

Diagnostic Protocols for Regulated Pests of Potato

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Potato Panel Members

- USA – Rick Zink, Chairperson
- USA – Will Wise
- Canada – Nathalie Bruneau
- Canada – Solke De Boer
- Mexico – Rene Hernandez
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Industry Contacts

- USA – Dan Moss
- Canada – Larry Buba
- Mexico – Hugo Gomez Arroyo

Bacterial Ring Rot TAG

- USA – Rick Zink, Chairperson
- USA – Amy Charkowski
- Canada – Solke De Boer
- Mexico – Alberto Flores Oliva

BRR TAG Accomplishments

Manuscript

“Procedure for Detection and Identification of *Clavibacter michiganensis* subsp. *sepedonicus*”

Revista Mexicana de Fitopatología

(De Boer *et al.*, 2005. 23:329-334)

Appendix 6 to RSPM No. 3

Detection and Identification of *Clavibacter michiganensis* subsp. *sepedonicus* (CMS)

Manuscript Addresses

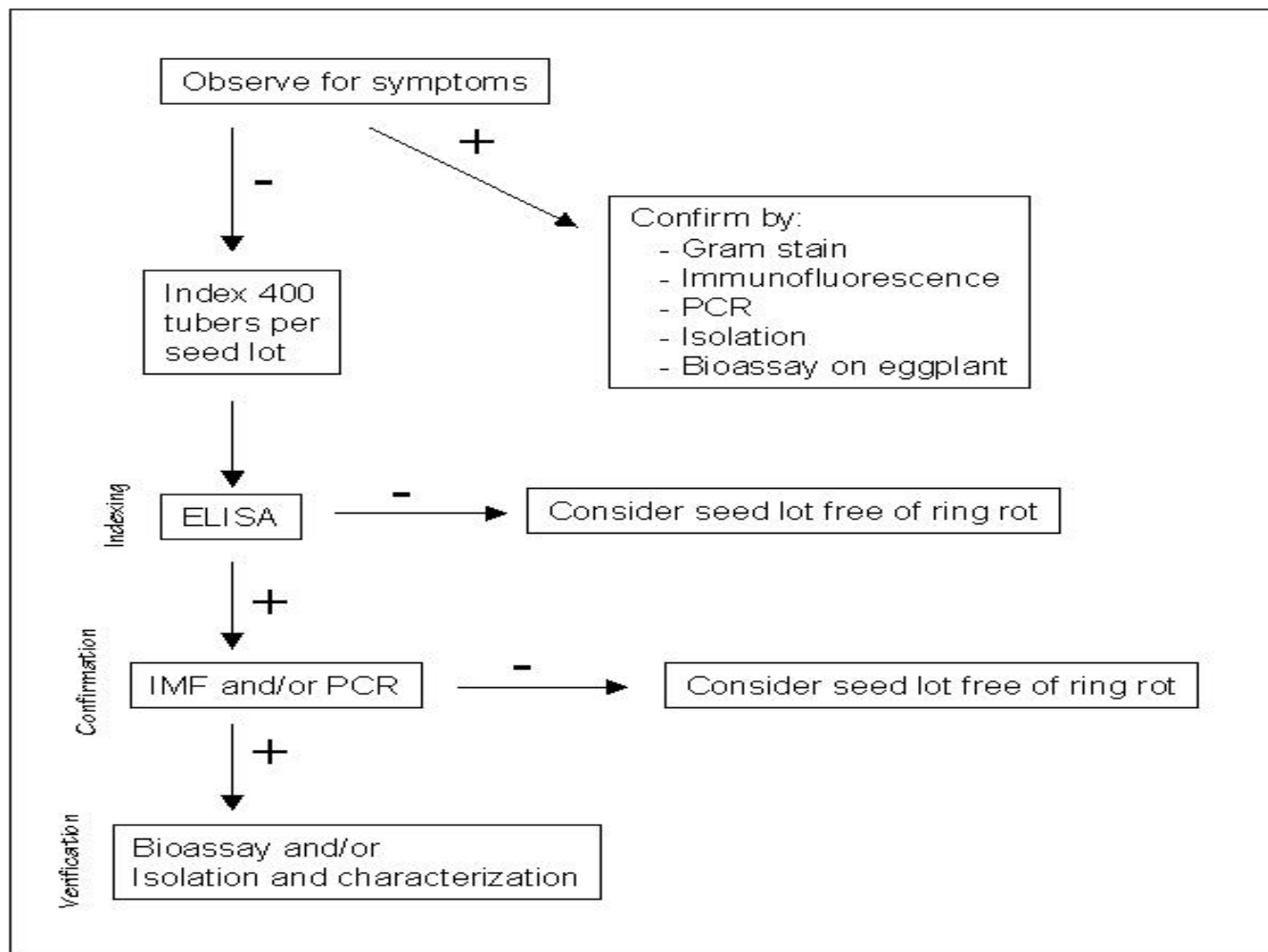
- Sample criteria
- Confirmation of ring rot symptoms
- Test protocols
- ELISA
- Immunofluorescence
- PCR
- Bioassay on eggplant
- Isolation and characterization
- Interpretation of results

