



# NAPPO

North American Plant Protection Organization  
Organización Norteamericana de Protección a las Plantas

## NAPPO Conference Call Report

Expert Group:	Seeds - ToBRFV	
Location:	Zoom meeting	
Date:	October 7, 2020	
Chairperson	Ed Podleckis (APHIS – PPQ)	
Participants:		
Pamela Ross (CFIA)	Huimin Xu (CFIA)	Nancy Osterbauer (APHIS – PPQ)
Vessela Mavrodieva (APHIS-PPQ)	Geoffrey Dennis (APHIS-PPQ)	Kevin Ong (Texas A&M)
Jessica Berenice (SENASICA)	José Manuel Cambrón (SENASICA)	Daniela Bocanegra (SENASICA)
Claudio Feulner (Canada Industry)	Ric Dunkle (US Industry)	Samantha Thomas (US Industry)
Marlene Ortíz (Mexico Industry)	Mario Puente (Mexico Industry)	Patricia Abad (APHIS-PPQ)
Nedelka Marín-Martínez (NAPPO)	Stephanie Bloem (NAPPO)	Alonso Suazo (NAPPO)

### Summary

Project:	A pilot for harmonization of diagnostic protocols for seed pests focused on ToBRFV.
General comments:	The Chairperson welcomed the EG members. Pamela Ross (CFIA) gave a brief introduction of Dr. Huimin Xu, a new member of the EG.
Item 1:	Summary of previous calls
Consensus:	<p>The Chairperson reminded the EG the following:</p> <ul style="list-style-type: none"> <li>• The EG work is a two-part project: <ul style="list-style-type: none"> <li>○ First, to develop a term of reference (ToR) for developing a process to harmonize diagnostic methods for the ToBRFV among the three NAPPO countries. The ToR document will be submitted to the NAPPO EC for approval.</li> <li>○ Second, implement a work plan. The result will be documented for the EG to draft a recommendation statement for the EC to consider.</li> </ul> </li> <li>• The initial discussions with the group to outline the objectives of the project.</li> <li>• The original “flowchart” presented to the EG and the decision to focus on the necessary steps in such flowchart.</li> <li>• It was decided to create two subgroups (SG) based on the flowchart and the project objectives. More information on</li> </ul>

	<p>the subgroups is provided in the June 10 conference call report available in the NAPPO website.</p> <p>The Chairperson thanked members of the subgroups for the work they have been conducting.</p>
Item 2:	Updates from the Seed EG subgroups
Consensus:	<p><b>Updates from subgroup 1-</b> Vessela Mavrodieva provided the following updates:</p> <ul style="list-style-type: none"> <li>• SG has had 3 conference calls.</li> <li>• The SG objective is to compile a list of available protocols for the detection of ToBRFV and decide which protocols can be considered for the ring tests.</li> <li>• Indicated that the SG compiled a list of 15 endpoint RT PCR protocols and several conventional PCR protocols and a digital droplet protocol (dd RT PCR) for consideration.</li> <li>• Indicated that validation data for each of the compiled assays were collected from all three NAPPO countries.</li> <li>• Informed that the SG decided to remove the dd RT PCR because it requires specialized equipment not available in every lab.</li> <li>• The subgroup started discussions on the desired characteristics of the RT-PCR assays: <ul style="list-style-type: none"> <li>○ Selected assays should have the best sensitivity and specificity.</li> <li>○ Consider assays based on two genomic regions.</li> <li>○ Avoid sequencing for final determination. Accurate results should be provided with the PCR only.</li> <li>○ Assays should be internationally accepted; however, only one internationally accepted assay is in the current list developed by the SG.</li> <li>○ Other internationally accepted assays were rejected because of deficiencies found by the SG (sensitivity and specificity).</li> </ul> </li> <li>• SG agreed on other criteria for assays to use in ring tests.</li> <li>• SG agreed that testing will be conducted in seeds and not plant tissues (from germinated seeds).</li> <li>• SG decided not to work on the harmonization of RNA extraction from seeds.</li> <li>• SG will develop a detailed check list for documentation of RNA extraction processes from seeds. Participant labs will fill this form (checklist) to provide sufficient details for the analysis of the results.</li> <li>• Selected assays should be cost effective and user friendly.</li> <li>• SG needs to discuss the controls used in the selected assays.</li> <li>• SG needs to discuss how many assays will be conducted</li> </ul>

	<p>by the selected laboratories.</p> <ul style="list-style-type: none"> <li>• SG needs to discuss laboratories resources to use the selected protocols.</li> <li>• SG needs to review the list of selected assays to generate a final list and needs to develop a list of performance characteristics to use for the assays.</li> </ul> <p>Following the updates from subgroup 1, Vessela presented the data sheets with specific information on the protocols that have been compiled.</p> <p><b>Updates from subgroup 2</b> – Geoffrey Dennis provided the updates:</p> <ul style="list-style-type: none"> <li>• Subgroup tasks <ul style="list-style-type: none"> <li>○ Compile a list of international standards and determine which standards are more appropriate to consider for the validation criteria. Geoff presented a list to the EG and described the subgroup categorization of the international standards. Feedback was received from Canada and Mexico.</li> <li>○ Put the information compiled from the selected international standard into a document. The subgroup is working on this document which will be presented to the expert group once it is completed. This document will provide ample documentation on the criteria that will be taken into consideration for protocols validation (number of samples tested, characteristics of the material to use, type of controls etc.). This information will be used to design the protocols validation master plan.</li> </ul> </li> </ul>
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**Next Steps**

Responsible Person	Action	Date
NAPPO Secretariat	Schedule a call with subgroups 1 and 2 to continue discussions on the outputs generated by each subgroup.	
	Share data on protocols including validation data on protocols considered by subgroup 1 or additional protocols not listed by subgroup 1.	

**Next Meeting**

Location:	Zoom meeting - Videoconference
Date:	November 10 from 11:30 a.m. to 1:00 p.m. EST

**Proposed Agenda Items**

1.	
2.	