that is to provide a “phytosanitary treatment toolbox” describing available phytosanitary treatments against *B. dorsalis*, the market access obtained with their use, any problems encountered with treatment efficacy and possible effects on the quality of the fruit.

In summarizing the agreed recommendations on future collaboration of the researchers present, the chairman concluded that the main mechanism for the continued work towards harmonization would be for the experts to collaborate within the PTTEG to address research concerns, pool experiences, and to supply relevant information to the Technical Panels (reviewed and consolidated by the PTTEG, where appropriate, such as sets of very similar treatment schedules).

9. Close of the meeting

[57] The host thanked the Secretariat for facilitating the meeting and the participants for their hard work on such an important topic. The Secretariat thanked the participants for their excellent work during the meeting, wished further success in this work and thanked the host and organizer for their outstanding hospitality and logistical arrangements.

Appendix 1 - Agenda

AGENDA ITEM	DOCUMENT NO.	PRESENTER
1. Opening of the meeting		
Welcome by the IPPC Secretariat		LARSON
Welcome remarks by MAFF Japan		FUKUSHIMA
Introduction of Participants		FUKUSHIMA
Election of the Chair		NIYAZI
Election of the Rapporteur	01_ECBD_2014_Dec_Rev	CHAIR
Adoption of the Agenda	38_ECBD_2014_Dec	CHAIR
Presentation – Overview of IPPC		LARSON
Meeting objectives and outputs		NIYAZI
2. Administrative Matters		CHAIR (CLARKE)
Documents List	02_ECBD_2014_Dec_Rev3	NIYAZI
Participants List	03_ECBD_2014_Dec_Rev	NIYAZI
Local Information	04_ECBD_2014_Dec_Rev	SUZUKI
Logistical Arrangements		SUZUKI
3. Systematics of *B. dorsalis* complex		CHAIR (CLARKE)
3.1 Current state of species within the *B. dorsalis* species complex	06_ECBD_2014_Dec 07_ECBD_2014_Dec 11_ECBD_2014_Dec 17_ECBD_2014_Dec	CLARKE
3.2 Molecular diagnostic method for *Bactrocera* spp.	16_ECBD_2014_Dec 27_ECBD_2014_Dec	CHOI
4. History and Development of Phytosanitary Treatments for the *B. dorsalis* Complex		CHAIR (CLARKE)
4.1 Overview of the development of phytosanitary treatments under the IPPC framework	39_ECBD_2014_Dec	NIYAZI
4.2 History of treatments developed to control the *B. dorsalis* complex including status of species within the complex relating to treatment efficacy and development of generic treatments	34_ECBD_2014_Dec	HALLMAN/ DOHINO
4.3 Current RPPO or NPPO approved treatments to control the *B. dorsalis* complex	33_ECBD_2014_Dec_Rev	DOHINO
4.4 Challenges with the data review and evaluation process		HALLMAN
5. Expert Presentations		CHAIR (CLARKE)
5.1 Background on the status of *B. dorsalis* in Africa and development of cold treatment	08_ECBD_2014_Dec 29_ECBD_2014_Dec 40_ECBD_2014_Dec	GROUT
5.2 Laboratory simulation of cold quarantine disinfestation of *B. dorsalis (=invadens)* in sweet oranges *NOTE: Speaker was unable to attend.*	15_ECBD_2014_Dec 26_ECBD_2014_Dec	UMEH

AGENDA ITEM	DOCUMENT NO.	PRESENTER
5.3 Efficacy of post-harvest hot water treatment in *B. dorsalis* on mango	14_ECBD_2014_Dec 25_ECBD_2014_Dec	CUGALA
5.3.B. Hot-water immersion: why is it an effective and inexpensive phytosanitary treatment proposed for mango fruits against *Bactrocera* species?	41_ECBD_2014_Dec 41a_ECBD_2014_Dec 41b_ECBD_2014_Dec 41c_ECBD_2014_Dec 41d_ECBD_2014_Dec 41e_ECBD_2014_Dec	HERNANDEZ
5.4 Large-scale mortality test for *B. papaya* using vapour heat treatment	13_ECBD_2014_Dec 24_ECBD_2014_Dec	MURDITA
5.5 Phytosanitary treatments for *B. dorsalis* in Vietnam	23_ECBD_2014_Dec 28_ECBD_2014_Dec	TU
5.6 Research on vapour heat treatment for disinfestation of fruit flies	22_ECBD_2014_Dec 36_ECBD_2014_Dec	SRIKACHAR
5.7 Cold treatments, methyl bromide fumigation and hot water treatments in 5 spp of *B. dorsalis* complex	21_ECBD_2014_Dec 37_ECBD_2014_Dec	MYERS
5.8 Recent phytosanitary research in Bactrocera species: systems-approach, radiotolerance under modified atmosphere, post-irradiation cold storage, cold tolerance in citrus	20_ECBD_2014_Dec 32_ECBD_2014_Dec	FOLLETT
5.9 Background and results of training programme for developing countries on thermal disinfestation treatments for fruit flies, incl. *B. dorsalis*	12_ECBD_2014_Dec 19_ECBD_2014_Dec	MIYAZAKI
5.10 Proposed steps for developing international standards for phytosanitary temperature treatments	18_ECBD_2014_Dec 31_ECBD_2014_Dec_Rev	DOHINO
6. Development of phytosanitary treatments for the *B. dorsalis* complex (Experimental design and other factors)		HALLMAN
Issues to be considered: • Identification of the *B. dorsalis* complex and ramifications for trade • Summary of the Expert Consultation on Cold Treatments relating to fruit flies • Measuring efficacy • Most tolerant life stage • Tolerance among species and species • Tolerance among populations of the same species, • Host conditions and effects on efficacy of the phytosanitary treatment (e.g. species, cultivar) • Effect of infestation methodology on efficacy • Acclimation ability • Sample size and confidence limits in phytosanitary research, and efficacy level calculation for treatments • Extrapolating from low test subject numbers • Other experimental conditions and their variations	09_ECBD_2014_Dec 10_ECBD_2014_Dec 30_ECBD_2014_Dec 35_ECBD_2014_Dec_Rev ECCT 2013 Meeting Report	
7. Operational conditions and commercial practicability		GROUT
7.1 Key operational issues identified by the Expert Consultation on Cold Treatments relating to fruit flies	42_ECBD_2014_Dec	NIYAZI

AGENDA ITEM	DOCUMENT NO.	PRESENTER
7.2 Issues to be considered: • The most suitable implementation technology approved by contracting parties (i.e. procedure for pre-treatment, treatment, post-treatment) • Possibility of generic treatments • Differences between treatments approved within bilateral agreements • Practical extension of treatment schedules from large scale trials into commercial application • Treatment starting point and "±" issue • Possibility of establishing a temperature threshold	43_ECBD_2014_Dec	GROUT
8. Overview and conclusions	-	CHAIR (CLARKE)/ LARSON
8.1 General and specific conclusions 8.2 Preparation of document about current treatments, current research, and research and submission issues 8.3 Next steps	44_ECBD_2014_Dec	
9. Close of the meeting	-	SUZUKI/ LARSON

