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Specialized Section on Standardization of Seed Potatoes

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Survey on bacterial testing methodologies — conclusions

Report on the survey on bacterial testing methodologies

Submitted by the secretariat

1. At its 2021 session, the Specialized Section reviewed preliminary results of the bacterial testing methodologies survey. The discussions were led by the delegate from the United States of America on behalf of the rapporteur's group (Finland, Israel, Netherlands, United Kingdom of Great Britain and Northern Ireland, United States of America).

2. The Specialized Section is invited to review an updated report prepared by the United States of America and discuss a possibility of issuing a position paper, which would be published on the UNECE website.

3. The document is submitted according to ECE/CTCS/2021/7 section V, ECE/CTCS/2021/2 Decision 2021-07-07, and A/76/6 (Sect. 20).

4. The UNECE Specialized Section on Standardization of Seed Potatoes completed a survey on testing methods for bacterial pathogens of potato that are associated with seed certification in March of 2021. There was a total of 51 responses received from 32 countries (Table 1.) 30 of the responses were complete and 21 were partially complete.

Table 1:

Respondents to the survey of testing methods for bacterial pathogens of potato that are associated with seed certification.

	Country
1	Australia
2	Belgium (2)
3	Bulgaria (5)
4	Croatia
5	Cyprus
6	Czech Republic
7	Denmark
8	Egypt
9	Estonia (2)
10	Finland
11	France (2)
12	Germany (2)
13	Greece
14	Italy
15	Japan
16	Latvia (5)
17	Lithuania
18	Luxemburg
19	New Zealand
20	The Netherlands
21	Republic of Ireland
22	Russian Federation
23	Serbia
24	Slovak Republic
25	Slovenia
26	South Africa
27	Sweden
28	Switzerland
29	Poland

	Country
30	United Kingdom (2)
31	United States of America (8)

5. The survey was designed to assess the importance of individual blackleg pathogens within *Pectobacterium* spp. and *Dickeya* spp., *Clavibacter michiganensis* subsp. *sepedonicus* (CMS), and *Ralstonia solanacearum* (Brown Rot), in different countries, and the methods used for diagnosis.

6. The overall response rate for the survey was very high at 51 respondents, but the full completion of the questions dropped significantly as more specific questions were posed, resulting in 31 complete sets of answers. In the case of blackleg, the response rate was higher for questions directed towards *Pectobacterium* spp. and dropped off slightly for questions on *Dickeya* spp. For *Pectobacterium* spp., *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum* were the most common species associated with blackleg and for *Dickeya* spp., *D. solanacearum* and *D. dianthicola* were the most frequently reported. Tubers were the most commonly tested tissue for blackleg, and enrichment or incubation was rarely used. PCR was the most common method to detect all blackleg pathogens and small sample sizes of 1-50 tubers were typically used. Most labs sample the heal/stolon end of the tuber and the samples are generally bulked. Most labs use published PCR primers and sequencing for *Pectobacterium* spp. and *Dickeya* spp. Sequencing is performed by 11 of the responding programs to identify species.

7. The response rate on questions on *Clavibacter michinganensis* subsp. *sepedonicus* (CMS) was lower than for blackleg and may be due the fact that only 55 per cent of the countries/states/provinces report presence of CMS. Two-thirds of the respondents indicated that testing for CMS was compulsory in their area and performed by their organization. Reliability of the test is highly important. Tubers are the primary sample material and stems and microplants are also sampled by many programs. Enrichment and incubation were used 21 and 16 per cent respectively with the most common assay being PCR. The most common sample size was 51-200 tubers. Plugs or peals were primarily taken on the stolen end. Immunofluorescence (IF) is used in some labs. Most programs use commercial kits for nucleic acid extraction rather than an in-house method, and use published primer sequences.

8. The response rate for questions on *Ralstonia solanacearum* (Brown Rot) was nearly identical to that for CMS, and 55 per cent of respondents indicated that brown rot was found in their country/state/province. Tubers comprised the most common sampled material and enrichment and incubation were only used 23 and 16 per cent of the time respectively. 51-200 was the most common sample size for tubers and 1-50 units were more commonly sampled for stems and microplants. For tubers, plugs take at the stolon end was the most common sample type. Commercial IF kits are commonly used for detection of *Ralstonia solanacearum* and in-house methods are employed in some programs. PCR for *Ralstonia solanacearum* is also performed and kits for nucleic acid extraction are commonly used as well as some in-house methods. The majority of labs pool plant material prior to PCR and published primer sequences are used for detection. Sequencing to identify species is performed by 10 of the respondents.

9. The most common use of lab results by the certifying authority is to provide growers information and as part of their certification program. 56 per cent of the respondents replied that *C. michiganensis* subsp. *sepedonicus* and *R. solanacearum* are zero tolerance pathogens in their country, state or province. In situations where the pathogen is classified as zero tolerance, it was more likely the lab had to receive accreditation to perform specific diagnostic assays, that the labs procedures were validated, and that the labs perform ring or proficiency tests. 88 per cent of the respondents indicated that they have internal quality control systems in place.

10. Common themes that emerge from these responses are that blackleg pathogens more commonly cause disease problems in potato than CMS or *Ralstonia*. The reliability of the assay is the most important feature for selecting a diagnostic method and PCR is overtaking ELISA and IF as the most common detection method for bacterial pathogens. Tubers are the most

frequently sampled material and the sample number increases when the pathogen is considered zero tolerance. Labs that conduct testing for zero tolerance pathogens are more likely to receive accreditation to perform the required assays, and there are more rigorous measures in place to ensure the accuracy of their performance.