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Draft survey on bacterial testing methodologies

Update on the survey on bacterial testing methodologies *

Submitted by the secretariat

At its 2020 session, the Specialized Section reviewed and updated the draft bacterial testing methodologies survey. The discussions were led by the delegate from the United States on behalf of the working group (Finland, Israel, Netherlands, United Kingdom, United States).

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 were 51 responses received from 32 countries (Table 1.) Of those, 30 of the responses were complete and 21 were only partially complete.

The Specialized Section is invited to review a first preliminary update on the survey, prepared by the United States.

The document is submitted according to ECE/CTCS/2019/10 section IV, ECE/CTCS/2019/2 Decision 2019-8.6, and A/75/6 (Sect. 20).

* Submitted on the above date to allow for additional survey completions.

Table 1:

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

	Complete	*Incomplete*
1	United States (x6)	United States (x3)
2	Poland	Egypt (x2)
3	France	Cyprus
4	Denmark	Serbia
5	Italy	New Zealand
6	Lithuania	Germany
7	Australia	Republic of Ireland
8	Germany	France
9	Belgium	Bulgaria
10	Finland	Latvia – incomplete (x3)
11	Croatia	Czech Republic
12	Latvia	Belgium
13	Switzerland	Estonia
14	Slovenia	X
15	Sweden	A (x2)
16	Greece	
17	Russian Federation	
18	The Netherlands	
19	Slovak Republic	
20	Estonia	
21	Luxembourg	
22	South Africa	
23	United Kingdom	

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.

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 30 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 9 of the responding programs to ID species.

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% 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 25 and 19% 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.

The response rate for questions on *Ralstonia solanacearum* (Brown Rot) was nearly identical to that for CMS, and 55% 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 15 and 12% 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 solanacearumi* 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 7 of the respondents.

The most common use of lab results by the certifying authority is to provide growers information and as part of their certification program. 57% 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. 90% of the respondents indicated that they have internal quality control systems in place.

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.