Appendix 2 - Participants list

	Participant role	Region	Name, mailing, address, telephone	Email address
✓	Expert	Africa	**Mr. Domingos Raquene CUGALA (Prof.)** Researcher, Faculty of Agronomy and Forest Engineering, Main University Campus Avenue Julius Nyerere, Building No.1, Maputo **MOZAMBIQUE**	dcugala@gmail.com dcugala@uem.mz
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✓	Expert	Asia	**Mr Duong Minh TU** Entomologist Director Plant Quarantine Diagnostic Centre (PQDC) Plant Protection Department (PPD) Ministry of Agriculture and Rural Development (MARD) 149, Ho Dac Di, Dong Da, Ha Noi, **VIET NAM** Tel/fax: (84) 4 3851 3746 Handphone: 0904 101 090	tudm.bvtv@mard.gov.vn duongminhtu60@gmail.com
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✓	Expert/Facilitator / Steering Committee		**Mr Guy HALLMAN** Research Entomologist Insect Pest Control Laboratory Reaktorstrasse 1 A-2444 Seibersdorf **AUSTRIA**	G.J.Hallman@iaea.org

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Appendix 3 – Documents list

DOCUMENT NUMBER	AGENDA ITEM	DOCUMENT TITLE (PREPARED BY)	DATE POSTED / DISTRIBUTED
01_ECBD_2014_Dec_Rev3	1.	Agenda	2014-12-05
02_ECBD_2014_Dec_Rev3	2.	Documents List	2014-12-05
03_ECBD_2014_Dec_Rev	2.	Participants List	2014-12-02
04_ECBD_2014_Dec_Rev	2.	Local Information	
06_ECBD_2014_Dec	3.1	One and the same: Integrative Taxonomic Evidence that *Bactrocera Invadens* (Diptera: Tephritidae) is the same species as the Oriental Fruit Fly Bactrocera dorsalis	2014-11-13
07_ECBD_2014_Dec	3.1	Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae.	2014-11-13
08_ECBD_2014_Dec	5.1	Cold Susceptibility and Disinfestation of *Bactrocera invadens* in Oranges	2014-11-13
09_ECBD_2014_Dec	6.	Development of Postharvest Phytosanitary Disinfestation Treatments	2014-11-13
10_ECBD_2014_Dec	6.	Requirements for Phytosanitary Treatments as Phytosanitary Measures	2014-11-13
11_ECBD_2014_Dec	3.1	Integrative taxonomy and resolution of pest species in the *Bactrocera dorsalis* species complex – Presentation: CLARKE, Australia	2014-11-13
12_ECBD_2014_Dec	5.9	Background and Results of Training Program for Developing Countries on Thermal Disinfestation Treatments for Fruit Flies, Including *Bactrocera dorsalis* – Presentation: MIYAZAKI, Japan	2014-11-13
13_ECBD_2014_Dec	5.4	Vapour Heat treatment against three species of fruit flies in Mango Dedong – Presentation: MURDITA, Indonesia	2014-11-13
14_ECBD_2014_Dec	5.3	Assessment of efficacy of hot water treatment for post harvest – Presentation: CUGALA, Mozambique	2014-11-13
15_ECBD_2014_Dec	5.2	Laboratory simulation of cold quarantine disinfection of sweet oranges – Presentation: UMEH, Nigeria	2014-11-13
16_ECBD_2014_Dec	3.2	An introduction to diagnostic method for *Bactrocera* species – Presentation: CHOI, Korea	2014-11-13
17_ECBD_2014_Dec	3.1	Abstract – CLARKE, Australia	2014-11-13
18_ECBD_2014_Dec	5.10	Abstract – DOHINO, Japan	2014-11-13
19_ECBD_2014_Dec	5.9	Abstract – MIYAZAKI, Japan	2014-11-14
20_ECBD_2014_Dec	5.8	Abstract – FOLLETT, USA	2014-11-14
21_ECBD_2014_Dec	5.7	Abstract – MYERS, USA	2014-11-14
22_ECBD_2014_Dec	5.6	Abstract – PHANKUM, SRIKACHAR, ORANKANOK, Thailand	2014-11-14

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23_ECBD_2014_Dec	5.5	Abstract – TU, Vietnam	2014-11-14
24_ECBD_2014_Dec	5.4	Abstract – MURDITA, Indonesia	2014-11-14
25_ECBD_2014_Dec	5.3	Abstract – CUGALA, Mozambique	2014-11-14
26_ECBD_2014_Dec	5.2	Abstract – UMEH, Nigeria	2014-11-14
27_ECBD_2014_Dec	3.2	Abstract – CHOI, Korea	2014-11-14
28_ECBD_2014_Dec	5.5	Phytosanitary Treatment for Oriental Fruit Fly in Vietnam – Presentation: TU, Vietnam	2014-11-14
29_ECBD_2014_Dec	5.1	Abstract – GROUT, South Africa	2014-11-14
30_ECBD_2014_Dec	6.	Characterization of Heated Air Treatments	2014-11-17
31_ECBD_2014_Dec_Rev	5.10	Proposed steps for developing international standards for phytosanitary temperature treatments – Presentation: DOHINO, Japan	2014-12-01
32_ECBD_2014_Dec	5.8	Recent Bactrocera Quarantine treatment research in Hawai'I – Presentation: FOLLET, USA	2014-11-17
33_ECBD_2014_Dec_Rev	4.3	Current RPPO or NPPO approved treatments to control the *Bactrocera Dorsalis* complex	2014-12-01
34_ECBD_2014_Dec	4.2	Introduction to the History of Phytosanitary Treatments developed to control the *Bactrocera dorsalis Complex*	2014-11-24
35_ECBD_2014_Dec_Rev	6.	Development of Phytosanitary Treatments for the *Bactrocera dorsalis Complex*	2014-12-02
36_ECBD_2014_Dec	5.6	Phytosanitary Treatmens for Bactrocera dorsalis complex in Thailand – Presentation: SRIKACHAR, Thailand	2014-11-24
37_ECBD_2014_Dec	5.7	Comparison of Phytosanitary Treatments among Bactrocera dorsalis Complex species – Presentation: MYERS, USA	2014-11-24
38_ECBD_2014_Dec	1	IPPC Overview	2014-11-25
39_ECBD_2014_Dec	4.1	Overview of the development of phytosanitary treatments under the IPPC framework	2014-11-25
40_ECBD_2014_Dec	5.1	Background on the status of *B. dorsalis* in Africa and the development of a cold treatment – Presentation: GROUT, South Africa	2014-12-01
41_ECBD_2014_Dec	5.3.B.	Hot-water immersion: why is it an effective and inexpensive phytosanitary treatment proposed for mango fruits against Bactrocera species? – Presentation: HERNANDEZ, Mexico	2014-12-01
41a_ECBD_2014_Dec	5.3.B.	Hot water treatments for mangoes against *C. capitata*	2014-12-01
41b_ECBD_2014_Dec	5.3.B.	Cooling methods for Ataulfo mangoes	2014-12-01
41c_ECBD_2014_Dec	5.3.B.	Hot water treatment for baby Ataulfo mangoes	2014-12-01
41d_ECBD_2014_Dec	5.3.B.	"Termosensibilidad de *Anastrepha* spp"	2014-12-01

DOCUMENT NUMBER	AGENDA ITEM	DOCUMENT TITLE (PREPARED BY)	DATE POSTED / DISTRIBUTED
41e_ECBD_2014_Dec	5.3.B.	Work plan to export mango from Mexcio to USA	2014-12-01
42_ECBD_2014_Dec	7.1	Key Operational Challenges	2014-12-03
43_ECBD_2014_Dec	7.2	Operational conditions and commercial practicability	2014-12-03
44_ECBD_2014_Dec	8.2	Outline of Paper – PhytoToolbox for *B. dorsalis*	2014-12-04