Appendix 6

Detection and Identification of CMS

- Sample collection and sample size
- Diagnostic methodologies
- ELISA – initial index test
- Immunofluorescence
- PCR – confirmatory test
- PCR primers
- Bioassay
- Isolation and characterization
- References

Scheme for Indexing Seed Potato Lots for CMS



Comments Submitted During Country Consultation on Draft Appendix 6

- Who are “persons officially designated by the exporting NPPO”
- What is meant by “qualified plant pathologist”
- NPPO vs. NAPPO
- Should have a standard for collection, transportation and storage of samples
- ELIAS or PCR for detection or confirmation

Nematology TAG

- USA – Lynn Carta, Chairperson
- USA – Zafar Handoo
- USA – Tom Powers
- Canada – Sylvia Miller
- Mexico – Ricardo Perez Zubiri
- Mexico – Angel Ramirez

Nematology TAG Accomplishments

Manuscript

“Guidelines for Isolation and Identification of Regulated Nematodes of Potato in North America” *Revista Mexicana de Fitopatología* (Carta *et al.*, 2005. 23:212-222)

Appendix 7 to RSPM No. 3

Guidelines for Identification of *Meloidogyne chitwoodi*, *Globodera rostochiensis*, *G. pallida*, *Ditylenchus destructor* and *D. dipsaci*

Manuscript Addresses

- *Taxonomy of Globodera rostochiensis and G. pallida, Meloidogyne chitwoodi, and Ditylenchus destructor and D. dipsaci*
- Ideal source material for species identification
- Symptoms, sample size and extraction
- Microscopy and archival methods: traditional, digital and DNA
- References for molecular identification
- Positive and negative PCR controls for each species
- Ideal primer sets for species combinations
- Interpretation of results

**Determination of *Meloidogyne chitwoodi*, *Globodera rostochiensis*,
Ditylenchus destructor and *D. dipsaci* from Potato tubers**

**Sample tubers in sample lot for nematodes
by washing, peeling and dissection of atypical tissue**

Meloidogyne

Globodera

Ditylenchus

Females
Swollen, necrotic tissue

Cysts and juveniles (J2)

Females, juveniles
Woolly, sunken, cracked

Identify by
Perineal pattern: striae,
lateral lines
Median bulb vesicles
Stylet length, knob shape

Identify by
Cyst morphology
J2 head, tail shape
J2 body, tail length

Identify by
Stylet length
Post-vulval sac length
Tail tip shape, length
Lateral lines

Photographs, measurements, voucher slides

Confirm by:
PCR

Confirm by:
PCR

Confirm by:
PCR

Verify by sequencing if ambiguous

Appendix 7 to RSPM No. 3

Nematode Identification

- Sample collection and sample size
- Diagnostic methodologies
- Microscopic morphology – primary test
- PCR – confirmatory test
- PCR primers and restriction enzymes
- References

Comments Submitted During Country Consultation on Draft Appendix 7

- No comments were received

Acknowledgments

- Dr. Lynn Carta, Research Plant Pathologist, USDA ARS
- Dr. Solke De Boer, Section Head for Potato Diseases, CFIA

Current Panel Activities

- Drafting Appendix 8, Requirements for Recognition of Pest Freedom for *Globodera pallida* and *G. rostochiensis*
- Diagnostics for PVY
- Required five year review of RSPM No. 3