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Appendix 4 – Abstracts of expert presentations

I. Background on the status of *B. dorsalis* in Africa and the development of a cold treatment

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Bactrocera dorsalis was recorded in Africa for the first time as *Bactrocera invadens* in 2003 in Kenya and within a year in the neighbouring countries of Tanzania and Uganda. Since then it has been found to occur in all countries south of the Sahara, although it is only permanently established in the northern, subtropical parts of South Africa. Coordinated attempts to monitor and control the pest were scarce in most countries other than some in East and West Africa. Although some pest risk assessments recommended appropriate postharvest treatments, no recommendations for specific treatments were being made and even now are difficult to find. A heat treatment for mangoes was recommended by Self et al. in 2012 and Guy Hallman published some small scale evaluations of heat and cold treatments in 2011. Apart from the research by South Africans in Kenya with the collaboration of *icipe*, very few postharvest research results are available. Most exports from East Africa are vegetables and other plants that are not hosts of *B. dorsalis* so these have been unaffected by the presence of the fruit fly. However, the export of avocadoes to South Africa from Kenya was stopped by the South African authorities. I started some research in 2008 with Sunday Ekesi at *icipe* where we refurbished a cold room that could be used for the cold treatment of citrus. We used Egyptian exported oranges that we purchased in Nairobi and infested them with *B. dorsalis* eggs from a laboratory culture. When 22,449 third instars were treated at 1.1°C (± 0.5) the results suggested that a period of 16 days could be effective in oranges. Results from the first replicate of 16,617 larvae showed no survivors but the second replicate of 23,536 larvae had three survivors. Further replicates were therefore conducted at a lower mid-point of 0.5°C and mean hourly maximum of 0.9°C (± 0.5), for 16 d. After treating 65,752 *B. dorsalis* third instars without survivors, the Japanese requirement of 99.99% mortality at the 95% confidence level was surpassed. The following treatment protocol for *B. dorsalis* larvae in oranges was therefore recommended: "Fruit pulp to be maintained at temperatures of 0.9°C (± 0.5) or lower for 16 consecutive days". Subsequent work by Ware et al. published in 2012 showed that a continuous cold treatment at 1.5°C (or lower) for 18 days provided sufficient phytosanitary security in Hass avocadoes. The technique used by myself and later by Ware et al. 2012 was based on a larval endpoint as this is the way that the first research on cold treatments for Japan was conducted in South Africa. This is a very labour-intensive method but assumes that a live larva will enclose as an adult which is what most fruit inspectors are concerned about.

II. Assessment of efficacy of hot water treatment for post harvest disinfestation of the invasive fruit fly, *Bactrocera dorsalis (=invadens)*, on mango

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The invasive fruit fly, *Bactrocera dorsalis (=invadens)*, recently introduced into Africa has become a major threat for the production and trade of fresh fruits including mango. Direct damage on mango of up to 94.5% due to *B. dorsalis* has been reported in Mozambique. The occurrence of *B. dorsalis* in Mozambique has led to the suspension of fruit and vegetable- exports to the country's major trading partners, causing severe financial losses to producers and a virtual cessation of investment. To overcome those restrictions and mitigate on the impact of *B. dorsalis*, the use of post-harvest phytosanitary treatment in Mozambique is viewed as a potential strategy. Therefore, the present proposal attempts to establish the extent of hot water treatment efficacy in controlling *B. dorsalis* on mango fruits. The experiments will be conducted at Ganel mango orchard located at Dombe, at central province of Manica, Mozambique. Two export mango varieties (Tommy Atkins and Kent) will be used. The study will be conducted in a completely randomized block design with 4 hot water temperatures with each temperature serving as a treatments as follows: T1= 47°C; T2 = 46.5°C; T3 = 46°C; T4 = Control (no treatment), arranged in 4 replications. A total of 10 to 20 mango trees containing fruits at the harvest stage will be selected and caged or covered with netting materials, and then the fruits will be exposed to 40 gravid female *B. dorsalis* (12-day old) for 24 hours. The mango plants will be left in the field caged for a period of at least 10 days. A total of 160 mango fruits will be harvested at 1 day, 7 and 10 days after infestation to ensure the presence of egg, first and second instar larvae, respectively. The fruits will then be counted, weighted and the number of visible oviposition wounds counted before fruits are submitted to hot water treatments at the above temperatures. The fruits will be divided into 4 groups of 40 fruits per treatment (temperature and control) corresponding to 10 fruits per replication. Depending on the temperature, mango fruits will be submitted to the treatments for 10, 11 and 12 minutes for the lowest temperature. The fruits will be placed separately in rearing containers and kept for at least 6 weeks for emergence of adult fruit flies. The number of emerged adults will be counted and identified to species level. Fruit fly infestation will be expressed as number of fruit flies/kg fruit. Data will be subjected to Analysis of variance (ANOVA) (Proc ANOVA) and treatment means will be compared using Student Newman Keuls (SNK) (P = 0.05). It is expected that at least one treatment temperature will be efficient in achieving Probit 9 for post harvest disinfestation of B. *dorsalis* in infested fruits.

Keywords: hot water, *Bactrocera dorsalis (=invadens)*, mango fruits

III. Laboratory Simulation of Cold Quarantine Disinfection of sweet oranges (*Citrus sinensis* Osbeck) against the new invasive fruit fly *Bactocera dorsalis*.

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The fruit industry in West Africa is seriously affected by the invasive fruit fly *Bactrocera dorsalis*. Thereby undermining laudable initiatives embarked upon by various governments to expand horticultural crop production in the sub region. Citrus and mango fruits targeted for export are usually prone to *B. dorsalis* infestation. This new trend has drastically affected export trade in the sub region due to strict regulations on the importation of infested fruits. The Agricultural Research Council of Nigeria through the Competitive Agricultural Research Grant Scheme (CARGS) funded a research project aimed at controlling fruit flies of economic importance along citrus and mango value chain. For the exportation of citrus, disinfection of fruit flies has been achieved with cold treatments in many countries. However, this varies with the variety of citrus used and the species of fruit fly involved. The development of heat or cold treatments usually requires testing a very large number of insects over several years. We bypassed this drudgery by adopting established cold disinfection temperatures for fruit flies with similar behaviors and modes of infestation to the new *B. dorsalis*. We therefore evaluated in the laboratory the appropriate cold temperatures and length of time to which fruits of sweet orange varieties Valencia Late and Agege can be exposed during export to prevent the survival of fruit fly larval stages while retaining fruit quality after exposure. Infestation of fruits and larval feeding were carried out naturally in the laboratory at the National Horticultural Research Institute and treatment assessment commenced when the simulated in-transit cold quarantine test temperatures of 2 ± 05°C or 3.5 ± 0.5°C were attained by the fruits in two separate freezing chambers. Based on emerging pupae after end point, all tested stages (egg, L1, L2 and L3) were totally killed by treatments at 2 ± 05°C or 3.5 ± 0.5°C for 30 days in the two sweet orange varieties. At 21 days of cold treatment, only individuals subjected to 2 ± 05°C treatment recorded total mortality in the two sweet orange varieties, while some L3 larvae survived. The two tested temperatures 2 ± 05°C or 3.5 ± 05°C caused no chilling injury nor affected the quality of the citrus fruits at 21 days of preservation. Very little chilling injury was recorded on fruits exposed to cold temperature of 2 ± 05°C for 30 days. However, the fruits met with commercially acceptable level of overall quality. Appearance of fruits was rated as good and water loss was minimal. Analyzed Vitamin C, total flavonoids, titratable acidity (% citric acid) and soluble solids (brix) met the acceptable levels for export.

IV. An introduction to diagnostic method for *Bactrocera* species

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The *Bactrocera dorsalis* complex of fruit flies, in the broad sense, comprises 68 species with varying distributions in Asia, Australia and the Pacific islands. Most of these species can be readily distinguished by morphological characteristics. However, five members (*B. dorsalis, B. papaya, B. philipinensis, B. carambolae*, and *B. invadens*) of this complex are difficult or impossible to separate based on morphology alone. Molecular techniques are best used to support or augment morphological identification. They can be used to identify early larva stages and eggs. We conducted a phylogenetic study of Tephritidae. For the molecular analysis using the mitochondrial COI and COII genes, 74 tephritid flies, nine *Bactrocera* species were analyzed using Pyrgotidae as an outgroup. We also introduction to identification procedure of *Bactrocera dorsalis* from dried persimmon intercepted under the plant quarantine inspection.

V. Large Scale Mortality Test of Bactrocera papayae (Diptera: Tephritidae) by Vapor Heat Treatment

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Bactrocera papayae is included in Bactrocera dorsalis complex. Based on the experiment held at Pest Forecasting Institute (PFI) in West Java, Bactrocera papayae was determined as the most heat tolerant species of 3 there species of fruit flies (*B.papayae, B.carambolae and B.cucurbitae*) and from all stages of B.papayae, Mature egg was determined as the most heat tolerant stage. The large scale mortality test was conducted to confirm 100% mortality on mature egg of *B. papayae*. VHT treatment with 47.0^{o}C of fruit core temperature and 30 minutes holding time proved that 100% of test insects or more than 30,000 test insects (40,708 test insects) were completely killed. Therefore, on commercial scale treatment, these conditions can be used as a standard to ensure the complete mortality of all stages of *B. carambolae, B. cucurbitae* and *B. papayae*. The VHT standard with 47.0^{o}C or above of fruit core temperature and 30 minutes holding time will be sufficient for quarantine security against 3 species of fruit flies in mangoes on the commercial treatment.

Key Words: Vapor heat treatment, *B.papayae*, fruit core temperature, holding time.

Materials and Methods

Test Insects

Test insect used for the test was mature egg of *B. papayae* as shown in Table below.

Parents insects for egg collection

	Species	Stage	Generation	Date of eclosion
Rep. 1	*Bactrocera papayae*	Adult	G-68	31-Oct-12
Rep. 2	*Bactrocera papayae*	Adult	G-68	31-Oct-12
Rep. 3	*Bactrocera papayae*	Adult	G-69	14-Nov-12
Rep. 4	*Bactrocera papayae*	Adult	G-69	14-Nov-12

Test insects for the treatment

	Species	Stage	Time from oviposition
Rep.1, 2, 3 and 4	*B. papayae*	Mature egg	27-28 hours

Test Fruits

Test fruit used in this test is mango 'Gedong' (*Mangifera indica*).. Weight of mango used for the test is 250-300g with 75-80% maturity at the treatment time.

Numbers of test fruits used in half load condition (the 1st and the 2nd replication) were 205 fruits; 75 fruits as treatment, 30 fruits as control, 88 fruits as filler and 5 fruits as sensor. For full load condition, 373 fruits; 100 fruits as treatment, 30 fruits as control, 229 fruits as filler and 7 fruits as sensor, were used. Number of the fruit for checking developmental stage and back up was same in both treatment condition, 2 fruits for checking developmental stage and 5 fruits for back up.

Preparation of Infested Fruits

Before inoculation, test fruits were washed, weighed and sorted. The eggs of fruit flies used in the test were the mature eggs of *B. papayae* taken from the rearing cage of fruit flies in the VHT laboratory in PFI, Jatisari. The eggs were collected by using the egging device for 1 hour.

In order to prepare for inoculation, the peels of the fruits were cut rectangularly (L 3 cm × W 2cm), except for one side. A hole with a diameter of 5 mm was made by a cork borer at the center of rectangular piece of the peel for the ventilation. The peel was turned, the certain amount of the pulp was removed, and shallow slits were made on the pulp as a place where eggs were inoculated. After the inoculation, the peer was closed with surgical tape, but the hole was not covered. The number of egg inoculated was 150 eggs in each mango.

Vapor Heat Treatment (VHT)

The VHT conditions are as below and VHT setting for program mode are shown as below.

VHT conditions:

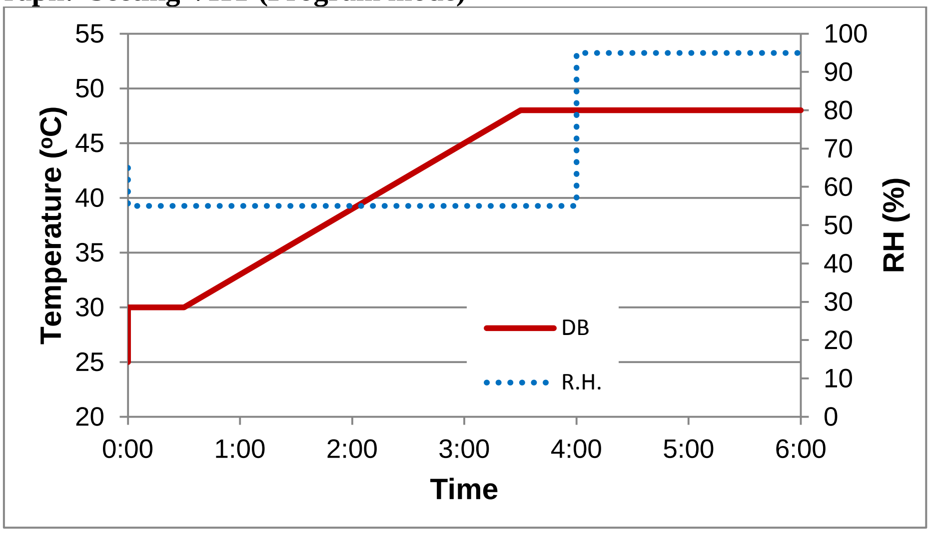
- VHT system: Program mode
- Temperature in VHT chamber (dry bulb): Keep 30.0 ^{o}C for 30 minutes and rise from 30.0 ^{o}C to 48.0 ^{o}C in 2 hours (48.0 ^{o}C is 1.0 ^{o}C higher than target fruit core temp.)
- Relative humidity (RH): Keep 55 % for 150 minutes and rise more than 95%
- Target fruit core temperature: 47.0 ^{o}C
- Holding time: 0, 10, 20, 30 minutes
- Water cooling: 10 minutes
- Air cooling: 30 minutes
- Load factor in the chamber VHT:
  - ➢ Half Load: 43,966.0 g/chamber (Rep.1)
    43,996.8 g/chamber (Rep.2)
  - ➢ Full Load: 88,369.9 g/chamber (Rep.3)
    88,666.0 g/chamber (Rep.4)

VHT setting for program mode in the large scale mortality test of *B. papayae* in VHT

Setting VHT (Program mode)

Step	DB(ºC)	R.H.(%)	Time (min.)	Remark
Step 0	30.0	55.0	0	
Step 1	30.0	55.0	30	
Step 2	48.0	55.0	120	
Step 3	48.0	55.0	(120)	Until fruit temp 42.ºC
Step 4	48.0	95.0	0	
Step 5	48.0	95.0	(120)	Until the end of holding time
Target fruit core temp.		above 47.0ºC		3 of 5 sensor fruits (haf load)
				4 of 7 sensor fruits (full load)
Holding time		30 minutes		
Cooling		Shower 10min./Fan 30min		

Graph: Seeting VHT (Program mode)



Before the treatment, all of the test fruits stored in the biotron were taken out and then the area of the air holes and the inoculation holes were wrapped with surgical tape. Before putting them into the VHT chamber, fruits consisting of treatment, filler and sensor fruits were placed in plastic containers.

The temperature of the infested fruits was monitored by inserting sensor into the sensor fruits.

For half load treatment, target temperature was determined by more than 3 of 5 fruits sensors had reached predetermined target temperatures, i.e., 47.0^{0}C, while for full load treatment, it was determined by more than 4 of 7 fruit sensors. After VHT treatment, water and air cooling were conducted in the same VHT system. The date of VHT treatment is shown as below.

Treatment date of the large scale mortality test

	Replications	Date of treatment
1	Replication 1 (Half load)	22-Nov-12
2	Replication 2 (Half load)	23-Nov-12
3	Replication 3 (Full load)	30-Nov-12

4	Replication 4 (Full load)	7-Dec-12

Cooling Methods and Storage of Treated Fruits

Two methods were used in the cooling process of the treatment, i.e., water cooling and air cooling. Water cooling was conducted after the infested fruits reached the target temperature for 10 minutes in the other VHT system. Air cooling was conducted after water cooling by using the electrical fans for 30 minutes in the VHT room.

After the air cooling, the surgical tape was removed from the treated fruits and then two slits were made on each treated fruit by a scalpel. In order to discharge the juice, produced by larvae in accordance with their growth, inside the treated fruits. That was to prevent larvae from drowning by the juice.

The treated fruit was placed in the box which was covered with a tissue at the bottom. The treated fruit was placed in the upright position so the juice flowed out from the slits. Before closing the plastic box with the lid, its top was also covered with a tissue. The treated fruits were stored in the biotron set at 28.0°C and kept for 4 days, on the other hand, untreated fruits (control) were kept for 5 days.

Confirmation of Development Stage

The dissection survey of fruit was conducted to ensure the developmental stage of the test insect. The dissection was carried out at the same time of vapor heat treatment. Two mangoes were prepared for every stage of insects. Based on the dissection survey of the test fruits, the developmental stage of the test insect in the test fruit was appropriate.

Handling of Untreated Fruits (Control)

The untreated fruits were handled in the same manner as the treated fruits and kept in the room temperature during the treatment.

Fruit Dissection

The dissection survey of the treated fruits and the untreated control fruits were conducted 4 days after the VHT treatment.

When the dissection survey of fruit was conducted, especially around the area where the eggs inoculated was checked carefully, after that, the whole of the the fruits was checked.

The test insects were checked if they were alive or not by using a stereomicroscope when it's necesarry, and counted.

The effective number of the test insects was estimated based on the number of the living insects in the untreated control fruits.

Data was corrected by Abbot's formula and also evaluated by using Logit analysis to determine the most thermotolerant stage. The effective number of the test insects was estimated based on the number of the living insects in the untreated control fruits.

Results and Discussions

The total of effective insects on the large scale mortality test was more than 30,000 test insects (40,708 test insects). The test showed that VHT treatment with 47.0^0 C of fruit core temperature and 30 minutes holding time, 100% of test insects were completely killed. Therefore, it could be said that the vapor heat treatment at 47.0^0 C or above of fruit core temperature with 30 minutes holding time achieves complete mortality of all stages of *B. carambolae*, *B. cucurbitae* and *B. papayae* and it also will be sufficient for the quarantine security.

Number of test fruits, no. of survivor and mortality in the large scale mortality test of *B. papayae* (Mature egg) in VHT

Replication	Load factor	Control		Treatment at 47.0 °C for 30 minutes			
		No. of fruits	No. of survivor	No. of fruits	Estimated no. of test insects	No. of survivor	Mortality (%)
1	Half load	30	3,332	75	8,330	0	100
2	Half load	30	3,545	75	8,862	0	100
3	Full load	30	3,633	100	12,110	0	100
4	Full load	30	3,422	100	11,406	0	100
Total	**-**	**120**	**13,932**	**350**	**40,708**	**0**	**100**

Fruits weight, number of survivor of test insects in the control plot in the large scale mortality test

Fruit no.	Rep. 1		Rep. 2		Rep. 3		Rep. 4	
	Fruits weigh (g)	No. of survivor *)	Fruits weigh (g)	No. of survivor *)	Fruits weigh (g)	No. of survivor *)	Fruits weigh (g)	No. of survivor *)
1	251.6	102	298.4	119	258.3	120	296.7	116
2	289.7	118	295.0	116	280.3	118	280.8	103
3	253.9	113	289.4	122	279.3	118	272.4	106
4	274.7	106	251.5	116	271.3	132	264.1	101
5	259.9	117	268.7	114	270.2	114	277.2	115
6	261.6	91	285.3	121	285.7	126	261.2	119
7	266.6	115	261.4	123	275.8	124	267.3	102
8	288.6	114	299.4	121	273.2	124	288.6	108
9	255.2	107	257.5	124	257.6	117	296.5	111
10	255.9	103	267.2	128	264.7	124	254.7	125
11	253.4	113	258.0	121	255.1	111	296.8	115
12	274.6	104	272.4	110	261.9	124	293.7	113
13	253.2	119	256.2	111	254.2	121	266.7	111
14	274.2	117	274.3	112	256.8	122	294.8	107
15	253.7	101	289.7	114	272.4	121	252.0	121
16	260.9	113	252.4	112	261.6	125	254.8	123
17	299.2	116	252.9	111	259.4	116	285.6	121
18	256.6	114	260.8	116	266.7	122	291.3	123
19	251.9	115	279.8	122	253.3	115	256.9	111
20	285.9	121	284.6	121	286.5	129	269.0	117
21	280.8	109	274.8	125	252.1	121	276.5	112
22	274.3	105	266.6	131	251.8	123	264.2	116
23	292.8	117	251.7	118	255.0	119	287.1	103
24	276.9	113	286.6	126	256.2	115	271.6	117
25	280.2	116	284.3	113	258.6	128	250.4	121
26	293.4	116	269.3	116	266.4	124	258.7	113
27	265.8	112	274.2	114	284.3	121	281.2	114
28	263.7	105	279.8	120	270.8	118	259.3	114
29	265.1	109	267.5	114	253.4	122	256.4	120
30	290.9	111	298.2	114	252.0	119	251.3	124
Total	**-**	**3,332**	**-**	**3,545**	**-**	**3,633**	**-**	**3,422**

*) Inoculated 150 eggs each mango fruit

VI. Phytosanitary treatments for *Bactrocera dorsalis* in Vietnam

Duong Minh TU, Vietnam

Distribution and host

Oriental fruit fly - *Bactrocera dorsalis* (OFF) is native for Vietnam and other countries in Asia (CABI, CPC 2014).

Bactrocera dorsalis was firstly recorded in the North of Vietnam in 1967-1968 with scientific name is *Chaetodacus ferrugineus* (Fabricius).

The national surveillance of fruit fly in Vietnam was carried out by Dick Drew, Le Duc Khanh and Ha Minh Trung (project code ICP/VIE/8823/1999-2000) confirmed of the presence of 30 fruit fly species in Vietnam. Oriental fruit fly is one of the most serious pests of fruit crops in Vietnam.

Damage and crop loss

The OFF is a very serious pest of fruit crops in Vietnam such as orange, mango, guava, dragon and pomelo fruit, etc.

Field control and management

Methyl Eugenol in combination with DDVP has been widely used to attract and kill OFF in the field in Viet Nam since 1980s. This attractant was also used for monitoring of OFF.

More recently, protein baits are being used to monitor and suppress OFF. Two beer waste protein plants were set up (one in the South and the other in the North) to produce protein bait for management of fruit fly in general and OFF in particular.

Quarantine treatments

In 1980s, Vietnam used to export big quantities of fresh orange fruits into the USSR but OFF was a quarantine pest in the USSR. To overcome this problem, fumigation with methyl bromide (without 2% of chloropicrin) was used.

The first study on application of vapor heat treatment for oriental fruit fly on dragon fruit was carried out in Vietnam from 1998 to 2001. The second study on application of vapor heat treatment for oriental fruit fly and other fruit fly species on dragon and mango fruits was carried from 2005 to 2008. Those studies produced data that was accepted by the importing countries as phytosanitary treatment for dragon and mango fruit.

In parallel, a study on application of irradiation against oriental fruit fly on dragon fruit was carried out in Vietnam from 2001 to 2003. The effective dose to quarantine OFF on dragon fruits, accepted by the importing country (USA), is 250 Gy.

In Vietnam, fresh fruits such as dragon fruit, mango fruit, longan fruit, rambutan fruit are being treated by vapor heat treatment (5 VHT plants) or irradiation (2 irradiation plants) before export into the United State, Japan, Chile and South Korea./.

VII. Control treatment of Oriental Fruit Fly *Bactrocera dorsalis* Hendel in Thailand

Saluckjit PHANKUM; Sunyanee SRIKACHAR; Watchreeporn ORANKANOK, Thailand

Sunyanee Srikachar, Saluckjit Phankum, Walaikron Rattanadechakul, Udorn Unahawutti, Pitawat Ongthonglang, Chamlong Chettanachitara, Monnipa Srimartpirom, Chutima Ormking, Chainarat Sonsiri, Jaruwan Chantra and Rachada Intarakhumheng

Department of Agricultural, Plant Protection Research and Development Office, Bangkok, Thailand

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The oriental fruit fly *Bactrocera dorsalis* Hendel and the guava fruit fly *Bactrocera correcta* Bezzi are the major insect pests of economic fruit crop in Thailand. Monetary estimates of fruit production and fruit fly damage are not available for most countries. However, Thailand may be taken as an example, with annual fruit production running at over 40,000 million Thai Baht and potential losses if fruit flies were not controlled are believed to exceed 3,000 million Thai Baht. It is therefore very important that quarantine entomologists can make rapid identifications of fruit flies intercepted with imported fruit produce, so that measures can be taken quickly to try to prevent the establishment of new fruit fly pests. For almost all pests, and especially for fruit flies, the earliest treatments were the application of heat or cold. Heat treatments especially heated air treatment is now being widely investigated and used in many countries. Heat treatment has the merit of effective fungicidal and insecticidal action, ease of application, and leave no chemical residues. Thailand was successfully development, modified vapor heat treatment as a quarantine treatment to disinfest fruit flies in 4 mango cultivars, 'Nang Klarngwan', 'Nam Dorkmai', 'Rad' and 'Pimsen Daeng' were heated with hot air at 50-80% RH from ambient temperature to 47 0C with high temperature air saturated with water vapor, and subsequently the center temperature was kept at 47 °C for 20 min.. In mangosteen, were heated at 46 °C for 58 min and pummelo were heated at 46 °C for 30 min.

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VIII. Comparison of Phytosanitary Treatments among *Bactrocera dorsalis* complex species

Presenter: Scott Myers, USA
Co-Authors: Emily Fontenot, Guy Hallman

The issue regarding the number of species within the *Bactrocera dorsalis* complex and the potential for varying susceptibility to phytosanitary treatments across this group is cause for concern for the US and its trading partners. To address this question we conducted several small scale experiments to evaluate the potential for treatment differences among this group to cold storage, methyl bromide fumigation and hot water treatments. Laboratory reared Tephritids of the following species were compared: *B. dorsalis*, *B. carambolae*, *B. invadens*, *B. philippinensis* and *B. papayae*. Fumigation experiments with methyl bromide were performed at 5 and 15°C in 10L chambers *in vitro* with 3rd instar larvae. Hot water dip treatments were conducted in a 46°C water bath using larvae treated in bags of artificial diet to simulate fruit and with infested mangoes. Cold treatments were conducted in a $0.9m^3$ environmental chamber at 1.1°C using third instar larvae reared in navel oranges. In each case multiple treatments were made across a range of doses/days to develop dose-mortality relationships for the species of interest. Regression models were used to estimate mortality and maximum likelihood and lethal dose ratio tests were used to compare efficacy of each treatment type across the species tested. Results provide evidence to support the use of generic treatment schedules for *B. dorsalis* complex.

IX. Abstract

Peter FOLLETT, USA

Fruits and vegetables grown in Hawaii are subject to federal quarantine regulations because of four exotic Bactrocera fruit flies— *Bactrocera cucurbitae* (Coquillet), melon fly, *Ceratitis capitata* (Wiedemann), Mediterranean fruit fly, and *Bactrocera dorsalis* (Hendel), oriental fruit fly, and *Bactrocera latifrons* (Hendel), solanaceous fruit fly (Diptera: Tephritidae)—and other quarantine pests. Phytosanitary treatments disinfest host commodities of fruit flies and other pests before they are exported to the U.S. mainland where the pests do not occur. Hawaii has approved quarantine treatments (irradiation, heat, and cold treatments, and systems approaches) for 25 different tropical fruits and vegetables. Recent projects with *Bactrocera* fruit flies will be discussed including development of a systems approach to exclude oriental fruit fly in exported avocados; the increased radio-tolerance in melon fly under low oxygen conditions produced by modified atmosphere packaging of fruit and implications for export protocols; the effect of post-irradiation cold storage in enhancing the efficacy and margin of security during export; and the comparative cold tolerance of melon fly and medfly in citrus. Communication with regulatory authorities is critical to the development and adoption of new export protocols and procedures.

X. Abstract of Proposed Presentation

Isao MIYAZAKI, Japan

The Japanese government has been providing assistance to developing countries in which fruit flies of economic importance are present to develop plant quarantine treatment techniques such as vapor heat and cold treatments for disinfestation of fruit flies in fresh fruit. As part of this technical cooperation, training programs focusing on thermal quarantine treatments have been conducted in Okinawa where oriental fruit fly and melon fly were eradicated by official control project. In addition to the training program, the Japan has supported developing countries under JICA technical cooperation project by dispatching experts and providing techniques such as a vapor heat treatment. In this presentation, I would like to introduce background and results of the cooperation.

The group training program, Thermal Treatment for Disinfestation of Fruit Flies course, has been implemented by the Japan International Cooperation Agency (JICA) with the cooperation of Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan at Naha Plant Protection Station in Okinawa Island since 1988. For the past 27 years, total number of participants has reached 139 from 40 countries.

The objective of the training program is that participants in developing countries acquire development methods of plant quarantine treatment suited to respective conditions of each country. In order to achieve this objective, participants attend lectures and conduct practices concerning taxonomy and rearing methods of fruit flies, thermal quarantine treatments, etc. In particular, practices of disinfestation trial are designed to simulate the procedure for development of vapor heat and cold treatments technique, using *Bactrocera dorsalis* and *B. cucurbitae* as test insects. The subjects of these practices are 1) preparation of infested fruits, 2) survey for larval development stage in fruit, 3) hot water treatment in a susceptibility trial to determine the most tolerant fruit fly species, 4) vapor heat treatment and cold treatment in susceptibility trials to determine the most tolerant development

stage, 5) vapor heat treatment in a small-scale trial to determine fruit core temperature and dwell time to obtain 100% mortality.
The program contributed to establishment of plant quarantine measures using thermal treatments against fruit flies in several countries. Consequently, those countries succeeded to access to global market in the trade of fresh fruits.

XI. Abstract of Proposed Presentation

Toshiyuki DOHINO, Japan

Temperature treatments of ISPM 28 are expected to provide the minimum requirements necessary to control a regulated pest. Although significant amount of work and efforts are made by the TPPT over a numbers of years, consensus is not reached and cold treatment is not adopted yet at the CPM. One of the major challenges to reach consensus among the member countries is the issue related to applicability of the treatment to a wide range of countries.
Treatment schedules for international standard should be established supported by sufficient numbers of disinfestation tests verifying their efficacy and applicability to those countries/areas where target fruit flies are present. For example, draft ISPM cold treatment for Medfly should be validated by several countries rather than only one country because Medfly distributes numbers of countries, which may cause possible regional differences in cold tolerance of Medfly.
Considering the above, I would like to suggest possible steps for developing the phytosanitary temperature treatments for international standards as follows:

1. Accumulation of mortality research data
Supporting mortality research data for the same treatment condition should be sought from different countries. If such data are obtained in several countries covering the distribution area of the fruit fly, these data should be accumulated and evaluated to develop a draft ISPM.

2. Validation of efficacy on the treatment condition (Accumulation of efficacy data)
In case only one data/publication is available, efficacy of the treatment condition should be validated in several countries using the most tolerant stage of the target fruit fly. In accordance with validation test plan drawn based on the guideline for development of disinfestation treatment, collaborating countries conduct validation tests. Those countries (collaborators) may be nominated taking into account the distribution of the fruit fly. Confirmation team composed by experts from the TPPT and member countries may check test procedures and efficacy of the treatment condition, if necessary. Financial resource for validation tests needs to be explored.

3. Development of draft ISPMs and implementation
The results of validation tests conducted by collaborators under the same treatment condition are evaluated by the TPPT to develop ISPMs. The experts who attended confirmation team are expected to contribute toward developing further ISPMs and implementation of developed ISPMs.

XII. The current state of species within the *Bactrocera dorsalis* species complex

Anthony Clarke, Australia
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Bactrocera dorsalis (Hendel), *B. papayae* Drew & Hancock, *B. philippinensis* Drew & Hancock, *B. carambolae* Drew & Hancock and *B. invadens* Drew, Tsuruta & White form a highly pestiferous sibling species complex. The native and invasive range of the complex includes Africa, Asia, the South Pacific and South America. The biological species status of the different taxa within the complex has been a topic of considerable debate and is of fundamental importance to market access discussions. As part of an FAO/IAEA CRP, an integrative taxonomic approach has been applied to delimiting the biological species within this complex. Research tools used included pre- and post-zygotic compatibility trials, genetic analysis, geometric morphometrics and traditional morphology.

Results from multiple, independent species delimitation tests are consistent with a hypothesis that *B. papayae*, *B. philippinensis* and *B. invadens* are the same biological species as *B. dorsalis*. Some differentiation in phenotype and genotype does occur across the species' range, but such differences are consistent with population level variation rather than species level variation. Genetic, mating and pheromone data for *B. carambolae* infer that it is unique biological species, albeit very closely related to *B. dorsalis*. The great bulk of this research has now been published in the international scientific literature, including a formal taxonomic revision. For the species within the scope of this Expert Consultation, i.e. *Bactrocera invadens*, *B. dorsalis* s.s., *B. papayae*, and *B. philippinensis*, only *B. dorsalis* remains a valid scientific name.

XIII. Hot water immersion: Why is an effective and inexpensive phytosanitary treatment proposed for mango fruits against *Bactrocera* spp?

Emilio Hernández, Mexico

Emilio Hernández, Marysol Aceituno-Medina, José Caro Corrales, Pablo Montoya and Jorge Toledo

Hot water immersion phytosanitary treatment for infested mango fruits with larvae of *A. ludens* was developed and approved since 1989, establishing that the 'Tommy Atkins', 'Keitt', 'Kent' and 'Haden' varieties of mango with weights between 500 and 700 g must be treated for 75 and 90 min, respectively. The mango cv. 'Ataulfo' and 'Manila' with weights between 375 and 570 g must treated for 65 and 75 minutes, respectively. Each treatment the water temperature must not be lower than 46.1°C and at the end of the treatment the fruit pulp temperatures shall be at least 45°C. While, the 'Ataulfo' mangoes infested with the Mediterranean fruit fly, *Ceratitis capitata,* required an immersion at 46.4-47°C for 95 min.

Treated fruit may be hydrocooled or air-cooled immediately after treatment. In this case, the previous hot water immersion treatment needs extended an additional 10 min. Otherwise; it shall become necessary to wait 30 min and then exposing the fruit to hydrocooling or air-cooling, in both cases the temperature must be not less than 21ºC. Although mango fruit can tolerate up to 46.1 °C for 110 minutes without affecting the quality, the immersion in hot- water reduces shelf life. Hot water treatment remains effective, but requires to be improved.

Packers need follow the proper cooling techniques after hot water immersion. Poor cooling after hot water treatment is one of the primary reasons for the poor mango fruits quality.

The hot water immersion treatment is the most popular in Latin America, because is effective, inexpensive and the investment is available to any producers and packers organization. The technology is not patented and the materials and equipment are easy access. The immersion hot water tanks can be built using cement or stainless steel, the size depends on the production quantity. The heating system may be by gas. The measurement and control of temperature is generic and is easily access. A potential problem is that it require large amount of water.

Where, the water is problem, the packers could use vapor-heat, forced hot-air treatment, forced hot-air with controlled atmosphere, and irradiation.

The irradiation is the most promising phytosanitary treatment, and it could be use for packers with a low production of fruits. In this case the producers or packers need are organized in cooperatives. They need the technical support to mobilize the fruits without any treatment to the facility for irradiation, located in a central and strategic place.

The heat treatments based on the lethal dose of temperature-time. The hot-water immersion, vapour heat and forced hot-air only need little adjust for other fruits and fruit flies specie.

The above mentioned are the reasons for the which the hot water immersion treatment is recommended for mangos infested with *Bactrocera* larvae.

Appendix 5 – Introduction to the History of Phytosanitary Treatments Developed to Control the *Bactrocera dorsalis* Complex (Prepared by Mr Guy HALLMAN)

The *Bactrocera dorsalis* species complex comprises almost 100 species of which several were considered of significant economic importance. However, observations made since many of these species were named in 1994 questioned the validity of a number of them culminating in the opinion of Schutze et al. (2014), based on wide-ranging data, that the economic species *B. invadens*, *B. papayae*, and *B. philippinensis* are junior synonyms of *B. dorsalis*. The main concern of this Expert Consultation is with these synonymous species.

Bactrocera dorsalis is the most studied tephritid after *Ceratitis capitata* regarding phytosanitary treatments. One of the earliest references is Koidsumi (1930) who found that relatively low doses of ionizing radiation prevented adult emergence of 3rd instars. It would be 65 years until irradiation was used as a commercial treatment against *B. dorsalis*.

A few years after this initial irradiation research, vapour heat was being studied as a treatment against the species in Taiwan (Koidsumi 1936). Models to predict the time required to kill immature stages in fruit were developed. Phytosanitary treatments against *B. dorsalis* were researched soon after the fly was found in Hawaii in 1946 with lengthy commercial vapour heat treatments at <45°C resulting. By the early 1950s ethylene dibromide and methyl bromide began replacing vapour heat for treating fruit at risk of infestation by *B. dorsalis* and other tephritids and research on treatments greatly declined (Hallman and Armstrong 1994).

By the late 1970s research with shorter vapour heat treatments was initiated in Japan because of problems with commodities tolerating fumigants (Sugimoto et al. 1983). Shortening the treatments was accomplished by forcing heated air through the fruit load instead of letting it passively penetrate the load as in earlier treatments. Japanese researchers continued to develop vapour heat treatments for a variety of fruits over subsequent years.

The loss of ethylene dibromide beginning in the United States in 1984 spurred new research to find alternatives to that effective fumigant, and more recent effort to reduce the use of methyl bromide as a phytosanitary treatment continues to fuel the need for further research into phytosanitary treatments against *B. dorsalis* and other quarantine pests (Heather and Hallman 2008). Results have been a generic phytosanitary irradiation treatment for all fruit and all Tephritidae and hot water, heated air, and cold treatments against *B. dorsalis* in various commodities.

In 1994 the naming of *B. papayae* and *B. philippinensis* created the need for phytosanitary treatments against these purportedly new economic species, with research being done on irradiation and vapour heat. The more recent designation of *B. invadens* as a new species rapidly invading central Africa created new urgencies for developing treatments against it, and cold treatments were developed (Grout et al. 2011).

The decision that *B. invadens* and other species of *Bactrocera* are junior synonyms of *B. dorsalis* does not negate the need for research on phytosanitary treatments to facilitate trade because *B. dorsalis* is invading new regions in Africa where treatments against it do not exist. This event argues for more holistic and harmonized approaches to developing phytosanitary solutions to quarantines. In response recent advances toward this end have included a project at FAO/IAEA in Seibersdorf, Austria, funded in part by USDA-APHIS, to compare treatments for efficacy across tephritid species and populations with the objective of developing more generic solutions and the creation of a Phytosanitary Treatment Expert Group at the urging of the IPPC where critical phytosanitary treatment issues can be addressed through discussion and collaborative research. Information on the latter is at https://www.ippc.int/partners/international-organizations/phytosanitary-temperature-treatments-expert-group

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Appendix 6 – Development for phytosanitary treatments for the *Bactrocera dorsalis* complex

The development of phytosanitary treatments is often done on an emergency basis because trade is suddenly threatened, requiring an urgent solution. Therefore, it may be done hastily with a number of assumptions and shortcuts made. These assumptions might not be acknowledged or possibly even understood by the researchers and are not always well documented. Possible effects of these assumptions on commercial efficacy of the treatment might never be tested.

The following table lists a number of differences between the commercial situation for which a phytosanitary treatment is developed and the research used to develop the treatment; this list is not exhaustive. Inherent in the research is the assumption that the differences do not reduce treatment efficacy.

The experts reviewed this list and present them in order of priority for future research.

Table 1. Differences between the Commercial Environment and the Research Supporting Phytosanitary Treatments

Priority	Factor	Environment	
		Commerce	**Research**
1	Pest	Feral	Usually colony
2	Varietal tests		
3	Pest populations from diff. geographical regions		
4	Treatment rate	Sometimes slower than research	Sometimes faster than commerce
5	Infestation	Natural	Sometimes artificial
6	Food	Host plant	Sometimes diet
7	Infestation rate	Usually very low	Often high
8	Host-material relationship to plant during pest development	Attached	Almost always harvested
9	Endpoint of efficacy	Amenable to regulatory process	Sometimes not consistent with regulatory process
10	Measurement of efficacy	No survivors upon inspection	Sometimes allows for survivors when inspection would occur
11	Host source	Broad	Often limited
12	Host growth stage when infested	Pre-harvest	Usually ready for harvest
13	Rearing temperature	Ambient, variable	Often moderate, constant

Few of these assumptions have been tested for their effect on efficacy, but of those that have, some have been shown to reduce efficacy. However, the important question is if they reduce efficacy sufficiently to jeopardize commercial application. Phytosanitary treatments are usually very robust because fresh horticultural commodities are not infested at the high rate for which efficacy is confirmed.

Nevertheless, some NPPOs have not approved treatments where certain untested assumptions have been made, such as using artificial infestation with diet-reared 3rd instars. Therefore, it behoves researchers to make as few assumptions as possible (both stated and not) and explain and justify those that are made.

If an assumption results in increased efficacy because the research situation uses insects that are more tolerant than the feral situation the higher dose could result in damage to the commodity or increased costs of treatment, which, although not jeopardizing efficacy, should be avoided.

Besides the assumptions made during the research, a number of other issues should be considered when developing phytosanitary treatments. The points below are not all-inclusive.

The most tolerant life stage of the pest should be determined *in situ* so that large scale confirmatory testing can focus on the most tolerant life stage. It is insufficient to do this testing *in vitro* as the natural occurrence of the stages in the commodity can affect relative tolerance among the stages.

To make treatments more broadly applicable, different cultivars and even species of commodities should be tested for efficacy. If there is no significant difference it can be argued that the treatment should be applicable across appropriate groups of host commodities.

The dose to be used in large-scale confirmatory testing must be determined. Often this is done via dose-response testing and analysis by a statistical regression model, such as probit 9. However, the ability of any model to predict the levels of efficacy required of phytosanitary treatments (near 100%) is suspect. An iterative approach might be preferable; i.e., the dose is increased until it demonstrates that the desired level of efficacy is reached.. This may not end up being a lengthier approach because it avoids the need for the dose-response testing required for a (suspect) predictive model.

Near export-quality commodities should be used for confirmatory testing. This may be difficult to accomplish when the most tolerant stage is 3rd instar larvae and achieving sufficient numbers in fresh commodities results in partially decomposed material. Modifications of this approach would need to be justified and tested for their effect on efficacy.

The number of organisms to be tested for confirmation of a treatment varies but seems to be coalescing around 30,000 individuals for Tephritidae. This may be difficult to achieve for hard-to-rear species or hard-to-infest commodities. It can be argued that confirmatory requirements for hard-to-infest hosts should not be as high as for easy-to-infest hosts. Nevertheless a researcher’s inability to work with an invasive species should not be an excuse for lightening research requirements if the invasive pathway of the species is deemed moderate to high risk.